Growth Compensation in *Labeo bata* (Hamilton) with Feed Deprivation and Restriction Protocols

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In

Fisheries Resource Management

By

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CERTIFICATE

*This is to certify that the work recorded in the thesis entitled **"Growth Compensation in Labeo bata (Hamilton) with Feed Deprivation and Restriction Protocols"** submitted by **Hiramoni Das** in partial fulfillment of requirements for the degree of **Master of Fishery Science (Fisheries Resource Management)** in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Science, is the faithful and bonafide research work carried out under my supervision and guidance .The results of the investigation reported in this thesis have not so far been submitted for any other Degree or Diploma.*

The assistance and help received during the course of investigation have been duly acknowledged.

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Details of Symbols

Symbols	Details
D_1	One day of feed deprivation
D_2	Two days of feed deprivation
D_3	Three days of feed deprivation
R_1	One week of restricted feeding
R_2	Two weeks of restricted feeding
R_3	Three weeks of restricted feeding
C	Control feeding
%	Percentage
g	Gram
Kcal	Kilo calorie
Kj	Kilo joule
°C	Degree Celsius
ppm	Parts per million
mm	Millimeter
mg	Milligram.
SD	Standard deviation

Chapter 1

INTRODUCTION

Fisheries sector in India contribute significantly to food and nutritional security by providing livelihood to approximately 14.49 million people in the country and thus playing an important role in the national economy. It has been recognized as a powerful income and employment generator as it stimulates growth of a number of subsidiary industries. It also plays an important role in improving the socio-economic condition of people by way of supplementing family income and generating gainful employment in the rural sector, particularly among the landless labourers, small and marginal farmers and women.

Global fish production from capture fisheries has remained relatively stable over the past two decades while fish production through aquaculture has progressively increased. India is the third largest producer of fish and the second largest producer of fresh water fish in the world. Presently, the annual fish production in India has been estimated at 8.03 million metric tones, of which nearly 5.07 million metric tones comes from Inland sector (Tripathi, 2011).

Though aquaculture production is increasing, the demands for fish and other aqua-food is rising steadily in our society, due to increase in population and supply is unable to cope with it. As a result, there arise several disparities such as uneven fish distribution, price hierarchy, over exploitation of fish resources etc leading to social and environmental issues. The panacea to such problems lies in biological management for sustainable production through optimum use of resources.

Labeo bata (Hamilton, 1822) is considered as an important candidate species in aquafarming. It is being cultured widely because of certain advantages such as thriving well in shallow waters, high market demand, good growth rate, omnivorous feeding tendency, acceptability to artificial diet etc. This minor carp is mainly cultivated in eastern India and it has good consumer demand. Generally they are hardy and are capable of tolerating wide fluctuations of temperature, oxygen, turbidity etc. Its taxonomical features reveal that it has cylindrical/elongated body, the dorsal profile being more convex than the ventral. Snout is blunt and often covered with pores. Mouth is inferior with thin lips, which is slightly fringed. Body colour generally silvery, darkest along the back. The most identifying character of the fish is the presence of small black spot on 5th and 6th scales on the lateral line (Rahman ,1989).Apart from its taxonomical features, they can identified by their shine.

Labeo bata naturally inhabits in river systems of Bangladesh, India, and Pakistan. In India this species is very common in West Bengal, Assam, Orissa, Bihar, Jharkhand, Uttar Pradesh etc. Moreover it is found in rivers, lakes, haors, flood plains, ponds and similar water bodies. It does not breed naturally in ponds and it breeds naturally in rivers during monsoon months. It is a benthopelagic and potamodromous species capable of attending maximum length of 61 cm (Fishbase 2009) whereas largest observed specimen was 290 mm in total length by Rahman (1989). This fish get mature in 9-10 months. Spawning occurs during July and August. Each individual spawns only once with the onset of monsoon season. Its average fecundity is 1, 92,785 (Rath, 2000).

Labeo bata is being cultured along with other carps in composite type of aqua-farming. Intensive production of this fish requires cost effective nutritionally complete formulated feed. Monoculture is also practiced by few farmers for short duration due to high market demand. Moreover, it is very tasty fish of all cultured local fishes of Bangladesh and they are generally consumed in fresh condition. It mainly breeds in aquatic plants and its comprise crustaceans and insects larvae in early stages (Bhuiyan, 1964). It is predominantly bottom feeder and exhibits herbi-omnivore feeding habit.

In the present study, *Labeo bata* have been selected for evaluation of growth following feed restriction and deprivation protocol. Growth is a reflection of the difference between the magnitude of synthesis and that of break down. It has been suggested that in the period following transfer from a restricted to a liberal feeding regime, the metabolic rate may not immediately return to the same level as that of a continuously fed animal.

Many species of fish undergo repeated periods of reduced food intake during the course of their lives. This period of food deprivation, which lead to growth retardation or loss of weight, do not appear to compromise the ability of fish to grow when they are returned to full rations .On the contrary, there is evidence to suggest that some fish may show a period of rapid weight increase, known as compensatory growth, following a period of feed restriction (Miglaves and Jobling, 1989). Compensatory growth is defined as a phase of unusually rapid growth, following a period of under nutrition (Hayward and Wang, 1997).

Compensatory growth (CG) is a phase of accelerated growth when favourable conditions are restored after a period of growth depression. It reduces variance in size by causing growth trajectories to converge and is important to fisheries management, aquaculture and life history analysis because it can offset the effects of growth arrests. Periods of food deprivation induce changes in the storage reserves particularly lipids of fish. Apart from the strong evidence for the restoration of somatic growth trajectories, it is a response to restore lipid levels (Ali and Wootton, 2003)

The success of culturing fish depends on maximizing cost effectiveness in the production process. It is known that inappropriate feeding practices in aquaculture may lead to over feeding which results in feed wastes in pond water and consequently higher production cost and contamination of aquatic environment. However insufficient feeding lead to poor growth and high fish mortalities which make losses in the aquaculture business (Eroldogan *et al.*, 2006). An important approach to reduce feed costs in commercial aquaculture is to develop proper feed management and husbandary strategies (Lovell, 1998). One potential way of reducing feed cost is to take advantage of the phenomenon of compensatory growth.

Compensatory growth in fish is not only of theoretical interest but it has application in aquaculture (Hayward and Wang, 1997) as appropriate exploitation of this phenomenon may result increased growth rate and feed efficiency. It provides a possible response to overcome the disadvantage that individuals' experienced reduced growth rate following a period of deprivation and under nutrition and subsequently can be recovered following suitable feed management measures. It is of interest in aquaculture because of an understanding of its dynamics may allow to design an appropriate feeding schedule which can improve growth rates (Zhu *et al.*, 2001)

The objectives of the present study are as follows

a) To determine compensatory growth following one day, two days and three days of feed deprivation (Starvation) followed by 2 days of satiation feeding.

b) To determine compensatory growth following one week, two weeks and three weeks of restricted feeding followed by usual feeding as the control. The feeding level during restricted feeding was 2 percent of body weight daily which matches to Maintenance ration (Hayward and Wang, 1997)

Chapter 2

REVIEW AND LITERATURE

Protein is the main dietary component for growth of fish. Protein serves as the source of both amino-acids and energy in fish. It is the most expensive constituent of fish feeds. Fishes are known to utilize protein preferentially to lipid or carbohydrate as an energy source. Therefore, it is important from nutritional, environmental and economic point of view to improve protein utilization for tissue synthesis rather than for energy purposes. The optimization of dietary protein/ digestible energy ratio (DP/DE) has proved to have an important role on protein and energy utilization (Kaushik, 1994). The increase of DE content of fish diets by lipid can cause protein sparing effect and therefore reduce nitrogen losses to the environment (Cho and Kaushik, 1990).

Optimum protein requirement varies in different species. Small size fishes require higher levels of protein for growth than larger one. Optimum dietary protein requirement of several cyprinid species have been determined under a variety of conditions at different stages of growth. It include the following: fry of *Ctenopharyngodon idella* at 41-43 percent (Dabrowski, 1977); fingerlings of *Puntius gonionotus* at 35 percent (Wee and Nagamsnae, 1987); fry of *Catla catla* at 47 percent (Singh and Bhanot, 1988); fry of *Aristichthyes nobilis* at 30 percent (Santiago and Reyes, 1991).Anjana and Das (2003), during their experiment on *Labeo bata* observed that the diet containing 36 percent protein registered best growth in comparison to other diets. Ghosh and Das (2004) found that 40 percent dietary protein could cause best growth in fingerlings of *Anabas testudineus.*

Mohmood *et al,* (2000) reported that the optimum protein requirement for fry of *Clarias batrachus* was 49.1 percent under the water temperature ranging from 27 to 30°C and observed the protein energy ratio was 128.7 mg protein/K cal energy and protein efficiency ratio was 0.44. Singh *et al* ,(2007) reported that the diet with 36 percent protein and 32.17 percent carbohydrate to be the best diet for *Cirrhinus mrigala* fry at 28-32°C .

Mookherjee (1944) revealed that, true herbivorous or true carnivorous are hard to find among fresh water fishes. Early stages of all types of carps feed on crustaceans, protozoan, insect larvae and algae which are converted into higher percentage of plant material in semi rotten condition and again with negligible proportion of aquatic algae in adult stages. *Labeo bata* is a herbi-omnivore minor carp and its food comprises crustaceans and insect larvae in early stages (Bhuiyan, 1964). It is predominantly bottom feeder in nature. Sarkar (2002) reported that the major food items in the gut of *Labeo bata* were decayed organic matter, semi-decayed organic matter, algae and phytoplankton, mud and sand, zoo-plankton and other miscellaneous items.

2.1 Water temperature for growth:

Water temperature is one of the main abiotic factors that control growth of poikilothermic organisms living in aquatic environment. Specifically, it has an effect on fish respiration, feed intake, digestion, assimilation and growth (Turker *et al.*, 2003). Boyd and Pillai (1984) reported that warm water fishes grow well in the temperature range of 25°C to 32° C. Jhingran (1982) found that carps grow well at the temperature range of 18 °C to 37°C. *Labeo rohita* attained significantly higher body weight and total length under water temperature between 24-26°C. (Kausar and Salim, 2006).Victor *et al.*, (2004) found that the water temperature at 29 ± 0.5°C was optimum for *Cyprinus carpio.* Degani (2006) observed that the growth of *Cyprinus carpio* at 23 ± 1 °C temperature was good. Hossain *et al.*, (2007) during their experiment found 27 to 28°C water temperature was suitable for growth of *Labeo bata.*

2.2 Starvation in Fishes:

Starvation is experienced in most species of fish during certain periods of every year largely due to environmental conditions. After a period of starvation, refeeding resulted increase in growth rate, body weight and also food conversion ratio. High dietary protein levels increased concentration of free amino acids in body, ammonia excretion, protein synthesis and gluconeogenic enzyme activities and decreased glycolytic enzyme activities. During prolong starvation, proteolytic activity in fish tissue increase with the amino acids produced being used for energy. Fasting fish generally utilize lipid reserves as an energy source in preference to protein and carbohydrate. A juvenile fish requires more energy per unit weight for metabolism and has the potential to grow faster than an adult fish. Therefore, juvenile fish need a higher ration.

Abdel-Hakim *et al.*, (2009) observed at the end of their experiment that juvenile Hybrid tilapia (*Oreochromis niloticus x Oreochromis aureus*) with deprived feeding for 1 or 2 days/ week had body weights nearly the same compared to the control. There were significant differences in feed intake (F_I), feed conversion ratio (FCR), protein utilization efficiency between the control group and the other treatment groups with better performance of the control group. In carcass analysis, the moisture, ash and protein contents were increased in fish deprived for 2 days compared to the control meanwhile ether extract (EE) and energy were decreased. It can be concluded that juvenile hybrid tilapia reared in fresh water showed good growth performance with reducing feeding costs in moderate feed restriction deprivation regime by skip of 1and 2 days of feeding per week.

Shoemaker *et al.*, (2003) reported that juvenile catfish, *Ictalurus Ictalurus* which were not fed for 2 and 4 weeks had a significant increase ($P < 0.05$) in gutted weight, wet weight ratio and decrease in other organ somatic indices such as gut index (GI) Mesenteric fat index (MFI) and Hepatosomatic index (HSI), Blood glucose, liver glycogen. GI and HIS are sensitive indicators for channel catfish deprived of feed (NF) for four weeks.

The Gibel carp (*Carassius auratus gibelio*) are able to show complete growth compensation following 1and 2 weeks of food deprivation (Qian *et al.*, 2000). Tian *et al.*, (2003) observed that the fish starved for 1 week reached the same weight as the control fish after re-feeding for 3 weeks indicating that complete compensatory growth occurred in barramundi, *Lates calcarifer*.

