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Biochemical composition and nutritional value of *Streptocephalus simplex* as live feed in ornamental fish culture



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Abstract A feed trial was conducted to evaluate the efficiency of *Streptocephalus simplex* as a live feed in freshwater ornamental fish culture. The efficiency of live feed was compared with that of artificial/pellet diet to determine the growth rate biochemical parameters and carotenoid concentration of *Carassius auratus* for a period of 45 days. As a result the proximate composition on the *S. simplex* indicates that they are rich in protein, lipids, essential amino acids and fatty acids. Availability of these growth factors was perfectly reflected on the successful growth rate of *C. auratus*. Moreover, presence of carotenoids viz., astaxanthin (36.4 ± 2.4), canthaxanthin (23.6 ± 1.7), and β -carotene (1.7 ± 0.6) has improved the intensity of the skin color of commercially important ornamental fish *C. auratus* after feeding with *S. simplex*.

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Introduction

Zooplankton are considered to be “living capsules of nutrition” for commercially important cultivable and ornamental species, as they are valuable sources of proteins, lipids, carbohydrates, vitamins, minerals, amino acids, fatty acids and carotenoids (New, 1998; Hernandez Molejon and Alvarez-Lajonchere, 2003; Rajkumar et al., 2008; Pronob et al., 2012). In the natural food web, they play a major role as diet for several invertebrates and vertebrate organisms and it is generally believed that the calorific value of zooplankton can

meet the nutritional requirements of fish (Evjemo Ove et al., 2003). In aquaculture practices, live food is difficult to sustain and requires considerable space and expense, on the other hand micro diets are easier to maintain and usually have lower production costs (Jones et al., 1993; Person et al., 1993). In spite of the difficulties found in practicing live feed culture, Wang et al. (2005) found that the survival was significantly higher in larvae fed with live food than in larvae fed the three formulated diets. Introduction of live zooplankton is therefore being investigated as an alternate to pond fertilization for increasing fish yields while avoiding water quality deterioration (Jha et al., 2007).

Different types of live feed are in practice, zooplankton viz., Rotifers, Brain shrimp, Fairy shrimp, Copepods, and Cladocerans stands in the top notch for demand. Among them large branchiopods (Brain shrimp & Fairy shrimp) as live feed

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are theoretically promising because of their high biomass, rapid growth and cyst production. Besides, they contain a high quantity of polyunsaturated fatty acids (PUFA) (Munuswamy et al., 1992) which reflects the level of triglycerides, a major source of metabolizable energy and are directly linked to the growth of punter organism (Sargent et al., 1990).

Fairy shrimp, the freshwater relatives of the more popular *Artemia*, offer interesting possibilities as live food in larval culture (Prasanth et al., 1994). Fairy shrimps are large branchiopods in the order Anostraca. These miniatures are abundantly available in temporary pools and ditches immediately after monsoon and post monsoon rains, cysts (eggs) of these organisms will survive drought for several years and hatch about 24 h after rains fill the pool where they live. Five species of fairy shrimps viz., *Streptocephalus dichotomus*, *Streptocephalus simplex*, *Streptocephalus echinus*, *Streptocephalus longimanus* and *Streptocephalus spinifer* have been reported till now in India. Since then attempts have been made to culture them in order to utilize them as alternative for pellet food (Velu and Munuswamy, 2003; Saengphan and Sanoamuang, 2009).

Fairy shrimps are probably more appropriate for freshwater fish and crustacean cultures that depend on live foods. Moreover, their high carotenoid content makes them a candidate for color enhancement in ornamental fish culture (Pronob et al., 2012). Due to array of problems in ornamental fish culture related to formulated diet, consequently the live feed remains as an important feed source. Several studies have proved the use of *Artemia* as an excellent diet in both marine and freshwater aquaculture (Sorgeloos, 1999; Tackaert and Sorgeloos, 1991) but studies related to different species of fairy shrimp are constrained. In this line, the present study has been conducted to compare and evaluate the effects of live food and formulated diets on the growth, survival, chemical composition and pigmentation of *Carassius auratus*, a fresh water ornamental fish.

Materials and methods

Reagents

Chemicals for extraction procedures and chromatographic analyses were of HPLC grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other commercially available chemicals and reagents were of analytical grade. Pellet feed was procured from local ornamental fish vendor.

