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Nutritional evaluation and culture of freshwater live food organisms on *Catla catla*

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Abstract

Feeding management plays a critical role in the success of fish culture. The current trend in fish culture is towards increased intensification whereby, provision of feeds becomes necessary and success depends significantly on the availability of well balanced nutritionally complete and cost effective compounded feeds. Micro algae and zooplankton play a vital role in the hatchery phase of many aquacultures as feed for larval and juvenile crustaceans and fish. Several algae are being investigated as potential source of protein for both livestock and human consumption. The food value of live food organisms for a particular fish species was primarily determined by its size and form. Nutrients are often the limiting resources in ecosystems and an environmental template of geological and climatic and ecological features ultimately delimits their availability. Nutritional quality of live food in aquaculture is important for survival and growth of larvae. A 5-d laboratory trial was conducted to evaluate live feed organisms on growth and survival of *Catla catla*. *Chroococcus turgidus* and *Mesocyclops aspericornis* were fed to fish throughout the study. Biochemical analysis was carried out on the live feed organisms and fish. The protein, lipid, carbohydrate and amino acids content of fish were quite variable when live food was consumed. The present study confirmed that alga and copepod could be suitable live food for *Catla catla* rearing because it meets their nutritive requirements.

Key words: *Chroococcus turgidus*, *Mesocyclops aspericornis*, *Catla catla*, Nutritional *values*

INTRODUCTION

Energy in pelagic ecosystems flows from phytoplankton to zooplankton through the classical food chain and microbial food webs. Energy flow at the primary producer and herbivore interface is influenced by both the quality and quantity of food available for zooplankton [3, 19, and 36]. Algal size, secondary metabolites, digestibility, elemental and biochemical composition have previously been used to explain food quality for zooplankton [16, 19]. Zooplankton growth in nature may depend on the quality of the food available as the phytoplankton community changes. Phytoplankton may stimulate zooplankton development by production of vitamin E (α- tocopherol) and releasing `odour' into water [29]. Feeding rates of zooplankton organisms are mainly dependent on food concentration, food quality and water temperature [15, 24]. It has been shown by several workers that feeding among copepods was related to chemo receptors (Friedman and Strickler [10, 23]; Paffenhofer *et al.,* [23]; mechanoreceptors by Strickler [36] and taste of the particular food Poulet and Marsot [25].

The food value of live food organisms for a particular fish species was primarily determined by its size and form. While a small food organism was desirable for fish larvae in term of digestibility, the use of larger organisms was more beneficial as long as the size of the food organisms did not interfere with the ingestion mechanisms of the predator [18]. Fish would take a long time to attain satiation if fed with smaller live food organisms, and this would result in poor growth due to inefficient feeding and waste of energy. Nutritional quality of live food in aquaculture is important for survival and growth of larvae [38]. Zooplankton is the valuable source of amino acids, fatty acids, minerals and enzymes [12]. Live zooplankton contains enzymes (amylase, protease, exonuclease and esterase), which play important role in larvae nutrition [8] and easily digestible. The live food organisms have a high food value as protein source of fish [22, 42].

The nutritional quality of any algal species depends on its cell size, digestibility, production of toxic substance and biochemical composition. Nutritional value of algae can vary according to the culture conditions like composition of culture medium [3, 5]. Copepods constitute an important component of food chain in aquatic systems. The nutritional quality of copepods is accepted to be highly satisfactory for larvae of prawn and finfish spaces [30, 41]. Furthermore, these copepods feed on algae which contain large amounts of free amino acids, and it is believed that amino acid release could be responsible for trypsin stimulation in post larvae [1].

In recent decades, aquaculture has become an increasingly important part of the world economy. One of the major problems associated with culture of live food is its unstable nature often exhibiting a rapid population decline immediately after a peak. So there is a need to standardize the technique. Except for a couple of publications [14, 20, 26, 40], very little work has been done on the aminoacid requirements of Indian major carps. In view of this, the present study was designated to investigate the nutritional requirements for the growth by fry of *Catla catla*.