Durairaja (2005) found that specific growth rate (length) and specific growth rate (weight) of *Clarias batrachus* exhibited a decline trend of variation during his experiment on feed deprivation. Minor variation was due to frequency of feeding in the weeks. Wang *et al.*, (2000) reported that hybrid tilapia(*Oreochromis mossambicus)* showed compensatory growth response during re-feeding following a period of feed deprivation and observed that hyperphagia was responsible for increased growth rate during compensatory growth.

Compensatory growth of juvenile barramundi, *Lates calcarifer* (Bloch, 1790) was investigated at 28°C for 8 weeks. Fishes were divided into four feeding groups including one group with continuous feeding (C) and three other groups with food deprivation for 1 week (S_1), 2 weeks (S_2) and 3 weeks (S_3), respectively. All starved fish resumed feeding in week 4. Changes in body weight, specific growth rate, feeding rate, food conversion ratio (FCR) and apparent digestion rate were examined weekly during the re-feeding period. Chemical compositions of fish were separately determined at the end of week 3 and week 8. Fish starved for 1 week reached the same weight as the control fish after re-feeding for 3 weeks, indicating that complete compensatory growth occurred though the specific growth rate in S_2 and S_3 fish was greater than that in the control fish after re-feeding, neither S_2 or S_3 fish reached the same body weight of the control fish at the end. The feeding rates of S_1, S_2 and S_3 fish were greater than those of control fish for a period of 2, 3 and 4 weeks respectively. No significant differences in feeding rates were found among S_1, S_2 and S_3 fish in the second week of re-feeding (Xiangli *et al.*, 2003).

Wu *et al.*, (2002) experienced four cycles of 1 week of feed deprivation followed by 2 weeks of feeding to satiation among four species, namely European minnows *Phoxinus phoxinus* (Cyprinidae), Three-spined sticklebacks *Gasteosteus acculeatus* (Gasterosteidae) , gibel carp *Carassius auratus gibelio* (Cyprinidae) and the longsnout catfish *Leiocassis longirostris* (Bagridae) and found that stickle back , carp and catfish showed significant increase in food intake following deprivation . But, the temporal pattern of consumption during the re-feeding periods differed between the four species. In stickle-back, daily feed intake over a refeeding period initially decreased, but then recovered. In minnows, intake tended to decline over a re-feeding period. Gibel carp showed an increase in daily intake on re-feeding and catfish had a weak tendency to show an initial decline, followed by an increase over a re-feeding period.

Feed intake and growth were studied in groups of turbot *Scophthalmus maximus L.* fed daily rations of 0.25 %, 0.38 % and 1 % of body weight per day for 41 days. Then, all groups were fed 1 % body weight per day for next 41 days. The two restricted rations resulted in reduced growth rates (30 % and 60 % of fully fed controls). The turbot became hyperphagic and displayed compensatory growth after the change from restricted to excess feeding, with compensatory growth being most marked among the fish that had been subjected to the most severe feed restriction. Fish fed restricted rations tended to have lower feed intake than controls. (Saether and Jobling, 1999).

Rueda *et al* (1998) , during their experiment found that the red porgies *Pagrus pagrus L.* fasted for 7, 14 or 28 days showed lost weight and had higher relative weights of eviscerated body than the control fish . Refeeding was accompanied by hyperphagia, which resulted in similar total food demand being shown by all groups. Deprived fish displayed compensatory growth and the final weights of these fish at the end of the experiment were similar to controls.

2.3 Hyperphagia

Hyperphagia is the main mechanism involved in the compensatory growth response, although increased food conversion efficiencies or behavioural adjustments might play a role (Ali *et al.*, 2003). Hyperphagia has been found in fish species during compensatory growth (Miglavs & Jobling, 1989; Russell & Wootton, 1992; Hayward *et al.*, 1997; Wang *et al.*, 2000; Xie *et al.*, 2001; Nikki *et al.*, 2004), whereas improved food conversion efficiency has not been widely observed in fish showing compensatory growth (Dobson & Holmes, 1984; Russell & Wootton, 1992; Qian *et al.*, 2000; Eroldog˘anv *et al.*, 2006). Many studies detected no significant differences in food conversion efficiency between the control group and the deprived groups during re-feeding periods (Kim & Lovell, 1995; Hayward *et al.*, 1997; Speare & Arsenault, 1997; Xie *et al.*, 2001). Hyperphagia was observed in the deprived fish after re-feeding, but there were no significant differences in food conversion efficiency. Thus, enhanced food intake during the re-feeding periods was the major cause for compensatory growth in transgenic carp. The proximate composition of the deprived fish at the end of the experiment was similar to that of the control fish, although the live masses diverged from controls(Fu *et al.*,2007).Similar patterns were also observed in other fish (Zhu *et al.*, 2004). It suggests that defense of body composition has priority over defense of the growth trajectory in fresh mass (Ali *et al.*, 2003). Hyperphagia was suggested to be the mechanism responsible for compensatory growth response.

Fu *et al.*, (2006) observed that in common carp (*Cyprinus carpio*) live masses of fish in the deprived groups were still significantly lower than those of control. During the re-feeding period, size- adjusted mean specific growth rates and mean feed intake were significantly higher in the deprived fish than in the controls, indicating a partial compensatory growth response in these fish. No significant differences were found in food conversion efficiency between the deprived and control fish during re-feeding, suggesting that hyperphagia was the mechanism responsible for increased growth rates. The proximate composition of the deprived fish at the end of the experiment was similar to that of the control fish.

Rubio *et al.*, (2010) reported that during the winter, seabass (*Dicentrarchus labrax*) exhibited significantly increased food intake, after both fasting periods 2 and 9 days. After the two days fasting period, significant hyperphagia (51 %) was observed, although this level of hyperphagia only lasted three days. After the second fasting period (9 days), fish exhibited pronounced hyperphagia of 79 %, the food intake on the first refeeding day reaching four times pre-fasting levels.

2.4 Compensatory growth of fishes:

Compensatory growth (CG) is a phase of accelerated growth when favorable conditions are restored after a period of growth depression.

Parimal *et al.*, (2006) observed that the fish (*Labeo rohita*) restricted of feed had statistically ($P < 0.05$) similar but numerically higher body weight than the control.

During re-alimentation period, weight gain, specific growth rate, feed intake, absolute growth rate (AGR), gross growth efficiency (GGE) or feed efficiency ratio (FER) and protein and energy retention efficiency were significantly higher in restricted fish than in the control indicating compensatory response in these fish. Restricted fish also showed hyper feed consumption activity during re-alimentation period. No significant (P<0.05) differences were found in digestibility of dry matter (DM), protein and energy as well as protein to lipid gain ratio between restricted and control fish during re-alimentation period. But, the tendency of restricted fish was found to have higher values of protein gain to lipid gain ratio than the control, suggesting that hyperphagia due to hyperactivity, of feed consumption was the mechanism for increased growth rate. Growth efficiency as well as protein and energy retention efficiency might be due to lower maintenance requirement during compensatory growth of rohu. This also suggested that compensatory growth obtained might be due to the protein growth rather than increased gut fat deposit or increased water up take, because body length proportionally increased with the body weight. Numerically complete compensation of body weight of Rohu was obtained perhaps due to the ability of rohu restricted of food to catch up in the body weight of control fish probably resulted from relative strong capacity for compensatory growth.

Sahin *et al.*, (1998) investigated on the effect of a period of starvation and subsequent re-feeding on the weight and length of seabass, seabream and rainbow trout reared at 17ppt salinity and at different temperature. The fishes were starved for 3 weeks and then fed *ad libtitum* by hand for 3 weeks in the 3 different studies. The study provided evidence of the adaption of the fish to starvation followed by what may be termed compensatory growth once feeding was resumed. The length changes of the fish indicate that the weight gains were due to growth rather than increases in gut fat deposits or increased water uptake.

Jiwyam (2010) conducted a 16 week trial in 15 indoor tanks to investigate the growth and compensatory response of juvenile *Pangasius bocourti*, Juvenile *P. bocourti* weighing a mean of 2 g, were fed a diet (40% protein) at 5 different ration levels (4%, 6%, 8%, 10% and 12 % of initial body weight) per day for 8 weeks (restricted ration period) and then re-fed a diet (25% protein) at normal ration (2%) for another 4 weeks (normal-ration period). The average final weight, specific growth rate and feed conversion efficiency were found out. At the end of restricted ration period, significant differences were found in growth parameters, average final body weight and specific growth rate among the five groups of fish receiving different rations ($P< 0.05$). The relationship between specific growth rate in wet weight and ration level was an asymptotic curve. Feed conversion efficiency decreased significantly with increasing ration levels. At the end of the normal ration period, there was no significant difference in final weight among the five groups which indicates complete compensation in the fish experiencing restricted feeding. There was an improvement in feed conversion efficiency in the juvenile *P. bocourti*, experiencing restricted feeding.

Baveeviae *et al.*, (2010) found in gilthead sea bream (*Sparus aurata*) that all feed restrictions resulted in compensatory growth in weight. When fed to satiation in the second half of the experiment, fish starved in the first half gained weight faster than the fish fed to satiation all the time.

Zhua *et al.*, (2004) observed that both the juvenile gibel carp, *Carassius auratus gibelio* and Chinese long snout catfish, *Leiocassis longirostis* showed compensatory growth in the re-feeding periods. There was no evidence of over-compensation with the deprived re-feeding protocols used in their study.

Nikkia *et al.*, (2003) reported that juvenile rainbow trout, *Oncorhynchus mykiss* subjected to fasting displayed compensatory growth and high growth rate during the recovery phage and it was achieved by hyperphagia rather than improved food conversion. Restricting feeding during the normal feeding season for catfish (May through October in the south-eastern United states) might have even more impact on growth and processing yield than winter feed restrictions since water temperatures are warmer and the fishes maintenance energy requirements are higher ,(Trucker and Robinson ,1990). Durairaja (2005) found that the specific growth rate (weight) of *Clarias batrachus* was highest in restricted feeding in compare to the usual feeding. The specific growth rate (weight) was fluctuated from 1.2430 to 5.8786 percent per day among different treatments and control group.

Yong oh *et al.*, (2008) observed that juvenile black rock fish fasted for 5-14 days can exhibited compensatory growth after re-feeding, but timing and degree vary depending on the duration of feed deprivation.

Gaylord and Gatlin (2007) during his experiment on Channel cat fish *(Ictalurus punctatus)* found that fish fed to apparent satiation during the first 4 week of the trial had a 41 % increase in body weight, while the fasted fish decreased in weight by 20%. During the subsequent refeeding period, previously unfed fish were not able to increase growth rates sufficiently to overcome weight loss imposed by the 4 week feed restriction. However, after 8 week of refeeding, total increase in body weight of the previously unfed fish was 179% of initial weight and similar to that of control fish which gained 231% of initial weight. Hepatosomatic index (HSI) and condition factor decreased rapidly during the fasting period and increased rapidly to control levels during subsequent refeeding. Muscle ratio showed little effect from the 4-week period of feed deprivation.It appears that non-feeding of channel catfish fingerlings for 4 week is too long to induce a compensatory growth response.

Amin *et al.*, (2002) observed that the Thai pangas (*Pangasius hypophthalmus*) responded to a change from a restricted to satiation feeding showing a higher daily feed demand compared to their counterparts raised on a liberal feeding regime. The total feed demand of fish in controll treatments was, however, much higher than the fish in the other three treatments like 1 day deprivation and 1 day feeding, 2 days of deprivation and 2 days of feeding and 5 days of feed deprivation and 5 days of feeding. Fish that fed to satiation on alternate day had similar body weight to the control and were longer than those exposed to 2 or 5 days of feed deprivation. There was no significant difference in specific growth rate of fish in the treatments. The higher FCR was found in the control where fish were fed to satiation twice a day. The study provided evidence that Thai pangas would be cultured in feeding regime with feeding every alternate day without any significant differences in fish size and final production. As farmers have to give less feed in the system they can manage water quality in a better way.

Chapter 3

MATERIALS AND METHODS

The present study on "Growth Compensation in *Labeo bata* following feed restriction and deprivation protocol was conducted in the Department of Fisheries Resource Management of Faculty of Fishery Sciences under West Bengal University of Animal and Fishery Sciences at Chakgaria campus of 24 South Paraganas district in West Bengal. The experiment was conducted for a period of 9 weeks from the 4th December, 2010 to 5th February, 2011. It has two components such as food deprivation and food restriction followed by re-alimentation or re-feeding.

3. Feed:

3.1. Feed ingredients:

Artificial diets were prepared using rice polish, groundnut oil cake, fish meal, wheat floor, vitamin-mineral mixture and Soya bean oil. All the ingredients were procured from the local market. The vitamin-mineral mixture used in the preparation of feed was Bevon manufactured by Cure Medicines (I) Pvt.Ltd. The composition of the vitamin-mineral mixture is presented in Table 1.

