Islam et al., *Journal of Innovation in Applied Research* (2023), 6 (1): 10-22
https://doi.org/10.51323/JIAR.2023/6/1/10-20

Journal of Innovation in Applied Research
eISSN: 2581-4206

ORIGINAL ARTICLE

Dietary influence of selected microalgae on carotenoid concentration, nutrition and biochemical composition of fry Nile tilapia

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Received: 11 November 2022
Accepted: 004 December 2022
First Published: 10 February 2023

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Abstract: Abstract : To study the effects, of *Tetraselmis* sp. and *Nannochloropsis* sp. microalgae on carotenoid concentration, nutrition and biochemical composition of fry Nile tilapia (primary weight 0.023) an 8-week feeding trial was conducted. Treatments were designed with five experimental diets: CF (0% microalgae which is control feed), N25 (25% *Nannochloropsis* sp.), T25 (25% *Tetraselmis* sp.), N50 (50% *Nannochloropsis* sp.) and T50 (50% *Tetraselmis* sp.) respectively. Two hundred seventy Nile tilapia fry were randomly assigned in 15 rectangular tanks in triplicate. Fish were sampled at the end of 8-week feeding trial to determine the total carotenoid concentration, nutrition and biochemical composition of Nile tilapia body tissue and significant ($p< 0.05$) changes of values were observed. The Highest protein concentration in whole fish muscle was recorded in control. The Highest lipid and carbohydrate were recorded in N50 and T50 treatment respectively. Comparatively, higher carotenoid concentration was revealed in N50 than control. Increasing dietary *Nannochloropsis* sp. and *Tetraselmis* sp. has also significantly ($p< 0.05$) increased the percentage of saturated fatty acids (SAFs), polyunsaturated fatty acids (PUFAs) like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) compared to control. Thus, this study revealed that microalgae can be a potential source for fish pigmentation, nutrition and biochemical composition.

Keywords: Nile tilapia; Microalgae; Fatty acids; Nutrition; Carotenoids

1. Introduction

Microalgae are unicellular photosynthetic organisms and capable of converting light energy, nutrients and carbon di oxide into new algal biomass. Polysaccharides, proteins, lipids, pigments and vitamins are some biologically valuable components which are produced by microalgae through the utilization of carbon di oxide with the help of sunlight(Gouveia et al., 2009; Bellou et al., 2014; Moha-Leon et al., 2018). A good amount of protein, carbohydrate and oil deposition in microalgae depends on algal strain and their growth environment(Draaisma et al., 2013). So, microalgae play a vital role in fish larval, molluscs and crustaceans direct nutrition(Muller-Feuga, 2000). Cultivation of photosynthetic microalgae have received great interest in the field of aquaculture due to their high nutritional profile which makes them good sources of high-quality proteins, sterols and PUFAs like, EPA, arachidonic acid, linoleic acid and DHA etc(Muller-Feuga, 2000; Hemaiswarya et al., 2010). Antioxidant content also present in microalgae(Hemaiswarya et al., 2010), are essential for increasing larval growth, development, survivality and metamorphosis(Muller-Feuga, 2000; Hemaiswarya et al., 2010). Nannochloropsis sp. are unicellular microalgae which possess only one chloroplast and have cell wall consisting with polysaccharide. They are considered as a high nutritional species widely used in aquaculture for hatchery grown herbivores, such as larval and juvenile bivalves(Okauchi, 2004).

It is recognized as the potential algae for industrial applications as a consequence of its capability to build up high levels of polyunsaturated fatty acid. Chlorophyll a, astaxanthin, zeaxanthin and canthaxanthin are some valuable pigment components which are found abundant in *Nannochloropsis* sp.(Lubian et al., 2000) and also regarded as a potential source of essential ω-3 PUFA, EPA (20:5ω-3)(Sukenik et al., 1990; Renaud et al., 1991; Renaud et al., 1994). Gbadamosi and Lupatsch (2018) concluded that fatty acid profile of Nannochloropsis salina increases the ω-3 PUFA in edible fish and enhances the essential nutrient components for diet of human beings. *Tetraselmis* sp. are unicellular flagellated chlorophytes with rapid growth rate and can withstand with broad range of temperature and pH (Khatoon et al., 2014). They are known to have a promising nutritional profile containing sufficient amount of protein, carbohydrate, lipid and fatty acids which are vital for cultured organisms. Different bioactive compounds such as vitamin E, carotenoids etc. are abundantly found in *Tetraselmis* sp.(Ismaiel et al., 2016). According to Pereira et al.(2019) *Tetraselmis* sp. contain PUFA, EPA and α-linolenic acids, which are important for different nutritional applications. *Tetraselmis chuii* is a widely used microalgae that is thought to be an excellent source of long-chain PUFAs, particularly EPA(Meseck et al., 2005; Zaki & Saad, 2010). Carotenoids are considered as one of the most important group of pigments which provides various colors like yellow, red and orange to fish, crustaceans, animals and plants skin and tissues (Kop & Durmaz, 2008).Different plant species, various fungi, heterotrophic bacteria and photosynthetic prokaryotes can produce lipophilic pigments regarded as carotenoids. They highly contribute in photosynthetic activity because carotenoid contains different pigments which harvests lights and prevents photo oxidation (Hirschberg, 2001; DellaPenna & Pogson, 2006; Walter & struck, 2011).Many fish deposit carotenoids in their integuments and gonads but in case of salmonids, they accumulate carotenoids in muscle. According to Torrissen and Nsevdal(1988) reported that, in matured fish species high quantity of carotenoids are observed in its integument and ovaries, almost 90% carotenoid was also observed as free form in fish flesh. Different aquatic species like fish and other animal species could not able to produce carotenoid pigments in their skin and muscle tissues as a result they are completely dependent on dietary carotenoids which regulates coloration in fish and other animal species. Teimouri et al. (2013) concluded that dietary supplements with Spirulina platensis increased pigmentation in rainbow trout.

