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Necessity of Dietary Mineral Supplements for the Growth of Freshwater Fish *Cyprinus carpio* and *Heteropneustes fossilis*

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Abstract

Thirty day feeding trials were conducted in 400-liter outdoor cement tanks separately for *Cyprinus carpio* and *Heteropneustes fossilis* to determine if mineral supplement is necessary for the growth of these freshwater fishes. Each of the species was reared under three dietary conditions: a basal diet with 31.5 % crude protein (T1), the basal diet containing 2% mineral supplement (T2) and the basal diet with an exogenous supply of live plankton at 5 ml concentrated suspension (containing about 800-1220 zooplankton) six days a week (T3). Both species showed a significant reduction in growth if the basal diet (T1) was not supplemented by minerals either as mineral mixture (T2) or through exogenous supply of plankton (T3). Deficiency of Co in the basal diet and in the test medium was found to be the reason for poor growth.

Introduction

Minerals are required by fish for various life processes (Stickney 1994). However, determination of dietary mineral requirement of fish is difficult because they have the ability to absorb minerals from the surrounding water in addition to the food ingested. Therefore, the dietary requirement of a fish species for a particular element depends to a large extent on the concentration of the element in the medium (Hepher 1990).

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At present, there is little information concerning the contribution of water-borne elements to the total mineral balance of fish (Hasan 2001). Concentration of the minerals in freshwater is also highly variable and often very scarce turning the fish dependent on the diet for the supply of the nutrients.

Animal and plant feed stuff used in the artificial feed formulation provide a significant quantity of minerals, which are often in excess of the estimated requirement (Gatlin and Wilson 1986; Storebakken et al. 2000). In the preparation of formulated feed for the Indian major carps and catfishes, minerals are generally mixed at 2-3% of the feed (Mishra and Mukhopadhyay 1996; Datta and Kaviraj 2003) with little attention on the requirements of the minerals for different species, availability of the minerals in the medium and in natural food and bioavailability of these minerals. Depending upon the feeding behaviour, these fish species obtain varying degrees of minerals from the natural sources. Live plankton constitutes an important source of the minerals to many of these fish species. Exogenous supply of plankton has been found to induce better growth of carp (Chakrabarti and Jana 1992). Lubzens et al. (1984) showed that providing rotifers in addition to artificial food improved the growth rate and survival of *Cyprinus carpio* larvae significantly. The objective of the present study was to determine if supply of mineral, either in the form of dietary supplement to the basal diet or in the form of exogenous supply of live plankton was necessary for the growth of the carp *Cyprinus carpio* and of the catfish *Heteropneustes fossilis*.

Materials and Methods

The 30-day feeding trials were conducted in 400-liter outdoor cement tanks separately for each of the two species of fish. The feeding trials for *Cyprinus carpio* were conducted during post monsoon period, when temperature of water ranged between 16.5 to 25.0°C, while those for *Heteropnesutes fossilis* were conducted during the summer when the water temperature ranged from 33.0 to 35.8°C. Fingerlings of these species were collected from local hatcheries and were acclimatized to laboratory conditions for one week before the trials. During acclimatization, the fish were fed *ad libitum* with a mixture of rice bran and mustard oil cake (1:1). The acclimatized fingerlings were stocked in outdoor tanks at 40 fish per tank for *C. carpio* and at 25 fish per tank for *H. fossilis*. Altogether nine tanks were used for each of the trials and these were arranged according to randomized block design (Gomez and Gomez 1984) so that there were three replicates for each of the three dietary conditions: a basal diet (T1), a basal diet supplemented by 2% mineral mixture (T2), and a basal diet with an exogenous supply of live plankton at 5 ml concentrated suspension (containing about 800-1220 zooplankton) six days a week (T3). Ingredients used in formulating the basal diet and its proximate composition are given in table 1. To maintain an exogenous supply of plankton on a regular basis, a culture of plankton was maintained in three separate tanks using cow dung as fertilizer. Food ration was provided at 5% of the body weight of the stocked fish. The fish were sampled every 10 days, weighed and the ration adjusted accordingly.