MATERIALS AND MTHODS

Microalgae culture

The microalga *Chroococcus turgidus* isolated from Porur Lake, Chennai, Tamilnadu, India was cultured using CFTRI medium [44]. The isolated microalga were introduced to 100 ml of the medium and maintained in 250 ml of conical flask. Culture was grown at 24 ± 1ºC in a thermostatically controlled room illuminated with cool white fluorescent lamps (Philips 40w light 6500k) at an intensity of 2000 lux in a 12:12 light : dark regime and continuously aerated. The microalgal cells were counted using an improved Neubauer haemocytometer (0.25 mm^2 x 0.1 mm) under Olympus microscope (Japan)

When the microalga growths reached the stationary phase, algal cells were harvested by a laboratory centrifuge Sigma 4-15 (Remi, India) (252 g) using 250mL bottles for 10 min. The micro algal cells were chilled to 4°C and stored for 2 days before being used as food for *Mesocyclops aspericornis* and *Catla catla*.

Mesocyclops aspericornis was reared for few generations in the laboratory condition with yeast and alga (*Chroococcus turgidus*) as feedings. Laboratory raised zooplankton consisting of adult male and female were introduced (inoculums of 50nos/l) in the culture tanks. After 21 days, only adults were selected for experimental studies.

Catla catla was collected from the Tamilnadu Fish Seed Farm, Poondi, Tamilnadu, India, using cast net and maintained in the laboratory in a glass aquarium tank and acclimated in aerated tap water (temperature 30±1º C; pH 8.0; DO ˜ 7.0 ± 0.3 mg/L) with continuous aeration for two weeks prior to experimentation. During this period, fishes were fed with a known amount of fish food. The water in the aquarium tank was siphoned off everyday to remove remains of the feed and faecal matter and made up with fresh aerated water.

Experiment procedure

Grazing experiments were conducted for a period of five days in the laboratory. Approximately 500 ml of reservoir water was collected on the surface and poured to 1 L container in seven numbers of triplicates were taken. *Chroococcus turgidus* was added at 174 x 10^4 cell mL^{-1} to zooplankton and fish as their feed once in a day. Zooplankton (*Mesocyclops aspericornis)* 100 nos. was added to fish at twice a day as its feed. Everyday observation was made regarding the grazing of zooplankton and fish. The number of the organisms used in the experiment were maintained and checked every day. For zooplankton and fish, number, length, width and weight of the organism was taken. At the end of the experiment phytoplankton, zooplankton (wild and cultured) and fish were examined for biochemical analysis.

Nutritional analysis

Protein, lipid and carbohydrates were determined by the following methods. The basic procedures of the analytical scheme were taken from Lowry *et al*., [17] for protein, Folch *et al*., [9], for lipids, Roe [28] for carbohydrates and amino acids (Yamamoto *et al*., [42]

Statistical analysis

Differences in treatment means were compared by Duncan's Multiple Range Test (Snedecor and Cochran, [35]. The results of the feeding experiments was tested for statistical significance ($P<0.05$) by one-way analysis of variance**.** T-test was used for further testing the similarity or difference between the variations of different biochemical constituents of the organism. All statistical analysis was carried out using Statistical Package for Social Science (SPSS 2002, version11.5).

RESULTS AND DISCUSSION

Microalgae and zooplankton play a vital role in the hatchery phase of many aquacultures as feed for larval and juvenile crustaceans and fish. The results obtained on biochemical profile of alga, copepod and fish (both experimental and control) are given in the tables 1, 2, 3 & 4.

Table. 1. Biochemical analysis of alga, *Chroococcus turgidus* in control

Organism	Carbohydrate	Protein	Lipid
Chroococcus turgidus (Control)	78.89 ± 0.26	17.80 ± 0.41	3.28 ± 0.39

Table. 2. Proximal carbohydrate level in control and treated organisms.

Organism	Mean	SD	t-value	p-value
Mesocyclops aspericornis (Control)	11.04	0.99	12.46	0.000**
Mesocyclops aspericornis (Treated) + ▪*Chroococcus turgidus*	18.57	0.34		
Catla catla (Control)	24.49	3.41	1.60	0.185NS
Catla catla + ▪*Chroococcus turgidus (Treated)*	21.19	1.06		
Catla catla (Control)	24.49	3.41	4.40	0.012*
Catla catla (Treated) + ▪*Mesocyclops aspericornis*	12.66	3.16		
Catla catla (Control)	24.49	3.41	5.55	0.005**
Catla catla (Treated) + ▪*Chroococcus turgidus* +	12.46	1.56		

▪*Mesocyclops aspericornis*				

Table. 3 Proximal proteins level in control and treated organisms.