Nile tilapia is considered as one of the most important farmed fish species around the world because of its high commercial significance. Nile tilapia is well accepted due to presence of high amount of nutrients like proteins, minerals and essential fats. Thus, this study is aimed to evaluate the dietary influence of selected *Nannochloropsis* sp. and *Tetraselmis* sp. microalgae on the pigmentation, nutrition and biochemical composition of Nile tilapia fry.

2. Materials and methods

2.1. Microalgae strain collection and study area

Tetraselmis sp. and *Nannochloropsis* sp. isolates were chosen and obtained at the Faculty of Fisheries live feed research corner laboratory at Chattogram Veterinary and Animal Sciences University. Seawater was obtained from the neighboring Sagorika Sea Beach (Kattoli in Chattogram) in order to make medium for microalgae cultivation. Seawater was brought into laboratory and stored for overnight in plastic tanks to settle down the solid particles of water. Initially, a filter bag was used for filtering the sea water avoiding the settled solid particles. After that, in a vacuum pump Whatman filter paper (GMF Circles 4.7 cm) was used for fine filtration and then filtered seawater was sterilized using autoclave for 15 minutes at 121°C. For microalgae cultivation filtered and sterilized seawater was used. Sufficient freshwater circulation facilities were ensured to conduct the feeding trial. For UV sterilization freshwater was stored into two 80 L (each) plastic containers so that sterilized water can be circulated into the entire culture system. This method was carried out for 8 weeks in the Chattogram Veterinary and Animal Sciences University's Faculty of Fisheries' Wet Laboratory, in accordance with the water requirement in the fish culture tank.

2.2. Microalgae mass cultivation

Tetraselmis sp. and *Nannochloropsis* sp. were mass cultured in separate 20 L clear plastic jars in indoors at 25°C. Following the proportions provided by Tompkins et al. (1995) Conway medium was prepared so that microalgae strains could grow within sufficient nutrient enrich environment. To prepare 1 L Conway media, 1 mL of solution A, 0.5 mL of solution B, and 0.1 mL of solution C were mixed together in filtered and sterilized saltwater. The microalgae culture volume was gradually increased from a starting stock culture volume of 20 mL to a final culture volume of 20 L. In each flask for batch cultivation, 20 mL of microalgal stock was combined with 30 mL of culture media. and the total culture volume was 50 mL. Then the volume of culture was scaled up gradually such as, 0.25 L, 0.5 L, 1 L, 2 L and at the end expanded up in plastic container of 20 L, following the batch cultivation method. During the exponential period of development(Islam et al., 2021) the cultures were shifted into next batch. Microalgae cells were centrifuged for 5 min. at 5000 rpm (HERMLE Z 206A, Germany), after reaching the microalgal growth into the stationary phase(Islam et al., 2021). The wet biomass was collected after centrifugation and then dried into oven for overnight at 60°C temperature. A hot air oven (JSR Korea's Natural Convention Oven LNO-150) was utilized for oven drying, and a mortar and pestle was employed to ground oven-dried microalgae biomass into small particles (0.4-0.5 mm in diameter). The powdered microalgae were then kept in a standard freezer at 4°C until further usage in feed preparation. The feeding trial was conducted when sufficient dried biomass of each microalga had been obtained through batch culture.

2.3. Experimental diets

Five experimental diets with different percentages of microalgal biomass (N25%, T25%, N50%, T50%) were prepared using local feed ingredients depicted in Table 1 and feed with no microalgae (0%) was used as control. All the feed components were grounded, sieved and oven dried during the process of feed preparation. Commercialized fish meal was replaced up to 25% and 50% with microalgal dried biomass of *Nannochloropsis* sp. and *Tetraselmis* sp. In the following Table 1 the proximate composition of five experimental diets for fry Nile Tilapia is given away.

Table 1. All feed components and nutritional level of experimental diets (dry weight basis).

Inclusion level (%)					
Feed components	**CF**	**N25**	**T25**	**N50**	T50
Commercialized fish meal	67.55	50.67	50.67	33.77	33.77
Nannochloropsis sp.	0.00	16.88	0.00	33.78	0.00
Tetraselmis sp.	0.00	0.00	16.88	0.00	33.78
Rice bran	9.65	9.65	9.65	9.65	9.65
Wheat flour	9.65	9.65	9.65	9.65	9.65
Corn flour	9.65	9.65	9.65	9.65	9.65
Vitamin and mineral mixture	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00
Molasses	0.50	0.50	0.50	0.5	0.5
Total	100	100	100.00	100.00	100.00
Nutritional level of experimental diets (%)					
Carbohydrate	20.43	22.33	23.19	23.18	26.22
Protein	40.00	36.03	38.00	33.45	36.90
Lipid	11.21	14.37	12.29	16.22	13.11

CF:indicates control feed with no replacement of fish meal; **N25**:indicates 25% replacement of fish meal with *Nannochloropsis* sp.; **T25**:indicates 25% replacement of fish meal

with *Tetraselmis* sp.; **N50**:indicates 50% replacement of fish meal with *Nannochloropsis* sp.; **T50**:indicates 50% replacement of fish meal with *Tetraselmis* sp.

