

**STUDY ON METHIONINE MODULATED BIOAVAILABILITY
OF INORGANIC ZINC IN COMMON CARP (*Cyprinus carpio* L.)
FED WITH DIETS CONTAINING TRICALCIUM PHOSPHATE**

Thesis

**Submitted to the
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in partial fulfillment of the requirements for the degree of**

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In

AQUACULTURE

(Minor Subject: Aquatic Environment Management)

By

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(L-2014-F-03-M)**



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2016

CERTIFICATE – I

This is to certify that the thesis entitled, “**STUDY ON METHIONINE MODULATED BIOAVAILABILITY OF INORGANIC ZINC IN COMMON CARP (*Cyprinus carpio* L.) FED WITH DIETS CONTAINING TRICALCIUM PHOSPHATE**” submitted for the degree of M.F.Sc., in the subject of **AQUACULTURE** (Minor Subject: **Aquatic Environment Management**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Ms. Jagdeep Kaur (L-2014-F-03-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

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

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CERTIFICATE – II

This is to certify that the thesis entitled, “**STUDY ON METHIONINE MODULATED BIOAVAILABILITY OF INORGANIC ZINC IN COMMON CARP (*Cyprinus carpio L.*) FED WITH DIETS CONTAINING TRICALCIUM PHOSPHATE**” submitted by **Ms. Jagdeep Kaur (L-2014-F-03-M)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.F.Sc.**, in the subject of **AQUACULTURE** (Minor Subject: **Aquatic Environment Management**) has been approved by the Student’s Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

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ABSTRACT

The study was conducted to study the methionine (MET) modulated bioavailability of inorganic zinc (Zn) as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, in common carp (*Cyprinus carpio*) fed with diets containing tricalcium phosphate (TCP). The experiment was carried out in the indoor glass aquaria (60× 30 ×30cm) with seven diets including one control (D₁) and six experimental diets (D₂ to D₇). All seven experimental diets were prepared by mixing casein, dextrin, gelatin, fish meal, sodium alginate, soybean meal, oil, carboxy methyl cellulose, Zn free mineral, vitamin mixture were 20%, 30%, 8%, 10%, 12%, 8%, 2%, 5%, 3% and 2%, respectively. D₁ i.e. control diet was prepared without adding Zn i.e., TCP and MET. D₂ and D₃ having 88.4 and 176.8 mg kg⁻¹ Zn without TCP and MET. D₄ and D₅ having 88.4 and 176.8 mg kg⁻¹ Zn and 2 % TCP without MET. D₆ and D₇ having 88.4 and 176.8 mg kg⁻¹ Zn, 2 % TCP and 1% MET. No significant difference ($P \leq 0.05$) in SGR, FCR and PER value of common carp (*C. carpio*) was recorded between the treatments (D₁ to D₇), however FCR value (ratio) was recorded minimum in diet D₇, while it was toward higher side in diets containing TCP without adding methionine (D₄ and D₅). PER value was also insignificant and was recorded maximum in D₇, while it was minimum in diets containing TCP without adding methionine (D₄ and D₅). Significantly higher ($P \leq 0.05$) concentration of Zn in flesh of fish fed with diet D₇ was recorded. Zn concentration in different tissue was improved in diets containing 1% methionine, while it was lowest in diets containing no methionine (D₄ and D₅). Zn concentration was significantly higher in diets D₂ and D₃ among dietary component variables. Similar pattern was also observed in liver and bone except Zn concentration in liver, where it was recorded significantly high in diet D₆.

Keywords: Common carp (*Cyprinus carpio*), Bioavailability, Methionine, Tricalcium phosphate, Inorganic zinc

Signature of Major Advisor

Signature of the Student

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Chapter -I

INTRODUCTION

Minerals are required for the normal life processes and all animals, including fish, need these inorganic elements for different physiological activities. Minerals serve as an essential requirement to the variety of functions, as both intra and extra cellular components. They are important structural components in both hard (bone, fin rays, scales, teeth and skeleton) and soft (proteins, phospholipids and nucleic acids) tissues and also play a vital role in the development of specialized cells like metalloproteins, serves as co-factor, activates variety of enzymes, besides regulating osmoregulation, acid-base balance and membrane potential. Mineral deficiencies can cause biochemical, structural and functional deformities. Their bioavailability to the animal depends on several factors, including the duration and degree of mineral deprivation. On the basis of quantity required; minerals are considered as macro and micro minerals. Ten minerals (calcium, phosphorus, magnesium, potassium, copper, iron, zinc, manganese, selenium and iodine) are considered as essential for the optimum fish growth and health (Davis and Gatlin 1996). Among these, zinc (Zn) is an essentially required micro mineral.

Zinc is an essential transitional metal that is directly or indirectly involved in a wide variety of physiological processes including growth, development, reproduction and immune function (Watanabe *et al.*1997). Zinc was discovered as an essential mineral for the growth of living organisms in 1869. In fish, its deficiency leads to poor growth, high mortality, erosion of fins and skin, low content in bone (Takeuchi *et al* 2002). Concentration of Zn varies from organ to organ and among organs highest concentration of Zn is reported in the choroid of the eye and optic nerve. Requirement of Zn is mainly fulfilled through food. Dietary requirement of Zn can be fulfilled by both organic and inorganic sources.

Common carp (*Cyprinus carpio L.*) is an important fresh water carp, cultured across the world. Early stages of the life cycle of organism are very crucial for its development. In cases of carps, fry stage is the transitional stage between the dependence of yolk and transfer to feeding on natural food. During early life cycle stages, common carp requires high amount of dietary protein, for this application of animal protein in the formulated diets is increasing. Waste from aquatic as well as

terrestrial animals is considered as an excellent source of protein for fish, moreover rendered (recycled) by-products from waste serve as cheap source of quality protein enriched with lysine, sulfur amino acids (methionine and cystine), histidine, arginine, phosphorous (El. Seyed 1998), but their application is limited due to low digestibility and restricted bioavailability of nutrients due to certain limiting factors (Cho *et al* 1982, Gill 2000).

Major bioavailable form of Zn sources are zinc picolinate, zinc ascorbate, zinc chloride, zinc sulphate, zinc carbonate, zinc citrates, zinc bisglycinate, zinc oxide and chelated zinc. In fish, two major routes for Zn uptake exist; one is waterborne and another one is oral. Waterborne Zn is absorbed mainly through the gills, while the gut assimilates particulate Zn from diet and imbibed water. Zn uptake through alimentary canal is still not well defined, while branchial uptake depends on absorption of Zn through an apical calcium channel in the ion transporting cells (Glover *et al* 2003).

Certain anti-nutritional factors (ANFs) present in both animal and plant feed resources, affect uptake and hence, bioavailability of Zn in fish. Tricalcium phosphate (TCP) in fish meal and phytate in most of plant feed resources inhibit Zn uptake in fish (Davis *et al* 1993). However, it is still not very clear how TCP inhibits the Zn uptake and more over for this calcium phosphate, calcium or phosphorous is responsible. TCP is calcium salt of phosphoric acid and is also known as tribasic calcium phosphate or bone phosphate of lime and is commonly used as anti-caking agent in food. In fish meal, TCP comes from skeletal part of fish.

Organic minerals are important source of trace minerals, because they protect trace elements from forming insoluble complexes (such as phytate and TCP) in the digestive tract and facilitates transport across the intestinal mucosa (Ashmead 1993). Bioavailability of any nutrient, including mineral, is an important criterion for its uptake and utilization. Improvement in bioavailability can be achieved through different pathways. One of the most common pathway is that the amino acids may act as Zn shuttle from dietary components having low Zn binding affinity to uptake from epithelial surface with higher affinity (Wapnir *et al* 1985, Ackland and McArdle 1990, Bobilya *et al* 1993). Methionine is the first limiting amino acid in many fish diets, especially those containing high levels of plant protein source (Mai *et al* 2006). Alternatively, the formation of an amino acid–Zn chelate may create a substrate for Zn transport across the epithelial surface (Wapnir *et al* 1983, Ashmead *et al* 1985).

Chelation refers to a bonding formed between a metal ion (mineral) and ligand (protein or amino acid). A mineral complex is a mixture of mineral and organic compound more over biological role of chelated trace minerals is important to convert it into chelated form. Chelates and complexes have the capability to compete with dietary inhibitors, making minerals more available to animals. Hence, mineral sources with higher availability should be considered during feed formulation. Chelated form of minerals with protein/amino acids, showing increased availability of mineral as compared to conventional (inorganic) sources (Ashmead 1993). Amino acids may increase bioavailability by removing chelated Zn from dietary Zn-binding constituents such as phytic acid and TCP. Copper (Cu) is an essential element and it enhances the enzymatic activity of the body. However it creates health hazard when present in large quantity (Yesser *et al* 2013). As a part of the enzyme copper – zinc superoxide dismutase (Cu /Zn SOD) help in protecting the cells and some compounds from the harmful effects of free radicals. Excess quantity of Cu causes toxicity to fish (FAO/WHO 1983). Being a wide cultivable fish species in India as well as in the world, common carp is still not well studied in fish species with respect to response to inhibitory effect of TCP; moreover the negative effect of TCP can be mitigate up to certain level through amino acid (methionine) modulation.

Keeping in view the above facts, the present work “Efficacy of methionine modulated availability of dietary inorganic Zn ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) in the fingerlings of common carp (*Cyprinus carpio L.*)” had been designed to study the following aspects:-

1. Uptake of dietary inorganic Zn.
2. Effect on uptake of dietary inorganic Zn with TCP as an inhibitory agent.
3. Methionine modulated uptake of dietary inorganic Zn with TCP as an inhibitory agent.
4. Uptake of Zn in different tissues of fish.

Chapter II

REVIEW OF LITERATURE

Available information with respect to water quality, nutritional requirement, importance and uptake of inorganic zinc in common carp (*C. carpio*) in presence of inhibitor (TCP) and promoter amino acid (Methionine) is presented below:

2.1 Water quality requirement for common carp

2.2 Nutritional requirement and feed utilization in common carp

2.3 Zinc requirement in fish

2.4 Effect of TCP on Zn uptake

2.5 Amino acid modulated bioavailability of Zn in fish

2.6 Copper toxicity in fish tissue

2.1 Water quality requirement for common carp

Fish, being an aquatic animal requires a suitable water quality for optimum growth and survival and health. For fish culture water quality is determined mainly by variables like temperature, transparency, turbidity, water colour, dissolved oxygen, carbon dioxide, pH, alkalinity, hardness, unionized ammonia, nitrite, nitrate, phosphate.

Water quality is an important parameter for the survival of any living organism. Jhingran (1985) reported that carp thrive well in the temperature range of 18.3- 37.8°C, while it can occur in temperature range of 3- 35°C (Froese and Pauly 2011). FAO (2000) documented that common carp can tolerate high water temperature i.e. up to 32°C without effect, but the optimum temperature for the growth is in between 20 – 25°C (Froese and Pauly 2011) while fish is active in feeding, when the water temperature is over 18-20°C. Oyugi *et al* (2012) suggested that the temperature play a significant and positive role on the foraging and growth of the juvenile. They also observed maximal foraging and growth of common carp in between 24 -28°C.

pH is an important limiting factor in fish culture. It indicates acid-base balance of water. Jhingran (1991) suggested that pH between 6.5 to 9.6 is suitable for the

growth of Indian major carp and exotic carp. pH values above 10.8 and below 5.0 is considered as rapidly fatal to cyprinids especially carps (Svobodova *et al* 1993). Water pH plays an important role to ensure good fish production and survival. For optimum fish culture, the pH between 6.5 to 9.0 is generally considered as most acceptable range (Zweig *et al* 1999). Heydarnejad (2012) suggested that the optimum range for survival and growth of common carp is pH 7.5 to 8.0.

Dissolved oxygen (D.O.) affects the growth, survival, distribution, behavior and the physiology of aquatic organisms (Solis, 1988). For optimum productivity of carp dissolved oxygen content $>5.0 \text{ mg l}^{-1}$ is required to be maintained in the ponds throughout the culture period (Swingle 1961, Boyd 1990). According to Banerjee (1967), D.O. between 3.0 to 5.0 mg l^{-1} in pond water is considered as unproductive, while for good production he suggested that dissolved oxygen level should be 5.0 mg l^{-1} or more. He also suggested that very high concentration of D.O. leads to a state of super saturation. Alikunhi *et al* (1952) suggested that the super saturated oxygenated water sometimes becomes lethal to fish fry especially during the rearing of spawn in nursery ponds. Ekubo and Abowei (2011) reported that fish can die if exposed to less than 0.3 mg l^{-1} D.O. for long period; they also suggested minimum D.O. concentration of 1.0 mg l^{-1} for survival and $>5.0 \text{ mg l}^{-1}$ D.O. to sustain fish for long period. Fish exposed to oxygen deficient water do not take food, collect near the water surface (Svobodova *et al* 1993).

Alkalinity is the ability of water to resist changes in pH and is a measure of the total concentration of bases in pond water including carbonates, bicarbonates, hydroxides, phosphates and borates, along with dissolved calcium, magnesium, and other compounds in the water (Stumm and Margan 1981). Moyle (1946) classified the water productivity for fish production on the basis of alkalinity value. According to him the range of total alkalinity as $0 - 20 \text{ CaCO}_3 \text{ mg l}^{-1}$ for low production, $20 - 40 \text{ CaCO}_3 \text{ mg l}^{-1}$ low to medium, $40 - 90 \text{ CaCO}_3 \text{ mg l}^{-1}$ medium to high and above $90 \text{ CaCO}_3 \text{ mg l}^{-1}$ productive. Boyd and Lichtkoppler (1979) suggested that water with $20 - 150 \text{ CaCO}_3 \text{ mg l}^{-1}$ total alkalinity contain suitable quantities of carbon dioxide to permit plankton production for fish culture. Santhosh and Singh (2007) proposed the ideal value of alkalinity for fish culture is in between $50 - 300 \text{ CaCO}_3 \text{ mg l}^{-1}$.

Hardness of water depends on the dissolved solids and pH. Hardness gives a measure of the total concentration of the divalent metallic cations like calcium,

magnesium and strontium. According to Bhatnagar *et al* (2004) hardness values less than 20 CaCO₃ mg l⁻¹ causes stress, 75 –150 CaCO₃ mg l⁻¹ is optimum for fish culture, while >300 CaCO₃ mg l⁻¹ is lethal to fish as it increases pH, resulting in non – availability of nutrients. Bhatnagar and Devi (2013) suggested the water quality criteria for pond water fish culture temperature 15–35°C, pH 7 – 9.5, D.O.≥ 5mg l⁻¹, alkalinity 50 –200 CaCO₃ mg l⁻¹, hardness > 20 CaCO₃ mg l⁻¹ as optimum range.

2.2 Nutritional requirement and feed utilization in common carp

In fish farming, feed is the major input among variable cost. Feed helps in fast growth and better health of fish. Optimum production from fish culture system can be achieved through quality feed with desirable quantity. The quality of feed is measured in terms of its nutritive value and digestibility, which mainly depends on type and level of feed stuff incorporated in the diet. Crude protein (CP) content in prepared feed is an important criterion of its nutritional value. Protein requirement of fish also varies with life stage. During early life stages it requires high protein diet while protein requirement of fish reduces with advancement of stage and feed quality has to be adjusted accordingly.

Fry is a critical stage in the life cycle of fish. Garling and Wilson (1976) suggested 25% -36% CP as optimum level in diets for warm water fishes, which however, varies with species, size and environmental factors; particularly temperature.

Sultana *et al* (2001) observed the effect of feeding frequency on the growth of common carp. A feeding trial was conducted with 33.34% CP for 45 days on the fry of common carp in aquaria. Daily ration with different feeding frequencies viz 2, 3, 4, 5 and 6 times @ 5% fish body weight (BW) were provided. At the end of experiment they recorded maximum growth in common carp, when fed 4 times daily. Feed conversion ratio (FCR) and protein efficiency ratio (PER) recorded ranging 1.22 and 1.78, 1.68 and 2.48, respectively.

Manjappa *et al* (2002) observed the growth performance of common carp fed with low protein diet (24% CP) with 0, 3, 6 and 9% fish oil. Young ones of common carp fed for 120 days @ 5% BW They observed the maximum growth at 24% CP with 6% fish oil combination, while Rath (2012) documented the protein requirement in carp spawn as 35 -40% with balanced combination of essential and non-essential

amino acids. Moreover the superlative P: E ratio for optimum growth of common carp is 108 mg kcal⁻¹.

Jader *et al* (2012) evaluated nutritional effect of three diets with CP 25%, 30% and 35% on common carp juveniles and reported highest growth performance with 30% CP diet, while least growth with 25% CP diet. FCR ranged from 2.27 (30% CP diet) to 3.01(25% CP diet), while least PER (0.79) was obtained, when fish was fed with 35% CP diet.

Mohapatra and Patra (2014) reported maximum growth and survival at 35.2% CP level, while comparing the diets with 27.1, 28, 30% and 35.2% CP levels fed @ 5% BW to common carp fry. They also suggested the use of animal protein, in the feed formulation for young ones of common carp.