Table 1: Composition of vitamin-mineral mixture:

Each 5 ml contains:

Cholecalciferol IP (as stabilizer)	200 IU	B-carotene dispersion 2.5 %	38 mg
Pyridoxine Hydrochloride IP	1 mg	Manganese	0.8 mg
Niacinamide IP	15 mg	Selenium	10 mcg
Cyanocobalamin IP	1 mcg	Lysine, Hydrochloride BP	30 mg
D- panthenol IP	2.5 mg	Iodine (as potassium Iodide IP)	50 mcg
Zinc	3 mg	Biotin USP	10 mcg
Chromium	10 mcg	Inositol	10 mg.

3.2 Proximate composition of formulated diet:

Proximate composition such as moisture, crude protein, crude fat and total ash of feed ingredients and formulated diet were analyzed immediately after preparation. The composition of different ingredients used for preparation of formulated diet is presented in Table 2. To determine moisture content, the sample was dried at 105°C for 30 minutes and then at 65°C till constant weight was obtained following the method of Boyd (1969). Total nitrogen content was estimated using Micro-Kjeldahl's method (AOAC, 1995) and crude protein was found out by multiplying Nitrogen content with the factor 6.25. The crude fat was estimated using the Soxhlet extraction apparatus by extracting the fat content of the sample for 6-8 hours with the Petroleum ether (boiling point 60-80°C). To determine the ash content, the sample was burnt at 550 ± 20°C for six hours in muffle furnace.

Table2: Proportion of ingredients used in formulated diet.

Ingredients	Formulated diet (%)
Fish meal	40
Wheat floor	10
Rice bran	17
Ground nut oil cake	30
Soyabean cake	2
Vitamin-mineral mixture	1

3.3 Preparation of experimental aquaria:

The experiment was carried out in 21 glass aquaria of size 60 x 30 x 30 cm^3 each. The aquaria were cleaned thoroughly using scrub and then dried for a week. They were filled with good quality tap water up to a depth of 20 cm. This depth was maintained throughout the experimental period. Half of the water in all aquaria along with excreta was removed by siphoning thrice in a week. Subsequently freshwater was filled to maintain the depth at 20 cm.

3.4.1. Stocking and rearing:

The fingerlings of *Labeo bata* were collected from Mukundopur on 15.11.2010. They were kept in two 500 liters rectangular tanks held at 20 to 22°C room temperature and fed a mixture of live Tubifex species and formulated diet for about two weeks prior to the experiment trial. Before onset of experiment, all the fishes were starved for one day. Then they were sorted out by size wise. Ten numbers of uniform size fingerlings (72.61 mm) were kept in each aquarium. There were total seven treatments and each treatment was tried in triplicate. Fishes of first three treatments (starvation) were fed under feed deprivation protocol and were designed as D_1, D_2 and D_3 respectively followed by usual feeding. Here the fishes were starved for one day, two days and three days respectively and then fed at the rate of 5 percent body weight for a period of two days in each feeding cycle. Thus the corresponding feeding cycles were three days (one day starvation + 2 days feeding), four days (2 days starvation + 2 days feeding), and five days (3 days starvation + 2 days feeding) for treatments D_1, D_2 and D_3 respectively. This experiment continued for the period of 60 days and terminated on 61^{st} day.

The second experiment was under feed restriction protocol and was designed as R_1, R_2 and R_3 respectively. Here, the fishes of three treatments were fed at 2% body weight (maintenance ration) every day for the period 1 week, 2 weeks and 3 weeks respectively. Subsequently, they were fed at the rate of 5 % body weight till end of 9^{th} week. Another group was control (C) in which fishes were fed daily at the rate of 5 % body weight. Aeration was provided in each aquarium during night hours. Fishes were fed once at 8 AM in a Petri dish placed at one corner of each aquarium. Excess feed was removed by siphoning after 4 hours of feeding. The restriction set of experiment continued for 63 days with corresponding termination on 64^{th} days. Water quality parameters such as temperature, P^H, alkalinity, hardness and dissolved oxygen contents of each aquarium were monitored at weekly intervals following standard methods (APHA, 1995).

3.4.2 Feed Deprivation Schedule

Date	Weeks	Days	D_1	D_2	D_3	Control
4/12/10	1	1	d	d	d	f
5/12/10		2	f	d	d	f
6/12/10		3	f	f	d	f
7/12/10		4	d	f	f	f
8/12/10		5	f	d	f	f
9/12/10		6	f	d	d	f
10/12/10		7	d	f	d	f
11/12/10	2	8	f	f	d	f
12/12/10		9	f	d	f	f
13/12/10		10	d	d	f	f
14/12/10		11	f	f	d	f
15/12/10		12	f	f	d	f
16/12/10		13	d	d	d	f
17/12/10		14	f	d	f	f
18/12/10	3	15	f	f	f	f
19/12/10		16	d	f	d	f
20/12/10		17	f	d	d	f
21/12/10		18	f	d	d	f
22/12/10		19	d	f	f	f
23/12/10		20	f	f	f	f
24/12/10		21	f	d	d	f
25/12/10	4	22	d	d	d	f
26/12/10		23	f	f	d	f
27/12/10		24	f	f	f	f
28/12/10		25	d	d	f	f
29/12/10		26	f	d	d	f
30/12/10		27	f	f	d	f
31/12/10		28	d	f	d	f
1/1/11	5	29	f	d	f	f
2/1/11		30	f	d	f	f
3/1/11		31	d	f	d	f
4/1/11		32	f	f	d	f
5/1/11		33	f	d	d	f
6/1/11		34	d	d	f	f
7/1/11		35	f	f	f	f
8/1/11		36	f	f	d	f

9/1/11	6	37	d	d	d	f
10/1/11		38	f	d	d	f
11/1/11		39	f	f	f	f
12/1/11		40	d	f	f	f
13/1/11		41	f	d	d	f
14/1/11		42	f	d	d	f
15/1/11		43	d	f	d	f
16/1/11	7	44	f	f	f	f
17/1/11		45	f	d	f	f
18/1/11		46	d	d	d	f
19/1/11		47	f	f	d	f
20/1/11		48	f	f	d	f
21/1/11		49	d	d	f	f
22/1/11	8	50	f	d	f	f
23/1/11		51	f	f	d	f
24/1/11		52	d	f	d	f
25/1/11		53	f	d	d	f
26/1/11		54	f	d	f	f
27/1/11		55	d	f	f	f
28/1/11		56	f	f	d	f
29/1/11	9	57	f	d	d	f
30/1/11		58	d	d	d	f
31/1/11		59	f	f	f	f
1/2/11		60	f	f	f	f

f= Usual feeding @ 5% body weight d= Deprivation (Starvation)

3.4.3 Feed Restriction Schedule:

Date	weeks	Days	R_1	R_2	R_3	Control
4/12/10		1	2	2	2	5
5/12/10		2	2	2	2	5
6/12/10	1	3	2	2	2	5
7/12/10		4	2	2	2	5
8/12/10		5	2	2	2	5
9/12/10		6	2	2	2	5
10/12/10		7	2	2	2	5
11/12/10		8	5	2	2	5
12/12/10		9	5	2	2	5
13/12/10	2	10	5	2	2	5
14/12/10		11	5	2	2	5
15/12/10		12	5	2	2	5
16/12/10		13	5	2	2	5
17/12/10		14	5	2	2	5
18/12/10		15	5	5	2	5
19/12/10		16	5	5	2	5
20/12/10	3	17	5	5	2	5
21/12/10		18	5	5	2	5
22/12/10		19	5	5	2	5
23/12/10		20	5	5	2	5
24/12/10		21	5	5	2	5
25/12/10		22	5	5	5	5
26/12/10		23	5	5	5	5
27/12/10	4	24	5	5	5	5
28/12/10		25	5	5	5	5
29/12/10		26	5	5	5	5
30/12/10		27	5	5	5	5
31/12/10		28	5	5	5	5
1/1/11		29	5	5	5	5
2/1/11		30	5	5	5	5
3/1/11	5	31	5	5	5	5
4/1/11		32	5	5	5	5
5/1/11		33	5	5	5	5
6/1/11		34	5	5	5	5
7/1/11		35	5	5	5	5
8/1/11		36	5	5	5	5
9/1/11	6	37	5	5	5	5

10/1/11		38	5	5	5	5
11/1/11		39	5	5	5	5
12/1/11		40	5	5	5	5
13/1/11		41	5	5	5	5
14/1/11		42	5	5	5	5
15/1/11		43	5	5	5	5
16/1/11	7	44	5	5	5	5
17/1/11		45	5	5	5	5
18/1/11		46	5	5	5	5
19/1/11		47	5	5	5	5
20/1/11		48	5	5	5	5
21/1/11		49	5	5	5	5
22/1/11	8	50	5	5	5	5
23/1/11		51	5	5	5	5
24/1/11		52	5	5	5	5
25/1/11		53	5	5	5	5
26/1/11		54	5	5	5	5
27/1/11		55	5	5	5	5
28/1/11		56	5	5	5	5
29/1/11	9	57	5	5	5	5
30/1/11		58	5	5	5	5
31/1/11		59	5	5	5	5
1/2/11		60	5	5	5	5
2/2/11		61	5	5	5	5
3/2/11		62	5	5	5	5
4/2/11		63	5	5	5	5

2 = Feeding @ 2 % body weight 5 = Feeding @ 5% body weight

PLATE-1: SET OF EXPERIMENT

3.5 Feed consumption :

A specific quantity of feed was given to fish in a Petri dish in everyday. After 3 hours of feeding, the left over feed was removed by siphoning. It was dried in the oven at 60°C for 8 hours. After drying it was kept in a polythene bag and maintained treatment wise. After one week, the total amount of left over feed of each aquarium was found out by taking its total weight in electronic balance. Feed consumption was calculated by subtracting the leftover feed from the quantity of feed given to fish. Mean daily feed consumption was calculated from the total feed consumption.

3.6 Growth sampling :

Fish sampling was done at seven days interval to record their growth. Five numbers of fishes were caught randomly from each aquarium during sampling to record their length and weight. The adhering water was soaked in blotting paper before taking weight. Fishes were weighed in electronic balance to record the total weight in grams and their individual length was measured in millimeters.

3.7 Growth formulas :

3.7. 1(a). *Specific Growth Rate (SGR)*

$$\text{Specific Growth Rate} = (\ln W_2 - \ln W_1) / (t_2 - t_1) \times 100$$

Where, W_1 = Weight of fish at time t_1

W_2 = Weight of fish at time t_2

The calculated values give the average percentage increase in body weight per day over the experimental period.

3.7.2. *Feed Conversion Ratio (FCR)*

The feed conversion ratio was calculated using the formula:

Feed conversion ratio = Dry weight of feed given (g)/ Wet weight increased (g)

This gives the amount of feed required to produce a unit weight of fish.

3.8 Proximate composition of fish muscle:

Proximate composition of body muscle of *Labeo bata* fingerlings was estimated both at the commencement and termination of the experiment. Muscle samples were collected from the trunk portion of the fishes avoiding bony parts. The muscle samples were dried in the hot air oven to obtain dry matter. The dried samples were analyzed for crude protein, crude fat, moisture and total ash following the standard procedure (AOAC, 1995). The caloric content was determined by multiplying the fat, protein and carbohydrate content with the energy factors 9.44, 5.64 and 4.11 respectively. (NRC, 1993).

3.9 Statistical Analysis:

Mean values of growth of fish for each treatment were calculated at regular intervals for their variation in length and wet weight. Differences between treatments were tested for significance using analysis of variance (ANOVA) technique (Snedocor and Cochran, 1968). Mean values of nutrients in the fish muscle were tested for significance of difference with t-test.

Chapter 4

Experiment Results

4.1. Proximate composition of diet ingredients

Proximate compositions of different ingredients used in formulated diet are presented in Table 3.

The highest moisture content was found in ground nut oil cake (6.21 percent) and the lowest in rice bran (3.22 percent). The average values in fishmeal and wheat flour were 3.53 percent and 4.6 percent respectively. The dry matter content of the ingredients ranged from 93.79 percent (ground nut oil cake) to 96.78 percent in rice bran.

The highest crude protein was found in fish meal (55.71 percent) and the lowest in rice bran (7.73 percent). The average values for ground nut oil cake and wheat flour were 43.00 percent and 12.18 percent respectively. The crude fat content was 4.04 percent, 8.72 percent, 6.59 percent and 3.5 percent in rice bran, ground nut oil cake, fish meal and wheat flour respectively.

