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Effects of dietary calcium level on growth performance and mineral contents in *Labeo bata* (Hamilton, 1822)

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Abstract

Twelve-week feeding experiment has been conducted to investigate the effects of dietary calcium level on growth performance and mineral contents in *Labeo bata* (Hamilton, 1822). Nine cages were prepared for rearing the fish and three practical diets were formulated containing graded Ca levels ranging from 0.2% to 1.28%. The diets were used to feed the fish in corresponding treatments of T₁, T₂ and T₃, respectively. Initially, the fish were fed 7% of their body weight which further reduced to the rate of 4% monthly. Stocking density among the treatments was 50 fishes m⁻³. Initial mean weight of the *L. bata* fish was 5.08±0.06 g. The mean value of final weight was found significantly higher in the fish group of T₃ (17.08±0.04 g) than that of T₁ (15.58±0.03 g). The weight gain in T₃ (12.04±0.05 g) was significantly higher than that of T₁ (10.50±0.10 g). The FCR value was much lower in T₃ (2.73±0.02) than that of T₁ (3.04±0.05). The mean value of SGR was found considerably higher in the fish group of T₃ (1.45±0.01) than that of T₁ (1.34±0.02) group. Results showed that the best final weight, weight gain, specific growth rate and FCR were obtained in fish group fed diet containing 1.28% Ca and it could be suggested that 1.28% Ca in the practical diet might improve the growth performance of the *Labeo bata* fish.

Keywords: *Labeo bata*, dietary calcium, growth performance, mineral contents

Introduction

Bangladesh, a country in South Asia, is deemed a riverine country consist of about 700 rivers including tributaries flow through the country constituting a waterway of total length around 24,140 km. With the world's largest flooded wetland, Bangladesh is considered as one of the most suitable regions for aquaculture and fisheries in the world. Fisheries sector is playing a significant role regarding employment generation, animal protein supply, earning foreign currency and poverty alleviation. Fisheries sector is contributing 2.01% of the total export earnings and 3.61% to the national GDP and 24.41% to the agricultural GDP [1]. About 17.80 million people are directly or indirectly involved in this sector and women employment is about 1.40 million. Fisheries sector achieves second position in agriculture sector and contributes 22.21% of total agricultural production. In the fiscal year 2016–2017, about 4.1 million metric ton fishes are obtained from water-bodies [1]. *Labeo bata* (Hamilton, 1822), locally called as “ilish bata” and/or “bhargon bata” belonging to the family of *Cyprinidae*, is one of the important minor carps in Bangladesh with great demand as table fish due to its deliciousness, flavor and less spine. This species is also an important source of carbohydrate, protein, fat, vitamins, minerals, iron and calcium [2]. In Bangladesh, it is found in Padma, Jamuna, Brahmaputra, Kangsha, Surma, Arialkha and Halda river system. It is also found in haors, baors, beels, canals and ponds of Bangladesh. Recently, a big portion of this fish is cultivated in may locally made pond due to its deliciousness and low cost production.

Calcium (Ca) is considered as one of the most important minerals for fish and is essential for normal growth, skeletal development and several physiological processes including the maintenance of acid–base equilibrium, osmoregulation, muscle contraction, bone mineralization, blood clot formation, nerve transmission etc. It also maintains cell membrane integrity, activation of several important enzymes that occurs in most diets consumed by fish [3-4]. It is noticed that few studies have been performed on the dietary Ca requirements of fish. Dietary Ca supplementation up to a certain level is not only benefited the performance of blue tilapia reared in Ca-free water but also fingerling scorpion fish reared in sea water [5-6]. In American cichlid, a positive effect of dietary Ca supplementation was found and in Atlantic salmon when dietary phosphorous (P) level was inadequate and in red sea bream at high