FCR is a numerical value used to measure the gross utilization of feed for growth in fish and other animals. It is also a measure of the efficiency or suitability of feed to animal (Teugels 1982), PER expresses the efficiency of protein utilization. Sultana *et al* (2001) reported 2.53 – 3.24 specific growth rate (SGR), 1.22 – 1.78 feed conversion ratio (FCR) and 1.68 – 2.48 protein efficiency ratio (PER) in common carp fry fed with 33.34% CP diet @ 5% BW for 45 days. Kiaalvandi *et al* (2011) reported 4.76 – 6.25 FCR and 0.38 – 0.47 PER in common carp juveniles (8.6 g) fed with 26 – 28% CP @ 5% BW daily, while Jader and Sulevany (2012) recorded 0.71 – 0.87SGR, 2.27 – 3.01FCR and 0.79 – 1.05 PER in juveniles of common carp, when fed with 25-35% CP diet.

2.3 Zinc requirement in fish

Zinc deficiency has been found to cause mortality, anorexia, poor growth, cataract, skin erosion and oxidative damage in fish such as rainbow trout, *Oncorhynchus mykiss*, channel catfish, *Ictalurus punctatus* and tilapia, *Oreochromis niloticus* (Ogino and Yang 1978, Gatlin and Wilson 1983, Eid and Ghonim 1994).

Zinc is required as micro mineral to the fish. Ogino and Yang (1978) evaluated the Zn requirements in rainbow trout (*O. mykiss*), fed with Zn incorporated feed in a semi- purified diet and suggest that minimum requirements to promote normal growth and to prevent Zn deficiency are between 15-30 mg kg⁻¹ as dietary Zn. Galtin and Wilson (1983) conducted the experiment using ZnS as Zn supplement with egg white based diet in channel catfish (*I. punctatus*) and concluded optimum

requirement of Zn in channel catfish is 20 mg kg⁻¹. Satoh (1991) evaluated the Zn requirement in common carp and suggested that for optimum growth, it requires 15-30 mg kg⁻¹ zinc. He also suggested that deficiency of Zn leads to poor growth, high mortality, erosion of fins and skin, low zinc content in bone.

Jeng and Lian (1994) compared the Zn absorption in the form of ZnCl₂ in common carp (*C. carpio*), silver carp (*Hypophthalmichthys molitrix*) and tilapia (*O. niloticus* X *O. aureus*). The Zn concentration in the basal diet was maintained at 28.3 mg l⁻¹. Under experimental conditions common carp absorbed 95% of its Zn from diet, whereas tilapia, grass carp and silver carp absorbed 85%, 78% and 70% of their Zn from diet, respectively.

Lall (2002) suggested that even higher levels of supplemental Zn can be added to practical diets to compensate for reduced bioavailability Zn from dietary phytate, calcium and phosphorus.

Clearwater *et al* (2002) assessed the bioavailability and toxicity of diet born copper (Cu) and Zn in different fish species and concluded that diet born Cu toxicity occurred at daily doses of >1mg kg⁻¹BW day⁻¹ for channel catfish (*I. punctatus*), 1-15 mg kg⁻¹ BW day⁻¹ for atlantic salmon (*Salmo salar*) and 30-45 mg kg⁻¹BW day⁻¹ for rainbow trout (*O. mykiss*), while Zn toxicity occurred at daily doses of 9-12 mg kg⁻¹ BW day⁻¹ for common carp (*C. carpio*), nile tilapia (*O. niloticus*) and guppy (*Poecilia reticulata*).

Rider *et al* (2009) evaluated the bioavailability of co-supplemented organic and inorganic Zn and selenium (Se) sources in white fish meal boned rainbow trout (*O. mykiss*) diet. The diets containing no added Zn or Se, an organic Se yeast, zinc proteinate and inorganic sodium selenite and zinc sulphate supplement were provided to feed rainbow trout. They concluded that the digestibility of ZnSO₄ (inorganic Zn) improved significantly greater retention than Zn- proteinate (organic zinc). Moreover, among tissue, highest concentration of Zn was recorded in the posterior intestinal tissue (404.8 mg kg⁻¹) and minimum in muscle (5.19 mg kg⁻¹).

Hasnat *et al* (2012) fed the gold fish (*Carassius auratus*) fry with five levels of zinc sulfate (ZnSO₄.7H₂O) @ 0, 30, 60, 90, 120 and 150 mg kg⁻¹ diet and reported increased growth with the dietary Zn supplementation up to 60 mg Zn kg⁻¹ diet and 60 mg kg⁻¹ supplementation growth rate of fish declined.

Ganesan and Karuppasamy (2015) observed the pattern of Zn accumulation in liver, kidney and gills of *Channa punctatus*, when exposed with $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ @ 35.37 mg/l concentration up to 60 days. At the end of experiment, Zn showed a highest accumulation ($p \geq 0.05$) in the liver ($46.45 \mu\text{g g}^{-1}$) and followed by kidney ($29.12 \mu\text{g g}^{-1}$) and gill ($19.42 \mu\text{g g}^{-1}$).

Welker *et al* (2016) studied the effect of Zn (0, 15, 30, 60, 120 mg kg^{-1} diet) with fish and plant protein source and concluded that Zn supplementation in the fish meal diet had no effect on growth performance. However, in plant based diet, growth increased significantly with Zn supplementation up to 30 mg kg^{-1} . During this investigation, the best FCR and SGR with Zn supplementation up to 60 mg kg^{-1} Zn in fish meal was reported.

2.4 Effect of TCP on Zn uptake

Satoh *et al* (1992) observed the effect of supplemental TCP on Zn and Mn availability to common carp. On the basis of feeding experiment to observe the effect of supplemental TCP on Zn and Mn bioavailability in common carp, the lowest growth was recorded in fish fed the diet without supplemental Zn and Mn and was improved by supplementation of Zn and Mn, irrespective of dietary TCP level. They concluded that the TCP inhibited both Zn and Mn bioavailability to common carp. Zinc (Zn) and Manganese (Mn) requirement of common carp were reconfirmed to be 15-30 and 13-15 mg kg^{-1} , respectively.

Paripatananont and Lovell (1995) reported that chelated Zn reduces the dietary requirement of Zn in channel catfish, *I. punctatus*. In their experiment Zn -methionine (Zn -Met) was used as chelated Zn source, while zinc sulphate heptahydrate (ZnS) was used as iron chelated source of Zn. At the end of experiment they concluded Zn- Met has approximately 3 times higher potency compared to ZnS in purified diets and 4.5 times than the potency of Zn in practical diet containing phytic acid for meeting the dietary Zn requirement in channel catfish.

2.5 Amino acid modulated bioavailability of Zn in fish

Apines *et al* (2003) studied the availability of amino acid- chelated trace elements in diets containing TCP and phytate to rainbow trout, *O. mykiss* and reported that whole body and bone Zn contents were significantly higher in fish fed the diets containing chelated amino acids. They also concluded that chelated amino acids as a

feed supplement has more advantage as compared to inorganic source of minerals. The amino acid- chelate showed a higher availability than inorganic compound even in the presence of TCP and phytate, which are normal components of plant protein sources in practical diets. Methionine is the first limiting amino acid in fish diet (Mai *et al* 2006).

Rui Ma *et al* (2014) reported that supplementation of dietary chelated or inorganic zinc improves the growth, feed utilization, bone zinc deposition and antioxidant responses in turbot (*Scophthalmus maximus*) in presence of 2% tricalcium phosphate.

2.6 Copper toxicity in fish tissue

Copper is one of the most toxic heavy metal to fish (Mance 1987) as well as Cu is required in trace amount for carrying out vital function in living organisms (Cousins 1985). As a part of the enzyme copper – zinc superoxide dismutase (Cu/Zn SOD) help in protecting the cells and some compounds from the harmful effects of free radicals. As per international standards (FAO/WHO 1983) maximum permissible limit (MPL) of Cu concentration in flesh is $30\mu\text{g g}^{-1}$. Peyghan *et al* (2003) reported the copper in liver (17.92 mg/100g) in muscles (2.2 mg/100g) in common carp when exposed to copper sulphate @ 10 mg l^{-1} for 30 days; without effect.

Chapter III

MATERIALS AND METHODS

3.1 Site of the Experiment

The present 90 days experiment was conducted to study the methionine modulated bioavailability of inorganic zinc (Zn) in the common carp (*C. carpio*) fingerlings fed with the diets containing tricalcium phosphate (TCP), in the Department of Aquaculture, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, from August to November, 2015.

3.2 Setting of the experimental aquaria

The experiment was carried out in the indoor glass aquaria (60× 30 ×30cm) in three tier system. A total 21 aquaria for 7 treatments, with 3 replicates, were set for the proposed study. Aquaria were filled with fresh underground water and each aquarium was connected with aeration facility.

3.4 Stocking of fish

After 2 days of filling water in the aquaria, each aquarium was stocked with pre-acclimatized 11 fingerlings (average length 6.02 cm and average weight 3.49 g @ 33 m⁻³) of common carp (*C. carpio*).

3.3 Preparation of experimental diets

For present study, seven experimental diets including one control (D₁) and six experimental diets (D₂ to D₇) were formulated (Table 1). All seven experimental diets were prepared by mixing different ingredients such as casein, dextrin, gelatin, fish meal, sodium alginate, soybean meal, oil, carboxy methyl cellulose, Zn free mineral, vitamin mix, zinc sulphate (ZnSO₄.7H₂O), TCP and DL-methionine. Percentage of casein, dextrin, gelatin, fish meal, sodium alginate, soybean meal, oil, carboxy methyl cellulose, Zn free mineral, vitamin mix were 20%, 30%, 8%, 10%, 12%, 8%, 2%, 5%, 3% and 2%, respectively. D₁ i.e. control diet was prepared without adding ZnSO₄.H₂O, TCP and methionine. D₂ and D₃ contained 88.4 and 176.8 mg kg⁻¹ ZnSO₄.H₂O without TCP and methionine. D₄ and D₅ contained 88.4 and 176.8 mg kg⁻¹ ZnSO₄.H₂O and 2 % TCP without methionine. D₆ and D₇ contained 88.4 and 176.8 mg kg⁻¹ ZnSO₄.H₂O, 2 % TCP and 1% methionine.

Table 1: Composition of experimental diets (%)

Ingredients	Diets						
	D1 Control (0mg Zn kg ⁻¹)	D2 (20mg Zn kg ⁻¹)	D3 (40mg Zn kg ⁻¹)	D4 (20mg Zn kg ⁻¹)	D5 (40mgZn kg ⁻¹)	D6 (20mg Zn kg ⁻¹)	D7 (40mg Zn kg ⁻¹)
Casein	D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
	20	20	20	20	20	20	20
Dextrin	30	30	30	30	30	30	30
Gelatin	8	8	8	8	8	8	8
Fish Meal	10	10	10	10	10	10	10
Sodium Alginate	12	12	12	12	12	12	12
Soybean Meal	8	8	8	8	8	8	8
Soybean Oil	2	2	2	2	2	2	2
Carboxy Methyl Cellulose	5	5	5	5	5	5	5
Zinc Free Mineral*	3	3	3	3	3	3	3
Vitamin Mix**	2	2	2	2	2	2	2
ZnSO₄.7H₂O(mg/kg diet)	0	88.4	176.8	88.4	176.8	88.4	176.8
Tri Calcium Phosphate***	0	0	0	2	2	2	2
DL Methionine***	0	0	0	0	0	1	1

*FeSO₄ .H₂O: 41.16, CuSO₄:0.51, CaCO₃: 20.58, KIO₃: 10.56, MgSO₄: 44.50, MnSO₄.H₂O: 2.29, NaCl: 17.15(g kg⁻¹ diet).

**Vitamin B2: 1.25g, Vitamin B6: 0.5g, Vitamin B12: 6.25 mg, Biotin: 12.5mg, Cal. Pantothenate: 1.25g, Niacinamide: 37.5g, Base: q.s. (g 100g⁻¹)

*** Added as over and above 100%

3.5. Proximate composition of experimental diets

The proximate analysis (moisture, crude protein, ether extract, ash, crude fiber and Nitrogen free extract) of feed ingredients/experimental diets (Table 2) was done on dry matter (DM) basis by following the methods of AOAC (2000).

3.5.1 Moisture

Approximately 10 g sample was taken in pre weighed petridish. Weight of petridish was further taken with sample and was kept in hot air-oven maintained at $100 \pm 2^{\circ}\text{C}$ to dry the sample for 6 hours. After drying, petridish was cooled in desiccator and weighed. The process of heating, cooling, and weighing was repeated until the difference in weight between two successive weights was less than 1 mg, the lowest weight was recorded.

Calculations

$$\text{Moisture content (\%)} = 100 - \left[\frac{W_2 - W_1}{W} \times 100 \right]$$

Where,

W_1 = Weight of empty crucible (g)

W_2 = Weight of crucible+dried sample (g)

W = Weight of sample (g)

3.5.2 Crude protein (CP)

The nitrogen content in diet was estimated quantitatively following Kjeldahl method [digestion with KEL PLUS (digestion unit) and distillation with KEL PLUS-Classic DX (distillation unit), Pelican Equipments]. The crude protein percentage was obtained by multiplying the nitrogen percentage by a factor of 6.25.

Reagents for digestion

- Concentrated sulphuric acid (H_2SO_4) – specific gravity 1.84
- Catalyst/digestion mixture - cupric sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and potassium sulphate (K_2SO_4) in the ratio 1:9.

Reagents for distillation

- a) 40% sodium hydroxide solution - 40 g NaOH dissolved in distilled water to make total volume of 100 ml.
- b) 15% sodium hydroxide solution - 15 g NaOH dissolved in distilled water to make total volume of 100 ml.
- c) 4% boric acid indicator solution - 40 g boric acid dissolved in 900 ml of distilled water. 10 ml of mixed indicator solution was added in it (*) and was maintained the final volume to 1 liter with distilled water.

*0.1 g methyl red and 0.5 g bromocresol green was dissolved in 100 ml of 95% alcohol.

Reagent for titration

- a) Standard 0.1 N H_2SO_4 solution- 2.6 ml of conc. H_2SO_4 dissolved in distilled water to make total volume of 100 ml.

Procedure for digestion

- i. Digestion system was preheated to 320°C.
- ii. Approximately 0.1 g of sample was taken in the digestion tube.
- iii. 3 g of digestion mixture and 10 ml of conc. H_2SO_4 was added to the sample in digestion tubes and were placed in the digestion block for digestion.
- iv. When the sample in the digestion tubes started boiling, temperature was further increased to 420°C and heated till colour of samples turned into green.
- v. Once the green colour appeared (approximately in 4 to 5 hours), tubes were removed and placed in the cooling stand.
- vi. During digestion, scrubber system (having 15% NaOH and distilled water) was kept running for absorption of fumes.

Procedure for distillation

- i. 10 ml of distilled water was added to the digestion tube with digested sample.
- ii. Digestion tubes and 250 ml conical flasks were loaded in distillation unit with preprogrammed automatic distillation unit.

Procedure for titration

- i. Contents in the conical flask were titrated with 0.1 N H₂SO₄ till wine colour appears and considered as end point.
- ii. Volume of H₂SO₄ used for the titration was recorded.
- iii. A blank was also run simultaneously to account for the nitrogen present in the reagents and that absorbed from the atmosphere.

Observations

Weight of the sample taken	-	W (0.1 g)
Normality of the standard H ₂ SO ₄ used	-	0.1 N
Initial burette reading	-	X ₁ ml
Final burette reading	-	X ₂ ml
Acid used in titration for sample	-	(X ₂ -X ₁) ml
Acid used in titration for blank	-	Y ml
Actual acid used	-	(X ₂ - X ₁) - Y = Z ml

Calculations

$$N_2 (\%) \text{ in the sample} = \frac{0.014 \times Z \times 0.1 \text{ N}}{W}$$

$$CP (\%) = N_2 (\%) \times 6.25$$

Where,

Z= ml of titrant used

W= weight of sample

3.5.3 Ether extract (EE) or crude fat

Ether extract was estimated by Soxhlet apparatus (SOCS PLUS, SCN 6, Pelican Equipment).

Reagent

Petroleum ether (Boiling point 60 – 80°C)

Procedure

- i. Dried empty beakers were weighed (W_1).
- ii. Approximately 1g prepared sample were kept in thimbles by placing cotton plug above and below the sample.
- iii. 80 ml of petroleum ether was poured into each beaker.
- iv. Thimbles were inserted in the thimble holder and placed in beakers having petroleum ether.
- v. All the beakers were loaded in the Soxhlet apparatus.
- vi. Cycle I (Extraction cycle) – Distillation was done at 100°C temperature for 1 hour.
- vii. Cycle II (Collection cycle) – Distillation was done at 140-160°C temperature for 30 minutes.
- viii. After cycle II, beakers were taken out (with 1-2 ml of remaining petroleum ether) from the apparatus and were further dried in hot air oven. After complete evaporation of petroleum ether, beakers were taken out and placed in a desiccator for cooling.
- ix. Beakers along with extracted fat were weighed (W_2).