Live feed

The fairy shrimps, *S. simplex*, were collected from temporary pools by towing plankton net, and transferred to the laboratory in live condition. Adult strains were maintained in plastic tubs under continuous aeration as described by Munuswamy et al. (1997).

Feeding trial

Juvenile goldfish (*C. auratus*) were stocked and acclimatized for two weeks prior experimentation. Before 48 h of the experiment, the juveniles were starved to empty their gut contents. Three tanks were randomly assigned for each diet (triplicate) and there were no significant differences in the fish length

and weight among replicates, at the start of the experiment (45.6 ± 0.11 mm, length; 2.94 ± 0.14 g, weight) ($n = 15$, $p > 0.05$). Water temperature was maintained at 27–28 °C, pH 7.5 and ammonium nitrogen at <0.5 mg L⁻¹ and aeration was supplied 24 h day⁻¹, photoperiod was controlled as 12 h dark:12 h light using a 40 W fluorescent light. Animal density was maintained as 2–3 animals L⁻¹ in a 6 L tank (30 cm × 15 cm × 15 cm) with daily water exchange up to 20% during the experimental period. For the feeding trial experiment, adult *S. simplex* and pellet diet were offered at 10% of body weight per day (Meade and Bulkowski, 1987), and dispensed 2 times a day in equal proportion.

Biochemical analyses

At the end of the experiment (45 days), fishes fed with pellet and *S. simplex* were sacrificed and subjected to various biochemical analyses such as carbohydrates (Kemp et al., 1954), total protein (Lowry et al., 1951), total lipid (Folch et al., 1957), ash (AOAC, 1995) and water content determination (Passoneau and Williams, 1953).

Amino acid analyses

To estimate the amino acid composition, wet samples were treated with 10% trichloroacetic acid (TCA) to precipitate the proteins. The protein precipitates were washed successively with 7% TCA, ethanol, chloroform–methanol (3:1) mixture and diethyl ether and by centrifugation the precipitate was collected. The sample proteins so obtained were hydrolyzed with 6 N HCl at 110 °C for 5 h. The acid hydrolysate was dried using speed vac concentrator. Amino acid composition was analyzed by High Performance Liquid Chromatography (HPLC) system (Hewlett-Packard model 1100 series, Hewlett-Packard, Palo Alto, CA, USA) equipped with a hypersil AA-003 silica based C18 column (200 mm × 2.1 mm) was used for the analyses using *O*-phthaldialdehyde (OPA), 2-mercaptoethanol reagent (Lee and Drescher, 1978). The solvents were at a flow rate of 0.50 ml/min at 40 °C [sodium acetate buffer (pH 7.20 ± 0.05) and acetonitrile buffer (pH 7.20 ± 0.05)] and analyzed at 338 nm. Individual amino acids were identified by comparing their retention times with those of amino acids standards, (HP 5062-2478, Hewlett-Packard, Palo Alto, CA, USA) run under identical conditions and expressed as percentage of total amino acids.

Fatty acid analyses

Total lipids were extracted from the samples by homogenizing in 5 volumes of chloroform/methanol (2:1, v/v) and measured gravimetrically according to the method of Folch et al. (1957). According to Morrison and Smith (1964), the lipids were converted into fatty acid methyl esters (FAMES) by saponification and methylation and then identified by gas chromatography. The fatty acid methyl esters were analyzed using a CHEMITO GC 8610 gas chromatograph equipped with BPX-70 (50% cyanopropyl 50% methylsiloxane) column (25 m length × 0.22 ID) and flame ionization detector. Nitrogen was used as the carrier gas and temperature programming for the oven was from 160 °C for 10 min and then to 180 °C at 1.5 °C/min which was then maintained for

5 min. Temperatures for the injector and detector were 200 and 300 °C, respectively. The data collection was done by Winchrom software and results were expressed in the percentages of total fatty acids.

Carotenoid content

Lyophilized samples were extracted in 10 ml 100% HPLC – grade acetone in a glass tissue homogenizer tube kept on ice, flushed with nitrogen and set aside for 0.5 h in the dark at 20 °C. Carotenoid peaks were identified by comparison to carotenoid standard (Sigma A9335). The absorption maxima were determined with a Spectromom-203 spectrophotometer as outlined by Czczuga and Czczuga (1998).

