EFFECT OF RESTRICTED FEEDING ON GROWTH PERFORMANCE AND PHYSIO-METABOLIC RESPONSES OF AMUR COMMON CARP (*Cyprinus carpio haematopterus*) FINGERLINGS

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By

Mr. Arka Chowdhury

DEPARTMENT OF AQUACULTURE
FACULTY OF FISHERY SCIENCES
WEST BENGAL UNIVERSITY OF ANIMAL AND FISHERY SCIENCES
5, BUDERHAT ROAD, CHAKGARIA CAMPUS, PANCHASAYAR,
KOLKATA-700094
WEST BENGAL, INDIA

2022

West Bengal University of Animal and Fishery Sciences

Faculty of Fishery Sciences

Department of Aquaculture

5-Budherhat Road, Chakgaria, P.O:- Panchasayar, Kolkata-700 094
(Main campus: 68 - Kshudiram Bose Sarani, Belgachia, Kolkata-700 037)

Prof. T.K. Ghosh
Professor, Department of Aquaculture

Ref. No: ----------
Date: ----------

CERTIFICATE

This is to certify that the work recorded in the thesis entitled **"Effect of restricted feeding on growth performance and physio-metabolic responses of amur common carp (*Cyprinus carpio haematopterus*) fingerlings"** submitted by **Mr. Arka Chowdhury** in partial fulfillment of requirement for the degree of **Doctor of Philosophy (Aquaculture)** in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is the faithful and bona-fide 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.

Date: 26/8/22

Chakgaria Campus
Kolkata- 700 094

(Prof. T. K. Ghosh)

Chairman
Advisory Committee

WEST BENGAL UNIVERSITY OF ANIMAL AND FISHERY SCIENCES

Faculty of Fishery Sciences

Department of Aquaculture

5-Budherhat Road, P.O. Panchyasayar, Chakgaria, Kol-700094
(Main Campus: 68, K.B. Sarani, Belgachia, Kolkata-700037)

APPROVAL SHEET

Approval of the examiners for the award of the degree of Doctor of Philosophy (Aquaculture)

We, the undersigned have been satisfied with the performance of **Mr. Arka Chowdhury,** in the viva–voce examination, conducted today, the 26th August '22 recommended that the thesis be accepted for the award of the degree of Doctor of Philosophy in Aquaculture.

Name	Signature
1. Prof. T.K. Ghosh Chairman of Advisory Committee	..... 26/8/22
2. Dr. U. K. Sarkar External Examiner	.....
3. Prof. S.K. Das Member of the Advisory Committee	..... 26/8/2022
4. Dr. S. Chowdhury Member of the Advisory Committee	..... 26.08.22
5. Prof. S. Batabyal Member of the Advisory Committee	..... 26/08/22

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Date: 26/08/22

Arka Chowdhury
.....

(Arka Chowdhury)

Place: Faculty of Fishery Sciences,
W.B.U.A.F.S, Chakgaria Campus,
Kolkata -700094

Dedicated to

my

Beloved Parents

and

Dear Sir

Prof. T.K. Ghosh

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbols and abbreviations	Details
TC	Control treatment
T1/1	Cycle of 1-day feeding and 1-day starvation
T1/2	Cycle of 1-day feeding and 2-days starvation
T2/1	Cycle of 2-days feeding and 1-day starvation
T2/2	Cycle of 2-days feeding and 2-days starvation
Tab.	Table
Fig.	Figure
%	Percentage
@	At the rate of
g	Gram
mg	Milligram
kg	Kilogram
L or l	Litre
µl	Microlitre
ml	Mililitre
dl	Deciitre
nM	Nano moles
cm	Centimetre
°C	Degree celcius
×	Multiple
<	Less than
>	Greater than
±	Plus minus
()	First bracket
:	Is to
min	Minute
SD	Standard deviation
AHP	Analytical Hierarchy Process

Abstract

This present study was designed to evaluate the competent restricted feeding strategy for amur common carp (*Cyprinus carpio haematopterus*) culture in terms of growth, physio-metabolic responses, and production performance. Fingerlings (2.28±0.27 g) were submitted to 5 different restricted feeding regimes: TC (daily feeding), T1/1 (cycle of 1-day feeding and 1-day starvation), T2/1 (cycle of 2-days feeding and 1-day starvation), T2/2 (cycle of 2-days feeding, 2-days starvation), and T1/2 (cycle of 1-day feeding and 2-days starvation). The result showed significantly similar weight gain in TC and T2/1. In other starved groups the fishgrowthwas much poor. 1-day starvation followed by 2 consecutive days of feeding (T2/1) had no significantly adverse effect on SGR when compared to the fishes fed daily, rather it supported improved FCE and PER. Amongst starved group the superior digestive capacity was evidenced in T2/1 with elevated protease and amylase activity. Periodic starvation had no significant impact on lipase activity in this study. Reduced hepatic LDH and MDH activity was also reported in T2/1 among the starved group, suggested reduced stress and might strengthen the growth of fishes in T2/1. Feed deprivation increased both ALT and AST activity in blood. In the current study, the test fish amur common carp exhibited an adoptive response to the oxidative stress caused by periodic starvation, which could explain why hepatic SOD, catalase and GPx activity increased and remained proportional to the intensity of starvation. Reduced blood glucose level, parallel to the degree of feed deprivation was also evidenced in the present research. Water and soil pH always had the tendency to be reduceddue to the acidification followed by microbial decomposition of uneaten/undigested feed and faeces. Treatments with higher feed supplement found to be rich in NH_3-N concentration and also evidenced with lower DO and elevated BOD levels. Soil organic carbon, available nitrogen and available phosphorus increased gradually in all the treatments till the end of the experiment.Finally, from the economic aspect this study can suggest the cycle of 2-days feeding and 1-day starvation as the best restricted feeding strategy for amur common carp having the highest net return without compromising the yield.

Keywords: amur common carp, periodic starvation, digestive capacity, oxidative stress, compensatory growth

Chapter -1

INTRODUCTION

Overconsumption and overpopulation lie beneath every single environmental and social problem we are facing today. The first and foremost condition of conservation is avoiding the extremes of forwardness and reservation. Once, Mark Bittman posed a question: "Can farm-raised salmon be organic when its feed has nothing to do with its natural diet, even if the feed itself is supposedly organic, and the fish themselves are packed tightly in pens, swimming in their own filth?" Actually, in the long run, spoon feeding teaches us nothing but the shape of the spoon. So, cutting food waste is a delicious way to save money and save the planet.

Aquaculture, or underwater agriculture, bears out about half of the animal-based protein requirements of the world population through the most cut-rate protein-rich product, fish (FAO, 2014). In the current global scenario, cumulative population and keeping a tight rein on natural resources, the rearing of fish in captive circumstances is contemplated as the most efficient and cheapest animal-sourced protein production system compared to other animal protein production sectors (Bjørkli, 2002). However, in the evolution perspective of up-to-dated intensive fish culture practices, feed utilization efficacy of fish is considered as an important controlling factor of aquaculture production efficiency which, also, resolves growth and nutrient deposition in fish carcass (Naylor, 2009, Hixson, 2014). On the other hand, acceptance of farm-raised fish by the consumer as a nutritious food is becoming progressively more controversial since fish encounter multiple stressors in captivity and the treatment with antibiotics in farming yields an inferior quality of farmed fish (Love, R.M., 1970; Føre, 2018). In the future, with high-pitched social consciousness and a health-concerned population, there must be an increase in demand for the production of superior quality farmed fish in an energy-saving manner. This is proving to be a crucial challenge for this rapid-growing aquaculture industry.

The most expensive constituent of an aquaculture enterprise is the feed, typically demanding the lion's share, which is more or less 40–60% of the functional variable cost governed by the intensity of production. Therefore, it is crucial for one to ensure that the fish receive the food they require, both qualitatively and quantitatively, but not in excess. Hence, a successful fish culture cycle requires optimisation of feeding practises to certify the growth rates that are most economically effective and feed utilisation competency.

But one of the key glitches challenged by fish culturists is the crave for acquiring balance between rapid fish growth and efficient use of the supplied feed. Conventional feeding rehearsals may lead to wastage of feed and reduced feed conversion ratio due to day-to-day and rhythmical variations in the appetite of fish. When feeding of fish is regulated by using self-feeders, fish can synchronise feed ingestion in relation to their energy requirements and feeding regimes. Nevertheless, a restriction of the time during which feed is made available may lead to reduced feed waste without any alteration of growth performance, provided that the feeding periods are in phase with their feeding rhythms. Recognizing nutrient requirements and instigating suitable feeding stratagems can achieve waste reduction and an upsurge in profit. Feed efficiency is essential in livestock farming in all-purpose and, sure enough, in the case of aquaculture. From a management standpoint, frequent feeding of fish may not be cost-effective or, we can say, easy on the pocket due to multiplied labour costs (Riche *et al.*, 2004). Therefore, it is equally significant to realise the growth pattern and nutritional requirements of fish as well as be aware of the best feeding strategies for a species (Jobling, 1983; Jørgensen *et al.*, 1996; Gokcek *et al.*, 2008). The theory assumes that there is a genetically preordained growth trail in all animals, and animals can sense rebounds from this trajectory and compensate for them by readjusting appetite and metabolism (Xie *et al.*, 2001). Compensatory growth represents readjustments of growth rate to minimize the discrepancy between achieve and desired growth rate caused by a period of under-nutrition.

Trimming down feed costs for culture practices in a practical approach can be triumphed by grabbing the advantage of the phenomenon of restricted feeding tactics and the concept of compensatory growth. In a far-reaching logic, feeding restriction speaks up for the compensatory growth that has been demonstrated in a variety of warm-water and cold-water fish species. There is a positive correlation between growth and feeding frequency (Riche *et al.*, 2004, Riche, 2008). However, Crampton (1991) demonstrated that it may not be obligatory to feed fish daily in order to achieve maximum growth rates. In 1994, De Silva and Anderson observed that beyond a certain level, surplus feeding consigns no influence on fish growth and results in deprived growth. Excess feed ingestion beyond what the fish really needs triggers a worse FCR. Expression of the phenomenon of compensatory growth utilizes a wide choice of feeding restrictions and re-feeding protocols amongst these fish species, habitually associated with a variety of

physiological responses (Ali *et al.*, 2016). Certain responses to the span of feed restriction upon re-feeding have been incorporated with hyperphagia, boosting feed efficiency and heightened growth rates. These responses increase interest of the fisheries managers in the study of compensatory growth as well as grabbing attention of fisher-folks. Feeding strategies like this could draw a check with management time of personnel, water quality factors, as well as fish-feeding activity and economic enhancement. During the compensatory phase, the perked up rates of weight gain are presumed to save extra rooms for metabolic energy as well as amino acid escalation for protein synthesis. Intensification of protein synthesis and elevated metabolic processes are adding fuel to the auxiliary demands for energy to endorse this growth. Compensatory growth is believed to be higher, greater the subjected period of restriction has been (Jobling and Koskela, 1997; Nikki *et al.*, 2004; Tian and Qin, 2003,2004).

Moreover, an important approach to bringing down feeding costs and thus amplifying profits in aquafarming systems is to schedule proper feeding management. The success of the feeding management strategies depends on the correspondence of the feed supply (feed provision) to demand (appetite and the nutritional requirements of fish) assimilating certain factors such as rate of feeding, feeding frequency, feeding duration, and the choice of feeding regime. Attempts have been driven to reduce feeding costs while increasing growth rates and making the best use of feed by capitalizing on digestive enzymes in the diet. Other investigated trials include the use of mixed feeding schedules of varying high and low dietary protein levels in feed and elevating the rate of feeding in fish (Kumar, Pankaj. *et al.*, 2013). Feeding routines by means of restriction and re-feeding strategies have been spelled out in many species as a simple, easy, practically applicable, and pocket-friendly way of cutting feeding costs. Some fish can convert an immense quantity of feed to body weight without any adversarial drift of their growth and vivid utilisation of nutrients under a restricted feeding regime than they do under conventional unrestricted daily feeding ration regime. Through restricted feeding strategies, the acquired growth in fish seems to be meticulously related to the duration of feeding and intensity of feeding restrictions imposed prior to re-feeding. Thus, it's very much necessary to identify the most suitable restricted feeding regimes that can accomplish compensatory growth of fish prior to the practical implications of commercial aquaculture practices. The time interval of feed deprivation that prompts compensatory growth also diverges among fish species.

Researchers have demonstrated that advancements in growth rates in response to a period of feed deprivation may be as simple as a rise in feed consumption driving the higher growth level. Such feeding strategies to provoke compensatory growth could be advantageous for aquaculture on a commercial scale in ways other than just boosting growth and feed efficacy. Feeding restriction and re-feeding have been illustrated for many groups of fishes, including cyprinids, gadoids, pleuronectids, molatids, cichlids, ictalurids, salmonids, and clariids. Furthermore, outcomes of feeding trials in fish farmers' ponds clearly demonstrated that the mixed feeding schedule of a low protein diet interchanged with a high protein diet resulted in improved growth, better feed utilisation and higher production than feeding sutchi catfish and silver carp with a high protein diet uninterruptedly. This was reflected as a feasible way of lessening feed costs. Mixed feeding schedules using diets containing low and high protein offered an increased and decreased nitrogen retention and loss, respectively, in tilapia and carps. The existence of rhythmic metabolic activities in fish points towards the fact that they may not require a similar amount of nutrient intake daily. It has been reported that a mixed-feeding schedule of substituting the high-dietary protein diet with a lower dietary protein level diet reduced the overall feeding cost without negotiating with the growth of tilapia.

Compensatory growth (CG) is an accelerated growth response observed in stunted fish seed after being subjected to a stressful condition under optimal culture conditions (Ali *et al.*, 2003). CG is considered as a promising tool to intensify aquaculture yield while cutting cost and it had been experimented far and wide in cultivable fish species because of its speedier growth rate and enhanced feed utilization potential (Jobling, M., 2010). It is vital to keep the fish in a catabolic phase, which suggests the activation of the CG response by regulating the endogenous energy reserves and endocrine profile in order to induce the CG response (Won *et al.*, 2013). In nature, many fish species endure a prolonged period of starvation during spawning migration and the winter season, so it is acceptable to keep the fish in feed deprivation for a prolonged period (Krogdahl *et al.*, 2005). Stunting (an excessive stocking and feed deprivation technique) is practised by fisher-folks to produce stunted fish that display CG response under suitable culture conditions (Mohapatra *et al.*, 2017). In general, larger fish may require prolonged time interval of stunting, to induce the 'nutritional stress,' than smaller fish to elicit a CG response (Jobling *et al.*, 1994).

Compensatory growth is a phase of remarkably speedy growth followed by a period of under-nutrition (Zhu *et al.*, 2001). The phenomenon has been reported in a wide range of fish species covering varied taxa (Dobson & Holmes, 1984; Russell & Wootton, 1992; Jobling *et al.*, 1994; Kim & Lovell, 1995; Hayward *et al.*, 1997; Saether & Jobling, 1999; Qian *et al.*, 2000; Wang *et al.*, 2000). Numerous studies on compensatory growth directed towards the time span of complete feeding restriction (Wieser *et al.*, 1992; Jobling and Koskela, 1996; Zhu *et al.*, 2001; Tian and Qin, 2003, 2004; Azodi *et al.*, 2016) and among them most try-outs were carried out on cold water fish species, whereas findings on warm water species are few (Schwarz *et al.*, 1985; Kim and Lovell, 1995; Hayward *et al.*, 1997). Many reports show that Hybrid tilapia, *Oreochromis mossambicus* × *Oreochromis niloticus*, showed evidence of compensatory responses following feed deprivation (Wang *et al.*, 2000, 2004, 2005, 2009; Gao *et al.*, 2015; Ye *et al.*, 2016), but apparently a great majority of cases also showed fish that were withheld on restricted feeding regimes failed to recover completely. Study on Gibel carp *Carassius auratus gibelio (L.)*, a warm-water omnivorous fish, reported that they were able to compensate fully for food deprivation for 1–2 weeks within 4 weeks of re-feeding (Qian *et al.*, 2000; Xie *et al.*, 2001). Following a period of feeding restriction, Gibel carp were observed to compensate for growth through elevated feed intake and feed conversion efficiency, but increased conversion efficiency was not acquired by rising digestibility or reducing activity. Then again, there is a lacuna in recognising the response of compensatory growth in milkfish. So, it is necessary to systematise and to standardise the optimum time and extent of growth-stunting for milkfish, with the aim of maximising fish production through stocking of stunted seed. Therefore, a few studies were conducted to evaluate the effects of growth inhibition and duration of stunting on the growth, digestive enzymes, and carcass quality of milkfish under pond conditions (Sawant *et al.*, 2019). Although until recently, fish were raised in ponds starved of artificial feeding, further upshots of its recent magnification are mandatory to increase the nutritional knowledge of this fish species.

Digestion is a key metabolic process and determines the nutrient availability for all biological functions, including growth, which is regulated by digestive enzymatic activity (Gisbert *et al.*, 2009). Many researchers narrated a noteworthy reduction and successful restoration in enzyme activities responsible for digestion of fish during feeding restriction and usual feeding conditions, respectively. In general, fish digestive enzyme

activities are distressed by aquaculture feeding practises such as starvation and re-feeding. Hence, sketching fish digestive enzyme activity in the stunting and post-stunting phases is necessary for recognising the growth pattern and feed utilisation of fish. Under optimal culture conditions, the morphometric alterations incorporated with compensatory growth performance of fish that suffered stunting affect the nutritional composition and carcass traits of post-stunted fish (Jobling, 2010). In the stunting phase, fish make use of the endogenous energy reserves, lipid and protein, which reduce the carcass nutrient content and the stunted fish become less nutritious (Weatherley and Gill, 1987). However, some previous studies registered a successful restoration of depleted nutrients in the compensatory growth phase of fish (Collins *et al.*, 1995; Zhu *et al.*, 2005). The nucleus of aquaculture globally has shifted predominantly towards elevating the nutritional quality of farmed fish due to the emerging health concerns and issues running in the contemporary world. In this present scenario, CG can be harnessed for refining the nutritional composition of cultured fish in order to meet the demand of the modern consumers (Jobling *et al.*, 1994).

It is very much pronounced from the various studies that, the discriminate restriction in feeding for one or two days presents an eminent opportunity to protect fish farmers against unfavourable situations such as feed scarcities or high cost of feed. This strategy offers opportunities for fisher-folks to cut down on the cost of production at every turn. Restricted feeding regimes may be a promising tool for increasing the productivity as well as proficiency of fish production. Satiation feeding may be responsible for the awful deterioration in water quality. By totally avoiding feeding or by limiting feed during the winter, producers and investors can save money on geosynclinal losses by reducing feed and labour costs and possibly lowering losses due to various diseases as well. Further research is desirable to determine the optimum interval of time to restrict feeding for different sizes of fish in order to take full advantage of the effects of compensatory growth and optimise disease resistance.

In this context, the present study was designed with Amur common carp (*Cyprinus carpio haematopterus*) as it's a very important fish species to be grown together with IMCs in a polyculture system. The objectives of this research are

i. To study the effect of restricted feeding regimes on growth performance and nutrient utilization of Amur common carp (*Cyprinus carpio haematopterus*) fingerlings

ii. To study the effect of restricted feeding regimes on physio-metabolic responses of Amur common carp (*Cyprinus carpio haematopterus*) fingerlings

iii. To investigate the water and soil quality parameters with regard to environmental health of the culture system under different feeding regimes.

Chapter -2

REVIEW OF LITERATURE

Feed cost engulfs the lion's share of the operational costs in aquaculture or aqua farming. Thus, approaches for reducing feed cost have been one of the major thrust areas in fish nutrition research. Fish feed accounts for about (60-70) % of the overall recurring expenditure in aquaculture, hence it is essential to adopt a feeding strategy which optimizes feed efficiency and production, and maximizes profitability (Ali *et al.*, 2006). Several fish species are exposed to a natural starvation phase during a part of the year and hence have acquired a remarkable competence to withstand prolonged periods of starvation. Many organisms show faster growth when recovering from starvation than when having access to constant food availability as an adaptation to such a lifestyle. When food supplies are amplified following a period of starvation or restricted feeding, fish may reflect a growth surge, often denoted as compensatory growth.

Successful cultured fish production therefore requires optimisation of feeding practices to ensure the most economically effective growth rates and feed utilisation efficiencies. One of the major challenges that fish culturists face is achieving a balance between rapid fish growth and efficient use of supplied feed. Regular feeding practises may result in feed waste and reduced feed conversion due to day-to-day and rhythmical variations in appetite. When fish are fed using self-feeders, they can control their feed intake based on their energy needs and feeding rhythms. However, a constraint of the time during which feed is made obtainable may lead to lessened feed waste without any shift in growth performance, offered that the feeding periods are in phase with their feeding tempos.

Reducing feed costs for culture practises in a practical way is possible by leveraging the experience of restricted feeding strategies. Restricted feeding promotes compensatory growth, which has been observed in a variety of warm-water and cold-water fish species. Numerous feed restriction and refeeding etiquettes have been applied to express the occurrence of compensatory growth among these fish species habitually with a variety of physiological responses. Some of the responses to the feed restriction period upon refeeding have included hyperphagia, increased feed efficiency, and faster growth rates. These responses have heightened interest in the investigation of compensatory growth as a powerful management tool in aquafarming. Such feeding strategies could improve personnel time management, water quality, fish feeding

activity, and economic performance. The improved rates of gain during the compensatory phase would be expected to place extra demands for metabolic energy as well as increase amino acid requirements for protein synthesis during this period. The added demands for energy would be expected to come from increased rates of protein growth and elevated metabolic processes to support this growth.

Moreover, an important approach to reduce feeding costs and thus increasing profits in aquaculture farming system is to develop proper feeding management. Feed management tactics must correspond feed supply (feed delivery) to demand (appetite and the nutritional requirements of fish) merging such factors as feeding rate, frequency, duration and the selection of feeding regime. Efforts have been channelized to reduce feeding cost, while increasing growth rate and maximising feed utilisation by including digestive enzymes in the diet. Other methods tested include the use of mixed feeding schedules of varying high and low dietary protein levels in feed and optimising the feeding rate. Feeding regimes based on restriction and re-feeding strategies have been demonstrated in many species as a simple, easy, practically applicable, and cost-effective way of reducing feeding costs. Several fish convert a greater portion of feed to body weight under a restricted feeding regime with no adverse effect on growth or nutrient utilisation than they do under an unrestricted diurnal feeding ration regime.

The duration and severity of feed restriction imposed before refeeding appeared to be closely related to growth in fish obtained through restricted feeding strategies. Thus, prior to the practical application of aquaculture, it is necessary to understand the appropriate feeding regimes that achieve compensatory growth of fish. The duration of feed deprivation that causes compensatory growth differs among fish species.

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 high-priced constituent of fish feeds. Fishes are known to utilize protein favourably to lipid or carbohydrate as an energy source. Hence, it is significant from nutritional, environmental and economic viewpoint to perk up protein utilization for tissue synthesis rather than for energy purposes. The optimization of dietary protein/ digestible energy ratio (DP/DE) has proved to have a crucial task on protein and energy utilization (Kaushik, 1994). The upsurge of DE content of fish diets by lipid can cause protein sparing effect and therefore reduce nitrogen shortfalls to the environment (Cho and Kaushik, 1990). The research works related to the present study are reviewed as below:

2.1. Principles:

Developing intensive production methods for a given species requires determining environmental preferences, including those pertaining to feeding. The evaluation of feed necessities should incorporate the daily feed ration in addition to the chemical composition of feed (predominantly protein and fat content), caloric content, size of pellets along with their shape, colour and texture (Higuera, 2001). The effects of rearing fish under intensive conditions are determined not only by the quantity and quality of feed but also by the method employed to deliver it (manual or automated feeding) and by the frequency of feeding (Alanärä *et al.*, 2001). Feeding frequency possibly have an influence on growth rate of fish, feed utilization efficiency, and intra-group size variation of fish (trends of dominance and hierarchy in fish stocks). This feature was analysed in numerous scientific investigations (e.g. Jobling, 1983; Alanärä, 1992; Johansen and Jobling, 1998; Brännäs and Linnér, 2000; Lambert and Dutil, 2001; Petursdottir, 2002). The results of investigations conducted on various fish species are not unequivocal. The influence of feeding frequency is not only species specific; it also depends on the given developmental stage (size) of the fish (Folkvord and Otterå, 1993; Linnér and Brännäs, 2001; Giberson and Litvak, 2003). In intensive rearing, particularly in recirculating methods, feeding frequency may affect fish growth mutually directly and indirectly as it can distress water quality as well as oxygen and ammonia concentrations (Phillips *et al.*, 1998).

Competition for food raises when it is limited or when feed restriction is applied (McCarthy *et al.*, 1992; Jobling, 1995). Under such circumstances growth of fish is often lessened and the significance of the trends of dominance and hierarchy enhances within the stock (Jobling, 1983). It seems that the consequences of feeding frequency on growth and intra-group size variability possibly be more superficial when restricted feeding is harnessed.

Practically, when fish are fed frequently with automated feeders, it is challenging to confirm that the circumstances will permit for each entity to feed to satiation during each feeding. During low frequency feeding a portion of the feed is not consumed by the fish and falls to the bottom (this occurs with fish that feed from the water column exclusively). In this instance, feeding frequency is augmented and the feed is served in pronounced portions of rations all the way through the daily cycle. Johansen and Jobling (1998) witnessed that the Atlantic salmon, *Salmo salar*, fed at a

lower frequency were less active. They also concluded that there were immense size variations amongst fish fed more recurrently by means of an automatic feeder. These investigators did not mention the effect of the feeding frequency on the average growth rate of fish.

The sustainability of aquaculture depends on cost effective exercises throughout production period. Feeding practice is one of the rituals that call for optimization in aquaculture, because overfeeding could steer to accelerate production costs and water pollution, however underfeeding could steer to reduced growth performance and poor economic gain (Eroldogan, Kumlu, & Akta, 2004). In tilapia farming, feed cost constitutes almost 60-70% of the total production cost (Borski *et al.*, 2011), and this has made it hard to convert the benefits of higher production associated with commercial feed into economic gains when fed fish are fed following traditional practices. In an endeavour to amplify aquaculture revenues, fish farmers have progressed numerous feeding management approaches, which trim down feed inputs (Cuvin-Aralar, Gibbs, Palma, Andayog, & Noblefranca, 2012), diminish water quality hazards along with labour cost (Blanquet and Oliva-Teles, 2010). Some of these approaches incorporate mixed feeding for instance substitute commercial pellets adding to farm-made feed (Akinwole & Faturoti, 2007), and restricted feeding, for example, feeding by body weight, or feed deprivation and refeeding cycles, with fish usually consumed to satiation in the course of refeeding period (Ali, Nicieza, & Wooton, 2003).

2.2. Feed restriction:

It entails offering the animals limited amounts of feed, normally lower than the amount that they are able to eat. Restricted feeding refers to restricting the amount of food while still ensuring nutritional adequacy. This denotes that the sum of energy merely has been restricted. Feed restriction is the highly recommended feed management approach in aquaculture (Yengkokpam *et al.*, 2013; Jobling, Meløy, dos Santos, & Christiansen, 1994; Quinton & Blake, 1990; Oh *et al.*, 2008). This approach is considered to exploit the benefit of a physiological phenomenon termed Compensatory growth, which is portrayed as an augmented growth rate stemming from a suitable refeeding course of the fish after an interval of restricted feeding or revealing to adverse circumstances for instance low oxygen, low temperature, and reproductive force (Ali *et al.*, 2003).

From a management standpoint frequent feeding (number feeding per day) of fish may not be economical due to increased labour costs (Riche *et al.*, 2004). So, one of the problems in fish production is to obtain a good balance between fish growth and food consumption. Therefore, equally important it is to know the growth and nutritional needs of fish, as knowing the best feeding strategies for a species (Jobling, 1993, Goddard, 1996, Jørgensen *et al.*, 1996, Gokcek *et al.*, 2008).

Growth and feeding frequency have a strong positive correlation (Riche *et al.*, 2004, Riche, 2008). However, Crampton (1991) demonstrated that feeding daily may not be necessary to achieve maximum growth rates. De Silva and Anderson (1995) also discovered that, after a certain point, excessive feeding has no effect on growth and results in deprived growth. Surplus consumption leads to a worse FCR, which is higher than what the fish actually requires.

Hyperphagy donated to the restoration of the energy shortfall triggered by the starvation interlude. Indeed, compensatory growth is an obvious reaction to hyperphagia (Ali and Wootton, 2003). Hyperphagia allows an animal, that has been subjected to a food restriction, to regain the weight that it would have gained if it had been fed without restriction. When the animal is subjected to a longer restriction span, compensatory growth is increased (Jobling and Koskela, 1997, Nikki *et al.*, 2004, Tian and Qin, 2003, Tian and Qin, 2004).

Most reports of compensatory growth have concentrated on the period of complete feed deprivation (Wieser *et al.*, 1992, Jobling and Koskela, 1996, Zhu *et al.*, 2001, Tian and Qin, 2003, Tian and Qin, 2004, Azodi *et al.*, 2016) and predominantly were executed on cold water species, whereas findings on warm water species are scarce (Schwarz *et al.*, 1985, Kim and Lovell, 1995, Hayward *et al.*, 1997). Hybrid tilapia *Oreochromis mossambicus* × *Oreochromis niloticus*, showed compensatory responses after restricted feeding period (Wang *et al.*, 2000, Wang *et al.*, 2004, Wang *et al.*, 2005, Wang *et al.*, 2009, Gao *et al.*, 2015, Ye *et al.*, 2016), but fish that were retained on feeding restriction schedules seized to entirely retrieve in the abundant number of cases. In spite of the pronounced possibility of tilapia production, information regarding the consequences of feeding strategies and management practices on fish performance is limited.

conversion was not dissimilar amongst the groups of fish throughout the whole study. Nevertheless, fish with restricted diets received more feed per body weight and gained more weight during the refining period than fish with unrestricted diets. Fish that were restricted to food intake and fish that were not fed at 3-week intervals weighed less than those that were fed continuously after the first 9 weeks of the study.

This report designates that channel catfish on feeding restriction for a comparatively brief time period show evidence of compensatory weight gain all through subsequent refeeding phase under optimum growing conditions if they are fed to optimum satiation all through the refeeding phase. Hence, if the aquaculturist is incapable to feed for a short span because of disease outbreak or undesirable pond circumstances, the fish can counterweigh for this deprivation if fed to satiation when feeding is recommenced. These investigations also propose that enhancements in growth rate and feed efficiency may arise only for a reduced time upon refeeding. Thus, additional study is required to determine what length of feeding deprivation period will boost compensatory gain.

2.2.2. Restricted feeding regimes during winter:

Winter feeding schedules are important because fish metabolism, feed intake, digestion, and immune responses decline at suboptimal temperatures, which could affect how efficiently channel catfish are produced. Because of this, and because intemperate winter weather often renders feeding fish in ponds hard, fish consume less in winter. Several reports have been accomplished to investigate if restricted feeding during the winter can be manipulated so that compensatory weight gain may be optimized the succeeding spring.

Two overwintering studies at Auburn University inspected the outcomes of no feeding, partial feeding (no feeding during December to February), and continuous feeding during cool weather on year 1 (initial weight 0.09 pound) and year 2 (initial weight 1.5 pounds) channel catfish. Each size class was stocked distinctly into ponds at commercial densities in October and harvested in late April. At the termination of the experiment, weight of the partially fed fish was not different from that of the uninterruptedly fed fish, although they were fed only about 60 percent as much feed. Non-fed fish lost weight and were notably undersized than the regularly fed and partially fed fish. This study found no benefit in weight gain when feeding channel

catfish in December, January and February, as fish that were not fed during that period showed compensatory gains in the following spring.

An analogous overwintering investigation was carried out at Texas A&M University in which stocking of channel catfish (initial weight 0.4 pound) were accomplished into six ponds in advance December. Whenever the water temperature rises above 55 degrees Fahrenheit, the fish in the three ponds are fed as much as they can eat. Fish in the other three ponds were not served feed in any way in the course of December to March. Feeding of unfed fish was resumed in early April and continued until August to coincide with a normal growth season. Fish fed during the winter months gain significantly more weight than those without food. After refeeding, nevertheless, no significant difference was observed in percentage weight gain, food conversion, or survival between regularly fed and formerly unfed fish. These studies denote that channel catfish not fed during the cold winter months (December, January and February) possibly will be able to show compensatory gains after refeeding in the spring.

Compensatory growth achieved from restricting winter feeding is dependent on various factors such as fish size, extent of feed deprivation, and severity of the winter. The practical implications of these findings are that, producers, who prefer not to feed their fish in the winter, may be able to obtain partial compensatory weight gain during the spring feeding course. Further investigation is required to conclude the optimum time span to deprive fish of supplementary feed in winter so that compensatory growth can be maximized in spring without reducing total yield.

2.2.3. Effect of restricted protein feeding on fish body composition, nutrient utilisation, and growth performance

Researchers have demonstrated that improvements in growth rates in response to a phase of restricted feeding may be as simple as a surge in feed consumption driving the higher plane of growth. Such feeding tactics to stimulate compensatory growth could be beneficial in commercial aquaculture in ways other than just enhancing growth and feed efficiency. For many groups of fish, including cyprinids, clariids, cichlids, gadoids, ictalurids, molatids, pleuronectids, and salmonids, feed restriction and re-feeding have been described.

Furthermore, feeding trial results in farmers' ponds clearly demonstrated and instructed that an admixed feeding schedule of a low protein di*et al*ternated with a high protein diet resulted in better growth, feed utilisation, and production than feeding sutchi catfish and silver carp a high protein diet continuously. This was pondered as a promising manner of cutting feed cost. Mixed feeding programs sourcing diets comprising low and high-protein offered an enhanced and declined nitrogen retention and deficiency, correspondingly, in tilapia and carps. The existence of rhythmic metabolic activities in fish implies that they perhaps not require a similar amount of nutrient intake daily. It has been reported that a mixed-feeding schedule of alternating the high-dietary protein diet with lower dietary protein level diet reduced the overall feeding cost without compromising growth of tilapia.

2.3. Starvation in Fishes

Many fish species suffer from natural starvation at certain times of the year and have therefore developed a remarkable ability to tolerate starvation at certain times. During starvation crucial activities are sustained at the sacrifice of accumulated energy reserves which of course, consequences in the gradual exhaustion of body tissue.