The maximum ash content was recorded fishmeal (12.64 percent) and minimum in wheat flour (3.72 percent). The average values for ground nut oil cake and rice bran were 7.86 percent and 12.24 percent respectively. The carbohydrate (nitrogen free extract) content of ingredients ranged from 76 .00 percent (wheat flour) to 21.53 percent (fish meal). The caloric content of ingredients varied from 3.60 Kcal/g in rice bran to 4.51 Kcal/g in groundnut oil cake. It was 4.12 Kcal/g in wheat flour and 4.41 Kcal/g in fish meal.

Table3. Proximate Composition (percent) of Feed ingredients*

Ingredients/Parameters	Rice bran	Groundnut Oil cake	Fish meal	Wheat flour
Moisture	3.22 ± 0.49	6.21 ± 0.52	3.53 ± 0.50	4.60 ± 0.51
Dry matter	96.78 ± 1.20	93.79 ± 1.10	96.47± 1.11	95.40 ± 1.12
Crude protein	7.73 ± 0.62	43.00 ± 0.70	55.71 ± 0.75	12.18 ± 0.50
Crude fat	4.04 ± 0.50	8.72 ± 0.56	6.59 ± 0.56	3.51 ± 0.50
Ash	12.24 ± 0.54	7.86 ± 0.53	12.64 ± 0.55	3.73 ± 0.53
NFE (Carbohydrate)	72.77	34.21	21.53	76.00
Caloric content (Kcal/g)	3.60	4.51	4.41	4.12

*Results are mean with SD.

4.2. Proximate composition of formulated diet

A proximate composition of formulated diet is given in Table 4. The moisture content and dry matter of it was 7.45 percent and 90.54 percent respectively. The crude protein, crude fat and total ash content of the diet were 35.10 percent, 7.51 percent and 9.84 percent respectively. The corresponding value of nitrogen free extract was 40.10 percent. The caloric value of the diet was 4.34 Kcal/g.

Table4. Proximate Composition (percent) of the Formulated diet.

Parameters	Diet (%)
Moisture	7.45 ± 0.50
Dry matter	90.54 ± 2.82
Crude protein	35.10 ± 0.64
Crude fat	7.51 ± 0.31
Ash	9.84 ± 0.27
Nitrogen free extract	40.10
Caloric content (kcal/g)	4.34

4.3. Water quality parameters

4.3.1. Temperature

The water temperature was recorded at weekly intervals and it is presented in Table 5. It was lowest 16.33°C on 5th week of the experiment and highest 23.50°C on 7th week of the experiment.

Table5.Average Values of water Temperature (°C) recorded in different treatments.

Sampling date	D_1	D_2	D_3	R_1	R_2	R_3	Control
4.12.10	19.33	19.33	19.33	19.33	19.33	19.33	19.33
11.12.10	23.33	23.33	23.00	23.33	23.33	23.33	23.33
18.12.10	21.50	21.67	21.50	21.50	21.33	21.50	21.50
25.12.10	18.00	18.33	18.33	18.33	18.33	18.50	18.33
1.1.11	17.50	17.50	17.33	17.50	17.50	17.50	17.50
8.1.11	16.33	16.33	16.50	16.67	16.67	16.67	16.67
15.1.11	17.33	17.33	17.33	17.50	17.33	17.33	17.33
22.1.11	23.33	23.33	23.50	23.67	23.33	23.33	23.33
29.1.11	23.00	23.00	23.00	23.00	23.00	23.33	23.33
5.2.11	22.50	22.50	22.50	22.50	22.50	22.50	22.50

4.3.2 Dissolved Oxygen

The values of dissolved oxygen content of water recorded in different aquaria are shown in Table 6. The lowest value (4.30 mg/l) was found on the 5th of the experiment in the treatment D_2 and the highest average value (6.91mg/l) was recorded on the 1st week of the experiment in the treatment R_3.

Table 6: Average Values of Dissolved oxygen (mg/l) recorded in different treatments

Sampling dates	D_1	D_2	D_3	R_1	R_2	R_3	Control
4.12.10	5.60	5.61	5.41	5.62	5.31	5.61	5.65
11.12.10	6.51	6.39	6.42	6.61	6.31	6.91	6.25
18.12.10	5.50	5.12	5.33	4.99	4.98	5.33	5.20
25.12.10	5.01	4.82	5.21	5.22	5.21	5.93	5.56
1.1.11	5.21	5.10	5.10	5.33	5.32	5.11	5.22
8.1.11	4.41	4.30	4.52	4.42	4.48	4.44	4.43
15.1.11	4.93	4.48	4.50	4.80	4.78	4.32	4.56
22.1.11	6.48	6.20	6.47	6.50	6.28	6.30	6.42
29.1.11	6.30	6.42	6.52	6.21	6.32	6.41	6.50
5.1.11	6.35	6.20	6.35	6.32	6.42	6.37	6.33

4.3.3 P^H

The values of P^H recorded in different aquaria at weekly intervals are given in Table 7. The P^H of water was ranged from 7.04 to 7.41 during the period of investigation.

Table7.Average P^H values recorded in different treatments.

Dates	D_1	D_2	D_3	R_1	R_2	R_3	Control
4.12.10	7.31	7.31	7.31	7.31	7.31	7.31	7.31
11.12.10	7.31	7.35	7.37	7.31	7.33	7.31	7.32
18.12.10	7.25	7.41	7.21	7.22	7.24	7.22	7.25
25.12.10	7.45	7.48	7.46	7.40	7.47	7.45	7.40
1.1.11	7.08	7.08	7.14	7.08	7.11	7.04	7.05
8.1.11	7.35	7.32	7.30	7.35	7.30	7.36	7.30
15.1.11	7.35	7.32	7.33	7.33	7.30	7.30	7.33
22.1.11	7.32	7.31	7.32	7.33	7.32	7.31	7.32
29.1.11	7.31	7.30	7.30	7.33	7.32	7.31	7.22
5.2.11	7.28	7.30	7.30	7.30	7.31	7.32	7.30

4.3.4 Total Alkalinity

The total alkalinity of water was recorded at weekly intervals in different set of experimental aquaria are shown in table 8. The lowest value (147.16 mg/l) was found on the 5^{th} week of the experiment in treatment D_3 and the highest average value (163.44 mg/l) was recorded on the 9^{th} week of the experiment in control.

Table 8. Average values of total Alkalinity (mg/l) recorded in different treatments.

Dates	D_1	D_2	D_3	R_1	R_2	R_3	Control
4.12.10	163.21	163.33	163.43	163.43	163.21	163.20	163. 40
11.12.10	154.33	154.43	152.44	152.43	152.43	151.20	155.20
18.12.10	154.33	154.33	155.17	154.33	153.17	154.33	152.33
25.12.10	157.13	157.19	157.13	155.20	154.33	157.13	156.17
1.1.11	155.17	156.33	156.27	156.27	155.17	155.13	155.13
8.1.11	148.23	148.17	147.16	145.33	146.33	147.43	147.34
15.1.11	156.45	156.43	154.33	154.45	154.23	154.33	155.43
22.1.11	160.21	160.17	159.24	159.33	159.42	159.44	159.56
29.1.11	162.33	162.45	161.30	161.12	161.23	161.45	161.51
5.1.11	161.23	161.12	160.34	160.44	160.21	160.32	163.44

4.3.5 Hardness

The hardness of water was recorded at weekly intervals in different set of experimental aquaria are shown in table 9. The lowest value (784 mg/l) was found on the 6th week of the experiment in treatment D_1 and the highest average value (988mg/l) was recorded on the 3rd week of the experiment in treatment D_3.

Table9.Average values of Hardness (mg/l) recorded in different treatments.

Dates	D_1	D_2	D_3	R_1	R_2	R_3	Control
4.12.10	852	899	923	855	902	823	843
11.12.10	982	926	982	984	974	932	926
18.12.10	892	900	915	823	984	926	936
25.12.10	913	945	988	975	967	959	924
1.1.11	885	864	826	879	892	848	846
8.1.11	892	926	884	846	928	872	864
15.1.11	784	846	794	926	848	864	848
22.1.11	794	796	824	846	826	848	892
29.1.11	982	926	848	932	876	862	823
5.1.11	926	956	932	922	952	956	922

4.4. Growth of fish under feed deprivation set of the experiment

4.4.1 Length

The length of fingerlings of *Labeo bata* recorded in different aquaria is given in table 10 and it is graphically represented in fig 1. The specific growth rate (length) is presented in Table 11 and fig2. The highest value (81.34 mm) for length was recorded in control and the lowest value (78.17mm) with treatment D_3. The corresponding values for D_1 and D_2 were 80.44 mm and 79.90 mm respectively. The specific growth rate (length) ranged from 0.100 to 0.314 percent per day among different treatments and control group.

Table 10: Average length of fish (mm) in deprivation set of experiment.

Week	Date	D_1	D_2	D_3	Control
0	4.12.10	72.61	72.61	72.61	72.61
1	11. 12.10	73.61	73.31	73.23	73.38
2	18. 12.10	74.81	74.31	74.12	74.01
3	25. 12.10	76.32	75.41	74.72	76.43
4	1.1.11	76.93	76.51	75.23	77.13
5	8.1.11	77.63	77.24	75.83	78.22
6	15.1.11	78.24	77.84	76.31	79.32
7	22.1.11	78.94	78.80	76.89	79.92
8	29.1.11	79.64	79.40	77.47	80.54
9	1.2.11	80.44	79.90	78.17	81.34

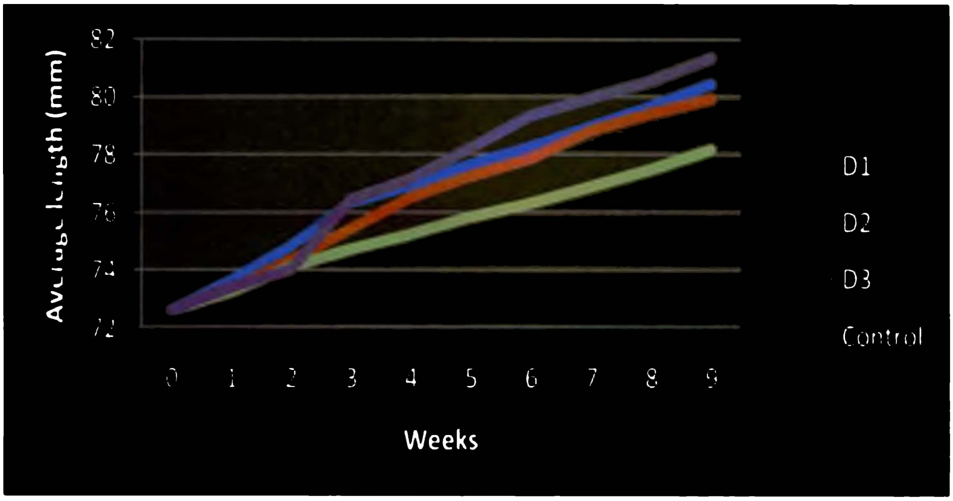

Fig 1: Average length of fish under feed deprivation.

Table11. Specific growth rate (length) of fish in deprivation set of experiment.

Week	D_1	D_2	D_3	Control
1	0.143	0.137	0.121	0.151
2	0.286	0.193	0.173	0.314
3	0.286	0.210	0.114	0.257
4	0.114	0.207	0.100	0.130
5	0.129	0.136	0.103	0.200
6	0.114	0.114	0.103	0.200
7	0.129	0.171	0.100	0.114
8	0.129	0.114	0.143	0.114
9 (60^{th} day)	0.143	0.142	0.129	0.143

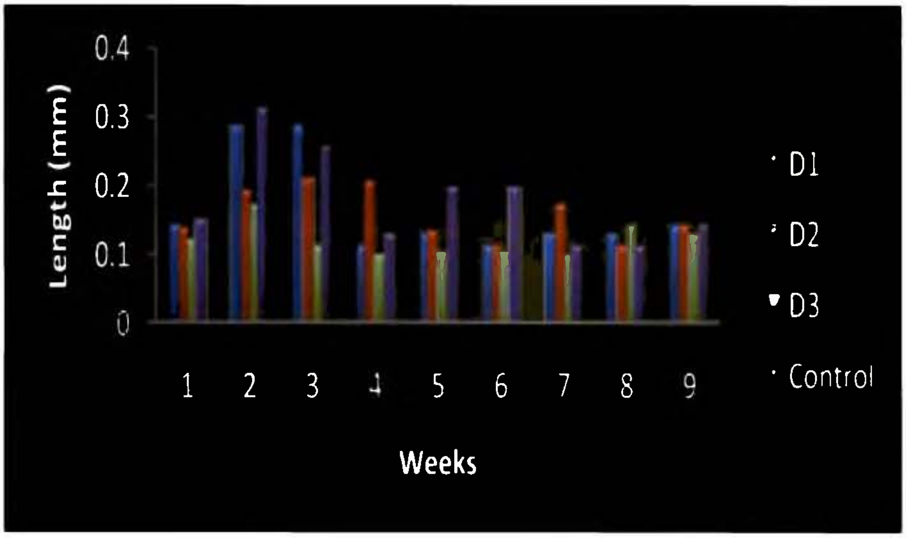

Fig 2.Specific growth rate (length) of fish in feed deprivation.