Statistical analysis

Biochemical and growth analyses were performed on three replicates and data were expressed as mean \pm standard deviation (SD). Differences between the diets and the experimental animals were analyzed statistically using a one-way analysis of variance (ANOVA). The statistical analysis was performed using statistical package for the social science (SPSS) 10.0 software (SPSS Inc. Chicago, IL, USA). The significant difference was determined with 95% confidence interval ($p < 0.05$).

Results

Feeding trails and biochemical analysis

The proximate composition of pellet diet and *S. simplex* ie. protein, carbohydrate and lipid content is shown in Table 1. Percentage of protein and lipids was high in *S. simplex* when compared to that of the pellet feed. It is observed during the experimental period that *C. auratus* actively feed on *S. simplex* than on pellet diet. The final body weight and specific growth rate of fishes were significantly higher in the group fed with *S. simplex*, 6.59 ± 0.39 g and 08.11 ± 0.02 than with the group fed with pellet diet 3.74 ± 0.44 g and 01.77 ± 0.01 (Table 2). An appreciable growth is observed in the length of fishes between the groups after 45 days of experimentation. There was no mortality observed during the entire experimental period in all the experimental groups, the fishes stocked survived and were healthy throughout the experimental period. None of the stocked animals showed any symptoms of stress, whitened by the feeding rate or microbial infections.

At the end of the experiment (45 days), fishes fed with pellet diet and *S. simplex* were sacrificed and subjected to various biochemical analyses and significant differences were found

Table 1 Proximate composition of pellet diet and *S. simplex*.

Component (%)	Pellet diet	<i>S. simplex</i>
Proteins	28.18 \pm 0.85	49.93 \pm 1.23
Lipids	11.93 \pm 1.15	15.03 \pm 1.10
Carbohydrates	35.38 \pm 1.13	08.86 \pm 0.95
Ash	14.26 \pm 1.59	11.60 \pm 0.57
Moisture	10.67 \pm 2.05	14.37 \pm 1.19

Mean \pm standard deviation, $n = 3$.

Table 2 Growth parameters of *C. auratus* fed with pellet feed and *S. simplex* before and after the experimental period.

Growth parameters	Pellet feed	Fed with <i>S. simplex</i>
Initial weight (g)	02.94 \pm 0.08	02.94 \pm 0.08
Initial length (mm)	45.61 \pm 0.11	45.61 \pm 0.11
Final weight (g)	03.74 \pm 0.44	06.59 \pm 0.39
Final length (mm)	58.19 \pm 0.15	75.14 \pm 0.37
Weight gain (%) ^a	80.27 \pm 1.82	124.14 \pm 2.15
Length increase (%) ^a	27.41 \pm 0.45	64.69 \pm 0.30
Specific growth rate ^b	01.77 \pm 0.01	08.11 \pm 0.02

Mean \pm SD, ($n = 3$; $p < 0.02$).

^a (Final – initial/initial) \times 100.

^b (Final – initial/no. of days) \times 100.

between various parameters (Table 3). High protein and lipid contents were recorded in the group fed with *S. simplex*, 15.2 ± 0.61 and 04.6 ± 0.15 than that of pellet group, 13.6 ± 0.51 and 03.4 ± 0.05 . On the other hand, ash content was high in the pellet fed (05.1 ± 0.34) group than in the *S. simplex* group (02.3 ± 0.07).

Amino acid analysis

Overall, the amino acid content in the pellet diet and *S. simplex* showed the presence of essential amino acids such as lysine (4.8 ± 0.1 and 13.7 ± 0.3), threonine (4 ± 0.2 and 5.3 ± 0.1), arginine (2.8 ± 0.1 and 5 ± 0.3), phenylalanine (2.2 ± 0.06 and 3.8 ± 0.3), leucine (1.8 ± 0.1 and 3.1 ± 0.2), and certain non-essential amino acids viz., glutamic acid (11.2 ± 0.7 and 12 ± 0.4), alanine (4.9 ± 0.5 and 9.6 ± 0.3) and aspartic acid (7.4 ± 0.4 and 8 ± 0.5) (Fig. 1a). As a general rule, the amino acid requirement of species closely mirrors the amino acid composition of their muscle tissue. More particular amino acid analysis on the tissue of the fish perfectly reflected their utilization of certain amino acids, high levels of arginine (2.1 ± 0.01 and 4.2 ± 0.3), lysine (2.4 ± 0.1 and 7.4 ± 0.3), threonine (3.7 ± 0.02 and 5.1 ± 0.1) and phenylalanine (0.8 ± 0.06 and 3.3 ± 0.3) are expressed in fish fed on pellet feed and *S. simplex* (Fig. 1b).