Weiser *et al.* (1992) conducted time dependant compensatory growth for three of the cyprinid species and described that, the longer the fish remain without food, the faster the metabolic response to the presentation of food, as if, with prolonged food shortage, an emergency machinery is brought into action to guarantee the most rapid and efficient exploration of new food source. Upon re-feeding, mass specific maintenance and routine rates of metabolism as well as relative growth rates increased rapidly. It was also observed in their experiment that the maximum compensatory growth of three cyprinids after starving four weeks with relative growth rates reached 30% per day throughout the first assessing interval after re-feeding.

2.4. Effect of feed Restriction and Starvation

Natural starvation occurs in most fish species at certain times of the year, owing largely to environmental conditions. Fish can withstand comparatively prolong interludes of starvation without severe consequences. After an interval of starvation, refeeding resulted increase in growth rate, weight of body and also food conversion ratio.

The specific effects of starvation on metabolism are determined by a number of factors, including the species under consideration and the preferential tissue for different routes of mobilisation. It's also important to distinguish between experimental and natural fasting, because natural fasting can be accompanied by additional factors like gonadal growth and low temperature.

Due to consumption of high dietary protein it increased the concentration of free amino acids in body, protein synthesis and gluconeogenic enzyme activities, ammonia excretion and also decreased glycolytic enzyme activities. During prolong starvation period proteolytic activity in fish tissue increase with the amino acids, which produce for using of energy. Fasting conditioned fish generally utilize reserved lipid as a source of energy in preference to protein and carbohydrate. A juvenile fish requires more energy per unit body 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 termination of their experiment that Juvenile Hybrid Tilapia (*Oreochromis niloticus x Oreochromis aureus*) unfed for 1 or 2 days/week gained body weights nearly the identical compared to the control group. There were substantial variations in Feed Intake (FI), Feed Conversion Ratio (FCR) and Protein Utilization Efficiency (PUE).

Nekoubin *et al.* (2012) studied on the influence of feeding frequency on growth performance, feed efficiency and survival rate of juvenile grass carp. Three troops of juvenile grass carps (an average weight 4.31±0.1g) were intended with three different feeding frequencies of one meal per day (D1), two meals per day (D2), and three meals per day (D3), with two replicates of each treatment for a total of 60 days of experimentation. The grass carp in an experimental treatment were fed by *Lemna* sp. with 20% of body weight. They found out the final body weight and specific growth rate (SGR) were significantly higher in-group D3 ($P<0.05$). Similar responses were observed for Body Weight Increase (BWI), Daily Growth Rate (DGR), Feed Conversion Ratio (FCR) and survivalists was significantly higher ($P<0.05$) in the treatment D3.

Eslamloo *et al.* (2012) studied on consequences of starvating and refeeding on growth performance, feed utilization and body composition of Tinfoil barb (*Barbonymus schwanenfeldii*) and found out that, the utmost final weight (5.30g ±

0.52g) and specific growth rate (1.91±0.17%) were observed in treatment T2 (which are deprived for 4 days and subsequently 12 days refed in cyclic manner). Diurnal feed consumption and overall feed consumption was extensively different among the deprived and the control fish ($P<0.05$). The lower most values of FCR (2.19± 0.40) and the higher most values of FCE (48.37 ± 7.52%) and PER (1.17 ± 0.18%) were witnessed in the T2.

Singh *et al.* (2005) conducted 8-week experiment on *Cirrhinus mrigala* fry with restricted feeding for 1, 2 and 4 weeks while the control fry were regularly served feed twice in a day to satiation. There were no notable changes in initial body weight among treatment groups at the start of the experiment but at the termination of the re-alimentation period, the weight of 1 week and 2-week fry were extensively higher than the control and 4-week fry. The feed conversion ratios of the one and 2-week fry were radically improved than in the control and 4 week groups. Survival in the control, 1 week and 2 week groups did not substantially differ from each other but were all ominously better than in the 4-week group.

Morshedi *et al.* (2016) researched the outcomes of starving and following refeeding on growth, body composition, haematological indices, feed utilization and several morphological and plasma biochemical parameters of Siberian sturgeon (*Acipenser baerii*) with average initial weight of 19.32 ± 0.62 g (mean ± SE). The fish were subjected to four feeding regimes: the control group (C) was fed four times in a day to apparent satisfaction during the course of the trial. Fish in the other three treatments were left without food for 2, 4, or 8 days followed by 8, 16, or 32 days of refeeding (T1, T2 and T3, respectively) in repeated cycles during an 80 days feeding trial. At the conclusion of the investigation, there were no noteworthy alternations in final weight (except for T2 group), growth and feeding performance, whole body composition, hepato-somatic index and digestive somatic index between the deprived and control fish ($P > 0.05$). Feeding approaches appeared to have a momentous influence merely on the protein content ($P < 0.05$). At the conclusion of the experimentation, protein content of T3 group fish was substantially poor than the other treatments ($P < 0.05$). The concentrations of total plasma protein and triglyceride were considerably greater in T3 group than those in T1, T2 and control groups ($P < 0.05$). Feed deprivation notably impacts on white blood cell (WBC) count at the termination of the trial ($P < 0.05$). The existing results indicated that Siberian sturgeon submitted to 2

days and 8 days of starvation and subsequent refeeding showed complete growth compensation. Hence, it could be decided that the degree of growth compensation governed by the time interval of feed deprivation. Likewise, this species might regain the growth performance and certain morphological and biochemical parameters trailing a phase of starvation.

Ali *et al.* (2016) reported the impact of distinct weekly feeding frequencies on Nile tilapia fingerlings, weighted 2.02 g, was ascertained throughout 12 weeks. This was accomplished by serving feed to the fish for 7 days per week, 6 days per week or 5 days per week. After this restriction-feeding period, all fish were served feed same as the control group (fed for 7 days in a week) for initial 26 days to examine the competence of the fish to balance the growth throughout the entire re-feeding phase. At the edge of the feed deprivation, the weights amongst the distinct treatments differed significantly, even though the importance was identified only at 7-days per week levels that exhibited the maximum final body weight contrasted to another 2 treatments. The diurnal feed ingestion, the feed conversion ratio and protein efficiency ratio did not portray significant differences in any way. Crude Protein Efficiency (CPE) and Gross Energy Efficiency (GEE) were influenced by the feeding frequency; representing elevated values in fish which were fed for 7 days per week. Growth consequences acquired throughout this re-feeding phase specify that Weight Gain (WG) and Specific Growth Rate (SGR) denoted a linear escalation from 7 to 5 days to week, i.e. with growing feed restriction phase; the fish may recompense the growth effectually, attempting to catch up with the weight of the control group.

Yengkokpam *et al.* (2014) inspected the sort of growth compensation on *Labeo rohita* fingerlings of average weight 3.75 ± 0.06 g around ten-week. Fingerlings were fed regularly (control), deprived of food for one (D1), two (D2) or three (D3) weeks and eventually refed to apparent satiation intended for 5 weeks. The starvation was performed in D3 group on 3rd, 4th and 5th week; D2 group on 4th and 5th week; D1 group on 5th week, and finally refeeding of each of all groups were commenced from 6th week ahead. Both the D1 and D2 groups matched in body weight with the control within 2 weeks and 4 weeks of refeeding, correspondingly, though the D3 group possessed significantly ($P < 0.05$) reduced body weight compared to the control following 5 refeeding weeks. Better growth efficiency was witnessed in all starved groups in only 1st week of refeeding. After 5 weeks of refeeding, feed ingestion in D1 group turned out

to be similar to the control; however D2 and D3 groups were still hyperphagic once the trial ended. Therefore, growth compensation was due to hyperphagia and improved growth efficiency. As a conclusion, absolute growth compensation was noticed in *L. rohita* fingerlings following restricted feeding of 1 and 2 weeks.

Azodi *et al.* (2015) examined to conclude the impacts of short-term deprivation and refeeding phases on growth, feeding executions and body composition of Rainbow trout (*Oncorhynchus mykiss*) around sixty days. Here three hundred trout fingerlings were subjected to 5 distinct feeding protocols; such as,

i. Control: uninterruptedly fed twice per day to utmost satiation;

ii. T1: deprived from feed for 1 day and re-fed for subsequent 2 days;

iii. T2: deprived from feed for 1 day and re-fed for subsequent 4 days;

iv. T3: deprived from feed for 3 day and re-fed for subsequent 12 days;

v. T4: deprived from feed for 4 day and re-fed for subsequent 16 days.

At the termination of the trial, growth occurrence, feed utilization, complete body ash and moisture contents were not significantly ($P>0.05$) different among the treatments. However, total body protein composition in T3 was significantly elevated compared to other treatments ($P<0.05$). A precise significant difference in entire body fat composition was spotted between T3 and control at the termination of the investigation ($P<0.05$). In conclusion, this investigation proposes that feeding protocols concerning deprivation (for 1 to 4 days) and re-feeding rotations are potential feed management aids for Rainbow trout cultivation.

Azodi *et al.* (2016) also studied the outcomes of diverse deprivation and refeeding phases on growth, feed utilization and entire body composition in Asian sea bass (*Lates calcarifer*) with an average primary body weight of 30.26±1.4 g (mean±SE). The fish were subjected to three distinct protocols:

i. The fishes of control group served feed two times per day to utmost satiation all through the trial (C),

ii. The fishes of 1st group were deprived of feed for 4 days and subsequently refed for around 16 days, the whole cycle was recapped two times (T1) and

iii. The fishes of 2nd group were deprived of feed for 8 days and subsequently refed for around 32 days (T2).

At the termination of trial, growth as well as feeding performance among different treatments didn't differ significantly ($p>0.05$). Daily feed ingestion was significantly advanced in the starved compared to the control ($p<0.05$). Moisture, lipid, ash content and Nitrogen Free Extract (NFE) content of carcass at the termination of various deprivation and refeeding phases amid the starved and control groups of fishes, didn't differ significantly ($p>0.05$). Feed deprivation had a momentous impact on the protein content on one sampling date throughout the investigational interval; protein matter in T2 on 8th day was significantly reduced than the control ($p<0.05$). Sea bass exhibited absolute compensation signifying a superior capability of the starved fish to mature satisfactorily to completely recompense for weight loss during deprivation. The outcomes proposed that feeding regime comprising deprivation-refeeding rotations might be an encouraging feed management strategy for the farming of this species.

In a carp-prawn grow-out production system, Mohanty (2015) investigated the effects of starvation on the occurrence of compensatory growth (CG) in Indian major carps, sediment loading, and water productivity. He said that the overall growth and crop performance were in the similar line in both T1 (regular feeding, 2 times a day) and T2 (4-week feeding followed by 2-week no feed). However, between T1 and T3 (8-week feeding followed by 2-week no feed), there was a significant ($P < 0.05$) variation in the overall growth and crop performance. This was perhaps as a result of the prolonged refeeding intervals after rotational starvation that in response magnificently activated growth compensation in T3 (CG Index: 98–104%). Treatment-wise sediment load ranged between 59.2 and 69.6 m^3 t1 biomass. Better the apparent feed conversion ratio (FCR), greater was the rate of sedimentation. Greater the feed input, greater were the water exchange required, as well as total water use and consumptive water use index. Besides, rotational starvation and refeeding assisted in sustaining water quality as a result of the deprived feed input (10.5% in T2 and 2.0% in T3), therefore lessens the input cost and enhances production efficiency. Keeping the growth and yield performance, water productivity and economic efficiency in view, T3 is considered the best feed management protocol followed by T2 and T1.

Durairaja (2005) found that specific growth rate (length) and specific growth rate (weight) of *Clarius batrachus* exhibited a decline trend of variation during his experiment. Minor variations were due to frequency of feeding in the weeks.

Sevgili *et al.* (2013) studied on effect of several extents of single-phase starvation on growth compensation in Rainbow Trout (*Oncorhynchus mykiss*) during summer environments (18.1°C and 12.5-14.5 hours duration of day). Five distinct treatments with triplicate tanks were in this manner: Control (C), satiate fed around 84 days; one (S1), two (S2), three (S3), and four (S4) weeks of feed deprivation; and later refeeding for the rest eight weeks of the trial. Deprivation phases tempted hyperphagia throughout refeeding though only S1 (one week of starvation) and S2 (one week of starvation) were competent to get closer to control. Repeated actions of ANOVA, analysis of variance recommended a convergence in body mass but not in structural body length. Organo-somatic indices of the deprived sets were substantially decreased at the termination of deprivation intervals and reconditioned to levels of the fish of the control within the initial two weeks of the refeeding phase. In a broad sense, deprivation lengthier than a week considerably lowered utmost digestibility of dry matter, lipid, and energy competed to the control group but did not impact protein and ash, and an absolute retrieval in the digestibility coefficients appeared within two weeks of satiate feeding. A linear upsurge in body moisture and a decline in lipid and lipid/lean body mass ratio were found with the intensity of deprivation phases; then again these divergences mostly faded at the ending of refeeding.

Wu *et al.* (2002) experienced four rounds of 1 week of feed restriction trailed by 2 weeks of feeding to satiation among four species, namely European minnows *Phoxinus phoxinus* (Cyprinidae), Three-spined stickle backs *Gasteosteus aculeatus* (Gasterosteidae), Gibel carp *Carassius auratus gibelio* (Cyprinedae) and Long-snout cat fish *Heiocassis longinostris* (Bagridae) and found that stickle back, carp and cat fish showed significant increase in food intake following deprivation. However the temporal prototype of ingesting throughout the refeeding phases contrasted among the four species. In Stickle back, daily feed ingestion across a refeeding cycle primarily declined, but afterward recovered. In minnows, ingestion leaned towards dropping across a refeeding cycle. Gibel carp exhibited an upsurge in diurnal ingestion on the refeeding and catfish had a weak inclination to display an early fall, shadowed by an escalation across a refeeding phase.

Bhat *et al.* (2011) investigated either deprived feeding (5% of the body weight or 1% of the body weight as maintenance necessity) or satiate feeding sectioned into three uniform feeds on Rainbow Trout (*Onchorhynchus mykiss*). The finest ($P<0.05$) growth feedback in terms of decisive body weight, weight gain percentage and Specific Growth Rate (SGR) was witnessed for feeding regime I (control), for fish fed to satiation all the way through. Feeding regime II exhibited significantly the best ($P<0.05$) growth feedback in terms of decisive body weight, weight gain percentage and Specific Growth Rate (SGR) compared to other feeding schedules. Growth Rate (GR) and Food Conversion Efficiency (FCE) in feeding regime II were noticeably greater throughout phase II compared to phase I. Growth rate of phase I (0-4 weeks) marginally augmented in feed deprived fish all through (I-II). Conversely, in phase II (4-8 weeks) fish were fed to satiation all through (I-II) exhibited a prompt rise in fortnightly growth feedback and better growth rates, consumption of feed and FCE.

Adakh *et al.* (2015) researched the influences of starving and refeeding rotations on growth activity and body biochemical composition of adolescent Sea bass. Throughout the extent of the investigation, the control group (C) was served fed to apparent satiation thrice daily. The feeding schedules of another three groups were planned as such: 2 days of deprivation / 8 days of satiate feeding (G1) (5 cycles), 5 days of deprivation / 20 days of satiate feeding (G2) (2 cycles) and 10 days of deprivation / 40 days of satiate feeding (G3) (1 cycle). After around 50 days, just group G1 expressed partial growth compensation. The precise difference amid the final weights (FW) of groups was noticed significant in terms of statistics ($P<0.05$). Specific Growth Rates (SGR) of starving groups were lesser compared to the control group ($P<0.05$). G1 group was concluded to possess the finest values of Feed Conversion Ratio (FCR) and Economic Conversion Ratio (ECR). There was no significant difference in Hepatosomatic index (HSI) among groups ($P>0.05$). Total fat (TF) content was least in G3 group ($P<0.05$). The partial growth compensation by G1 group bestowed potentials for economic optimization.

Eroldoganv *et al.*, (2006) assessed the effects of distinct series of starving and refeeding regimes for 7 weeks on growth performance and feed ingestion in Gilthead sea bream, *Sparus aurata*, weighed 14 g. After 7 weeks of rotated cycles; all the groups were served feed to utmost satiation for following 3 weeks. Three groups were starved for 2, 4 or 7 days (S2, S4 and S7, respectively) and later referred till their corresponding

feed ingestion contrasted by less than 20% of apparently fed control fishes till the ending of the week 7, whereas a 4th group (S7/Rf14) gone through three rotations, each comprising of 1 week of feed restriction trailed by 2 weeks of satiate feeding. Control (C) group were fed to utmost satiation all through the experiment. The fish were served feed a Sea bream diet (450 g/kg crude protein) in accordance to the regimes, two times daily for 7 weeks.

Aderolu *et al.* (2011) studied on compensatory growth effects on previously started fingerlings and adolescents of African cat fish (*Clarius garipinus*) and reported no major disparity in the FCR and voluntary feed intake in juveniles as well as fingerlings. Fish fed three times a day documented the maximum mean weight gain but consistency was observed across the various feeding frequencies in the juvenile fish. The specific growth rate value of 0.57 and 0.53% per day was recorded respectively for the fingerlings and juveniles, fed four times a day. No major difference was noted in overall economic parameters evaluated for the juveniles among the feeding levels. They concluded that after feeding once a day for 8 weeks, a fish could still catch up with others fed at a higher feeding frequency when reverted to satiation feeding.

Turano *et al.* (2008) found that fingerlings of Hybrid Striped Bass (HSB) *Morone chrysops* × *M. saxatilis* had experienced periods of 2 or 4 weeks feed deprivation followed by refeeding for a similar duration resulted in partial growth compensation.

The partial growth compensation observed in group held gibel carp, *Carassius auratus gibelio* (Bloch), following 3 and 4 weeks of restricted feeding (Zheng *et al.*, 2006).

Shoemaker *et al.* (2003) reported that juvenile cat fish, *Ictalurus ictalurus* which were not given feed for 2 and 4 weeks had a major escalation ($P<0.05$) in gutted weight wet weight ratio and decline in other organo somatic indices such as Gut Index (GI), Mesenteric Fat Index (MFI) and Hepatosomatic Index (HIS) Blood glucose, liver glycogen. GI and HIS are sensitive indicators for channel cat fish deprived of feed (NF) for four weeks.

Tian *et al.* (2003) reported that the fish kept starving for 1 week attained the equal weight as the control fish after refeeding for 3 weeks representing that complete growth compensation stricken in barramundi, *Lates calcarifer*.

Qian *et al.* (2000) reported that the gibel carp (*Carassius auratus gibelio*) are competent to exhibit complete compensatory growth succeeding 1 and 2 weeks of feed deprivation.

Wang *et al.* (2000) reported that hybrid tilapia (*Oreochromis mossambicus*) showed compensatory growth response during refeeding after an interval of food restriction and observed that hyperphagia was accountable for enhanced growth rate during compensatory growth.

Zhu *et al.* (2001) observed that the growth rate throughout the compensatory growth phase was greater than that of control, which fed continuously and allows the Size of the feed deprived fish to have converged wholly (full compensation) or partially (partial compensation) on that of control. Three-spined Stickle back, *Gasterosteus aculeatus* showed compensatory growth following phases of restricted feeding of 1 or 2 weeks and subsequent refeeding. The compensatory growth feedback of the Stickle back showed a lag of a week before developing in the refeeding phase.

Zhu *et al.* (2004) had reported a lag in the growth compensation in *Leuciscus cephalus* often 1 or 2 weeks of starvation. Full compensation was reported in Rainbow trout, *Oncorhynchus mykiss* and partial compensation in Arctic charr, *Salvelinus alpines*. Complete compensatory growth response was reported in *Chalcarburmus chalocoides*. Minnows, *Phoxinus phoxinus* had compensated fully for 1 or 2 weeks of refeeding.

2.5. Hyperphagia

Hyperphagia, abnormally increased appetite for and consumption of food, is the common phenomenon used to explain growth compensation of fish (Cho, 2011). It is the key process concerned in terms of compensatory growth response, although increased food conversion efficiencies or behavioural adjustments might play a role (Ali *et al.*, 2003). Hyperphagia has been recognized in fish species all through compensatory growth (Miglavs & Jobling, 1989; Russell & Wootton, 1992; Hayward *et al.*, 1997; Wang *et al.*, 2000; Xie *et al.*, 2001; Nikki *et al.*, 2004), while progressed food conversion efficiency has not been broadly studied in fish exhibiting growth compensation (Dobson & Holmes, 1984; Russell & Wootton, 1992; Qian *et al.*, 2000; Eroldoganv *et al.*, 2006). Many studies detected no noteworthy divergences in food

conversion efficiency among the control group and the food-deprived groups during re-feeding periods (Kim & Lovell, 1995; Hayward *et al.*, 1997; Xie *et al.*, 2001).

Rubio *et al.* (2010) reported that during the winter, Sea bass (*Dicentrarchus labrax*) showed ominously enhanced food ingestion, following both starving intervals of 2 and 9 days. Next to the two-days of starving course, noticeable hyperphagia (51%) had been perceived, even though that level of hyperphagia merely persisted three days. After the second starving course of 9 days, fish showed prominent hyperphagia (79%), the food ingestion on the very first re-feeding day attaining four times of pre-starving levels.

Prabhakar *et al.* (2006) organized an investigation to estimate the suitability of feed deprivation tailed by the realimentation for Rohu culture. Five distinct feeding approaches were attempted by varying the time extent of starvation and realimentation. The results indicate that feed-deprived fish of the T_1S and T_2S groups exhibit growth compensation with hyperphasia, improved feed conversion ratio, specific growth rate and obviously protein efficiency ratio.

Hyperphagia was detected in the starved fish after refeeding, but there were no major alterations in food conversion efficiency. Therefore, improved food ingestion during the refeeding cycles was the major cause for growth compensation in transgenic carp. The proximate composition of the limitedly fed fish at the termination of the investigation was analogous to that of the control fish, although the live masses diverged from control (Fu *et al.*, 2007). Similar patterns were also observed in other fish (Zhu *et al.*, 2004). It suggests that defence of body composition has priority over defence of the growth trajectory in fresh mass (Ali *et al.*, 2003). Hyperphagia was suggested to be the process accountable for compensatory growth response.

Fu *et al.* (2006) observed that in Common carp (*Cyprinus carpio*) live masses of the fish in the deprived groups were still significantly lesser than those of control. Throughout the refeeding cycle, size adapted mean Specific Growth Rates (SGRs) and mean feed ingestion were notably elevated in the starved fish compared to the control groups, denoting partial growth compensation feedback in these fish. Food Conversion Efficiency among the starved and control fish throughout refeeding phase did not differ significantly, signifying that hyperphagia was the procedure accountable for enhanced

growth rates. The proximate composition of the starved fish at the termination of the investigation was analogous to the fish of the control.

2.6. Disease resistance:

Another pragmatic implication of deprived feeding is its influence on the health of the fish. Traditional belief is that fish fed over the winter days are healthier and with improved ability to survive and disease outbreaks occur in the spring. Recent research suggests that this may not always be the case. In the previously mentioned study at Auburn University which examined winter feeding regimes, disease resistance also was examined. Following winter feeding, fish in each size class from the continuous, partial, and starved groups were removed and infected with *Edwardsiella ictaluri*, the causative agent of enteric septicaemia of catfish. Mortality due to enteric septicaemia was higher in the unfed year-1 fish; however, in the unfed year-2 fish, mortality caused by enteric septicaemia was least. No difference was noticed between the mortality rates of partially fed and continuously fed fish from both age groups. These results denote that while food deprivation accelerates the susceptibility of small Channel catfish to disease, it also boosts the immunity as well as disease resistance of large fish.

2.7. Compensatory Growth

Compensatory growth (CG) is a phase of growth acceleration that occurs when favourable conditions are restored following a period of growth depression. Compensatory Growth (CG) is a period of accelerated somatic growth that alleviates growth-stunting conditions, resulting in a faster growth trajectory than a cohort that was previously unaffected by adverse conditions. This phenomenon was inspiringly recorded almost a century ago (Osborne and Mendel, 1916), and the term "Compensatory growth (CG)" coined 40 years later (Bohman, 1955). CG has been observed in every vertebrate class, including humans (Prader *et al.*, 1963; Boersma and Wit, 1997; Sapolsky, 1998), other mammals (Bohman, 1955; Wilson and Osbourn, 1960; Mersmann *et al.*, 1987; Ryan, 1990), birds (Wilson and Osbourn, 1960), reptiles (Bjorndal *et al.*, 2003; Radder *et al.*, 2007), and amphibians also (Alford and Harris, 1988; Vonesh and Bolker, 2005), but most broadly in fish (Ali *et al.*, 2003). Regardless of the variety of plants and animals that can show CG, the fundamental process that control feedback are not properly recognized. CG decreases variability in size by incorporating growth trajectory and is significant for fisheries and aquaculture,

fisheries management and life history study as it can counteract growth inhibition effects (Ali and Wotton, 1998). Compensatory growth can be categorized as Over compensation (Hayward, Noltie, & Wang, 1997), Complete-compensatory (Jobling, Koskela, & Winberg, 1999) or Partial compensation (Paul, Paul, & Smith, 1995), and it is influenced by the species, the time-extent and acuteness of the mechanism (Tian & Qin, 2004).

2.7.1. Compensatory growth of fishes

Compensatory growth is defined as a phase of unusually rapid growth resulting a phase of under nutrition (Hayward *et al.*, 1997). Compensatory growth was widely reported in Salmonids (Jobling and Miglavs, 1993; Nikki *et al.*, 2004; Limbu and Jumanne, 2014), Cyprinids (Russell and wootton, 1992; Zhu *et al.*, 2004; Das, 2011), cat fishes (Gaylord and Gatlin, 2001; Jiwyam, 2010).

Depending on the extent of compensation, compensatory growth can be categorized into three types as follows.

i. **Over compensation**: In which numerous rotations of restriction and refeeding give rise to a weight increase that, surpasses that of fish fed regularly (Hayward *et al.*, 1997).

ii. **Full compensation**: In which fish formerly exposed to feed deprivation / restriction upon refeeding attain the similar body mass no less than the fish regularly fed (Johansen *et al.*, 2001; Tian and Qin, 2003).

iii. **Partial compensation**: In which feed restricted fish exhibit accelerated growth after resumption of normal feeding, but do not attain the similar body weight as the fish fed regularly (Jobling and Siikavuopio, 1993; Paul *et al.*, 1995; Tian and Qin, 2004).

Growth compensation is a suitable substitute to recover growth rate in fish across the proper selection of where food deprivation periods are tracked by phases of satiate feeding. This practice, if well accomplished, can balance for the dropped growth throughout food deprivation and may be a prospect to regain the spent growth once the food supply restarts.

Compensatory growth commonly leads to a phase of food deprivation (Dobson and Holmes, 1984, Hayward *et al.*, 1997). Fish submitted to former nutritional deprivation perhaps partially (Miglavs and Jobling, 1989, Jobling, 1993) or absolutely (Johansen *et al.*, 2001, Maclean and Metcalfe, 2001) revert to the weight and even can compete with those who haven't been exposed to feed deprivation (Dobson and Holmes, 1984, Kim and Lovell, 1995).

Baveeviae *et al.*, (2010) found in Gilthead sea bream (*Sparus aurata*) that all feed restrictions lead to growth compensation in weight. Fish starved in the initial half and consumed to utmost satiation in another half of the experimentation, attained body mass more rapidly compared to those fed satiate all along.

Absence of growth compensation feedback in Gilthead sea bream (*Sparus aurata*) adolescents resulting starving and following refeeding was studied by Peres *et al.*, (2011). They conducted an experiment for 10 weeks with three distinct feeding practices, which were:

1) Control (c), fishes fed to superficial satiation two times in a day, 6 days in a week, throughout the entire investigation period.

2) One-week or two-weeks unfed groups (groups U1 and U2).

3) Refed for the rest 8 weeks of the trial as control group.

Initial body weight was of 58g. They found out that deprivation designed for one to two weeks tempted substantial shortfalls of body mass which relatively greater than the control group. Perivisceral lipids, eviscerated body lipid as well as energy matters were gradually reduced in fish starving for 2 weeks. Food ingestion along with feed efficiency had been equal in each group. Identical consistency amid growth trajectory slopes among the control group and the other starved groups were observed, directing no conjunction of growth in starved groups regarding that or the control group. A total recovery of the whole body composition, various organ indices, liver and viscera composition was noticed at the termination of the trial. They concluded that the gilthead sea bream adolescents were unable to recompense growth following the food deprivation imposed, and underneath the investigational circumstances pertained brief one to two weeks of feed deprivation phases are not worthwhile as management tactic to be practiced in Gilthead sea beam production.

Wang (2009) recorded that cyclical restricted feeding and refeeding fails to enhance compensatory growth in Nile tilapia, *Oreochromis niloticus* having four feeding treatments and with three replicates such as: 1) control (c) fish, satiate fed two times daily throughout the experiment, 2) four cycles of one week deprivation and two weeks of refeeding (S1F2), 3) two cycles of two weeks of restriction and four weeks refeeding (S2F4) and one cycle of four weeks of restriction and eight weeks of refeeding (S4F8). Results of the experiment indicated that survival of fish was noticeably lessened in S4F8 treatment than that of other treatments. Specific growth rate as well as feed intake of fish in cyclical feed treatment was greater than that of control fish. Body weight was lower in the S1F2, S2F4, and S4F8 than control fish. There was no momentous difference in lipid, protein and moisture content among all treatments.

Pegu (2010) reported that the fish unfed for one day followed by two days of satiation feeding attained the identical weight as the control fish, in the fingerlings of *Oreochromis niloticus* denoting that absolute growth compensation arisen in this fish.

Jiwyam (2010) investigated the growth and growth compensation feedbacks of adolescent *Pangasius bocourti*, a 16-weeks experiment was performed in fifteen indoor tanks. Juvenile *P. bocourti*, an average of 2 g weight, were served a diet, containing 40% protein at five diverse ration points (correspondingly 4%, 6%, 8%, 10% and 12% of the initial body weight) per day constantly for 8 weeks (deprived-ration phase) and then regained a diet, containing 25% protein at regular ration level for next 8 weeks (normal-ration phase). At the termination of the deprived-ration phase, substantial differences were obtained in growth parameters, mean final body weight and specific growth rate amongst the five groups of fish getting various rations ($p<0.05$). Feed conversion efficiency fell considerably with cumulative ration points. At the termination of the regular-ration phase, there was no such momentous difference in ultimate weight amongst five groups, which denotes absolute compensation in the restrictedly fed fish. There was an improvement in feed conversion efficiency in the juvenile *P. bocourti*, experiencing restricted feeding. Based on the stated outcomes, it can be determined that a ration of 8% of body mass per day is most ideal for better growth and feed conversion efficiency of adolescent *P. bocourti*.

Singh *et al.* (2005) conducted 8-week experiment on *Cirrhinus mrigala* fry with restricted feeding for 1, 2, and 4 weeks while the control fry were regularly fed two times regularly to satiation. Initial body weight among treatment groups at the start of

the experiment did not significantly differ but at the termination of the re-alimentation period, the weight of 1 week and 2-week fry were notably greater comparatively to the control and 4-week fry. The Feed Conversion Ratios (FCRs) of the 1-week and 2-week fry had been noticeably improved than those of the control and 4-week groups. There were no significantly difference in the survival of the control, 1-week and 2-week groups but they all exhibited appreciably higher survivability than in the 4-week group.

Eroldogan *et al.* (2008) observed the influence of deprived feeding regimes on growth performance and food utilization of juvenile Gilthead seabream (*Sparus aurata).* Four distinct feeding protocols were tried, such as,

i. Control fed for uninterruptedly 48 days without deprivation (C),
ii. Starvation for one day and subsequently refed for two days (S1),
iii. 50% satiate feeding for two days and subsequently refed up to apparent satiation intended for two days (R2) and
iv. 50% satiate feeding for six days and subsequently refed up to apparent satiation intended for another six days (R6).

Results of the experiment indicated that all the fish subjected to rotational deprived feeding protocols were not able to attain reaching to the control group. The highest feed ingestion was noticed in fish in control regime and feed intake of fish in other treatments (S1, R2 and R6) did not differ significantly. The specific growth rate of fish, belong to the control, was considerably greater than individuals in other treatments, which did not differ significantly from each other. PER was significantly affected by feed restriction; it was found highest in S2 treatment compared to the other treatments. Fish; belong to R2, acquired least feed conversion ratio (FCR) than the control. The expenditure of feed necessitate to harvest 1 kg biomass (ECI) was least in R2 than other treatments. Body protein composition of fish in R6 was substantially lessened compared to control; S1 and R2 while moisture, and lipid and ash content did not significantly differ to control.

Effect of rotational feeding on growth compensation exhibited by hybrid Striped bass *(Morone chrysops* x *M. saxitilis*), which is a food fish, and water quality parameters in fish production ponds was studied by Turano *et al.* (2008). They conducted an experiment for eighteen weeks with three treatments viz: 1) control (C) satiate feeding throughout experiment, 2) cycles of three-week deprivation and three weeks feeding (3/3) and 3) cycles of three-week deprivation and six weeks feeding (3/6)

and found that, survival of fish among all the treatments did not differ significantly. Specific growth rate, showed by fish 3/3 in the treatment was substantially better compared to the control and (3/6) treatments. Growth compensation was noticed in both periodic feeding regimes; nevertheless, the feedback was inadequate for the fish to entirely reclaim the lost body weight.

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

Gaylord and Gatlin (2001) during his experiment on Channel cat fish (*Ictalurus punctatus*) found that in the previous 4 weeks of the experiment, the body weight of the fish fed for apparent gratification increased by 41%, while the weight of the starving fish decreased by 20%. Throughout the succeeding refeeding phase, formerly starved fish were unable to significantly accelerate the growth rate to compensate the loss in body mass executed by the 4-week food deprivation. Conversely, following 8 weeks of refeeding, overall upsurge in body weight of the formerly starved fish was 179% of primary weight and analogous to the fish of the control which attained 231% of primary weight. Hepatosomatic Index (HSI) and condition factor reduced promptly throughout the starving phase and augmented swiftly catching up the control levels throughout following refeeding. Muscle ratio exhibited slight influence since the 4-week period of restricted feeding. It appears that non-feeding of Channel catfish fingerlings for 4-week is prolonged to stimulate a growth compensation feedback.