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dietary *P* levels [7-9]. Researchers also reported that dietary *Ca* supplement was essential for redlip mullet (*Chelon haematocheilus*); Japanese flounder (*Paralichthys olivaceus*) and scorpion fish (*Sebastiscus marmoratus*) [10]. Some studies stated that excess dietary *Ca* has negative effects in many fish species [11]. It has also been reported that dietary *Ca* may not be needed such as black sea bream (*Sparus macrocephalus*) [12]. It is known that, dietary *Ca* supplementation in diet have no impact on whole body composition, such as fingerling scorpion fish, juvenile jade perch (*Scortum barcoo*), Atlantic cod (*Gadus morhua*) [13-14]. The *Ca* requirement of most fish, such as red sea bream (*Pagrus major*), black sea bream (*Acanthopagrus schlegeli*), common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), chum salmon (*Oncorhynchus keta*), goldfish (*Carassius auratus*) and guppy (*Poecilia reticulata*) is satisfied by absorption from the surrounding water or from feed ingredients in practical and purified diets [15-17]. Report showed that *Ca* deficiency lowers the growth and feed conversion rate in scorpion fish (*Sebastiscus marmoratus*), catfish (*Ictalurus punctatus*), tiger puffer (*Takifugu rubripes*) as well as excess dietary *Ca* depresses *P*, *Mg* and *Zn* absorption whilst *Mg*, *Zn*, *Fe* and *Mn* deposition in vertebrae and scales [18-20]. Moreover, *Ca* deficiency also indirectly inhibits bone mineralization in Atlantic salmon (*Salmo salar*) by inhibiting *P* absorption [3]. Although, there are some research conducted on the effects of dietary *Ca* levels on the growth performance of different fish species but this types of investigations on *L. bata* fish are not yet to be done in Bangladesh. Therefore, the present study has been conducted to investigate the effects of dietary *Ca* level on the growth performance and mineral contents in the fish *L. bata* (Hamilton, 1822). The main target of this research is to supply *Ca* mineral into human body of the rural peoples of Bangladesh through the fish *L. bata* since the fish is very cheap and abundant.

Materials and Methods

The experiment has been conducted for a period of 12 weeks from the month September to November. Nine cages were installed in the experimental pond situated on the north side of the Agricultural Faculty Building of Rajshahi University, Bangladesh. A location map of the experiment is shown in Fig.1. The experiment was performed with three different treatments of feed composition of *Labeo bata* namely T₁, T₂ and T₃ each with three replications. The assigned treatments were described in the following section.

Cage construction, installation and management

Nine cube shaped (1m×1m×1m) cages were constructed using iron rod frame, black nylon net (8-10 mm mesh size) and nylon twine. In cages, one edge of upper side was kept open and tied with nylon threads to facilitate various management activities including supplying of feed, sampling of fishes. Cages were installed of keeping 15 cm above the surface of pond water which permits each cage having a volume of 0.85 m water. One cage installed in each cage one after another with the help of two horizontal bamboo poles. Pictorial presentation of the detail experimental cages, feeding and weight measurement of fish is shown in Fig. 2. Cage walls were cleaned fortnightly to facilitate unhindered water flow by removing unused feed, algae or dirt with the help of nylon brush. The cages were also checked to identify damage.

Seed source, stocking and feeding

The *bata* fish were collected from Rajshahi local area. Fishes were brought to the experimental site through oxygenated polythene bag. Fishes were acclimatized by keeping the transport bags float in the fish cage for about 30 minutes, after then the fish were allowed to get out freely from the bags. The fishes were randomly released into three replicates of three treatments. Fish were stocked early in the morning when the water was cool. The stocking rate was 50 fishes m⁻³ for treatments T₁, T₂ and T₃, respectively. The feed composition for three treatments i.e. T₁, T₂ and T₃ are summarized in Table 1. Contents of *Ca* level determined by atomic absorption spectrophotometric method in the corresponding diets are diet-1=0.20 ppm, diet-2=1.15 ppm and diet-3=1.28 ppm. Fishes of 5.08±0.06 g initial mean weight were stocked for the experiment. Fishes were fed twice a day at 09:00 am and 04:00 pm. The fish were initially fed at a rate of 7% of their body weight and rate was reduced to 4% gradually monthly. A number of water quality parameter such as temperature (°C), pH, dissolve oxygen (mg L⁻¹) and free carbon dioxide (mg L⁻¹) were measured monthly at 8:00~9:00 am.