4.4.2 Weight:

The weight of fingerlings of *Labeo bata* recorded in different aquaria is given in Table 12 and Fig 3. The specific growth rate is presented in Table 13 and Fig 4. The highest value (5.32g) for average weight was recorded in control and the lowest value (4.84g) was recorded with treatment D_3. The intermediate values of 5.16g and 4.98 g was recorded in treatments D_1 and D_2 respectively. The specific growth rate (weight) ranged from 0.214 to 0.771percent per day among different treatments and control group. The specific growth rate (length) and specific growth rate (weight) of fish exhibited an irregular trend of variation during experiment. Minor variations were due to frequency of feeding in different weeks.

Table12A. Average weight of fish (g) in feed deprivation.

Weeks	Date	D_1	D_2	D_3	Control
0	4.12.10	3.89	3.89	3.89	3.89
1	11.12.10	4.02	3.99	3.99	4.05
2	18.12.10	4.24	4.10	4.09	4.27
3	25.12.10	4.42	4.24	4.19	4.44
4	1.1.11	4.52	4.38	4.29	4.62
5	8.1.11	4.67	4.51	4.40	4.77
6	15.1.11	4.81	4.63	4.50	4.87
7	22.1.11	4.91	4.75	4.68	5.03
8	29.1.11	5.03	4.87	4.77	5.17
9	1.2.11	5.16	4.98	4.84	5.32
Weight gain		1.27	1.09	0.95	1.43
Comparative weight gain		88.81%	76.22%	66.43%	100%

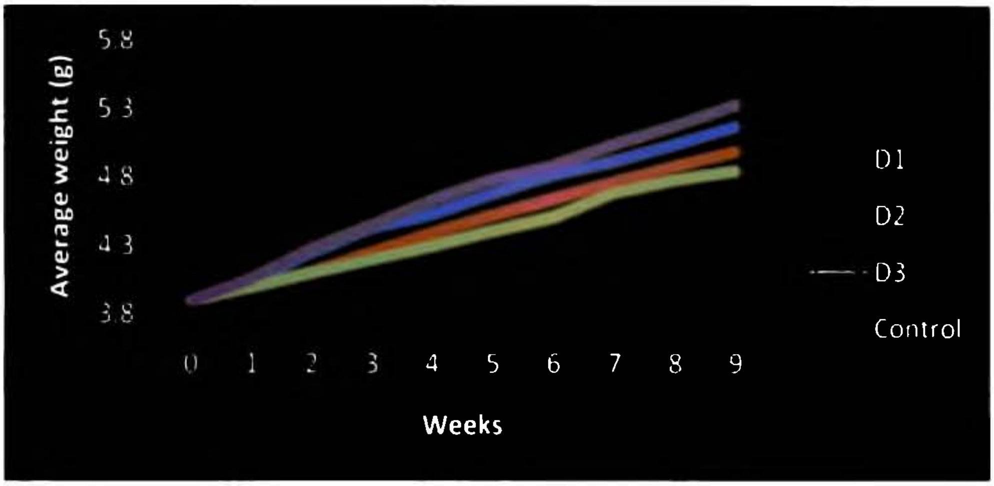

Fig 3: Average weight of fish under feed deprivation:

Table 12 B. Average weight gain (g) of fish in feed deprivation

Week	D_1		D_2		D_3		Control	
	Weight gain	Days of feeding	Weight gain	Days of feeding	Weight gain	Days of feeding	Weight gain	Days of feeding
1	0.13	4	0.10	3	0.10	2	0.16	7
2	0.22	5	0.11	3	0.10	3	0.23	7
3	0.18	5	0.14	4	0.10	3	0.12	7
4	0.10	4	0.14	4	0.10	2	0.20	7
5	0.15	5	0.13	3	0.11	4	0.17	7
6	0.14	5	0.12	3	0.10	2	0.16	7
7	0.10	4	0.12	4	0.08	3	0.16	7
8	0.12	5	0.12	4	0.08	3	0.14	7
9	0.13	3	0.11	2	0.07	2	0.15	7

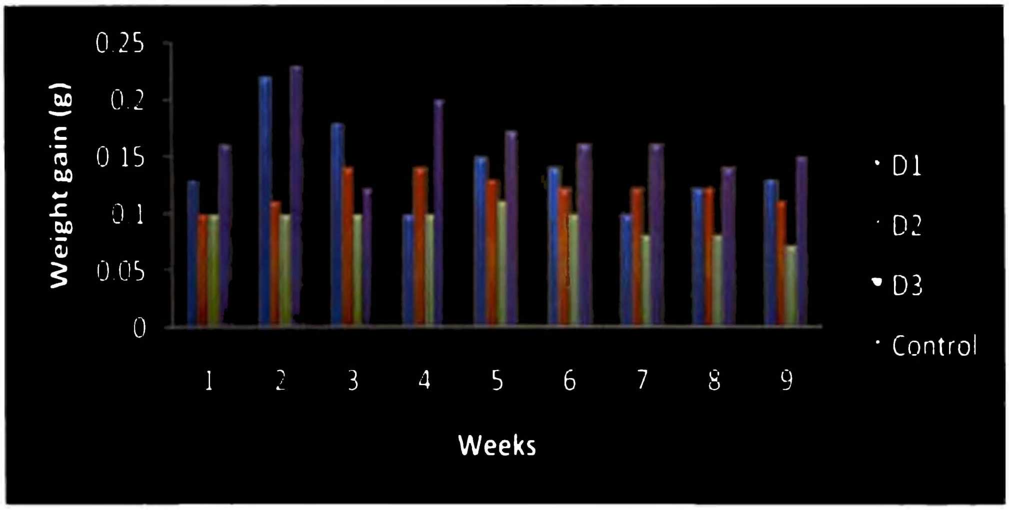

Fig 4: Average weight gain (g) of fish in feed deprivation.

Table13. Specific growth rate (weight) of fish in deprivation set of experiment.

Week	D_1	D_2	D_3	Control
1	0.471	0.300	0.300	0.571
2	0.771	0.457	0.214	0.757
3	0.586	0.486	0.429	0.557
4	0.329	0.457	0.331	0.571
5	0.457	0.414	0.300	0.457
6	0.429	0.386	0.229	0.300
7	0.286	0.357	0.557	0.457
8	0.343	0.357	0.271	0.400
9	0.371	0.314	0.214	0.400

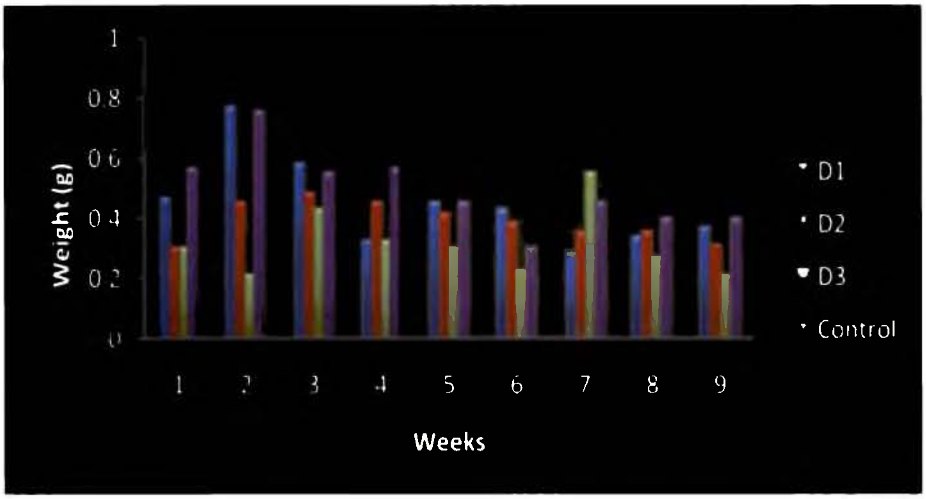

Fig5: Specific growth rate (Weight) under feed deprivation.

4.5. Feed consumption

The total number of days of feeding with treatments D_1, D_2, D_3 and control were 40, 30, 24 and 60 days respectively. The values of total feed consumption (%) of D_1, D_2, D_3 and control are presented in Table 14 and fig 6. It was found that the feed consumed by the fishes in the treatments D_1, D_2 and D_3 were 74.92, 63.27and 47.08 percentage of control feeding respectively.

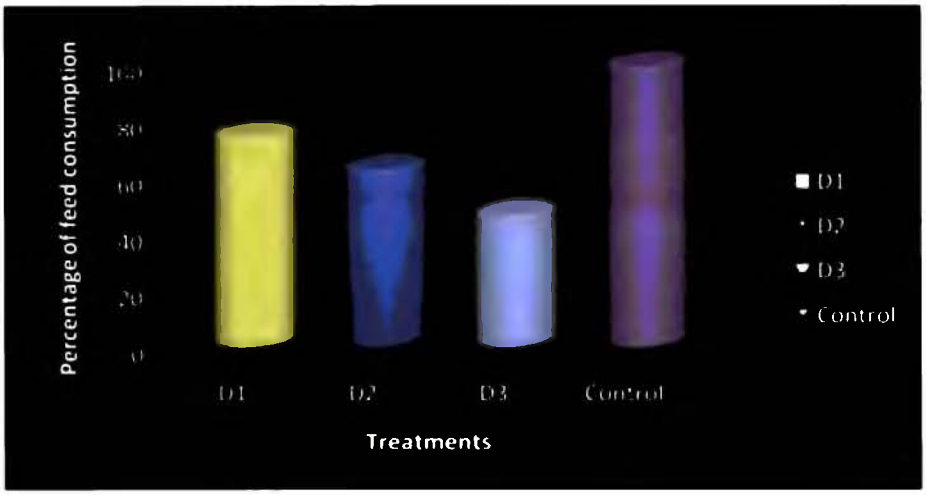

Fig6: Total feed consumption (%) of D_1, D_2, D_3 and Control.

Table14. Feed Utilisation in Different Treatments (Deprivation Protocol)

Sl no	1st Week	2nd	3rd	4th	5th	6th	7th	8th	9th	Total days of feeding	Feed consumption (% of Control
D1											
DF	4	5	5	4	5	5	4	5	3	40	74.92
D2											
DF	3	3	4	4	3	3	4	4	2	30	63.27
D3											
DF	2	3	3	2	4	2	3	3	2	24	47.08
C											
DF	7	7	7	7	7	7	7	7	4	60	100

DF-days of feeding

4.6. Proximate composition of fish muscle under feed deprivation/ starvation set of experiment

The results of proximate composition of the flesh of the fingerlings of *Labeo bata* at the beginning and at the end of the experiment are presented in Table 15. Protein content varied from 14.49to 15.54 %, lipid from 1.98 to 2.78% and ash content from 1.62 to 2.48 % in fish flesh during termination of the experiment.

Table15.Proximate composition of fish muscle under feed deprivation set of experiment.

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	NFE
Initial	76.54 ± 1.24	14.49 ± 0.54	2.18 ± 0.57	2.48 ± 0.48	4.31
D1	75.18 ± 1.72	15.34 ± 0.58	2.72 ± 0.43	1.92 ± 0.62	4.84
D2	75.12 ± 1.90	14.48 ± 0.37	2.51 ± 0.52	2.04 ± 0.56	5.85
D3	76.26 ± 1.13	14.36 ± 0. 46	1.98 ± 0.46	1.62 ± 0.36	5.48
Control	75.24 ± 1.81	15.54 ± 0.54	2.78 ± 0.54	2.38 ± 0.54	4.06

4.7. Growth of fish under restricted feeding set of experiment

4.7.1 Length

The average length of fingerlings of *Labeo bata* recorded in different aquaria is given in Table 16 and Fig 7. The specific growth rate (length) is presented in Table 17 and Fig 8. The average value (length) of 81.06 mm, 80.84 mm and 79.02 mm were recorded for R1, R2 and R3. The specific growth rate (length) ranged from 0.114 to 0.286 percent per day among different treatments and control groups.