Cui and Wang (2007) studied sequential alterations in body weight; body composition as well as metabolic activities appeared in Gibel carp *Carassius auratus gebelio* during food deprivation of 56 days. During the study they found that both fresh and dry mass of the fish decreased over the deprivation period with fresh mass depleted faster than dry mass. Concentration of lipid, protein and energy in whole body decreased, while percentage water and ash concentration increased during deprivation.

Heide *et al.* (2006) inspected the consequences of short span deprivation phases on growth feedback and crude composition of fish-fillet of adolescent Halibut. Fish were served satiate feed two times per day or fasted in four feeding regimes in this manner; feeding (F) uninterruptedly for 99 days (Control); 11-days of starving (S), subsequently 20 days F, 14 days S, 22 days F, 11 days S and 21 days F (Starvation A);

16 days S, 28 days F, 16 days S and 39 days F (Starvation B); 32 days S and 67 days F (Starvation C). Every tank comprised 70 fish with an initial mean weight (SD) of 195.7 (46.6) g. All deprived groups exhibited partial compensatory growth throughout refeeding, presenting an escalation in diurnal feeding rate along with a propensity headed for augmented feed conversion competence than the control group. The extent of the refeeding phases was, conversely, inadequate to permit the fasting Halibut get closer to the body weight of fish in the control even though rate of growth in the end sampling stage up to day 99 was appreciably better in the starving groups compared to the control. The outcomes directed that the trial was terminated in a stage till the fish were experiencing growth compensation. The consequences also specified that up to 32 days of starving and following feeding might not distress the crude composition of fish-fillet of adolescent Halibut.

According to Mirea *et al.* (2013), the consequence of rotational feeding on growth compensation on Nile tilapia, where feed ingestion and body weight gain related very significantly, however, feed conversion efficiency differ among fish submitted to the various treatments, finally showed better end result in the second treatment subjected with 2 days of starving period. Fish submitted to starving exhibited growth compensation, and improved growth rate throughout the retrieval period was attained by hyperphagia, noticeably compared to enhanced feed conversion efficiency.

Cui *et al.* (2006) observed compensatory growth of grouped-held Gibel carp, *Carassius auratus gebelio* (Bloch), following feed deprivation. In this experiment, five feeding protocols were determined: one distinct group of fish was served feed to utmost satiation during the trial as control, however the residual four groups of fish were restrictedly fed for one, two, three and four weeks throughout the initial four weeks, subsequently satiate feeding continued from week five to ten. Outcomes of the trial indicated that body mass of the feed deprived fish decreased as deprivation proceeded during weeks one to four. At the termination of the trial, body mass weight of the restrictedly fed fish was still lesser comparatively to the control. Feed intake in the fish deprived one and two weeks did not differ significantly from that of the control during five to ten weeks. SGR between the control and fish deprived one and two weeks during week's five to ten, did not differ significantly. At the termination of the trial, entire body protein content between control and deprived fish did not differ significantly. The fish deprived two, three and four weeks had a lower whole body lipid and higher ash

and moisture contents than the control. Lipid/LBM was lower in the fish deprived three and four weeks than the control. In the recent trial, partial compensatory growth arisen in grouped-held Gibel carp resulting three and four weeks of feed deprivation.

Parimal *et al.* (2006) recorded that restrictedly fed fish (*Labeo rohita*) had statistically ($P<0.05$) similar but numerically higher body weight comparatively to the control. During re-alimentation period, weight gain, Specific Growth Rate (SGR), feed intake, Absolute Growth Rate (AGR), Gross Growth Efficiency (GGE) or Feed Efficiency Ratio (FER) as well as protein and energy retention efficiency were considerably greater in restricted fish comparatively to 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 noticed in ability to digest dry matter along with body protein and energy as well as protein gain to lipid gain ratio between fish of restricted and control groups throughout 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, which was the mechanism for increased growth rate. Growth efficiency as well as protein and energy retention efficiency could be as a result of lower maintenance requirement during compensatory growth of Rohu and this also recommended that growth compensation obtained could be caused by the protein growth rather than increased gut fat deposit or increased water uptake, because body length proportionately 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 mass of control fish probably resulted from relative strong capacity for compensatory growth.

Hussein (2012) examined growth performance in fingerlings as well as adolescents of African Catfish (*Clarias gariepinus*) after various feeding frequencies. Fish were initially served feed one, two, three and four times per day throughout a time span of 10 weeks afore satiate feeding restarted for 12 weeks. Feed Conversion Ratio (FCR) and voluntary feed intake at the adolescent as well as fingerling phases did not differ significantly. Fish served feed 3 times per day documented the maximum average weight gain however the various feeding frequencies in the adolescent fish did not differ significantly.

Turkmen *et al.* (2011) investigated the growth compensation feedback exhibited in European sea bass (*Dicentrarchus labrax*). Sea bass has quick feedback to rotational starving/refeeding and 25% deprived feeding ratio is deficient to appeal growth compensation feedback in Sea bass.

Gao and Lee (2012) inspected growth compensation in Nile Tilapia *Oreochromis niloticus* in physical size and live body weight in retort to various restriction phases and refeeding cycles. Four treatments had been appointed indiscriminately to fish in all total 12 glass tanks, with every single treatment implemented in triplicate. To begin with, Control was served feed to utmost satiation trice a day all the through the investigation. The rest of three treatment sets were deprived of feed for 1 week (S1), 2 weeks (S2), or 4 weeks (S4) respectively and subsequently refed till the termination of the investigation. Considering the investigation, no momentous differences were witnessed amid S1, S2, and the Control in average weight or length, whereas the weight and length of S4 were significantly reduced. Relative condition factors of the three deprived sets reduced substantially till the termination of the starving time span but regained quickly as soon as refeeding commenced. The Specific Growth Rates in Weight (SGRW) of the three deprived sets of fish regained rapidly upon refeeding and were considerably advanced comparatively to the control set, however, these dissimilarities faded progressively till the termination of the trial. Specific Growth Rates in Length (SGRL) among the control and the other three deprived sets following refeeding, did not differ significantly. Each one of the three sets exhibited hyperphagia for a brief extent of time upon refeeding. The feeding efficacy among the four groups did not differ statistically.

In the case of *Catla catla*, an investigation was conducted to estimate the effect of offered cycling regimes on growth, survival, feed conversion ratio, economic conversion index, and body composition. *C. catla* fingerlings were randomly assigned to one of four feed cycling regimes: control (daily fed), 2D2F, 4D4F, 6D6F, and 8D8F (2, 4, 6, 8 days deprivation and 2, 4, 6, 8 days feeding, respectively). Body mass weight gain as well as Specific Growth Rate (SGR) in 4D4F, 6D6F and 8D8F were significantly lower than control and 2D2F feed cycling regime. The 8D8F feed cycling regime significantly improved the Feed Conversion Ratio (FCR) and Economic Conversion Index. Feed cycling regimes influenced body composition and the RNA/DNA ratio significantly. Protein, lipid, and RNA/DNA ratios were significantly

lower in 6D6F and 8D8F feed cycling regimes than in control and 2D2F feed cycling regimes. Finally, *Catla catla* fingerlings grown under 2D2F feed cycling regimes demonstrated the ability to compensate for growth without compromising growth parameters (Nagar and Patidar, 2015).

Llameg and Serrano (2014) evaluated diverse rotational feeding protocols in Milkfish (*Chanos chanos*) adolescents whether or not compensatory growth (CG) possibly would be stimulated that could progress growth performance, feed efficiency, body chemical composition or any blend of those three feedbacks. Fish were submitted arbitrarily to five distinct feeding protocols, to be exact, a control group (C), fed regularly, one-day starving trailed by 6 days of refeeding (1:6, days fasted: days fed), 2:5, 5:223, or 7:21 rotational feeding. Fish, belonged to 1:6 and 2:5 groups were assigned to 8 rotations though fish of 5:23 and 7:21 groups for 2 rotations all through around 56 days of investigational span. Milkfish adolescents fed uninterruptedly without starving (control group) showed statistically analogous mean body weight with the fish submitted the 1:6 and 2:5 feeding routines whereas those belonged to the 7:21 group significantly showed the least mean body weight and steadily maintained the least values till the ending of the investigation. Specific Growth Rate (SGR) of Milkfish possessed the control routine and the fish fed under 1:6 and 2:5 feeding routines showed significantly advanced compared to those starved for 5 or 7 days and subsequently refed (viz. 5:23 and 7:21, correspondingly). Food Conversion Ratio (FCR) as well as Protein Efficiency Ratio (PER) values were firmly and significantly the finest in Milkfish assigned the 2:5 feeding protocol whereas the fishes assigned in the control and the residue of the rotationally fed fish showed lessen values however were statistically alike to each other. Survivability, Condition Factor (CF), Hepatosomatic Index (HSI), and Viscero-Somatic Index (VSI) were not affected by the rotational feeding protocols. As a conclusion, rotational feeding was prosperous in prompting a CG feedback in Milkfish fingerlings under the circumstances of the recent report. Fish submitted in the 2:5 rotating feeding routine brought about statistically analogous SGR with the control and improved whole FCR in comparison with other treatments and control of fish. Based on the results of this study, complete growth compensation could be elicited in 1:6 and 2:5 cyclic feeding regimens.

By utilizing this event in Tilapia, the growth performance and feed efficacy can be augmented (Wang *et al.*, 2000, Wang *et al.*, 2009, Gao *et al.*, 2015). This too

appeared to be instance in the current investigation, after an extended interval of deprived feeding (12 weeks) that triggered substantial differences in the mean body weights, those differences were reduced at the termination of the re-feeding phase. There was no significant difference in the final weight of Tilapia among all treatments however fish with no restriction in feed exhibited an elevated weight compared to starving animals, only when initial weight was used as covariate, the differences disappeared. This outcome signifies partial growth compensation; meanwhile the starved animals did not attain the similar weight of the regularly fed animals.

Tian and Qin (2004) concluded that absolute growth compensation arises only in fish undergoing a modest restricted feeding regime. Complete growth compensation was stated after a 16-day restriction routine in Minnows (*Phoxinus phoxinus*) (Russell and Wootton, 1992), whereas, in Rainbow trout after a 3-week restriction routine (Dobson and Holmes, 1984, Quinton and Blake, 1990). Also Ali and Jauncey (2004) revealed that African catfish (*Clarias gariepinus*) exhibits partial growth compensation retorts at swapping interludes of feeding schedules.

An acute decline in FCR (1.10) later being (1.30) at the 1st phase was clarified by the better growth rate complemented by better feed conversion ratio during the re-alimentation phase, those are especially growth compensation indicators (Ali and Wootton, 2003). Similarly, PER has amplified approving the rise in Weight Gain, concerning that fish subjected in all the treatments being fed the same protein quantity.

Two probable clarifications of the growth compensation documented by numerous researchers are the hyperphagia or a blend of hyperphagia and advanced feed efficacy (Ye *et al.*, 2016). The recent research approved the outcome from a former research on growth compensation of Tilapia resulting starvation, that the hyperphagia was the chief process for growth compensation in this species (Wang *et al.*, 2000, Wang *et al.*, 2004, Tian and Qin, 2004).

Thus, Nile tilapia submitted to 1 or 2 starving days exhibited absolute growth compensation and higher growth rate throughout the recovery period when assigned for specific span equal or lengthier than the extent of restricted feeding phase, however to equal the final body weight of the fish which were fed regularly, the re-feeding phase should have been advanced.

Inspite of widespread experiments on growth compensation in fish, ambiguous outcomes have been documented. For instance, absolute growth compensation was documented in *Lates calcarifer* (Tian & Qin, 2004), over-compensatory growth in hybrid Sunfish (*Lepomis cyanellus* x *Lepomis macrochirus*) (Hayward *et al.*, 1997), and no compensatory growth was stated in *Cyprinus carpio* (Schwarz, Plank, Kirchgessner, 1985). Likewise, inconsistent varying outcomes were recorded in Tilapia species such as absolute compensatory growth in *Oreochromis niloticus* (Cuvin-Aralar *et al.*, 2012; Passinato *et al.*, 2015), a restricted ability in hybrid Tilapia (*O. mossambicus* x *O. niloticus*) (Wang, Cui, Yang, & Cai, 2000; Gabriel, Omoregie, Tjipute, Kukuri, & Shilombwelwa, 2017), and an absence of compensation in *Oreochromis niloticus* (Gao, Wang, Hur, & Lee, 2015). Sufficient information is necessary to clarify these inconsistent conclusions between and among the fish species cultured under various trial systems and feeding regimes. Since, growth compensation is of concern in aquaculture, and a compassionate understanding of its dynamics may permit the design of feeding schedules that enhance yield, cut feed cost, labour cost, and diminish water quality problems (Xie, Cui, Yang, & Liu, 1997; Wang *et al.*, 2000). In spite of these, investigation of short-term cycles feed restriction and refeeding impact on growth activity, feed utilization, and body muscle composition of *O. mossambicus* is one of the rare reports on growth compensation in *O. mossambicus* that had brief feed deprivation/refeeding rotations, which is displayed to be effectual at encouraging growth compensation in fish (Tian & Qin, 2004).

Successful aquaculture aims at production maximization following a cost-effective method. The hugest expenses are those correlated with supplementary feed and feeding. Producers and investigators are relentlessly looking for latest manners of dropping these expenses. One approach of trimming down feed expenses is to take benefit of growth compensation. Study has revealed that numerous animals (adding in some fish) temporarily starved will exhibit growth more speedily when feeding is restarted and "catch-up" with animals which were regularly fed. This phenomenon, acknowledged as Compensatory Growth, can be established by a rise in both growth rate and feed utilization efficiency throughout the refeeding phase. Advancement of feeding schedules that acquire benefit of compensatory growth will advance the efficacy of fish growth.

2.7.2. Compensatory Growth (CG) paradigm during fasting and refeeding

Compensatory growth has been demonstrated in both individually housed and grouped fish, typically after growth depression has been induced by complete or partial food deprivation. Partial, full and over compensation have all been evoked in fish; although over compensation has only been demonstrated when cycles of deprivation and satiation feeding has been imposed. Individually housed fish have demonstrated that CG is partly a response to hyperphagia, when food consumption rates are significantly higher than in fish that have not experienced growth depression. The severity of the growth depression lengthens the hyperphagic phase rather than increasing the maximum daily feeding rate. Many studies found that growth efficiencies were higher during CG. Changes in metabolic rate and swimming activity have yet to be shown to play a role.

Food deprivation causes changes in fish storage reserves, particularly lipids. CG is a response to restore lipid levels, in addition to the strong evidence for the restoration of somatic growth trajectories. Although several neuro peptides, including neuropeptide Y, are probably involved in the control of appetite, their role and the role of hormones, such as growth hormone (GH) and insulin like growth factor (IGF), in the hyperphagia associated with CG are still unclear.

The benefits of CG are most likely related to the size-dependent mortality, fecundity, and diet of teleosts. If environmental factors allow, these size dependencies favour recovery from the effects of growth depression. High growth rates may also impose costs, including adverse effects on future development, growth, reproduction and swimming performance. In the presence of predators, hyperphagia may lead to riskier behaviour. CG's evolutionary consequences are largely unexplored. An understanding of why animals grow at rates below their physiological capacity, an evaluation of the costs of rapid growth and the identification of the constraints on growth trajectories represent major challenges for life history theory.

A wide variety of teleosts are capable of undergoing CG responses in response to the removal of various growth-stunting conditions or their combination, such as suboptimal temperature, crowding, or other stressful environments, and feed restriction, the latter reflecting the condition most commonly studied (Ali *et al.*, 2003). A CG response has been reported in salmonids (Jobling *et al.*, 1993; Maclean and Metcalfe, 2001; Nikki *et al.*, 2004), cyprinids (Russell and Wootton, 1992; Wieser *et*

al., 1992), perciformes (Hayward *et al.*, 1997; Turano *et al.*, 2007, 2008; Ferrando *et al.*, 2009), flatfish (Cho, 2005; Heide *et al.*, 2006), sticklebacks (Zhu *et al.*, 2003), catfish (Gaylord and Gatlin, 2000), cichlids (Wang *et al.*, 2000), and gadids (Jobling *et al.*, 1994). While the degree of growth compensation achieved depends on species, it is nonetheless typically characterized by hyperphagia, improved feed conversion efficiency, and elevated specific growth rate (SGR). Although not often assessed, a critical feature of individuals undergoing CG is that their SGR is higher relative to similar-sized cohorts (i.e., SGR normalized to body mass) that were never subjected to stunting conditions (Skalski *et al.*, 2005; Picha *et al.*, 2006). Considering that CG results in enhanced growth rate and feed efficiency, it is not surprising that commercial production appears to be the driving impetus behind investigations into CG in fish, as the majority of studies to date involve cultivated species. Compared to conventional methods of fish farming that deploy a constant regimen, incorporation of rearing protocols that induce CG shows promise of reducing the amount of feed needed to grow at least some species of fish commercially.

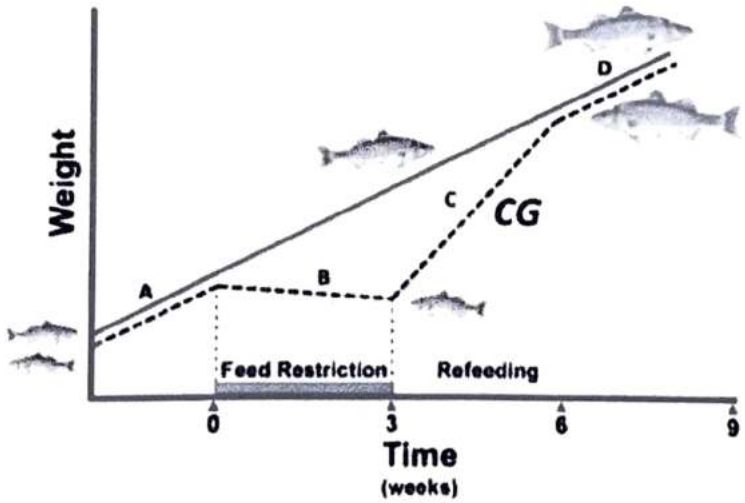


Fig. 2.1. Compensatory Growth (CG) paradigm during fasting and refeeding (dashed line) compared to constant growth rate in fed controls (solid line). (Source: Endocrine regulation of compensatory growth in fish, Won, Eugene T., and Russell J. Borski, 2013)

Fig 2.1. indicates that Normal Growth (**A**) is disrupted by feed restriction (hatched bar), which results in a decline in the growth trajectory (**B**) and a size disparity compared to control animals fed a constant regimen. When feeding resumes, hyperphagia and enhanced growth axis activity drive a hyperanabolic phase (**C**) marked by a steeper growth curve than that of constantly fed animals. The CG response potentially allows stunted animals to fully compensate for lost growth opportunity and re-converge in size with constantly fed controls before the growth rate returns to normal (**D**).

Because most fish exhibit indeterminate growth and many are susceptible to seasonal changes in growth rate associated with natural variations in temperature and prey availability, they tend to exhibit a robust capacity for CG (Mommsen, 2001). Hence, they can apparently serve as valuable subjects for evaluating the metabolic and endocrine mechanisms that may contribute to anabolic processes generally, and hyper-anabolism specifically. Acknowledging the diversity of fish in which CG has been documented and the complexity of the response itself, few attempts have been made to consolidate what is known about the endocrine mechanisms that underlie the response; however, the cumulative research on isolated components of CG provides insightful information from which to extrapolate a fundamental framework. The CG response, in particular, can be divided into catabolic (e.g., during fasting, stress, or low temperature) and anabolic (during realimentation or a return to more favourable conditions) phases, each of which elicits distinct and sequential endocrine responses. The goal of this review is to assign potential roles to select appetite and growth-regulatory hormones during the catabolic and (hyper)anabolic phases of the CG response in teleosts, with a focus on growth hormone (GH), insulin-like growth factors (IGFs), cortisol, somatostatin, neuropeptide Y (NPY), ghrelin, and leptin.

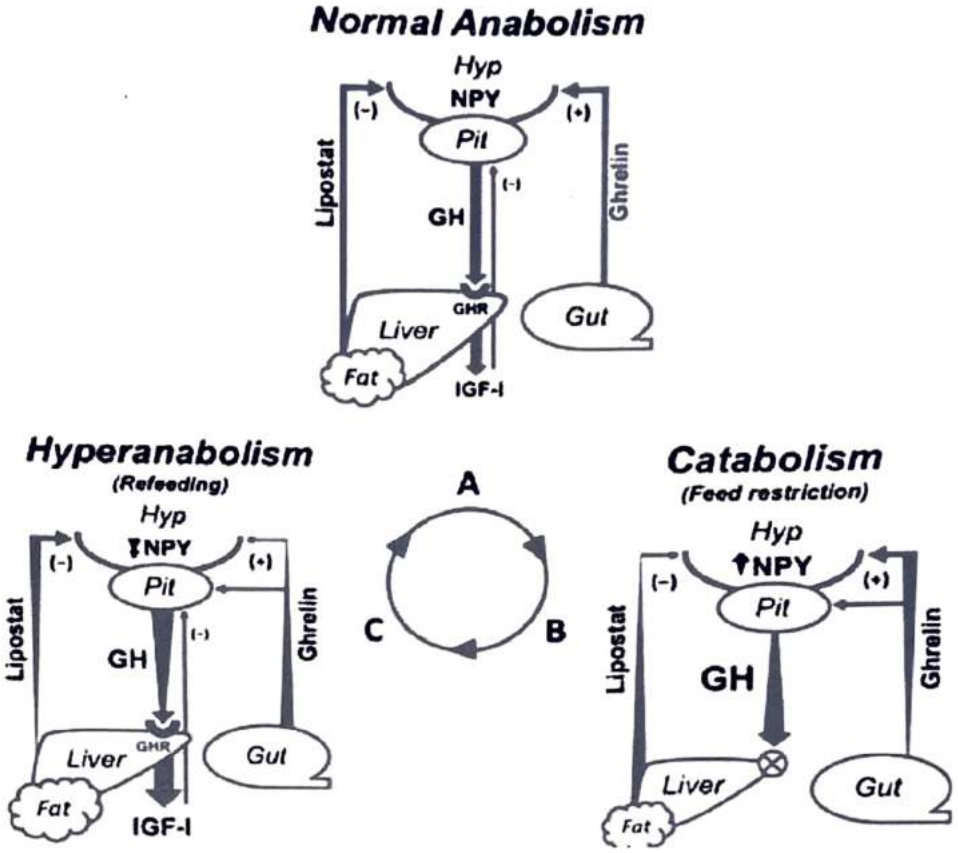


Fig. 2.2. Endocrine regulation of growth and appetite during normal anabolism, catabolism, and hyperanabolism (CG) resulting from feeding status. (Source: Endocrine regulation of compensatory growth in fish, Won, Eugene T., and Russell J. Borski, 2013)

Growth is regulated by the GH/IGF axis; GH secreted into circulation by the pituitary binds its receptor (GHR) to stimulate hepatic IGF-I production, which systemically drives somatic growth and exerts negative feedback on GH secretion. Lipolysis is an alternate function of GH during catabolism. Peripheral signals from a lipostatic mechanism (anorexigenic), possibly leptin, and ghrelin (orexigenic) regulate energy intake by modulating NPY and other neuropeptides in the central feeding centre. Ghrelin also functions as a GH secretagogue. Arrows show the direction of regulatory pathways; widening/narrowing of arrows represents a dynamic increase/decrease in a component over the duration of a particular metabolic state. (A) During regular feeding, energy homeostasis is maintained by matching energy intake and expenditure. Peripheral signals counter-regulate appetite centrally. Growth is regulated by nominal levels of circulating GH, which stimulates IGF-I production via hepatic GHRs. (B)

Fasting necessitates catabolic processes to provide energy for basal metabolism. Rising ghrelin production stimulates both appetite and circulating GH levels. Elevated lipolytic GH levels exploit stored energy reserves, decreasing lipostatic signalling. Reduced hepatic GHR expression desensitizes the liver to GH-induced IGF-I production. (C) Refeeding signifies the switch from catabolic to anabolic processes. Temporally elevated orexigens carried over from fasting drive hyperphagia. The return to positive energy status is characterized by the resumption of hepatic GH sensitivity and a steep rise in circulating IGF-I levels, which promotes accelerated growth. Eventually, the repletion of energy reserves and negative feedback from IGF-I returns GH and appetite to nominal levels, marking the return to normal growth rates. (PIT, pituitary; HYP, hypothalamus; NPY, neuropeptide Y; GH, growth hormone; GHR, growth hormone receptor; IGF-I, insulin-like growth factor I).

2.7.3. Sequence of phenomena throughout catabolic and anabolic states heading to Compensatory Growth

Compensatory growth is an episode of fast-tracked growth succeeding the mitigation from growth-arresting states, in basic term 'Fasting', which potentially lets an organism to subsidize for lost growth prospect. The reaction can be split into a catabolic state, when growth is hindered as well as reserved energy is run off, and a hyper-anabolic state, when growth regains an advanced rate. The chronological description of physiological alterations and endocrine activities in fish all through these metabolic states, and how they may eventually attain Compensatory Growth owing to improved feeding, substrate assimilation, and rapid growth, is discussed next.

Throughout the precedent catabolic state, Orexigens encourage appetite as endogenous reserved energy is exhausted. Ghrelin and Cortisol promote GH production and boost circulating GH levels so as to free energy-storing lipids.Yet, hepatic GH resistance and the growth-inhibitory properties of Cortisol and Somatostatins dominate IGF production under the cause that circumstances are adverse for growth. Decreased negative response from IGF-I further helps to increase GH levels. Hormonal changes therefore require an important phase of catabolism which predominates in fish for hyperphasia and makes the growth axis over-potential.

During refeeding, CG is fueled by the hyperphagic influx of exogenous energy and substrates. Changes in metabolic substrate absorption result in food being

assimilated with higher efficiency, partly due to residual GH levels. Hepatic GHR is restored and GH sensitivity returns, tailed by a steep increase or even additional compensation in IGF-I production. The production of IGF during refeeding is affected by a decrease in growth-inhibitors. Substrate and energy accessibility, improved assimilation efficacy, and enhancement of the growth axis culminate in a hyperanabolic, speedy growth phase till a lipostat-like process begins the restore of basal appetite and a growth axis profile, which represents a regular growth trajectory.

Hence, Compensatory growth is controlled by chronological endocrine responses throughout apparent metabolic states. Teleosts have been the focus of plentiful CG studies; nevertheless, our perception of their endocrine processes during these events is usually limited to observations of a few model species or extrapolation from studies of higher vertebrates. Although not included in any way, this theoretical combination of recorded hormone activity during catabolic and anabolic conditions is intended to provide a basic framework for the regulation of endocrine secretion of CG in fish and possibly higher vertebrates. The significance of specific hormones, time spans, and even the aptitude of a CG response probably depends on the species, size, and stage of life. Nevertheless, the variables concerned in this review are assumed to be of fundamental importance for the CG of fish, even though the degree of their relevance may vary depending on the specific species.

2.8. Key findings from the literature on restricted feeding and compensatory growth

According to various studies, the discriminate restriction to feed for one or two days provides an opportunity to protect fish farmers from unfavourable situations such as feed shortages or high feed costs. This strategy provides opportunities for fish farmers to reduce production costs. Restricted feeding schedules may be effective tools for increasing the efficiency of fish production. By completely not feeding or by depriving feed throughout the winter, producers be able to save money by trimming down feed as well as labour costs, and possibly decreasing disease fatalities too. Broaden research is required to establish the optimum interval of time for feed deprivation for diverse sizes of fish in order to maximise the effects of compensatory growth and optimise disease resistance. Satiation feeding can result into acute water quality deterioration.

A review of the available literature indicates that the impact of feeding frequency on fish growth varies considerably. With the intention to stipulate for the highest growth rate in salmonids, diurnally one to three rations are adequate to consume them to optimum satiation (Jobling, 1983a; Ruohonen *et al.*, 1998), while other species for instance the African catfish, *Clarias lazera*, achieve the utmost growth rate when fed continuously (Hogendoorn, 1981). Even in closely related species from the same salmonid family such as Arctic charr, *Salvelinus alpinus;* and rainbow trout, *Oncorhynchus mykiss;* the impression of feeding frequency can differ diametrically. An improvement in the Arctic charr growth rate was observed when the fish were served feed at a higher frequency (8 rations versus 32; Linnér and Brännäs, 2001). However, a decrease in feeding frequency improved the growth rate of rainbow trout. A study by Alanärä (1992) also confirmed that frequent feeding with an automatic feeder had a negative effect on rainbow trout. This species exhibits a prompt increase in activity during feeding; this may suggest that frequent feeding is a stress factor that elicits great expenditures of energy thus reducing the fish growth (Alanärä, 1992). Arctic charr, however, do not react as spontaneously or aggressively and feeding is definitely calmer (Brännäs and Alanärä, 1992). Observations of the pikeperch feeding behaviour indicate that this species is calm and is not aggressive even when a restricted feeding regime is applied. The behaviour of this species possibly implies that the feeding frequency does not affect the zootechnical parameters analysed in this study. It should be kept in mind that feeding schedules can directly and indirectly determine the growth rate of fish (e.g. by lowering water quality). Single ration feeding produces a substantial, short-term decrease in water quality that can be a significant stress factor (Giberson and Litvak, 2003). The maximum ammonia excretion and oxygen consumption and fluctuations in these parameters in the daily feeding cycle when one ration is delivered quickly can be significantly higher than when feed is delivered more frequently. This was confirmed in European perch, *Perca fluviatilis*; (Zakęś and Demska-Zakęś, 2002) and in pikeperch (Zakęś, 1999).

Fish that are served feed seldom can acclimatize to such circumstances by ingesting greater quantities of feed during every single course of feeding. If such a regime is applied for a prolonged period of time, this can lead to improved gut capacity and hyperhagia (Jobling, 1982; Ruohonen and Grove, 1996). The fish that are fed more often consume a generous amount of feed; conversely, when the interval between meals is brief, the food passes through the digestive tract faster, resulting in less efficient

digestion (Liu and Liao, 1999). This is why determining the optimum feeding frequency (number of rations and the interval between them) is of such practical importance. The efficacy of feed utilization (feed conversion ratio) by juvenile pikeperch did not depend on the feeding frequency applied in the present study. This indicates that the efficiency of feed utilization was not affected by the feed ration or particularly by the duration of feeding of fish that were fed once. Nor was the time interval between subsequent feedings (three ration regime) found to influence it. Studies conducted on salmonid fishes indicated that an hour of feeding was adequate for the fish to eat to satiation (e.g. Elliott, 1975). In the current report, the fish were served feed for three hours (one feed ration) or three times for one hour (three feed rations). Thus, it can be assumed that the applied feeding schedule met the nutritional necessities of juvenile pikeperch. Studies of walleye (Phillips *et al.*, 1998), Atlantic sturgeon, *Acipenser oxyrinchus* (Giberson and Litvak, 2003), and sunfish hybrids (female green sunfish, *Lepomis cyanellus* × male bluegill, *L. macrochirus*) (Wang *et al.*, 1998) also showed that the feeding frequency did not affect the feed conversion ratio.

The feeding frequency did not ensure a major effect on body weight variations within the juvenile pikeperch groups. Nor was it found to increase the phenomena of hierarchy and domination within the fish stocks. It is important that this refers to both experiments in which the daily feed rations differed significantly (in excess and restricted feeding). It is documented that improved feeding rivalry, as in other cases, is manifested by the establishment of a hierarchy in the stock, specifically when the feed is restricted (e.g. McCarthy *et al.*, 1992; Jobling, 1995). However, the feeding regime pertained in the investigations did not have an impact on the dissimilarity in juvenile pikeperch size (the value of the coefficient CvBW). This might indicate that the pikeperch do not exhibit strong stock domination or hierarchy. This hypothesis was confirmed by earlier studies in which, among other factors, the impact of the daily ration feed size on the results of rearing was tested. But in this study significant differences between the groups were mentioned in the growth rate, condition, and chemical composition of the fish, no differences were determined in the final CvBW coefficient (Zakęś *et al.*, 2003). The feeding frequency was not found to have a substantial impact on the values of the body weight variability coefficient in walleye (3 to 30 feed rations daily; BW = 28.2 g; Phillips *et al.*, 1998) or in Atlantic salmon fed at frequencies of 3, 9 or 27 rations daily (average BW depended on the experiment – 225–1 218 g; Thomassen and Fjaera, 1996). It should be emphasized that in the recent study,

the coefficient of body weight variability was highly differentiated in the replication of each experimental group. The influence of feeding frequency – CvBW was characteristic of each rearing tank. Therefore, the average values of the coefficient CvBW did not fluctuate significantly statistically. In this case, the so-called "tank effect" might come into play here (Dwyer *et al.*, 2002).

The degree of dominance that arises within a stock can be concluded by its concentration. If this factor is correctly modeled, the incidence of food competition and dominance in fish stocks can be reduced in a fish stock (Jobling and Baardvik, 1994). In a report of the effect of feeding frequency on Arctic charr, Jobling (1983) stated that decreased feeding frequency of fish, retained at reduced stocking densities, can let fall their growth rate and augmented intra-group body weight variability. Applying high stocking density can lowered the degree of antagonistic behaviour, which led to more efficient feeding. As a result, growth rate had improved as well as stock heterogeneity had reduced (Jobling and Baardvik, 1994). The stocking density used in the present study should be considered as low (from 2.5 to 17.5 kg/m^3). In the light of the studies cited above, the stocking densities used in the current study could cause increased antagonism among the fish. It should be emphasized that the point feed delivery method with the automatic feeder used in the current study created advantageous conditions for dominant individuals to monopolize the feed (Jobling, 1994).

Another study on Compensatory growth in *O. mossambicus* for the course of feed deprivation / refeeding phases and Partial compensatory growth (though subjected to deprived feeding and refeeding regimes, when fish cannot attain the same body mass comparing to those fed regularly) (Paul *et al.*, 1995), was recounted in fish that were submitted to 2-days deprivation / 4-days refeeding regime. In compliance with this study, Christensen and McLean (1998) reported that compensatory growth was as well demonstrated in the same fish (*O. mossambicus*). Furthermore, Abdel-Hakim, Abo State, Al-Azab, and El-Kholy (2009) registered an overall compensatory growth in hybrid tilapia (*O. niloticus* x *O. aureus*) deprived for feed once and twice a week, correspondingly. They further reported that moderate restricted feeding schedule (1 and 2 days per week) exhibited a noteworthy drop in supplementary feeding costs. Additionally, complete compensatory growth was witnessed in Nile tilapia (Passinato *et al.*, 2015; Cuvin-Aralar *et al.*, 2012; Gao & Lee, 2012) and *Lates calcarifer* (Tian & Qin, 2004) imperilled to various restricted feeding / refeeding regimes, respectively.