2.4. Fish source along with experimental design

Nile tilapia fish fry were taken from a commercial tilapia hatchery called "Niribili Tilapia Hatchery" (located in Cox's Bazar). To conduct the feeding trial experiment for 8 weeks, 270 Nile tilapia fish fries (0.023$\pm$0.0001 g of mean individual weight) of 14 days old were used. Fries were allocated randomly in 15 transparent, glass aquariums (45$\times$30$\times$30 cm) with a capacity up to 30 L water. To conduct this experiment five treatments were designed namely CF, T25, T50, N25, N50 in triplicate form. Prior to stocking, the fish were acclimatized in a large tank under laboratory conditions for two days. To maintain optimum O_2 level in rearing tank constant air circulation was supplied using aerator pump with perforated air stone in each tank. Fish were fed commercial starting feed for two days during acclimation. Towards the end of the conditioning period, according to the experimental design, 18 fish per tank were stocked encompassing 18 L water in each rearing tank. Diet prepared with *Nannochloropsis* sp. and *Tetraselmis* sp. were fed to Nile tilapia fry, in addition to a control feed containing no algae replacements. Prepared 5 categories of diet were grounded and given to the fish at 8 AM, 11 AM, 2 PM, and 5 PM on a day, based on 15% according to their body weight. Fish waste and residual meals have been regularly sifted out from the bottom of each aquarium. Daily, 1/3 of the culture water in the experimental tank was replaced with new and purified (UV-sterilized) tap water. Physical parameters (temperature, pH, O_2) were checked daily and chemical parameters (TAN, NO_2-N, SRP) were analyzed weekly to check the variables of rearing environment.

2.5. Determination of carotenoid concentration in fish tissue

After feeding experiment for 8-weeks, fish were sampled, and three fish were collected from each tank to measure the pigment density and the level of color enhancement of fish tissue due to microalgae fed diet. Fish were starved for 24 h before sampling. Olson(1979) method of pigment extraction and determination was followed. Excluding the alimentary canal and head, 1 gram body tissue of Nile tilapia fish was removed as a sample from each tank and stored in a screw-capped transparent glass vials with a volume of 10 mL. The glass vial was then filled with sodium sulphate (2.5 g anhydrous Na_2SO_4). Then the sample was homogenized with hand-homogenizer and 5 mL chloroform was added in that vial and left at 0^0C for overnight. When a clean layer of 1-2 cm was produced above the caked residue, optical density was measured using a spectrophotometer (T80 UV/VIS spectrophotometer, UK) at 380 nm, 450 nm, 470 nm, and 500 nm to compare the maximum reading range for computation. Before being read, 0.3 ml chloroform aliquots from each glass vial were collected and mixed to 3 mL ethanol at a concentration of 100%. Similarly, for comparison a blank was prepared. For the computation, the wavelength with the highest absorption was utilized. The maximum wave length absorbance was calculated by following formula:

$$Total\ carotenoids\ (\mu g/g) = \frac{\lambda_{max}\ (nm)}{sample\ weight\ (g)\ \times 0.25} \times 10$$

where, dilution factor =10; extinction coefficient= 0.25

2.6. Sample préparation

At first, whole fish samples were oven dried for overnight (Natural Convention Oven LNO-150, JSR Korea) at 40^0C. Oven dried fish samples were grounded using a mortar and pestle to form into powdered format for the analyses of protein, carbohydrate, lipid content and fatty acids of Nile tilapia fish.

2.7. Chemical analyses of proximate composition

To assess the protein content, the Lowry et al.(1951) method was followed. In addition to solution preparation, 25 mL of deionized water were combined in and placed into a test tube with 5 mg of oven-dried and powdered fish sample. From the prepared sample solution about 0.5 mL aliquot was taken out. Reactive 1 and 2 solution were prepared previously so that mixed reagent

can be made. Protein content of fish samples were evaluated by the addition of 1 N sodium hydroxide, subsequently addition of alkaline copper solution and Folin reagent. Using bovine serum albumin at different concentrations, standard solutions was prepared. The absorbance of the prepared sample solution was measured using a spectrophotometer (T80 UV/VIS spectrophotometer, UK) at a wavelength of 750 nm. Carbohydrate analysis was carried out using the technique developed by Dubois et al. (1956). The samples were tested by adding 1 mL of 5% phenolic solution and 5 mL of concentrated sulphuric acid. The absorbance of the prepared sample solution was measured using a spectrophotometer (T80 UV/VIS spectrophotometer, UK) at a wavelength of 488 nm. According to the sulfuric acid-charring method of Marsh and Weinstein(1966), lipid analysis was conducted. Then followed the carbonization technique using tripalmitin as standard, after the extraction of lipids according to Bligh and Dyer(1959) methods. At 200°C for 15 minutes carbonization was performed. The optical density measurement for each solution was done with a wavelength of 375 nm using a spectrophotometer (T80 UV/VIS spectrophotometer, UK).

