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Effects of feeding frequency and *Spirulina* on growth performance, skin coloration and seed production on kenyi cichlids (*Maylandia lombardoi*)

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Abstract Kenyi cichlids belong to mbuna group which is specific to Lake Malawi. Gender discrimination is easy because males have yellow, females have blue colors and their spawning efficiency is good. Cichlid producers prefer kenyi cichlids in recent years due to reproduction performance and coloring of kenyi. In this study, effects of *Spirulina*-based diet and feeding frequency on coloration, seed production, growth and survival on kenyi cichlids (*Maylandia lombardoi*) were investigated for 112 days. The study was carried out in a recirculating system which has 100 L each tank and 12 fiberglass tanks with three replicates. Ten fish (3 months old, mean body weight 2.00 ± 0.05 g and mean total length 4.51 ± 0.42 cm) were randomly placed in each tank. Experimental groups were designed with commercial granule (C) and commercial granule *Spirulina* (S) feeds. In the present study, two feeding frequencies were applied: one feeding daily at 09:00 (namely C1, S1) and three times daily at 09:00, 12:00 and 17:00 (namely C3, S3). The growth and seed production of cichlid fed three times daily were significantly higher compared to fish fed one feeding daily, irrespective of feed source ($P < 0.05$). Moreover, the specific growth rate of cichlid fed *Spirulina*-based diet was significantly elevated compared to fish fed non-*Spirulina*-based diet. The *Spirulina*-based diets affected skin coloration giving a bluish hue and a typical chroma values for the females of kenyi cichlid. In conclusion, growth performance, seed production and skin coloration of kenyi cichlid fed *Spirulina* diets three times daily enhanced under the study condition.

Keywords Cichlidae · Pigmentation · Feeding frequency · Microalgae · Water quality

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Introduction

In recent years, aquarium sector becomes a developing sector around the world (Alderton 2008). Many species have been imported and exported to lots of countries for fulfilling the demands from aquarists. Farmers emphasized ornamental fish culturing for satisfying these demands (Çelik et al. 2011). Total import, export and production amounts of ornamental fish (both fresh and saltwater) reached 50,000 tons in 2011 from 25,000 tons in 2000 with 100 % increase. Also, the total production value in aquarium sector reached to 717 million $ with considerable increase in 2011 (FAO 2011).

Aquarium industry is developing rapidly in the sense that it is no longer a hobby today. As a result of these developments, lots of potential species (fish, invertebrates, plants, etc.) have been interested from aquarists. Most of freshwater fish (90 %) dealt in the aquarium industry have been provided from the aquaculture, while remaining are captured from the nature (Türkmen et al. 2011). Cichlids, livebearers, labyrinth fish and cyprinids are among ornamental fish in the most important groups. Kenyi cichlids which are usually fed as omnivores dealt with in this study belong to mbuna group which is specific to Lake Malawi (Naish and Ribbink 1990). It showed semi-aggressive behavior with their and other species in artificial environments. In the cichlid reproduction behavior, female kenyi choose conspecific males for their visual cues such as brilliant color. Gender discrimination is easy because males have yellow, females have blue colors and their spawning efficiency is quite good (Smith 2000). For these features, kenyi cichlids have been preferred over cichlid producers in recent years.

Energy requirements for metabolism are provided by the consumption of feed in fish as in other creatures. Feeding frequency varied according to fish life stages. An increase of feeding rate or frequency enhances the production efficiency by improving growth and reducing feed waste (Johnston et al. 2003; Montajami et al. 2012), whereas a lacking feeding frequency leads to poor growth performance of ornamental fish (Priestley et al. 2006). Also, excessive feeding undesirably affects water quality. Most ornamental fish are grown in closed aquarium systems where water is at least partially recycled (Güroy et al. 2012). Therefore, ideal feeding frequency should be determined for optimum of growth and water quality in ornamental fish.