Fatty acid analysis

Fatty acid profile of the tissue of *C. auratus* fed with pellet diet and *S. simplex* observed significant differences between the percentages of saturated and unsaturated fatty acid composition (Table 4). A comparatively high percentage of unsaturated fatty

Table 3 Proximate composition of the tissue of *C. auratus* fed with pellet feed and *S. simplex* after the experimental period.

Component (%)	Pellet diet	<i>S. simplex</i>
Proteins	13.6 \pm 0.51	15.2 \pm 0.61
Lipids	03.4 \pm 0.05	04.6 \pm 0.15
Carbohydrates	07.6 \pm 0.13	07.4 \pm 0.83
Ash	05.1 \pm 0.34	02.3 \pm 0.07
Moisture	70.1 \pm 1.45	70.4 \pm 1.85

Mean \pm standard deviation, $n = 3$.

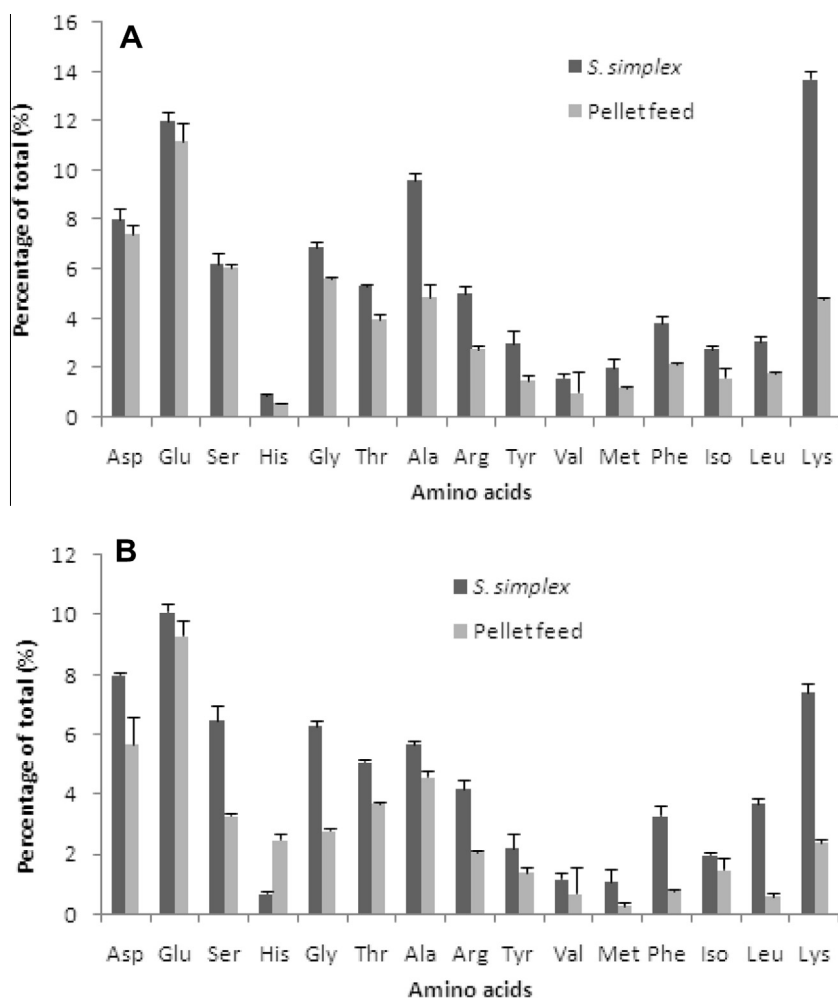


Figure 1 Amino acid profile of the experimental diet (A) and tissue of *C. auratus* fed on experimental diet (B). Results were plotted as mean \pm standard deviation, $n = 3$.

acids *viz.*, palmitoleic acid (4.4 ± 0.04 and 5.4 ± 0.03), linoleic acid (11 ± 0.01 and 13.5 ± 0.01) was seen and a special note has to be made regarding the presence of eicosapentaenoic acid (16.7 ± 0.05 and 29.6 ± 0.05) and docosahexaenoic acid (10.6 ± 0.01 and 13.6 ± 0.05). It is observed that the fatty acid profile of tissue of *C. auratus* fed on *S. simplex* reproduced a good percentage of fatty acids than the group fed with pellet diet.