Growth monitoring and harvesting

At least 10 fishes from each cage were randomly sampled at six-week basis by partially lifting the cage and removing fish with a dip net. On each sampling day, fish from cages were weighed and measured using a weight balance. The purpose was to determine fish growth in weight and to adjust the ration. Fishes were handled carefully to avoid stress during sampling. After the completion of the study, the fishes were entirely harvested from the experimental cage. The following parameters were used to monitor the growth during sampling and after harvesting. The final weight was taken at the time of harvesting and was expressed as gram (g). Weight gain, specific growth rate (SGR, % bwd⁻¹) and feed conversion ratio (FCR) were calculated by using the following equations (1) (2) and (3), respectively:

$$\text{Weight gain (g)} = \text{Mean final weight (g)} - \text{Mean initial weight (g)} \text{----- (1)}$$

$$\text{SGR (\% bwd}^{-1}\text{)} = [\ln (\text{final weight}) - \ln (\text{initial weight})] / \text{culture period (days)} \times 100 \text{----- (2)}$$

$$\text{FCR} = \text{Feed fed (dry weight)} / \text{Live weight gain} \text{----- (3)}$$

Mineral analysis

At first, the fishes were sampled and dissected their edible parts using stainless stile knife and quickly wrap with plastic bags. The bags were frozen in icebox until digestion. The samples were then dried overnight on oven at 105°C. Digestion of the fish and the feed materials were performed by wet ashing digestion method. Mineral contents in the fish and feed samples were determined by atomic absorption spectrophotometer (SHIMADZU, AA-6800). Experimental procedure and measurement of the mineral is described here [21]. For the statistical analysis of the collected data, one way analysis of variance was performed using computer software SPSS (Statistical Package for Social Science, Version 20) and differences between means were tested using the Duncan test. Significance was assigned at the 0.05 level.

Results

Water quality measurement

The variations in the mean values of different water quality parameters with different treatments in the three months interval are presented in Table 2. The mean values of water temperature were found to be ranging from 26.23 ± 0.21 °C to 32.47 ± 0.06 °C. The minimum value was recorded with the treatment T₁ in November. The maximum value was recorded with the treatment T₂ in September. Water temperature was not varied significantly among the treatments in the three different months. The mean values of dissolved oxygen (DO, mg L⁻¹) were found to be ranging from 5.26 ± 0.25 to 5.53 ± 0.15 mg L⁻¹. The minimum value was recorded with the treatment T₃ in September and the maximum value was recorded with the treatment T₂ in October. Among the treatments, no significant difference was found in the different months. The mean values of free CO₂ mgL⁻¹ were found to be ranging from 2.93 ± 0.15 to 3.18 ± 0.21 mg L⁻¹. The minimum value was recorded with the treatment T₁ in October and the maximum value was recorded with the treatment T₂ in November and no significant difference was found among the treatments in different months. The mean values of pH were found to be ranging from 7.13 ± 0.23 to 7.27 ± 0.15 . The minimum value was recorded with the treatment T₃ in September. The maximum value was recorded with the treatment T₁ in October. In the case of pH, also no significant difference was found among the treatments in different months. During the three months experimental observation there was no major difference found in all the water quality parameters.

Growth performance measurement

Growth performance of experimented fish in terms of final weight, weight gain, SGR (specific growth rate), FCR (food conversion ratio) etc. were calculated and the results are shown in Table 3. The average initial weights of fish under different treatments were 5.08 ± 0.08 g, 5.11 ± 0.05 and 5.04 ± 0.06 g for the treatments T₁, T₂ and T₃, respectively. It has been observed that there was no significant difference in the initial weight of fish in different treatments. The mean values of final weights were found to be 15.58 ± 0.03 g, 16.58 ± 0.04 and 17.08 ± 0.04 g in the treatments T₁, T₂ and T₃, respectively. From the Table 3, it is seen that weight increments were noteworthy among the three treatments. The highest final weight 17.08 ± 0.04 g was found in T₃ and the lowest final weight 15.58 ± 0.03 g was found in T₁ treatment. The mean values of weight gain were found to be of 10.50 ± 0.10 g, 11.46 ± 0.04 and 12.04 ± 0.05 g in the treatments of T₁, T₂ and T₃, respectively. The highest mean weight gain was observed in T₃ (12.04 ± 0.05 g) and lowest in T₁ (10.50 ± 0.10 g). A major difference was found among the treatments.

