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Effect of vitamin C and mineral enriched diet on growth and survival of *Labeo rohita* fry

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ABSTRACT

The present study was conducted to understand the effect of vitamin C and mineral enriched diet on growth, survival and mineral accumulation in *Labeo rohita* fry. The study was conducted for a period of 60 days and it was found that the growth and survival rate increased when local ingredients were fortified with commercially available and commonly used vitamin-mineral mixture, vitamin C and selected mineral elements (Zn, Cu and Mn). The best survival rate (76%) was recorded when feed was supplemented with all the three components (vitamin-mineral mixture, vitamin C and mineral supplement), followed by vitamin-mineral mixture + vitamin C (75.3%) and vitamin-mineral mixture. The Zn, Cu and Mn fortification in the diet also reflected higher deposition of these three minerals in the whole body as well as 38.4% reduction in the cost of production and 152.6% higher revenue generation compared to commercial feed (control).

Keywords: Copper, *Labeo rohita,* Manganese, Vitamin C, Zinc

Introduction

Aquaculture is a rapidly growing sector in India with an annual growth rate of over 7%, in which freshwater aquaculture contributes over 95% of the total annual aquaculture production of 5.77 million t (Jayasankar, 2018). The total fish and seed production during 2019-20 was 14.16 million t and 52,170.6 million fry, respectively where the inland sector contributed around 73.7% of the total fish production (HBFS, 2020). Freshwater aquaculture, mainly confined to carp culture, has evolved from a household activity being practised in West Bengal and Odisha to that of an industry (Ayyappan and Jena, 2003). In the last decade, growth of fish production was very encouraging in all the states of India compared to other sectors, but still, there is a large gap between demand and supply. One of the major problems of poor production and productivity is the non-availability of quality fish seed in required quantities.

In the rearing of Indian major carp, inadequate availability of quality seed was the major constraint being faced by the farmers followed by deficiency of water in critical periods and the high cost of supplementary feed (Vignesh *et al.*, 2017). The Indian major carps (IMCs) and Chinese carps do not breed in confined water, hence they are induced to breed by administering hormone injections. Despite development in various aquaculture technologies, the survival rate of spawn and fry is one of the most crucial factors determining sustainability of carp seed hatcheries. The average survival rate of carp fry hardly exceeds 30-40% under pond conditions (Ghosh *et al.*, 2002; Nandi, 2015). When carp seed is reared in two stages (*i.e.,* nursery and rearing), the survival of the fry is 30 and 50%, respectively, with rice bran and oil cake being used as larval feed (Basavaraja, 2007). To reduce the cost of production many farmers even restrict their feeding to rice bran only and use oil cakes during last phase of the production cycle (Ramakrishna *et al.,* 2013). Because of poor recovery and other technical issues, many hatchery owners are forced to abandon hatchery operation as the production is not cost-effective. Shivakumar *et al.* (2014) reported that the margin of fish farming was narrow over the years due to high input, labour and fertiliser costs.

The concentration of minerals in the body of an aquatic organism depends on the food sources, environment, species, stage of development and physiological status of the animal (Halver and Hardy, 2002; Adewumi, 2018). However, in many cases, minerals need to be supplemented in the diet. According to Satoh *et al.* (2001), fish meal incorporated diet for yellowtail and flounder was not sufficient to supply enough minerals necessitating supplementation of minerals to improve growth performance. Similarly, vitamin-mineral mixture is also essential to achieve better growth, protein efficiency ratio and apparent net protein utilisation (Mazid *et al.,* 1997). Vitamin C (ascorbic acid) is an essential nutrient for living organisms but it is not synthesised inside the fish body, hence, it has to be supplemented in the diet. Dietary vitamin C supplementation is reported to enhance growth and immune response in fish (Barros *et al.,* 2014; Liua *et al.,* 2016; Okhionkpamwonyi and Edema, 2017; Abdel Rahman *et al.,* 2018; Ibrahim *et al.,* 2020). Due to higher growth and metabolic rates, it is suggested that larval fish could deplete the storage of vitamins faster than juveniles and therefore, vitamin requirement for larval fish is higher than those for juveniles (Dabrowski, 1992).