Table16. Average length of fish (mm) with feed restriction

Weeks	Date	R_1	R_2	R_3	Control
0	4.12.10	72.61	72.61	72.61	72.61
1	11.12.10	73.91	73.62	73.15	73.38
2	18.12.10	74.94	74.52	74.01	75.01
3	25.12.10	76.44	75.91	74.62	76.43
4	1.1.11	77.44	76.81	75.32	77.13
5	8.1.11	78.19	77.81	76.12	78.22
6	15.1.11	79.16	78.87	77.12	79.32
7	22.1.11	79.86	79.54	77.82	79.92
8	29.1.11	80.46	80.14	78.42	80.54
9	5.2.11	81.06	80.84	79.02	81.34

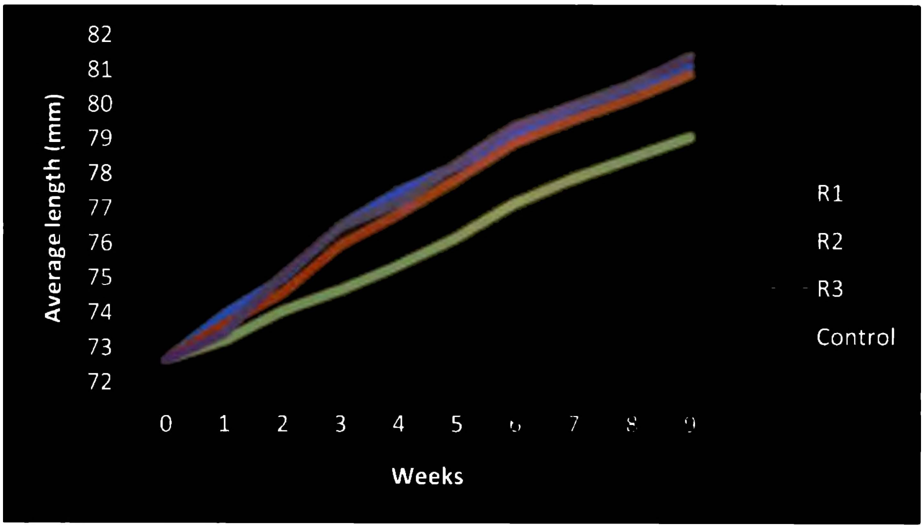

Fig 7: Average length of fish under feed restriction

PLATE-2: FINGERLINGS OF *LABEO BATA*

Table 17: Specific growth rate (length) of fish in feed restriction

Week	R_1	R_2	R_3	Control
1	0.257	0.200	0.114	0.151
2	0.201	0.172	0.154	0.314
3	0.286	0.271	0.114	0.257
4	0.201	0.157	0.143	0.130
5	0.129	0.186	0.143	0.200
6	0.171	0.201	0.185	0.200
7	0.129	0.114	0.128	0.114
8	0.114	0.114	0.114	0.114
9	0.114	0.114	0.114	0.143

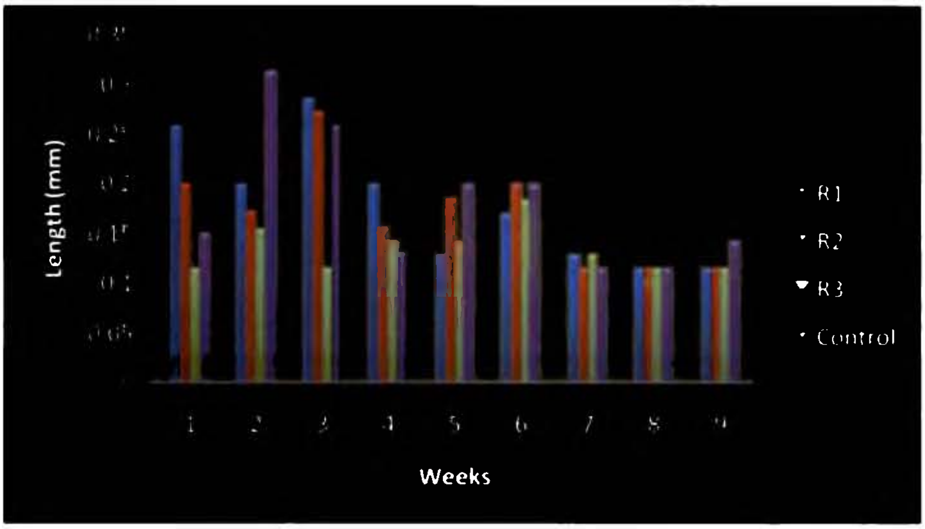

Fig 8: Specific growth rate (length) of fish in restriction set of experiment

4.7.2. Weight

The average weight of fingerlings of *Labeo bata* recorded in different aquaria is given in Table 18A and Fig9. The specific growth rate is presented in Table 19 and Fig 11. The highest value (0.22 g) for average weight was recorded in the treatment R_1 in the 2^{nd} week of the experiment and the lowest value (0.10 g) with treatment R_3 (Table18B and Fig10). The specific growth rate (weight) ranged from 0.286 to 0.771 percent per day among different treatments and control group.

Table18A. Average weight of fish (g) in feed restriction.

Week	R_1	R_2	R_3	Control
0	3.89	3.89	3.89	3.89
1	4.02	3.99	3.99	4.05
2	4.24	4.10	4.10	4.27
3	4.44	4.23	4.20	4.44
4	4.65	4.38	4.31	4.62
5	4.78	4.54	4.43	4.77
6	4.89	4.69	4.54	4.87
7	4.99	4.84	4.62	5.03
8	5.11	4.96	4.70	5.17
9	5.21	5.07	4.77	5.32
Weight gain	1.32	1.18	0.88	1.43
Comparative weight gain	92.31%	82.52%	61.54%	100%

Fig9: Average weight of fish (g) in feed restriction set of experiment.

Table18B: Weight gain (g) in fish during feed restriction

Weeks	R1		R2		R3		Control	
	Weight gain	Days of feeding	Weight gain	Days of feeding	Weight gain	Days of feeding	Weight gain	Days of feeding
1	0.13	7	0.10	7	0.10	7	0.16	7
2	0.22	7	0.11	7	0.11	7	0.23	7
3	0.20	7	0.15	7	0.10	7	0.12	7
4	0.21	7	0.16	7	0.11	7	0.20	7
5	0.13	7	0.16	7	0.12	7	0.17	7
6	0.11	7	0.15	7	0.11	7	0.16	7
7	0.10	7	0.15	7	0.08	7	0.16	7
8	0.12	7	0.12	7	0.08	7	0.14	7
9	0.10	7	0.11	7	0.07	7	0.15	7

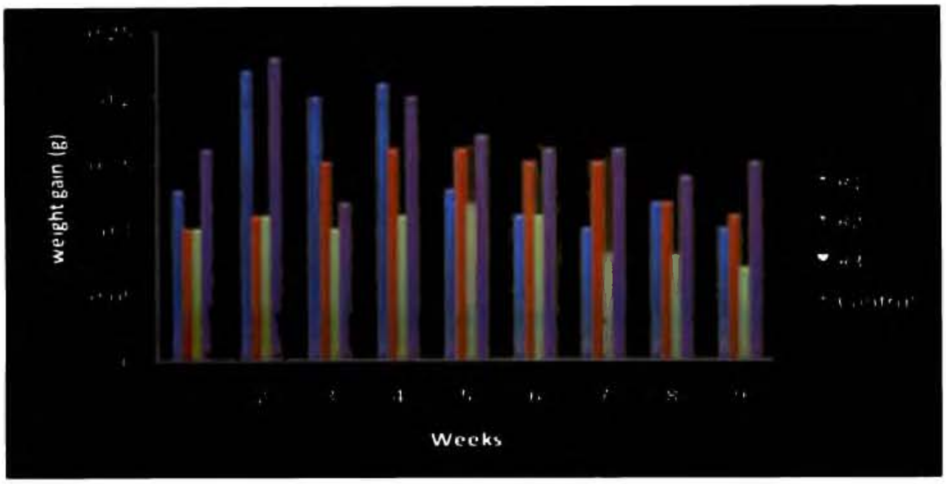

Fig 10: Average weight gain (g) during feed restriction.

Table 19: Specific growth rate (weight) of fish in feed restriction

Week	R_1	R_2	R_3	Control
1	0.471	0.371	0.371	0.571
2	0.771	0.385	0.385	0.757
3	0.657	0.442	0.343	0.557
4	0.657	0.500	0.371	0.571
5	0.400	0.514	0.386	0.457
6	0.329	0.457	0.357	0.300
7	0.286	0.457	0.243	0.457
8	0.349	0.343	0.257	0.400
9	0.286	0.314	0.200	0.400

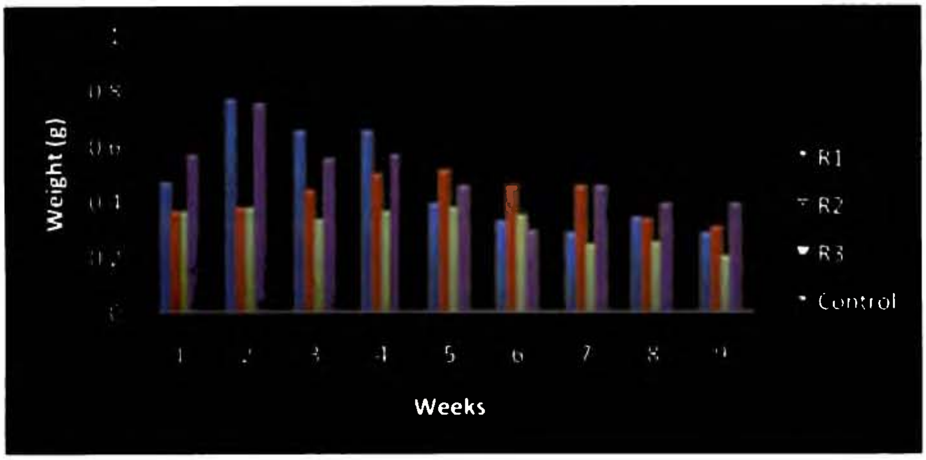

Fig11: Specific growth rate (weight) of fish in feed restriction set of experiment.

4.8. Total feed consumption

The feed consumption was recorded during the course of experiment. The values of total consumption of feed are presented in Table 20 and Fig 12. The numbers of days of feed restriction were 7, 14 and 21 days in treatments R_1, R_2 and R_3 respectively during the course of experiment. The percentage of feed consumption ranged from 89.15, 85.53 and 74.94 in treatment R_1, R_2 and R_3 of the control feeding

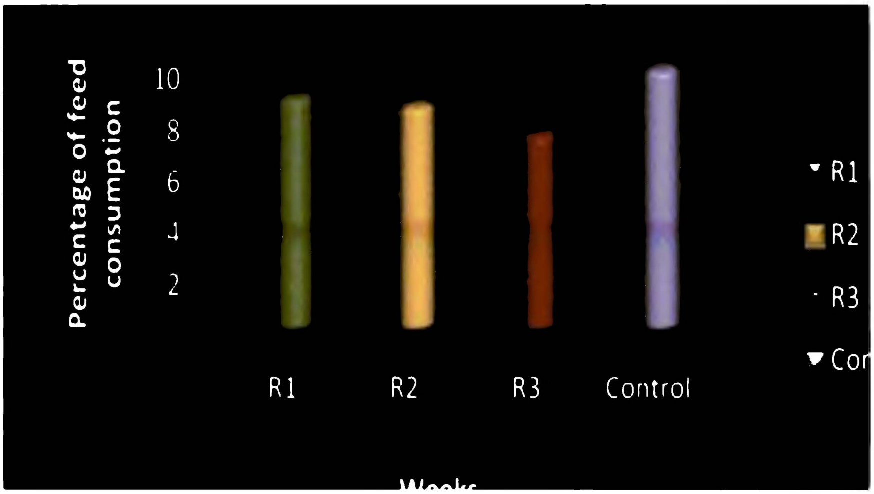

Fig12: Total feed consumption (%) of R_1, R_2, R_3 and Control.

Table 20: Feed utilization in different treatments (Restricted protocol)

Sl no	1st Week	2nd	3rd	4th	5th	6th	7th	8th	9th	Total days of feeding	% of Control
R1											
Feed	2%	5%	5%	5%	5%	5%	5%	5%	5%	63	89.15
R2											
Feed	2%	2%	5%	5%	5%	5%	5%	5%	5%	63	85.53
R3											
Feed	2%	2%	2%	5%	5%	5%	5%	5%	5%	63	74.94
C											
Feed	5%	5%	5%	5%	5%	5%	5%	5%		60	100

2%-Feeding@ 2% body weight *5%-Feeding@5% body weight*

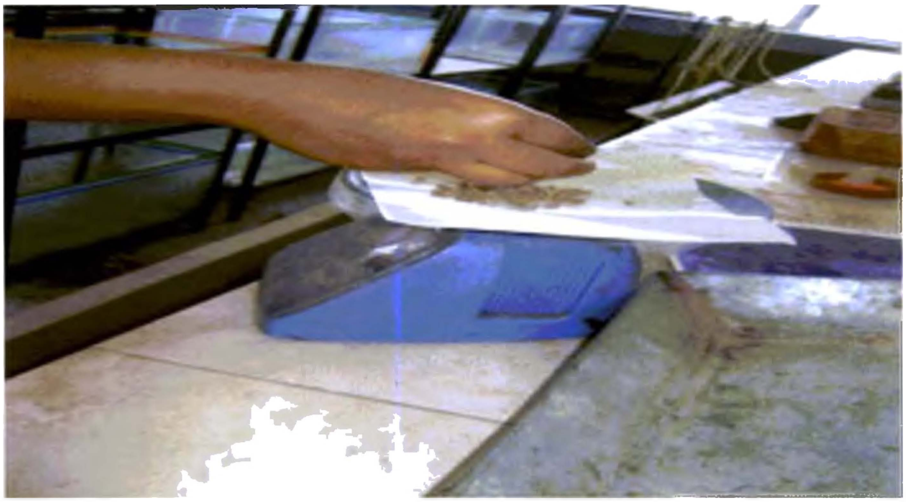

PLATE-3: WEIGHING OF FEED

PLATE-4: FISHES ARE FED IN A PETRI DISH

4.9. Proximate composition of fish muscle under feed restriction

The result of proximate composition of the flesh of fingerlings of *Labeo bata* at the beginning and end of the experiment are presented in the Table 21.