Table 1. Ingredients constituents and proximate composition of the basal diet

Ingredient constituents (%)		Proximate composition	
Mustard oil cake	35.10	Dry matter (DM) %	89.50
Rice bran	08.78	Protein (% DM)	31.50
Wheat flour	17.55	Lipid (% DM)	3.75
Fishmeal	36.57	Ash (% DM)	17.00
Vitamin premix[a]	02.00	Moisture	10.50

[a]Vitamin mixture (Ambiplex, Brihans Lab, Pune)

Chemical analyses and data collection

Proximate analyses of the basal diet were performed following AOAC procedures (AOAC 1990) as follows: moisture was determined by oven drying at 105°C for 24h; crude protein (Nitrogen X 6.25) was determined by Kjeldahl digestion; total lipid was determined gravimetrically after extracting the residue with 40-60°C petroleum ether for 7-8 h in a Soxhlet apparatus, and ash was determined by ignition at 550°C in a muffle furnace to a constant weight. Concentration of the minerals, namely copper (Cu), manganese (Mn), zinc (Zn), cobalt (Co), iron (Fe) and calcium (Ca), in the diet, trial medium and in samples of plankton was determined by flame atomic absorption spectrophotometer (Varian Spectra AA240) following the analytical procedures of Guhathakurta and Kaviraj (2000). Water quality parameters in the experimental tanks were checked every week during the trials (APHA 1995).

Fish mortality was recorded every day and dead fish, if any, was removed from the tank. All fish from each outdoor tank were sampled at the end of the trial and % increase in weight, specific growth rate (SGR) and food conversion ratio (FCR) were determined. Data from each trial were subjected to one way ANOVA followed by least significant difference (LSD) test between the treatments (Gomez and Gomez 1984).

Results

Concentrations of the minerals (Cu, Mn, Zn, Co, Fe and Ca) in diets, plankton and the test medium are given in table 2. The basal diet contained all these minerals at high concentration except Co. Addition of 2% mineral mixture significantly increased the concentrations of all these minerals in the basal diet. Concentrations of the minerals were higher in plankton than in water. Concentrations of Mn, Zn, Fe and Ca were very high in plankton and moderately high in water. The Cu was detected in trace quantity in water but in moderately high quantity in plankton sample, while Co was not detectable in water and was present in trace quantity in the plankton samples.

Table 2. Concentration of minerals in diets, plankton and water samples. Values are means of three replicates

Source	Cu	Mn	Zn	Co	Fe	Ca
Basal diet ($\mu g \bullet g^{-1}$)	9.67 ± 4.37	87.50 ± 20.46	72.62 ± 29.19	1.11 ± 0.92	1900 ± 388	12816 ± 1343
Basal diet with mineral mixture* ($\mu g \bullet g^{-1}$)	12.75 ± 4.60	125.83 ± 25.04	90.00 ± 10.40	106.29 ± 11.25	2725 ± 1047	14605 ± 530
Plankton ($\mu g \bullet g^{-1}$)	21.9 ± 2.13	988.25 ± 37.77	164.1 ± 6.31	5.45 ± 0.22	5619 ± 296	2384 ± 19
Test medium ($\mu g \bullet ml^{-1}$)	0.97 ± 0.01	23.73 ± 3.09	8.25 ± 4.00	ND	31.67 ± 6.66	306 ± 38

*100g mineral mixture (2% of basal diet) contained Calcium lactate (1.68g); $CaSO_4.2H_2O$ (13.441g); $CaCl_2.2H_2O$ (67.1g); KH_2PO_4 (4.2g); KCl (3.7g); NaCl (4.37g); Na_2CO_3 (1.01g); $MgSO_4.7H_2O$ (1.68g); $FeSO_4.7H_2O$ (0.67g); $ZnSO_4$ (0.034g); KI (0.54g); $CoCl_2.6H_2O$ (0.034g); $MnSO_4$ (0.07g).
ND = Not detectable

Growth parameters of the fish are summarized in table 3. Significant variation in growth was found between the dietary treatment groups for both the species of fish. Both species of fish showed a significant reduction in growth when fed the basal diet (T1) alone as compared to those fed mineral supplemented basal diet (T2) or those fed basal diet with an exogenous supply of plankton (T3). There was no significant difference in the growth of *Heteropneustes fossilis* between T2 and T3 dietary conditions, while growth of *Cyprinus carpio* was significantly higher in T3 as compared to T2 dietary condition. There was significant variation in mortality of fish between the dietary groups. But mortality of *C. carpio* was always less than 10% while those of the *H. fossilis* ranged from 7.2 to 31%. The alkalinity, free CO_2, ammonia and nitrate nitrogen level of water were under control in both trials.