Organism	Mean	SD	t-value	p-value
Mesocyclops aspericornis (Control) *Mesocyclops aspericornis (Treated)*+ ▪*Chroococcus turgidus*	75.26 78.84	0.50 1.38	4.22	0.014*
Catla catla (Control) *Catla catla* + *(Treated)* ▪*Chroococcus turgidus*	69.86 65.91	4.35 1.28	1.51	0.206NS
Catla catla (Control) *Catla catla (Treated)* + ▪*Mesocyclops aspericornis*	69.86 77.25	4.35 1.21	2.84	0.047*
Catla catla (Control) *Catla catla (Treated)* + ▪*Chroococcus turgidus* + ▪*Mesocyclops aspericornis*	69.86 79.38	4.35 0.94	3.71	0.021*

Table 4 Proximal lipid levels in control and treated organisms.

Organism	Mean	SD	t-value	p-value
Mesocyclops aspericornis (Control)	13.69	1.15	9.28	0.001**
Mesocyclops aspericornis (Treated) +	2.91	1.65		
▪*Chroococcus turgidus*				
Catla catla (Control)	5.64	1.08	7.38	0.002**
Catla catla +	12.55	1.21		
▪*Chroococcus turgidus (Treated)*				
Catla catla (Control)	5.64	1.08	3.86	0.018*
Catla catla (Treated) +	8.41	0.61		
▪*Mesocyclops aspericornis*				
Catla catla (Control)	5.64	1.08	2.27	0.050*
Catla catla (Treated) +				
▪*Chroococcus turgidus* +	8.14	1.13		
▪*Mesocyclops aspericornis*				

Values are ± S.D (three replicates) ▪ Feed

* Significant at 1 % level

* * Significant at 5 % level; NS Non significant (> 0,05)

The carbohydrate composition of the control alga (*C.turgidus*) was 78.89 ± 0.26%, copepod (*M. aspericornis*) 11.04 ± 0.99% and fingerlings of *C.catla* were 24.49 ± 3.41%. Copepod fed with alga, the carbohydrate gained is 18.57 ± 0.34%**.** The difference in the carbohydrate content between alga and copepod was significant

($p<0.05$). Carbohydrates in the form of polysaccharide glycogen are also an important form of energy storage in zooplankton [11]. These results differ from that of Groeger *et al*., [11] who recorded that copepod (*Argyrodiaptomus furcatus*) had carbohydrate levels above 27% (DW), showing that all the diets studied were efficient with the result of this component.

Fingerlings of the *C.catla* fed with alga showed reduced carbohydrate level (21.19 ± 1.06%). Similarly, fingerling of *C.catla* fed with copepod also showed a reduced carbohydrate level of 12.66 ± 3.16%. The difference in carbohydrate composition between copepod and fish was significant ($p<0.05$). Fingerling of *C.catla* fed with mixed diet also showed reduced carbohydrate level of 12.46 ± 1.56%. The variation in the carbohydrate content between them was highly significant ($p<0.01$). In fish, the carbohydrate is present in lower quantities in the form of glycogen, sugar and their derivatives. It was observed by Santhanam *et al*., [31] that, ten to fifty percent of carbohydrates in fish feed enhanced the growth of fish through their protein sparing action.

Fishes, in general, utilize dietary carbohydrates poorly. The optimum level of dietary carbohydrate should enhance the maximum growth and feed efficiency [34]. Based on the results of the present study, it was estimated that carbohydrate requirement is about 12% for *C.catla*.

The lipid composition of the alga (control) was 3.28 ± 0.39%; copepod 13.69 ± 1.15% and fingerlings of *C.catla* was 5.64 ± 1.08% (Table. 4)**.** The copepod fed with alga (*C.turgidus*) which showed reduction in lipid content (2.91 ± 1.65%). The variation in the lipid content between the two organisms were highly significant ($p<0.01$). The fingerlings of the *C.catla* fed with alga showed increased level of the lipid (12.55 ± 1.21% of the total dry weight). The variation in the lipid level between the two organism were highly significant ($p<0.01$). Similarly, in the fingerlings of the *C.catla* fed with copepod, (*M.aspericornis*) the lipid level increased to 8.41 ± 0.61% of the total dry weight.