Many minerals such as iron, manganese, copper, zinc, calcium and magnesium have been found to play vital roles in several body functions of fish for their normal life processes. Satoh *et al.* (1983a) reported that the supplementation of dietary Zn, Mn, Cu and Mg to fish meal diets are essential for common carp. Fish fed diets inadequate in vitamin C, zinc, copper or iron usually exhibit retarded growth, poor feed utilisation and anaemia (John *et al.*, 1979; Halver, 1989; Lall, 1989). Many of these minerals act as cofactors for metabolism and to achieve accelerated growth, these must be provided in formulated diets (Adewumi, 2018). Zn is an integral part of metalloenzymes, such as carbonic anhydrase and alkaline phosphatase and takes part in regulating many processes of carbohydrate, lipid and protein metabolism (Chen *et al.*, 2014). Dietary Zn levels have been found to influence growth performance, feed utilisation and haematological parameters (Moazenzadeh *et al*., 2017, 2018). It has been reported that Zn and Mn supplementation are necessary in order to improve growth, body composition and mineral retention when gilthead seabream are fed diets containing high levels of plant ingredients (Domingueza *et al.,* 2019).

Farm-made aquafeeds play an important role in the production of carps; more than 97% of the carp feeds used by Indian farmers are farm-made aquafeed (Jaysankar and Mohanta, 2019). The main reason for using farm-made feed is the higher cost and scarce availability of pelleted feed, especially on the farm site. In the present study, we wanted to improve the quality of locally and commonly used feed ingredients with selected feed supplements. There is limited information on the use of vitamin C and mineral in the locally formulated fish feed. Hence, an attempt has been made to study the impact of vitamin C and mineral enriched diets on the performance of *Labeo rohita* (rohu) fry.

Materials and methods

Experimental design

The study was carried out in ICAR-Research Complex for Eastern Region at Patna, Bihar, India for 60 days. Rohu fry (25 days post-hatch; 2.52±0.16 cm, 0.17±0.022 g) were collected from the Institute farm, acclimatised in tank conditions for one week, stocked at 50 nos. per FRP tank of 500 l capacity and fed with any of the following feeds in triplicates. Round the clock aeration was provided in all the tanks using a portable aerator. Five treatment groups used were: F1: Commercial feed (control); F2: Rice bran and oil cake at 1:1 ratio; F3: 99% F2 diet+vitamin-mineral mixture (1%)[1*]; F4: 99% F2 diet+vitamin-mineral mixture (0.9%)+vitamin C (0.1%)[2*] and F5: 99% F2 diet+ vitamin-mineral mixture (0.9%)+vitamin C (0.1%)+minerals[3*] (*i.e,* Zn: 30 mg kg^{-1} feed; Cu: 3 mg kg^{-1} feed and Mn: 10 mg kg^{-1} feed).

[[1*]Composition of vitamin-mineral mix (Agrimin) (quantity kg^{-1}): Vitamin A-7,00,000 IU; Vitamin D_3-7 0,000 IU; Vitamin E-250 mg; Co-150 mg; Cu-1200 mg; I-325 mg; Fe-1500 g; Mg-6000 g; K-100 mg; Na-5.9 mg; Mn-1500 g; Zn-9600 mg; DL-methionine -1000 mg, Ca-25.5%, P-12.75%

[2*]Vitamin C: Rovimix Stay-C 35 (Roche)

[3*]Minerals: Zn: 30 mg kg^{-1} feed (Ogino and Yang, 1979); Cu: 3 mg kg^{-1} of feed (Ogino and Yang, 1980) and Mn: 10 mg kg^{-1} feed (Ogino and Yang, 1980; Satoh et al., 1987)]

Feed preparation

All the ingredients (rice bran and mustard oil cake) were purchased from the local market and powdered. Proximate composition of rice bran and oil cake was analysed. Protein, fat and ash content of rice bran were 12.13±1.03, 11.76±1.42 and 9.43±0.10%, respectively and that of mustard oil cake were 28.72±0.40, 9.50±1.0 and 10.85±0.88%, respectively. The commercial sinking pellet used as control feed in the present experiment (F1) contained 22% crude protein and 5% crude fat. These feed were also powdered using a mixer-grinder. As per the requirements for a week, these two ingredients were mixed in equal proportions and stored in moist-free condition. Every day other ingredients like vitamin mix, vitamin C and minerals (inorganic form *ie.* $ZnSO_4$, $MnSO_4$ and $CuSO_4$) were mixed thoroughly as per the treatments and applied in the tanks. Feeding was done in the morning (09 00 hrs) and afternoon hours (14 00 hrs) at the rate of 10% of the body weight. Weekly 50% water exchange was done through siphoning. Feeding was done @ 6 kg for the first 30 days and @ 10 kg subsequently until day 60 for one lakh fish seed (fry) (Bhaumik, 1991).