2.8. Analyses of biochemical composition

The direct methylation techniques of Divakaran and Ostrowski(1989) was followed to prepare fatty acid methyl esters (FAMEs). To begin, the total lipid content of the sample was determined using the Soxhlet device. As a lipid extraction solvent, diethyl ether ($(C_2H_5)_2O$) was used, and the last stage of lipid extraction was carried out at 60°C. This extracted lipid served as the final lipid sample for the FAMEs study. For the examination of the FAMEs of the samples, a gas liquid chromatograph (GC-2010 Plus, Shimadzu, Japan) with a high sensitivity FID and clean detector gas flows and a capillary pillar (0.25 μm of film width, 0.25 mm of inner diameter, 25 m of length) was employed. The injection volume was 1 μL. The column temperature was: firstly (50-280)^{0}C at 3^{0}C/min and then (45-180)^{0}C at 2^{0}C/min. Compared to retention periods of established standards, individual FAME peaks were identified. Initially, the levels of each fatty acid were expressed as ppm and then transformed into percentages of total fatty acids (TFAs).

2.9. Statistical analysis

Microsoft Excel was utilized to compute the mean and standard error mean (SEM) of the acquired data. The significance level ($p< 0.05$) of total carotenoid concentration, protein, carbohydrate, lipid, and fatty acid concentrations were then determined using one-way analysis of variance (ANOVA). Finally, the Multiple Range Test of Duncan was performed using IBM SPSS software (v.26) to compare the means of each treatment.

3. Results

3.1. Total carotenoid concentration of fish tissue

In this study, microalgae were proven to be an efficient color enhancer and values of five treatment CF, N25, N50, T25 and T50 varied significantly ($p <0.05$). Between the *Nannochloropsis* sp. and *Tetraselmis* sp. microalgae fed diet *Nannochloropsis* sp. exhibited amazing color enhancing performance in Nile tilapia fish tissue in comparison to *Tetraselmis* sp. Among 380 nm, 450 nm, 470 nm and 500 nm wave length, the highest absorption was recorded in 450 nm wavelengths. As a result, absorption values in 450 nm wavelengths were used for the determination of total carotenoid concentration. The highest concentration of total carotenoid content in wet weight was documented in N50 (3.27 ± 0.04 μg/g) and the lowest concentration was recorded in CF (0.87 ± 0.03 μg/g) (Figure 1). Correspondingly, the total carotenoid concentration in T50, N25 and T25 treatment also showed significantly ($p <0.05$) higher values compared to CF (Figure 1).

3.2. Fish nutrition

After the end of feeding trial, the protein, lipid and carbohydrate percentages of Nile tilapia (*Oreochromis niloticus*) fry of each treatment were determined by chemical analysis process and

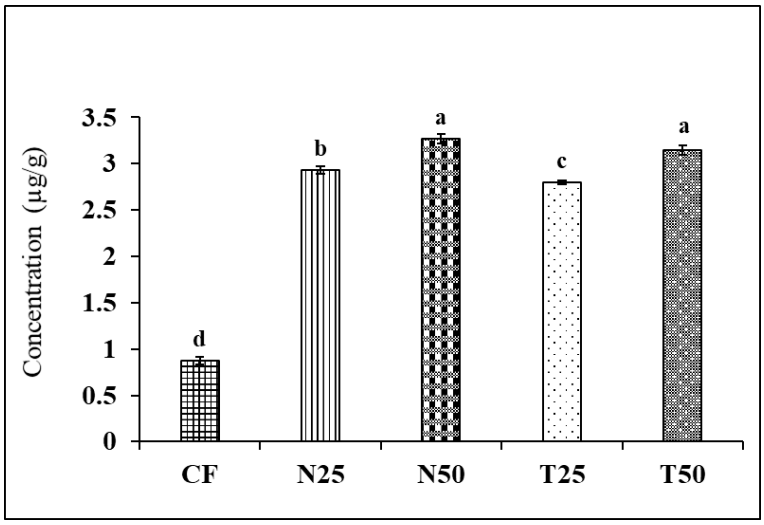

Figure 1. The effect of different concentrations of *Tetraselmis* sp. as well as *Nannochloropsis* sp. fed diet on total carotenoid concentration of Nile tilapia (*Oreochromis niloticus*) fish tissue. Values are as mean ± SE (standard error) (n=3, p< 0.05).

there were significant changes (p <0.05) in the whole fish sample documented (Figure 2). The observed values for protein, lipid, carbohydrate was ranged from 26.07% to 32.16% (Figure 2.A), 12.4% to 19.8% (Figure 2.B) and 9.59% to 12.78% (Figure 2.C) consequently. In this study, the highest protein, lipid and carbohydrate nutrient value was documented in CF (32.16±0.06%), N50 (19.8±0.12%) and T50 (12.78±0.02%) accordingly. The lowest value of protein is achieved in N50 (26.07±0.06%), lipid in CF (12.4±0.12%) and carbohydrate in CF (9.59±0.02%) treatment consequently.

