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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 trail 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 Anostracan. 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*-ophthaldialdehyde (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

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