Estimation of minerals in fish

After completion of the experiment, six fishes from each treatment were dried in an electric hot air oven between 55-60°C until the samples had constant weight. After drying, samples were ground uniformly. These dried samples were used for estimation of minerals. The samples were digested with concentrated nitric acid and analysed for various minerals using Atomic absorption spectrophotometer (AOAC, 2000).

Analysis of water quality parameters

Water quality parameters including temperature, pH, dissolved oxygen, total alkalinity and total hardness were measured according to standard procedures (APHA, 1998). Water exchange was carried out periodically to maintain optimum water quality.

Estimation of different biological parameters

Monthly sampling was carried out and weight gain percent, survival rate and specific growth rate (SGR% per day) was calculated using the following formulae:

$$\text{Weight gain (\%)} = \frac{\text{(Final weight - Initial weight)}}{\text{(Initial weight)}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{Final nos. of fish harvested}}{\text{Initial nos. of fish stocked}} \times 100$$

$$\text{Specific growth rate (\% per day)} = \frac{\text{Ln of final weight - Ln of initial weight}}{\text{Experimental days}} \times 100$$

Statistical analysis

The experimental results were subjected to one-way ANOVA using Statistical Package, SPSS version 11. Duncan's multiple range test (DMRT) was carried out for *post hoc* comparison of means at 5% probability level.

Results and discussion

In the present experiment, *L. rohita* fry were reared in FRP tanks for 60 days. Table 1 illustrates the water quality parameters during the study, which did not vary significantly between the treatments.

Absolute growth and survival of *L. rohita* fry after 60 days of feeding is illustrated in Fig. 1. Survival rate of experimental fish was the highest in F5 group (76%) followed by F4 (75.3%) and F3 (71.5%) although they did not differ significantly ($p>0.05$). In contrast, the lowest survival rate was observed in F1 (62.7%) feed (control). This indicates that supplementation of vitamin C and mineral can significantly influence survival rate of rohu. Similar to the present study, Okhionkpamwonyi *et al.* (2017) also observed higher survival rate of *C. gariepinus* with increasing supplementation of dietary vitamin C.

Similarly, the final weight of fish was taken at the end of 60 days of culture that showed higher growth in the F5 followed by F4, F3, F2 and F1 treatments. Percentage weight gain was the highest at F5 (1752%) followed by F4 (1450%) and lowest was at F1 (Fig. 2). Specific growth rate also followed a similar pattern. This implies that there is a positive effect of vitamin C and mineral supplementation on weight gain in fishes. It has been reported that vitamin C supplementation improves fish growth and influences important blood parameters that indicate the health status of fish (Fazio *et al*., 2019). Similarly, Khanh *et al.* (2018) also reported that final weight gain and specific growth rate (SGR) of fish in all the vitamin C supplemented treatments were significantly higher than the control.

All aquatic animals like fish require inorganic elements or minerals for their normal life processes and they have the ability to absorb some inorganic elements from their diets as well as from the aquatic environment they live in. As the test animal in the present study is at its young stage, it potentially can accumulate higher concentration of minerals in their body to achieve higher metabolic performance, better growth and survival. To see the impact of the incorporation of minerals (Mn, Zn and Cu) in the diet, the whole body minerals (dry weight basis) were estimated from individual fishes at the end of 60 days of rearing (Table 2). From the study, it was found that bioaccumulation pattern of minerals such as Mn, Zn and Cu was significantly affected ($p<0.05$) by the feed given to them.

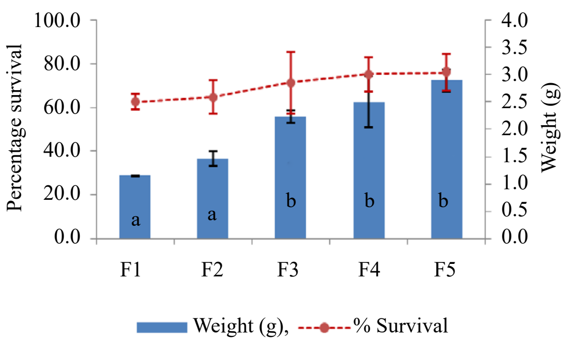

Fig. 1. Growth and survival of *L. rohita* fed with different feed formulations at the end of 60 days of culture. Data are expressed as mean±SE. Bars with different alphabets differ significantly ($p<0.05$)