3.3. Biochemical composition

The result of percent (%) total fatty acid composition of whole Nile tilapia fish tissue is presented in the following Table 2. The observed values indicating that percentages of different fatty acids are significantly affected (p< 0.05) by dietary microalgae compared to control feed. Nile tilapia fish fed with control feed obtained the lowest percentage of SFAs such as decanoic acid (C10:0), palmitic acid (C16:0), heptadecanoic acid (C17:0), heneicosanoic acid (C21:0), behenic acid (C22:0), tricosanoic acid (C23:0) and lignoceric acid (C24:0) in comparison to treatment N25, N50, T25 and T50, although lowest percentage of total SFA was observed in T50 (32.87±0.01%) and highest value was recorded in N25 (35.49±0.09%) treatment. In case of total monounsaturated fatty acid (MUFA), 25% *Nannochloropsis* sp. based diet exhibited the lowest total MUFA. In regards, highest percentage of PUFA, EPA (C20:5n-3) was observed in N50 (22.42±0.02%) treatment. Elevated concentration of *Nannochloropsis* sp. in fish diet has significantly increased the EPA (C20:5n-3) content in fish tissue compared to other treatment while in case of other PUFAs elevated concentration of microalgae did not significantly increase the PUFA content numerically. The Highest total PUFA was recorded in T50 (52.02±0.06%) treatment in comparison to other treatment.

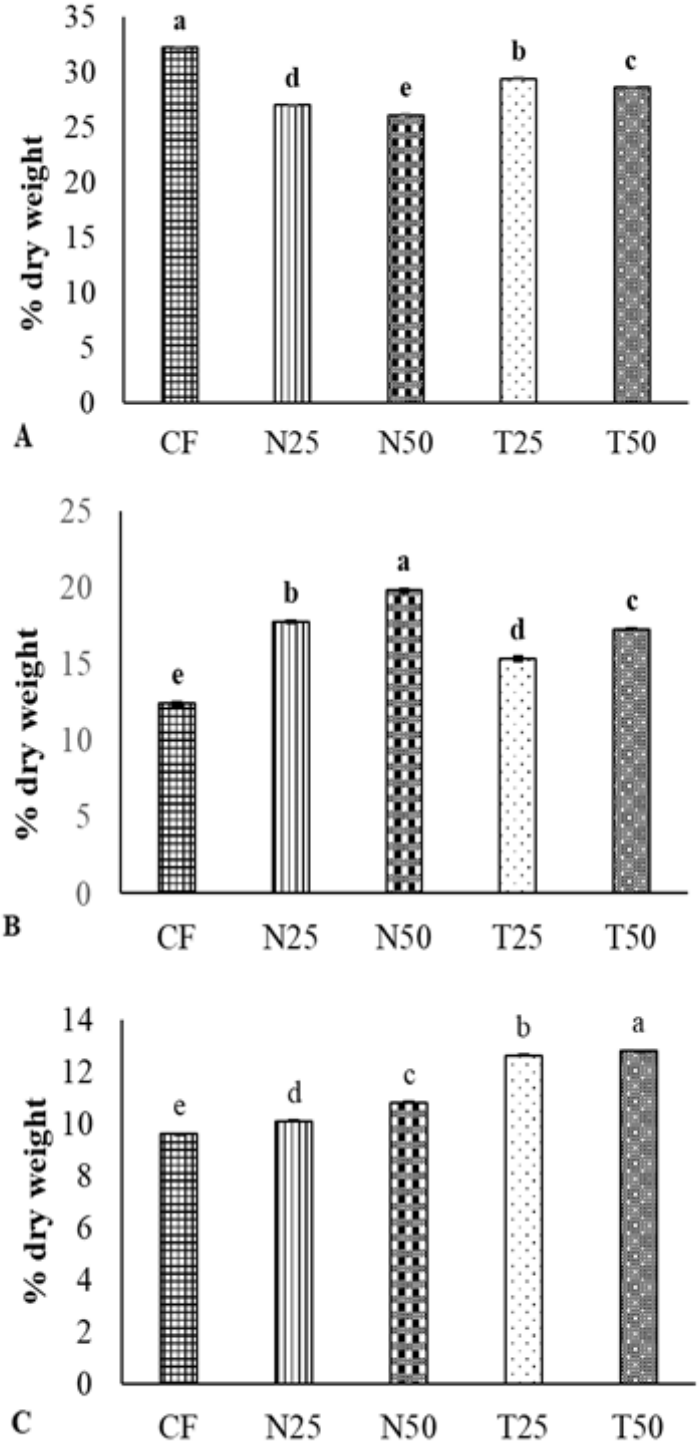

Figure 2. The effect of different concentrations of *Tetraselmis* sp. as well as *Nannochloropsis* sp. fed diet on protein (A), lipid (B), carbohydrate (C) composition of whole Nile tilapia (*Oreochromis niloticus*) fish tissue. Values are as mean ± SE (standard error) (n=3, p< 0.05).