Observations

Amount of sample taken	–	W (Approx.1g)
Weight of empty beaker	–	W_1
Weight of beaker + EE	–	W_2
Weight of EE or crude fat	–	$W_2 - W_1$

Calculations

$$\text{EE (\%)} = \frac{W_2 - W_1}{W} \times 100$$

3.5.4 Ash

Procedure

- i. Clean and dried crucibles were accurately weighed and approximately 1g of sample was taken in the crucible and charred on heater till completely burnt (smokeless).
- ii. After burning, crucible with sample was kept in Muffle furnace at 550°C for 3 hrs.
- iii. Weight of crucible with ash was recorded after cooling it.

Calculations

$$\text{Ash (\%)} = \frac{W_2 - W_1}{W} \times 100$$

Where,

W_2 = Weight of crucible + ash (g)

W_1 = Weight of empty crucible (g)

W = Weight of sample (g)

3.5.5 Crude fiber (CF) - The crude fiber content in the sample was determined by FIBRA-PLUS, FES 4, Pelican Equipments.

Reagents

- a) 1.25 % H_2SO_4 - 0.7 ml conc. H_2SO_4 dissolved in distilled water to make final volume of 100 ml.
- b) 1.25 % NaOH - 1.25g NaOH pellets dissolved in distilled water to make total volume of 100 ml.

Procedure

- i. 1 g of sample was weighed (W g) and transferred to oven dried sintered glass crucibles.
- ii. Crucibles were placed into the metal adapters of FIBRA PLUS hot extraction unit.
- iii. Acid wash
 - 150 ml of 1.25 % H_2SO_4 was poured into each extractor from top.

- Instrument was switched on and initial temperature was set at 500 °C.
 - When boiling started, temperature was reduced to 400 °C.
 - Sample was allowed to boil for 40 minutes in acid and after this, acid was drained and sample was washed twice or thrice with distilled water.
- iv. Alkali wash
- 150 ml of 1.25 % NaOH was poured into each extractor from top.
 - Instrument was switched on and initial temperature was set at 500 °C.
 - When boiling started, temperature was reduced to 400 °C.
 - Sample was allowed to boil for 40 minutes in alkali and after this, alkali was drained and sample was washed twice or thrice with distilled water.
- v. After alkali wash, crucibles were taken out and dried in hot air oven to make the samples moistures free.
- vi. Hot crucibles were cooled down to room temperature using a desiccator.
- vii. Crucibles were weighed (W_1 g).
- viii. All the crucibles were placed in the muffle furnace at 330 °C for ashing.
- ix. Hot crucibles were cooled down to room temperature after ashing using a desiccator and weighed (W_2 g).

Calculations

$$CF (\%) = \frac{W_1 - W_2}{W} \times 100$$

3.5.6 Nitrogen free extract (NFE)

It was obtained by subtracting the sum of percentage of CP, EE, CF and ash from 100

Calculations

$$NFE (\%) = 100 - (\%CP + \%EE + \%CF + \%Ash)$$

Table 2: Proximate composition (%) of formulated diets (on DM basis)

Component	Diets						
	D1	D2	D3	D4	D5	D6	D7
Moisture	8.88	9.20	8.76	8.98	9.12	8.66	8.54
Crude protein	36.68	35.42	35.84	37.12	36.28	38.66	39.14
Crude fat	2.12	2.68	2.48	1.98	2.22	2.44	2.56
Ash	9.14	10.88	10.58	9.28	11.00	10.62	11.24
Crude fiber	3.41	3.40	3.52	3.43	3.42	3.52	3.50
NFE	39.76	38.41	38.81	39.20	37.95	36.10	35.01
Zn (mg kg ⁻¹)	31.80	58.80	63.80	57.40	61.60	54.60	62.80

*Variation in proximate composition may be due to addition of TCP in D4, D5 and TCP with methionine in D6 and D7.

3.6 Feeding of fish

Fish were fed daily with experimental diets, twice (9:00 am and 4:00 pm) @ 2% of fish body weight (BW) for 90 days. Amount of feed was adjusted at every sampling according to increase in fish BW.

3.7 Observations recorded

- Physico-chemical parameters (temperature, pH, dissolved oxygen, total alkalinity, phenolphthalein alkalinity, methyl orange alkalinity, total hardness) were recorded at weekly interval, while calcium, magnesium and sodium content in water were recorded fortnightly throughout the experiment.
- Survival and growth (length and weight) of fish recorded at every fortnightly throughout the experiment.
- Biochemical changes in the flesh were estimated through estimating total proteins, total lipids, ash, moisture and total carbohydrates at the start and termination of the experiment.

- Zinc concentrations in flesh, liver and bone were estimated at the start and termination of the experiment.
- Calculation of growth parameters such as total body length gain (TBLG), net weight gain (NWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish were determined at the termination of experiment.

3.8 Physico-chemical parameters of water

Water samples were collected at weekly intervals in the morning hours for analysis of various physico-chemical parameters. The physico-chemical parameters of the water quality were estimated as standard method described in APHA, 2005.

3.8.1 Temperature – Water temperature (°C) was recorded with the help of digital thermometer (METTLER TOLEDO: MODEL FG2-1). The working range of digital thermometer was 0-50°C with $\pm 0.1^\circ\text{C}$.

3.8.2 pH – pH was recorded by using digital pH meter (METTLER TOLEDO: MODEL FG2-1).

3.8.3 Dissolved oxygen – Dissolved oxygen of water was estimated by modified Winkler's method.

Reagents

1. Manganese sulphate: 480 g $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ was dissolved in distilled to 1000 ml and filtered.
2. Alkali iodide-azide reagent : 500 g NaOH and 150 g KI was dissolved in distilled water and diluted to 1000 ml. 10 g sodium azide, (NaN_3) added and dissolved in 40 ml distilled water.
3. Concentrated Sulphuric acid : specific gravity 1.84
4. Starch indicator: 2.0 g of soluble starch powder and 0.2 g salicylic acid as preservative was dissolved in distilled water. This solution was poured into 100 ml boiling distilled water and allowed to boil for a few minutes, cooled and then used.
5. Sodium thiosulphate stock solution (0.1N): 24.82 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ was dissolved in distilled water and preserved by adding 0.4 g solid NaOH and diluted to 1000ml.

6. Standard sodium thiosulphate (0.025N): 250 ml stock $\text{Na}_2\text{S}_2\text{O}_3$ solution was dissolved to 1000 ml with freshly boiled and cooled distilled water.

Procedure

- i. Samples were collected in 250 ml BOD bottles.
- ii. To this, 1.0 ml of MnSO_4 was added and followed by addition of 1.0 ml of alkali-iodide-azide reagent up to the brim.
- iii. The contents were then mixed well by inverting the bottle 2-3 times and precipitate was allowed to settle leaving 150 ml clear supernatant.
- iv. The precipitate was further dissolved by adding 1.0 ml of concentrated sulphuric acid.
- v. 50.0 ml of this solution was taken in a conical flask and titrated against standard 0.025 N $\text{Na}_2\text{S}_2\text{O}_3$ solution using starch solution (2 ml) as an indicator.

Calculations:

$$\text{D.O. (mg l}^{-1}\text{)} = \frac{T \times N \times 8 \times 1000}{50}$$

Where,

T = Volume of titrant ($\text{Na}_2\text{S}_2\text{O}_3$) used in ml

N = Normality of $\text{Na}_2\text{S}_2\text{O}_3$ solution

3.8.4 Hardness - Hardness of water was determined by EDTA titration method.

Reagents

1. Buffer solution: 16.9 g NH_4Cl was dissolved in 143 ml NH_4OH solution. 1.25 g magnesium salt of EDTA was added to obtain sharp change in colour of indicator and diluted to 250 ml. 780 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was dissolved in 50 ml distilled water. This solution was further added to above solution (NH_4Cl in NH_4OH) and diluted to 250 ml.
2. Eriochrome black T indicator: 0.5 g of dye mixed with 100 g NaCl to prepare dye powder.
3. Mureoxide indicator (for calcium hardness): Ground mixture of 200 mg of mureoxide was prepared with 100 g of solid NaCl.

4. Sodium hydroxide (2N) for calcium hardness: 80 g NaOH was dissolved and diluted to 1000 ml.
5. Standard EDTA solution (0.01 M): 3.723 g EDTA sodium salt was dissolved and diluted to 100 ml.

Procedure

3.8.4.1 Total hardness

- i. 50ml of well mixed sample was taken in a conical flask.
- ii. To this sample, 1-2 ml of buffer solution was added.
- iii. A pinch of Eriochrome black T was added in it. The resulting sample was titrated with standard EDTA (0.01M) till wine red colour changes to blue and is considered as an end point.
- iv. The volume of EDTA used (ml) was recorded as (C).

Calculations

$$\text{Total hardness (CaCO}_3\text{mg l}^{-1}\text{)} = \frac{C \times 1000}{\text{Sample (ml)}}$$

3.8.4.2 Calcium hardness

- i. 50 ml of well mixed sample was taken in 250 ml conical flask.
- ii. 1.0ml of NaOH and a pinch of Mureoxide indicator were added to it.
- iii. It was titrated immediately with EDTA till pink colour changed to purple.
- iv. The volume of EDTA used was recorded as (C₁).

Calculations

$$\text{Calcium hardness (mg l}^{-1}\text{)} = \frac{C_1 \times 1000}{\text{Sample (ml)}}$$

Where,

C₁= volume of EDTA used for titration

3.8.4.3 Magnesium hardness

Magnesium hardness was calculated as:

Total hardness – Calcium hardness

3.8.5 Alkalinity – Alkalinity of water was determined by volumetric method.

Reagents

1. Standard H_2SO_4 (0.02 N): 0.1 N H_2SO_4 was prepared by diluting 3 ml conc. H_2SO_4 to 1000 ml.
2. Phenolphthalein indicator: 0.5 g was dissolved in 500 ml of 95% ethyl alcohol and further 500 ml of distilled water was added. 0.02 N NaOH was added dropwise till faint pink colour appeared (pH 8.3).
3. Methyl orange indicator: 0.5 g was dissolved and diluted to 1000 ml with CO_2 free distilled water (pH 4.3- 4.5).

Procedure

- i. 50 ml sample was taken in a conical flask and 2-3 drops of phenolphthalein indicator were added to it.
- ii. Pink colour developed and it was titrated with 0.02N H_2SO_4 till disappearance of the pink colour. The volume of H_2SO_4 used was noted (A).
- iii. 2-3 drops of methyl orange were added to the same flask, and it was again titrated till yellow colour changed to orange. The volume of H_2SO_4 used was noted (B).

Calculations

Phenolphthalein alkalinity and methyl orange alkalinity was calculated as:

Phenolphthalein alkalinity, as $\text{mg CaCO}_3 \text{ l}^{-1} = A \times 1000 \text{ ml}^{-1} \text{ sample} = X$

Methyl Orange alkalinity, as $\text{mg CaCO}_3 \text{ l}^{-1} = B \times 1000 \text{ ml}^{-1} \text{ sample} = Y$

Total alkalinity, as $\text{mg CaCO}_3 \text{ l}^{-1} = X + Y$

3.8.6 Sodium (Na) – Na of water was estimated by flame emission photometric method.

Reagents

1. Deionised distilled water
2. Stock Na solution: 2.542 g of NaCl dried at 140°C was dissolved and diluted in 1000 ml distilled water.
3. Intermediate Na solution: 10 ml of stock Na solution was diluted with 100 ml distilled water.

4. Standard Na solution: 10 ml of intermediate Na solution was further diluted with 100 ml distilled water.

Procedure

Direct-intensity measurement:

- i. A blank was prepared and a Na calibration standard in stepped amounts in the following applicable ranges: 0 to 1.0, 0 to 10, or 0 to 100 mg l⁻¹.
- ii. The operation was repeated with both calibration standards and samples enough times to secure a reliable average reading for each solution. A calibration curve was constructed from the Na standards.
- iii. The Na concentration of sample was determined from the calibration curve recorded at 589 nm on photo flame meter.

Calculation

For direct reference to the calibration curve $\text{mg Na l}^{-1} = (\text{mg Na l}^{-1} \text{ in portion}) \times D$

Where,

D = dilution ratio

3.9 Survival and growth of fish

Survival and growth of fish in each treatment was determined by comparing the number fish recovered at the end of experiment with that of the total fish stocked. At fortnight interval, fish sampling was done by taking out fish from aquarium to record total body length (TBL) and body weight (BW) of fish. TBLG, NWG, SGR, FCR and PER were calculated according to the following formulae:

$$\text{TBLG (cm)} = \text{Average final TBL (cm)} - \text{Average initial TBL (cm)}$$

$$\text{NWG (g)} = \text{Average final BW (g)} - \text{Average initial BW (g)}$$

$$\text{SGR (\% increase in BW day}^{-1}\text{)} = \frac{\ln \text{ Final BW (g)} - \ln \text{ Initial BW (g)}}{\text{Culture days}} \times 100$$

Where, ln is natural log

$$\text{FCR} = \frac{\text{Feed given (g)}}{\text{Weight gain (g)}}$$

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

3.10 Proximate analysis of fish flesh

Flesh samples of fish flesh were collected after dissecting the fish.

3.10.1 Moisture

- i. Dried and clean crucibles were weighed.
- ii. Approximately 1.0 g sample was taken in the crucible and dried in an oven at 100°C for overnight, till the constant weight was obtained.
- iii. Moisture was calculated by using the following formula.

Calculation

$$\text{Moisture content (\%)} = 100 - \left[\frac{W_2 - W_1}{W} \times 100 \right]$$

Where,

W_1 = Weight of empty crucible (g)

W_2 = Weight of crucible+dried sample (g)

W = Weight of flesh sample (g)

3.10.2 Total protein-Total protein of flesh was estimated according to the methods of Lowery *et al* (1951).

Reagents

- a) Tris HCL buffer-Tris buffer (0.05 M) of pH 7.6 was prepared by mixing 50 ml of 0.2 M Tris with 38.4 ml of 0.2 M HCL and adjusting the volume to 200ml with distilled water.
- b) Reagent A - 2 g sodium carbonate in 100 ml of 0.1 N sodium hydroxide.

- c) Reagent B - 0.5 g copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 100ml of 1% of sodium citrate.
- d) Reagent C (Folin's reagent) - Alkaline copper sulphate (50ml of Reagent A +1ml of Reagent B).
- e) Reagent D- Diluted Folin's reagent was prepared by adding double volume of distilled water in Folin's reagent.
- f) Standard solution -100 mg of Bovine Serum Albumin (BSA) in 100 ml of distilled water.

Procedure – 100 mg of tissue was homogenized with 5 ml Tris-HCL buffer to extract protein. The extract was centrifuged and supernatant was collected. From this, an aliquot of 0.25 ml was taken to which 0.75 ml distilled water was added to make 1 ml solution. To this solution, 5ml of reagent C was added. The contents were mixed well and 0.5 ml of reagent D was added. After 30 minutes, absorbance of the blue color developed was read on spectrophotometer (T90 +UV/VIS) at 520 nm. The value of protein content in sample was calculated using the BSA standard curve.

3.10.3 Total lipid – Total lipids in the flesh was determined by method of Folch *et al* (1957).

Reagents

- a) Sodium sulphate
- b) Chloroform
- c) Methanol
- d) 0.9% Saline- 0.9g NaCl in 100 ml distilled water.

3.9.3.2 Procedure

Extraction– One gram of flesh was mixed in pestle and mortar along with anhydrous Sodium sulphate (Na_2SO_4). Extraction was done in extraction medium (chloroform: methanol in 2:1 v/v) in ratio of 1:20 w/v) i.e. for 1g of sample 20 ml of extraction medium was used. The contents were then transferred to airtight glass stoppered flask and shaken on an electric shaker for 2 hours. The contents were then filtered by using sintered funnel (G3) under vacuum. To remove water soluble impurities, 0.9% saline (added as $1/5^{\text{th}}$ of volume filtrate) was added in separating funnel, and shaken it for 15

minutes and were allowed to stand for 30 minutes for separation of solution into two layers.

The pure lipid fraction settled on lower side of separating funnel was collected. The upper layer containing water soluble impurities was again washed with 10 ml with chloroform to obtain residual lipids. The lipid fraction was then placed in pre-weighted glass beaker, till the solvent was evaporated completely. Total lipid content was estimated by the following methods

3.9.3.3 Calculations

$$\text{Total lipid (\%)} = \frac{W_2 - W_1}{W} \times 100$$

Where,

W_1 = Weight of empty crucible (g)

W_2 = Weight of crucible + lipid (g)

W = Weight of flesh sample (g)

3.10.4. Ash

- i. Clean and dried crucibles were accurately weighted.
- ii. 1.0 g of sample was taken in the pre-weighted crucibles and was kept over the heater till it was completely burn.
- iii. Crucibles were further kept in a muffle furnace at 550° C for 3 hours to burn the sample completely.

