Indian Journal of Animal Sciences **79** (11): 1185–1188, November 2009

Comparative growth performance and proteolytic enzyme activity of Indian major carp larvae, fed with live food and refrigerated-plankton food

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Received: 20 February 2009; Accepted: 2 May 2009

ABSTRACT

Indian major carp *Catla catla* (0.79±0.01 mg) larvae were cultured under 2 feeding regimes of live food (LFS) and refrigerated-plankton food (RPFS) in recirculating systems separately for 30 days. Survival of larvae was 18% higher in LFS compared to RPFS. Final average weight was significantly higher in fish fed with live food compared to the refrigerated-plankton fed fish. Specific growth rate was significantly higher in former compared to latter one. RNA/DNA of catla was nearly two-fold higher in live food fed fish compared to the refrigerated-plankton food fed fish. Food was utilized in a better way in LFS compared to RPFS is evident from the significantly lower value of FCR in the former compared to the latter system. Specific proteolytic activity was significantly higher in LFS (0.82±0.02 mg tyrosine/mg protein/h) compared to RPFS (0.457±0.01 mg tyrosine/mg protein/h). The presence of more digestive enzymes, especially proteolytic enzyme in live food fed catla resulted into the proper digestion of ingested food compared to the fish fed with refrigerated-plankton food. Prevalence of better water quality in terms of significantly lower values of ammonia, nitrite, phosphate and COD in LFS compared to the RPFS was conducive for the better performance of catla in the former feeding scheme. Reduction in nutrient contents like protein and lipid as well as leaching of essential nutrients especially free amino acids from refrigerated-plankton may be responsible for the poor performance of catla larvae in RPFS compared to LFS.

Key words: Catla, Live food, Proteolytic enzyme, Refrigerated-plankton food, RNA/DNA

Successful mass production of larvae of carp *Catla catla* (catla) has great economic importance. High mortality, stunted growth rate, skeletal deformities are some of the common problems encountered in carp culture (Jena *et al*. 1998). The tiny size and the fragility of the larvae and their dependence on live food organisms are usually reported as limiting factors. Zooplankton have been greatly appreciated as natural food in the nursery of Indian major carps (Sharma and Chakrabarti 1999, 2003). Culture of live food throughout the year, their storage and transportation are major constrains. Amali and Solomom (2001) reported preserved zooplankton as a suitable food for the rearing of *Clarias gariepinus* larvae. Frozen zooplankton is a cheap and readily available alternative to live *Artemia* as a co-feeding weaning diet (Herbert and Graham 2003). The present investigation aims to evaluate the possibility of substituting live plankton by refrigerated-plankton as a sole food for catla during early development.

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MATERIALS AND METHODS

Larval rearing: Catla larvae (0.79±0.01 mg) were cultured under 2 feeding regimes of live plankton food (LFS) and refrigerated-plankton food (RPFS) in recirculating systems separately. The stocking density was 125 larvae/ 15 litre aquarium. Three replicates were used for each feeding scheme. In the LFS, plankton were supplied at the rate of 150 mg/aquarium (dry weight) thrice daily. In the RPFS, same amount of plankton were kept in the refrigerator for 12 h and were directly supplied to the larvae. Among the various plankters, *Ceriodaphnia cornuta* was the dominant species, contributing 72% of the plankton population. Other zooplankton were *Mesocyclops* spp. (8%), *Brachionus* spp. (18%) and rest were phytoplankton. Biochemical assays showed that protein contents of live food and refrigerated-plankton food were 47.65±0.33 and 40.22±0.62%, respectively; 12.77±0.71 and 8.75±0.56% lipid were found in the former and latter, respectively. After 30 days, fish were harvested and used for various assays.

FCR and SGR estimations: Food conversion ratio (FCR) and specific growth rate (SGR) were calculated using the equations:

$$FCR = W_{dt}/ (W_2–W_1) \text{ and}$$
$$SGR = 100 \times (\ln W_2–\ln W_1)/t$$

where, W_{dt} was the dry weight of food, W_1 and W_2 were the initial and final weights of fish, respectively and t is the time in days.

RNA and DNA estimations: The nucleic acids were determined by pentose analysis (Schneider 1957). RNA and DNA were determined by Orcinol reaction and Diphenylamine reaction, respectively.