Skin coloration is one of the most important marketing criteria in the ornamental fish trade (Güroy et al. 2012). Dietary additives such as essential fatty acids, alpha tocopherol, ascorbic acid and carotenoids influenced the reproduction performance and coloring of fish (Güroy et al. 2012). Enhanced growth performance, health, nutritional performance and coloration have been declared with dietary small amount microalgae inclusion (1–5 %) in a range of fish species (Güroy et al. 2011, 2012; Mustafa and Nakagawa 1995). *Spirulina* is a microalgae commercially used as a supplementary food in human nutrition and finfish diets due to its rich source of protein, indispensable fatty acids, essential amino acids, vitamins and minerals (Spolaore et al. 2006; Nakagawa and Montgomery 2007; Güroy et al. 2012). The effects of dietary *Spirulina* on the growth performance and skin coloration have been studied for ornamental finfish species, including red swordtail, *Xiphophorus helleri* (James et al. 2006), goldfish, *Carassius auratus* (James et al. 2009; Vasudhevan and James 2011), yellow tail cichlid, *Pseudotropheus acei* (Güroy et al. 2012), and three-spot gourami, *Trichopodus trichopterus* (Khanzadeh et al. 2016). Therefore, the aim of the present study was to evaluate the effects of *Spirulina* and feeding frequency on performance, skin coloration and seed production on kenyi cichlids *Maylandia lombardoi*.

Materials and methods

Rearing systems and fish

Twelve 100-L circular tanks (diameter: 50 cm, height: 55 cm), within a 4000-L recirculation freshwater system, were annexed to a 650-L sand filter system. The water flow rate was 5 L/min and water quality was monitored daily. Water temperature was maintained at 26.5 ± 0.06 °C, dissolved oxygen at 8.12 ± 0.06 mg/L, pH at 7.98 ± 0.06 and ammonium at 0–0.5 mg/L. The system was housed in a climate-controlled laboratory with controlled photoperiod (12-h light: 12-h dark). Kenyi cichlids were obtained from a commercial facility in Istanbul, Turkey, and transported to the Ornamental Fish Unit of Armutlu Vocational School, University of Yalova, Turkey. Prior to the start of the feeding trial, fish were transferred to a 100-L aquarium and fed a commercial tropical fish diet (42 % protein, 5 % lipid, 1.1 % fiber, 7 % ash; AHM Marin) for 2 weeks for acclimation.

Kenyi cichlids (3 months old, mean body weight 2.00 ± 0.05 g and mean total length 4.51 ± 0.42 cm) were randomly distributed among 12 tanks (100 L volume of each tank) at a density of 10 fish per tank with three replicate tanks for each dietary treatment. Ten fish were placed in each tank with 2 male:8 female sex ratio. Daily feed intake was recorded to calculate feed conversion ratio (FCR). Growth and FCR were monitored biweekly by collectively weighing the fish from each aquarium. All fish were anesthetized with clove oil (0.001 mL/L).

Experimental design and diet

A two (feed source) × two (feeding frequency) factorial design with three replications was used. The experimental diets used granule (C) (AHM Marin Natural Cichlid Granulate) and granule *Spirulina* (S) (AHM Marin Tanganyika Green Granulate) feeds (Table 1). Rations of the both cichlid diets were same and include fish meal, de-hulled soybean meal, wheat meal, squid meal, krill meal, fish oil and other dietary additives (vitamin premix, mineral premix, antioxidants). *Spirulina*-based diet was formulated containing 20 % inclusion level by substituting fish meal on an equal weight basis. The crude protein contents of *Spirulina* and fish meal are 66 and 69 %, respectively. Two feeding frequencies were applied in this experiment: one feeding daily at 09:00 (namely C1, S1) and three times daily at 09:00, 12:00 and 17:00 (namely C3, S3). Fish were hand-fed to apparent

Table 1 Chemical composition of the granule and *Spirulina* feeds (%, dry matter)