Ash (%) was calculated by the following formula

Calculations

$$\text{Ash (\%)} = \frac{W_2 - W_1}{W} \times 100$$

Where,

W_2 = Weight of crucible + ash (g)

W_1 = Weight of crucible + sample (g)

W = Weight of sample (g)

3.10.5 Total carbohydrates as NFE

It was obtained by subtracting the sum of percentage of Moisture, CP, EE and ash from 100

Calculations

Total carbohydrates (%) = $100 - (\% \text{moisture} + \% \text{CP} + \% \text{EE} + \% \text{Ash})$

3.11 Zn and Cu concentration in flesh, liver and bone

Fish flesh, liver and bone samples were collected for the analysis of Zn and Cu concentration.

Requirements

Apparatus:-

1. Atomic absorption spectrometry (Elico).
2. Hollow cathode and electrode with discharge lamps (Elico) for Zn, and Cu.
3. Hot plate.
4. Glass wares like Volumetric Flask (250 ml) and measuring cylinder (25, 50 and 100 ml) were obtained from Borosil TM and plastic wares like wide mouth bottle of size 30 , 60 and 125 ml were obtained from Tarsons TM. Polythene zip bags-used to store the dried fish muscle tissue.

Reagents

Analytical grade (AR) chemicals were used for the sample preparation and analytical purpose.

Laboratory procedure

Cleaning of glass and plastic wares

All the glass and plastic wares including pestle mortar and silica crucibles were first washed with water and detergent, then rinsed in water to remove detergent, washed with 3% HNO₃ acid and finally dried in hot air oven (Jorhem and Engman 2000).

Sampling drying

The deep frozen samples were thawed at room temperature. After washing the dish with deionized water, flesh tissue was removed by using scalpel blade. The fish

flesh tissue was placed in silica crucible and dried in oven at 105°C until it reached constant weight preferably for 6 hr (Jorhem and Engman 2000). The dried muscle tissue was homogenized by grinding in pestle and mortar and was stored in desiccators after packing it in plastic zip bags.

Hot plate digestion of fish

Fish samples were digested on hot plate with little modification according to following procedure as mentioned in FAO, 1983.

1. 1g dried and homogenized fish muscle tissue was weight by digital weighing balance and poured into conical flask or beaker.
2. 16 ml of conc. HNO_3 (69%) and 4 ml of HClO_4 (69%) was added into the conical flask or beaker containing sample and were kept overnight at room temperature.
3. The samples were then digested on hot plate at 120°C till its turns to clear solution and after completion of digestion; digested samples were cooled to room temperature.
4. Digested samples were filtered through Whatman no.1 filter paper and diluted with deionised water in 50 ml measuring cylinder to make final volume up to 50 ml and further transfer to the plastic bottles (50 ml) for quantification against known standard.

Preparation of working solution for AAS

Preparation of working standard from stock solutions of Zn and Cu

Initially 1 mg l^{-1} of Zn and Cu were prepared by dissolving 100 μl of stock standard solution of these elements in 100 ml deionized water in volumetric flask. Volume of stock standard requirement was calculated using following formula

$$N_1V_1 = N_2V_2$$

Where

N_1 – concentration of solution -1

V_1 – volume of solution -1

N_2 – concentration of solution -2

V_2 – volume of solution-2

Different concentrations of the standards were prepared from 1 mg kg^{-1} of working solution for the calibration of AAS.

Quantification of minerals

Elements (mg kg^{-1}) = (C) (d.f.)

Where,

C is the concentration of the element in the sample solution on mg l^{-1} (converted as mg kg^{-1} on the basis of standard prepared mixing weight in solution)

d.f. is the dilution factor.

$$\text{Dilution factor} = \frac{\text{Volume of diluted sample solution in ml}}{\text{Volume of aliquot taken for dilution in ml}} \times 100$$

3.11. Statistical analysis:

The data recorded during the experimental period was statistically analysed by one way ANOVA using software SPSS 16.00. Duncan's multiple range tests was used to determine the differences among treatments and within treatment.

Chapter IV

RESULTS AND DISCUSSION

Results of the present study are presented and discussed under the following heads:

4.1 Physico-chemical parameters of water

4.2 Survival and growth of fish

4.3. Biochemical composition of fish flesh

4.4 Zinc and copper concentration in fish flesh, liver and bone

4.5 Conclusion

4.1 Physico-chemical parameters of water

Physico-chemical parameters of water play an important role in the biology, physiology and other metabolic activities of the fish. For fish culture, water quality is determined mainly by variables like temperature, transparency, turbidity, water colour, dissolved oxygen, carbon dioxide, pH, alkalinity, hardness, unionized ammonia, nitrite, nitrate and phosphate. Such parameters are greatly dependent interactions between biotic and abiotic (including feed amount, nutritional level and minerals) factors. Supplementary feeding improves fish growth but composition, digestibility and physical characteristics of feed may have a significant effect on the water quality (Sumagaysay 1998 and Verbeeten *et al* 1999). During the present study, water quality with respect to temperature, pH, dissolved oxygen, alkalinity, hardness, calcium, magnesium and sodium in different treatments is presented below.

4.1.1 Temperature

The water temperature (°C) in all treatments (D₁ to D₇) ranged between 27.1 to 31.6 during the experimental period (August to November) and the differences were significant. In between treatments, temperature of water ranged between 27.3–30.6, 27.4–30.6, 27.4 – 31.2, 27.2 –30.2, 27.8–31.6, 27.1–31.0 and 27.4–30.3 °C in treatments D₁ (control), D₂, D₃, D₄, D₅, D₆ and D₇, respectively (Table 3& Fig. 1). Although, some significant ($P \leq 0.05$) changes were observed within the treatments during the experimental period, but differences with respect to mean temperature values among different treatments were insignificant.

Table 3: Water temperature (°C) in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	27.7 ^{ab,34} ±0.44	27.4 ^{b,3} ±0.15	28.1 ^{ab,45} ±0.32	28.2 ^{ab,2} ±0.26	28.2 ^{ab,56} ±0.29	28.2 ^{ab,4} ±0.26	28.4 ^{a,45} ±0.27
	7	30.4 ^{ab,12} ±0.82	30.6 ^{ab,1} ±0.45	29.9 ^{b,123} ±0.37	30.0 ^{ab,1} ±0.54	31.6 ^{a,1} ±0.23	31.0 ^{ab,1} ±0.63	29.5 ^{b,1234} ±0.21
	14	29.5 ^{a,1} ±0.64	30.5 ^{a,1} ±0.23	30.5 ^{a,12} ±0.26	30.2 ^{a,1} ±0.15	30.2 ^{a,234} ±0.11	30.0 ^{a,12} ±0.10	29.6 ^{a,1234} ±0.39
	21	29.6 ^{a,12} ±0.63	29.7 ^{a,1} ±0.14	30.1 ^{a,123} ±0.51	30.1 ^{a,1} ±0.17	30.5 ^{a,12} ±0.26	29.8 ^{a,12} ±0.29	30.3 ^{a,1} ±0.76
September	28	30.4 ^{a,12} ±0.37	30.1 ^{a,1} ±0.32	28.9 ^{a,34} ±0.63	29.8 ^{a,1} ±0.51	30.5 ^{a,23} ±0.48	29.9 ^{a,12} ±0.37	30.3 ^{a,12} ±0.46
	35	27.3 ^{c,4} ±0.33	27.7 ^{abc,23} ±0.26	28.7 ^{a,34} ±0.43	27.5 ^{bc,2} ±0.17	27.8 ^{abc,6} ±0.34	28.0 ^{abc,4} ±0.48	28.6 ^{ab,345} ±0.34
	42	29.4 ^{bc,12} ±0.30	29.9 ^{ab,1} ±0.58	31.2 ^{a,1} ±0.25	29.6 ^{abc,1} ±0.24	29.2 ^{bc,2345} ±0.97	28.0 ^{c,4} ±0.48	28.8 ^{bc,234} ±0.52
	49	30.3 ^{a,12} ±0.43	30.4 ^{a,1} ±0.23	29.8 ^{a,23} ±0.49	29.8 ^{a,1} ±0.47	29.6 ^{a,234} ±0.20	29.9 ^{a,12} ±0.63	29.2 ^{a,234} ±0.25
October	56	30.6 ^{a,1} ±0.26	30.2 ^{a,1} ±0.29	30.0 ^{a,123} ±0.23	29.8 ^{a,1} ±0.16	29.6 ^{a,234} ±0.49	29.9 ^{a,12} ±0.52	29.6 ^{a,1234} ±0.52
	63	30.3 ^{a,12} ±0.40	30.1 ^{a,1} ±0.20	29.7 ^{a,23} ±0.52	29.4 ^{ab,1} ±0.63	29.1 ^{ab,3456} ±0.20	28.3 ^{b,4} ±0.21	29.2 ^{ab,1234} ±0.14
	70	29.8 ^{a,12} ±0.37	28.5 ^{b,2} ±0.29	29.7 ^{ab,23} ±0.15	29.5 ^{ab,1} ±0.60	29.1 ^{ab,56} ±0.26	29.6 ^{ab,23} ±0.29	29.6 ^{ab,1234} ±0.52
	77	29.3 ^{a,12} ±0.40	28.0 ^{abc,23} ±0.83	27.7 ^{bc,45} ±0.64	27.2 ^{c,2} ±0.61	28.7 ^{ab,456} ±0.75	28.4 ^{abc,34} ±0.52	27.4 ^{bc,5} ±0.30
November	84	28.9 ^{a,23} ±0.96	28.0 ^{ab,23} ±0.83	27.4 ^{b,5} ±0.15	27.3 ^{b,2} ±0.51	28.8 ^{a,3456} ±0.70	27.1 ^{b,4} ±0.11	27.5 ^{b,5} ±0.45
	91	29.6 ^{a,12} ±0.52	29.9 ^{a,1} ±0.52	29.6 ^{a,23} ±0.49	29.8 ^{a,1} ±0.16	30.0 ^{a,234} ±0.23	30.2 ^{a,12} ±0.29	29.9 ^{a,123} ±0.41
Mean		29.6 ^a ±0.18	29.3 ^a ±0.19	29.4 ^a ±0.18	29.1 ^a ±0.18	29.5 ^a ±0.17	29.2 ^a ±0.19	29.1 ^a ±0.16

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

**Values with different numerical superscripts differ significantly within column ($P \leq 0.05$)

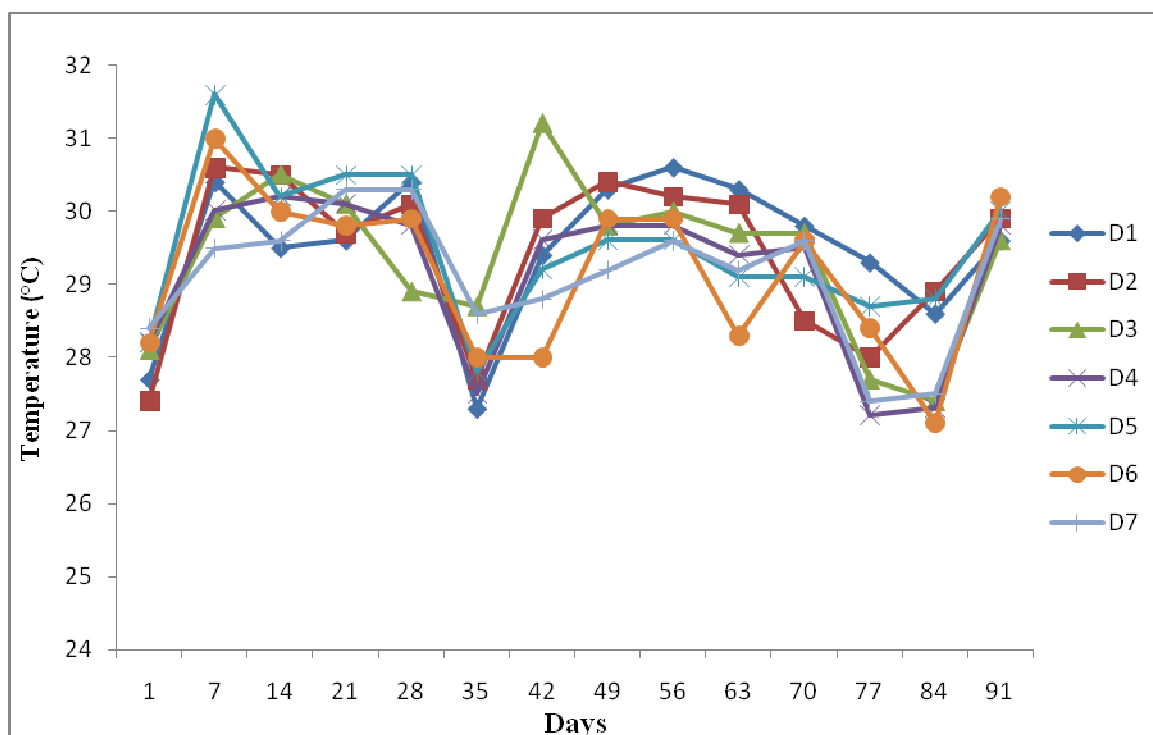


Fig 1: Changes in water temperature (°C) in different treatments during the experimental period

Fish are cold blooded animals and their rate of metabolism is directly influenced by water temperature. Temperature range for optimum growth of fish varies with species and so does the upper and lower temperature tolerance limit (Summerfelt 1998). Water temperature variations (27.1 to 31.6°C) during present study were well within the optimum range of 26-32°C documented by Swingle (1969), Boyd (1990), Jhingran (1991) and Bhatnagar and Devi (2013) for warm water fish. Oyugi *et al* (2012) suggested that temperature had a significant and positive effect on the foraging and growth of juvenile. They reported maximal foraging and growth of common carp between 24 –28°C.

4.1.2 Water pH

The water pH in all treatments (D₁ to D₇) ranged between 8.04 to 8.84 during the experimental period and the differences were significant. The pH of water ranged between 8.22 – 8.84, 8.09 – 8.84, 8.04 – 8.80, 8.18 – 8.66, 8.12 – 8.81, 8.21 – 8.72 and 8.12 – 8.77 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively (Table 4 & Fig.2) and differences among treatments were significant (D₁≥D₆=D₂=D₇=D₅≥D₃=D₄), during the experimental period (P ≤ 0.05).

Table 4: Water pH in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	8.81 ^{a,12} ±0.04	8.58 ^{b,23} ±0.02	8.46 ^{c,234} ±0.01	8.41 ^{cd,12} ±0.04	8.34 ^{de,345} ±0.02	8.30 ^{ef,45} ±0.05	8.25 ^{f,45} ±0.04
	7	8.74 ^{ab,123} ±0.08	8.84 ^{a,1} ±0.01	8.52 ^{b,234} ±0.14	8.55 ^{ab,12} ±0.10	8.50 ^{b,23} ±0.08	8.50 ^{b,1234} ±0.06	8.65 ^{ab,12} ±0.09
	14	8.84 ^{a,1} ±0.03	8.78 ^{ab,12} ±0.05	8.68 ^{ab,12} ±0.28	8.61 ^{b,12} ±0.01	8.73 ^{ab,12} ±0.06	8.67 ^{ab,12} ±0.08	8.77 ^{ab,1} ±0.14
	21	8.76 ^{a,123} ±0.08	8.53 ^{bc,34} ±0.06	8.55 ^{b,23} ±0.03	8.23 ^{d,12} ±0.03	8.36 ^{cd,345} ±0.04	8.22 ^{d,5} ±0.02	8.21 ^{d,45} ±0.07
September	28	8.22 ^{c,5} ±0.01	8.32 ^{c,45} ±0.05	8.80 ^{a,1} ±0.04	8.60 ^{ab,12} ±0.10	8.41 ^{bc,34} ±0.09	8.65 ^{a,12} ±0.02	8.60 ^{ab,123} ±0.03
	35	8.60 ^{a,1234} ±0.15	8.41 ^{a,345} ±0.14	8.52 ^{a,234} ±0.17	8.28 ^{a,12} ±0.25	8.20 ^{a,45} ±0.02	8.31 ^{a,45} ±0.15	8.56 ^{a,123} ±0.08
	42	8.31 ^{bc,5} ±0.05	8.26 ^{c,56} ±0.07	8.31 ^{bc,3456} ±0.02	8.18 ^{c,2} ±0.05	8.49 ^{b,23} ±0.01	8.72 ^{a,1} ±0.06	8.32 ^{bc,345} ±0.13
	49	8.54 ^{ab,34} ±0.02	8.65 ^{ab,123} ±0.08	8.43 ^{b,2345} ±0.15	8.66 ^{ab,1} ±0.11	8.79 ^{a,1} ±0.04	8.64 ^{ab,123} ±0.04	8.12 ^{c,5} ±0.03
October	56	8.25 ^{bcd,5} ±0.05	8.09 ^{c,6} ±0.06	8.47 ^{a,234} ±0.03	8.28 ^{bc,12} ±0.10	8.12 ^{cd,5} ±0.02	8.21 ^{bcd,5} ±0.02	8.36 ^{ab,2345} ±0.02
	63	8.58 ^{bc,234} ±0.05	8.56 ^{bc,23} ±0.07	8.04 ^{d,7} ±0.02	8.46 ^{c,12} ±0.15	8.81 ^{a,9} ±0.03	8.70 ^{ab,1} ±0.02	8.45 ^{c,234} ±0.03
	70	8.76 ^{a,123} ±0.08	8.53 ^{b,34} ±0.06	8.46 ^{bc,234} ±0.01	8.38 ^{bc,12} ±0.01	8.29 ^{c,345} ±0.03	8.34 ^{bc,45} ±0.13	8.45 ^{bc,234} ±0.04
	77	8.56 ^{a,34} ±0.08	8.31 ^{a,45} ±0.02	8.20 ^{a,567} ±0.02	8.28 ^{a,12} ±0.25	8.52 ^{a,23} ±0.17	8.41 ^{a,345} ±0.14	8.60 ^{a,123} ±0.15
November	84	8.56 ^{a,34} ±0.08	8.28 ^{a,56} ±0.01	8.16 ^{a,67} ±0.03	8.50 ^{a,12} ±0.22	8.37 ^{a,345} ±0.16	8.46 ^{a,234} ±0.12	8.49 ^{a,1234} ±0.14
	91	8.39 ^{bc,45} ±0.03	8.45 ^{bc,345} ±0.12	8.28 ^{c,4567} ±0.04	8.38 ^{bc,12} ±0.01	8.46 ^{bc,3} ±0.01	8.53 ^{b,1234} ±0.06	8.76 ^{a,1} ±0.08
Mean		8.58 ^a ±0.03	8.47 ^{ab} ±0.03	8.42 ^b ±0.03	8.41 ^b ±0.03	8.46 ^{ab} ±0.03	8.48 ^{ab} ±0.03	8.47 ^{ab} ±0.03