Up to date, mechanisms for compensatory growth are poorly understood in fish, despite numerous studies. However, various reports implied that compensatory growth in fish may be an outcome of depleted basal metabolism (Fu, Xie, & Cao, 2005), augmented feed ingestion (hyperphagia) (Xie *et al.*, 2001), or improved feed utilization indices such as FCR and FER (Foss *et al.*, 2009; Adakli & Tasbozan, 2015) resulting interlude of starvation or intermitted feeding. Improved feed utilization parameters have been observed in many fish including hybrid tilapia (*O. niloticus* x *O. aureus*) (Abdel-Hakim *et al.*, 2009), Nile tilapia (Passinato *et al.*, 2015), and even in shellfish such as *Fenneropenaeus chinesis* (Zhang, Zhang, Li, & Gao, 2010) exposed to deprived feeding and refeeding regimes. Improvements to these parameters have been attributed to increased fish digestion during refeeding (Bolasina, Perez and Yamashita, 2006). For example, *Labeo rohita* (Yengkokpam *et al.*, 2013), and Atlantic salmon (Krogdahl & Bakke-Mckellep, 2005), subjected to deprivation of feeding and refeeding regimes, have showed increased digestive activity. Meanwhile, Zhang *et al.* (2010) reported *Fenneropenaeus chinesis* juveniles exhibited higher protease activities during refeeding phase and observed enhanced FER and feed intake parameters and improved growth performance contrasted to the control group. Therefore, the current investigation reported lower FCR and higher FER in fish subjected to a 2-days feed deprivation /4-days refeeding cycle competed to those fed daily. This may be an outcome of enhanced digestive enzymes activities throughout the refeeding phase as exhibited in earlier reports (Yengkokpam *et al.*, 2013; Krogdahl & BakkeMckellep, 2005). This is also a hint that the short-term feed deprivation / refeeding cycle may be an effective tool to reduce the amount of feed without negotiating the fish farm production output.

Similar to growth performance and feed utilization parameters, mixed results were obtained for body composition in fish subjected to feed restriction / refeeding regimes. Studies on channel catfish (*Ictalurus punctatus*) (Gaylord & Garlin, 2000), gilbel carp (Xie *et al.*, 2001), hybrid striped bass, *Morone chrysops* x *Morone saxatilis* (Turano *et al.*, 2007) failed to report significant effect of feeding management strategies on body composition. Though, Adakli and Tasbozan (2015) reported a noteworthy decline in the total fat *Decentrarchus labrax* starved for 10 days and re-fed 40 days, compared to the control (fed on daily basis). In comparison, various studies have reported lower lipid content in the body of fish, victim of starvation / refeeding regime (Tian & Qin, 2004; Oh, Noh, & Cho, 2007; Peres *et al.*, 2011; Zhu *et al.*, 2001). These fish were unable to restore lipid and protein content utilized during starvation period to

support basal metabolism and survival as explained by (Adakli & Tasbozan (2015). This is an indication that severe or long-term feed deprivation or refeeding cycles can result in less fattening and higher energy consumption in fish.

In conclusion, short-term restricted feeding and refeeding cycles had influence on growth performance, feed utilization, muscle composition parameters of *O. mossambicus*, and 2-days deprivation /4-days refeeding cycle appears to be better among deprivation treatment groups. However, further studies on economic aspects, water quality parameters, and physiological responses of fish following restricted feeding and refeeding protocols are deemed necessary.

To evaluate the compensatory growth, a 10 month pond experimentation was supervised in which three species of Indian major carps, viz. *Catla catla* (presently *Labeo catla*), *Labeo rohita* and *Cirrhinus mrigala* (1 : 1 : 1), were stocked at 7500 ha^{-1} and the fish were subjected to diverse feed deprivation and refeeding regimes, viz. Control (C): Regularly fed for 10 months; Treatment 1 (T1): Initial supplementary feeding for 2 months+ No supplementary feeding for 1 month + Refeeding for 7 months; Treatment 2 (T2): Initial supplementary feeding for 2 months + No supplementary feeding for 2 months + Refeeding for 6 months; and Treatment 3 (T3): Initial supplementary feeding for 2 months + No supplementary feeding for 3 months + Refeeding for 5 months in triplicate ponds. At the end of the trial, 100% increase of growth compensation was attained in T2 for all the three carp species. Amongst all the treatments, high weight gain, PER and PPV and low AFCR were also noticed in T2, resulting in the highest fish production. Restricted feeding of fish had a significant effect ($P < 0.05$) on the overall body chemical composition. From this study, it was concluded that in 10 months of carp cultivation in fertilized pond, T2 would be the superior and most economical feeding strategy (K. N. Mohanta, Kausalyaganga, CIFA, Bhubaneswar, 2016).

The influence of deprived feeding on growth, feed efficacy and body composition was examined in adolescents of Gilthead sea bream, *Sparus aurata*. Juvenile fishes, weighted 6.4 g, were stocked right away into 12 tanks at a density of 16 fish in each tank. Four distinct feeding protocols were tried on triplicate sets of adolescent fish:

(1) control regularly served feed for 48 days,

(2) feed deprivation for 1 day and subsequently refed for 2 days (S1),

(3) 50% satiate feeding for 2 days and subsequently refed to utmost satiation for 2 days (R2), and

(4) 50% satiate feeding for 6 days and subsequently refed to utmost satiation for next 6 days (R6).

Outcomes implied that all fish submitted to rotational deprived feeding protocols were incapable to catch up with control group. The Specific Growth Rate (SGR) of fish in the control group was notably greater in comparison of S1, R2, and R6, and there was no significant difference between them. Protein efficiency and protein productive value were appreciably greater in R2 as competed to control, S1, and R6 groups. Fish in R2 had least Feed Conversion Ratio (1.12) as competed to the control group (1.17). Body protein composition in R6 was lesser compared to the control, S1, and R2, while there were no significant difference in moisture, lipid, and ash content contrasted to the control (Article in Journal of the World Aquaculture Society 39(2):267 - 274 · March 2008).

The observation that fish may adjust its growth rate to recompense the depleted weight gain throughout phases of feed deprivation has inspired investigation in this zone, owing to its prospective application as management strategy in fish farm. Indeed, CG can be treated as an implement to augment growth (Eroldogan *et al.*, 2008; Huang *et al.*, 2008; Johansen *et al.*, 2001; Maclean and Metcalfe, 2001; Qian *et al.*, 2000; Zhu *et al.*, 2005) or utmost utilization of feed (Eroldogan *et al.*, 2008; Huang *et al.*, 2008; Jobling *et al.*, 1994; Wang *et al.*, 2000); to lessen fish size dissimilarities (Ali *et al.*, 2003) or to enrich water quality and ease work labour and cut feeding costs (Blanquet and Oliva-Teles, 2010; Reigh *et al.*, 2006). Moreover, growth compensation may be applied to influence body composition, for example by inhibiting extreme lipid accumulation throughout finishing phase, consequently creating it a functional tool to improve meat quality to a great extent for human consumption (Hayward *et al.*, 1997; Jobling *et al.*, 1994; Eroldogan *et al.*, 2006; Grigorakis and Alexis, 2005; Heide *et al.*, 2006; Turchini *et al.*, 2007).

The influence of the number of feeding days per week on growth of Nile tilapia, assessing the impact of feed deprivation at weekends (Saturday and Sunday), on fish growth and feeding efficacy was revised. Outcomes revealed that feeding throughout

the week have influenced impressively on the decisive body weight, weight gain and SGR. These outcomes do not approve the observations documented by the previously cited investigators, De Silva and Anderson (1995), in which experimentation, an excess feeding has no stimulus on growth, however is in compliance with those of Riche *et al.* (2004) who witnessed a positive correlation between feeding frequency and growth as stated earlier.

Similarly, Okumus and Bascinar (2001) observed the influences of the number of feeding days per week and starving at weekends on the growth of Rainbow trout (*Oncorhynchus mykiss*) and gave a demonstration that feeding for 7 days per week is needed for attaining the greatest growth. In the mentioned report, either CF, HSI or VSI has not been influenced by the feeding frequency, this denotes that the liver and the viscera are in absolutely normal state and no abnormal accumulation has been displayed, this inspection is in agreement with others stated in numerous fish species (Miglavs and Jobling, 1989, Rueda *et al.*, 1998). Furthermore, C. Fatness reported a propensity to be declined with rising time extend of feed restriction. Numerous authors recommended that in short-range starvation visceral fats and muscle fats are exploited as sources of energy (Weatherley and Gill, 1987) and this possibly will be sanctioned by the considerably lower values of EUE acquired at T2 and T3 levels. Feeding protocols modifies feed utilisation and therefore affects body composition of fish (Lovell, 1992, Adebayo *et al.*, 2000).

Even though reducing the number of feeding days from 7 to 5 days per week was gone together with by a linear decline in protein and fat contents and linear rising in the ash content, and there was no significant differences documented. Moisture content has not monitored a specific rule but there was an immense drive to be enhanced with rising interval of starving, denoting that there was a substitute of muscle lipids by water. Also, Wang *et al.* (2000) established that Tilapia body moisture and ash content had a tendency to be greater, whereas lipid and protein contents had a tendency to be lesser as the time extent of feed restriction enhances.

2.9. Amur common carp (*Cyprinus carpio haematopterus*)

Common carp (*Cyprinus carpio*) is a species of the largest and most diverse fish family Cyprinidae (Nelson, 1995). Its natural habitat ranges from Western Europe to China, Korea, Japan, and Southeast Asia; from Siberia at 60°N to the Mediterranean Sea and

India (Gross, Kohlmann, & Kersten, 2002). The common carp was also the first fish species to be domesticated in China, around the 5th century BC, around the same time it was being cultivated in Europe during the height of the Roman Empire (Balon, 2006). To date, there is no agreement on the origin of common carp; some researchers believe it originated in the Caspian and Aral Sea regions, from which it spread east and west (Balon, 1995). Others believe that the common carp originated in Eastern Asia, where it was domesticated before spreading to Europe during the Greco-Roman period (Zardoya & Doadrio, 1999).

The common carp is an important fish species, which is mostly grown together with IMCs in polyculture systems. Although the best growth is obtained at water temperature of 23-30°C. The Amur wild carp is an ancient form that evolved from Asian carp (*Cyprinus carpio haematopterus*, Amur-China type of wild carp) and later spread to Western Asian water bodies. Over hundreds of years, this carp adapted to the local environmental conditions in the Amur River and became established there. In 1982, it was transferred from the Russian National Fisheries Research Institute to the Fish Culture Research Institute in Hungary, where it was used to develop an improved breed of Amur common carp (Hugarian strain) (Bakos and Gorda, 2001).

There are several drawbacks to the existing stock of common carp in India, including the fact that the fish matures in six months and breeds naturally in the pond (Jhingran, 1982). This phenomenon causes competition for food and space, which slows growth. Furthermore, the gonads account for 20-30% of body weight in common carp (Rao and Rao, 1983). To address these flaws, efforts have been made to breed all-male or sterile common carp populations (Rao and Rao, 1983; Das *et al.*, 1990). Because the existing stock of common carp's growth performance has been a major concern in existing culture systems, the genetically improved breed of common carp, Amur (Hungarian strain), was first introduced in India in the year 2000.

A comprehensive research was commenced to assess the present genetic status of existing common carp stocks and to develop appropriate strategies for stock improvement of this species under Department of International Development (DFID) - Aquaculture and Fish Genetics Research programme. It was a collaborative programme among University of Stirling, University of whales, UK and erstwhile University of Agricultural Sciences, Bangalore and Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka (Basavaraju *et al.*, 2003).

In this programme, common carp stocks from various topographical origin viz. Hungary (Amur and P3), Vietnam (SV), Indonesia (RJ) and India (FRS and LBRP) were evaluated in different culture systems and environments to initiate a breeding programme for identifying an improved stock with faster growth rate and delayed maturity. The study over an extent of 6 years revealed that the Amur strain performed consistently superior to all other stocks and crosses including local stocks in all the trials across all the culture systems and environments. The increase in weight of Amur over local stocks ranged from 13.2% to 50.1% along with a mean increase of 27.3%). After rigorous evaluation and protocols, Amur common carp were released by KVAFSU for commercial production and supply of its seed to hatcheries for multiplication purpose and to farmers for grow out purpose (Basavaraju and Reddy, 2013).

Chapter - 3

MATERIALS AND METHODS

The present study on **"Effect of restricted feeding on growth performance and physio-metabolic responses of amur common carp (*Cyprinus carpio haematopterus*) fingerlings"** was conducted in the Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Panchasayar, Kolkata-94 (22°28'46"N 88°24'4"E) by utilising the indoor and outdoor experimental facilities.

The experiment was executed in both in-vitro (controlled condition) and in-vivo (actual pond condition) trial with a duration of 120 days (22th May, 2019 to 18th September, 2019).

3.1. Preparation of experimental setup

3.1.1. In-vitro experimental setup

The in-vitro experiment was carried out in 15 numbers of cemented cylindrical cisterns of 200 litre capacity each. The cisterns were cleaned thoroughly and dried for a week. After that each cistern was provided with agricultural soil base (15 cm) and filled with 180 litre of good quality ground water, which was maintained throughout the experimental period. Evaporation loss was fully compensated once in a week. Water exchange @ 25% through siphoning was performed fortnightly from each cistern after collection of sample water.

3.1.2. In-vivo experimental setup

For the in-vivo experiment 5 numbers of symmetrical earthen ponds of 150 m^2 surface area and 1.5 m depth were selected from the hatchery complex of Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences and each pond was divided in 3 equal parts by setting two nylon nets (0.5 mm mesh size) inside to get the results in triplicate. The ponds were fully drained and dried for 2 weeks. After that the soil was ploughed manually and the ponds were filled with water maintaining 1 m water depth. All the ponds were surrounded by nylon mosquito net and covered with monofilament bird fence.

3.2. Stocking and rearing of fish

1000 numbers of amur common carp *(Cyprinus carpio haematopterus)* fingerlings of 2.28±0.27 g were procured from FFRTC, Kulia, Nadia. The fishes were treated with 2% Potassium permanganate ($KMnO_4$) solution for 3 to 4 minutes as prophylactic measures and stocked in two rectangular cemented tanks (5000 litres capacity each) for acclimatization (7 days). The fishes were fed with commercial pelleted fish feed (1.5 mm) containing 28% crude protein @ 5% of the body weight daily. Feeding was stopped before 24 hrs of stocking in the experimental cisterns and ponds. After measuring the average weight(g) and length(cm), 12 nos. and 50 nos. of Amur common carp fingerlings were stocked in each replicate of treatment of in-vitro and in-vivo trial respectively.

3.3. Experimental design

The present study was carried out in 5 different batches of treatment following Randomized Block Design (RBD), where the first batch TC was practised with daily feeding and considered as control. The second batch T1/1 was provided with a cycle of 1 day feeding and 1-day deprivation. In the third batch T2/1, a cycle of 2 days feeding and 1-day deprivation was practised. Cycle of 2 days feeding, 2 days deprivation and 1 day feeding, 2 days deprivation were carried out in the fourth (T2/2) and fifth (T1/2) batches respectively.

3.4. Feeding

Feeding was done @ 5 % of the body weight. The daily ration was divided into two parts and was fed at 08:30 AM and 4:30 PM respectively as per feeding schedule.

3.5. Collection of samples:

Water sample was collected at 15 days intervals from each of the treatments at a fixed hour of the day (09:00 AM) following all the necessary precaution.

The soil sample from each of the treatments was collected at 15 days intervals from two different places of the soil bed using a mini hand grab sampler. Then they were mixed, air dried, pulverized with the mortar and pestle and sieved through a 150 µm mesh size sieve and stored in labelled polythene packets for analyses.

Length and weight of the fishes were measured at 30 days intervals. At least 5 nos. of fishes and 10 nos. of fishes from each replicate were selected for sampling in case of in-vitro and in-vivo treatment respectively.

At the end of the trial only from the in-vivo experiment the blood and tissue samples were collected, preserved and analysed to study the physio-metabolic responses of the test fish Amur common carp.

3.6. Proximate Analysis

Percentage composition of the test fishes and the diet was analysed following standard methods (AOAC, 2005) at Department of Fish Processing Technology, Faculty of Fishery Sciences, Chakgaria campus, W.B.U.A.F.S.

3.6.1. Moisture

The moisture content was determined by taking a known weight of the sample in the Petri dish and drying it in a hot air oven at 100°C till a constant weight was achieved. The difference in weight of the sample gave the moisture content, which was calculated by using the following formula.

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample - Dried weight of sample}}{\text{Wet weight of sample}} \times 100$$

3.6.2. Crude protein (CP)

Protein content (%) was determined by Kjeldahl method in a Kjeltec Autosampler system (AOAC, 2005). The crude protein percentage was obtained by multiplying the Nitrogen percentage by a factor of 6.25 because, average nitrogen content of fish protein is 16%, so 1g nitrogen = 100/16 = 6.25g protein.

3.6.3. Crude fat

The crude fat content of dried experimental diets samples was estimated by Soxhlet apparatus using petroleum ether (Boiling point 55±5°C) as the solvent (AOAC, 2005). The calculation was made as follows.

$$\text{Fat (\%)} = \frac{\text{Initial weight of sample - Weight of extracted sample}}{\text{Initial weight of the sample}} \times 100$$

3.6.4. Crude fibre

The AOAC, 2005 method was used to determine the crude fibre content of a dry sample. After boiling with 1.25 percent dilute H_2SO_4, the sample was washed with water, boiled again with 1.25 percent dilute sodium hydroxide, and the remaining residue after digestion was taken as crude fibre. The calculation was done as follows:

$$\text{Crude fibre (\%)} = \frac{\text{Loss in weight on ignition}}{\text{Weight of sample}} \times 100$$

3.6.5. Ash

Ash content was estimated by taking a known weight of dried samples in a silica crucible and placing it in a muffle furnace at 550°C ± 20°C for 6 hours. The calculation was done as follows:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.6.6. Carbohydrate (Nitrogen free extract)

The carbohydrate (Nitrogen Free Extract or NFE) was determined following the difference method (Hastings, 1976).

Nitrogen Free Extract (NFE %) = 100 – (% crude protein + % crude fat + % crude fibre + % ash).

3.7. Analyses of physico - chemical parameters of water

The physico-chemical parameters of water such as temperature, pH, dissolved oxygen, total alkalinity, total hardness, ammonia-nitrogen (NH_3-N) and phosphate-phosphorous (PO_4-P) were estimated in triplicate by standard methods as described below.

3.7.1. Temperature

Water temperature (°C) was measured by centigrade thermometer (Range: 0°C to 50°C) at 9.00 h fortnightly (APHA, 1995).

3.7.2. pH

pH of water samples was measured by a digital pH meter (Systronics-VI) (APHA, 1995).

3.7.3. Dissolved oxygen (DO)

Dissolved oxygen of water samples was estimated following the Winkler's method (APHA, 1995). Water was collected in a 300 ml BOD sampling bottle, air bubbles were avoided, and 1 ml of Winkler's A (Manganous sulphate solution) and 1 ml of Winkler's B (Potassium iodide) were added. When the precipitate settled to a level, 1 ml of concentrated sulphuric acid (H_2SO_4) was added, and the colour changed to golden yellow. A 50 ml sample was placed in a conical flask and a few drops of starch indicator were added. Then the sample was titrated against Sodium thiosulphate (N/40 $Na_2S_2O_3$) till the sample became colourless. The volume of the titrant was recorded and the result was expressed as mg/L. Dissolved oxygen content was calculated as follows:

$$\text{DO (mg/L)} = \frac{0.2 \times \text{ml. of } Na_2S_2O_3 \text{ used}}{\text{Volume of sample taken (ml)}} \times 1000$$

3.7.4. Total alkalinity

Total alkalinity was calculated through titration method (APHA, 1995). Water sample (50 ml) was taken in a conical flask, and then 2-3 drops of phenolphthalein indicator were added to it. If pink colour developed, sample was titrated against 0.02 N H_2SO_4 till the colour disappears. Then the burette reading was noted down and to that sample, 2 drops of methyl orange indicator were added and the solution turned into orange colour. The titration was continued with 0.02 N H_2SO_4 until the orange colour turned into pink. If pink colour did not appear with phenolphthalein indicator, the sample was titrated against 0.02N H_2SO_4 after adding methyl orange as an indicator. The total burette reading was noted down and result was expressed as mg/L. Total alkalinity content was calculated as follows:

$$\text{Total alkalinity (mg/L)} = \frac{\text{Volume of } H_2SO_4 \text{ used (ml)}}{\text{Volume of sample taken (ml)}} \times 1000$$

3.7.5. Total hardness

The total hardness of water samples was measured by titrating the 50 ml of sample against EDTA (Ethylene di-amine tetra acetic acid) after adding ammonia buffer and Eriochrome black T (APHA, 1995) as indicator. The value was expressed in mg/L. Total hardness content was calculated as follows:

$$\text{Total hardness (mg/L)} = \frac{\text{Volume of EDTA used (ml)}}{\text{Volume of sample taken (ml)}} \times 1000$$

3.7.6. Biochemical oxygen demand (BOD_1)

Biochemical oxygen demand (BOD) was estimated following the method as described in APHA (1995). The initial dissolved oxygen (DO) value was estimated by Winkler's method immediately after collection of the water sample. Another sample was kept in the BOD incubator for 24 hours and final DO was estimated by the same method. BOD_1 value was calculated by subtracting final DO value from initial DO value and expressed as mg/L.

3.7.7. Ammonia-nitrogen (NH_3-N)

Total Ammonia-nitrogen of water sample was analyzed by the phenate method (APHA, 1995). 25 ml of sample was taken in a 50 ml conical flask. Then 1 ml phenol solution followed by 1 ml sodium nitroprusside solution and 2.5 ml oxidizing solution was added and mixed thoroughly. The sample was covered with plastic wrap or paraffin wrapper film and kept in the darkroom at 22 to 27°C temperature for at least 1 hour for colour development. The colour was stable for 24 hours. Simultaneously a blank as standard was prepared. The absorbance of the samples was measured with a spectrophotometer at 640 nm wavelength and compared with standard curve. The concentration was expressed as mg/L.

3.7.8. Nitrate-nitrogen (NO_3-N)

The concentration of nitrite was measured through a double beam UV-vis-spectrophotometer (CECIL CE-4002) at 410 nm wavelengths using Brucine method (EPA, 2009).

3.7.9. Phosphate-phosphorous (PO_4-P)

Phosphate-phosphorous was estimated spectrophotometrically following the stannous chloride method (APHA, 1995). Filtered water sample of 10 ml was taken in a 25 ml test tube and 2 drops of phenolphthalein indicator were added. If the solution turned pink, a strong acid solution (H_2SO_4 + HNO_3) was added dropwise until the pink colour disappears. Then 0.4 ml of ammonium molybdate [$(NH_4)_6$ Mo_7O_{24}] reagent and 0.05 ml of stannous chloride ($SnCl_2$) indicator were added subsequently and mixed thoroughly till blue colour developed. The absorbance of the solution was taken after 10 minutes and before 12 minutes of addition of stannous chloride through spectrophotometer at 690 nm wavelength. The Phosphate-phosphorous concentration of the water sample was computed from the standard curve and the result was expressed as mg/L.

3.8. Analyses of physico-chemical parameters of soil

The physico-chemical parameters of soil such as pH, organic carbon, available phosphorus, available nitrogen was estimated in triplicate by standard methods as described below.

3.8.1. pH

The pH was determined with a digital pH meter (Systronics-VI) using 1:2 suspensions of soil and water (APHA, 1995).

3.8.2. Organic carbon

For estimation of organic carbon, air-dried powdered sediment sample (1 g) was digested with 1 N $K_2Cr_2O_7$ (10 ml) and concentrated H_2SO_4 (20 ml) and kept for 30 minutes at dark. The digested sample was then diluted with 200 ml distilled water and 10 ml ortho-phosphoric acid and 1 ml di-phenyl amine indicator was added. It was then titrated against 1 N ferrous ammonium sulphate (Mohr's salt) until brilliant green colour appeared (Walkley and Black, 1934).

3.8.3. Available phosphorus (P_2O_5)

Available phosphorus was determined using 1:20 soil to Olsen's extractant (0.5 N $NaHCO_3$ adjusted to pH 8.5) (Olsen, 1954) followed by Dickman and Bray's (1940) Chlorostannous reduced molybdophosphoric blue colour method in hypochloric acid system as described by Jackson (1967).

3.8.4. Available nitrogen

Available soil nitrogen was determined by alkaline potassium permanganate method (Subbiah and Asija, 1956). 10 g soil sample was placed in a distillation tube, and 100 ml of 0.38% potassium permanganate (KMnO4) and 10 ml of 20% sodium hydroxide (NaOH) were added. The soil was distilled for 5 min in a Kjeltec apparatus, and the distillate was captured in a conical flask containing 20 ml of 0.02N sulfuric acid (H_2SO_4). The residual acidity in the distillate was titrated with 0.02N sodium carbonate (Na_2CO_3). A blank was carried through the same procedure. The concentration of available soil N was calculated using the variations in titre volumes between samples and a blank.

3.9. Collection of blood and serum

Each fish was anesthetized with clove oil (Apollo Pharmacy) @ 50 µl per litre of water before taking blood from fish. Blood was withdrawn from vena caudalis by using dispovan single use 2ml syringe, left overnight to clot in the undisturbed condition in 4°C. Then the supernatant was collected using micro-pipette and centrifuged at 2000 rpm at 4°C for 30 minutes using a cooling centrifuge to allow precipitation of blood cells. The serum samples were collected and stored at -20°C for analysis.

3.10. Biochemical analysis

3.10.1. Blood Glucose

Blood glucose was estimated by the method of Nelson and Somogyi (1945) as described by Nohl-Oser (1965) by using Arkray glucose 500 blood glucose diagnostic kit (Annapurna Scientific Instrument Pvt. Ltd). 14 nos. of test tubes were taken and labelled as blank (B), standard (S) and test (T) as per requirement. For blank (B) the test tube was filled with distilled water. In standard (S), test tube was filled with 1 ml of working solution. In test (T), each test tube was filled with 0.01 ml of standard solution and 0.01 ml of serum sample. All test tubes were shaken well and incubated at 37°C for 30 minutes. The absorbance of standard (S) and test (T) was measured against blank (B) in a spectrophotometer at 505 nm.

$$\text{Glucose in mg/dl} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 100$$

3.10.2 Total serum protein

Total proteins in the serum were measured by Biuret Method using Total Protein Kit (Arkray). Protein, an alkaline medium, binds with cupric ions found in the biuret reagent to create a complex that is blue-violet in colour and whose absorbance is inversely proportional to the amount of protein in the sample.

14 nos. of test tubes were taken and labelled as blank (B), standard (S) and test (T) as per requirement. 1.0 ml of biuret reagent was taken in each tube. In the blank (B), 0.02 ml of distilled water was added. In standard (S), 0.02 ml of protein standard was added and in test (T), 0.02 ml of serum sample was added. All test tubes were shaken well and incubated at 37^0C for 10 minutes. The absorbance of standard (S) and test (T) was measured against Blank (B) within 60 minutes in 550 nm wavelength using a spectrophotometer.

$$\text{Total Proteins in g/dl} = \frac{\text{Absorbance of test (T)}}{\text{Absorbance of standard (S)}} \times 8$$

3.10.3. Albumin

Serum albumin was estimated by bromocresol green binding method (BCG method) using the Autozyme Albumin kit (Annapurna Scientific Instrument Pvt. Ltd). Serum albumin in the presence of bromocresol-green under acidic conditions forms a green coloured complex. The absorbance of the complex is proportional to the albumin concentration in the serum.

14 nos. of test tubes were taken and labelled as blank (B), standard (S) and test (T) as per requirement labelled. 1 ml of BCG reagent was taken in each test tube. In the blank (B), 0.01 ml of distilled water was added. 0.01 ml of albumin standard was added into standard (S) and 0.01 ml of serum sample was added into test (T). It was shaken well and incubated at room temperature for 1 minute. The absorbances of standard (S) and test (T) were measured immediately against blank (B) in a spectrophotometer at 600 nm.

$$\text{Albumin in g/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 5$$

3.10.4. Globulin

Globulin content was calculated by subtracting albumin values from total serum protein.

Globulin in g/dl = Total protein in g/dl - Albumin in g/dl

3.10.5 Albumin-globulin ratio

Albumin-Globulin ratio was calculated by dividing albumin values by globulin values.

$$\text{A/G ratio} = \frac{\text{Albumin in g/dl}}{\text{Globulin in g/dl}}$$

3.11. Enzyme assay

3.11.1. Tissue collection and preparation of homogenate

At the last day of experiments, fish were anesthetized with clove oil @ 50 µl per litre of water and after sedation fish were sacrificed for collection of gills, liver, and intestine. Samples were carefully collected, weighed from each treatment in triplicate. They were homogenized in a chilled 0.25 (M) sucrose solution to prepare 5% (1:19 w/v) homogenate. The tube was continuously kept in ice to avoid enzyme reactions. The homogenate was centrifuged at 12000 rpm for 10 minutes at 4^0C to obtain the supernatant that was used for enzyme analysis. All the supernatants were stored at -20^0C until further analysis.

3.11.2. Digestive enzyme activity

3.11.2.1. Amylase activity

The reducing sugars produced due to the action of glucoamylase and alpha amylase on carbohydrates was estimated using Di-nitro Salicylic Acid (DNS) method (Rick and Stegbauer, 1974). The reaction mixture consists of 1% (w/v) starch solution, phosphate buffer, and the tissue homogenate. The reaction mixtures were incubated at 37°C for 30 minutes. DNS was added after incubation and kept in a boiling water bath for 5 minutes. After cooling, the reaction mixture was diluted with distilled water and absorbance was measured at 540 nm. Maltose was used as the standard. Amylase activity was expressed as micromole (mM) of maltose released from starch per minute at that temperature.

3.11.2.2. Protease activity

The protease activity was determined by the casein digestion method (Drapeau, 1974). The enzyme reaction mixture consisted of 1% (w/v) casein in 0.05M Tris-phosphate buffer (pH 7.8) and incubated for 5 minutes at 37°C. Then tissue homogenate was added. Ten minutes later, the reaction was stopped by adding 10% TCA (Trichloro Acetic Acid) and the whole content was filtered. By adding tissue homogenate right before stopping the reaction with TCA and without incubating it, the reagent blank was created. The amount of enzyme required to release acid-soluble fragments corresponding to $\Delta 0.001$ A_{280} per minute at 37°C and pH 7.8 was used to define one unit of enzyme activity.

3.11.2.3. Lipase activity

The lipase activity was assayed by the method of Cherry and Crandell (1932). Two test tubes labelled as a test (T) and control (C) were taken. Each of the two tubes was added with 3ml of the distilled water and 1ml of the homogenate. One of the tubes (C) was placed in boiling water served to inactivate the lipase in control. Then 0.5ml of buffer solution (phosphate buffer pH 7.0) and 2 ml olive oil emulsion was added to both the tubes, shaken well and incubated at 37°C for 24 hours. Then 3ml of 95% alcohol and 2 drops of phenolphthalein solution were mixed. Each of the tubes was titrated with 0.05N NaOH up to the appearance of a permanent pink color. The volume (ml) of N/20 NaOH solution required for 100 mg intestinal or liver tissue in the experimental tube subtract the volume (ml) of N/20 NaOH solution required for the same amount of intestinal or liver tissue in the control tube represented the units of intestinal or liver lipase activity per gram tissue.

3.11.3. Enzymes of Protein Metabolism

3.11.3.1. Alanine aminotransferases (ALT)

ALT test kit, Modified UV (IFCC), Kinetic assay; Span Diagnostics Ltd., India was used to measure alanine aminotransferases (ALT) activity in blood. The activity was expressed as IU/L.

3.11.3.2. Aspartate aminotransferases (AST)

Aspartate aminotransferases (AST) activity was estimated by AST test kit, Modified UV (IFCC), Kinetic assay; Span Diagnostics Ltd., India. The activity was expressed as IU/L.

3.11.4. Enzyme for carbohydrate metabolism

3.11.4.1. Lactate dehydrogenase (LDH) activity:

The LDH activity was assayed in liver tissues by the method of Wroblewski and Ladue (1955). Total 3ml of the reaction mixture comprised of 2.7ml of 0.1M phosphate buffer (pH 7.5), 0.1ml of NADH solution (2 mg NADH dissolved in 1ml of phosphate buffer solution), 0.1ml of tissue homogenate and 0.1ml of sodium pyruvate. The reaction was started after addition of substrate sodium pyruvate. The OD was recorded at 340nm at 30 sec intervals for 2 min. The enzyme activity was expressed as units/ mg protein/ min at 25 °C where 1 unit was equal to Δ 0.01 OD/ min at 37°C.

3.11.4.2. Malate dehydrogenase activity

The MDH activity was assayed in liver by the method of Ochoa (1955). Total 3 ml of the reaction mixture comprised of 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of NADH solution (2 mg NADH dissolved in 1 ml of phosphate buffer solution), 0.1 ml of tissue homogenate and 0.1 ml of freshly prepared oxaloacetate solution (2 mg oxaloacetate dissolved in 2 ml chilled distilled water). The reaction was started after addition of oxaloacetate solution as substrate. The OD was recorded at 340nm at 30 sec intervals for 2 min. The enzyme activity was expressed as units/mg protein/min at 25°C where 1 unit was equal to Δ 0.01 OD/ min at 37°C.

3.11.5. Oxidative Stress Enzyme

3.11.5.1. Assay of superoxide dismutase

Superoxide dismutase (SOD) was determined following methods adopted by Misra and Fridovich (1972) with some modifications. Aliquot of 30 µl enzyme from the tissue homogenate pipetted into a cuvette containing 1.5 ml, 0.05 (M) carbonate-bicarbonate buffer (pH -10.2), with 10^{-4} (M) EDTA. After running a blank with distilled water instead of sample, the cuvette with the buffer and sample mixture was set in its position and 0.5 ml, 0.01 (M) epinephrine (molecular weight-183.2 g) was pipetted into the mixture. Immediately, changes in absorbance at 480 nm for 30 minutes at 30 seconds interval were recorded using UV-spectrophotometer. Each treatment was carried out in triplicate. SOD was expressed in unit of activity, where one unit of activity is the amount of protein required to give 50% inhibition of epinephrine auto-oxidation.