4. Discussion

4.1. Total carotenoid concentration of fish tissue

Fish skin color is an essential predictor of consumer acceptance, and carotenoids are responsible for fish coloration. Carotenoids must be supplemented in the diet of farmed species because fish cannot synthesize them on their own (Gupta et al., 2007). It has been proposed that microalgae such as *Nannochloropsis* sp. and *Tetraselmis* sp. can support the aquaculture sector with vital vitamins, pigments, and polyphenols(Brown et al., 1999; Pataroa et al., 2019; Kokkali et al., 2020). Similar results were also obtained in the current study. Carotenoids-rich *Nannochloropsis* sp. and *Tetraselmis* sp.(Di Lena et al., 2018) showed considerable significance as natural fish colorants and confirmed the existence of a significant quantity of total carotenoids in muscle tissue of Nile tilapia (Figure 1). Although gradual increase in total carotenoid concentration was noticed in both *Nannochloropsis* sp. and *Tetraselmis* sp. fed treatment than control however highest performance was exhibited by N50 treatment. Carotenoids, are naturally occurring pigments that can serve as provitamin A and are found in microalgae at a level of 0.1-0.2 percent dry matter, are generated de novo predominantly by photosynthetic organisms(Becker, 1994). Though there are significant differences in the results of carotenoid contents in *Nannochloropsis* sp. and Tetraselmis sp. fed treatment, similar results obtained in the study of Tulli et al.(2012) and a change in skin pigmentation was detected after feeding *Tetraselmis suecica* containing meals to juvenile sea bass. Greenish pigmentation was found to be enhanced in fish fed diets including microalgae, like what was seen in *Carassius Carassius* (Gouveia & Rema, 2005). Teimouri et al.(2013) also found that 10% addition of *Spirulina platensis* has resulted in maximum carotenoid deposition in the skin and fillet tissue of rainbow trout (*Oncorhynchus mykiss*) as a natural pigment source. Another study found that eating a diet high in carotenoids can lower the incidence of free radical-related disorders including atherosclerosis and cancer(Gouveia et al., 2008).

4.2. Fish nutrition

A microalgal diet's nutritional value is directly proportional to its capacity to offer needed macro and micronutrients to the intended animal consumer and fish exhibit its nutritional status in muscle tissue according to the nutrition provided into its diet. In the current study the results of proximate composition of protein, lipid and carbohydrate (Figure 2A-2C) of Nile tilapia *Oreochromis niloticus* is significantly affected by different diet composition. One of the primary reasons for considering microalgae as a nontraditional source of protein is their high protein content(Becker, 2007) but in the current study among the experimental fish, *Nannochloropsis* sp. and *Tetraselmis* sp. based diet, treatment with commercial fish meal-based diet (Control Feed) has shown better protein percentage (Figure 2.A) and this might be due to presence of high fibre content in higher percentage in microalgae which has reduced the digestibility of microalgae-based diet in fish body(Sarker et al., 2018). Sørensen et al.(2021) also found similar results in his study with fish and confirmed that fish meal-based diet has shown higher protein percentage in the whole-body composition of Atlantic Salmo salar than *Nannochloropsis oceanica* and *Tetraselmis* sp. fed diet. Tibbetts(2018) documented that, *Nannochloropsis* sp. has a protein composition that ranges from 18 to 48 % and lipid composition ranges from 2 to 68 % besides *Tetraselmis* sp. contain protein and lipid ranges from 27 to 54 % and from 3 to 45 % correspondingly. Between *Nannochloropsis* sp. and *Tetraselmis* sp. comparatively higher protein nutrition obtained in T25 and the highest lipid content among all the treatment was recorded in N50 treatment (Figure 2.B). Carbohydrates are the cheapest energy sources for fish diets and gradual increase in carbohydrate content than control was noticed in this study (Figure 2.C). Carbohydrates are added in aquaculture diets, even though they have a negligible contribution in fish nutrition and growth, to minimize feed costs and for their binding action increasing the water stability of diets. The study of Radhakrishnan et al.(2015) is in agreement with the current study and documented that 50% replacement of fish meal with *Chlorella vulgaris* microalgae can increase the carbohydrate content in *Macrobrachium rosenbergii* post larvae. For the economic production of healthy and high-quality product good nutrition is essential and in this regard this study suggests reducing the inclusion of microalgae(Sarker et al., 2020) to get better pro-

Table 2. The fatty acid content of Nile tilapia whole fish (% of total fatty acid). Values (n=2) with different small uppercase within individual column are as mean ± SE (standard error) and statistically significant (p< 0.05)