Carotenoid content

Carotenoid complex revealed a high level of carotenoids $112.21 \pm 2.1 \mu\text{g}^{-1}$ dry weight in the fish group fed with *S. simplex*, whereas a significantly low level of carotenoids in the group fed with pellet diet, $101.64 \pm 1.7 \mu\text{g}^{-1}$ dry weight was seen. In the present investigation it is revealed that astaxanthin (36.4 ± 2.4), canthaxanthin (23.6 ± 1.7), and β -carotene (1.7 ± 0.6) were predominant carotenoids observed in the fishes fed with fairy shrimp and significantly low level carotenoids of the same were expressed in the other group (Fig. 2).

Discussion

Live feeds are being utilized as nursery/weaning/maturation diets and they also improve energy balance which results in

maturation, quick growth, coloration and physiological conditions (Mitchell, 1991; Munuswamy et al., 1997; Velu and Munuswamy, 2007). As illustrated in Table 2 fish growth performance parameters regarding the average final weights of *C. auratus* fed by pellet feed and *S. simplex* were 03.74 ± 0.44 and 06.59 ± 0.39 g, respectively. Significant differences among the treatments continued during the experimental period. The final weight of *C. auratus* increased linearly day by day and comparatively more in all the fishes after feeding the *S. simplex*. These results may be due to that fairy shrimps are more digested than pellet feed. It is always experimentally proved that, the final weight of punter fish species depends on the initial weight and management techniques (Shaker, 2008; Shaker et al., 2008), while the live food *viz.*, rotifers (Wilcox et al., 2006), brine shrimps (Smith et al., 2004) are more suitable for different fish species.

The nutritive value of the feed is important as it reflects on the consumer organisms (Bubinas and Lozys, 2000). Estimation of biochemical composition of zooplankton is important in understanding their physiological function, metabolic rate, nutritive value and energy transfer (Jha et al., 2007). Moisture, ash, fat, and protein contents showed significant ($p < 0.05$) differences among the experimental diets (Table 3). Moisture contents were similar in both the groups

Table 4 Fatty acid composition of the tissue of *C. auratus* fed with pellet diet and *S. simplex*.

Fatty acid		Tissue of <i>C. auratus</i> fed with	
		Pellet feed	<i>S. simplex</i>
Myristic acid	14:0	7.9 ± 0.03	5.9 ± 0.05
Palmitic acid	16:0	10.6 ± 0.01	19.4 ± 0.09
Stearic acid	18:0	1.7 ± 0.01	0.7 ± 0.01
Arachidic acid	20:0	2.8 ± 0.02	4.06 ± 0.05
Behenic acid	22:0	1.8 ± 0.07	3.2 ± 0.01
Lignoceric acid	24:0	0.01 ± 0.01	0.08 ± 0.01
Palmitoleic acid	16:1 <i>n</i> – 7	4.4 ± 0.04	5.4 ± 0.03
Oleic acid	18:1 <i>n</i> – 9	4.0 ± 0.05	5.7 ± 0.01
Linoleic acid	18:2 <i>n</i> – 6	11.0 ± 0.01	13.5 ± 0.01
α-Linolenic acid	18:2 <i>n</i> – 3	0.3 ± 0.04	0.5 ± 0.02
Eicosapentaenoic acid	20:5 <i>n</i> – 3	16.7 ± 0.05	29.6 ± 0.05
Docosahexaenoic acid	22:6 <i>n</i> – 3	10.6 ± 0.01	13.6 ± 0.05

Mean ± standard deviation, *n* = 3.

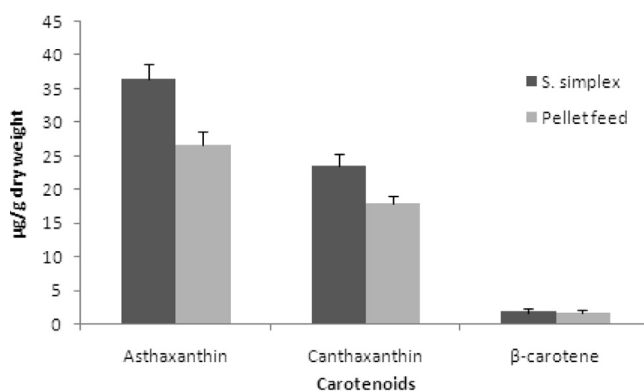


Figure 2 Carotenoids content observed in the tissue of *C. auratus* fed on experimental diet expressed in µg/g dry weight. Results expressed as mean ± SD; *n* = 3 (*p* < 0.05).