	Granule	*Spirulina*
Moisture	6.29	6.44
Crude Protein	40.34	40.02
Crude lipid	5.08	4.98
Crude ash	6.92	7.01
Nitrogen free extract	47.66	47.99
Gross energy (MJ/kg)	19.77	19.78

Nitrogen-free extracts (NFE) were calculated as NFE = 100 − (% protein + % lipids + % ash). Gross energy was calculated using the conversion factors of 23.7 kJ/g for protein, 39.5 kJ/g for lipid and 17.2 kJ/g for carbohydrates satiety 7 days per week for the 112-day experimental period. Feed intake was recorded biweekly. Fish individually weighed at the start and the end of feeding trial and bulk-weighed fortnightly.

Seed production

The seed production of kenyi cichlids fed their respective diet was monitored over 112 days. Brooding females were checked for spawning activity daily. Whenever present, eggs were gently removed from the buccal cavity of brooding fish, and the spawning rate was monitored over 14 days.

Color measurement

All female were individually weighed and measured for skin color using a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd., Asaka, Japan) before commencement of the feeding trial to establish baseline measurements (week 0) and then every 2 weeks for the 16-week period. The measurements were performed on left surface (10 mm) of body area and caudal region of each fish. Skin coloration of body and caudal areas was determined by the front and end of dorsal fin vertically, respectively. The Chroma Meter was set to take absolute measurements in the L^*, a^*, b^* measuring mode (CIE 1976) using D65 illuminate. L^* is the lightness variable (where white: 100 L^* and black: 0 L^*), a^* is the red chromaticity coordinates where $+a^*$ stands for red, and $-a^*$ stands for green, and b^* is the yellow chromaticity coordinates where $+b^*$ stands for yellow, and $-b^*$ stands for blue. The hue (H_{ab}) and chroma (C_{ab}) values were calculated according to a^* and b^* values. Hue and Chroma values are calculated by the equation, $H_{ab} = \arctan(b^*/a^*)$ and $C_{ab} = (a^{*2} + b^{*2})^{1/2}$, respectively (Hunt 1977).

Determination of total ammonia–nitrogen (TAN) excretion

After the feeding trial, fish were starved for a period of 3 days to ensure evacuation of food from the gut. On the morning of the fourth day, tanks were thoroughly cleaned and fish in all tanks were fed the appropriate diet to apparent satiation. Thirty minutes postprandial, water flow to each aquarium was discontinued, uneaten food was removed, and a baseline TAN excretion level was analyzed with the ammonia salicylate method using NH_3–N reagent kits from Hach Lange and measured in a spectrophotometer (DR-2800, Hach, Loveland, CO, USA). At 12 h postprandial, an additional sample was taken and analyzed and the TAN levels were determined by subtracting the baseline value of each tank.

Fatty acid analysis

Diets were extracted according to the procedure of Folch et al. (1957) with chloroform/methanol (2:1 v/v). The fatty acid profile of experimental diets is detailed in Table 2. The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14 % boron trifluoride–methanol (AOAC 2000). Fatty acids were analyzed using a flame ionization gas chromatograph equipped with an Omegawax 250 capillary column (30 mL × 0.25 mm internal diameter), a FID detector and a split injection system with nitrogen carrier gas. Injector port and detector

Table 2 Fatty acid composition of experimental diets (%)

	Granule	*Spirulina*
C14:0	7.85	6.37
C15:0	0.35	0.25
C16:0	21.42	20.82
C17:0	1.02	0.89
C18:0	2.63	3.01
C20:0	3.89	2.44
C21:0	0.10	0.06
C22:0	0.01	0.16
C23:0	0.50	0.34
Total SFA	37.78	34.35
C16:1n7	6.56	6.11
C18:1n9	24.13	25.75
C20:1n9	0.36	0.34
C22:1n9	0.82	1.16
C24:1n9	0.01	0.11
Total MUFA	31.88	33.47
C18:2n6	11.73	14.93
C18:3n6	1.96	1.98
C18:3n3	1.34	1.62
C20:3n3	0.46	0.36
C20:5n3	8.02	8.11
C22:6n3	5.52	4.65
Total PUFA	29.03	31.65
Total n6	13.69	16.91
Total n3	15.34	14.74