Table1. Water quality parameters from the tanks under different treatments

Treatment	Temperature (^{0}C)	pH	DO (ppm)	Carbon dioxide (ppm)	Alkalinity (ppm)	Hardness (ppm)
F1	16.00 – 22.2	7.85±0.08	5.34±0.17	0.07±0.1	243.33±1.76	230.67±4.81
F2	16.40-22.40	7.85±0.09	6.19±0.42	0.00	246.67±1.33	228.67±8.97
F3	16.39-22.31	7.80±0.10	5.74±0.23	0.00	239.33±6.96	212.00±5.29
F4	16.20-22.43	7.88±0.06	6.31±0.14	0.03±0.02	246.67±3.53	220.67±5.81
F5	16.14-22.36	7.73±0.09	6.13±0.05	0.08±0.01	264.00±15.01	227.33±8.35

Table 2. Average mineral concentrations in the whole body of *L. rohita* (dry weight) at the end of 60 days of rearing

Treatment	Iron (mg kg^{-1})	Manganese (mg kg^{-1})	Copper (mg kg^{-1})	Zinc (mg kg^{-1})	Calcium (mg g^{-1})	Magnesium (mg g^{-1})
F1	34.66±2.93	2.27±0.71^{a}	0.81±0.27^{a}	18.87±2.32^{ab}	1.13±0.08	0.17±0.02
F2	29.67±2.28	2.45±0.72^{ab}	4.32±0.36^{c}	18.78±1.47^{ab}	1.27±0.22	0.18±0.02
F3	31.59±2.31	3.60±0.17^{ab}	3.71±0.94^{bc}	18.94±1.45^{ab}	1.23±0.16	0.17±0.01
F4	31.31±3.27	4.01±0.27^{b}	3.42±0.66^{bc}	16.82±1.04^{a}	1.23±0.08	0.16±0.02
F5	35.6±2.93	4.02±0.71^{b}	2.10±0.27^{ab}	22.20±2.32^{b}	1.55±0.08	0.21±0.02

Data are expressed as mean±SE (n=6). Different superscripts, in the same column indicate significant difference (Duncan's multiple range test, p<0.05).

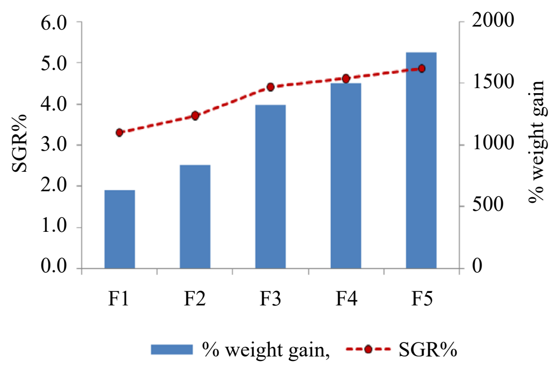

Fig. 2. Specific growth rate (SGR %) and percentage weight gain of *L. rohita* under different feed formulations at the end of 60 days of culture

The presence of iron in the fish muscle, though was not significantly affected ($p>0.05$), the lowest was recorded when only rice bran and oil cake (F2) was given and the highest was recorded when both vitamin C and minerals were added (F5). The bioavailability of iron is influenced not only by its chemical form but also by interactions between iron and other dietary components and ascorbic acid enhances iron absorption (Halver and Hardy, 2002). Lall (2002) mentioned that the presence of reducing substances in the diet (*e.g.,* ascorbic acid) can enhance the ability of fish to absorb iron. Similarly, Lynch and Cook (2008) reported that ascorbic acid is a powerful enhancer of nonhaeme iron absorption. Similar to the present study, Paul *et al.* (2016) reported that Fe content in *L. rohita* ranges between 17.5-42.3 mg kg^{-1}. Chandrashekar and Deosthale (1993) also reported that iron in muscle (wet tissue) of fishes ranges between 5-18 mg kg^{-1}, which is close to the present study, while high iron content was also reported (68.6-163 µg g^{-1}) in different fish species Uluozlu *et al.* (2007).