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

** Values with different numerical superscripts differ significantly within column ($P \leq 0.05$)

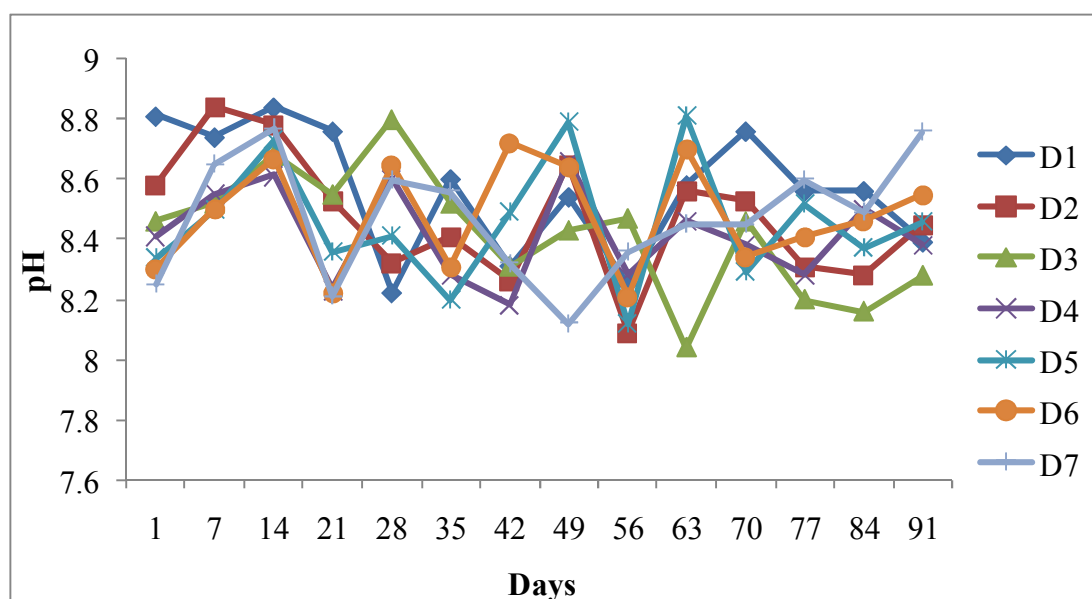


Fig 2: Changes in water pH in different treatments during the experimental period

For optimum fish culture, the acceptable range of water pH is 6.5 to 9.0 (Zweig *et al* 1999). Parmeswaran *et al* (1971) and Jhingran (1991) have also recommended water pH between 6.5 to 9.6 for optimum growth of Indian major carp and exotic carp. Heydarnejad (2012) suggested the best range for survival and growth of common carp is pH 7.5 to 8.0. Sub-optimal as well as higher pH (<4.0 or >10.5) causes stress, disease, reduced growth or even leads to death (Bhatnagar *et al* 2004). In present study, water pH recorded in the different treatments remained well within the recommended values.

4.1.3 Dissolved oxygen (D.O.)

D.O. (mg l^{-1}) in all treatments (D_1 to D_7) ranged between 6.40 to 10.60 during the experimental period and the differences were significant. D.O. in water ranged between 6.93 – 9.86, 6.93 – 9.86, 7.60 – 9.60, 6.40 – 9.86, 6.53 – 9.53, 6.66 – 10.60 and 6.53 – 10.20 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively (Table 5 & Fig. 3). Although, some significant ($P \leq 0.05$) changes were observed within the treatments during the experimental period, but differences with respect to mean D.O. values among different treatments were insignificant.

The level of oxygen gas dissolved in water (D.O.) is vital for the survival of most aquatic organisms as it provides them with oxygen to carry out cellular respiration. Oxygen requirement of fish depends on number of factors like temperature, pH, CO_2 , metabolic rate of fish moreover oxygen requirement of fish increases with temperature (Svobodova *et al* 1993).

Table 5: Water dissolved oxygen (mg l⁻¹) in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	8.00 ^{a,1} ±1.00	8.00 ^{a,1} ±0.30	7.80 ^{a,1} ±0.46	7.93 ^{a,12} ±0.33	9.53 ^{a,1} ±1.68	7.33 ^{a,2} ±1.33	8.66 ^{a,123} ±1.40
	7	9.60 ^{ab,1} ±0.80	9.20 ^{ab,1} ±1.28	8.40 ^{ab,1} ±1.61	6.80 ^{b,2} ±0.40	7.20 ^{b,12} ±0.80	10.60 ^{a,12} ±0.46	7.73 ^{ab,123} ±1.66
	14	7.84 ^{a,1} ±0.57	9.86 ^{a,1} ±1.50	9.33 ^{a,1} ±1.06	9.86 ^{a,1} ±1.53	8.40 ^{a,12} ±1.05	8.93 ^{a,12} ±1.48	9.80 ^{a,1} ±1.48
	21	9.86 ^{a,1} ±0.35	8.00 ^{a,1} ±1.22	7.73 ^{a,1} ±1.16	8.70 ^{a,12} ±0.92	7.40 ^{a,12} ±0.87	7.86 ^{a,12} ±0.83	7.73 ^{a,123} ±0.92
September	28	9.40 ^{a,1} ±0.34	6.93 ^{a,1} ±1.40	8.00 ^{a,1} ±1.38	8.53 ^{a,12} ±1.15	7.53 ^{a,12} ±1.30	8.53 ^{a,12} ±1.66	8.00 ^{a,3} ±2.11
	35	6.93 ^{a,1} ±1.33	7.33 ^{a,1} ±0.74	8.13 ^{a,1} ±0.63	6.40 ^{a,2} ±1.20	6.53 ^{a,2} ±0.46	6.66 ^{a,2} ±0.66	6.53 ^{a,123} ±0.70
	42	7.33 ^{a,1} ±0.93	8.00 ^{a,1} ±0.69	9.60 ^{a,1} ±0.46	8.73 ^{a,12} ±0.37	8.13 ^{a,12} ±0.35	9.06 ^{a,12} ±1.13	8.66 ^{a,123} ±0.93
	49	9.33 ^{a,1} ±1.53	8.93 ^{a,1} ±0.58	8.80 ^{a,1} ±0.83	9.46 ^{a,1} ±0.61	8.13 ^{a,12} ±1.04	8.80 ^{a,12} ±0.23	10.20 ^{a,1} ±0.84
October	56	8.66 ^{a,1} ±0.66	7.76 ^{a,1} ±0.93	7.60 ^{a,1} ±0.61	8.00 ^{a,12} ±0.46	7.73 ^{a,12} ±0.93	8.26 ^{a,12} ±0.93	7.06 ^{a,23} ±0.13
	63	8.00 ^{a,1} ±1.15	8.26 ^{a,1} ±0.26	8.26 ^{a,1} ±1.18	7.73 ^{a,12} ±0.74	8.63 ^{a,12} ±0.29	8.13 ^{a,12} ±0.157	8.66 ^{a,123} ±0.66
	70	8.66 ^{a,1} ±0.66	8.30 ^{a,1} ±1.46	9.40 ^{a,1} ±0.70	7.76 ^{a,12} ±0.67	7.46 ^{a,12} ±0.37	7.66 ^{a,12} ±0.76	8.53 ^{a,123} ±0.33
	77	9.86 ^{a,1} ±0.48	9.63 ^{a,1} ±0.63	8.00 ^{a,1} ±0.92	8.66 ^{a,12} ±0.81	9.40 ^{a,12} ±0.58	8.53 ^{a,12} ±0.74	9.63 ^{a,12} ±0.30
November	84	8.66 ^{a,1} ±0.81	7.33 ^{a,1} ±1.33	8.66 ^{a,1} ±2.09	6.63 ^{a,2} ±0.88	7.83 ^{a,12} ±0.31	8.73 ^{a,12} ±0.29	9.36 ^{a,12} ±0.66
	91	8.26 ^{a,1} ±0.93	8.00 ^{a,1} ±0.69	7.60 ^{a,1} ±0.83	7.73 ^{a,12} ±0.53	7.60 ^{a,12} ±0.61	7.76 ^{a,12} ±0.93	8.66 ^{a,123} ±0.66
Mean		8.64 ^a ±0.24	8.25 ^a ±0.25	8.38 ^a ±0.25	8.06 ^a ±0.21	7.96 ^a ±0.21	8.35 ^a ±0.25	8.49 ^a ±0.22

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

** Values with different numerical superscripts differ significantly within column ($P \leq 0.05$)

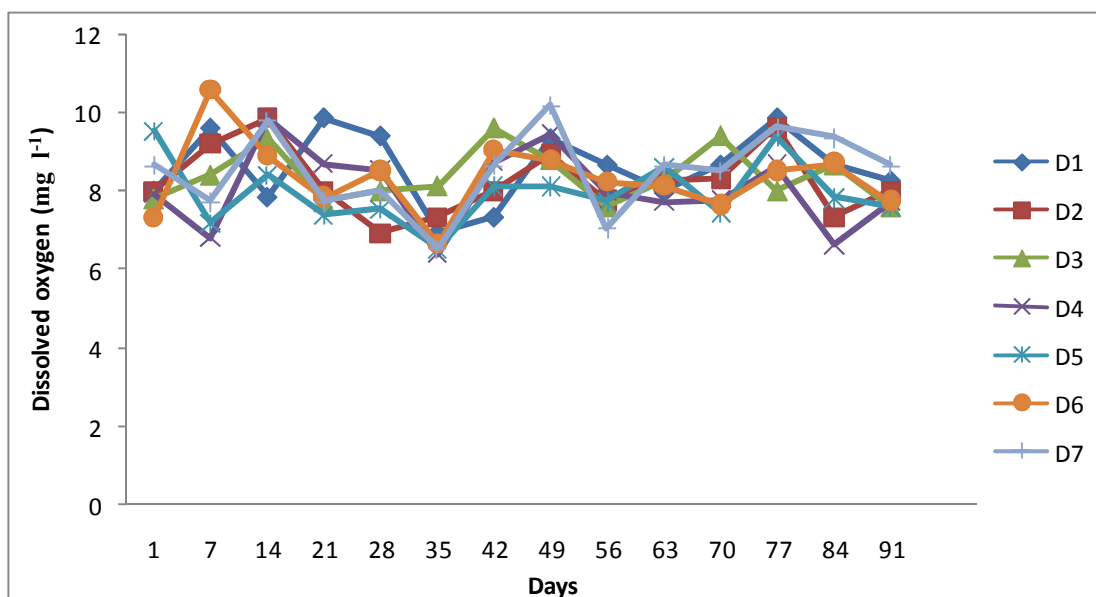


Fig 3: Changes in dissolved oxygen (mg l^{-1}) in different treatments the during the experimental period

In present study, D.O. recorded in the different treatments remained well above than the minimum recommended concentration i.e. 5 mg l^{-1} . For optimum productivity of carps D.O. content $\geq 5.0 \text{ mg l}^{-1}$ is required to be maintained in the ponds throughout the culture period (Swingle 1961, Banerjea 1967, Boyd 1992). Dissolved oxygen levels lower than 3.0 mg l^{-1} causes stress, increased susceptibility to disease, poor feed conversion efficiency, retarded growth, and even death. Ekubo and Abowei (2011) reported that fish can die if exposed to less than 0.3 mg l^{-1} dissolved oxygen for long period; they also suggested that minimum concentration of 1.0 mg l^{-1} for survival; to sustain fish for long period 5.0 mg l^{-1} or more is essential.

4.1.4 Total alkalinity

Total alkalinity ($\text{CaCO}_3 \text{ mg l}^{-1}$) in all treatments (D_1 to D_7) ranged between 171.33 to 320 during the experimental period and the differences were significant. Total alkalinity in water ranged between 234.00 – 320.00, 173.33 – 303.33, 193.33 – 296.67, 222.67 – 292.00, 206.00 – 306.67, 171.33 – 290.00 and 180.00 – 296.00 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively (Table 6 & Fig. 4). Although, some significant ($P \leq 0.05$) changes were observed within the treatments during the experimental period, but differences with respect to mean total alkalinity values among different treatments were insignificant.

Alkalinity is the measure of total concentration of bases in pond water including carbonates, bicarbonates, and hydroxides ions in the water, which provides a good buffering capacity to the diurnal pH changes.

Table 6: Water total alkalinity (CaCO₃mg l⁻¹) in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	268.67 ^{a,23} ±13.90	173.33 ^{b,5} ±6.66	193.33 ^{b,3} ±4.80	222.67 ^{ab,4} ±17.60	206.00 ^{ab,2} ±45.70	171.33 ^{b,2} ±15.07	180.00 ^{b,4} ±2.30
	7	320.00 ^{a,1} ±18.00	292.00 ^{ab,12} ±8.32	288.67 ^{ab,12} ±17.60	288.00 ^{ab,12} ±3.05	289.33 ^{ab,1} ±15.30	264.67 ^{b,1} ±12.40	285.33 ^{ab,12} ±9.33
	14	271.20 ^{a,23} ±7.31	269.33 ^{a,1234} ±10.90	264.00 ^{a,12} ±42.10	292.00 ^{a,1} ±8.32	281.00 ^{a,1} ±18.80	282.00 ^{a,1} ±9.45	234.00 ^{a,3} ±17.00
September	21	274.67 ^{a,23} ±19.60	284.67 ^{a,123} ±38.90	234.33 ^{a,23} ±7.44	246.67 ^{a,34} ±13.70	290.67 ^{a,1} ±27.80	261.33 ^{a,1} ±12.70	296.00 ^{a,1} ±22.70
	28	305.00 ^{a,12} ±27.90	303.33 ^{a,1} ±8.81	286.67 ^{a,12} ±3.33	266.67 ^{a,123} ±6.66	296.67 ^{a,1} ±20.20	286.67 ^{a,1} ±44.80	263.33 ^{a,123} ±8.81
	35	277.33 ^{a,123} ±11.60	280.00 ^{a,1234} ±0.00	290.67 ^{a,12} ±14.10	287.33 ^{a,12} ±4.37	272.00 ^{b,1} ±8.00	290.00 ^{a,1} ±0.00	281.67 ^{a,12} ±4.40
October	42	266.67 ^{a,23} ±6.66	260.00 ^{a,1234} ±12.80	268.67 ^{a,12} ±39.20	223.33 ^{a,4} ±6.66	254.00 ^{a,12} ±4.00	256.00 ^{a,1} ±11.00	266.67 ^{a,123} ±12.00
	49	263.3 ^{bc,23} ±6.66	263.33 ^{bc,1234} ±10.40	243.33 ^{c,123} ±8.81	262.67 ^{bc,123} ±13.90	306.67 ^{a,1} ±8.81	286.67 ^{ab,1} ±14.50	273.33 ^{abc,12} ±20.00
	56	264.67 ^{b,23} ±10.00	243.67 ^{b,234} ±6.33	258.67 ^{b,12} ±1.33	262.67 ^{a,123} ±13.90	306.67 ^{a,1} ±8.81	253.33 ^{b,1} ±6.66	236.67 ^{b,3} ±8.81
November	63	266.67 ^{a,23} ±13.30	233.33 ^{a,4} ±13.30	265.33 ^{a,12} ±14.40	234.00 ^{a,34} ±18.10	252.67 ^{a,12} ±8.19	264.67 ^{a,1} ±12.70	253.33 ^{a,23} ±7.51
	70	260.00 ^{a,23} ±11.50	242.00 ^{a,34} ±13.30	238.00 ^{a,123} ±9.84	270.00 ^{a,123} ±10.50	246.67 ^{a,12} ±3.33	250.00 ^{a,1} ±15.20	268.33 ^{a,123} ±9.27
	77	270.00 ^{ab,23} ±10.00	281.33 ^{ab,1234} ±11.60	296.67 ^{a,1} ±6.66	253.67 ^{b,234} ±7.31	260.00 ^{b,12} ±11.50	261.67 ^{b,1} ±14.20	285.00 ^{ab,12} ±5.00
Mean	84	234.00 ^{b,3} ±17.00	269.00 ^{ab,1234} ±14.30	294.67 ^{a,12} ±8.74	288.67 ^{a,12} ±0.66	283.33 ^{a,1} ±8.81	278.67 ^{a,1} ±18.50	274.67 ^{a,12} ±7.42
	91	236.67 ^{c,3} ±8.81	253.33 ^{bc,234} ±6.66	286.67 ^{a,12} ±3.33	262.67 ^{ab,123} ±13.90	258.67 ^{bc,12} ±1.33	235.00 ^{c,1} ±2.88	264.67 ^{ab,123} ±10.00
Mean		269.60 ^a ±4.58	260.62 ^a ±5.77	264.83 ^a ±5.92	261.50 ^a ±4.31	271.74 ^a ±5.70	260.14 ^a ±5.87	261.64 ^a ±5.04