Fatty acids	CF	N25	N50	T25	T50
C8:0	1.27 ± 0.01^{b}	1.48 ± 0.06^{a}	1.56 ± 0.04^{a}	1.22 ± 0.01^{b}	1.29 ± 0.01^{b}
C10:0	0.65 ± 0.02^{b}	0.92 ± 0.02^{a}	0.98 ± 0.07^{a}	0.68 ± 0.02^{b}	0.68 ± 0.01^{b}
C12:0	4.72 ± 0.07^{a}	3.56 ± 0.03^{d}	3.39 ± 0.04^{d}	4.50 ± 0.08^{b}	4.22 ± 0.03^{c}
C13:0	0.47 ± 0.02^{a}	0.25 ± 0.03^{c}	0.35 ± 0.01^{b}	0.12 ± 0.01^{d}	0.15 ± 0.01^{d}
C14:0	5.29 ± 0.04^{c}	5.76 ± 0.05^{a}	5.61 ± 0.02^{b}	5.37 ± 0.05^{c}	5.03 ± 0.03^{d}
C16:0	15.35 ± 0.02^{b}	16.47 ± 0.05^{a}	15.76 ± 0.06^{a}	16.79 ± 0.13^{b}	15.85 ± 0.09^{b}
C17:0	0.12 ± 0.01^{d}	0.27 ± 0.01^{b}	0.31 ± 0.03^{c}	0.17 ± 0.02^{a}	0.23 ± 0.00^{c}
C18:0	3.44 ± 0.11^{a}	2.94 ± 0.04^{b}	3.08 ± 0.01^{b}	2.54 ± 0.07^{c}	2.47 ± 0.04^{c}
C20:0	0.67 ± 0.01^{a}	0.65 ± 0.01^{ab}	0.69 ± 0.03^{a}	0.54 ± 0.03^{c}	0.58 ± 0.01^{b}
C21:0	0.11 ± 0.00^{d}	0.52 ± 0.05^{a}	0.57 ± 0.02^{a}	0.22 ± 0.01^{c}	0.34 ± 0.02^{b}
C22:0	0.36 ± 0.02^{c}	1.10 ± 0.04^{a}	1.18 ± 0.05^{a}	0.68 ± 0.02^{b}	0.79 ± 0.01^{b}
C23:0	0.06 ± 0.01^{d}	0.22 ± 0.01^{b}	0.28 ± 0.03^{a}	0.10 ± 0.01^{d}	0.16 ± 0.01^{e}
C24:0	0.66 ± 0.04^{d}	1.33 ± 0.01^{a}	1.34 ± 0.01^{a}	0.89 ± 0.05^{c}	1.08 ± 0.03^{b}
Total SFA	33.18 ± 0.03^{d}	35.49 ± 0.09^{a}	35.09 ± 0.12^{b}	33.80 ± 0.11^{c}	32.87 ± 0.01^{e}
C16:1	3.02 ± 0.08^{b}	3.58 ± 0.05^{a}	3.71 ± 0.04^{a}	2.90 ± 0.07^{b}	2.98 ± 0.02^{b}
C18:1	7.17 ± 0.11^{c}	7.47 ± 0.03^{b}	7.35 ± 0.04^{b}	9.91 ± 0.05^{a}	9.75 ± 0.03^{a}
C20:1	0.41 ± 0.06^{b}	0.67 ± 0.06^{a}	0.76 ± 0.02^{a}	0.42 ± 0.01^{b}	0.50 ± 0.05^{b}
C22:1	6.86 ± 0.06^{a}	2.23 ± 0.05^{c}	2.47 ± 0.03^{b}	1.48 ± 0.05^{e}	1.78 ± 0.05^{d}
C24:1	0.05 ± 0.00^{d}	0.13 ± 0.00^{a}	0.16 ± 0.02^{a}	0.09 ± 0.01^{c}	0.10 ± 0.00^{b}
C18:2n-6	10.84 ± 0.05^{d}	11.43 ± 0.04^{c}	11.50 ± 0.07^{c}	18.74 ± 0.05^{a}	17.62 ± 0.05^{b}
C20:3n-6	5.45 ± 0.04^{a}	4.91 ± 0.00^{b}	4.73 ± 0.07^{c}	3.75 ± 0.04^{e}	4.31 ± 0.02^{d}
C20:4n-6	0.55 ± 0.05^{c}	1.79 ± 0.03^{b}	1.20 ± 0.01^{a}	1.30 ± 0.10^{c}	1.64 ± 0.01^{b}
C18:3n-3	16.48 ± 0.12^{a}	8.37 ± 0.15^{c}	8.26 ± 0.05^{c}	10.18 ± 0.04^{b}	10.16 ± 0.02^{b}
C20:5n-3	15.12 ± 0.06^{d}	22.32 ± 0.01^{a}	22.42 ± 0.02^{a}	16.22 ± 0.05^{c}	16.65 ± 0.05^{b}
C22:5n-3	0.57 ± 0.03^{c}	1.49 ± 0.03^{a}	1.42 ± 0.10^{a}	0.91 ± 0.07^{b}	1.17 ± 0.01^{b}
C22:6n-3	0.29 ± 0.01^{b}	0.11 ± 0.01^{d}	0.15 ± 0.00^{c}	0.31 ± 0.00^{b}	0.47 ± 0.01^{a}
Total MUFA	17.52 ± 0.03^{a}	14.09 ± 0.08^{e}	14.43 ± 0.09^{d}	14.80 ± 0.07^{c}	15.11 ± 0.06^{b}
Total PUFA	49.30 ± 0.00^{d}	50.43 ± 0.17^{c}	50.48 ± 0.21^{c}	51.40 ± 0.04^{b}	52.02 ± 0.06^{a}
Total n-3	32.47 ± 0.14^{a}	32.29 ± 0.10^{a}	32.25 ± 0.07^{a}	27.62 ± 0.03^{c}	28.45 ± 0.03^{b}
Total n-6	16.83 ± 0.14c	18.13 ± 0.07^{b}	18.23 ± 0.15^{b}	23.78 ± 0.01^{a}	23.57 ± 0.03^{a}

tein nutrition in whole fish composition.