Manganese is also an important mineral and is widely distributed in fish. It functions either as a co-factor activating a large number of enzymes that form metal-enzyme complexes or as an integral part of certain metalloenzymes in carbohydrate, lipid and protein metabolism (Halver and Hardy, 2002). The deletion of Mn from the fish meal diet caused cataracts together with short body dwarfism and depressed growth (Satoh *et al.,* 1983b). Accumulation of manganese in the present study was significantly affected ($p<0.05$) in fish under different diets. In the present study, Mn was found progressively increasing and the highest accumulation in the fish body was recorded in F4 and F5 diet. This indicates that supplementation of vitamin C alone as well as vitamin C and Mn together increased body accumulation of Mn significantly. Tan *et al.* (2012) reported that whole-body Mn concentrations increased as dietary Mn supplementation level increased. Similar to the present study, Debnath *et al.* (2014) reported that Mn content in *L. rohita* was 3.9 mg kg^{-1} while Paul *et al.* (2016) reported Mn levels of 4.8-8.4 mg kg^{-1} in the same species. As such Mn content in IMC ranges between 2.5-8.4 mg kg^{-1} (Paul *et al.,* 2016) while fish of Black and Aegean Seas between 1.28-7.40 µg g^{-1} (Uluozlu *et al.,* 2007).

Copper is also an essential trace metal necessary for the growth and many enzymatic activities of all living organisms. It has been reported that dietary Cu or/ and vitamin C improved growth and health of red sea bream (Basuini *et al.*, 2017). In another study, carp fed diets without Cu supplement showed reduced growth and cataract formation (Satoh *et al.*, 1983a). The optimal level of copper in the diet as determined for several fish ranges from 3 to 5 mg Cu kg^{-1} diet (Watanabe *et al.*, 1997). In the present study, bioaccumulation of copper was also significantly affected ($p<0.05$) due to differential diet composition. However, unlike Mn, the highest Cu accumulation was recorded in F2 and thereafter the concentration progressively decreased and reached lowest at F5. Similar to the present study, Chandrashekar and Deosthale (1993) reported that presence of copper in the muscle of fishes ranges between 0.22-1.069 mg kg^{-1}, while Rao *et al.* (2014) reported 0.31 mg kg^{-1} in *L. rohita*. It has been reported that vitamin C has an antagonistic effect on copper metabolism (Pekiner and Nebioglu, 1994). In one of the study, Milne and Omaye (1980) recorded two to three fold decrease in liver copper concentration in guinea pig when diet was supplemented daily with vitamin C. Moreover, high dietary zinc intake was also found to depress copper absorption and increase faecal excretion of copper (Hoffman *et al.,* 1988). In the present study, the significant decrease in Cu recorded in the F5 treatment, inspite of the incorporation of copper in the diet (F5 feed) may be because of higher supplementation of vitamin C as well as zinc in the diet.

Zinc plays an important role in regulating oxidative stress, immunity and intervenes in reproductive processes and Zn supplementation is necessary to increase Zn levels in the whole body (Dominguez *et al.*, 2019). Although fish can uptake Zn directly from water, ambient waterborne Zn concentration in most freshwaters are insufficient to meet their metabolic requirements and hence Zn supplementation in the diet is necessary (Watanabe *et al.*, 1997, Luo *et al.*, 2011). In the present study, Zn was more or less the same in all treatments but significantly higher in F5 (Table 2). This indicates that dietary vitamin C and Zn supplementation has a positive effect on the accumulation of Zn in the whole body. Fishes tried to mobilise a higher concentration of Zn in their body and thereby achieve higher growth rate and better survival (F5 treatment) (Fig. 1). It has been reported that Zn in muscle of fishes ranges between 11-32 mg kg^{-1} (Chandrashekar and Deosthale, 1993) and 47.2-73.4 µg g^{-1} on dry weight basis (Begum *et al.*, 2005). Similar to the present study, Paul *et al.* (2016) and Debnath *et al.* (2014) reported that Zn content in *L. rohita* was 19.4-20.8 and 23.5 mg kg^{-1} respectively. Relatively lower range of Zn (6.98 mg kg^{-1}) in muscle of *L. rohita* has also been reported (Rao *et al.,* 2014).

Calcium was not supplemented in the feed as generally Ca from the feed ingredients of practical diets supplies sufficient Ca to meet the requirements of most finfish (Halver and Hardy, 2002). Similar to Zn, calcium present in the fish ($p>0.05$) was also more or less same in all treatments but was marginally higher at T5. This might be due to higher mobilisation of calcium by the fish for the formation of bone and skeleton to cope up with the better growth rate achieved in T5 treatment. Paul *et al.* (2018) studied the nutrient profile of five freshwater fish species and found that the calcium content ranged from 150 to 255 mg 100^{-1} g of fish which was slightly higher compared to the present study. Debnath *et al.* (2014) reported that calcium content in *Labeo* sp. is around 0.06% (600 mg kg^{-1} of fish), which is close to the present study.