* Values with different alphabetical superscripts differ significantly within row (P ≤ 0.05)

** Values with different numerical superscripts differ significantly within column (P ≤ 0.05)

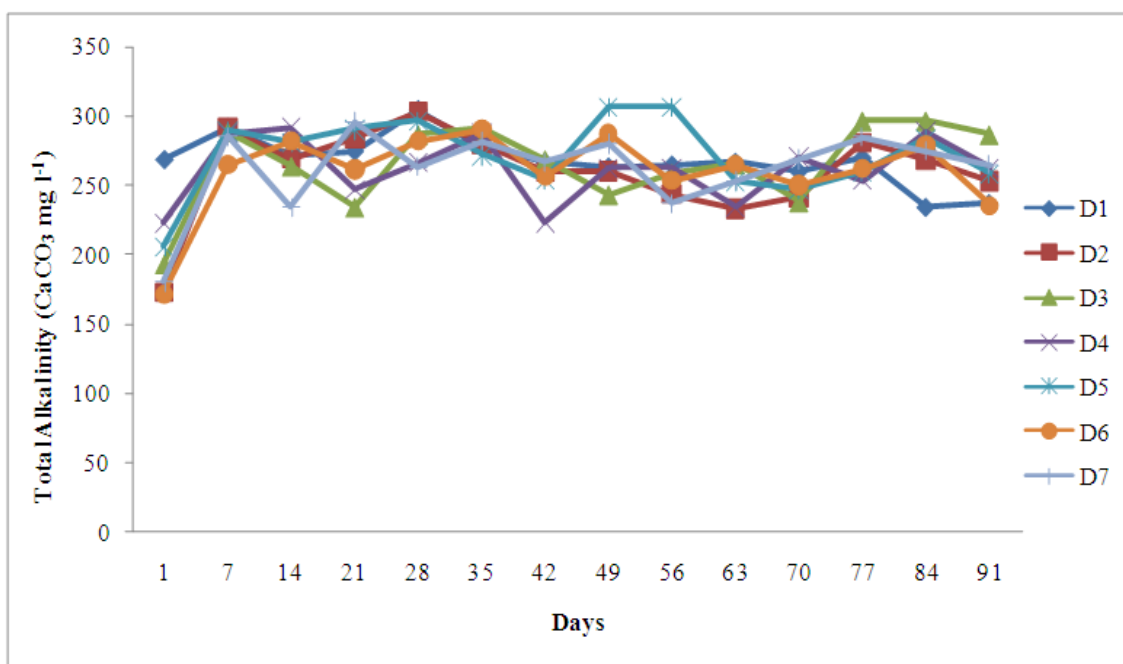


Fig 4: Changes in total alkalinity (CaCO₃mg l⁻¹) in different treatments during the experimental period

Water having total alkalinity less than 50 CaCO₃ mg l⁻¹ is poor in buffering capacity and less productive. Total alkalinity between 50- 300 CaCO₃ mg l⁻¹ is optimum for carp culture (Boyd and Tucker 1998). Santhosh and Singh (2007) proposed the ideal value of alkalinity for fish culture is between 50 - 300 CaCO₃ mg l⁻¹. Bhatnagar and Devi (2013) also suggested the total alkalinity 50 -200 CaCO₃ mg l⁻¹ as an acceptable range for fish culture. In present study, total alkalinity in different treatments was recorded well within the recommended range.

Total alkalinity consist of two type of alkalinity, one is due to carbonates and hydroxyl ions is called phenolphthalein alkalinity, while another one is due to bicarbonates is called methyl orange alkalinity.

4.1.5 Phenolphthalein alkalinity

Phenolphthalein alkalinity (CaCO₃ mg l⁻¹) in all treatments (D₁ to D₇) ranged between 9.33 to 33.3 during the experimental period and the differences were significant. In between treatments, phenolphthalein alkalinity in water ranged between 11.6 – 27.0, 15.0 – 33.3, 15.3 – 29.3, 12.0 – 31.6, 9.33 – 30.6, 12.6 – 27.3 and 12.6 – 29.6 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively (Table 7& Fig. 5). Although, some significant ($P \leq 0.05$) changes were observed within the treatments during the experimental period, but differences with respect to mean phenolphthalein alkalinity values among different treatments were insignificant.

Table 7: Water phenolphthalein alkalinity (CaCO₃mg l⁻¹) in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	23.3 ^{a,1} ±8.81	26.6 ^{a,12} ±6.66	24.6 ^{a,1} ±7.85	22.3 ^{a,12} ±6.83	30.0 ^{a,1} ±10.0	15.0 ^{a,1} ±2.88	23.3 ^{a,1} ±3.33
	7	24.0 ^{ab,1} ±3.05	33.3 ^{a,1} ±6.66	20.0 ^{ab,1} ±0.00	12.0 ^{b,2} ±1.15	19.3 ^{ab,123} ±5.81	17.0 ^{b,1} ±1.57	26.0 ^{ab,1} ±7.02
	14	27.0 ^{a,1} ±6.63	27.3 ^{a,12} ±4.05	20.6 ^{a,1} ±5.20	31.6 ^{a,1} ±6.00	17.3 ^{a,123} ±2.66	20.0 ^{a,1} ±2.30	21.6 ^{a,1} ±4.40
	21	18.6 ^{a,1} ±1.33	27.3 ^{a,12} ±7.33	20.0 ^{a,1} ±5.77	15.3 ^{a,12} ±2.90	28.6 ^{a,12} ±4.66	20.6 ^{a,1} ±5.81	25.0 ^{a,1} ±8.65
September	28	21.6 ^{ab,1} ±1.66	27.3 ^{a,12} ±2.66	18.3 ^{ab,1} ±1.66	20.0 ^{ab,12} ±5.77	24.6 ^{ab,123} ±2.90	20.0 ^{ab,1} ±5.77	13.3 ^{b,1} ±3.33
	35	22.6 ^{ab,1} ±4.80	18.6 ^{ab,12} ±1.33	17.3 ^{ab,1} ±3.71	22.6 ^{ab,12} ±8.19	9.33 ^{b,3} ±0.66	27.3 ^{a,1} ±3.71	26.3 ^{a,1} ±4.09
	42	14.0 ^{a,1} ±3.05	16.6 ^{a,2} ±3.33	29.3 ^{a,1} ±5.20	15.6 ^{a,12} ±3.48	16.6 ^{a,123} ±3.33	23.3 ^{a,1} ±3.33	18.6 ^{a,1} ±9.68
	49	19.3 ^{a,1} ±6.35	18.6 ^{a,12} ±5.92	22.3 ^{a,1} ±1.45	22.6 ^{a,12} ±8.19	17.3 ^{a,123} ±3.71	21.6 ^{a,1} ±1.66	25.0 ^{a,1} ±5.00
October	56	14.6 ^{bc,1} ±2.90	26.6 ^{ab,12} ±3.33	15.3 ^{bc,1} ±5.33	29.3 ^{a,12} ±5.20	18.0 ^{abc,123} ±2.00	24.0 ^{abc,1} ±3.05	12.6 ^{c,1} ±3.71
	63	16.6 ^{ab,1} ±4.37	15.0 ^{ab,2} ±2.88	24.3 ^{ab,1} ±4.33	20.6 ^{ab,12} ±5.20	30.6 ^{a,1} ±6.35	12.6 ^{b,1} ±2.40	29.6 ^{ab,1} ±5.77
	70	17.3 ^{a,1} ±3.71	20.6 ^{a,12} ±6.35	21.3 ^{a,1} ±6.90	20.0 ^{a,12} ±5.03	13.3 ^{a,23} ±3.33	23.3 ^{a,1} ±3.33	28.3 ^{a,1} ±6.00
	77	11.6 ^{b,1} ±1.66	19.6 ^{ab,12} ±3.17	20.0 ^{ab,1} ±5.77	17.0 ^{ab,12} ±1.52	18.0 ^{ab,123} ±6.00	22.3 ^{a,1} ±5.04	28.3 ^{b,1} ±1.66
November	84	21.3 ^{a,1} ±1.33	19.3 ^{a,12} ±4.66	24.0 ^{a,1} ±8.32	24.0 ^{a,12} ±3.05	17.3 ^{a,123} ±3.71	26.0 ^{a,1} ±8.08	21.3 ^{a,1} ±5.92
	91	16.6 ^{a,1} ±3.33	18.0 ^{a,12} ±1.15	26.0 ^{a,1} ±4.00	24.0 ^{a,12} ±3.05	22.0 ^{a,123} ±4.16	21.6 ^{a,1} ±7.26	20.6 ^{a,1} ±5.81
Mean		29.6 ^a ±1.23	22.5 ^a ±1.31	21.6 ^a ±1.28	21.2 ^a ±1.39	20.1 ^a ±1.41	21.0 ^a ±1.15	22.3 ^a ±1.45

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

** Values with different numerical superscripts differ significantly within column ($P \leq 0.05$)

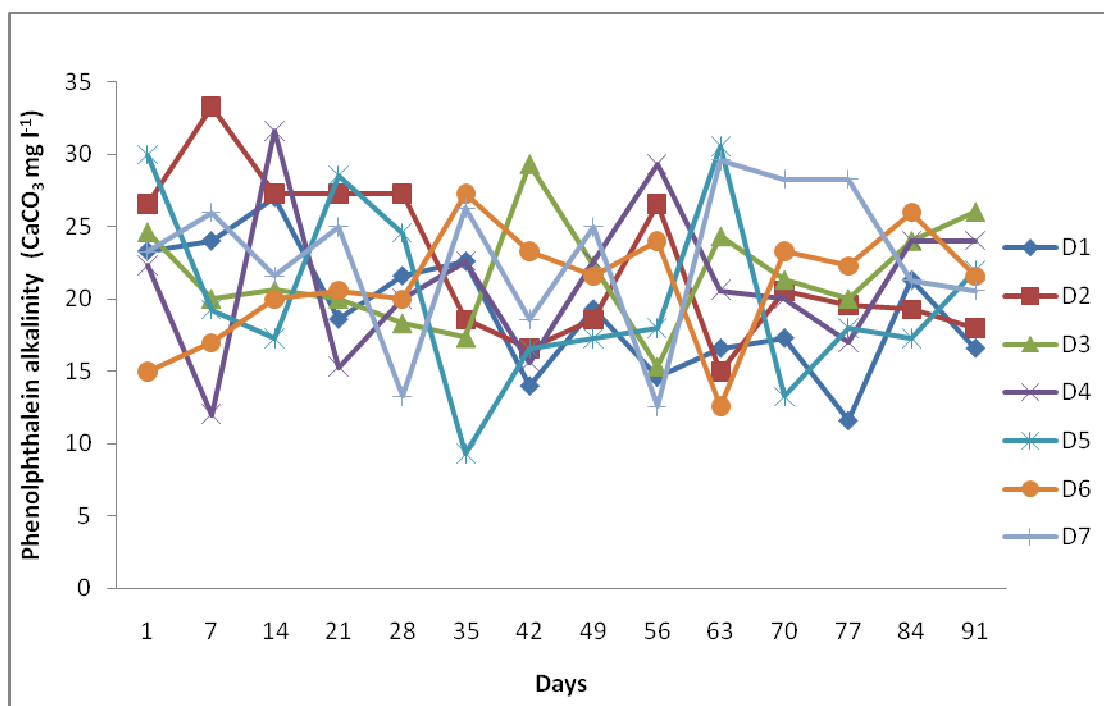


Fig 5: Changes in water phenolphthalein alkalinity (CaCO₃mg l⁻¹) in different treatments during the experimental period

4.1.6 Methyl orange alkalinity

Methyl orange alkalinity (CaCO₃mg l⁻¹), in all treatments (D₁ to D₇) ranged between 143.33 to 296.00 during the experimental period and the differences were significant. In between treatments, methyl orange alkalinity in water ranged between 212.67 – 296.00, 143.33 – 274.00, 168.67 – 276.67, 200.33 – 276.00, 176.00 – 289.33, 156.33 – 266.67 and 156.67 – 271.00 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively (Table 8 & Fig. 6). Although, some significant ($P \leq 0.05$) changes were observed within the treatments during the experimental period, but differences with respect to mean methyl orange alkalinity values among different treatments were insignificant.

Table 8: Water methyl orange alkalinity (CaCO₃mg l⁻¹) in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	245.33 ^{a,23} ±16.30	143.33 ^{b,3} ±8.81	168.67 ^{b,3} ±4.66	200.33 ^{ab,4} ±11.20	176.00 ^{b,3} ±37.80	156.33 ^{b,2} ±12.30	156.67 ^{b,4} ±1.76
	7	296.00 ^{a,1} ±15.00	258.67 ^{ab,12} ±14.80	266.67 ^{ab,1} ±17.60	276.00 ^{ab,1} ±3.05	270.00 ^{ab,12} ±10.20	247.67 ^{b,1} ±11.30	259.33 ^{ab,12} ±10.34
	14	244.20 ^{a,23} ±9.30	242.00 ^{a,12} ±11.10	243.33 ^{a,123} ±42.50	260.33 ^{a,12} ±13.80	263.67 ^{a,12} ±21.07	262.00 ^{a,1} ±9.01	212.33 ^{a,3} ±15.34
	21	256.00 ^{a,123} ±19.60	257.33 ^{a,12} ±32.30	214.33 ^{a,123} ±12.10	231.33 ^{a,234} ±13.30	262.00 ^{a,12} ±23.10	240.67 ^{a,1} ±10.60	271.00 ^{a,1} ±14.10
	28	286.67 ^{ab,12} ±29.50	274.00 ^{a,1} ±8.71	268.33 ^{ab,1} ±4.40	246.67 ^{ab,12} ±3.33	272.00 ^{ab,12} ±17.40	266.67 ^{ab,1} ±39.2	250.00 ^{b,12} ±10.00
September	35	252.27 ^{a,123} ±8.96	261.33 ^{a,12} ±1.33	273.33 ^{a,1} ±10.40	264.67 ^{a,12} ±4.80	262.67 ^{b,12} ±8.35	262.67 ^{a,1} ±3.71	255.33 ^{a,12} ±2.90
	42	252.67 ^{a,123} ±8.96	243.33 ^{a,12} ±12.70	239.33 ^{a,123} ±37.30	207.67 ^{a,34} ±9.93	237.33 ^{a,2} ±3.71	232.67 ^{a,1} ±13.80	254.67 ^{a,12} ±13.60
	49	237.33 ^{b,23} ±8.96	244.67 ^{b,12} ±13.80	227.67 ^{b,123} ±11.30	240.00 ^{b,23} ±5.77	289.33 ^{a,1} ±10.60	265.00 ^{ab,1} ±13.20	255.00 ^{ab,12} ±15.00
	56	250.00 ^{b,123} ±11.50	217.00 ^{c,2} ±6.24	243.33 ^{ab,123} ±6.66	236.00 ^{ab,23} ±11.30	288.67 ^{a,1} ±9.40	229.33 ^{bc,1} ±5.20	230.67 ^{bc,23} ±5.81
October	63	253.33 ^{a,123} ±16.70	218.33 ^{a,2} ±15.80	175.00 ^{a,23} ±77.20	213.33 ^{a,34} ±18.50	222.00 ^{a,2} ±2.00	252.00 ^{a,1} ±12.00	233.33 ^{a,23} ±11.30
	70	242.67 ^{ab,23} ±11.50	221.33 ^{ab,2} ±12.30	216.67 ^{b,123} ±3.33	250.00 ^{a,12} ±5.77	233.33 ^{ab,2} ±3.33	226.67 ^{ab,1} ±13.30	240.00 ^{ab,123} ±5.77
	77	258.33 ^{ab,123} ±9.27	261.67 ^{ab,12} ±10.90	276.67 ^{a,1} ±12.00	236.67 ^{b,23} ±8.81	245.33 ^{ab,12} ±3.71	244.00 ^{ab,1} ±14.00	248.33 ^{ab,12} ±11.60
November	84	212.67 ^{b,3} ±18.20	245.67 ^{ab,12} ±7.21	269.33 ^{a,1} ±16.30	264.67 ^{a,12} ±2.40	266.00 ^{a,12} ±6.00	252.67 ^{ab,1} ±26.5	253.33 ^{ab,12} ±12.00
	91	220.00 ^{abc,3} ±11.50	235.33 ^{abc,12} ±5.69	260.67 ^{a,12} ±6.35	238.67 ^{abc,23} ±10.90	236.67 ^{abc,2} ±4.05	213.33 ^{c,1} ±5.03	244.00 ^{ab,123} ±5.81
Mean		250.38 ^a ±4.65	237.43 ^a ±5.67	238.81 ^a ±8.00	240.45 ^a ±4.01	251.79 ^a ±5.58	239.69 ^a ±5.62	239.6 ^a ±4.76