3.11.5.2. Assay of catalase

Catalase activity was assayed following methods described by Takahara *et al.* (1960). A mixture of 2.5 ml, 50 milimole phosphate buffer (pH- 7) and 30 µl enzyme samples from tissue homogenate was prepared in a cuvette and was set in UV-spectrophotometer at 240 nm. Freshly prepared 1 ml, 30% H_2O_2 was pipetted into the mixture and immediately, changes in absorbance for 3 minutes at 15 seconds interval were recorded for each treatment in triplicate. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of H_2O_2 solution. Catalase enzyme specific activity was expressed as unit of CAT, where one unit is explained as the nanomole (nM) of H_2O_2 decomposed per minute per milligram protein.

3.11.5.3. Assay of Glutathione peroxidise (GPx)

Hepatic GPx activity was estimated following the method of Hafeman *et al.*, 1974. Tissue homogenate (100 µl) was treated for 6 minutes at 37°C with 5 mM glutathione (100 µl), 1.2 mM H_2O_2 (100 µl), 25 mM NaN_3 (100 µl), and 2.1 ml of 1 M phosphate buffer (pH 7.0). The reaction was stopped by adding 1.65 percent phosphoric acid (2.0 ml) and centrifuging the mixture at 3000 g for 10 minutes. The supernatant (2.0 ml) was combined with 0.4 M Na_2HPO_4 and 1 mM DTNB prepared in buffer (1 ml). After a 10-minute incubation at room temperature, the yellow colour was measured at 412 nm. GPx activity was measured in nano moles H_2O_2 decomposed per minute per mg protein.

3.12. Growth Study

Sampling was done randomly at an interval of 30 days to assess the body wet weight and length increment of test fishes. Body weight was mesured in an electric balance with precision of 0.01g. Total length (from the tip of the anterior most part of the body to the tip of the caudal fin) was measured by using 30 cm ruler to the nearest 0.1 cm. Growth performance was estimated using the following formula.

3.12.1. Body weight gain

Body weight gain was calculated by subtracting initial body weight from final body weight.

Body weight gain (g) = Final weight (g) - Initial weight (g)

3.12.2. Percentage of Weight gain

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

3.12.3. Specific growth rate (SGR)

$$\text{SGR (\% day}^{-1}\text{)} = \frac{\text{Ln (Final Weight) - Ln (Initial Weight)}}{\text{Number of Day}} \times 100$$

3.12.4. Feed conversion efficiency (FCE)

$$\text{FCE} = \frac{\text{Net weight gain (wet weight)}}{\text{Feed given (dry weight)}}$$

3.12.5. Protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Net weight gain (wet weight)}}{\text{Protein fed}}$$

3.13. Economic Analysis

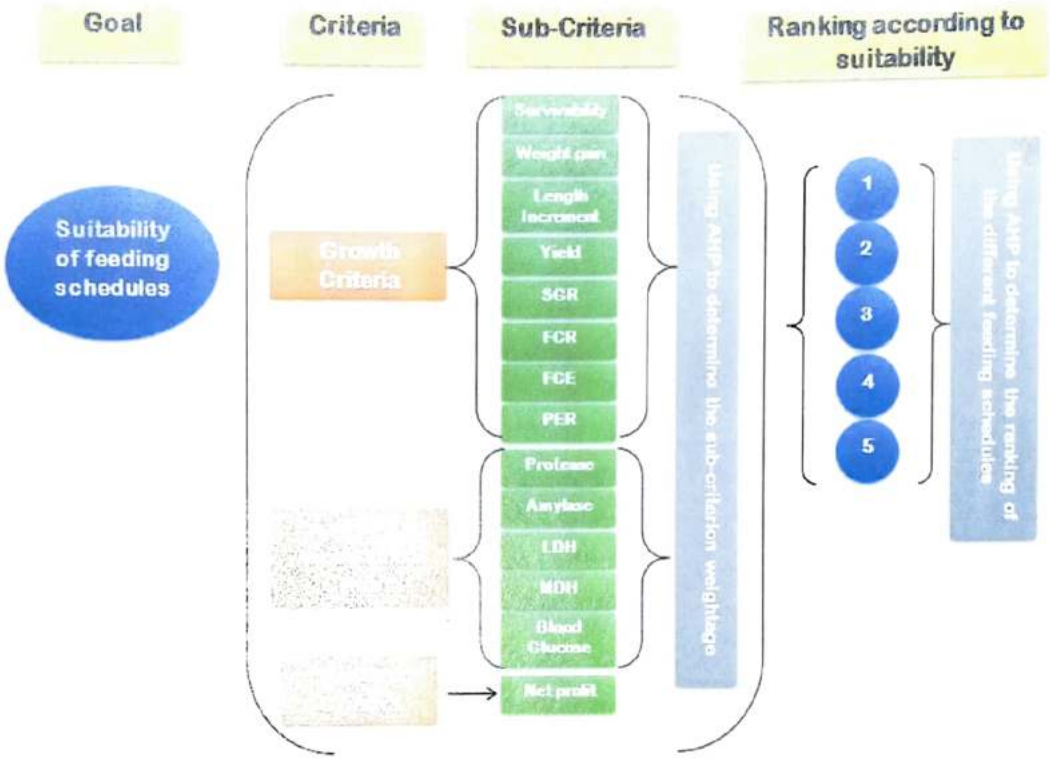
A simple algebraic equation was used to analyze the economics of this study. Economical analysis was performed based on the local market prices of inputs and selling prices of fish to compare the net return and profit margins among different treatments. The equation was used as follows:

Net return = Sales revenue – (Fixed cost + Variable cost + Interest on input)

3.14. Suitability Analysis

Using Thomas L Saaty (1988) technique the weightage and ranking of each criterion were estimated. Pair-wise Comparison Matrix (PCM) was made on the growth parameters. As well as, weightages of enzymatic/biochemical sub criteria and economic sub criterion were also estimated.

Another PCM was created to set the priority among growth, enzyme/biochemical assay, and economics. Finally, performance score was evaluated as the sum of the weighted normalized value of each criterion of the final Weighted Decision Matrix. The whole process is summarized below:



3.15. Statistical Analysis

One-way analysis of variance (ANOVA) following was used to test significant differences ($P<0.05$) in multiple comparisons in the above parameters in various treatments and expressed as arithmetic mean ± standard deviation (SD). Duncan's Multiple Range test (DMRT) was done to measure specific differences between pairs of means. Correlation coefficient was calculated to assess the correlation between variables. All analyses were performed using the statistical software SPSS 16 version.

Photographs of different activities

Plate 3.1. Acclimatization of amur common carp seed

Plate 3.2. In-vivo experimental setup

Plate 3.3. In-vitro experimental setup

Plate 3.4. Length measurement of amur common carp

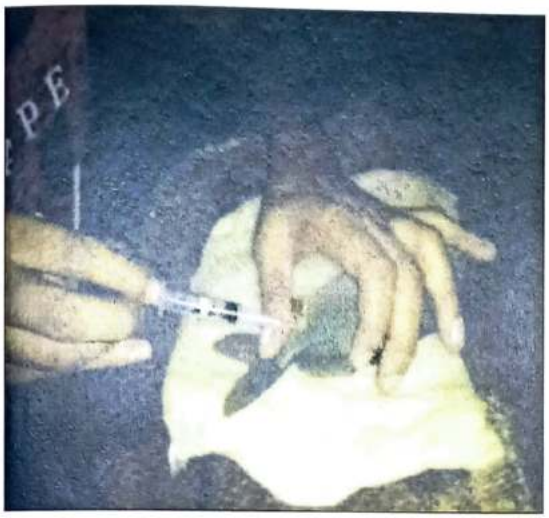
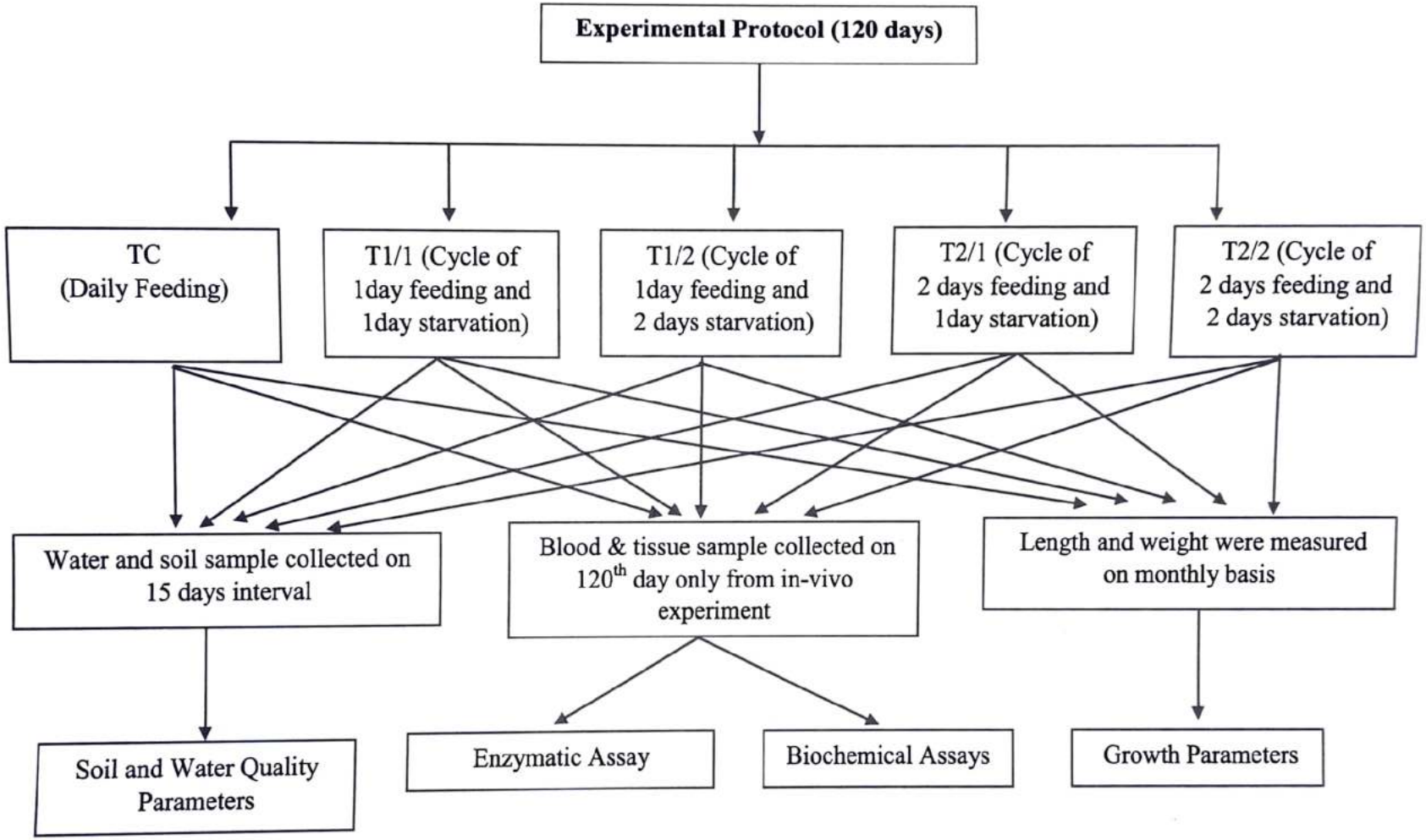


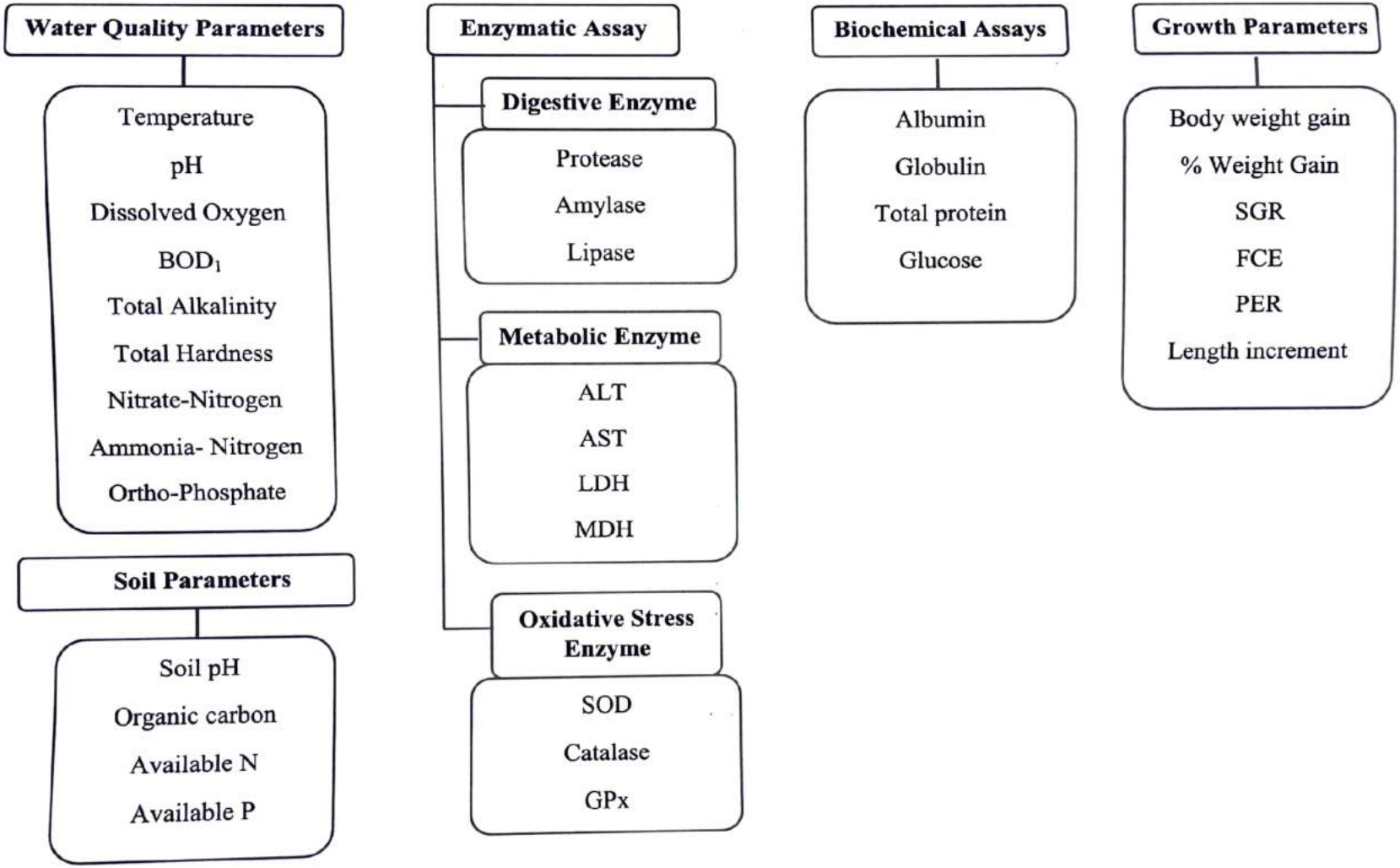
Plate 3.5. Collection of blood

Plate 3.6. Collection of tissue sample

Plate 3.7. Preparation of blood and tissue sample for analysis

Plate 3.8. Estimation of different enzyme activity


Experimental Protocol (120 days)
TC (Daily Feeding)
T1/1 (Cycle of 1day feeding and 1day starvation)
T1/2 (Cycle of 1day feeding and 2 days starvation)
T2/1 (Cycle of 2 days feeding and 1day starvation)
T2/2 (Cycle of 2 days feeding and 2 days starvation)
Water and soil sample collected on 15 days interval
Blood & tissue sample collected on 120th day only from in-vivo experiment
Length and weight were measured on monthly basis
Soil and Water Quality Parameters
Enzymatic Assay
Biochemical Assays
Growth Parameters


Water Quality Parameters
Temperature
pH
Dissolved Oxygen
BOD1
Total Alkalinity
Total Hardness
Nitrate-Nitrogen
Ammonia- Nitrogen
Ortho-Phosphate
Soil Parameters
Soil pH
Organic carbon
Available N
Available P
Enzymatic Assay
Digestive Enzyme
Protease
Amylase
Lipase
Metabolic Enzyme
ALT
AST
LDH
MDH
Oxidative Stress Enzyme
SOD
Catalase
GPx
Biochemical Assays
Albumin
Globulin
Total protein
Glucose
Growth Parameters
Body weight gain
% Weight Gain
SGR
FCE
PER
Length increment

Chapter- 4

RESULTS AND DISCUSSION

4.1. Proximate composition of experimental diet and fish

In the present study, the commercial diets fed to amur common carp was found to contain 27.73% crude protein, 6.42% crude lipid, 6.22% crude fibre, 1.86% ash, 5.80% moisture content and 51.97% nitrogen free elements (NFE) on dry matter basis.

The proximate analysis of the experimental fish was done to determine their treatment wise biochemical compositions (Table 4.1).

The moisture contents in Amur common carp of various treatment groups in descending order were like 77.26 ± 1.32 %, 74.14 ± 0.65 %, 71.79 ± 0.38 %, 70.09 ± 1.44 %, and 70.01 ± 1.38 % in T1/2, T2/2, T1/1, T2/1 and TC respectively having overall significant differences ($P<0.05$) among the treatments.

The ash content levels (%) were found 1.56 ± 0.04 %, 1.16 ± 0.10 %, 0.64 ± 0.08 %, 1.14 ± 0.05 %, and 0.65 ± 0.04 % in TC, T1/1, T1/2, T2/1 and T2/2 respectively having the highest mean value in TC followed by T1/1, T2/1, T2/2, and T1/2 with significant differences ($P<0.05$) among the treatments.

It has been observed that feed deprivation had a significant impact on the body lipid content of the test fish amur common carp. Lower body lipid content was evidenced with the severity of starvation. However highest mean crude fat content was found in TC (11.49 ± 0.07 %), followed by T2/1 (11.16 ± 0.51 %), T2/2 (9.10 ± 0.08 %), T1/1 (7.2 ± 0.05 %) and T1/2 (6.63 ± 0.10 %,) respectively with significant difference ($P<0.05$) among the treatments.

Protein is the major organic component in the fish tissue. Protein converts into amino acids which subsequently synthesise new proteins during growth and reproduction or to replace the existing proteins for maintenance. Like body lipid content, periodic starvation had a significant influence on muscle protein content of the test fish also. With the severity of starvation, lower muscle protein content was noted. The highest mean crude protein content was found in TC (19.85 ± 0.51 %) followed by T2/1 (18.1 ± 1.01 %), T1/1 (16.93 ± 1.34 %), T2/2 (15.62 ± 0.67 %) and T1/2 (15.47 ± 1.34 %) having significant differences ($P<0.05$) among the treatments.

Studies on channel catfish (*Ictalurus punctatus*) (Gaylord & Gatlin, 2000), gibel carp (Xie *et al.*, 2001), hybrid striped bass, *Morone chrysops* x *Morone saxatilis* (Turano *et al.*, 2007) were failed to determine any significant effect of feeding management strategies on body composition. Whereas, Adakli and Tasbozan (2015) discovered a significant reduction in total fat content of *Decentrarchus labrax* starved for 10 days and refed for 40 days when compared to the control (fed daily). Several studies have found that fish subjected to starvation/refeeding regimes have lower body lipid content (Tian & Qin, 2004; Oh, Noh, & Cho, 2007; Peres *et al.*, 2011; Zhu *et al.*, 2001). Lower muscle composition parameters in feed deprived *Oreochromis mossambicus* were reported by Gabriel *et al.* (2018). Significantly lower crude fat and crude protein content were evidenced in fishes subjected to 2-days deprivation/ 2-days refeeding and 2-days deprivation/ 3-days refeeding cycle, when compared to the control. But no significant difference was reported between 2-days deprivation/ 4-days refeeding and daily feeding group. These findings are consistent with the current study, which presented significantly lower muscle protein and body lipid content in T1/1, T1/2, T2/2 compared to the control and T2/1. The fishes subjected to T1/1, T1/2, and T2/2 were failed to restore lipid and protein content used during the starvation period to support basal metabolism and survival, as explained by Adakli & Tasbozan (2015). This suggests that in fish, severe or long-term feed deprivation/refeeding cycles can result in less fattening and higher energy consumption.

Table 4.1. Proximate composition of amur common carp fingerlings (mean ± SD)

Treatment	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Protein (%)
TC	70.01 ± 1.38^{a}	1.56 ± 0.04^{c}	11.49 ± 0.07^{d}	19.85 ± 0.51^{c}
T1/1	71.79 ± 0.38^{a}	1.16 ± 0.10^{b}	7.2 ± 0.05^{b}	16.93 ± 1.34^{ab}
T1/2	77.26 ± 1.32^{c}	0.64 ± 0.08^{a}	6.63 ± 0.10^{a}	15.47 ± 1.34^{a}
T2/1	70.09 ± 1.44^{a}	1.14 ± 0.05^{b}	11.16 ± 0.51^{d}	18.1 ± 1.01^{bc}
T2/2	74.14 ± 0.65^{b}	0.65 ± 0.04^{a}	9.10 ± 0.08^{c}	15.62 ± 0.67^{a}

Column wise altered superscript statistically implies significant difference ($P < 0.05$).

4.2. Growth and nutrient utilization parameters

4.2.1. Body weight gain

Body weight gain is nothing but the difference between initial and final weight. In this present study it has been evidenced that body weight gain of amur common carp fingerlings was severely impacted by feed deprivation in both in-vitro and in-vivo experiment.

In case of in-vitro treatment the highest mean body weight gain was found in TC (26.68 ± 1.85 g), followed by T2/1 (25.11 ± 2.16 g), T1/1 (19.54 ± 1.2 g), T2/2 (18.87 ± 1.07 g) and T1/2 (14.05 ± 0.61 g) respectively (Tab. 4.2 and Fig. 4.1).

In case of in-vivo treatment the overall highest mean value was found in TC (204.76 ± 15.55 g) which is 5.87%, 29.06%, 32.57% and 74.07% higher than T2/1 (193.4 ± 16.65 g), T1/1 (158.65 ± 7.49 g), T2/2 (154.45 ± 9.51 g) and T1/2 (117.63 ± 4.09 g) respectively (Tab. 4.3 and Fig. 4.1).

In both experiment an overall significant difference ($P<0.05$) among the treatment groups was noted, but no significant difference was found between TC and T2/1, as well as between T1/1 and T2/2.

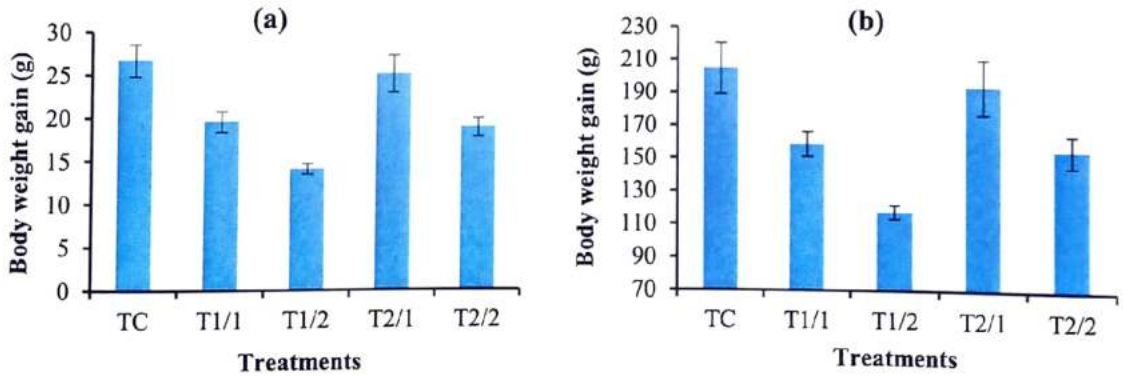


Fig. 4.1. Body weight gain of amur common carp fingerlings in different treatments of in-vitro (a) and in-vivo (b) experiment (mean ± SD)

4.2.2. Percentage of weight gain

Percentage of weight gain of amur common carp fingerlings followed the same trend of total body weight gain and exhibited the overall highest mean value in TC (1112.61 ± 81.69 %) followed by T2/1 (1053.53 ± 91.11 %), T1/1 (832.26 ± 39.5 %), T2/2 (792.45 ± 8.69 %) and T1/2 (599.62 ± 52.4 %) respectively (Tab. 4.2 and Fig. 4.2) having significant differences ($P<0.05$) among the treatment regimes in case of in-vitro experiment.

In case of in-vivo experiment the overall highest mean value of percentage of weight gain was achieved in TC (8536.48 ± 642.21 g) which is 5.19%, 26.28%, 31.60% and 70.19% higher than T2/1 (8114.86 ± 689.94 g), T1/1 (6760.13 ± 257.69 g), T2/2 (6486.52 ± 103.98 g) and T1/2 (5015.67 ± 323.26 g) respectively. However, the differences among the treatments remained significant ($P<0.05$) throughout the experiment (Tab. 4.3 and Fig. 4.2).

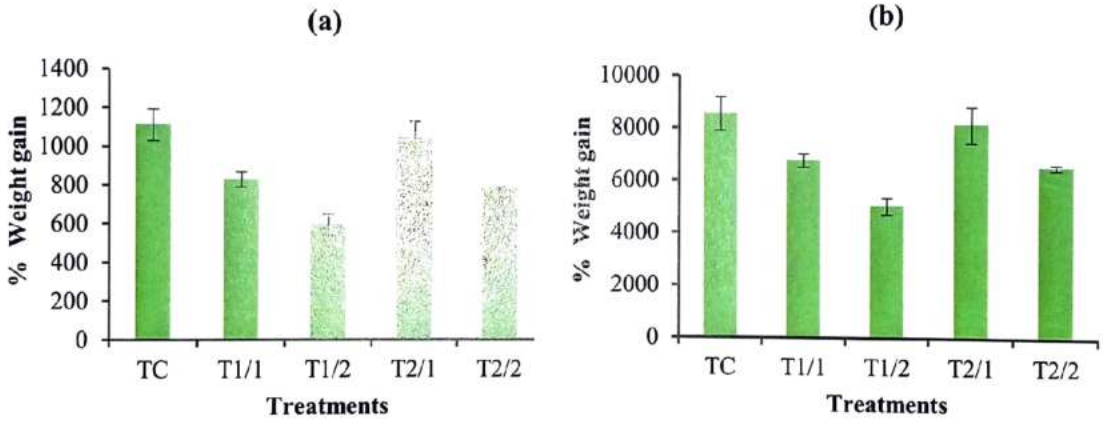


Fig. 4.2. Percentage of weight gain of amur common carp fingerlings in different treatments of in-vitro (a) and in-vivo (b) experiment (mean ± SD).

4.2.3. Length increment

In this present study the length increment of amur common carp fingerlings was also markedly influenced by feed deprivation in both in-vitro and in-vivo experiment.

Treatment wise length increment of the test fish in case of in-vitro experiment is presented in Tab. 4.2 and Fig. 4.3. The highest increment in length was found in TC (7.33 ± 0.28 cm) followed by T2/1 (7.09 ± 0.34 cm), T1/1 (6.16 ± 0.2 cm), T2/2 (6.02 ± 0.14 cm) and T1/2 (5.06 ± 0.18 cm) respectively having significant difference ($P<0.05$) among the treatment groups.

The highest increment in length in case of in-vivo experiment was also observed in TC (20.06 ± 0.67 cm) which is 2.56 %, 11.94 %, 13.46 % and 28.43 % higher than T2/1 (19.56 ± 0.75 cm), T1/1 (17.92 ± 0.36 cm), T2/2 (17.68 ± 0.42 cm) and T1/2 (15.62 ± 0.26 cm) respectively (Tab. 4.3 and Fig. 4.3). Statistical difference in terms of length increment remained significant ($P<0.05$) among the treatment groups.

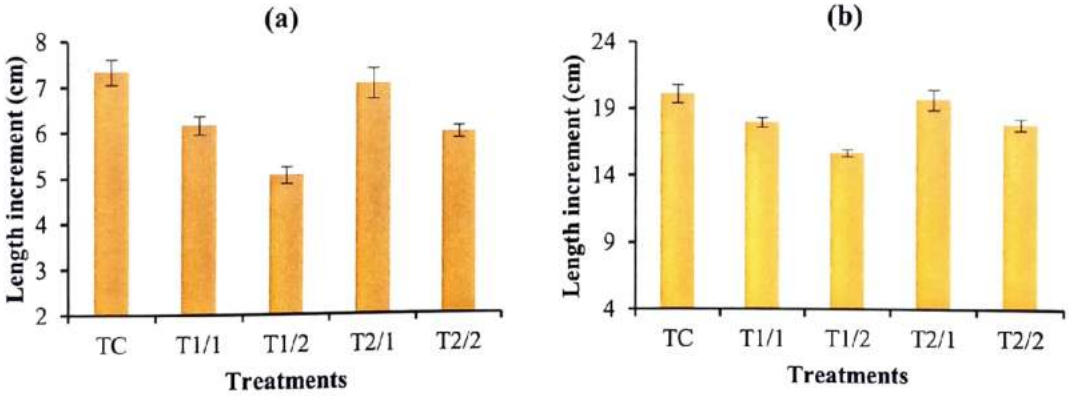


Fig. 4.3. Length increment of amur common carp fingerlings in different treatments of in-vitro (a) and in-vivo (b) experiment (mean ± SD).

4.2.4. Specific growth rate (SGR)

SGR is simply the percentage increase in size per day. It can be expressed as % day^{-1}. As periodic starvation had a significant impact on body weight gain, it was almost certain that it had an impact on SGR as well.

Treatment wise SGR of the test fishes in case if in-vitro experiment is portrayed in Tab. 4.2 and Fig. 4.4. The overall highest mean value of SGR was found in TC (1.97 ± 0.06) which is 2.60 %, 15.20 %, 17.26 % and 36.80 % higher than T2/1 (1.92 ± 0.08), T1/1 (1.71 ± 0.06), T2/2 (1.68 ± 0.05) and T1/2 (1.44 ± 0.03) respectively. However, there were significant differences ($P<0.05$) among the treatment groups in case of SGR of amur common carp fingerlings. But TC and T2/1 exhibited significantly similar SGR.

In case of in-vivo experiment (Tab. 4.3 and Fig. 4.4) the overall highest mean value of SGR was found in TC (3.71 ± 0.06) followed by, T2/1 (3.67 ± 0.07), T1/1 (3.59 ± 0.02), T2/2 (3.51 ± 0.04) and T1/2 (3.28 ± 0.05) respectively with significant differences ($P<0.05$) among the treatment groups. But no significant differences remained among TC. T2/1 and T1/1.

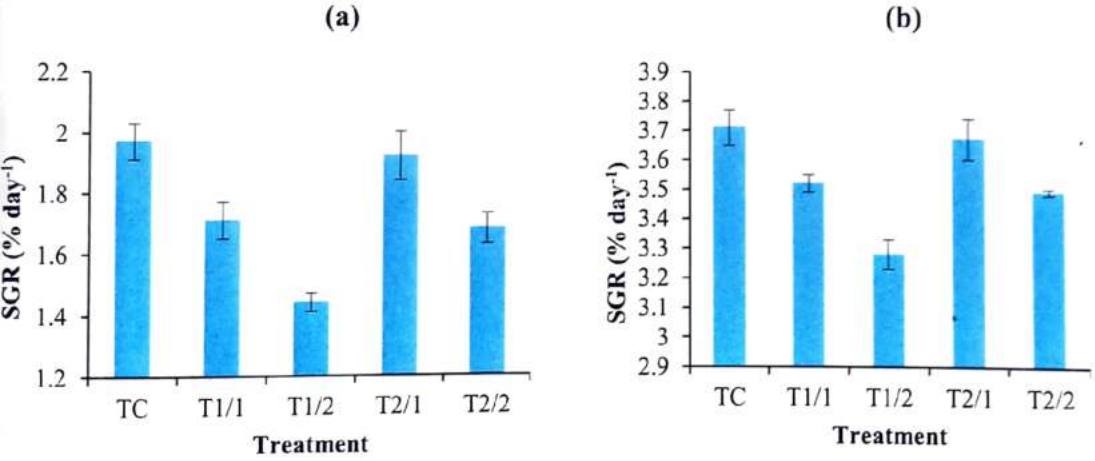


Fig. 4.4. SGR value of amur common carp fingerlings in different treatments of in-vitro (a) and in-vivo (b) experiment (mean ± SD).

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Treatment wise SGR of the test fishes in case if in-vitro experiment is portrayed in Tab. 4.2 and Fig. 4.4. The overall highest mean value of SGR was found in TC (1.97 ± 0.06) which is 2.60 %, 15.20 %, 17.26 % and 36.80 % higher than T2/1 (1.92 ± 0.08), T1/1 (1.71 ± 0.06), T2/2 (1.68 ± 0.05) and T1/2 (1.44 ± 0.03) respectively. However, there were significant differences ($P<0.05$) among the treatment groups in case of SGR of amur common carp fingerlings. But TC and T2/1 exhibited significantly similar SGR.