Enzyme assay: Fish were sampled at 8 a. m. before morning feeding. Digestive tract from individual fish was taken out, homogenized in 10 volumes (v/w) of ice-cold distilled water and centrifuged at 4°C at 10000×g. The supernatants were taken for analysis. Proteolytic activity was measured following the method of Anson (1938) and Kunitz (1947) using denatured casein substrate. The reaction mixture consisted of 1 ml of substrate solution, 1 ml of 0.1 M phosphate buffer (pH 7.6), 1 ml of $CaCl_2$ and 1 ml of crude enzyme extract. Reaction was stopped after 1 h of incubation using 3 ml of 5% TCA solution. After 10 min, the precipitate was removed by centrifugation; diluted Folin-Ciocalteu reagent was added to the supernatant and absorbance was measured at 650 nm. Concentration was obtained from a standard curve of tyrosine. Protein was estimated by the method of Lowry *et al*. (1951). Specific proteolytic activity was given as mg of liberated tyrosine/ mg of protein/h at 37°C.

Water quality : Water quality parameters were monitored at weekly interval. Water temperature ranged from 25.8–27°C throughout the study period. The pH of water was measured with a pH meter. Dissolved oxygen was determined with the help of a dissolved oxygen meter. Ammonia, nitrite, phosphate and chemical oxygen demand (COD) were measured following standard methods (APHA 1995).

Statistical analysis: Differences in fish growth, survival, RNA/DNA, enzyme activity and water quality parameters were assessed by one-way ANOVA and Duncan's Multiple Range test, DMR (Montgomery 1984). The level of significance was accepted at P<0.05.

RESULTS AND DISCUSSION

Survival and growth of fish: The survival rate of catla in LFS was significantly (P<0.05) higher compared to RPFS. 18% higher survival of fish was found in the former compared to the latter feeding scheme. Perch *Perca fluviatilis* larvae were fed with live *Artemia* and frozen *Artemia*, and higher mortality rate of 8%/d was recorded between days 7 and 10 in the latter group compared to the former (Fiogbe *et al*. 1995). Twenty-days-old Asian sea bass *Lates calcarifer* were fed with live or frozen *Moina macrocopa* and minced fish-by-catch, survival and specific growth rate of fish were significantly higher in the former compared to latter treatment (Fermin *et al*. 1994). The final average weight of catla was also significantly (P<0.05) higher in the group fed with live food (61.57±0.92 mg) compared to the fish fed with refrigerated-plankton food (42.92±0.45 mg). Milkfish fry were reared for 30 days using live and frozen *Moina macrocopa* and *Brachionus plicatilis.* Fry fed with live *M. macrocopa* showed higher growth and survival and yields significantly higher than fry fed with frozen food. On the other hand, there were no significant differences in growth and survival rates in fry fed live or frozen *B. plicatilis* (Villegas and Lumasag 2007). The growth of *Coregonus lavaretus* L. larvae fed with live zooplankton was significantly higher compared to the fish fed with frozen zooplankton (Kleifeld-Kriebitz and Rosch 2007).

Specific growth rate was significantly (P<0.05) higher in LES (6.30±0.023) compared to the RPES (5.78±0.01). African catfish *Heterobranchus longifilis* fed with live in the present study *Artemia* nauplii showed significantly higher final mean weight and specific growth rate compared to the fish fed with frozen food. But there was no significant difference in the survival rate of fish (Kerdchuen and Legendre 1994). RNA/DNA of catla was nearly two-fold higher in live food fed fish (3.15±0.07) compared to the refrigerated-plankton food fed fish (1.66±0.05). Significantly (P<0.05) higher RNA/DNA of fish in LFS was correlated with the larger size of fish in this system compared to RPFS. Increased RNA/DNA of herring *Clupea herengus* was correlated with the age or length of the larvae (Clemmesen 1994).