Cavaletto *et al*., [4] observed that triacylglycerides are the main lipid reserves used by the majority of freshwater zooplankton. Vanderploeg *et al*., [39] observed that copepods are able to survive for a long period at low temperatures without any food intake, probably because they maintain low metabolic rates fuelled by high phospholipid content. These compounds used not only for survival during periods of low food abundance but also as energy reserves to be allocated to the eggs. Safiullah Aman and Altaff [30] reported 6.27 ± 1.25% is the lipid level required for copepod (*M. aspericornis*). The quality of food available for zooplankton has been shown to be an important factor, and the quality of algal lipid present may vary under different regimes. However, at too high levels, dietary fat may reduce fish growth, mostly due to reduction in fish consumption [33].

In the fingerling of *C.catla* fed with mixed diet, the lipid level increased by 8.14 ±1.13% (Table.4). The variation in the lipid content between these two organisms were significant ($p<0.05$). The lipid requirement of any cultivable fish depends largely on its digestibility, quality and amount of essential fatty acids present in the diet. Furthermore, the fatty acids and esters of glycerols are used by fish for long term energy requirements, more particularly during the period of extensive swimming and inadequate food supply. Freshwater fish have high levels of poly unsaturated fatty acids (PUFA) compared to their marine counterpart [31]. Hence, marine fishes require more PUFA rich diet.

Fatty acids play a vital role in maintaining structural and functional integrity of fish/prawn cell membranes. Zooplankton contain high levels of arachidonic acid and which help in the growth and survival of larvae as documented by Bell *et al.*, [2] and Sargent *et al.*, [32]. The higher lipid content recorded in the present study suggests high nutritive value of *M. aspericornis*. In the present study revealed that *C.catla* fingerlings were able to store significant quantities of lipid in their body, but were not able to utilize this energy source to improve growth when the dietary lipid was increased from 5.64 ± 1.08 to 12.55 ±1.21%.

The protein composition of the control alga was 17.80 ± 0.41%; copepod 75.26±0.50% and fingerlings of *C.catla* was 69.86 ± 4.35%) (Table.3). In the copepod fed with alga (*C.turgidus*) the protein content increased to 78.84±1.38%. The change in the protein content between the two organisms was significant ($p<0.05$). In the fingerlings of *C.catla* fed with alga the protein content reduced to 65.91 ±1.28%. The fingerlings of *C.catla* fed on copepod (*M.aspericornis*) showed increased protein content (75.25 ± 1.21%). The change in the protein content between the two organisms were significant (p<0.05). Similarly, in the fingerling of *C.catla* fed with mixed diet, the protein level increased to 79.38 ± 0.94 %. Statistically the variation between the two organisms were significant (p<0.05).

Copepods contain high protein content and have good aminoacid profile (Table.5). Copepod feeding on alga (*C.turgidus*) showed better growth and survival (100%). Similar results were also reported by Karul *et al*., (1991). But, in the case of fish fed on alga showed reduction in protein content (65.91 ± 1.28%) which may be due to lack of some amino acids.

Table. 5 Amino acid composition (% dry matter) of alga (*C.turgidus*)

Amino acid	**Algae *C. turgidus* (Control)**
Histidine	1.15
Methionine	0.10
Isoleucine	0.11
Leucine	56.58
Total EAA	57.94
Glutamine	11.29
Serine	3.09
Glycine	27.65
Total NEAA	42.03
Total	99.97

Fishes derive much of their energy from proteins. The growth of fish in terms of muscle formation depends on the high percentage of protein intake. The protein requirements of cultureable fish depend on size, age, stocking density, oxygen supply and the presence of toxicants. Crucial to aquaculture industry is the successful rearing of frys and fingerlings of economic importance, which entirely depends on the feed, provided to them, and the role of copepods as a successful starter feed has been very well established. Only dietary protein concentration has a significant influence on fish performance. The present study suggests that protein content increases with increasing food level. Based on the results of the present study, it is estimated that protein requirement of *C.catla* is about 65.91 ±1.28%.