Magnesium is an also essential element and participates in many enzymatic reactions during metabolism. Though there was no significant ($p>0.05$) effect in the accumulation of Mg in whole body, the pattern of accumulation was similar to Ca and the highest Mg concentration was also recorded at F5 treatment. In the present study, overall concentration of minerals in fishes were also higher in all treatments, this may be due to the fact that smaller size fish has a faster growth rate. From the present study, it can be stated that vitamin C and minerals (Mn, Zn and Cu) influences the accumulation pattern of minerals in fish. A higher concentration of minerals in treatment F4 and T5 may be due to the ability of fish to mobilise higher minerals (except cupper) in their body which will help them to achieve optimum metabolism which ultimately helps them to achieve growth and survival. Similar to the present study, Chandrashekar and Deosthale (1993) reported that presence of Mg in muscle of fishes ranges between 290-543 mg kg^{-1}.

A basic economic analysis for the production of early fingerling from fry is illustrated in Table 3. Here it has to be mentioned that generally feed alone contributes around 50-60% of the overall cost of production. The present analysis was done just to see how varying feed supplementation can change the overall cost of production and ultimately the revenue. From the analysis it was found that though the production cost of one lakh early fingerling is relatively similar in F3, F4 and F5, when we consider the weight gain in fry and number of fish per kg, F5 was found the best treatment compared to control. Cost of production was maximum for F1 feed and the trend is like F1>F2>F3>F4>F5. Similarly, revenue was also calculated and found that from selling one lakh seed, revenue increased in opposite direction than that of the cost of production and maximum revenue was generated in F5. The trend of revenue generated was F5> F4> F3> F2> F1. It was found that vitamin C incorporated diet (F4) alone increased the revenue by 118.5% and when feed was enriched with vitamin C and minerals (F5), revenue increased by 152.6% compared to control (F1). It is well understood that the ultimate goal of aquaculture enterprises is to make sustainable profits. In many aquaculture operations today, feed costs account for over half of the total variable operating costs and since

Table 3. Estimated economic performance of different formulated feeds fed to *L. rohita* for a culture period of 60 days

Treatment	Feed cost kg^{-1} (₹)	Total feed required (kg) for 60 days	Production cost of one lakh early fingerlings (₹)	Actual production cost of one lakh early fingerlings based on survival rate (₹)	Percentage increase in cost of production from control (F1)	Number of fish per kg, based on final body weight (no. kg^{-1})	Estimated total fish seed production from one lakh seed (kg)	*Revenue generated from one lakh seed (₹ in lakhs)	% Increase in revenue from control (F1)
F1(control)	34.0	0.72	24.48	39.06	0.00	874	114.42	0.23	0.0
F2	22.5	0.72	16.20	25.05	35.87	683	146.42	0.29	28.0
F3	24.0	0.72	17.28	24.22	37.99	450	222.22	0.44	94.2
F4	25.0	0.72	18.00	23.89	38.83	400	250.00	0.50	118.5
F5	25.4	0.72	18.29	24.06	38.40	346	289.02	0.58	152.6

*Present selling market price of fish seed - ₹200 kg^{-1} (Bihar).

commercial fish feeds are quite expensive, making good quality farm-made feeds might be more economical and results in more profit (Jantrarotai and Jantrarotai, 1993). Peteri *et al.* (1991) reported that profit over investment for production of fingerlings is around 141%. Rice bran and mustard oil cake are the most commonly used feed ingredients, used by the farmers for feeding fish. In the present study, incorporation of vitamin C and minerals along with rice bran and mustard oil cake was found more remunerative in terms of generating higher revenue from selling fish seed apart from influencing higher growth and survival of fish.

It can be concluded from the present study that Zn, Mn and Cu supplementation in the diet are necessary in order to improve growth, survival and mineral retention in *L. rohita* fry. Still many farmers are practicing farm-made feed for feeding fishes in the nursery, rearing as well as in composite fish culture system. From this study, it can be suggested that the incorporation of minerals and vitamin mixture in the locally available ingredients can significantly improve the performance of the farm made feed and ultimately the income of the farmer. However, further studies are necessary to identify other important minerals and their role as well as requirement in diet especially in the early life stages of fishes to maximise survival and productivity.

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