Initial analysis of the fish muscle revealed 76.54 percent moisture, 14.49 percent crude protein, 2.18 percent crude fat 2.48 percent ash and 4.31 percent NFE in it. After being fed with formulated diet for 63 days, there was change in the proximate composition of fish flesh. The crude protein content ranged from 13.74 to 15.54 percent, crude fat content 2.01to 2.78 percent, total ash content 2.18 to 2.71 percent, NFE content 4.06 to 5.18 percent in fish muscles of different treatments during the experiment.

Table21 : Proximate composition of fish muscle (percent) under feed restriction

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	NFE
Initial	76.54 ± 1.24	14.49 ±0.54	2.18 ±0.50	2.48 ±0.50	4.31
R1	75.24±0.92	14.40 ±0.58	2.62 ±0.53	2.71 ±0.50	5.03
R2	76.39±0.92	14.34 ±0.53	2.51 ±0.52	2.41 ±0.52	4.35
R3	76.89±0.95	13.74 ±0.50	2.01 ±0.50	2.18 ±0.50	5.18
Control	75.24 ± 0.94	15.54 ±0.54	2.78 ±0.50	2.38 ±0.50	4.06

4.10. Statistical analysis

The average weight gain and specific growth rate per week were subjected to one way analysis of variance (ANOVA) and the results are given below.

Table22 (a): One way analysis of variance (ANOVA) for Average Length in feed deprivation.

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	3	20.83379	6.944596	1.341571*
Within Treatments	32	165.6469	5.176467	
Total	35	186.4807		

*Non significant

Critical Difference = 2.191

Average of treatments

C = 77.29
D_1= 76.917
D_2= 76.533
D_3= 75.458

Table22 (b): One way analysis of variance (ANOVA) for Average Weight in feed deprivation.

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	3	0.530822	0.176941	1.335157*
Within Treatments	32	4.240778	0.132524	
Total	35	4.7716		

*Non significant

Critical Difference = 0.0464

Average of treatments

C = 4.643
D_1= 4.567
D_2= 4.434
D_3= 4.364

Table23 One way analysis of variance (ANOVA) for weight gain in feed deprivation.

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	3	0.018853	0.006284	8.036708*
Within Treatments	32	0.025022	0.000782	
Total	35	0.043875		

*Significant at P<0.01

Critical Difference = 0.023

Average of treatments

C =0.166

D_1=0.146

D_2=0.128

D_3=0.102

Table24: One way analysis of variance (ANOVA) for Specific growth (Weight) in feed deprivation.

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	3	0.19898	0.066327	5.043332*
Within Treatments	32	0.420843	0.013151	
Total	35	0.619822		

*Significant at P<0.01

Critical Difference = 0.110

Average of treatments

C = 0.497

D_1= 0.449

D_2= 0.411

D_3= 0.296

Table 25 (a): One way analysis of variance (ANOVA) for Average Length in feed restriction

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	3	18.7497	6.249899	1.049937*
Within Treatments	32	190.4846	5.952644	
Total	35	209.2343		

*Non significant

Critical Difference = 2.349

Average of treatments

C = 77.39
R_1= 77.407
R_2= 77.067
R_3= 75.821

Table 25 (b): One way analysis of variance (ANOVA) for Average Weight in feed restriction

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	3	0.614875	0.204958	1.454056*
Within Treatments	32	4.5106	0.140956	
Total	35	5.125475		

*Non significant

Critical Difference = 0.362

Average of treatments

C = 4.643
R_1= 4.622
R_2= 4.469
R_3= 4.355

Table26: One way analysis of variance (ANOVA) for weight gain in feed restriction

Source of Variation	Degrees of freedom(df)	Total sum of square	Mean sum of square	F ratio
Between Treatments	3	0.017208	0.005736	5.146417*
Within Treatments	32	0.035667	0.001115	
Total	35	0.052875		

*Significant at P<0.01

Critical Difference = 0.032

Average of treatments

C = 0.166

R_1= 0.147

R_2= 0.146

R_3= 0.106

Table27: One way analysis of variance (ANOVA) for weight gain after 3 weeks in feed restriction

Source of Variation	Degrees of freedom(df)	Total sum of square	Mean sum of square	F ratio
Between Treatments	3	0.013358	0.002097222	2.936*
Within Treatments	8	0.012133	0.0042	
Total	11	0.025492		

*Significant at P<0.05

Critical Difference = 0.122

Average of treatments

C = 0.170

R_1= 0.183

R_2= 0.120

R_3= 0.103

Table28: One way analysis of variance (ANOVA) for Specific growth (weight) in feed restriction

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	3	0.11397	0.03799	2.588197*
Within Treatments	32	0.469701	0.014678	
Total	35	0.583671		

*Not significant

Critical Difference = 0.117

Average of treatments

C = 0.497

R_1= 0.467

R_2= 0.452

R_3= 0.347

Table29: One way analysis of variance (ANOVA) for Specific growth (weight) after 3 weeks in feed restriction

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	3	0.18593	0.061977	6.651829*
Within Treatments	8	0.074538	0.009317	
Total	11	0.260468		

*Significant at P<0.01

Critical Difference = 0.182

Average of treatments

C = 0.628

R_1= 0.633

R_2= 0.399

R_3= 0.366

Chapter 5

DISCUSSION

5.1 Evaluation of formulated diet:

Aquaculture is a feed based farm activity with over 60% of the operational cost contributed by feed sources alone (Pandian *et al.*, 2001). The cost of feed is largely influenced by the level and sources of protein which is the most expensive component of a fish diet. It is the major dietary component which influences growth of fish. Insufficient as well as excess level of protein in feed is not desirable, the former result in poor growth, while the latter would be wasted by diverting for energy. Hence, dietary protein level in fish feed needs to be optimized accurately (Singh *et al.*, 2006). Many studies have been done on the protein requirement of carps and found that diet containing 26 to 45 % crude protein gives satisfactory growth. (Ayyappan, 2006).

The present experimental diet containing 35.10% protein, 7.51% fat and 4.34 Kcal/g energy is considered suitable for growth of *Labeo bata* and it matches well with earlier studies (Anjana and Das, 2004).

5.2 Water quality:

5.2.1 Temperature:

Water temperature is a controlling factor in the bio-chemical, molecular and metabolic process of aquatic organisms. Specifically, water temperature has an effect on respiration, feed intake, digestion, assimilation and growth of fish (Turker *et al.*, 2003). Jhingran (1982) found that carps grow well at the temperature range of 18 °C to 37°C. Boyd and Pillai (1984) reported that warm water fishes grow well in the temperature range of 25°C to 32°C. Kausar and Salim (2006) reported that, *Labeo rohita* attained significantly higher body weight and total length under water temperature between 24 to 26° C. Victor *et al.*, (2004) found that the water temperature within 29 ± 0.5°C was optimum for *Cyprinus carpio*. Degani (2006) observed that the growth of *Cyprinus carpio*, at 23 ± 1°C temperature was good. Hossain *et al.*, (2007) during their experiment found water temperature, suitable for growth of *Labeo bata* was 27 to 28°C.

In the present study, water temperature exhibited a decline trend followed by an increasing trend of variation. This was due to seasonal condition prevailing in the locality. The temperature of water recorded during the present study was 16.33°C to 23.50°C, which is conducive for the growth of *Labeo bata.*

5.3 Dissolved Oxygen:

Of all the dissolved gases in water, oxygen is the most important for the survival of organisms under aquaculture. Phytoplanktons prepare carbohydrate using CO_2 and sunlight and release oxygen. This the major source of oxygen in the water. Low dissolve oxygen level is responsible for more fish kills, either directly or indirectly. Oxygen consumption of fish is directly linked to size, feeding rate, activity level and temperature. Low DO level in water causes stress in fish resulting in reduced appetite, poor growth and an increase in susceptibility to infectious diseases. Symptoms include gulping of air at the water surface, stress coloration and an increase in swimming activity. Its level should be maintain at saturation or at least 5 ppm to have good growth in fishes (Thermofisher, journal) .Concentration of dissolved oxygen less than 3 ppm in water is lethal to fishes.

Hossain *et al.*, (2007) reported that dissolved oxygen of 5.4 to 6.2 mg/l is suitable for the growth of *Labeo bata.* Victor *et al.*, (2004) found that dissolved oxygen range between 5.6 to7.2 mg/l was good for the growth of the *Cyprinus carpio.* Basu (1949) during his experiment on the oxygen requirement of the Indian fishes, *Catla catla*, *Labeo rohita*, *Labeo bata* and *Cirrhinus mrigala* found that fishes can survive over 24 hours in low concentrations of oxygen of the order of 1 ppm in water.

In the present study, dissolve oxygen content in different treatment varied from 4.30 to 6.91 mg/l exhibiting an irregular trend of variation. It can be attributed to aeration as well as variation in temperature. Dissolve oxygen level of 4.30 to 6.91 mg/l found in present study is suitable for growth of *Labeo bata.*

5.4 P^H

It is an important limiting factor in fish culture. It indicates the acid base balance of the water. The ideal P^H for the growth of fishes is between 7.5 and 8.5. When water is very alkaline (P^H>9), ammonium in water is converted to toxic ammonia, which can kill fish. On the other hand acidic water (P^H<5) may affect the metabolic rates of fish. Hossain *et al.*, (2007) found P^H range 7.2to7.8 is suitable for the growth of *Labeo bata.* Victor *et al.*, (2004) found that P^H range within 7.1 to 7.5 has given good result in the growth studies of *Cyprinus carpio.* In the present study P^H values of 7.04 to7.91 were in the suitable range for the growth of *Labeo bata.*

5.5 Alkalinity:

Alkalinity is an important parameter for the growth of fishes. Tuladhar (2003) found that alkalinity range from 153 to 190 mg/l was good for the growth of carps. Hossain *et al.*, (2007) reported that alkalinity in the range from 380 to 410 mg/l is suitable for the growth of *Labeo bata.* In the present study the total alkalinity ranged from 147.16 to 163.44 mg/l which is suitable for the growth of *Labeo bata.*

5.6 Hardness:

Hardness of water depends on the dissolved solids and P^H. Proper liming can rectify the hardness. The ideal value of hardness for fish culture is 30 to180 mg/l. Tuladhar (2003) found that hardness ranged from 149 to 209 mg/l was good for the growth of carps. Hossain *et al* (2007) found hardness ranged from 450 to 510 mg /l is quite good for the growth of *Labeo bata.* Hardness ranged from 784 to 1015mg/l during the present course of investigation which was found to be at higher side. It can be attributed to local conditions of underground water. It might have caused relatively low growth of fish during the experiment.

The purpose of monitoring water quality parameters was to know whether such parameters are within optimum range for the growth of fish or not. In the present study, it was found that temperature, dissolved oxygen, P^H and alkalinity of water had fluctuated with little variations and the values were under suitable range. However, hardness of the water was found to be quite high and thus it was not at desired level, which might have caused adverse effect to growth of fish.

6.1. Growth compensation in *Labeo bata* under feed deprivation:

At the beginning of the experiment, there was no significant difference in weight of fishes among different treatments of D_1, D_2, D_3 and control group. Fish of different treatments exhibited various rates of growth during the period of experiment. Specific growth was found different in various treatments. It might be due to duration of feeding, feed consumption and environmental factors.

It was found that growth of fish in the treatment D_1 was relatively faster in the 2^{nd} and 3^{rd} weeks of experiment which might be due to hyperphagia. Rubio *et al.*, (2010) observed significant hyperphagia (51%) after the two days fasting and 79% after the second fasting period (9 days) on sea bass (*Dicentrarchus labrax).*Similarly, Wang *et al.*, (2000) reported that hybrid tilapia, *Oreochromis mossambicus* showed CG response during re-feeding following a period of feed deprivation and observed that hyperphagia was responsible for increased growth rate .Hyperphagia is the main mechanism involved in the compensatory growth response, although increased food conversion efficiencies or behavioural adjustments might play a role (Ali *et al.*, 2003). Hyperphagia is found in fish species during compensatory growth (Nikki *et al.*, 2004). It was observed in the deprived fish after re-feeding (Fu *et al.*, 2007).