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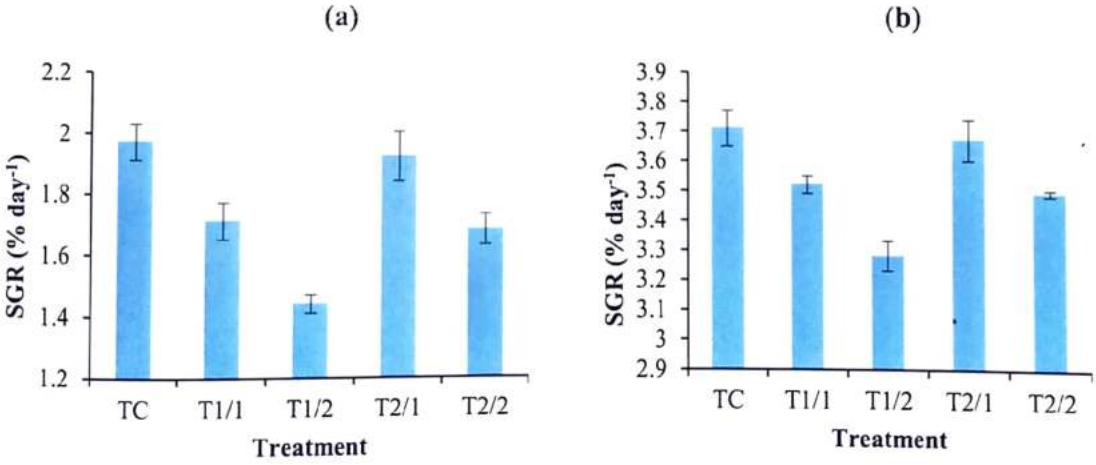

Fig. 4.4. SGR value of amur common carp fingerlings in different treatments of in-vitro (a) and in-vivo (b) experiment (mean ± SD).

4.2.5. Feed conversion efficiency (FCE)

Feed conversion efficiency is the efficiency with which animals turn feed into meat and other food products. In this present investigation, the feed conversion efficiency (FCE) was truly affected by feeding strategy. All the starved groups exhibited higher FCE than control group.

The treatment wise FCE for in vitro experiment is presented in Tab. 4.2 and Fig. 4.5. However, the highest mean FCE value was found in T1/2 (0.97 ± 0.12) followed by T1/1 (0.73 ± 0.07), T2/2 (0.73 ± 0.1), T2/1 (0.61 ± 0.1) and TC (0.47 ± 0.05) respectively having significant ($P < 0.05$) differences among the treatment groups, but no significant difference was found between TC and T2/1, as well as among T1/1, T2/1 and T2/2.

In case of in-vivo trial also the highest mean FCE value was achieved in T1/2 (2.24 ± 0.28) followed by T2/2 (1.59 ± 0.21), T1/1 (1.57 ± 0.15), T2/1 (1.24 ± 0.19) and TC (0.90 ± 0.07) respectively (Tab. 4.3 and Fig. 4.5). The overall differences among the treatment groups remained significant ($P < 0.05$), but similar to in-vitro trial here also no significant difference was found between TC and T2/1, as well as among T1/1, T2/1, and T2/2.

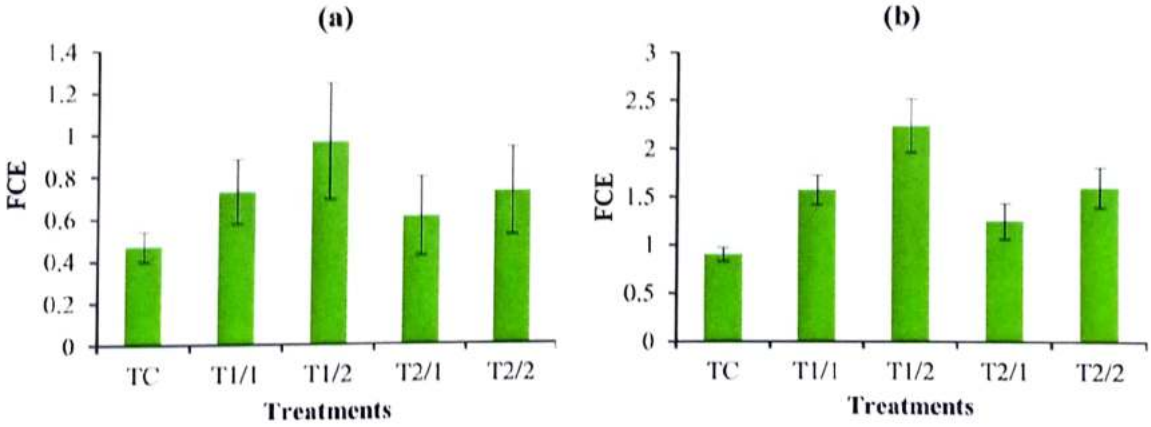

Fig. 4.5. FCE of amur common carp fingerlings in different treatments of in-vitro (a) and in-vivo (b) experiment (mean ± SD)

4.2.5. Feed conversion efficiency (FCE)

Feed conversion efficiency is the efficiency with which animals turn feed into meat and other food products. In this present investigation, the feed conversion efficiency (FCE) was truly affected by feeding strategy. All the starved groups exhibited higher FCE than control group.

The treatment wise FCE for in-vitro experiment is presented in Tab. 4.2 and Fig. 4.5. However, the highest mean FCE value was found in T1/2 (0.97 ± 0.12) followed by T1/1 (0.73 ± 0.07), T2/2 (0.73 ± 0.1), T2/1 (0.61 ± 0.1) and TC (0.47 ± 0.05) respectively having significant ($P<0.05$) differences among the treatment groups, but no significant difference was found between TC and T2/1, as well as among T1/1, T2/1 and T2/2.

In case of in-vivo trial also the highest mean FCE value was achieved in T1/2 (2.24 ± 0.28) followed by T2/2 (1.59 ± 0.21), T1/1 (1.57 ± 0.15), T2/1 (1.24 ± 0.19) and TC (0.90 ± 0.07) respectively (Tab. 4.3 and Fig. 4.5). The overall differences among the treatment groups remained significant ($P<0.05$), but similar to in-vitro trial here also no significant difference was found between TC and T2/1, as well as among T1/1, T2/1, and T2/2.

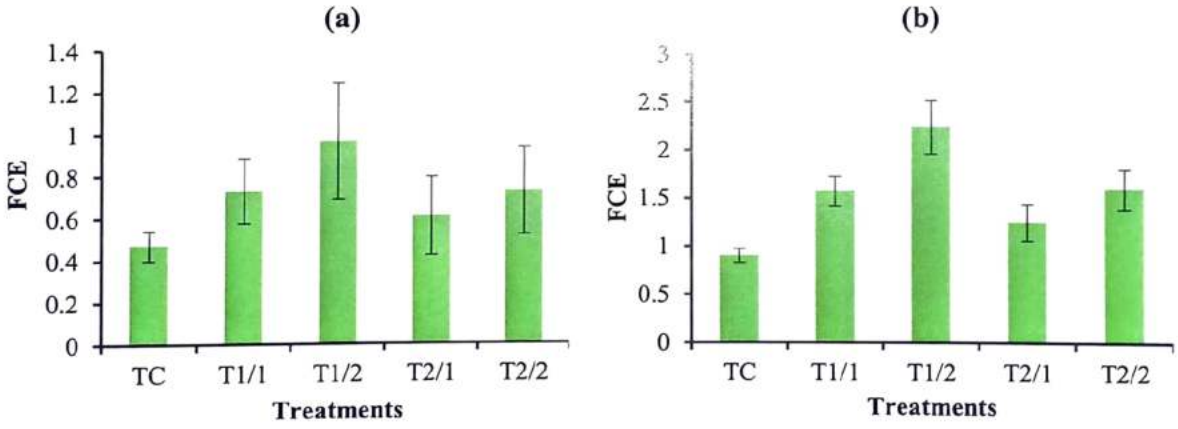

Fig. 4.5. FCE of amur common carp fingerlings in different treatments of in-vitro (a) and in-vivo (b) experiment (mean ± SD)

4.2.6. Protein efficiency ratio (PER)

PER can be defined as the weight gain of test group divided by the amount of protein consumed by the test group (WHO/FAO Expert Consultation, 1991). It is used to measure the quality of the protein in the feed.

Treatment wise PER of in-vitro trial is presented in Tab. 4.2 and Fig. 4.6. The highest mean PER value was obtained in T1/2 (3.47 ± 0.44) followed by T1/1 (2.6 ± 0.23), T2/2 (2.59 ± 0.36), T2/1 (2.19 ± 0.35) and TC (1.68 ± 0.18) respectively with an overall significant difference ($P<0.05$) among the treatments, but no significant difference remained between TC and T2/1, as well as among T1/1, T2/1 and T2/2.

In case of in-vivo experiment the highest mean PER values were found in T1/2 (7.99 ± 0.99) followed by T2/2 (5.68 ± 0.77), T1/1 (5.6 ± 0.55), T2/1 (4.44 ± 0.67) and TC (3.22 ± 0.25) respectively (Tab. 4.3 and Fig. 4.7). The statistical differences among the treatment groups remained noteworthy ($P<0.05$) in case of PER. During the experiment, the PER value was found to be significantly similar in TC and T2/1, as well as in T1/1, T2/1, and T2/2.

In this present investigation PER value was always found to be parallel with the degree of starvation.

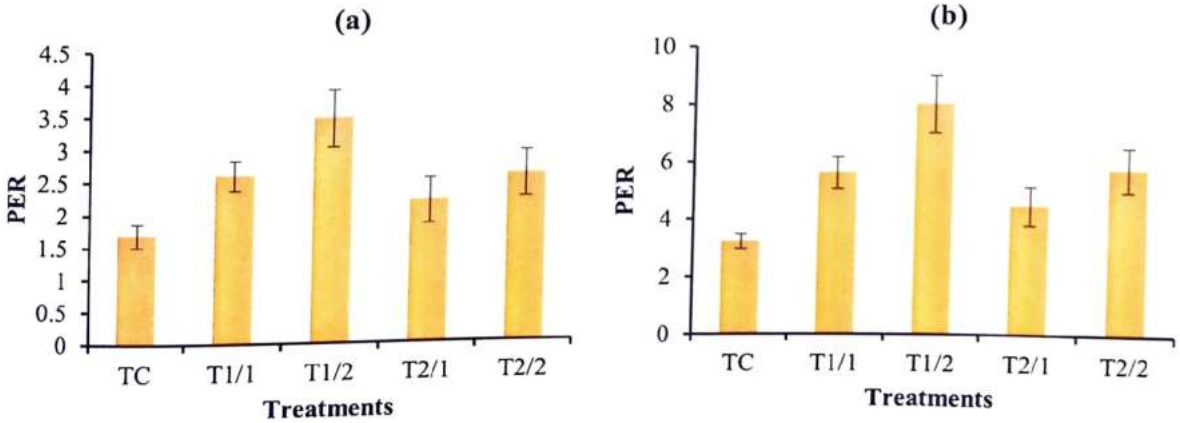

Fig. 4.6. PER values in different treatments of in-vitro (a) and in-vivo (b) experiment (mean ± SD)

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In case of in-vivo experiment the highest mean PER values were found in T1/2 (7.99 ± 0.99) followed by T2/2 (5.68 ± 0.77), T1/1 (5.6 ± 0.55), T2/1 (4.44 ± 0.67) and TC (3.22 ± 0.25) respectively (Tab. 4.3 and Fig. 4.7). The statistical differences among the treatment groups remained noteworthy ($P<0.05$) in case of PER. During the experiment, the PER value was found to be significantly similar in TC and T2/1, as well as in T1/1, T2/1, and T2/2.

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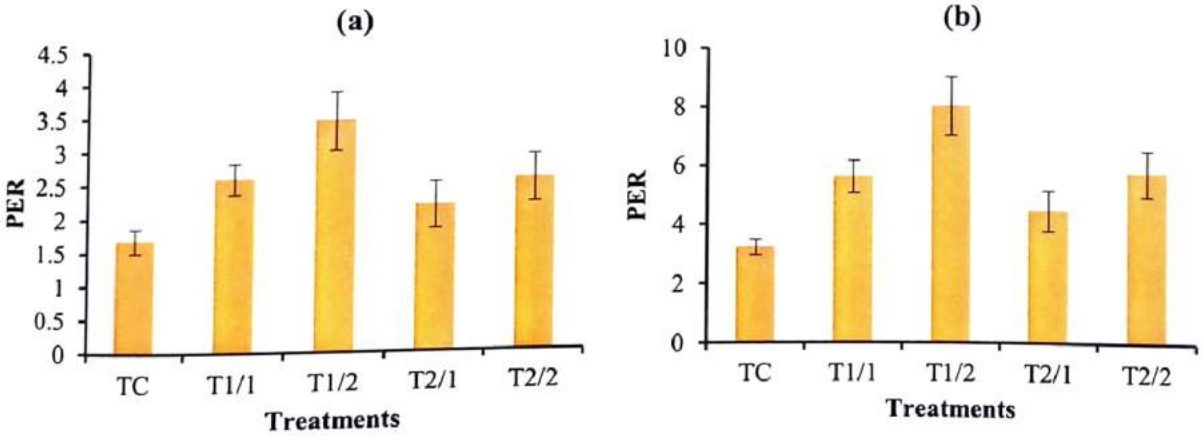

Fig. 4.6. PER values in different treatments of in-vitro (a) and in-vivo (b) experiment (mean ± SD)

4.2.7. Survival rate

In case of in-vitro experiment, no significant difference ($P>0.05$) was found among the treatments. But the overall highest survival rate was observed in TC followed by T2/1 and T2/2. The lowest mean value was achieved in both T1/1 and T1/2 (Tab. 4.2).

But in case of in-vivo experiment starvation had a significant impact on survival of amur common carp fingerlings. However, the highest mean survival rate was achieved in T2/1 (97.33 ± 2.31 %), followed by TC (96.67 ± 1.15 %), T1/1 (94.67 ± 1.15 %), T2/2 (93.33 ± 1.15 %), and T1/2 (91.33 ± 1.15 %) (Tab. 4.3). According to Jhingran (1991) 10% mortality in regular stocking of carps is usually considered normal and acceptable. In this present study the survival rate of amur common carp was always found around 90%.

Growth functions of amur common carp in both in-vitro and in-vivo experiment after 120 days showed that maximum body weight gain was accomplished in both TC and T2/1 feeding regimes. Superior growth of fish in TC was apparent because of receiving continuous and maximum amount of diet. But the similar weight gain in T2/1 indicated that 1-day food deprivation after 2 consecutive days of feeding (feed deprivation @ 33.33 % of the total culture period) had no significant impact upon growth and seemed to be readily compensated by amur common carp fingerlings upon refeeding. Whereas in T1/1, T1/2 and T2/2 poor compensatory growth was found in this present study. Pegu (2010) reported complete compensatory growth of *Oreochromis niloticus* subjected to 1-day starvation followed by two days of satiation feeding. Results indicated that feed deprivation up to 33.33 % of the total culture period can be compensated, but more than that really have adverse effect on growth. Quite similar result was found by Gabriel *et al.* (2018) in case of *Oreochromis mossambicus,* where short-term feed deprivation and refeeding cycles (2-days starvation /2-days refeeding, and 2-days starvation /3-days refeeding) had impact on growth performance and nutrient utilization of *O. mossambicus*, but 2-days starvation /4-days refeeding cycle (feed deprivation @ 33.33 % of the total culture period) appeared to be the best among deprived treatment groups.

Contrary to our present study, best compensatory growth was found in 1-day deprivation and 1-day feeding group by Rohul Amin *et. al.* (2012) in case of *Pangasinodon hypopthalmus* might be due to lack of such feeding group having more than 1 day of refeeding followed by 1 day of starvation.

So, it can be assumed that 1 or 2 days of cyclical short-term feed deprivation and overall 1/3 of the entire culture period of food deprivation can be fully compensated upon refeeding.

O. Mossambicus (Christensesn and Mclean, 1998), Nile tilapia (Gao and Lee, 2012; Passinato *et al.*, 2015), and other fish species like *Centropomus parallelus* (Ribeiro and Tsuzuki, 2010) and Sparus aurata (Ribeiro and Tsuzuki, 2010) also showed poor compensatory growth in fish subjected to longer periods of deprivation (Peres *et al.*,2011).

According to Xie *et al.* (2001), compensatory growth is brought on by an increase in feed intake brought on by hyperphagia. In our present study, it has been evidenced that both the nitrogenous and phosphorus content available in water as well as in soil are found to be significantly lower in the deprived treatment groups which might ensure lesser wastage of feed when compared to daily feeding group due to the improved feed intake resulted by extreme hyperphagia in fishes. The higher feed intake and extreme hyperphagia may also be proved by the quick feed receiving tendency of the fishes submitted to deprived feeding groups observed during feeding, which might trigger the compensatory growth in T2/1.

Several studies advocated that compensatory growth in fish could be caused by low basal metabolism (Fu, Xie, and Cao, 2005) or perfected feed utilization indices like FCR and FCE (Foss *et al.*, 2009; Adakli and Tasbozan, 2015) after a period of starvation or intermittent feeding. An increase in digestive capacity of fish during the refeeding is attributed to the improvement in these parameters as reported by Bolasina *et. al.* (2006). Increased digestive activitiy was found in *Labeo rohita* (Yengkokpam *et al.*, 2013), and Atlantic salmon (Krogdahl and Bakke-Mckellep, 2005) subjected to feed deprivation and refeeding regimes. In the meantime, Zhang *et al.* (2010) found greater protease activity in *F. chinesis* juveniles after refeeding, as well as enhanced FCE and feed intake metrics and superior growth performance in contrast to the control group. Accordingly, lower FCR and higher FCE were reported in fish subjected to 1-day starvation followed by 2 days of feeding compared to those fed daily in the present study. This could be due to increased digestive enzyme activity during the refeeding interval, as evidenced by previous research (Yengkokpam *et al.*, 2013; Krogdahl and BakkeMckellep, 2005). This also suggests that short-term feed deprivation/refeeding cycles could be an effective way to reduce feed consumption without impacting fish farm output.

Table 4.2. Different growth parameters of amur common carp fingerlings in different treatments of in-vitro experiment (mean±SD, n=15)

Treatment	Survival (%)	Weight Gain (g)	SGR (% day^{-1})	% Weight Gain	Length Increment (cm)	FCE	PER
TC	94.45 ± 4.81^{a}	26.68 ± 1.85^{c}	1.97 ± 0.06^{c}	1112.61 ± 81.69^{c}	7.33 ± 0.28^{c}	0.47 ± 0.05^{a}	1.68 ± 0.18^{a}
T1/1	86.11 ± 4.82^{a}	19.54 ± 1.2^{b}	1.71 ± 0.06^{b}	832.26 ± 39.5^{b}	6.16 ± 0.2^{b}	0.73 ± 0.07^{b}	2.6 ± 0.23^{b}
T1/2	86.11 ± 4.82^{a}	14.05 ± 0.61^{a}	1.44 ± 0.03^{a}	599.62 ± 52.4^{a}	5.06 ± 0.18^{a}	0.97 ± 0.12^{c}	3.47 ± 0.44^{c}
T2/1	91.67 ± 8.34^{a}	25.11 ± 2.16^{c}	1.92 ± 0.08^{c}	1053.53 ± 91.11^{c}	7.09 ± 0.34^{c}	0.61 ± 0.1^{ab}	2.19 ± 0.35^{ab}
T2/2	88.89 ± 4.82^{a}	18.87 ± 1.07^{b}	1.68 ± 0.05^{b}	792.45 ± 8.69^{b}	6.02 ± 0.14^{b}	0.73 ± 0.1^{b}	2.59 ± 0.36^{b}

Table 4.3. Different growth parameters of amur common carp fingerlings in different treatments of in-vivo experiment (mean±SD, n=30)

Treatment	Survival (%)	Weight Gain (g)	SGR (% day^{-1})	% Weight Gain	Length Increment (cm)	FCE	PER
TC	96.67 ± 1.15^{c}	204.76 ± 15.55^{c}	3.71 ± 0.06^{d}	8536.48 ± 642.21^{c}	20.06 ± 0.67^{c}	0.90 ± 0.07^{a}	3.22 ± 0.25^{a}
T1/1	94.67 ± 1.15^{bc}	158.65 ± 7.49^{b}	3.59 ± 0.02^{a}	6760.13 ± 257.69^{b}	17.92 ± 0.36^{b}	1.57 ± 0.15^{b}	5.6 ± 0.55^{b}
T1/2	91.33 ± 1.15^{a}	117.63 ± 4.09^{a}	3.28 ± 0.05^{c}	5015.67 ± 323.26^{a}	15.62 ± 0.26^{a}	2.24 ± 0.28^{c}	7.99 ± 0.99^{c}
T2/1	97.33 ± 2.31^{c}	193.4 ± 16.65^{c}	3.67 ± 0.07^{d}	8114.86 ± 689.94^{c}	19.56 ± 0.75^{c}	1.24 ± 0.19^{ab}	4.44 ± 0.67^{ab}
T2/2	93.33 ± 1.15^{ab}	154.45 ± 9.51^{b}	3.51 ± 0.04^{b}	6486.52 ± 103.98^{b}	17.68 ± 0.42^{b}	1.59 ± 0.21^{b}	5.68 ± 0.77^{b}

* In both table 4.2 and 4.3 column wise altered superscript statistically implies significant difference ($P < 0.05$)

4.3. Enzyme assay

4.3.1. Digestive enzyme assay

4.3.1.1. Protease activity

The protease activity of different treatment groups is depicted in Tab. 4.4 and Fig. 4.7. The activity is expressed as micromoles of tyrosine released/min/mg protein. Periodic starvation significantly influenced the protease activity in the present experiment and the relationship was such that higher the degree of starvation, lower the protease activity. However, the highest mean value of protease activity was obtained in T2/1 (24.76 ± 0.74) where the cycle of 2 consecutive days of feeding and 1 day of starvation was practised, and that was followed by TC (19.97 ± 1.06), T1/1 (11.2 ± 1.17), T2/2 (10.71 ± 0.86) and T1/2 (8.38 ± 1.35) respectively.

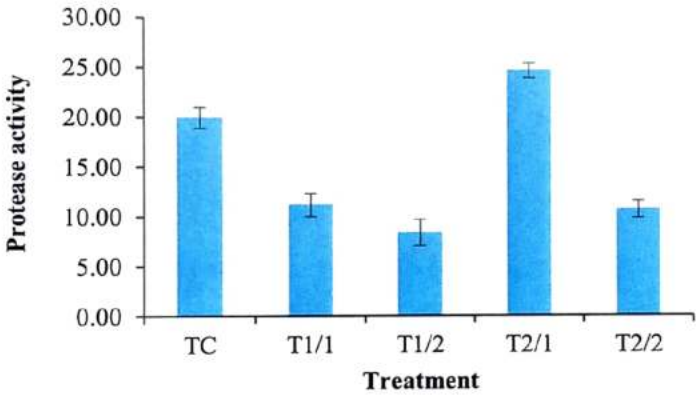

Fig. 4.7. Protease activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

4.3.1.2. Amylase activity

Amylase activity is expressed as micromoles of maltose released/min/mg protein. Likewise, protease activity periodic starvation showed similar significant influence over the amylase activity also in the present experiment. The highest mean value was observed in T2/1 (13.38 ± 0.86) followed by TC (10.86 ± 0.82), T1/1 (9.53 ± 0.87), T2/2 (9.12 ± 0.63) and T1/2 (7.61 ± 0.76) respectively (Tab. 4.4 and Fig. 4.8). There was significant difference ($P<0.05$) in the amylase activity among the treatment groups.

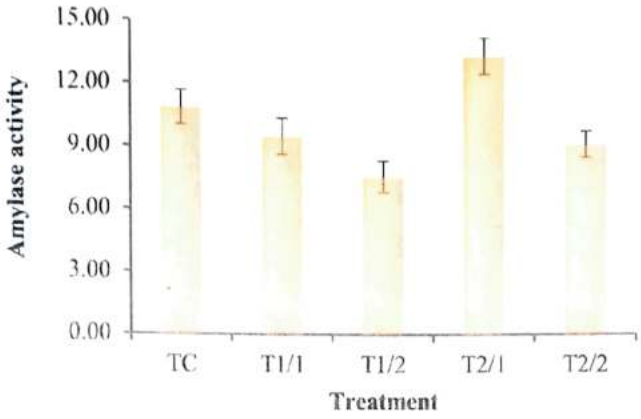

Fig. 4.8. Amylase activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

4.3.1.3. Lipase activity

Lipase activity is expressed as units/mg protein. Periodic starvation had no significant impact on lipase activity in the present study, and that's why, no significant difference ($P>0.05$) was found in lipase activity among the treatment groups. However, the treatment wise lipase activity values were 0.346 ± 0.021, 0.333 ± 0.006, 0.332 ± 0.007, 0.347 ± 0.006 and 0.335 ± 0.009 in TC, T1/1, T1/2, T2/1 and T2/2 respectively (Tab. 4.4 and Fig. 4.9).

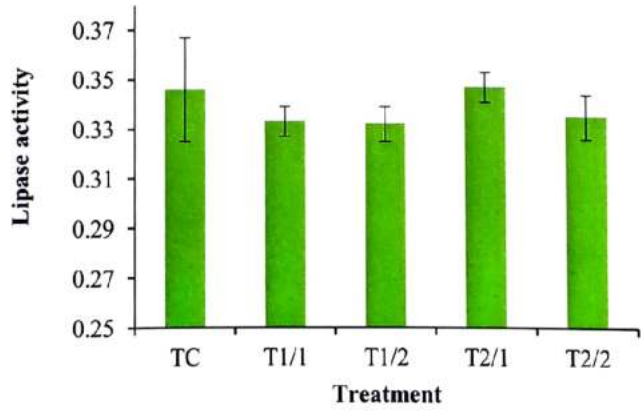

Fig. 4.9. Lipase activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

Table. 4.4. Digestive enzyme activity of amur common carp fingerlings in different treatments (mean ± SD).

Treatment	Protease activity[1]	Amylase activity[2]	Lipase activity[3]
TC	19.97 ± 1.06^{c}	10.86 ± 0.82^{c}	0.346 ± 0.021^{a}
T1/1	11.2 ± 1.17^{b}	9.53 ± 0.87^{bc}	0.333 ± 0.006^{a}
T1/2	8.38 ± 1.35^{a}	7.61 ± 0.76^{a}	0.332 ± 0.007^{a}
T2/1	24.76 ± 0.74^{d}	13.38 ± 0.86^{d}	0.347 ± 0.006^{a}
T2/2	10.71 ± 0.86^{b}	9.12 ± 0.63^{b}	0.335 ± 0.009^{a}

Column wise altered superscript statistically implies significant difference ($P < 0.05$).

[1] activity expressed as micromoles of tyrosine released/min/mg protein

[2] activity expressed as micromoles of maltose released/min/mg protein

[3] activity expressed as units/mg protein

The breakdown of large nutrients into small absorbable subunits in the animal's digestive tract is heavily reliant on the enzymes available (Cho, 1987). It has been found that starvation reduces digestive enzyme activity, which can be restored to a great extent upon refeeding (Krogdahl and Bakke-Mckellep, 2005). In our present study, it has been evidenced that the digestive enzyme activities were markedly higher in T2/1, where 1 day of starvation followed by 2 consecutive days of feeding was practised and this result might suggest the improved digestive capacity of amur common carp in T2/1 compared to the other treatments. Elevated protease and amylase activity suggested that the test fishes had a superior ability to digest protein and carbohydrate, respectively, and this might assign to compensatory growth in T2/1, which is corroborated with Yengkokpam *et al.*, 2013. However, the enzyme activity of all the three digestive enzymes exhibited strong positive correlation with weight gain of amur common carp (Tab. 4.5).

Table. 4.5. Correlation between weight gain and digestive enzyme activity of amur common carp fingerlings

	Weight gain	Protease	Lipase	Amylase
Weight gain	1			
Protease	0.846135	1		
Lipase	0.642169	0.60758	1	
Amylase	0.761025	0.90652	0.438691	1

4.3.2. Metabolic enzyme assay

4.3.2.1. Lactate dehydrogenase (LDH) activity

Hepatic LDH activity of amur common carp fingerlings in different treatment groups is presented in Tab. 4.6 and Fig. 4.10. LDH activity is expressed as Unit/min/mg protein. In the present study LDH activity exhibited a direct relationship with the severity of starvation. The highest mean hepatic LDH activity was observed in T1/2 (4.45 ± 0.46) followed by T2/2 (2.56 ± 0.11), T1/1 (2.56 ± 0.11), T2/1 (2.44 ± 0.1) and TC (1.25 ± 0.1) respectively having highly significant differences (P<0.001) among the treatment groups.

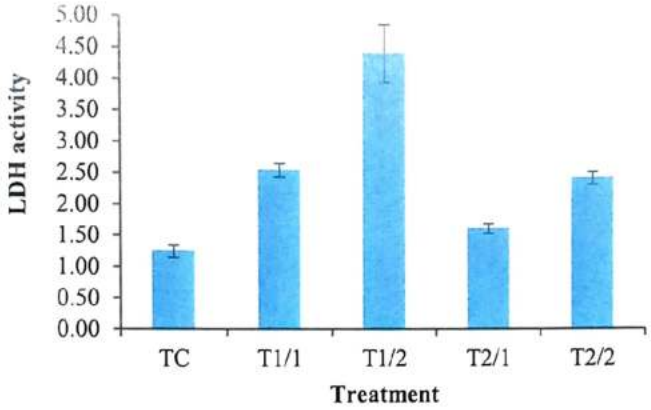

Fig. 4.10. LDH activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

4.3.2.2. Malate dehydrogenase (MDH) activity

MDH activity in the liver tissue of test fishes in different treatment groups are presented in Tab. 4.6 and Fig. 4.11. The activity is expressed as Unit/min/mg protein. Likewise, LDH activity the MDH activity also flashed a direct relationship with feed deprivation in the present experiment, means higher the degree of starvation, higher the MDH activity was. The overall highest mean hepatic MDH activity was obtained in T1/2 (5.27 ± 0.22) followed by T2/2 (4.48 ± 0.14), T1/1 (4.46 ± 0.34), T2/1 (4.06 ± 0.16) and TC (3.59 ± 0.23) respectively having highly significant differences (P<0.001) among the treatment groups.

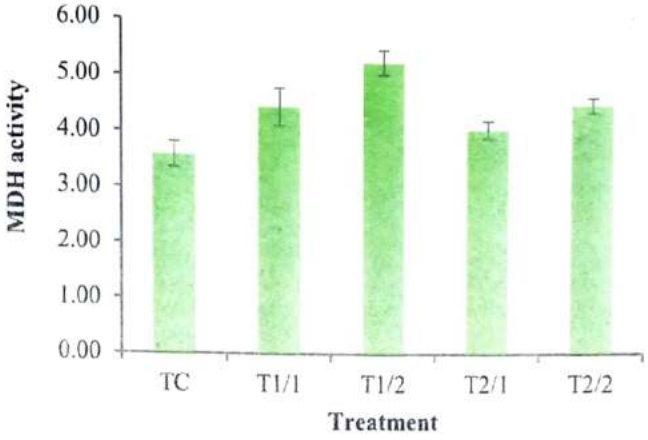

Fig. 4.11. MDH activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

LDH converts lactate to pyruvate in the presence of coenzyme NADH, which is converted to NAD+. Lactate dehydrogenase is the terminal enzyme of the glycolysis pathway. Hence, lactate dehydrogenase helps in maintaining the glycolysis cycle by supplying NAD+. In the presence of enough oxygen, pyruvate enters into the Krebs cycle, but when there is an oxygen shortage in the tissue, pyruvate is converted to lactate (Murray *et al.*, 2000). Generally, LDH activity increases in stress viz. starvation, reproductive stress etc (Vijayaraghavan & Rao 1986). The fishes in the T2/1 group showed significantly ($P<0.05$) lower LDH activity in liver, when compared to other starved treatment groups might be due to reduced stress. During stress, elevated energy requirement of fishes is more leads to increased activity of key enzymes of Krebs cycle in liver such as LDH and MDH for energy production. Augmented MDH activity indicates increased energy demand in fishes. In this present study LDH and MDH were negatively correlated with growth in terms of body weight gain of amur common carp (Tab. 4.7). Reduced hepatic LDH and MDH activity in T2/1 group suggest reduced stress, which might strengthen the growth.

4.3.2.3. Alanine transaminase (ALT) activity

Serum ALT activity of amur common carp fingerlings was significantly impacted by feed deprivation and exhibited a direct relationship with the severity of starvation. Although the lowest mean ALT activity was found in TC (12.25 ± 0.25) but among the starved group the lowest ALT activity was achieved in T2/1 (14.28 ± 0.23) followed by T1/1

(16.39 ± 0.18), T2/2 (17.56 ± 0.38), and T1/2 (18.65 ± 1.12) having highly significant difference (P<0.001) among the treatments (Tab. 4.6 and Fig. 4.12).

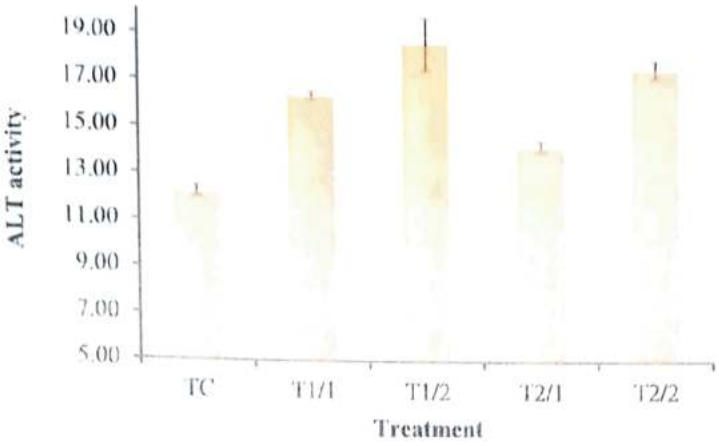

Fig. 4.12. ALT activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

4.3.2.4. Asparate transaminase (AST) activity

Similar to ALT activity, serum AST activity was also notably affected by feed deprivation and all the feed-deprived groups experienced increased AST activity in blood parallel to the degree of feed deprivation. The lowest mean AST activity was found in TC (50.63 ± 0.27). Amongst starved group T2/1 flashed the lowest ALT activity (53.5 ± 0.44), which was followed by T2/2 (63.42 ± 0.29), T1/1 (70.19 ± 0.96), and T1/2 (101.09 ± 0.39) having highly significant difference (P<0.001) among the treatments (Tab. 4.6 and Fig. 4.13).