* Values with different alphabetical superscripts differ significantly within row (P ≤ 0.05)

** Values with different numerical superscripts differ significantly within column (P ≤ 0.05)

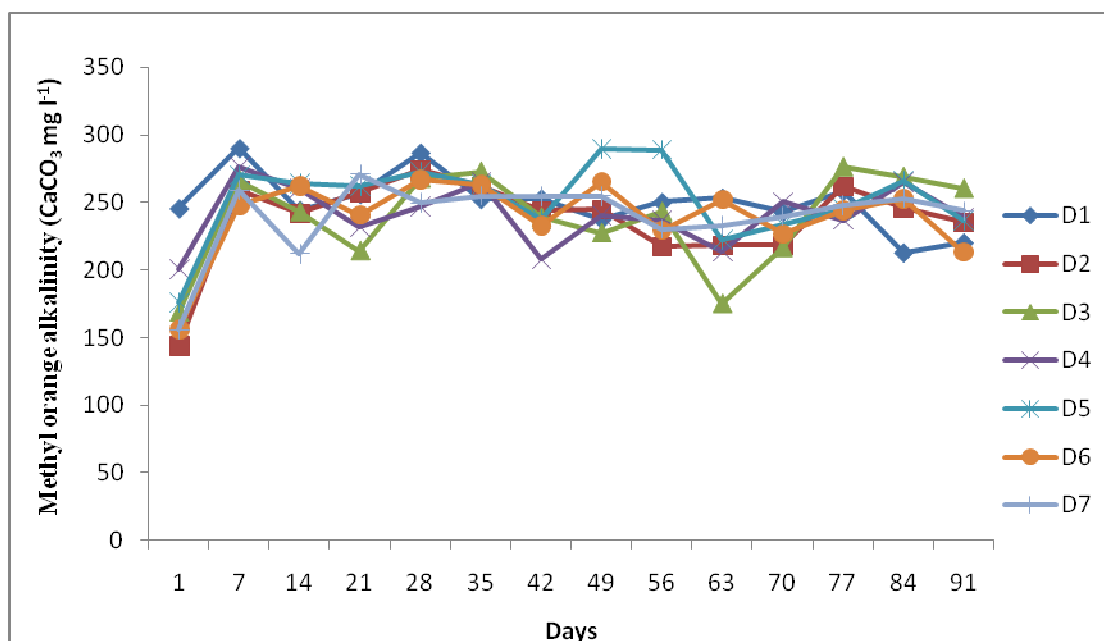


Fig 6: Changes in water methyl orange alkalinity ($\text{CaCO}_3\text{mg l}^{-1}$) in different treatments during the experimental period

4.1.7 Total hardness (CaCO_3)

Total hardness ($\text{CaCO}_3 \text{ mg l}^{-1}$) in all treatments (D_1 to D_7) ranged between 214.67 to 370.67 during the experimental period and the differences were significant. In between treatments, total hardness in water ranged between 232.67 – 340.00, 214.67 – 370.67, 224.00 – 338.67, 216.00 – 348.00, 233.33 – 338.67, 244.67 – 326.00 and 223.33 – 320.67 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively (Table 9 & Fig. 7). Although, some significant ($P \leq 0.05$) changes were observed within the treatments during the experimental period, but differences with respect to mean total hardness values among different treatments were insignificant.

Hardness represents the overall concentration of divalent salt (Ca, Mg etc.); but these are essentially required for the biological processes of aquatic animals; including scale and bone formation in fish (Wurts and Durborow 1992). Bhatnagar *et al* (2004) suggested the optimum value of hardness for fish culture in between 75- 150 $\text{CaCO}_3 \text{ mg l}^{-1}$, while Stone and Thomforde (2004) proposed the desirable range of hardness in between 50- 150 $\text{CaCO}_3\text{mg l}^{-1}$. Bhatnagar and Devi (2013) suggested the hardness $>300 \text{ CaCO}_3 \text{ mg l}^{-1}$ as stressed conditions for fish during culture. In present investigation, values estimated for the total hardness were record higher than the suggested values for fish rearing, but no ill effect of high concentration of hardness on fish in terms of behavioural change was recorded during the experimental period.

Table 9: Total hardness (CaCO₃mg l⁻¹) in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	232.67 ^{ab,4} ±3.71	214.67 ^{b,7} ±12.70	246.67 ^{ab,345} ±17.60	246.67 ^{ab,23} ±8.76	233.33 ^{ab,5} ±4.80	255.33 ^{a,23} ±13.40	223.33 ^{ab,5} ±13.3
	7	340.00 ^{a,1} ±11.50	326.67 ^{a,12} ±17.00	338.67 ^{a,1} ±19.30	314.67 ^{a,12} ±17.90	338.67 ^{a,1} ±11.70	294.67 ^{a,123} ±31.10	320.67 ^{a,1} ±4.66
	14	274.00 ^{bc,123} ±19.30	254.67 ^{bc,4567} ±32.70	224.00 ^{c,5} ±6.11	348.00 ^{a,1} ±14.00	288.67 ^{ab,12345} ±20.50	297.00 ^{ab,123} ±7.23	296.00 ^{ab,123} ±18.10
	21	274.67 ^{a,234} ±19.60	298.00 ^{a,2345} ±29.20	234.33 ^{a,45} ±7.44	246.67 ^{a,23} ±13.70	290.67 ^{a,12345} ±27.80	261.00 ^{a,23} ±12.70	296.00 ^{a,12} ±22.70
September	28	290.00 ^{a,123} ±20.00	242.67 ^{a,567} ±17.90	247.33 ^{a,345} ±46.30	288.00 ^{a,123} ±17.00	313.00 ^{a,123} ±21.00	288.00 ^{a,123} ±11.70	279.00 ^{a,23} ±7.81
	35	320.00 ^{c,12} ±33.40	302.67 ^{abc,234} ±11.80	324.00 ^{a,12} ±8.00	216.00 ^{bc,3} ±9.85	303.33 ^{abc,1234} ±21.60	277.33 ^{abc,123} ±10.70	298.67 ^{ab,12} ±15.30
	42	326.67 ^{a,1} ±17.60	320.00 ^{a,12} ±10.00	306.67 ^{a,123} ±1.76	326.66 ^{a,12} ±11.30	326.66 ^{a,12} ±18.50	326.00 ^{a,1} ±12.00	320.00 ^{a,1} ±15.20
	49	270.00 ^{a,234} ±0.00	282.67 ^{a,23456} ±2.40	270.67 ^{a,2345} ±9.68	270.00 ^{a,123} ±8.08	273.33 ^{a,2345} ±6.66	273.33 ^{a,123} ±20.90	284.00 ^{a,123} ±5.00
October	56	268.00 ^{a,234} ±6.11	260.67 ^{a,34567} ±8.66	243.33 ^{a,45} ±15.02	250.00 ^{a,23} ±0.00	271.67 ^{a,2345} ±1.66	254.00 ^{a,23} ±19.70	262.00 ^{a,234} ±6.11
	63	264.00 ^{a,34} ±3.05	236.00 ^{b,67} ±7.02	250.00 ^{ab,345} ±11.50	240.00 ^{b,23} ±8.32	250.00 ^{ab,45} ±0.00	263.33 ^{a,23} ±6.66	248.67 ^{ab,345} ±4.66
	70	265.33 ^{ab,34} ±12.90	280.00 ^{ab,23456} ±10.50	263.00 ^{ab,345} ±7.00	290.67 ^{a,123} ±10.90	263.00 ^{ab,345} ±31.50	244.67 ^{ab,3} ±16.50	237.00 ^{b,45} ±4.35
	77	312.00 ^{a,123} ±10.50	316.00 ^{a,23} ±21.10	305.33 ^{a,123} ±24.60	302.67 ^{a,123} ±11.60	290.67 ^{a,12345} ±14.10	312.00 ^{a,12} ±7.570	279.00 ^{a,23} ±7.00
November	84	296.00 ^{bc,123} ±18.10	370.67 ^{a,1} ±12.60	288.67 ^{bc,1234} ±20.50	348.00 ^{ab,1} ±14.00	262.67 ^{c,345} ±24.60	254.67 ^{c,23} ±32.70	274.67 ^{c,23} ±6.48
	91	272.00 ^{a,234} ±7.57	254.00 ^{a,4567} ±19.70	246.67 ^{a,345} ±8.81	250.00 ^{a,23} ±0.00	266.00 ^{a,345} ±8.32	266.00 ^{a,23} ±8.32	266.67 ^{a,234} ±6.66
Mean		287.95 ^a ±5.64	282.81 ^a ±7.42	270.67 ^a ±6.72	281.29 ^a ±8.96	283.64 ^a ±5.95	275.60 ^a ±5.29	277.60 ^a ±4.98

* Values with different alphabetical superscripts differ significantly within row (P ≤ 0.05)

** Values with different numerical superscripts differ significantly within column (P ≤ 0.05)

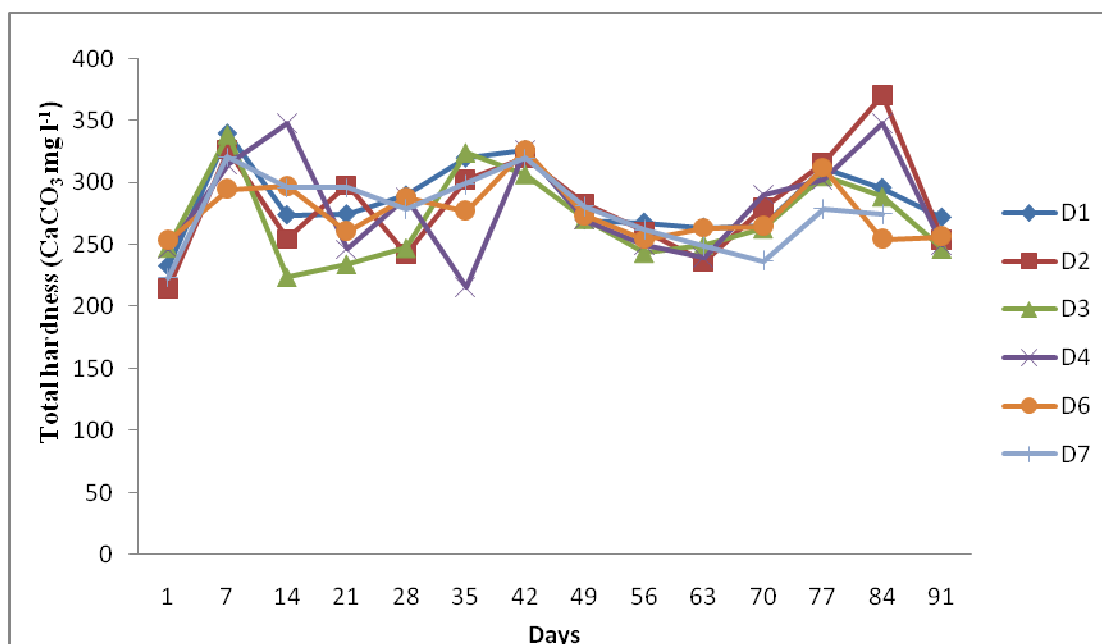


Fig 7: Changes in total hardness (CaCO₃ mg l⁻¹) of water in different treatments during the experimental period

4.1.8 Calcium hardness

Water calcium (mg l⁻¹) in all treatments (D₁ to D₇) ranged between 8.45 to 19.6 during the experimental period and the differences were significant. In between treatments, water calcium ranged between 16.2 – 18.2, 11.2 – 19.6, 12.5 – 19.6, 16.8 – 19.6, 15.6 – 19.3, 8.70 – 19.6 and 8.45 – 19.0 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively (Table 10 & Fig. 8) and among the treatments were significant (D₄=D₅=D₆≥D₁=D₇=D₃≥D₂)(P ≤ 0.05).

Excess calcium (Ca⁺⁺) contributes to water hardness. The contribution of Ca⁺⁺ in pond water should be less than 100 mg l⁻¹. Ca⁺⁺ less than 5 mg l⁻¹ doesn't support life. The acceptable range of free calcium is 25 – 100 mg l⁻¹ (63 – 250 mg l⁻¹ CaCO₃ hardness) (Wurts and Durborow 1992). Bhatnagar and Devi (2013) suggested 4 - 160 mg l⁻¹ calcium content in water as acceptable range, while 25- 100 mg l⁻¹ as desirable range for fish culture. Calcium content in water recorded during present study falls within the acceptable range.

Table 10: Calcium hardness (mg l⁻¹) in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	16.8 ^{a,12} ±0.00	14.0 ^{a,12} ±2.80	16.2 ^{a,1} ±0.57	19.6 ^{a,1} ±2.79	19.3 ^{a,1} ±3.65	12.8 ^{a,1} ±0.26	19.0 ^{a,1} ±2.22
	15	16.8 ^{a,12} ±0.00	14.0 ^{a,12} ±2.80	14.0 ^{a,1} ±2.80	16.8 ^{a,1} ±0.00	16.8 ^{a,1} ±0.00	14.0 ^{a,1} ±2.80	16.8 ^{a,1} ±0.00
September	30	18.2 ^{a,1} ±1.39	12.8 ^{a,12} ±0.26	19.3 ^{a,1} ±3.65	17.9 ^{a,1} ±1.09	15.6 ^{a,1} ±4.05	19.6 ^{a,1} ±2.79	16.8 ^{a,1} ±0.00
	45	16.8 ^{ab,12} ±0.00	15.4 ^{ab,12} ±3.70	12.5 ^{b,1} ±0.95	18.2 ^{ab,1} ±0.30	18.1 ^{ab,1} ±2.04	19.5 ^{a,1} ±1.76	16.7 ^{ab,1} ±1.66
October	60	16.8 ^{a,12} ±0.00	11.2 ^{b,2} ±1.39	19.6 ^{a,1} ±1.39	18.7 ^{a,1} ±1.24	17.6 ^{a,1} ±1.27	19.6 ^{a,1} ±3.70	16.8 ^{a,1} ±0.00
	75	17.0 ^{bc,12} ±0.26	16.8 ^{bc,12} ±0.00	16.2 ^{d,1} ±0.57	16.8 ^{c,1} ±0.00	17.9 ^{a,1} ±1.09	8.70 ^{ab,1} ±0.02	8.45 ^{c,1} ±0.03
November	91	16.2 ^{a,2} ±0.57	19.6 ^{a,1} ±2.79	15.6 ^{a,1} ±4.05	17.9 ^{a,1} ±1.09	19.3 ^{a,1} ±3.65	15.6 ^{a,1} ±2.67	16.8 ^{a,1} ±0.00
	Mean	16.9 ^{ab} ±0.22	14.8 ^b ±0.94	16.2 ^{ab} ±0.93	18.0 ^a ±0.45	17.8 ^a ±0.88	17.2 ^a ±1.05	16.8 ^{ab} ±0.48

* Values with different alphabetical superscripts differ significantly within row (P ≤ 0.05)

** Values with different numerical superscripts differ significantly within column (P ≤ 0.05)

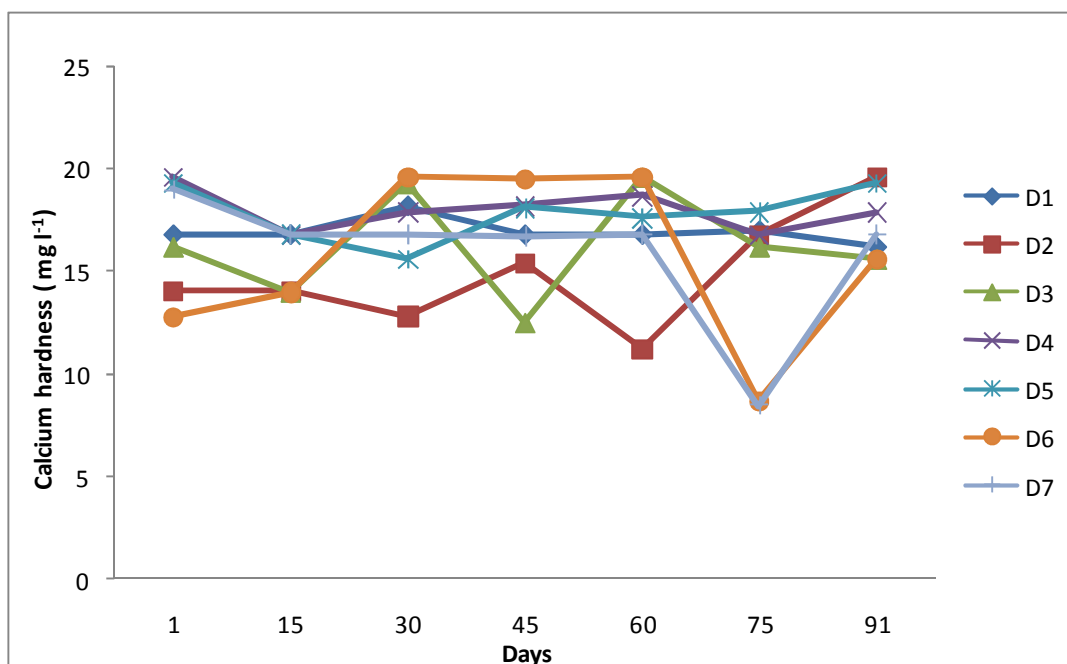


Fig 8: Changes in calcium hardness (mg l⁻¹) of water in different treatments during the experimental period

4.1.9 Magnesium hardness

Magnesium hardness (mg l⁻¹) in all treatments (D₁ to D₇) ranged between 50.0 to 86.3 during the experimental period and the differences were significant. In between treatments, magnesium in water ranged between 53.0 – 71.0, 55.8 – 73.9, 54.3 – 75.8, 56.1 – 85.2, 55.3 – 74.9, 51.7 – 86.3 and 50.0 – 73.6 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively (Table 11& Fig. 9). Although, some significant ($P \leq 0.05$) changes were observed within the treatments during the experimental period, but differences with respect to mean magnesium content values among different treatments were insignificant.