The mean values of specific growth rate (SGR, % bwd⁻¹) were found to be ranging from 1.34 ± 0.02 to 1.45 ± 0.01 . The minimum value of specific growth rate was recorded with the treatment T₁ whereas the maximum value of specific growth rate was recorded with the treatment T₃. Significant difference was found among the treatments for the mean values of specific growth rate. The mean feed conversion ratios (FCR) in different treatments were 3.04 ± 0.05 , 2.85 ± 0.03 and 2.73 ± 0.02 in the treatments of T₁, T₂ and T₃, respectively. There significant variation among the FCR values was observed among the treatments. The highest FCR (3.04 ± 0.05) value was observed in the T₁ and the lowest FCR (2.73 ± 0.02)

value was observed in the treatment T₃. The mineral contents results obtained in this study are summarized in Table 4. The Ca, Zn, Fe and Mn of the whole body were found to be slightly altered after feeding with the diets. The values of Ca, Zn, Fe and Mn contents in fish group of T₁ were found to be 4.91 ppm, 0.16, 1.55 and 0.19 ppm. In the fish group T₂ were 4.97 ppm, 0.15, 1.31 and 0.19 ppm and in the fish group T₃ were 4.47 ppm, 0.11, 1.13 and 0.15 ppm, respectively.

Discussion

Water temperature influences all metabolic and physiological activities and life processes such as feeding, reproduction, movement and distribution of aquatic organisms greatly. In this experiment, the mean values of water temperature were found to be ranging from 26.23 ± 0.21 °C to 32.47 ± 0.06 °C (Table 2). The minimum mean value (26.23 ± 0.21 °C) was recorded with the treatments T₁ in November and it might be due to the decrease in the bright sunshine. The maximum mean value (32.47 ± 0.06 °C) was recorded with treatments T₂ in September and it might be due to the influence of air temperature for bright sunshine. Some other research groups also found similar water temperature to be ranging from 20.5–36.5 °C in a fresh water pond [22-23].

The single most important water quality parameter in aquaculture is the dissolved oxygen (DO) content. In this experiment, the DO mean values were found to be ranging from 5.26 ± 0.25 mg L⁻¹ to 5.53 ± 0.15 mg L⁻¹. The minimum mean value (5.26 ± 0.25 mg L⁻¹) was recorded with the treatment T₃ in September and the maximum mean value (5.53 ± 0.15 mg L⁻¹) was recorded with the treatment T₂ in October. It has been reported that the DO concentration ranging from 5.1 to 6.7 mg L⁻¹ would be a good productive range and however, the DO concentration was found to be good matching with our results [24].

In this experiment, the mean values of free CO₂ were found to be ranging from 2.93 ± 0.15 to 3.18 ± 0.21 mg L⁻¹. The minimum mean value (2.93 ± 0.15 mg L⁻¹) was recorded with the treatment T₁ in October and the maximum (3.18 ± 0.21 mg L⁻¹) mean value was recorded with the treatment T₂ in November.

pH is an important factor in aquaculture and is also called the productivity index of a water body. In this experiment, the mean values of pH were recorded to be ranging from 7.13 ± 0.23 to 7.27 ± 0.15 . The minimum mean value (7.13 ± 0.23) was recorded with the treatment T₃ in September. The maximum mean value (7.27 ± 0.15) was recorded with the treatment T₁ in October. During the experimental period, pH of water of the experimental pond were slightly alkaline, which indicated a good pH condition for fish culture.