Table 3. Mean (±SD) values of different growth parameters of the fish reared under different dietary conditions. Different superscript indicates significant difference between mean (LSD at 5% level)

Test fish	Treat-ments	Mortality (%)	% increase in weight	FCR[1]	SGR[2] (% /day)
Cyprinus carpio	T1 (Basal diet)	12.1 ± 5.2^a	50.05 ± 8.93^b	3.06 ± 0.54^b	1.35 ± 0.19^b
Initial length 40.66±2.65mm	T2	8.8 ± 0.4^b	89.08 ± 25.97^a	1.77 ± 0.49^a	2.10 ± 0.45^a
Initial weight 0.80±0.14g	T3	15.2 ± 5.3^a	142.38 ± 30.71^c	1.45 ± 0.34^a	2.93 ± 0.43^a
Heteropneustes fossilis	T1 (Basal diet)	31 ± 3.4^a	54.42 ± 21.43^b	5.73 ± 1.89^b	1.43 ± 0.44^b
Initial length 133.55±7.66mm	T2	7.2 ± 10.1^b	119.36 ± 30.46^a	1.75 ± 0.51^a	2.59 ± 0.48^a
Initial weight 8.1±1.36g	T3	20 ± 28.3^c	115.00 ± 28.25^a	2.51 ± 0.69^a	2.53 ± 0.45^a

[1] FCR = Dry weight of feed given / increase in weight of the fish
[2] SGR = {(ln final weight – ln initial weight)/days on trial}

Discussion

Information on the mineral requirement of fish is not complete and sometimes highly variable. While requirement is known for a few minerals for the common carp or carp in general, little is known about the requirement of minerals by *H. fossilis*. Zinc requirement of carp has been found to be 15 to 30 mg/kg of diet (Ogino and Yang 1979) while requirement of manganese and copper for common carp has been demonstrated as 13 $mg \bullet kg^{-1}$ and 3 $mg \bullet kg^{-1}$, respectively (Ogino and Yang 1980). Average requirement of calcium for fish is very high and is stated to be 5 $g \bullet kg^{-1}$ diet (Hepher 1990). But most of the natural feed ingredients used in the formulation of practical diet supply sufficient calcium to meet the requirement of calcium for most finfish (Hasan 2001). Basal diet used in the present investigation contained Zn, Mn, Cu and Ca at levels much higher than those required by common carp and many other species of finfish (Hepher 1990; Hasan 2001). Concentrations of Zn, Mn and Cu in water and plankton were also considerably high in respect to requirements by common carp and most other finfish (Hepher 1990).

Requirement of Fe is not known either for *C. carpio* or *H. fossilis*. Iron requirement has been reported as 30 mg•kg^{-1} diet for catfish (Gatlin and Wilson 1986). Maximum requirement of Fe (170 mg•kg^{-1}) is demonstrated for Japanese eel (Nose and Arai 1979). The basal diet used in the present investigation contained Fe at a level much higher than this. In addition, the water and the plankton contained a very high concentration of Fe. Average requirement of cobalt for fish is stated to be 5 to 10 mg•kg^{-1} (Hepher 1990). Concentration of cobalt was not detectable in water while the concentration of this mineral in the basal diet was lower (1.1 mg•kg^{-1}) than the average requirement (5-10 mg•kg^{-1}) of fish (Hepher 1990). Therefore, concentration of cobalt made a marked difference between the groups fed basal diet and mineral supplemented diet, while other minerals were available in excess even in the basal diet. In the present day culture system finfish are often exposed to excess dietary mineral supplements, which have mixed effects on fish. Rainbow trout and common carp tolerated 1,700 to 1,900 mg Zn•kg^{-1} of diet without any adverse effect on growth or survival (Jeng and Sun 1981; Wekell et al. 1983). Although 0.2% Ca supplement (from Ca lactate or tri calcium phosphate) of the diet resulted in significantly higher growth of Japanese flounder, a high level of Ca (2.5%) of the mineral decreased the growth of the fish (Hossain and Furichi 2000). Similarly up to 500 mg•kg^{-1} of haem-bound iron supplement in the diet did not produce any significant impact on the growth of the Atlantic salmon; but 1500 mg•kg^{-1} supplement of the same iron retarded the growth and resulted in complete loss of ascorbic acid in the diets (Andersen et al. 1997). The excess mineral present in the basal diet used in the present investigation apparently did not produce any adverse impact on the fish. But deficiency of cobalt in the basal diet might have resulted in poor growth in both species of fish tested. It is revealed from the present study that dietary supplement of Co is most essential for both carps and catfishes.

Enhancement of growth in *Cyprinus carpio* and *Heteropneustes fossilis* by exogenous supply of plankton reflects the preference of plankton as natural food by these two fishes. Fingerlings of *C. carpio* have a wide food spectrum, but are reported to feed intensively on zooplankton if it is available in the medium (Chondar 1990). *H. fossilis* is also known to feed on zooplankton at the juvenile stage but switches over to detritus or other food items available to it (Thakur 1991). Table 2 indicates that zooplankton is a major source of Co and exogenous supply of zooplankton could compensate the deficiency of cobalt if feeding habit permits the fish to accept the zooplankton as food. However, selective preference of *C. carpio* for the zooplankton as compared to *H. fossilis* rendered a better growth of the former in T3 diet as compared to T2.

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