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

temperatures were maintained at 250 and 260 °C, respectively. The column temperature program was held at 140 °C for 5 min and then elevated at a rate of 3 °C/min to 200 °C. Total run time was 60 min per sample. Fatty acids were identified by comparing their retention times of the authentic standard fatty acid standards (Sigma-Aldrich, St. Louis, MO, USA).

Sampling and chemical analyses

Proximate analyses of the diets were performed using standard methods (AOAC 2000). Dry matter was measured by drying at 105 °C until a constant weight was achieved; crude lipid was determined by ether extraction, crude protein by the Kjeldahl method after acid digestion using a Behr system, and crude ash by incineration at 525 °C for 12 h in a muffle furnace. Crude fiber was determined by acid alkali hydrolysis and ignition of the dried sample for 3 h. Nitrogen-free extracts (NFE) were calculated as NFE = 100 − (% protein + % lipids + % ash). Gross energy was calculated using the conversion factors of 23.7 kJ/g for protein, 39.5 kJ/g for lipid and 17.2 kJ/g for carbohydrates (Brett and Groves 1979).

Evaluation of growth performance

The feed conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) were calculated as follows: FCR = feed intake/wt gain, SGR = 100 × ([ln final fish wt] − [ln initial fish wt])/experimental days, PER = (weight gain/dietary protein intake).

Statistical analysis

All data were subjected to one- and two-way analysis of variance (ANOVA) when interaction between the two factors found differences; Duncan's multiple range test was used to rank groups using Statgraphics Centurion XVI (Manugistics Inc., Rockville, MD) statistical software (Zar 2001). Differences were considered significant at 5 %.

Results

The kenyi cichlids readily accepted the experimental diets. There were no observed health problems throughout the experiment. The growth and seed production for kenyi cichlid fed different feeding frequency (one feeding daily or three times daily) and feed source (non-*Spirulina* or *Spirulina*) are given in Table 3. There is no statistical difference in survival rate (90.0–96.6 %).

In two-way ANOVA, interaction was not significant for all parameters in fish groups. Dietary *Spirulina* inclusion and feeding frequency were both significant factors on growth parameters such as specific growth rate (SGR) and feed intake (FI). Feeding frequency was a significant factor on final mean weight (FMW), final mean total length (FMTL) and average seed production (ASP).

The final mean weight and specific growth rate of cichlid fed three times daily were significantly higher compared to cichlid fed one feeding daily, irrespective of feed source. Also, the highest FMW was found in fish fed with the S3. The FMTL of C1 group was significantly lower compared to the other treatments ($P < 0.05$). All the experimental diets were well accepted by fish; furthermore, FI tended to be elevated with both increasing feeding frequency and incorporation of dietary *Spirulina*, and fish fed S3 group (11.93 g/fish) had significantly highest FI values compared to fish fed the other groups (6.96–10.42 g/fish). No significant differences in feed conversion ratio (FCR) and protein efficiency ratio (PER) were observed among all groups. Feeding frequency significantly affected the ASP of the kenyi cichlid (Table 3). The ASP of fish varied from 35 to 99, with the all three times daily feeding groups superior ($P < 0.05$) than those fed the one time daily groups. The total ammonia–nitrogen (TAN) excretion of kenyi cichlid fed with *Spirulina* diet reduced compared to fish fed the non-*Spirulina* diet, irrespective of feeding frequencies (Fig. 1). The skin coloration for female kenyi cichlids fed different feeding frequency and feed source is detailed in Tables 4 and 5 for body and caudal regions, respectively. There are no significant differences among the groups in the initial and final of both caudal and body regions with respect to lightness (L^*) ($P > 0.05$). The highest red/green tonality (a^*) of skin from the final body was found in fish fed all *Spirulina*-based diets irrespective of feeding frequency. Redness of the final body S3 was higher than those of the initial body S3 ($P < 0.05$). The yellow/blue tonality (b^*) of the body final S3 was significantly lower than that of C1 and C3 ($P < 0.05$). Yellowness of the final body C1 was