The dietary proteins, such as aminoacids are of primary importance for the effective utilization and for successful growth and the amino acid patterns should meet the requirement of the organism. The percentage composition of aminoacid profile of control and experimental organisms are represented in the Tables 5, 6, 7, 8 & 9.

Table.6 Amino acid composition of (% dry matter) of copepod (*M.aspericornis)* fed Laboratory cultured alga (*C. turgidus)*

Amino acid	***M. aspericornis* (Control)**	***M.aspericornis* + *C.turgidus* (Treated)**
Threonine	0.17	-
Methionine	9.79	-
Isoleucine	3.51	-
Lysine	-	86.64
Leucine	60.18	-
Histidine	-	0.01
Tyrosine	1.67	-
Total EAA	75.32	86.65
Glutamine	13.47	0.57
Serine	11.24	9.86
Glycine	0.03	0.01

Alanine	0.06	-
Asparagine	-	2.88
Total NEAA	24.80	13.32
Total	100.12	99.97

Table. 7 Amino acid composition of (% dry matter) of fish (*C.catla)* fed laboratory cultured alga (*C.turgidus)*

Amino acid	***C. catla*** **(Control)**	***C. catla*** **+** ***C. turgidus*** **(Treated)**
Leucine	33.18	-
Threonine	-	0.01
Valine	-	0.02
Methionine	-	0.01
Lysine	-	26.19
Tyrosine	0.01	0.01
Total EAA	33.19	26.24
Glutamine	2.95	7.84
Serine	39.27	1.89
Asparagine	24.57	63.95
Glycine	-	0.03
Total NEAA	66.79	73.71
Total	99.98	99.95

Table.8 Amino acid composition of (% dry matter) of fish (*C.catla)* fed Laboratory cultured copepod (*M.aspericornis)*

Amino acid	***C. catla*** **(Control)**	***C. catla* + *C. turgidus* + *M.aspericornis* (Treated)**
Leucine	33.18	-
Histidine	-	2.44
Methionine	-	0.01
Lysine	-	59.66
Tyrosine	0.01	0.04
Total EAA	33.19	62.15
Glutamine	2.95	33.56
Serine	39.27	1.94
Asparagine	24.57	0.46
Glycine	-	0.04
Alanine	-	1.89
Total NEAA	66.79	37.89
Total	99.98	100.04

Table.9 Amino acid composition of (% dry matter) of fish (*C.catla*) fed Laboratory cultured alga (*C.turgidus)* and copepod (*M.aspericornis)*

Amino acid	***C. catla*** **(Control)**	***C. catla*** **+** ***M.aspericornis*** **(Treated)**
Arginine	-	0.05
Leucine	33.18	-
Histidine	-	-
Methionine	-	-
Lysine	-	64.07
Tyrosine	0.01	-
Total EAA	33.19	64.12
Glutamine	2.95	12.21
Serine	39.27	19.33
Asparagine	24.57	4.24
Glycine	-	-
Alanine	-	-
Total NEAA	66.79	35.85
Total	99.98	99.97

Copepod fed on alga, the essential aminoacid lysine increased and histidine was present in trace amounts, which were not there in control copepod. The level of non-essential aminoacids such as glutamic acid, serine, glycine and aspartic acid decreased significantly when fed with algal diet. Reed and D'Abramo [27] reported that a suitable protein source would be the most likely fulfillment of aminoacid requirements of *Macrobrachium rosenbergii*. Lysine is an essential aminoacid used as a supplement to food stuffs to increase the biological value of low value plant proteins. The success of fish culture largely depends on the availability of the essential aminoacids, namely arginine, histidine, isolucine, leucine, lysine, methionine, phenylanaline, threonine, tryptophan and valine. A fish cannot synthesis these amino acids and these are to be chiefly obtained from the diet.

In the fingerlings of *C.catla* fed on alga, the level of non-essential aminoacids increased. The leucine disappeared and threonine, valine, methionine and lysine were formed. The level of lysine was high (26.19%). The quantitative dietary requirement of aminoacid lysine for *C.catla* is 3.55% as reported by Ravi and Devaraj [26]; common carp, 5.7% Nose, [21]. In the present study the level of lysine increased when *C. catla* was fed with algal diet.