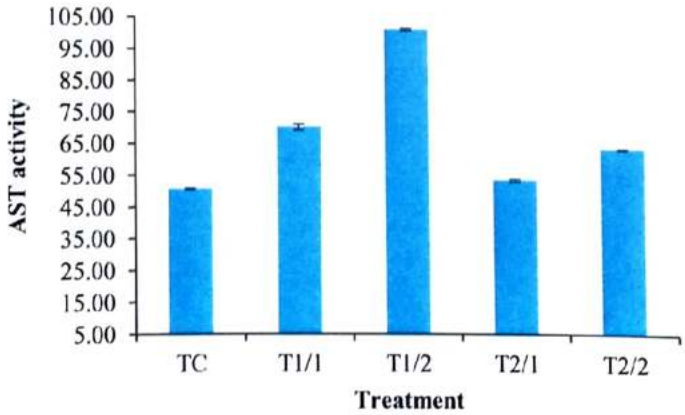

Fig. 4.13. AST activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

Both ALT and AST are linked to parenchymal cells in the liver. Due to their association with cell necrosis of the liver and skeletal or cardiac muscle, starvation, and vitamin E deficiency, these are commonly measured clinically as markers for liver health (Pakhira *et al.*, 2015). When liver cells are harmed, ALT and AST leak into the bloodstream. They are therefore a superior indicator of acute liver damage (Coppo *et al.*, 2003). In our study, serum ALT and AST levels increased with the severity of starvation, possibly indicating liver damage and starvation stress. However, the values of correlation co-efficient revealed that ALT and AST had strong negative correlation with body weight gain of amur common carp (Tab. 4.7).

Table 4.6. Metabolic enzyme activity of amur common carp fingerlings in different treatments (mean ± SD)

Treatment	LDH[1]	MDH[1]	ALT[2]	AST[2]
TC	1.25 ± 0.1^{a}	3.59 ± 0.23^{a}	12.25 ± 0.25^{a}	50.63 ± 0.27^{a}
T1/1	2.44 ± 0.1^{b}	4.46 ± 0.34^{b}	16.39 ± 0.18^{c}	70.19 ± 0.96^{d}
T1/2	4.45 ± 0.46^{c}	5.27 ± 0.22^{c}	18.65 ± 1.12^{e}	101.09 ± 0.39^{e}
T2/1	1.63 ± 0.08^{a}	4.06 ± 0.16^{b}	14.28 ± 0.23^{b}	53.5 ± 0.44^{b}
T2/2	2.56 ± 0.11^{b}	4.48 ± 0.14^{b}	17.56 ± 0.38^{d}	63.42 ± 0.29^{c}

Column wise altered superscript statistically implies significant difference ($P < 0.05$).

[1] activity expressed as units/min/mg protein

[2] activity expressed as IU/L blood

Table. 4.7. Correlation between weight gain and metabolic enzyme activity of amur common carp fingerlings

	Weight gain	LDH	MDH	ALT	AST
Weight gain	1				
LDH	-0.9188	1			
MDH	-0.87891	0.943506	1		
ALT	-0.89234	0.829692	0.853529	1	
AST	-0.90068	0.980039	0.902865	0.808163	1

4.3.3. Oxidative stress enzyme assay

4.3.3.1. Superoxide dismutase (SOD) assay

In the present experiment, the hepatic SOD activity of amur common carp was significantly impacted by starvation. Treatment wise hepatic SOD activity is presented in Tab. 4.8 and Fig. 4.14. The significant lowest SOD activity was found in T2/1(12.41 ± 0.75) followed by T1/1 (14.14 ± 1.6), TC (20.2 ± 3.03), T2/2 (24.47 ± 1.9), T1/2 (43.95 ± 1.56). Though the difference in SOD activity among the treatments remained highly significant (P<0.001) but no significant differences was found between T1/1 and T2/1.

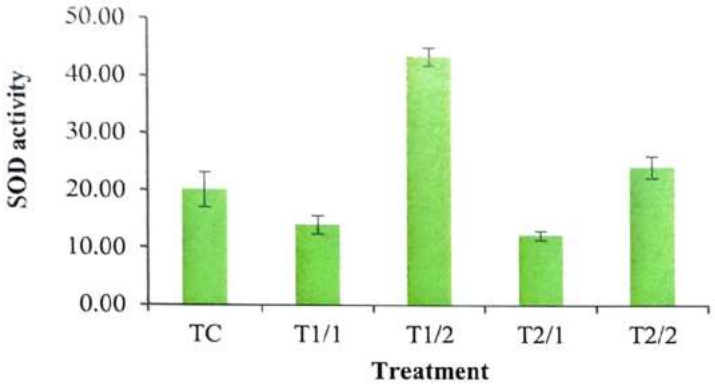

Fig. 4.14. SOD activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

4.3.3.2. Catalase assay

In case of catalase activity all the feed deprived groups experienced an elevated value in the liver tissue of amur common carp except T2/1 (Tab. 4.8 and Fig. 4.15) when compared to control. In ascending order, the lowest hepatic catalase activity was evidenced in T2/1 (0.38 ± 0.07) followed by TC (1.61 ± 0.2), T1/1 (1.67 ± 0.32), T2/2 (3.13 ± 0.75), and T1/2 (4.12 ± 0.62). However, the statistical difference remained highly significant (P<0.001) among the treatments.

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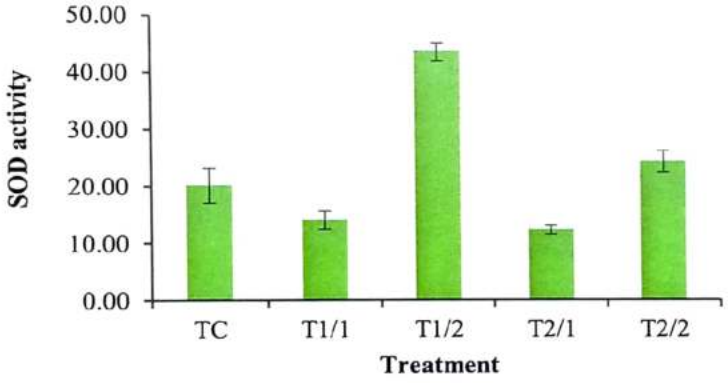

Fig. 4.14. SOD activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

4.3.3.2. Catalase assay

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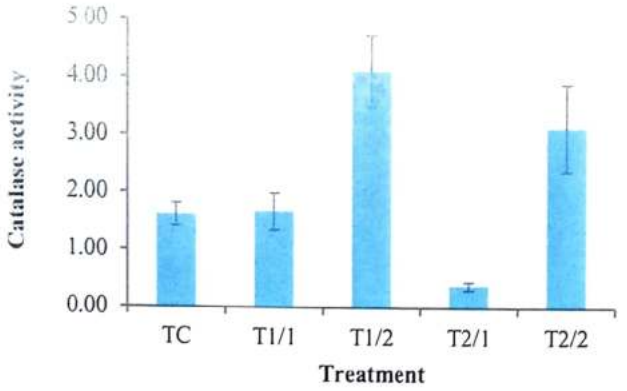

Fig. 4.15. Catalase activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

4.3.3.3. Glutathione peroxidase (GPx) assay

The interaction between periodic starvation and oxidative stress influenced the hepatic GPx activity of amur common carp (Tab. 4.8 and Fig. 4.16). GPx activity exhibited a direct relationship with the degree of feed deprivation. However, the lowest activity was found in TC (1.32±0.26) followed by T2/1 (2.06±0.21), T1/1 (2.53±0.27), T2/2 (3.95±0.29), T1/2 (5.09±0.37). One-way anova revealed highly significant difference ($P<0.001$) among the treatments.

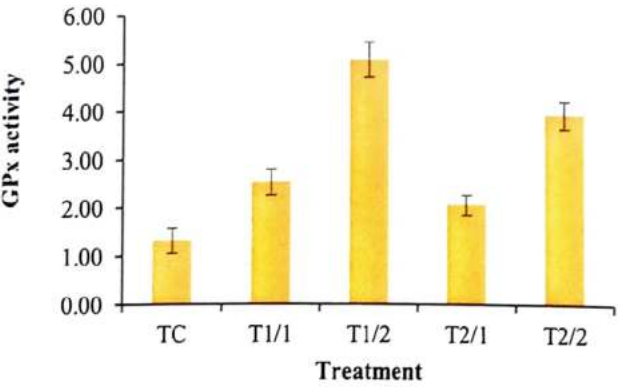

Fig. 4.16. GPx activity of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

Three anti-oxidative enzymes, Viz. superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) are indications of oxidative stress in fish (Pandey *et al.*, 2003; Pascual *et al.*, 2003). SOD plays an anti-oxidative role by detoxifying O_2^- and releasing H_2O_2, while catalase reduces the H_2O_2 generated. Glutathione peroxidase (GPx), an intracellular antioxidant enzyme converts H_2O_2 to water enzymatically in order to limit its harmful effects. In the present study, the test fish amur common carp exhibited adoptive response to the oxidative stress resulted by periodic starvation and maybe that's why the hepatic SOD, catalase, and GPx activity were increased and remained parallel with the intensity of starvation. But higher SOD and catalase activity in daily feeding group might be due to environmental stress. However, the growth of amur common carp exhibited a strong negative correlation with the enzyme activity of all three anti-oxidative enzyme (Tab. 4.9).

Table 4.8. Anti-oxidative enzyme activity of amur common carp fingerlings in different treatments (mean ± SD)

Treatment	SOD[1]	Catalase[2]	GPx[2]
TC	20.2 ± 3.03^{b}	1.61 ± 0.2^{b}	1.32 ± 0.26^{a}
T1/1	14.14 ± 1.6^{a}	1.67 ± 0.32^{b}	2.53 ± 0.27^{bc}
T1/2	43.95 ± 1.56^{d}	4.12 ± 0.62^{d}	5.09 ± 0.37^{e}
T2/1	12.41 ± 0.75^{a}	0.38 ± 0.07^{a}	2.06 ± 0.21^{b}
T2/2	24.47 ± 1.9^{c}	3.13 ± 0.75^{c}	3.95 ± 0.29^{d}

Column wise altered superscript statistically implies significant difference ($P < 0.05$).

[1] activity expressed as Unit

[2] activity expressed as nM of H_2O_2 decomposed/min/mg protein

Table. 4.9. Correlation between weight gain and anti-oxidative enzyme activity of amur common carp fingerlings

	Weight gain	SOD	CAT	GPx
Weight gain	1			
SOD	-0.73773	1		
CAT	-0.80353	0.870912	1	
GPx	-0.8978	0.811931	0.825553	1

4.4. Biochemical assay

4.4.1. Blood glucose

Blood glucose level of amur common carp in different treatment groups are presented in Fig. 4.17. In the present study, starvation had a notable influence on blood glucose level of amur common carp. However, the highest blood glucose level was found in TC (84.78 ± 5.87 mg/dl) followed by T2/1 (76.81 ± 4.29 mg/dl), T1/1 (64.38 ± 1.9 mg/dl), T2/2 (56.16 ± 1.2 mg/dl), and T1/2 (48.35 ± 1.74 mg/dl) having significant difference ($P<0.05$) treatment groups.

As glucose is a vital fuel for many tissues, it is especially crucial that glucose levels be maintained during starvation (Gillis and Ballantyne, 1996). Blood glucose levels may biochemically serve as a reliable predictor of starvation stress (Mahajan & Dheer, 1983). Each species has a different level of glucose homeostasis during starvation. The ability to mobilise tissue reserves has been directly linked to the maintenance of glycaemia during food restriction (Moon *et al.*, 1989; Figueiredo-Garutti *et al.*, 2004; Larsen *et al.*, 2001). When the blood glucose level is too low, it means the cells became energy-starved. In this present study, lower blood glucose level was reported in all the starved groups when compared to control, which might refer more energy demand of the fishes subjected to feed depravation. However decreased blood glucose level in starved groups was also evidenced by Navarro *et al.* (1992) in case of *Salmo trutta fario*, and Pottinger *et al.* (2003) in case of *Oncorhynchus mykiss*.

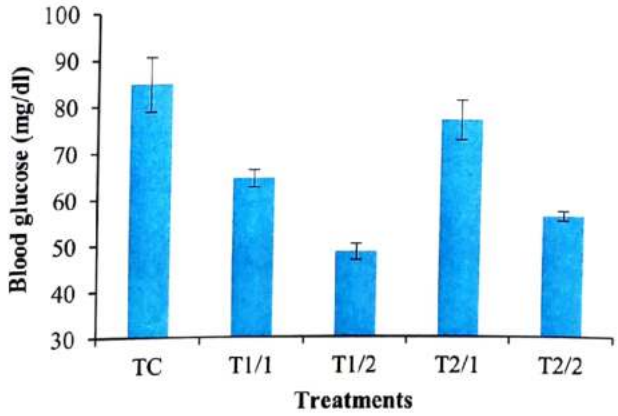

Fig. 4.17. Blood glucose level of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

4.4.2. Total serum protein (TP)

Proteins are the most vital component of serum. Serum proteins are classified into two groups: albumin and globulins. At the end of the experiment it has been evidenced that total serum protein level in amur common carp fingerlings significantly differed ($P<0.05$) among the treatment groups. The highest mean serum protein was achieved in T2/2 (4.18 ± 0.03 mg/dl), followed by T1/2 (4.14 ± 0.05 mg/dl), T1/1 (4.09 ± 0.03 mg/dl), T2/1 (3.45 ± 0.09 mg/dl), and TC (3.12 ± 0.05 mg/dl). However, no significant difference remained among T1/1, T1/2, and T2/2. Total serum protein level of amur common carp in different treatment groups are presented in Tab. 4.10 and Fig. 4.18.

4.4.3. Serum albumin

The highest level of albumin in serum was found in T2/2 (1.85 ± 0.02 mg/dl) followed by T1/1 (1.59 ± 0.02 mg/dl), T1/2 (1.45 ± 0.01 mg/dl), T2/1 (1.44 ± 0.01 mg/dl), and TC (0.96 ± 0.02 mg/dl). However, the statistical difference among the treatment groups was noteworthy ($P<0.05$). Treatment wise serum albumin level is flashed in Tab. 4.10 and Fig. 4.18.

4.4.4. Serum globulin

Serum globulin level also significantly differed ($P<0.05$) among the treatment groups. The highest level of globulin in serum was found in T1/2 (2.69 ± 0.06 mg/dl) followed by T1/1 (2.51 ± 0.04 mg/dl), T2/2 (2.33 ± 0.02 mg/dl), TC (2.16 ± 0.07 mg/dl), and T2/1 (2.01 ± 0.1 mg/dl). Treatment wise serum globulin level is presented in Tab. 4.10 and Fig. 4.18.

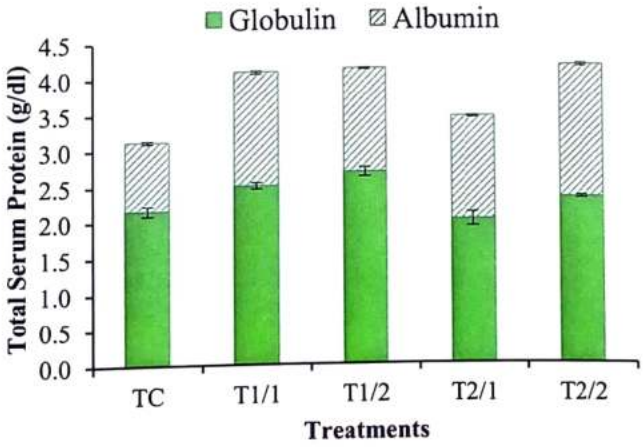

Fig. 4.18. Serum protein level of amur common carp fingerlings in different treatments of in-vivo experiment (mean ± SD)

4.4.5. Albumin-Globulin ratio (A:G ratio)

A:G ratio of amur common carp certainly differed significantly ($P<0.05$) among the treatment groups. However, the overall highest value was obtained in T2/2 (0.79 ± 0.01) followed by T2/1 (0.72 ± 0.04), T1/1 (0.63 ± 0.02), T1/2 (0.54 ± 0.02), and TC (0.45 ± 0.02). Treatment wise serum globulin level is portrayed in Tab. 4.10.

The liver produces albumin, which acts as an osmotic force to keep the vascular space's fluid volume constant. The albumin is a protein transporter and readily accessible protein reserve (Anderson *et al.*, 1979). Most of the serum biochemical active protein in the blood is derived from the gamma globulin fraction. Gamma globulins and other globulins are necessary to keep the immune system healthy. A strong innate response in fishes is thought to be an increase in albumin and globulin levels (Wiegertjes *et al.*, 1996). Since the gamma fraction accounts for the majority of globulin, it can be assumed that subsequently increased globulin levels in treatment groups may indicate enhanced immune response, as well as the preparatory stage for stress mitigation in amur common carp. Stress is the most common cause of elevated TP levels (Haney *et al.* 1992). The lower albumin-globulin ratio in the treatment groups may be due to less albumin protein synthesis and more globulin conversion to cope with immunological function. The presence of high levels of TP and globulin suggested a secondary infection. Stress is the most common cause of elevated TP levels (Haney *et al.* 1992). The lower albumin-globulin ratio in the treatment groups may be due to less albumin protein synthesis and more globulin conversion to cope with immunological function. The presence of high levels of TP and globulin in T1/1, T1/2 and T2/2 suggested a secondary level of stress.

Table 4.10. Serum protein profile of amur common carp fingerlings in different treatments (mean ± SD)

Treatment	Serum Protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	A:G ratio
TC	3.12 ± 0.05^{a}	0.96 ± 0.02^{a}	2.16 ± 0.07^{b}	0.45 ± 0.02^{a}
T1/1	4.09 ± 0.03^{c}	1.59 ± 0.02^{c}	2.51 ± 0.04^{d}	0.63 ± 0.02^{c}
T1/2	4.14 ± 0.05^{c}	1.45 ± 0.01^{b}	2.69 ± 0.06^{e}	0.54 ± 0.02^{b}
T2/1	3.45 ± 0.09^{b}	1.44 ± 0.01^{b}	2.01 ± 0.1^{a}	0.72 ± 0.04^{d}
T2/2	4.18 ± 0.03^{c}	1.85 ± 0.02^{d}	2.33 ± 0.02^{c}	0.79 ± 0.01^{e}

Column wise altered superscript statistically implies significant difference ($P < 0.05$).

4.5. Water quality

4.5.1. Surface water temperature

According to Wootton (1996) temperature is an important component for aquaculture production, which regulates various physiological activities of aquatic animals. Temperature has a direct effect on the important factors like food requirement, food conversation efficiency, oxygen demand and growth (Adhikari, 2011) and ultimately, the health of cultured animals.

During the 120 days' experiment the surface water temperature ranged from 23°C - 29°C in case of in-vitro experiment and 25°C – 32°C in case of in-vivo experiment.

Ayyappan *et al.* (2006) stated that under favorable condition optimum temperature range for various warm water fishes ranges between 24°C to 30°C. Majhi, P (2013) indicated that 29 ± 0.5°C is ideal for the growth of Common carp. Whereas Singh, S. (2019) found the maximum daily feeding rate of Amur common carp at 28°C temperature. So, the range of surface water temperature found in the present study was always conducive to the growth of amur common carp fingerlings.

4.5.2. pH

The value of water pH, recorded at fortnightly interval from different ponds under observation and presented in Fig 4.19. The pH of water ranged from 6.89 to7.69 in case of in-vitro treatment having significant difference ($P<0.05$) among the treatments. In case of in-vivo treatment the water pH ranged from 7.64 to 7.93. But here no significant difference ($P>0.05$) found among the treatment groups.

The value of pH always remained in ideal range for carp culture in all the treatments till the end, but it has been observed that water pH always had the tendency to be reduced might be due to the acidification followed by microbial decomposition of uneaten/undigested feed and faeces. Tucker and D'Abramo (2008) recommended that addition of easily decomposable organic matter produce carbon dioxide in water following decomposition and reduce pH.

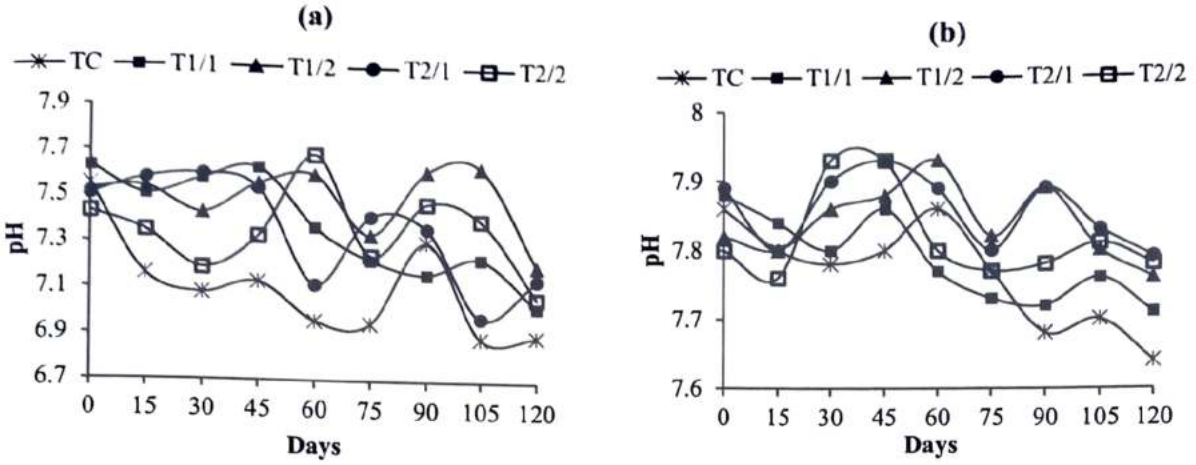

Fig. 4.19. Temporal changes of pH of water in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.5.3. Dissolved oxygen (DO)

In the present study the dissolved oxygen varied significantly ($P<0.05$) among the treatment groups in both in-vitro and in-vivo experiment. It was noticed that where there was more feeding, the less DO in water was (Fig. 4.20). But the level of DO always remained in the desirable range, as desirable DO concentration for carp culture was recommended to be more than 4 mg/l by Jena and Das (2011), which might trigger better growth of fishes in the present study. The overall least mean DO concentration was found in TC (Fig. 4.21), where daily satiate feeding was practiced might be due to the increased respiratory oxygen demand of fishes triggered by increased metabolic rate.

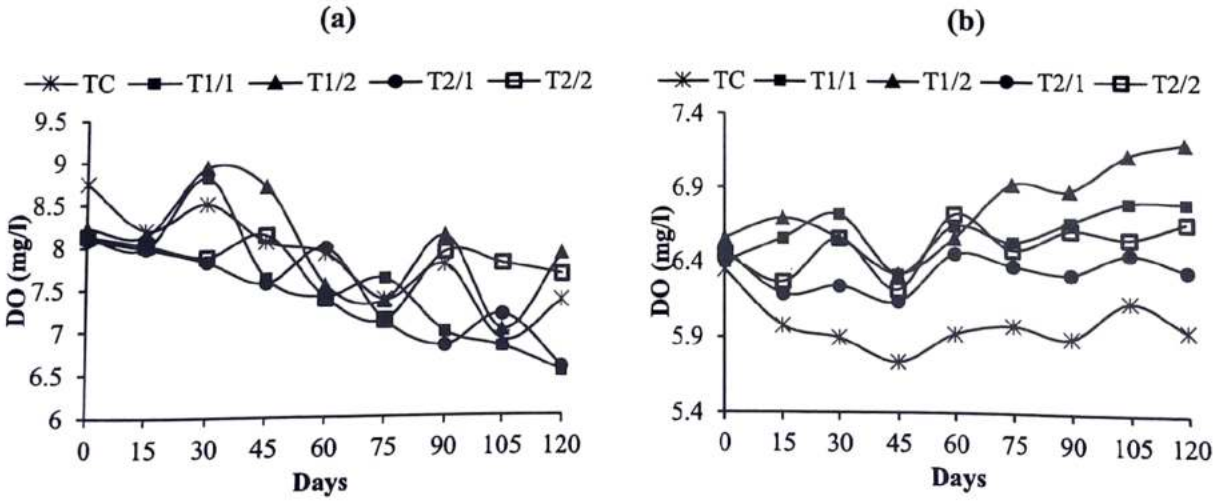

Fig. 4.20. Temporal changes of DO of water in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

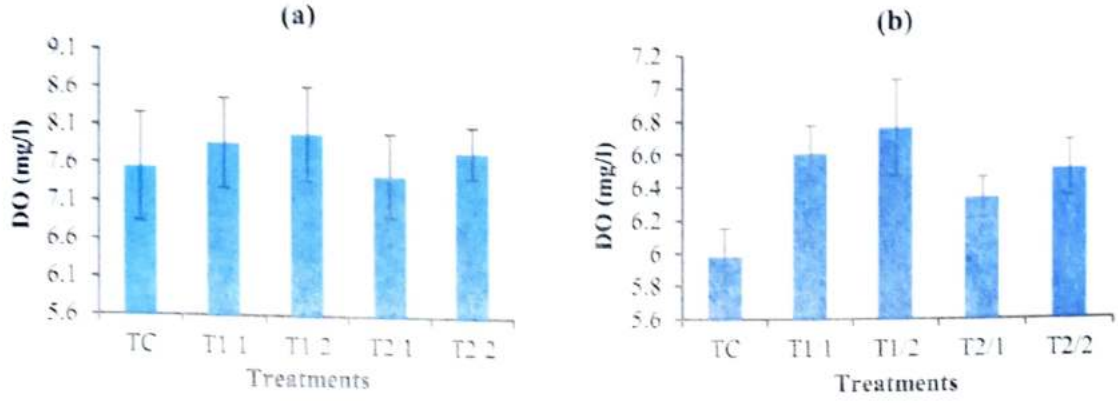

Fig. 4.21. Mean DO concentration of water in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.5.4. Biochemical oxygen demand (BOD_1)

Contrarary to DO, the BOD_1 was found to be higher in those treatments where the feeding was more (Fig. 4.22) and varied significantly ($P<0.05$) among the treatment groups in the present study in both in-vitro and in-vivo experiment.

According to Timmons & Lorsodo, (1994) accumulation of solid wastes in aquaculture system and the aerobic bacterial activity to decompose them generally increase the biochemical oxygen demand within the culture column and deplete oxygen. Not only that, according to Penn *et al.* (2009) oxygen demand for nitrification is also a major contributor to BOD.

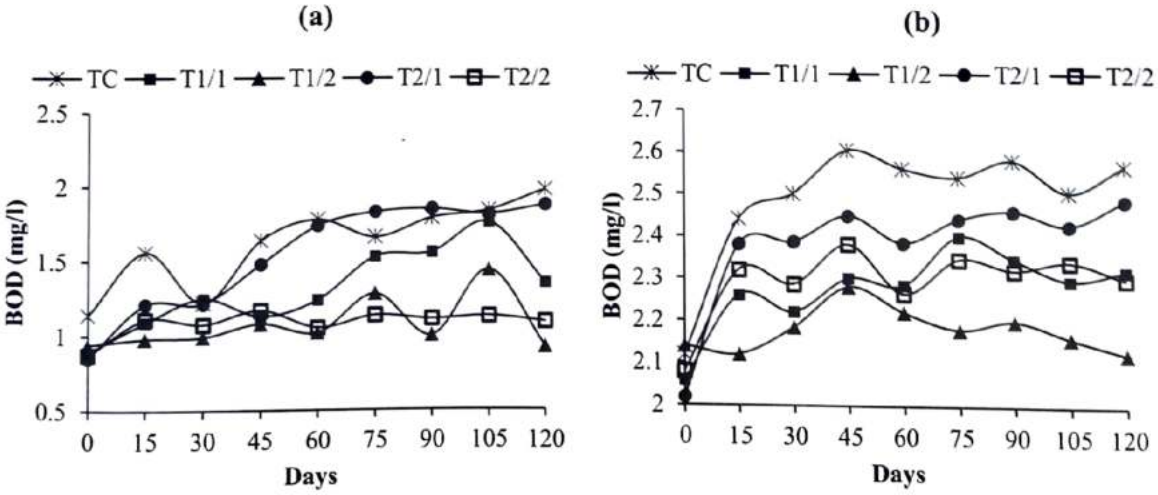

Fig. 4.22. Temporal changes of BOD_1 of water in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.5.5. Total alkalinity

The temporal trend of total alkalinity of water modestly decreased in all the treatments up to the end of both the in-vitro and in-vivo experiment (Fig. 4.23). The treatment difference remained highly significant ($P<0.005$) among the treatment groups.

According to Wurts and Durborow (1992) alkalinity between 75 to 200 mg/l, but not less than 20 mg/l is optimum in any aquaculture pond. Bhatnagar *et al.* (2004) suggested that 80-200 mg/l is pleasant for fish and >300 mg/l is undesirable due to non-availability of carbon dioxide. In this present study total alkalinity always remained pleasant for fish growth.

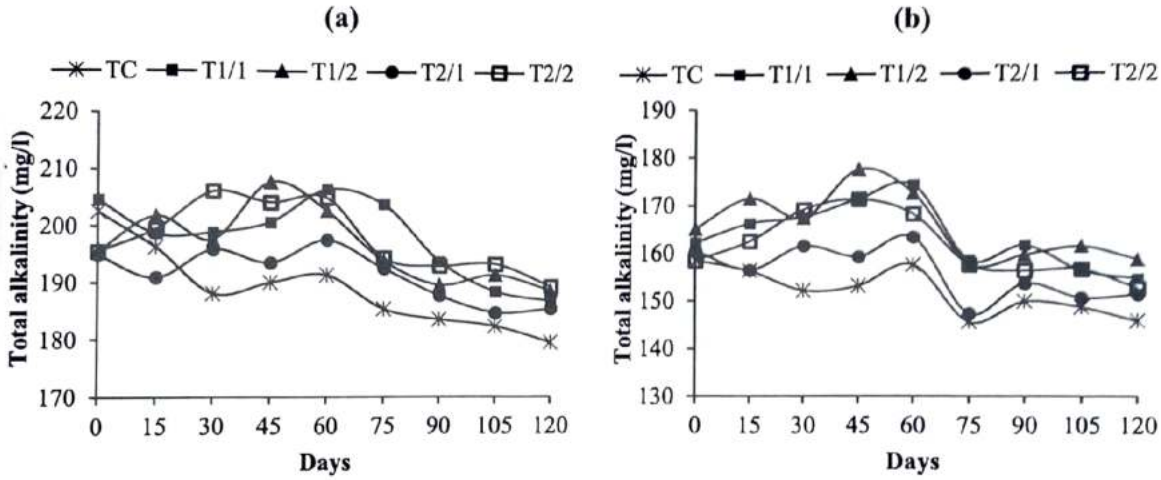

Fig. 4.23. Temporal changes of total alkalinity of water in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.5.5. Total hardness

In this 120 days' trial, the hardness of water showed a minute decreasing trend in all the treatments up to the end (Fig. 4.24). But the statistical difference among the treatment groups was found not significant ($P>0.05$) in both in-vitro and in-vivo experiment.

According to Bhatnagar *et al.* (2004) 75-150 mg/l total hardness is optimum for aquaculture. In this present study the values total hardness was found to be much higher, which might affect the overall growth of the test fishes but did not affect the survivability.

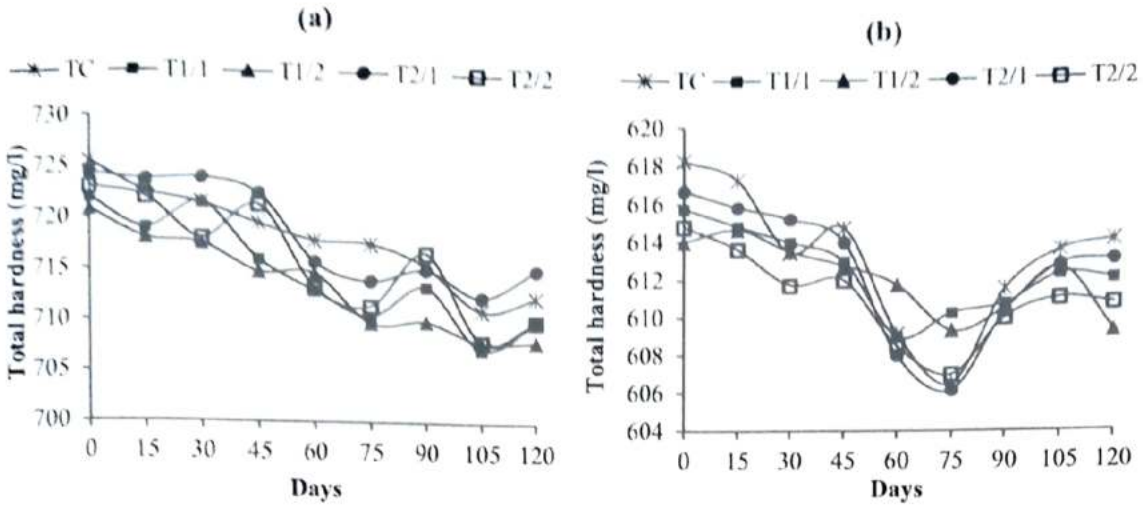

Fig. 4.24. Temporal changes of total hardness of water in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.5.6. Ammonia-Nitrogen (NH_3-N)

In both in-vitro and in-vivo experiment NH_3-N concentration in water gradually increased in all the treatments with a significant treatment difference ($P<0.05$). It has been evidenced that NH_3-N concentration in water varied with the feeding rate and amount of feed provided (Fig. 4.25). Treatments with higher feed supplement found rich in NH_3-N concentration.

For any aquafarm ammonia is usually recommended to be below 1 mg/l (Ajani *et al.*, 2011) and in this study NH_3-N never crossed it. So, the NH_3-N concentration had no adverse effect on growth and survival of amur common carp fingerlings in any way.