Table 3 Growth and reproduction performance of kenyi cichlids after 16 weeks of feeding on experimental diets ($n = 3$)

	Granule		*Spirulina*		Two-way ANOVA		
	One feeding daily	Three times daily	One feeding daily	Three times daily	Feeding frequency	Feed source	Interaction
Initial mean weight (g)	2.05 ± 0.02	2.05 ± 0.02	2.04 ± 0.01	2.04 ± 0.01	0.6430	0.3637	0.8158
Final mean weight (g)	7.81 ± 0.38[a]	10.87 ± 0.37[b]	8.09 ± 0.23[a]	12.02 ± 0.39[c]	0.0000	0.0752	0.2515
Initial mean total length (cm)	4.53 ± 0.01	4.50 ± 0.02	4.52 ± 0.02	4.50 ± 0.01	0.1516	1.0000	0.8265
Final mean total length (cm)	7.66 ± 0.10[a]	8.52 ± 0.18[b]	8.40 ± 0.12[b]	8.90 ± 0.38[b]	0.0227	0.0649	0.4132
Survival rate (%)	90.00 ± 5.77	96.67 ± 3.33	90.00 ± 5.77	96.67 ± 3.33	0.1950	1.0000	1.0000
Feed conversion ratio (FCR)	1.21 ± 0.04	1.18 ± 0.02	1.23 ± 0.01	1.20 ± 0.01	0.2438	0.5676	0.9488
Specific growth rate (SGR)	1.19 ± 0.04[a]	1.49 ± 0.02[b]	1.24 ± 0.02[a]	1.58 ± 0.03[b]	0.0000	0.0435	0.4226
Feed intake (FI)	6.96 ± 0.20[a]	10.42 ± 0.35[b]	7.46 ± 0.22[a]	11.93 ± 0.39[c]	0.0000	0.0105	0.1316
Protein efficiency ratio (PER)	2.05 ± 0.07	2.10 ± 0.04	2.03 ± 0.02	2.09 ± 0.02	0.2277	0.7887	0.9084
Average seed production (ASP)	37.00 ± 10.97[a]	61.00 ± 9.64[ab]	35.67 ± 11.61[a]	99.67 ± 30.75[b]	0.0419	0.3420	0.3099

In the same line, different letters indicate statistical significant differences ($P < 0.05$) among the treatments

±Means standard error

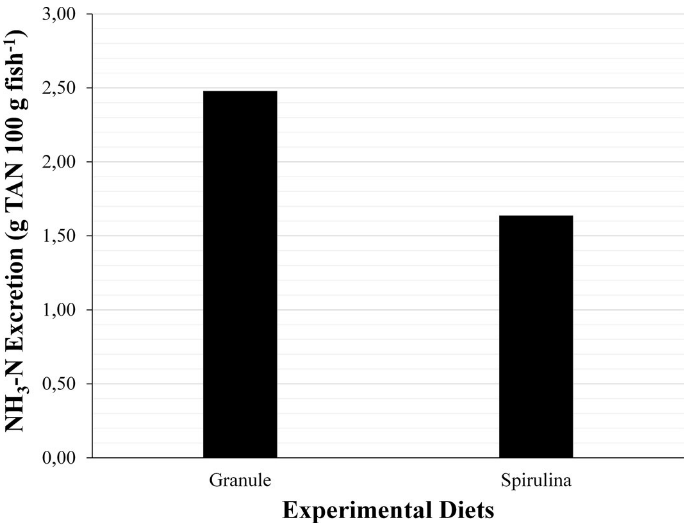

Fig. 1 Total ammonia–nitrogen (TAN) excretion of kenyi after 12 h of feeding on experimental diets

higher than those of the initial body C1 ($P < 0.05$). The red/green tonality ($a*$) of the caudal final C3 was significantly lower than that of S1 and S3 ($P < 0.05$). The yellow/blue tonality ($b*$) of the caudal final S3 was significantly lower than that of C3 ($P < 0.05$). Yellowness of the final caudal C1, S1 and S3 were higher than those of the initial body C1, S1 and S3 ($P < 0.05$).