In aquatic animal, Ca is accounted as one of the most important minerals and is essential for normal growth. In this present study, growth performance of *L. bata* was varied with the inclusion level of Ca. Growth performance of *bata* fish fed with 0.2% (T₁), 1.15% (T₂) and 1.28% (T₃) Ca for 12 weeks showed that there was an increasing trend with increasing level of Ca. The highest mean final weight (17.08 ± 0.04 g) was found with the treatment T₃ and fairly similar mean final weight (16.58 ± 0.04 g) was found with the treatment T₂ whereas lowest mean final weight (15.58 ± 0.03 g) was found with the treatment T₁ (Table 3). So, fish fed with the Ca-deficient diet showed low final weight and the best final weight was observed in fish group fed 1.28% dietary Ca enriched diet. This finding is similar to Liang *et al.*, who found that the final weight increased with the increasing

dietary Ca level of 0.41% to 1.26% in bighead carp [25]. Andrews reported that 1.5% calcium incorporated in the diet of channel catfish (*Ictalurus punctatus*) showed the highest final weight [18]. Kalantarian stated that Ca in diets at the range of 0.95–1.61% could not significantly affect the final weight [26].

The highest mean weight gain (12.04±0.05 g) was found with the treatment T₃ and fairly similar mean weight gain (11.46±0.04 g) was found with the treatment T₂ whereas lowest mean weight gain (10.50±0.10 g) was found with the treatment T₁. Therefore, the fish fed with the Ca-deficient diet showed low weight gain and the best weight gain was observed in fish group fed 1.28% dietary Ca enriched diet. This finding is similar to Liang *et al.* who found that weight gain increased with the increasing dietary Ca level of 0.41% to 1.26% in bighead carp [25]. Lawal indicated that 2% inclusion level of dicalcium phosphate as optimum requirement for the highest mean weight gain of *C. gariepinus* [27]. The results in this study are in good agreement with those obtained by O'Connell and Gatlin working on the effects of dietary calcium on weight gain and mineral composition of the blue tilapia (*Oreochromis aureus*) [28]. They explained that significantly (P<0.05) greater weight gain was observed for fish fed the calcium-supplemented diets compared to those fed diets without supplemental calcium. In contrast, Fontagné revealed that there was no significant effect of dietary P (0 to 1.6%) or Ca (0 or 1%) supplementation on weight gain of rainbow trout (*Oncorhynchus mykiss*) fry [29].

The highest mean specific growth rate (SGR) (1.45±0.01) was found with the treatment T₃ and fairly similar mean SGR (1.40±0.01) was found with the treatment T₂ whereas lowest mean SGR (1.34±0.02) was found with the treatment T₁. Therefore, fish fed with the Ca-deficient diet showed low specific growth rate and the best specific growth rate was observed in fish group fed 1.28% dietary Ca enriched diet. Liang found that the specific growth rate (0.86±0.03, 0.96±0.02, 1.00±0.01, 1.04±0.04 and 1.11±0.03%) which increased with the increasing dietary Ca level of 0.41, 0.72, 0.93, 1.15 and 1.26% in bighead carp [25]. Lawal indicated 2% inclusion level of dicalcium phosphate as optimum requirement for the highest SGR (2.13% day⁻¹ respectively) of *Clarias gariepinus* [27]. Cheng worked on the effects of dietary calcium, phosphorus and calcium/phosphorus ratio on the growth and tissue mineralization of *Litopenaeus vannamei* reared in low-salinity water (2%). They found that in the presence of 1% supplemental Ca, shrimp fed diets containing 1.22% EAP (2% total P) had better specific growth rate. On the contrary, supplementation of 2% Ca to the basal diets yielded a significant reduction in specific growth rate, indicating that dietary Ca should be reduced to minimize dietary P supplementation [30].

The mean value of FCR was found to be ranging from 2.73±0.02 to 3.04±0.05. The highest FCR (3.04±0.05) was found with the treatment T₁ and lowest FCR (2.73±0.02) was found with the treatment T₃ (Table 3). However, the best FCR was observed in fish group fed 1.28% dietary Ca enriched diet. In this study, it has been found that in the presence of 1% supplemental Ca, shrimp fed diets containing 1.22 % EAP (2% total P) had better FCR. Indeed, the present study clearly showed that the fish fed diet with 1.28 % incorporation of Ca performs excellently well FCR compared to the other treatments.