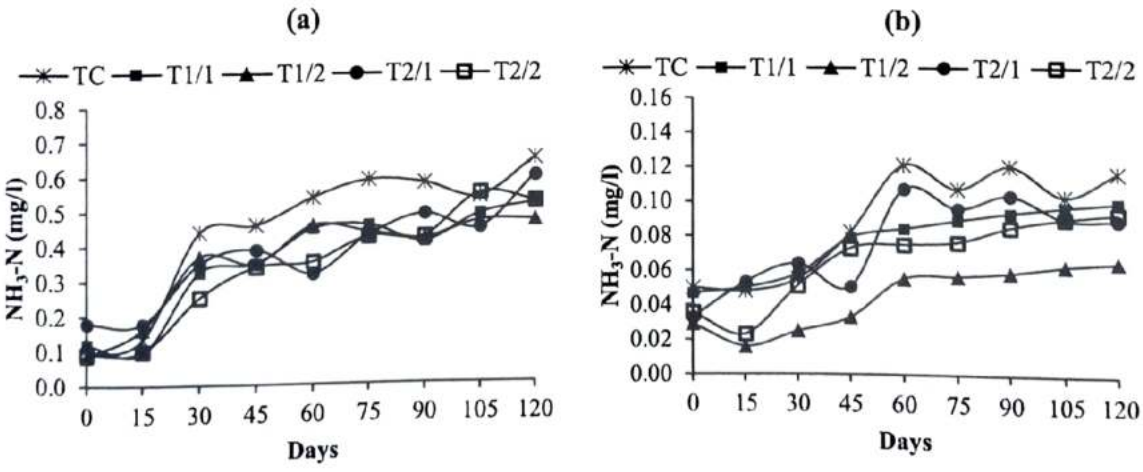

Fig. 4.25. Temporal changes of NH_3-N concentration of water in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.5.7. Nitrate-Nitrogen (NO_3-N)

NO_3-N concentration in water showed a slight increasing trend in all treatments till the end in both in-vitro and in-vivo experiment (Fig. 4.26). However, there was no significant difference ($P>0.05$) among the treatment groups of in-vivo experiment. But in case of in-vitro trial, the statistical difference among the treatments was found significant ($P<0.05$).

Unutilized feed particles and faeces typically increase nitrogenous compounds in water, causing stress in cultured fish (Akinwole *et al.*, 2016). This nitrogen is released into water as ammonia, and further decomposed to nitrite and nitrate (Dauda *et al.*, 2014; Piedrahita, 2003). Even at concentrations as high as 200 mg/l, nitrate, the byproduct of ammonia oxidation, is not toxic to the majority of fish species. In the present study NO_3-N concentration remained within optimal range, hence it might have no adverse effect on amur common carp fingerlings.

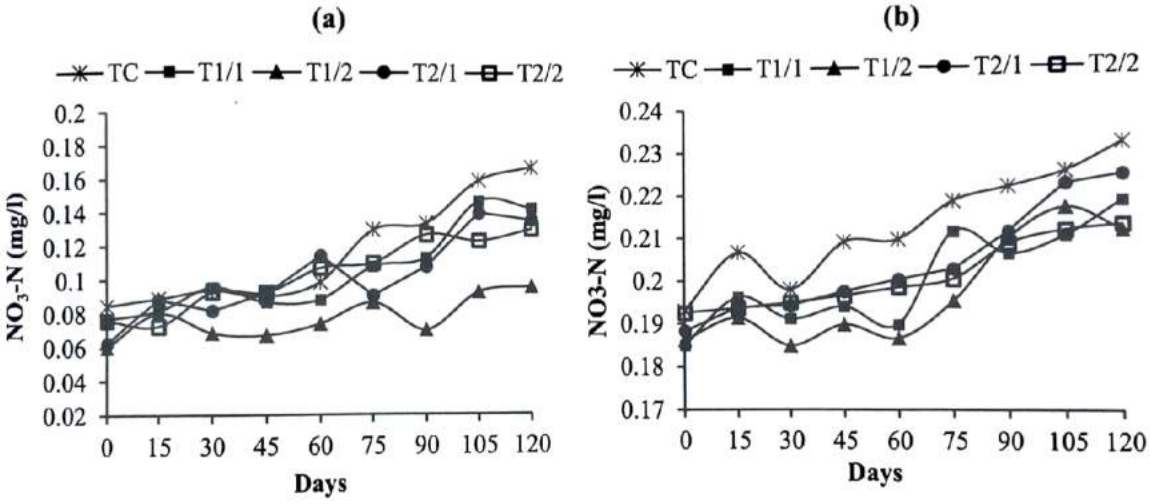

Fig. 4.26. Temporal changes of NO_3-N concentration of water in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.5.8. Ortho Phosphate (PO_4-P)

In the present study, the PO_4-P concentration of water gradually increased in all the treatments of both in-vitro and in-vivo experiment. The treatment difference was not significant ($P>0.05$) among the treatment groups. It has been evidenced that the PO_4-P concentration of water rationally increased with the feeding rate and amount of feed provided.

This increment in ortho-phosphate level might be due to the accumulation of fish excreta and degradation of uneaten feed, which is in corroboration with Van Bussel *et al.*, (2013). According to Boyd and Tucker (2012) the phosphate in feed is transformed into particulate organic phosphate through fish faecal phosphate and triggers the production of dissolved organic phosphate followed by ortho-phosphate.

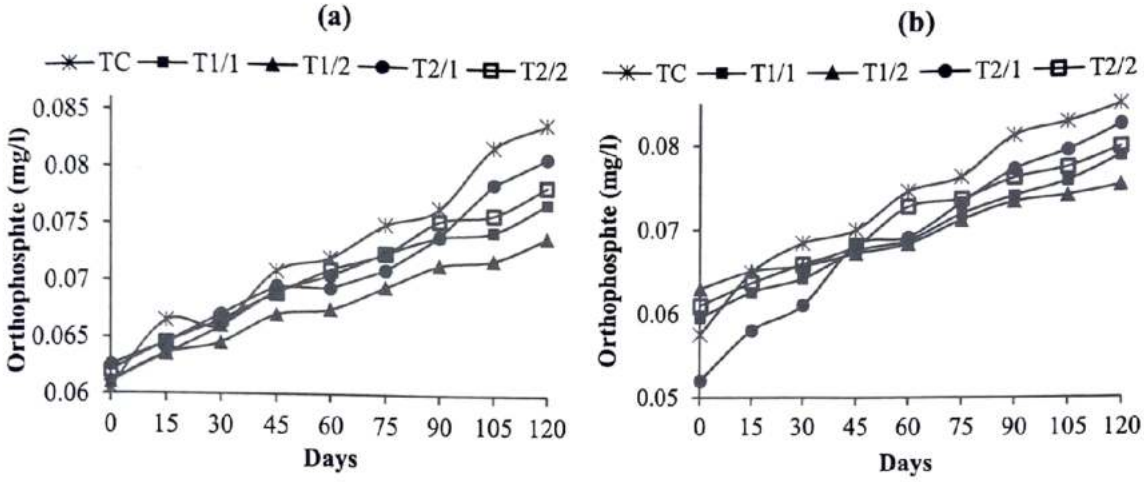

Fig. 4.27. Temporal changes of PO_4-P concentration of water in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

Table 4.11. Different water quality parameters in different treatments of in-vitro experiment (mean±SD)

Parameters	Treatments				
	C	T1/1	T1/2	T2/1	T2/2
pH	6.89-7.55	7.03-7.63	7.21-7.64	6.98-7.58	7.07-7.69
DO (mg/l)	7.54 ± 0.71	7.87 ± 0.59	8 ± 0.62	7.46 ± 0.55	7.79 ± 0.34
BOD_1 (mg/l)	1.62 ± 0.28	1.31 ± 0.27	1.08 ± 0.09	1.54 ± 0.37	1.07 ± 0.17
Total alkalinity (mg/l)	188.87 ± 7.2	198.13 ± 6.97	196.64 ± 6.45	191.57 ± 4.57	197.91 ± 6.04
Total hardness (mg/l)	718.18 ± 4.67	714.75 ± 5.21	713.52 ± 4.72	718.57 ± 4.9	716.01 ± 5.52
NH_3-N (mg/l)	0.447 ± 0.201	0.358 ± 0.155	0.359 ± 0.141	0.376 ± 0.138	0.34 ± 0.166
NO_3-N (mg/l)	0.116 ± 0.032	0.104 ± 0.026	0.077 ± 0.012	0.101 ± 0.025	0.103 ± 0.021
PO_4-P (mg/l)	0.073 ± 0.008	0.07 ± 0.005	0.068 ± 0.004	0.071 ± 0.006	0.071 ± 0.006

Table 4.12. Different water quality parameters in different treatments of in-vivo experiment (mean±SD)

Parameters	Treatments				
	C	T1/1	T1/2	T2/1	T2/2
pH	7.64-7.86	7.71-7.88	7.76-7.93	7.79-7.93	7.78-7.94
DO (mg/l)	5.98 ± 0.17	6.6 ± 0.17	6.76 ± 0.29	6.33 ± 0.12	6.5 ± 0.17
BOD (mg/l)	2.49 ± 0.15	2.28 ± 0.1	2.18 ± 0.05	2.38 ± 0.14	2.29 ± 0.09
Total alkalinity (mg/l)	151.87 ±4.91	163.57 ± 6.73	165.66 ± 7.04	155.93 ± 5.59	161.36 ± 6.68
Total hardness (mg/l)	613.15 ± 3.7	612.43 ± 2.22	612.04 ± 1.98	612.44 ± 3.6	611.04 ± 2.4
NH_3-N (mg/l)	0.089 ± 0.031	0.077 ± 0.02	0.045 ± 0.019	0.076 ± 0.026	0.067 ± 0.025
NO_3-N (mg/l)	0.213 ± 0.013	0.2 ± 0.012	0.197 ± 0.012	0.204 ± 0.013	0.201 ± 0.008
PO_4-P (mg/l)	0.073 ± 0.009	0.069 ± 0.006	0.069 ± 0.004	0.069 ± 0.01	0.071 ± 0.006

4.6. Soil quality

4.6.1. Soil pH

Alike water pH, soil pH also had the tendency in all the treatments of both in-vitro and in-vivo experiment to be reduced gradually till the end of the 120 days' study (Fig. 4.28). The reason might be the acidification of the soil, followed by microbial decomposition of uneaten/undigested feed particle and faecal matter. However, the soil pH always remained in the ideal range for carp culture. On condition in-vitro and in-vivo experiment it ranged from 6.08 to 6.83 and 6.18 to 6.94 respectively.

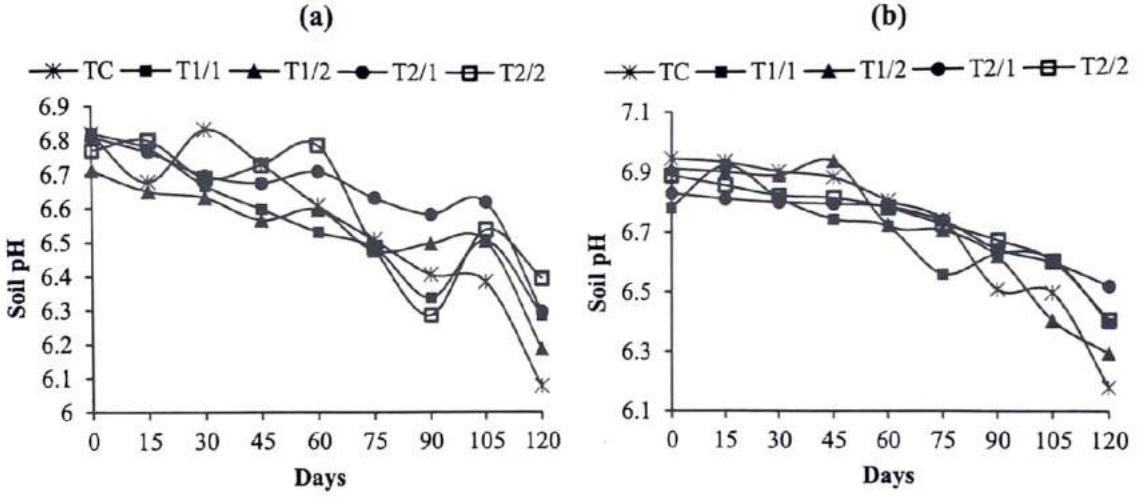

Fig. 4.28. Temporal changes of soil pH in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.6.3. Soil organic carbon

Soil organic carbon refers only to the carbon component of soil organic matter. In both in-vitro and in-vivo experiment it has been evidenced that organic carbon in soil increased gradually in all the treatments till the end of the experiment (Fig. 4.29) having significant difference ($P<0.05$) among the treatments.

The considerable amount of carbohydrate present in the uneaten feed and faecal matter might enriched the soil with organic carbon following decomposition which is supported by Annongu and Joseph, 2008.

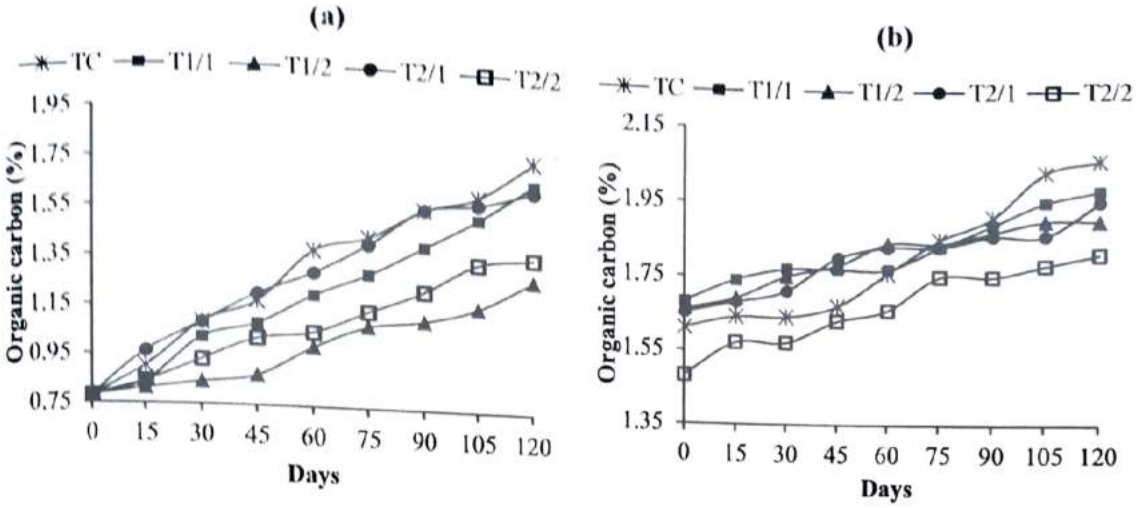

Fig. 4.29. Temporal changes of soil organic carbon in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.4.2. Soil available nitrogen

Soil available nitrogen reflects mineral nitrogen released from organic matter by soil microorganisms. Soil available nitrogen increased progressively in all the treatments till the end of both the in-vitro and in-vivo experiment (Fig. 4.30) having no significant difference ($P>0.05$) among the treatments.

Accumulation of solid organic waste (dead plankton cells, fish faecal solids and uneaten feed) in the surface layer of the pond bottom mud might contribute the available nitrogen in the soil continuously following their decomposition and mineralization (Boyd and Tucker, 2012).

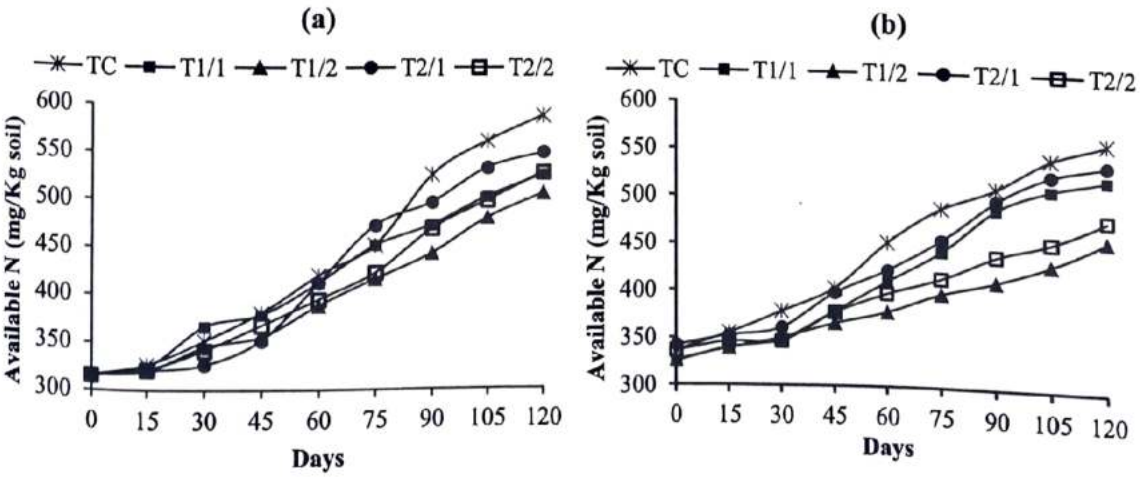

Fig. 4.30. Temporal changes of soil available nitrogen in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.6.4. Soil available phosphorus

The fraction of total phosphorus in soil that is readily available for absorption by plants is referred to as available phosphorus. In both in-vitro and in-vivo experiment of the present study, soil available phosphorus exhibited a gradual increasing trend in all the treatments till the end (Fig. 4.31) with significant statistical difference ($P<0.05$) among the treatments.

According to Tang *et al.*, (2012) commercial fish feed are usually high in phosphorus content (about 20 to 25 kg $Ca(H_2PO_4)_2 \cdot H_2O$ per ton feed). Large amounts of uneaten feed and faeces released into the aquatic environment enriched the soil continuously with phosphorus content.

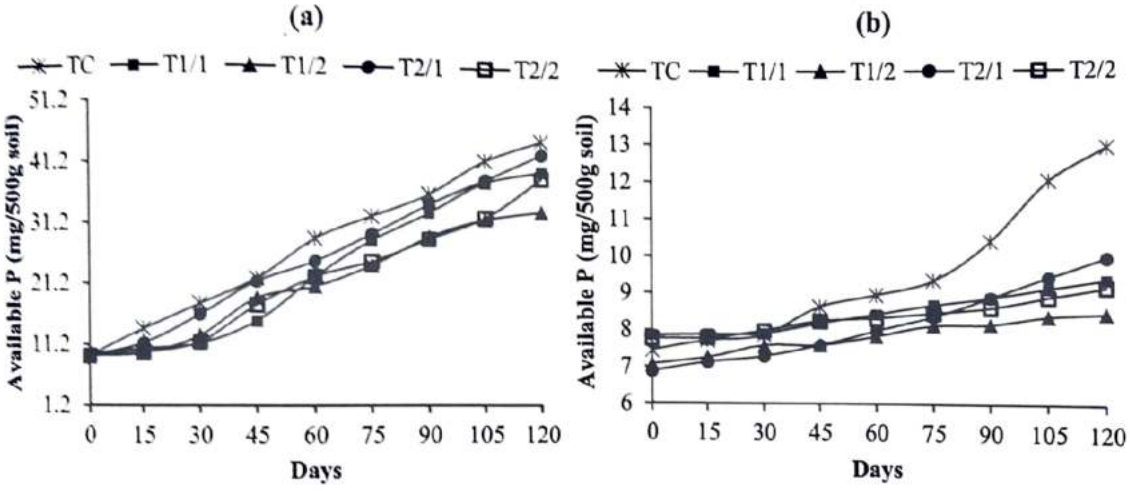

Fig. 4.31. Temporal changes of soil available phosphorus in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.6.4. Soil available phosphorus

The fraction of total phosphorus in soil that is readily available for absorption by plants is referred to as available phosphorus. In both in-vitro and in-vivo experiment of the present study, soil available phosphorus exhibited a gradual increasing trend in all the treatments till the end (Fig. 4.31) with significant statistical difference ($P<0.05$) among the treatments.

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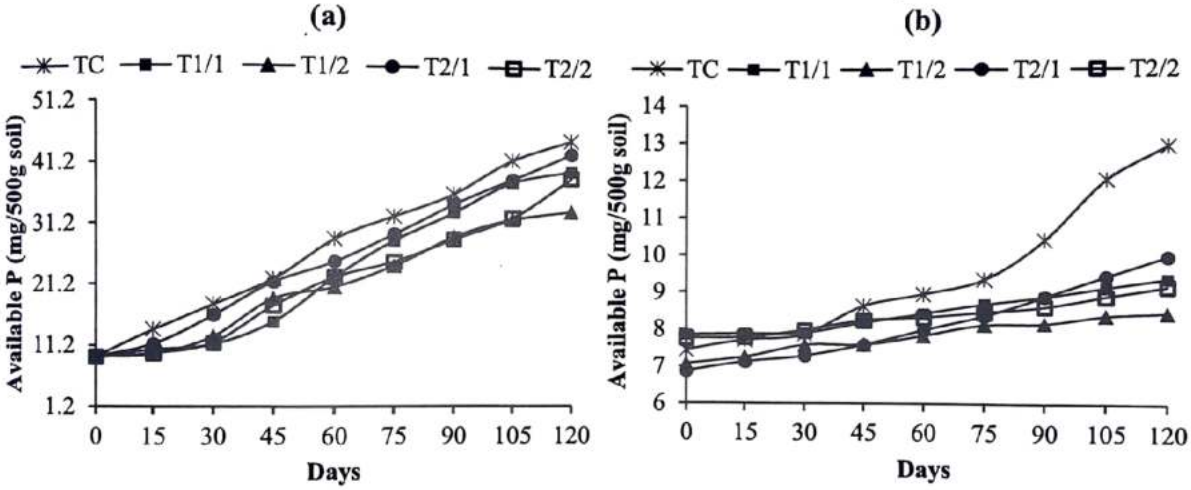

Fig. 4.31. Temporal changes of soil available phosphorus in different treatments employed of in-vitro (a) and in-vivo (b) experiment.

4.7. Production economics

The economics of the pond culture of amur common carp under different feeding strategies is summarized in Table 4.13. Results indicated no significant difference in fish yield between TC and T2/1. Although there was a positive correlation (r = 0.945) between total amount of feed and fish yield, the net return was not parallel to the fish yield. Significantly the highest net return was achieved in T2/1, which was 39.64%, 60.71%, 83.81% and 206.46% higher than TC, T1/1, T2/2 and T1/2 respectively.

Table 4.13. Production economics of amur common carp culture under different feeding regimes for 120 days

Variables	Treatment				
	TC	T1/1	T1/2	T2/1	T2/2
Yield (Kg/ha)	2002.82 ± 156.29^{c}	1524.12 ± 72.69^{b}	1095.76 ± 36.25^{a}	1903.56 ± 128.73^{c}	1464.33 ± 103.74^{b}
Selling Price (Rs. /Kg)	90 ± 0	80 ± 0	75 ± 0	90 ± 0	80 ± 0
Feed Provided (kg/Ha)	2233.11 ± 174.26^{d}	978.49 ± 46.67^{b}	495.07 ± 16.38^{a}	1556.19 ± 105.24^{c}	988.36 ± 70.02^{b}
Total Feed Cost (Rs./Ha)	89324.27 ± 6970.47^{d}	39139.77 ± 1866.68^{b}	19802.64 ± 655.14^{a}	62247.48 ± 4209.53^{c}	39534.37 ± 2800.92^{b}
Total Operation Cost (Rs./Ha)	128657.6 ± 7084.29^{d}	78006.43 ± 1826.72^{b}	58669.31 ± 761.41^{a}	101380.81 ± 4096.9^{c}	78467.71 ± 2895.4^{b}
Input interest @ 2.7% per year	3473.76 ± 191.28^{d}	2106.17 ± 49.32^{b}	1584.07 ± 20.56^{a}	2737.28 ± 110.62^{c}	2118.63 ± 78.18^{b}
Total Cost (Rs./Ha)	132131.36 ± 7275.57^{d}	80112.61 ± 1876.04^{b}	60253.38 ± 781.97^{a}	104118.09 ± 4207.52^{c}	80586.33 ± 2973.58^{b}
Sales Revenue (Rs./Ha)	180253.56 ± 14066.18^{c}	121929.6 ± 5815.15^{b}	82181.8 ± 2718.88^{a}	171320.4 ± 11585.65^{c}	117146.13 ± 8299.53^{b}
Net Profit (Rs./Ha)	48122.2 ± 6790.67^{c}	41816.99 ± 3960.06^{bc}	21928.42 ± 1939.15^{a}	67202.31 ± 7378.26^{d}	36559.8 ± 5327.21^{b}
Rate of Return on Operation Cost (%)	36.3 ± 3.23^{a}	52.14 ± 3.79^{b}	36.37 ± 2.74^{a}	64.43 ± 4.4^{c}	45.25 ± 4.91^{b}

*Currencies are given in Indian National Rupees (1 USD = 78.98 INR as on 28/06/2022).
Row wise altered superscript statistically implies significant difference ($P < 0.05$)

Highest net return in T2/1 was achieved by minimizing feed cost and achieving almost the same yield as daily feeding. On the other hand, the else deprived treatment groups are meant to be commercially unfeasible due to very limited profit or net return.

4.8. Suitability analysis of feeding strategies

Suitability of the feeding strategies was analysed by Analytical Hierarchy Process (AHP), a multi-criteria decision-making tool, following the method as described by Satty (1988).

Table 4.14. Pair-wise comparison matrix for assessing relative importance of different growth factors regarding suitability of the different feeding strategies

	Survival	Weight Gain (g)	Length Increment (cm)	Yield (Kg/ha)	SGR	FCR	FCE	PER	Criteria Weight (CW)
Survival	1	1	1	1	1	2	2	2	**0.154**
Weight Gain (g)	1	1	1	1	1	2	2	2	**0.154**
Length Increment (cm)	1	1	1	1	1	2	2	2	**0.154**
Yield (Kg/ha)	1	1	1	1	1	2	2	2	**0.154**
SGR	1	1	1	1	1	2	2	2	**0.154**
FCR	0.5	0.5	0.5	0.5	0.5	1	1	1	**0.077**
FCE	0.5	0.5	0.5	0.5	0.5	1	1	1	**0.077**
PER	0.5	0.5	0.5	0.5	0.5	1	1	1	**0.077**

$\lambda_{max} = 8$; Consistency Index (CI) = 0.00000; Consistency Ratio (CR) = 0.00000

In case of enzyme and biochemical assay, weightage of 0.2 was assigned for each sub-criterion, as they were concerned equally significant for the assessment. Since, only net profit was selected as economic criterion, there was no need for weightage assignment.

Table 4.15. Pair-wise comparison matrix for assessing priority among growth, enzyme/biochemical assay, and economics

	Growth criteria	Economic criteria	Enzyme criteria	Criteria Weight (CW)
Growth criteria	1	2	3	**0.538961**
Economic criteria	0.5	1	2	**0.297258**
Enzyme criteria	0.333333	0.5	1	**0.163781**

$\lambda_{max} = 3.0092096$; Consistency Index (CI) = 0.004605; Consistency Ratio (CR) = 0.007939

Consistency ratios (CR) were found to be within limit (<0.10). So, the weightage estimated are valid and used for further calculation.

Table 4.16. Performance score and ranking of different treatments derived from AHP analysis

Treatment	Performance score	Rank
T2/1	0.918	1
TC	0.828	2
T1/1	0.739	3
T2/2	0.704	4
T1/2	0.610	5

The performance score and rank of different treatments derived from AHP analysis is displayed in Tab. 4.16. The ranking achieved by AHP is visually interpreted as per Performance Score. The performance score reflects the degree of suitability of feeding schedules in terms of growth, enzyme/biochemical assay, and production economics. However, the highest performance score was achieved by T2/1 followed by TC, T1/1, T2/2, and T1/2 respectively. Hence, according to the result of AHP, T2/1 treatment is ascertained as most suitable restricted feeding strategy for amur common carp culture, whereas T1/2 treatment is ascertained as relatively least suitable.

Chapter - 5

SUMMARY AND CONCLUSION

Effects of different restricted feeding strategies on growth performance and physio-metabolic responses of amur common carp (*Cyprinus carpio haematopterus*) fingerlings have been studied successfully. The whole work embodied in this thesis consisting of 7 chapters. Viz. Introduction, Review of Literatures, Materials and Methods, Results and Discussion, Future Scope of Research, Summary and Conclusion, and Bibliography. Some interesting observations have been made on the subject and many valuable pieces of information have come out during the period of investigation. The main outcome of this work is summarized herein: Feed deprivation had a significant impact on body composition of amur common carp. Lower muscle protein and body lipid content was evidenced with the severity of starvation.

- The highest fish growth was achieved in TC, where regular feeding was concerned. But among the deprived groups the growth of fishes in terms of body weight gain and length increment in T2/1 was significantly similar when compared to control. In other deprived groups the growth was much poor.
- Improved feed utilization indices like FCE and PER were evidenced in all the feed deprived groups when compared to control, where regular feeding was concerned.
- Periodic starvation significantly influenced the protease and amylase activity, but not lipase activity. In our present research, the digestive enzyme activities were markedly higher in T2/1. Elevated protease and amylase activity suggested that the test fishes had a superior ability to digest protein and carbohydrate respectively, and this might assign to compensatory growth in T2/1.
- Both hepatic LDH and MDH activity have been found to be significantly thematic with feeding strategies. They showed a direct relationship with starvation.
- ALT and AST activity were also significantly affected by periodic feed deprivation. The feed-deprived groups experienced increased ALT and AST activity in blood parallel to the degree of feed deprivation.
- In the present study, the test fish amur common carp exhibited adoptive response to the oxidative stress resulted by periodic starvation and maybe that's why the

hepatic SOD, catalase and GPx activity were increased and remained parallel with the intensity of starvation.

- Water and soil pH always had the tendency to be reduced might be due to the acidification followed by microbial decomposition of uneaten/undigested feed and faeces.
- Treatments with higher feed supplement evidenced with lower DO and elevated BOD level.
- The NH_3-N concentration in water gradually increased and varied parallel to the feeding rate and amount of feed provided. Treatments with higher feed supplement found to be rich in NH_3-N concentration.
- Soil organic carbon, available nitrogen and available phosphorus increased gradually in all the treatments till the end of the experiment.
- Although there was a positive correlation ($r = 0.945$) between total amount of feed and fish yield, the net return was not parallel to the fish yield. Significantly the highest net return was achieved in T2/1.

Finally, according to the visual interpretation based on the performance score derived from AHP analysis, it can be concluded that cycle of 1-day starvation followed by 2 consecutive days of feeding (T2/1) is the most suitable feeding strategy for pond culture of amur common carp among all the restricted feeding strategies in this present study. Whereas, the cycle of 1-day feeding followed by 2-days starvation (T1/2) is ascertained as the least suitable.

Chapter - 6

FUTURE SCOPE OF RESEARCH

The present study on **"Effect of restricted feeding on growth performance and physio-metabolic responses of amur common carp (*Cyprinus carpio haematopterus*) fingerlings"** though emerged with several important findings, was limited with few shortcomings. Due to lack of infrastructural facilities few parameters, which could not be studied during the experiment but highly desired to be addressed in future studies are described below:

i. The haematological changes in amur common carp due to starvation should be studied, so as to investigate their impacts on growth.
ii. Histological assessment of cellular changes following starvation should also be included.
iii. Finally, the changes in gene expression due to feed deprivation should also be addressed.

Chapter - 7

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Chapter - 8

CURRICULUM VITAE

Communicated Paper

← Submissions Being Processed for Author

Page: 1 of 1 (1 total submissions)

Results per page 10

Action		Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status	
Action Links		AQUACULTURE-D-22-01508	Effects of periodic starvation on growth, production performance and physio-metabolic responses of pond cultured amur common carp (Cyprinus carpio haematopterus) fingerlings	May 02, 2022	May 17, 2022	Under Review	

Page: 1 of 1 (1 total submissions)

Results per page 10

Mr. Arka Chowdhury

Faculty of Fishery Sciences,
West Bengal University of Animal and Fishery Sciences
5 Buderhat Road, Chakgaria, P.O- Panchasayar,
Kolkata-700094

Contact No: 9007064529 / 8240343705

E-mail: arka406@gmail.com

Career Objective

i) To be an entrepreneur
ii) To promote scientific aquaculture practices in rural areas

Areas of Interest

- Fisheries & Aquaculture
- Nutrition & feed Technology

Education

SL No.	Name of the Exam.	Board/ University	Year	Total Marks	Marks achieved	% of marks
1.	Madhyamik (10^{th})	W.B.B.S.E	2009	800	705	88.13 %
2.	Higher Secondary (10+2)	W.B.C.H.S.E	2011	500	408	81.60 %
3.	B.F.Sc (10+2+4)	W.B.U.A.F.S	2015	10 points	8.04	80.40 %
4.	M.F.Sc (Aquaculture)	W.B.U.A.F.S	2017	10 points	8.48	84.80 %
5.	Ph.D (Aquaculture)	Pursuing at WBUAFS				

Training, Seminar & Webinar Attended

Sl. No.	Name of the Training/ Seminar	Name of the Institution	Duration
1.	Training on Marine Awareness Course	Sea Explorers' Institute, Kolkata	02 days
2.	On-farm Fisheries Training Programme	F.F.R.T.C, Kalyani, Nadia	21 days
3.	Training on Various Aspect of Aquaculture practice & Hatchery management	Govt. Fish Technological Station, Junput	13 days
4.	Training on "An Overview of Brackishwater Aquaculture"	Kakdwip Research Centre, CIBA	05 days
5.	On-farm Training Programme	West Bengal University of Animal & Fishery Sciences	11 days
6.	Training on "Concept Building and Basic Statistical Analysis For Inland Fisheries Management"	ICAR - CIFRI, Barrackpore	07 days
7.	Two Days National Seminar on "Advancement in Green Technology For Controlling and Managing Current Environmental Pollution"	University of Kalyani	-
8.	International E-conference on "Current Trends in Environment Conservation & Management as Adoptive Measures for Climate Change"	International Journal of Environmental and Technological Sciences (iJETs)	-
9.	ISEE National Seminar on "Integrated Farming System for Enhancing Farmers' Income and Nutritional Security"	West Bengal University of Animal & Fishery Sciences	03 days
10.	Workshop on "Innovation & Entrepreneurship" at WBUAFS, Kolkata	DSIR-TOCIC-IIT-Kharagpur, CSIR-CGCRI and CSIR-CMERI	01 day
11.	National Webinar on "Strategies of Small-scale Aquafarming During Covid 19 and Beyond"	Department of Aquaculture, Faculty of Fishery Sciences, WBUAFS	-

IT Skill

Sound knowledge in application of Windows, MS Office & Inrenet Application.
Studied 'ICT' as a scheduled course of B.F.Sc.

Personal Dossier

- Date of Birth: 26th November, 1993
- Sex: Male
- Nationality: Indian
- Marital Status: Unmarried
- Language Known: i) Bengali- Read, Write & Speak
  ii) English- Read, Write & Speak
  iii) Hindi- Speak
- Extra-curricular Activity: Singing, Song Composing & Playing Guitar.
- Permanent Address: Vill- Sastipur, P.O-Batanal, P.S-Arambagh, Dist- Hooghly, Pin-712615, West Bengal

Contact No: 9007064529 / 8240343705

E-mail: arka406@gmail.com