Discussion

The present study was performed to evaluate the effects of *Spirulina* and feeding frequency for kenyi cichlid. In the feeding trial, feeding frequency (three times daily) and *Spirulina*-based diet had clear beneficial effects on the growth or/and seed production, and skin coloration of kenyi cichlid, respectively. The SGR of cichlid fed three times daily were significantly higher compared to cichlid fed one daily feeding, irrespective of feed source. The optimum feeding frequency may vary with dietary protein and energy levels, feeding time, species and size of fish (Güroy et al. 2006; Silva et al. 2007). In the present study, growth performance improves increase with feeding frequency. This result is in agreement with that of James and Sampath (2004) who find growth enhancement when betta, *Betta splendens*, were fed higher feeding frequency (three times daily) for 77 days. A study with juvenile red pacu, *Colossoma macropomum,* by van der Meer et al. (1997) found fish fed higher feeding frequency (five times daily). James and Sampath (2003) declared that final mean weight of red swordtail, *X. helleri*, was superior in fish fed three times daily than in those receiving fewer meals. Kasiri et al. (2011) reported that final live weight and SGR of twice daily and four times daily were significantly higher than those of the once daily and the every other day in angelfish, *Pterophyllum scalare*. A study on the feeding frequency of goldfish, *C. auratus*, showed a feeding frequency of four times daily resulted in the most efficient food utilization when compared with one, three and six feedings times daily under

Table 4 Color (L^*, a^*, b^*), hue and chroma parameters in body of female kenyi cichlids fed experimental diets for 16 weeks ($n = 9$)

Body	Granule		*Spirulina*		Two-way ANOVA		
	One feeding daily	Three times daily	One feeding daily	Three times daily	Feeding frequency	Feed source	Interaction
Initial L^*	53.93 ± 0.71	52.69 ± 3.31	57.38 ± 1.28	56.06 ± 0.89	0.5123	0.1049	0.9827
Final L^*	57.32 ± 2.33	55.78 ± 2.08	53.34 ± 2.70	53.92 ± 1.44	0.8316	0.2182	0.6426
Initial a^*	−0.52 ± 0.13	−0.73 ± 0.82	−0.62 ± 0.19	-1.06 ± 0.41^{A}	0.5080	0.6630	0.8108
Final a^*	-0.56 ± 0.58^{a}	-0.89 ± 0.11^{a}	0.16 ± 0.56^{b}	$0.42 \pm 0.28^{b;B}$	0.9371	0.0458	0.5026
Initial b^*	-12.83 ± 0.07^{A}	−11.83 ± 1.52	−10.87 ± 0.75	−12.46 ± 0.99	0.7732	0.5192	0.2229
Final b^*	$-6.75 \pm 0.56^{b;B}$	-7.87 ± 0.47^{b}	-10.86 ± 1.91^{ab}	-12.49 ± 0.84^{a}	0.3026	0.0095	0.8426
Initial H_{ab}	1.53 ± 0.01	0.45 ± 0.98	1.51 ± 0.02	1.48 ± 0.04	0.2895	0.3320	0.3173
Final H_{ab}	0.43 ± 0.99	0.75 ± 0.72	0.52 ± 1.01	−0.49 ± 1.03	0.7217	0.5593	0.5011
Initial C_{ab}^*	12.84 ± 0.06	11.91 ± 1.49	10.89 ± 0.74	12.53 ± 0.47	0.7238	0.5072	0.2203
Final C_{ab}^*	6.83 ± 0.49^{a}	5.75 ± 2.17^{a}	10.89 ± 1.91^{ab}	12.50 ± 0.85^{b}	0.8647	0.0076	0.4036