Minerals are required for the normal life processes and fish

need these inorganic elements. Change in inorganic dietary-Ca had no significant effect on minerals such as Ca, Fe, Mn, and Zn of the whole body of *bata fish*. Fish may derive these minerals from the diet and also from ambient water. All forms of aquatic animals require inorganic elements or minerals for their normal life processes. Many essential elements are required in such small quantities that it is difficult to formulate diets and maintain an environment that is low in minerals to demonstrate a mineral deficiency. In this study, it was found that change in inorganic dietary-Ca had no significant effect on Ca, Fe, Mn, and Zn of the whole body of *bata fish*. The Ca, Zn, Fe and Mn of whole body were slightly changed. The values of Ca, Zn, Fe and Mn contents in fish group of T₁ were found to be of 4.91 ppm, 0.16, 1.55 and 0.19 ppm. In T₂ experiment, contain was as 4.97 ppm, 0.15, 1.31 and 0.19 ppm. In experiment T₃ the quantity was 4.47ppm, 0.11, 1.13 and 0.15 ppm.

Finally, result of this study reveals that growth performance of *L. bata* were significantly (P<0.05) higher in fish group of T₃ fed diet containing 1.28% Ca than that of T₂ (1.15% Ca) and T₁ (0.2% Ca) and there was no significant difference in whole body mineral contents of *L. bata* in different treatments. It is stated that the efficient and economically effective fish husbandry depends on fish nutrition and diet development. This research suggests that 1.28% Ca in the practical diet could improve growth performance of *L. bata*. This result will be helpful for researchers, fish farmers, and feed factories and fish nutritionists, overall for the country.

Table 1: Feed composition of three treatments T₁, T₂ and T₃ for the *Labeo bata* fish

Parameters	Treatments		
	T ₁	T ₂	T ₃
Fish meal (%)	5.0	5.0	5.0
Mustard oil cake (%)	30.0	30.0	30.0
Soybean meal (%)	32.0	32.0	32.0
Wheat meal (%)	26.0	23.0	22.0
Soybean oil (%)	5.0	5.0	5.0
Vitamin-C (%)	0.5	0.5	0.5
Vitamin premix (%)	1.0	1.0	1.0
Mineral premix (%)	0.5	0.5	0.5
Calcium lactate (%)	0.0	3.3	4.4
Total	100.0	100.0	100.0

Table 2: Variation in the mean values of water quality parameters under different treatments in the three months interval period

Month	Parameters	Treatments		
		T ₁	T ₂	T ₃
1 st Month	Temperature (°C)	32.40±0.36 ^a	32.47±0.06 ^a	32.43±0.45 ^a
	DO (mg/l)	5.27±0.25 ^a	5.26±0.25 ^a	5.33±0.25 ^a
	CO ₂ (mg/l)	2.93±0.16 ^a	3.07±0.47 ^a	3.00±0.26 ^a
	pH	7.17±0.21 ^a	7.23±0.25 ^a	7.13±.23 ^a
2 nd Month	Temperature (°C)	28.70±0.20 ^a	28.67±0.15 ^a	28.63±0.12 ^a
	DO (mg/l)	5.43±0.40 ^a	5.53±0.15 ^a	5.43±0.38 ^a
	CO ₂ (mg/l)	2.93±0.15 ^a	2.98±0.11 ^a	2.97±0.15 ^a
	pH	7.27±0.15 ^a	7.13±0.12 ^a	7.23±0.25 ^a
3 rd Month	Temperature (°C)	26.23±0.21 ^a	26.30±0.26 ^a	26.27±0.25 ^a
	DO (mg/l)	5.43±0.06 ^a	5.37±0.32 ^a	5.33±0.15 ^a
	CO ₂ (mg/l)	3.17±0.15 ^a	3.18±0.21 ^a	3.15±0.05 ^a
	pH	7.17±0.15 ^a	7.17±0.15 ^a	7.20±0.10 ^a

Figures in each row with superscript are not significantly different (P<0.05) from each other