Almost full growth compensation was achieved during 3^{rd} week in the treatment D_1. Similar result was found by Pegu (2010), who reported that the fish starved for 1 day followed by two days of satiation feeding reached the same weight as the control fish, in the fingerlings of *Oreochromis niloticus niloticus* indicating that complete compensatory growth occurred in this fish. Qian *et al.*, (2000) reported that Gibel carp, *Carassius auratus gibelio* (Bloch) are able to show complete growth compensation following 1 and 2 weeks of food deprivation. In the case of treatment D_2, there was relatively high growth during 3^{rd} and 4^{th} weeks and it might be due to hyperphagia in fish. In such case partial growth compensation was achieved during end of 5^{th} week. In the treatment D_3, there was relatively slow growth in comparison to control. It indicated that fish in such treatment could not compensate growth to match with the control. Similar result was found by Kim and Lovell (1995), who reported that more extended feed deprivation, prohibited the channel catfish from attaining a final weight equal to control fish.

In the present study, it was found that mean body weight of the fish of treatment $D_1$was numerically lower than Control but not different statistically. There was significant difference (df = 3, 32 F= 8.037, $P<0.05$) in weight gain of fish in different treatments. No significant difference was found between the treatment D_1 and control in weight gain. It indicated that one day of feed deprivation followed by two days of satiation feeding can compensate the growth in *Labeo bata*. However, there was significant difference ($P<0.05$) in growth of fish in treatment D_2 and D_3 compared to control. So, it can be inferred that two and three days of feed deprivation followed by two days of satiation feeding can not compensate the growth in *Labeo bata*.

6.2 Feed consumption:

The total number of days of feeding with treatment D_1, D_2, D_3 and control were 40, 30, 24 and 60 days respectively. It was found that the feed consumed by the fishes in the treatments D_1, D_2 and D_3 were 74.92, 63.27and 47.08 percent of control feeding respectively. The variation in feed consumption might be due to duration of feeding and hyperphagia in fish.

Though feed consumption was significantly ($P<0.05$) lower in D_1 in comparison to control, there was no significance difference in weight gain in fish. However, the weight gain in treatment D_2 and D_3 was found to be significantly lower with significant reduction in feed consumption. So, it can be concluded that, one day of feed deprivation followed by two days of satiation feeding can significantly reduce the feed consumption (expenditure in feed) without causing significant reduction in weight gain in *Labeo bata* and can be tested in field condition in order to reduce the operational cost due to feeding.

There was significant difference in feed consumption in treatment D_1 compared to control without compromising the growth. So, such protocol can reduce the expenditure on feed to the extent of 25.08 percent. Though feed consumption was quite low in treatment D_2 and D_3 but the growth of fish was significantly (P<0.05) lower than control. So, such protocol is not advisable due to low growth.

6.3 Proximate composition of fish muscle under feed deprivation series of the experiment

Moisture level decline over initial values in different treatments which might be due to higher growth of a fish. Protein level increased over initial values in all treatments except D_3. There was significant difference in protein level between treatments D_3 (t=2.881, P<0.05) and D_2 (t=2.804, P<0.05) compared to control ,which can be attributed to utilization of protein for energy purposes due to inadequate feed. So protein could not utilized properly for growth. There was no definite trend of variation in lipid content in different treatments. There was a significant variation in lipid content in treatment D_3 and control which might be due to more utilization of lipid in D_3 for body activities due to deprivation. It was also directly correlated with period of deprivation in different treatments. The ash content exhibited irregular trend of variation in different treatments during the termination of experiment.

Xiao *et al.*, (2010) found that crude protein and crude lipid content of muscle in fish in S_1 (One day feed deprivation in 1 week duration) and C (fed regularly) were significantly higher (P<0.05) than other treatments deprived for two, three and four days in 1 week experiment, while significant differences was found on moisture and ash content (P<0.05). The concentration of total protein, non-esterified fatty acids, triglycride and cholesterol in serum decreased significantly with increasing starvation days (P<0.05).

7.1 Growth compensation in *Labeo bata* under restricted feeding schedule:

In the present study, the weight gain of fish in the treatment R_1 was relatively more during 2^{nd}, 3^{rd} and 4^{th} weeks and it can be attributed to hyperphagia . Similar conditions were found in fish of R_2 treatment during 3^{rd} to 7^{th} weeks. However, the weight gain of fish in treatment R_3 was quite less compare to R_1 and R_2 and Control treatments. Effect of feed restriction on weight gain was evaluated by ANOVA techniques after 3 weeks of experiment. It was found that there was significance difference (df=3,32 F=, 2.936, P<0.05) in weight gain of the fish which can be attributed to less quantity of feeding due to restriction. Contrary to R_1, R_2, and R_3 treatment, the fish of control group had resulted relatively higher growth and it was due to satiation feeding.

Saether and Jobling (1999), reported that the turbot (*Scophthalmus maximus L*) also became hyperphagic and displayed compensatory growth after the change from restricted to excess feeding.However, complete growth compensation was achieved during 3rd week in this treatment. Bavcevic *et al.*, (2010) reported that in gilthead seabream (*Sparus aurata*), all feed restrictions resulted in compensatory growth in weight. When fed to satiation in the second half of the experiment, fish starved in the first half gained weight faster than the fish fed to satiation all the time. Similarly, Parimal *et al.*, (2006) found that the fish (*Labeo rohita*) restricted of feed had statistically (P< 0.05) similar but numerically higher body weight than the control.

Yong oh *et al.*,(2008) reported that juvenile black rock fish fasted for 14 days (2 weeks) can exhibit compensatory growth after re-feeding. In the treatment R_3, there was relatively slow growth in comparison to control. It indicated that fish in such treatment could not exhibit compensatery growth to match with control. There was significance difference (df= 3, 32 F=2.588, P<0.05) in weight gain of fish in different treatments during the period of investigation. The growth of fish was numerically less in R1 and R2 but statistically not significant from the control. It indicated that such fish could compensate the growth after feed restriction. However weight of fish in treatment R3 is significantly different (P<0.05) from control indicating it could not compensate the growth due to three weeks of restricted feeding.

7.2 Feed consumption:

It was found that feed consumption was directly related with duration of restriction. There was significant difference (P<0.05) in feed consumption of R_1 and R_2 compare to control without any compromise with growth statistically. So, such restriction protocol can be tested in field conditions. It can save feed cost to the extent of 10.85%and 14.47% in treatments R_1 and R_2 respectively. So, it can be inferred that one and two weeks of feed restriction can reduce the feed cost substantially without hampering the growth of fish.

7.3 Proximate composition of fish muscle under feed restriction schedule:

Artificial feeding is one of the important factors influencing the organoleptic characteristics of cultured carps (Ghittino, 1972). Flesh characteristics of fish such as nutritional value and organoleptic properties influence the market price of fish and thus the profit in fish culture. Quality of feed influence the carcass composition and hence the flesh quality of the final product (Anjana and Das, 2004).

In the present study the proximate composition of fish muscle such as moisture, crude protein, crude fat and ash were analysed in R_1,R_2,R_3 and control during beginning and end of the experiment. Moisture content of fish declined in different treatments (except R_3) over initial values and it could be due to higher growth of a fish. Protein content was found to be related with days of feed restriction. It was maximum in control and minimum in treatmentR_3.There was significant difference ($t=2.853 P<0.05$) in protein content in R_3 compared to control which indicated protein was used for energy rather than growth causing less weight to fish. Lipid content follows the same trend of variation as that of protein in different treatments. The minimum value in the treatment R_3 can be attributed to its utilization for body activities. According to Ogata *et al.*, (2002) the feed efficiency decreased as body weight increased. They found that wild strain (slow growing) of rainbow trout had significantly ($P<0.05$) lower lipid level in the body than domestic strain (fast growing) and this might be due to genetic factors, amount of feed intake or body size. The ash content exhibited an erratic variation in different treatments. It was relatively high in the treatment R_1 and R_2, which might be due to more growth in fish. Turker (2006) tested three restricted protocols, such as two days intervals (TDI), every other day (EOD) and Twice a day (TD). At the end of the experiment, he found that restricted feeding influenced the chemical composition of the fish. The lipid, moisture and protein content of fish increased when the fish were fed twice a day. Zamal and Ollevier (1995) found that percentage of ash content in the muscle of unfed juvenile catfish increased significantly after 6 days. This increase could be due to a decrease of protein and fat.

Myung *et al.*, (1995) found that dressing yield and body composition (total fat, protein, moisture) were the same for all the treatments at the end of the trial. Immediately following the period of feed deprivation, body fat in all restricted fed fish was lower than in the control fish. But within three weeks on full feeding, body fat in these fish increased to levels equals to that of the control fish. It indicated that Channel cat fish in production ponds can partially or completely recover in weight gain and body composition from of limited feeding provided they are subsequently fed to satiety.

Feed accounts for a lion's share in operational cost in aquaculture. So, there is need for efficient feed utilization in fish through biological management. Compensatory growth can be considered one of such management measures which can address maximum utilization of feed through deprivation, restriction and subsequent hyperphagia with satiation feeding. Furthermore, it can also reduce environmental pollution due to wastage of feed .In the present study it was observed that feeding protocol with two weeks of feed restriction can save the feed cost and wastage without hampering fish growth.

Chapter 6

SUMMARY AND CONCLUSION

Compensatory growth of fish is a rapid or faster growth than normal rate resulting from re-feeding after fasting or feed restriction. It has been reported in many fish species and it varies depending on fish species, fish size, water temperature, feed allowance, dietary nutrient composition, duration of feeding trial and feeding regime(Cho and Jo ,2008).Compensatory growth of fish is usually accompanied by an increase in food intake after re-feeding, called hyperphagia (Oh *et al.*,2008). The present study was designed to study the compensatory growth response of *Labeo bata* with following objectives.

1. Determination of compensatory growth following one day, two days and three days of feed deprivation (starvation) followed by 2 days of satiation feeding.
2. Determination of compensatory growth following one week, two weeks and three weeks of restricted feeding followed by usual feeding as the control.

The experimental diet contained 35.10% protein, 7.51% fat and 4.34 Kcal/g energy which is suitable for the growth of *Labeo bata*.

The water quality parameters monitored during the experimental period showed that they were well within conducive limits except the hardness of water .

Two set of experiments on compensatory growth were planned for the present study. First one is deprivation set (D_1,D_2,D_3) of experiment. Here the fishes were deprived for one day, two days, three days respectively followed by two days of satiation feeding (5% of body weight). It continued for 60 days. Second set was on restricted (R_1,R_2,R_3) feeding. Here the fishes were maintained under restricted feeding (2%of body weight) for a period of one week, two weeks and three weeks respectively. Subsequently they were fed with usual feeding of 5% body weight till end of experiment up to 9th week (63 days). Fishes in control were fed daily at 5% of their body weight.

The growth of fish in terms of length and weight were recorded at weekly intervals in all the treatments. The amount of feed consumption was also determined at regular intervals in different aquaria depending on weight of fish.

There was no significant difference in average weight of fish in treatment D_1 even though feed consumption being significant. However, the final average growth in the treatments D_2 and D_3 are significant .There was significant difference ($P<0.05$) in protein levels in treatments D_2 and D_3 compared to control which was attributed to its utilization for energy rather than growth due to deprivation. So, it can be concluded that, one day of feed deprivation followed by two days of satiation feeding can significantly reduce the feed consumption (expenditure in feed) without causing significant reduction in weight gain in *Labeo bata* and it can be tested in field condition in order to reduce the operational cost due to feeding.

It was found that there was significant difference ($P<0.05$) in feed consumption of R_1 and R_2 compare to control without any compromise with growth statistically. So, such restriction protocol can be tested in field conditions. It can save feed cost to the extent of 10.85%and 14.47% in treatments R_1 and R_2 respectively. So, it can be inferred that one and two weeks of feed restriction can reduce the feed cost substantially without hampering the growth of fish.

There was significant difference ($t=2.853$, $P<0.05$) in protein content in R_3 compared to control indicating protein was used for energy rather than growth causing less weight to fish. Protein and lipid content is minimum in the treatment R_3 which can be attributed to its utilization for body activities.

It can be inferred from the present study that the feed deprivation and restriction protocols like the tretments D_1, R_1 and R_2 can be tested in field conditions in order to reduce the cost of feed without compromising the growth in the fingerlings of *Labeo bata*.

Chapter 7

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