In the same line, different small letters indicate statistical significant differences ($P < 0.05$) among the treatments

In the column line, different upper case letters indicate statistical significant differences ($P < 0.05$) final values compared to initial values

±Means standard error

Table 5 Color (L^*, a^*, b^*), hue and chroma parameters in caudal region of female kenyi cichlids fed experimental diets for 16 weeks ($n = 9$)

Caudal	Granule		*Spirulina*		Two-way ANOVA		
	One feeding daily	Three times daily	One feeding daily	Three times daily	Feeding frequency	Feed source	Interaction
Initial L^*	61.30 ± 0.72	61.20 ± 1.67	63.59 ± 1.15	62.48 ± 2.21	0.7059	0.2817	0.7525
Final L^*	57.81 ± 1.18	57.11 ± 1.74	56.16 ± 1.88	56.39 ± 0.96	0.8786	0.4494	0.7613
Initial a^*	−0.36 ± 0.18	−0.61 ± 0.23	−0.49 ± 0.09	−0.79 ± 0.35	0.2730	0.5189	0.9335
Final a^*	-0.84 ± 0.29^{ab}	-1.61 ± 0.37^{a}	-0.02 ± 0.43^{b}	-0.29 ± 0.30^{b}	0.1779	0.0161	0.5090
Initial b^*	-4.04 ± 0.14^{A}	−3.20 ± 0.79	-3.74 ± 0.72^{A}	-5.04 ± 1.22^{A}	0.7821	0.3748	0.2273
Final b^*	$-7.64 \pm 0.13^{ab;B}$	-5.94 ± 1.83^{b}	$-8.84 \pm 1.27^{ab;B}$	$-10.38 \pm 0.69^{a;B}$	0.9459	0.0416	0.2024
Initial H_{ab}	1.48 ± 0.04	1.34 ± 0.09	1.44 ± 0.02	1.37 ± 0.10	0.2047	0.8970	0.6373
Final H_{ab}	1.46 ± 0.04	1.20 ± 0.21	−0.53 ± 0.99	0.49 ± 1.02	0.6108	0.0983	0.3993
Initial C_{ab}^*	4.06 ± 0.15	3.29 ± 0.76	3.77 ± 0.72	5.15 ± 1.16	0.7074	0.3441	0.2075
Final C_{ab}^*	7.70 ± 0.12^{ab}	6.32 ± 1.54^{a}	8.86 ± 1.27^{ab}	10.39 ± 0.68^{b}	0.9439	0.0380	0.2059

In the same line, different small letters indicate statistical significant differences ($P < 0.05$) among the treatments

In the column line, different upper case letters indicate statistical significant differences ($P < 0.05$) final values compared to initial values

±Means standard error

the conditions of this study (Priestley et al. 2006). The SGR in Nile tilapia (34 g), *Oreochromis niloticus*, fed three times daily higher than one time daily (Riche et al. 2004).

In the present study, the SGR of fish fed *Spirulina*-based diet was significantly improved compared to fish fed non-*Spirulina*-based diet. It has been recognized as a suitable dietary feed additive/ingredient for numerous finfish species. Dietary *Spirulina* could influence the growth performance of finfish species due to its high protein, essential amino acids and fatty acids, vitamins and minerals (Nakagawa and Montgomery 2007). For instance, in cichlids, it has earlier been declared that 10 % (Güroy et al. 2012), 20 % (Ungsethaphand et al. 2010) and 40 % (Olvera-Novoa et al. 1998) of fish meal could be substituted with *Spirulina* without causing any adverse effects on growth of yellow tail cichlid, *P. acei* and tilapia, *Oreochromis mossambicus*, respectively. James et al. (2006) reported that dietary addition of 8 % *Spirulina* significantly enhanced growth performance of the red swordtail, *X. helleri*. Also, James et al. (2009) declared that the growth performance of dietary *Spirulina* +300 mg vitamin E group was significantly improved compared to control and other experimental diets. Dietary *Spirulina* has been recognized as potential feed ingredients/additives for finfish diets since it has excellent protein content, essential amino acids, fatty acids and vitamins. Also, it has no cell wall, which causes enhanced digestion and absorption in fish.