Table 3: Variation in the mean values of growth parameters under different treatments after six, twelve weeks and during the study period feeding

Feeding period	Parameters	Treatments		
		T ₁	T ₂	T ₃
6 th week feeding	Initial weight (g)	5.08±0.08 ^a	5.11±0.05 ^a	5.04±0.06 ^a
	Final weight (g)	10.14±0.02 ^c	10.49±0.07 ^b	10.73±0.09 ^a
	Weight gain (g)	5.06±0.09 ^c	5.38±0.04 ^b	5.69±0.15 ^a
	FCR	2.95±0.10 ^a	2.79±0.03 ^a	2.61±0.10 ^b
	SGR (% b wd ⁻¹)	1.64±0.04 ^b	1.71±0.01 ^b	1.80±0.05 ^a
12 th week feeding	Initial weight (g)	10.14±0.02 ^c	10.49±0.07 ^b	10.73±0.09 ^a
	Final weight (g)	15.58±0.03 ^c	16.58±0.04 ^b	17.08±0.04 ^a
	Weight gain (g)	5.44±0.01 ^c	6.08±0.08 ^b	6.35±0.12 ^a
	FCR	3.13±0.00 ^a	2.90±0.05 ^b	2.84±0.08 ^b
	SGR (% bwd ⁻¹)	1.02±0.00 ^b	1.09±0.02 ^a	1.11±0.03 ^a
During the study period feeding	Initial weight (g)	5.08±0.08 ^a	5.11±0.05 ^a	5.04±0.06 ^a
	Final weight (g)	15.58±0.03 ^c	16.58±0.04 ^b	17.08±0.04 ^a
	Weight gain (g)	10.50±0.10 ^c	11.46±0.04 ^b	12.04±0.05 ^a
	FCR	3.04±0.05 ^a	2.85±0.03 ^b	2.73±0.02 ^c
	SGR (% bwd ⁻¹)	1.34±0.02 ^c	1.40±0.01 ^b	1.45±0.01 ^a

Figures in each row with different superscript are significantly different (P<0.05) from each other

Table 4: Whole body mineral contents of *L. bata* fish for 12 weeks feeding obtained by atomic absorption spectrophotometry method

Minerals	Concentration of minerals (ppm)		
	Diet-1	Diet-2	Diet-3
Ca	4.91	4.97	4.47
Fe	1.55	1.31	1.13
Mn	0.19	0.19	0.15
Zn	0.16	0.15	0.11



Fig 1: Study area map and the red circle indicated the sampling point.

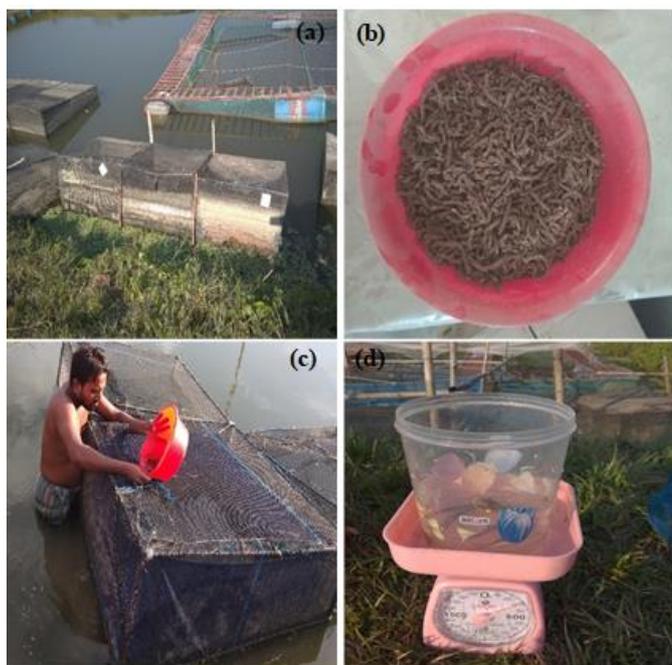


Fig 2: Pictorial presentation of (a) experimental cages, (b) prepared feed sample, (c) Stocking of fish and (d) weighing of fish sample

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