but a sharp increase of ash percentage was recorded in the fishes fed with pellet diet. From the data presented in Table 3, it is evident that the chemical composition showed that *C. auratus* fed by *S. simplex* had significantly (*p* < 0.05) higher contents of protein and fat than fish species fed by pellet feed. These results may be due to the high protein content in *S. simplex* than pellet feed diets (Table 1). These results indicate that the use of zooplankton as a live food for different fish species increased protein and fat contents, and in the mean time decreased ash content. These findings suggested the use of *S. simplex* as a live food for fish to improve the quality of fish.

The success of fish culture largely depends on the availability of the essential amino acids as fish cannot synthesize these amino acids and these are to be chiefly obtained from the diet (Murugesan et al., 2010). During the present analysis, totally 15 amino acids were observed in experimental diets with lysine, threonine, glutamic acid, alanine, aspartic acid, serine, glycine and arginine being prominent among them (Fig. 1). Only very limited information is available on the amino acid content of *S. simplex*, but the role of amino acids in the growth and maintenance of fish growth is well understood. *S. simplex* has a high amount of individual amino acids compared to that of pellet feed. The amino acid profile of gold fish fed on this live feed also perfectly reflected the utilization of amino acids available

in the diet. Methionine plays a vital role in the promotion of fish growth (Nose, 1979). Hence, the comparatively high percentage of methionine in *S. simplex* (Fig. 1a) would have contributed to the increase in the specific growth rate of goldfish fed. According to Yurkowshi and Tabachek (1979), methionine is said to be the first limiting amino acid in natural food organisms of fishes. Chen et al. (1996) reported that amino acids viz., phenylalanine, glycine, histidine and alanine reduce the oxidative stress in the organisms and promote the growth of the organism. And presence of arginine in *S. simplex* would have stimulated the release of hormones such as somatotropin and insulin in goldfish and enhance the size of the fish (Eddy et al., 1974). Velu and Munuswamy (2007) also reported the significance of essential amino acids in the brain shrimp and fairy shrimp with regard to the response of supplementation as nutritive diet to goldfish.

Like all other vertebrates, fishes also require essential fatty acids especially polyunsaturated fatty acids (PUFA) for their growth and development, hormonal balance, precursors for prostaglandins and other eicosanoids (Sargent et al., 1995, 1997). These fatty acids are unsaturated fatty acids and must be provided through diet (Bell et al., 1986; Tocher, 2010; Pozernick and Wiegand, 1997). The fatty acid profile of the tissue of *C. auratus* fed with adult *S. simplex* showed a high level of both PUFA and HUFA such as 18:2*n* – 6, 20:5*n* – 3, 20:6*n* – 3. Compared to this group the fatty acids levels are low in *C. auratus* fed with pellet diet. Consumption of *S. simplex* must have been the reason for the high accumulation of PUFA, after the experimental period which reflects its significance. Linoleic acid (18:2*n* – 6) has a significant biological role (Stickney et al., 1985) moreover they act as precursor for arachidonic acid. Furthermore, DHA comprises around 13.6% and is needed for optimal cognitive and visual development in the fishes (Neuringer et al., 1988).

Commercial value of ornamental fishes is increased by the intensity of skin color and it is due to various carotenoid pigments (Velu and Munuswamy, 2007). *C. auratus* pigmentation can be enhanced through carotenoid rich diets (Paripatananont et al., 1999). In the present study, the fish fed on *S. simplex* expressed more coloration compared to that of fish fed on the pellet diet; this may be due to the presence of astaxanthin, canthaxanthin and β-carotene in the zooplankton.

Conclusion

Feeding habits of fishes are different among the species but all fishes require protein rich food for their better growth, efficient breeding and survival (Mandal et al., 2009). The present investigation assessed the nutritive value of adult *S. simplex* as a diet in ornamental fish culture. It is once again experimentally proved that, artificial feeds are no match to live food organisms in terms of acceptance, nutritional and other factors. Moreover, the presence of carotenoids makes the fairy shrimps a suitable diet in ornamental fish culture.

Conflict of interest

The authors declare that they have no conflict of interest.

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