The quantitative requirement of aminoacid threonine for *C.catla* is 0.95% Ravi and Devaraj [26]. In the present study the level of threonine (0.01%) decreased. The quantitative requirement of aminoacid valine for *C.catla* is 3.55% [26]. In the present study the level of valine (0.02%) decreased. The quantitative requirement of aminoacid methionine for *C.catla* is 1.42% [26]. In the present study the level of methionine (0.01%) decreased. Methionine plays a vital role in the growth of fishes and hence, by providing the alga (*C.turgidus*) as feed, the essential aminoacid methionine can be transferred to fishes that in turn will promote their growth.

Fingerlings of *C.catla* fed on copepod (*M.aspericornis*) showed increase in essential aminoacids. The quantitative dietary requirement of aminoacid arginine of *C.catla* is 1.92% [26]. In the present study the level of arginine of *C.catla* decreased to 0.05%. Dabrowski and Rusieki [6] reported that arginine content decreased as the size of daphnids decreased. High levels of arginine in live feed organisms may stimulate concerted release of hormones such as somatotropin and insulin as reported by Eddy *et al*., [7]. The quantitative dietary requirement of aminoacid lysine of *C.catla* is 2.50% [26]. In the present study the level of amino acid lysine increased (64.12%). Fingerlings of *C.catla* fed with mixed diet showed increase in essential aminoacids. The quantitative requirement of aminoacid histidine for *C.catla* is 2.35% [26]. In the present study the growth data indicated that the gaining of weight improved significantly with increasing levels of dietary histidine (2.44%). The quantitative requirement of amino acid methionine for *C.catla* is 1.42% [26]. In the present study the level of methionine (0.01%) decreased. The quantitative dietary requirement of amino acid lysine of *C.catla* is 2.50% [26]. According to Yurkowski and Tabachek [43], methionine is said to be the first limiting amino acid in natural food organisms of fishes. In the present study the level of aminoacid lysine (59.66%) increased. The quantitative dietary requirement of aminoacid tyrosine for *C.catla* was 0.04%. The nutritional requirements of *C.catla* depend on the protein quantity and quality of alga and copepod. It is not yet completely clear which diet is preferable for zooplankton. Food quality is a crucial factor for these organisms, since they store large lipid reserves from the medium depending on their ability to assimilate nutrients from algae.

Copepod plays a very important and diverse role in freshwater aquaculture. Even though the trend to develop artificial feed is fast replacing the live feed, some outstanding quality of live feed cannot be overlooked. The natural food is free from pollution, and has good conversion efficiency. If the locally available species are used, they will be very effective and economical too. This idea is well supported by the experiments conducted.

C.catla plays a major role in aquaculture. But seed production currently is facing shortage due to high demand. If the availability of *C.catla* seed increases and if growth rates can be further improved through genetic enhancement, the contribution of *Catla catla* to aquaculture production in the Indian subcontinent will increase, with a decline in the importance of silver carp in carp polyculture systems. *C.catla* also has good potential for value added products for export.

The advantages of live feed are superior for it contains nutritional value and promotes normal growth and good pigmentation. They supply optimum lipid and protein for fast-growing fish and shrimp larvae. They improve stress resistance in finfish and shell fish larvae. Live food organisms induce visual and chemical stimuli in larvae and enhance their feeding. Live food is a source of exogenous digestive enzymes that improve digestion of prey in early-stage fish larvae when their gut is not fully functional.

CONCLUSION

Most commercial fish feed ingredients are costly and their non-availability poses problem for fish farmers to take up aquaculture practice. So a lot of research has been undertaken to find out suitable alternate cheaper ingredients of wide availability to boost culture. In the present study, variations in nutritional composition among planktons were observed. Even though alga are cheaper source of protein, lipid and other nutrients and culturing them is easy, they are yet to be considered as a major food item for fish. For commercial use of algae as fish feed, suitable commercial methods are to be developed to protect the fatty acid profile of algae. Standardization of mass culture of some specific algae, which have the ability to synthesize long-chain n3-fatty acids, is an important aspect in aquaculture. The present study recommends that alga (*C.turgidus*) and copepod (*M.aspericornis*) can be exclusively used as an important alternate or supplementary feed for commercial seed production of *C.catla*.

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