4.3. Biochemical composition

Fatty acids can be saturated, mono unsaturated and poly unsaturated types. Among different types of fatty acids, the ω-3 and ω-6 PUFA series are two important PUFA groups that are regarded as the most important bioactive molecules for living species(Li et al., 2014). Microalgae contains a variety of nutritional components, including minerals, essential fatty acids ω-3 and ω-6, and other beneficial elements (Tokusoglu & Ünal, 2001). Among PUFAs, notably for EPA and DHA, omega-3 fatty acids are universally acknowledged and proved to be beneficial(Siriwardhana et al., 2012; Tocher, 2015) in prevention or treatment of a variety of illnesses of human beings which includes blinding retinal conditions, cardiac illness, diabetes, malignance and dementia(SanGiovanni & Chew, 2005; Macchia et al., 2013; Shahidi & Ambigaipalan, 2018). EPA and DHA are mostly found in wild fish (Tocher, 2015). Nevertheless, in aquaculture, to obtain PUFAs specially EPA as well as DHA for cultured fish species fish oil along with fish meal is considered as unsustainable source. Because of the rapid expansion of aquaculture, fish oil and fish meal supply will be insufficient in the future to meet the increasing demand. To partially replace fish meal and fish oil alternatives such as soybean oil and soybean meal have frequently been employed because of their ease of cultivation, harvest, availability (Wang et al., 2014) and tolerance to microbes. In this aspect microalgae also can be a potential substitute to mitigate the essential fatty acid supply demand. In the current study, the addition of microalgae *Nannochloropsis* sp. and *Tetraselmis* sp. in diet has shown a significant positive impact on the fatty acid composition of Nile tilapia whole fish (Table 2) and desired results are obtained in this study. According to the presented data the, most abundant SFA, palmitic acid; MUFA, oleic acid was found in T25 treatment. Essential PUFAs, like linoleic acid (18:2n-6), EPA (C20:5n-3) and DHA (C22:6n-3) were found dominant in T25, N50 as well as T50 treatment, respectively. Findings of this study are consistent with the findings of Mohammadi et al.(2015) and he revealed that palmitic acid, oleic acid, alpha-linoleic acid, EPA are consequently the most prevalent SFA, MUFA, PUFA and highly unsaturated fatty acid (HUFA) in *Tetraselmis chuii* among all types of fatty acids. According to the experiment of Servel et al. (1994) as well as Schneider et al. (1995) the most abundant PUFA, EPA was found in the genus *Nannochloropsis* sp. which was also confirmed in the study of Tonon et al. (2002). The maintenance of ω-6 to ω-3 PUFAs are regarded as important for human health for their physical development, homeostasis and psychological health (Simopoulos, 2011). In this experiment Nile tilapia (*Oreochromis niloticus*) fish received SFAs, MUFAs and essential PUFAs from *Nannochloropsis* sp. and *Tetraselmis* sp. inoculated diet. Previous researchers namely, Takeuchi et al.(2002) fed *Spirulina* sp. to Nile tilapia, Dos Santos et al.(2019) fed *Schizochytrium* sp. to Nile tilapia, Hossain et al. (2017) fed *Chlorella vulgaris* and *Spirulina platensis* to Nile tilapia, Gbadamosi and Lupatsch(2018) fed *Nannochloropsis salina* to Nile tilapia and found elevated level of PUFAs in fed fish. According to previous studies, both *Nannochloropsis* sp. and *Tetraselmis* sp. microalgae are amazing source of some essential fatty acids as well as long chain PUFAs, especially EPA (Servel et al., 1994; Schneider et al., 1995; Meseck et al., 2005; Zaki & Saad, 2010; Gbadamosi & Lupatsch, 2018) which is confirmed in the current research. In this regard, our data show that *Nannochloropsis* sp. and *Tetraselmis* sp. may be useful in enhancing the fatty acid profile of whole fish composition. As a result, for the improvement of the deposition of human health-facilitating fatty acids, the addition of *Nannochloropsis* sp. and *Tetraselmis* sp. microalgae into fish diet is a smart strategy to boost the nutritional value of whole fish tissue.

5. Conclusion

Total carotenoid concentration, nutritional and biochemical composition of fish mainly depends on the diet provided to fish. The current study revealed that, inclusion of *Nannochloropsis* sp. and *Tetraselmis* sp. microalgae in diet has positively influenced the total carotenoid concentration, lipid, carbohydrate and valuable fatty acids deposition in fry Nile tilapia (*Oreochromis niloticus*) whole muscle tissue. Lower protein digestibility due to higher inclusion of microal-

gae, which is a plant protein source has noticed. This study suggests lowering the inclusion of plant protein source, microalgae to improve the digestibility and protein nutrient deposition. However, observing positive impacts of those two potential microalgae in fish pigmentation and nutrient profile, microalgae can be useful in fish farming.

Acknowledgement

This study was supported by the Krishi Gobeshona Foundation Project ID: TF 100-F/21 and Chattogram Veterinary and Animal Sciences University grants through University Grant Commission.

Conflicts of interest

The authors state that they have no known conflicting financial interests or personal ties that may have seemed to affect the work presented in this study.

Author contribution

Kafia Islam Amira:methodology design, mass culture, feeding experiment, data collection, data analysis and original draft manuscript preparation. **Mohammad Redwanur Rahman and Suchandan Sikder**: conceptualization, supervision, validation, review and editing. **Helena Khatoon**:critical review of the draft and draft manuscript submission, funding allocation. **Jinat Afruj**: data collection and data analysis. **Mohammad Jabedul Islam**: supported in mass culture, feeding experiment. **Foujia Jamal**: contributed in mass culture, manuscript preparation. All the authors commented on the draft manuscript.

Funding

The research was supported through the fund provided by Chattogram Veterinary and Animal Sciences University (CVASU) and University Grant Commission (UGC).

Data availability

The corresponding author will provide the data that supports the findings of the study on reasonable request.

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Citation information

Cite this article as: Islam, K., A., Khatoon, H., Rahman, M. R., Sikder, S., Afruj, J., Islam, M. J., & Jamal, F. (2023). Dietary influence of selected microalgae on carotenoid concentration, nutrition and biochemical composition of fry Nile tilapia. *Journal of Innovation in Applied Research, 6 (1)*, 10-22. doi: 10.51323/JIAR.2023/6/1/10-20

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