Sombatjinda et al. (2014) carried out a water quality study with *Spirulina* mat that was co-cultured with Pacific white shrimp (*Penaeus vannamei*) postlarvae in a closed recirculating system. They found that the *Spirulina* showed total ammonia–nitrogen excretion rates decrease, compared with the system without the mat. The similar study was carried out with black tiger shrimp (*P. monodon*), and they declared the same results for *Spirulina* (Chuntapa et al. 2003). Our findings for TAN excretion were supported by these previous researches that *Spirulina* is enhancing water quality.

In this trial, the ASP of kenyi cichlid fed all three times daily feeding groups higher than those fed the one time daily groups, irrespective of *Spirulina*-based diets. In the present study, feed intake increased with feeding frequency. In general, food limitation could affect seed production (Bhujel et al. 2007). Hatefi and Sudagar (2013) found that one feeding daily group not reached maturity and spawn compared to 2, 3, 4 and 5 feeding frequencies groups in angelfish, *P. scalare*. This could be explained that increase in ration level with the have produced more seed production (Chong et al. 2004; Kithsiri et al. 2010).

Güroy et al. (2012) stated that dietary *Spirulina* enhanced yellow tail cichlid, *P. acei* seed production. Dietary microalgae sources improved the seed production in various ornamental fish include goldfish, *C. auratus* (James et al. 2009; Vasudhevan and James 2011), red swordtail, *X. helleri* (James et al. 2006) and basa fish, *Pangasius bocourti* (Meng-Umphan 2009). In the trial, seed production of kenyi cichlid did not directly affect the dietary inclusion of *Spirulina*. Fatty acid (FA) composition of eggs was not analyzed in this study, but researches indicated that dietary FA profile reflected eggs FAs (Tocher, 2010). *Spirulina* is a good source of n-6 fatty acid, especially of linoleic acid and γ-linolenic acid which was higher in the *Spirulina* diet. Linoleic acid significantly enhanced egg quality, especially in freshwater fish. Therefore, ASP of the S3 group is higher than those in G3 group, although there is no statistical difference in all groups. The current study showed that *Spirulina* increased pigmentation in kenyi cichlids. Many factors such as dietary lipid, dietary vitamin A or E, dietary ingredients/additives, tank color, genetic factors, fish size and species can influence the fish pigmentation (Rotllant et al. 2003; Booth et al. 2004; Kelsh 2004). In the present study, *Spirulina*-based diets affected skin coloration giving a bluish hue and a typical chroma values for the females of kenyi cichlid.

Spirulina is a high level of xanthophyll, β-carotene and zeaxanthin, a type of carotenoid. Therefore, *Spirulina* affected skin pigmentation of various fish. Similar results were achieved by Matsuno et al. (1980), Gouveia et al. (2003), James et al. (2009), Alagappan et al. (2004), James et al. (2006), Güroy et al. (2012) and Sun et al. (2012) who observed that *Spirulina* inclusion to the diets enhanced pigmentation in red tilapia, goldfish, blue gourami, red swordtail, yellow tail cichlid, showa koi, respectively.

One of the dietary ingredients/additives is microalgae which are known to have a positive role in the nutrient metabolism, antioxidant activity, immune system and pigmentation of fish that could improve growth/seed production and coloration. The results clearly show that feeding frequency (three times daily) and *Spirulina*-based diet had a significant effect on the growth or/and seed production, and coloration of kenyi cichlid, respectively.

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