Significantly higher survival and growth of larvae in the live food system was related to the better utilization of food, which was evident from the lower value of food conversion ratio in this system compared to the refrigerated-plankton food system (Table 1). FCR was significantly (P<0.05) higher in RPFS compared to LFS. Specific proteolytic activity was significantly (P<0.05) higher in LFS (0.82±0.02 mg tyrosine/

Table 1. Means and SE of percentage survival, final average weight, feed conversion ratio (FCR), specific growth rate (SGR), RNA/DNA and specific proteolytic enzyme activity of *Catla catla* larvae fed live food and refrigerated-plankton food. Means not sharing same letter are significantly (P<0.05) different

	Live food	Refrigerated-plankton food
Survival (%)	82.0±1.22[a]	64.0±0.95[b]
Average weight (mg)	61.56±0.92[a]	42.92±0.45[b]
FCR	1.77±0.20[b]	2.57±0.20[a]
SGR	6.30±0.02[a]	5.78±0.01[b]
RNA/DNA	3.15±0.07[a]	1.66±0.05[b]
Specific proteolytic activity (mg tyrosine/ mg protein/h)	0.82±0.02[a]	0.45±0.01[b]

mg protein/h) compared to RPFS (0.457±0.01 mg tyrosine/ mg protein/h). The presence of more digestive enzymes, especially proteolytic enzyme in live food fed catla resulted into the proper digestion of ingested food compared to the fish fed with refrigerated-plankton food. The peristaltic movement of the ingested live food may be contributed in the secretion of neuropeptides in the digestive system of fish, which is lacking in the fish fed with refrigerated-plankton food. Moreover, the secretions of the digestive juices are related to the development of the digestive system. As the growth of catla was higher in the LFS compared to the RPFS, definitely the digestive system was well developed in the former compared to the latter one. Sharma and Chakrabarti (1999) showed that there was direct relation between the length of the digestive system and the amount of proteolytic enzymes in common carp *Cyprinus carpio* larvae (r=0.95). The gilthead seabream *Sparus aureta* larvae fed with rotifers, cultivated with freeze-dried microalgae showed a thinner digestive epithelium, lower number of lipid vacuoles in the enterocytes of anterior intestine and specially proteic vesicles in the posterior intestine compared to the larvae fed with rotifers, cultivated with live microalgae (Navarro and Sarasquete 1998). Significantly higher amylase and proteolytic enzyme activities were reported in live food fed common carp *Cyprinus carpio* larvae compared to the refrigerated-plankton food fish (Sharma and Chakrabarti 2000).

Water quality: The pH of water ranged from 8.27–8.44 and 8.38–8.59 in LFS and RPFS, respectively, during the study period. In both these systems, dissolved oxygen level was maintained above 5 mg/liter with the help of aerators throughout the study period. Significantly (P<0.05) higher level of ammonia was recorded in the RPFS compared to LFS (Table 2). Ammonia level was higher during the first week of culture and then gradually reduced regardless of feeding schemes. Similarly, nitrite level was also minimum during the later part of the culture period. Phosphate and COD showed opposite trends; values were minimum during the first week of the culture and then gradually increased with the passage of time. Phosphate level was significantly (P<0.05) higher in RPFS compared to LFS. COD was recorded as 199±10 and 180±9 mg/litre in RPFS and LFS, respectively. COD was significantly (P<0.05) higher in the former feeding scheme compared to the other.

Table 2. Means and SE of water quality parameters in recirculating culture systems of *Catla catla* larvae under two feeding regimes of live food and refrigerated-plankton food. Means not sharing same letter are significantly (P<0.05) different.

Parameters (mg/litre)	Live food	Refrigerated-plankton food
Dissolved oxygen	5.77±0.500[a]	5.41±0.02[b]
Ammonia	0.002±0.0007[b]	0.003±0.0003[a]
Nitrite	0.007±0.001[b]	0.008±0.001[a]
Phosphate	0.203±0.003[b]	0.239±0.001[a]
COD	180±9[b]	198±10[a]

Prevalence of better water quality in terms of significantly lower values of ammonia, nitrite, phosphate and COD in LFS compared to the RPFS was conducive for the better performances of catla in the former feeding scheme.

The study of biochemical composition showed that there were 7 and 4% reduction in the amounts of protein and lipid in the refrigerated-plankton food during 12 h storage period. The reduced nutritional quality was one of the major reasons for the poor performance of the fish in this treatment. Besides this, the leaching of essential nutrients, especially free amino acids was also responsible for the significant differences in survival and growth of catla cultured under these two different feeding regimes. Dietary amino acid imbalance results into increased amino acid oxidation and decreased growth rate (Tacon and Cowey 1985, Mohanty and Kaushik 1991). The leaching factor may be partially avoided by increasing the frequency of food supply and thus by reducing the amount of refrigerated-plankton food, deliver at each meal, and the time interval between food supply and ingestion by the larvae. Further study is required in this direction.

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