Table11: Magnesium hardness (mg l⁻¹) in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	53.0 ^{c,3} ±5.89	71.4 ^{a,12} ±0.64	75.8 ^{a,1} ±3.20	57.1 ^{a,3} ±1.10	59.5 ^{a,1} ±7.21	61.3 ^{a,34} ±2.31	59.5 ^{a,23} ±1.49
	15	68.7 ^{abc,12} ±4.86	58.4 ^{c,23} ±8.61	68.8 ^{abc,12} ±7.34	85.2 ^{ab,1} ±1.49	66.0 ^{c,1} ±4.98	86.3 ^{a,1} ±2.46	67.7 ^{bc,12} ±4.41
September	30	66.0 ^{ab,123} ±5.04	55.8 ^{ab,3} ±4.36	55.3 ^{b,12} ±10.5	65.5 ^{ab,3} ±4.18	74.9 ^{a,1} ±6.40	65.1 ^{ab,23} ±3.03	69.0 ^{ab,12} ±2.38
	45	71.0 ^{a,1} ±0.76	73.9 ^{a,1} ±2.73	71.4 ^{a,12} ±0.64	74.9 ^{a,2} ±2.71	74.9 ^{a,1} ±4.00	74.4 ^{a,2} ±2.77	73.6 ^{a,1} ±3.58
October	60	53.7 ^{a,3} ±1.75	56.5 ^{a,3} ±0.72	54.3 ^{a,2} ±3.67	56.1 ^{a,3} ±0.29	55.6 ^{a,1} ±2.16	56.9 ^{a,34} ±5.06	59.5 ^{a,23} ±1.49
	75	55.8 ^{a,23} ±4.36	60.3 ^{a,12} ±3.00	55.8 ^{a,2} ±1.62	62.6 ^{a,3} ±3.28	57.3 ^{a,1} ±7.47	51.7 ^{a,4} ±4.38	50.0 ^{a,3} ±2.03
November	91	55.8 ^{a,23} ±4.36	65.1 ^{a,123} ±3.03	74.9 ^{a,1} ±6.40	65.5 ^{a,3} ±4.18	55.3 ^{a,1} ±10.5	55.8 ^{a,34} ±4.36	66.0 ^{a,12} ±5.04
Mean		60.6 ^a ±2.08	63.0 ^a ±1.99	65.2 ^a ±2.70	66.7 ^a ±2.31	63.3 ^a ±2.75	64.5 ^a ±2.75	63.6 ^a ±1.91

* Values with different alphabetical superscripts differ significantly within row (P ≤ 0.05)

** Values with different numerical superscripts differ significantly within column (P ≤ 0.05)

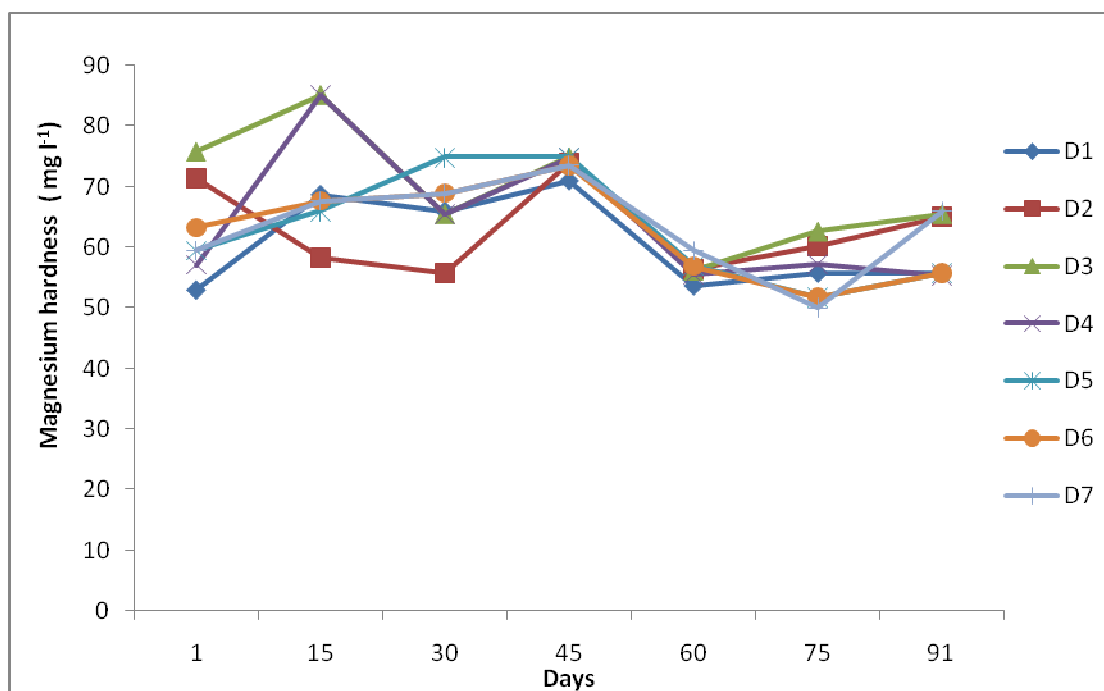


Fig 9: Changes in magnesium hardness (mg l^{-1}) of water in different treatments during the experimental period

4.1.10 Water sodium

Water sodium (mg l^{-1}), in all treatments (D_1 to D_7) ranged between 11.01 to 11.18 during the experimental period and the differences were significant. In between treatments, sodium in water ranged between 11.02 – 11.06 in D_1 and D_2 , 11.02 – 11.07 in D_3 and D_5 , 11.01– 11.04 in D_4 , 11.02 – 11.09 in D_6 and 11.02 – 11.18 in D_7 (Table 12 & Fig. 10). Although, some significant ($P \leq 0.05$) changes were observed within the treatments during the experimental period, but differences with respect to mean sodium content values among different treatments were insignificant.

Low and constant sodium concentration in water indicates that the water quality with respect to salinity remained unaffected even though sodium salt as NaCl was added as mineral source in diets.

Water quality with respect to temperature, pH, dissolved oxygen, alkalinity, hardness, calcium hardness, magnesium hardness and sodium recorded in different treatments varied within the desirable range suggested for fish rearing by Boyd (1990) and Bhatnagar and Devi (2013). Although significant differences ($P \leq 0.05$) within treatment recorded but no significant differences between treatments recorded except pH and calcium hardness. Differences within treatment may be due to change in season.

Table 12: Water sodium (mg l⁻¹) in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	11.03 ^{b,2} ±0.01	11.06 ^{b,1} ±0.00	11.03 ^{b,3} ±0.01	11.03 ^{b,1} ±0.01	11.05 ^{b,12} ±0.02	11.09 ^{b,1} ±0.04	11.18 ^{a,1} ±0.01
	15	11.04 ^{a,2} ±0.11	11.04 ^{a,12} ±0.01	11.04 ^{a,23} ±0.00	11.04 ^{a,1} ±0.01	11.04 ^{a,12} ±0.01	11.02 ^{a,2} ±0.00	11.03 ^{a,2} ±0.00
September	30	11.02 ^{b,2} ±0.00	11.04 ^{b,12} ±0.00	11.02 ^{b,3} ±0.00	11.03 ^{b,1} ±0.01	11.07 ^{a,1} ±0.00	11.04 ^{b,23} ±0.02	11.02 ^{b,2} ±0.00
	45	11.06 ^{a,1} ±0.00	11.02 ^{c,2} ±0.00	11.03 ^{bc,3} ±0.00	11.04 ^{ab,1} ±0.01	11.03 ^{bc,2} ±0.00	11.04 ^{ab,23} ±0.00	11.02 ^{c,2} ±0.00
October	60	11.02 ^{b,2} ±0.00	11.02 ^{ab,2} ±0.00	11.02 ^{ab,3} ±0.00	11.04 ^{a,1} ±0.00	11.04 ^{a,12} ±0.01	11.02 ^{b,2} ±0.00	11.02 ^{ab,2} ±0.11
	75	11.02 ^{b,2} ±0.00	11.04 ^{b,12} ±0.00	11.06 ^{a,12} ±0.00	11.01 ^{d,1} ±0.00	11.03 ^{b,2} ±0.00	11.04 ^{b,12} ±0.00	11.02 ^{c,2} ±0.00
November	91	11.02 ^{b,2} ±0.00	11.04 ^{b,12} ±0.02	11.07 ^{a,1} ±0.00	11.03 ^{b,1} ±0.01	11.02 ^{b,2} ±0.00	11.04 ^{b,12} ±0.00	11.02 ^{b,2} ±0.00
Mean		11.03 ^a ±0.00	11.03 ^a ±0.00	11.04 ^a ±0.00	11.03 ^a ±0.00	11.04 ^a ±0.00	11.03 ^a ±0.00	11.03 ^a ±0.01

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

** Values with different numerical superscripts differ significantly within column ($P \leq 0.05$)

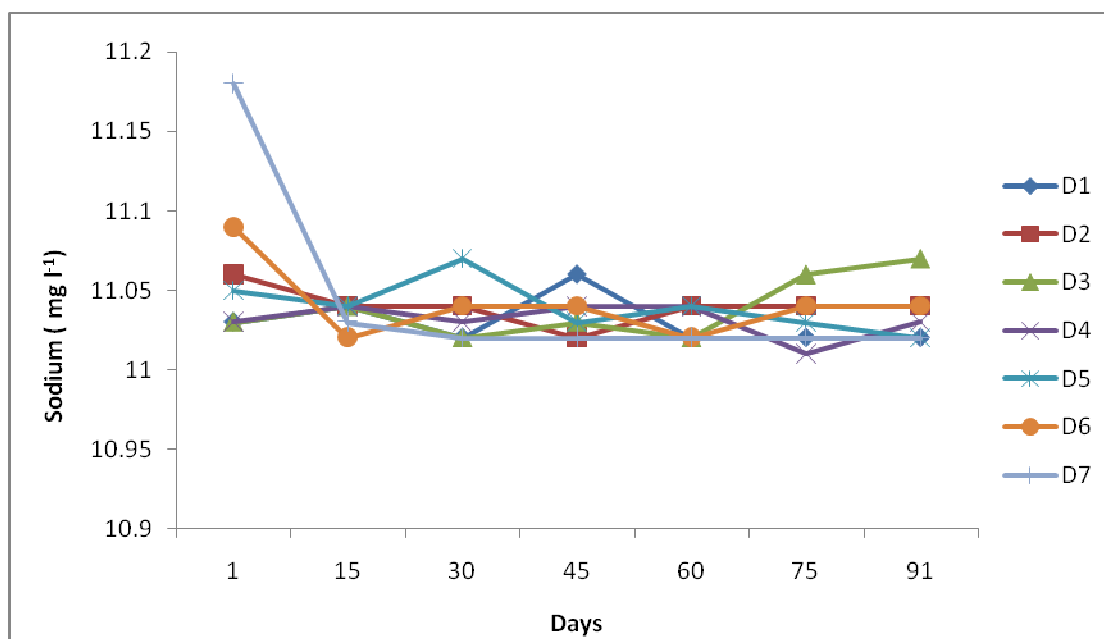


Fig 10: Changes in water sodium (mg l^{-1}) in different treatments during the experimental period

4.2 Survival and growth of fish

4.2.1 Fish survival

During the experimental period, survival (%) of fish ranged between 90.9 to 100% in all treatments (D_1 to D_7). At the end of experiment, lowest survival 90.9% was recorded in D_3 , D_4 and D_5 followed by 93.9 in D_2 , 96.9 in D_1 , D_7 and 100 in D_6 respectively (Table 14). Survival rate is one of the most important indicator of favorable conditions for organism.

4.2.2 Growth

The growth in fish stocked during experimental period was assessed in terms total body length (TBL, cm) and body weight (BW, g). At the end of experiment, total body length gain (TBLG), net weight gain (NWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish for each treatment was calculated.

4.2.3 Body length

At the end of experiment, TBLG (cm) in all treatments (D_1 to D_7) ranged between 2.21 to 3.58 during the experimental period. TBLG was 2.62, 2.30, 3.01, 3.58, 2.21, 2.79 and 2.85 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively (Table 13 & Fig.11, 13) and differences among the treatments were significant ($D_4 \geq D_3 = D_7 = D_6 \geq D_1 \geq D_2 = D_5$) ($P \leq 0.05$).

Table 13: Total body length and TBLG (cm) of fish during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	6.19 ^{ab,3} ±0.20	6.07 ^{ab,4} ±0.15	5.84 ^{ab,4} ±0.20	5.56 ^{b,4} ±0.16	6.35 ^{a,4} ±0.24	6.16 ^{ab,3} ±0.24	5.98 ^{ab,5} ±0.15
	15	7.05 ^{a,2} ±0.26	6.74 ^{a,3} ±0.18	6.52 ^{a,3} ±0.23	6.85 ^{a,3} ±0.25	6.76 ^{a,4} ±0.23	6.71 ^{a,3} ±0.24	6.37 ^{a,5} ±0.15
September	30	7.33 ^{a,2} ±0.23	7.17 ^{a,2,3} ±0.22	7.44 ^{a,2} ±0.24	7.30 ^{a,2,3} ±0.26	7.41 ^{a,3} ±0.22	7.50 ^{a,2} ±0.22	6.96 ^{a,4} ±0.13
	45	7.59 ^{a,2} ±0.23	7.46 ^{a,2} ±0.18	7.93 ^{a,2} ±0.16	7.67 ^{a,2} ±0.25	7.76 ^{a,2,3} ±0.16	7.81 ^{a,2} ±0.22	7.55 ^{a,3} ±0.22
October	60	8.34 ^{ab,1} ±0.18	8.31 ^{ab,1} ±0.17	8.54 ^{a,1} ±0.13	8.47 ^{ab,1} ±0.23	8.07 ^{ab,1,2} ±0.18	8.15 ^{ab,2} ±0.20	7.93 ^{b,2,3} ±0.20
	75	8.43 ^{abc,1} ±0.22	8.28 ^{bc,1} ±0.13	8.75 ^{ab,1} ±0.12	8.94 ^{a,1} ±0.25	8.35 ^{bc,1} ±0.12	8.08 ^{c,2} ±0.21	8.11 ^{c,2} ±0.13
November	91	8.80 ^{abc,1} ±0.17	8.37 ^{b,1} ±0.11	8.86 ^{abc,1} ±0.10	9.15 ^{a,1} ±0.21	8.56 ^{bc,1} ±0.78	8.96 ^{ab,1} ±0.27	8.84 ^{abc,1} ±0.19
TBLG*		2.62 ^b ±0.12	2.30 ^b ±0.21	3.01 ^{ab} ±0.40	3.58 ^a ±0.43	2.21 ^b ±0.21	2.79 ^{ab} ±0.22	2.85 ^{ab} ±0.59

*TBLG= Total body length gain

** Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

*** Values with different numerical superscripts differ significantly within column ($P \leq 0.05$)

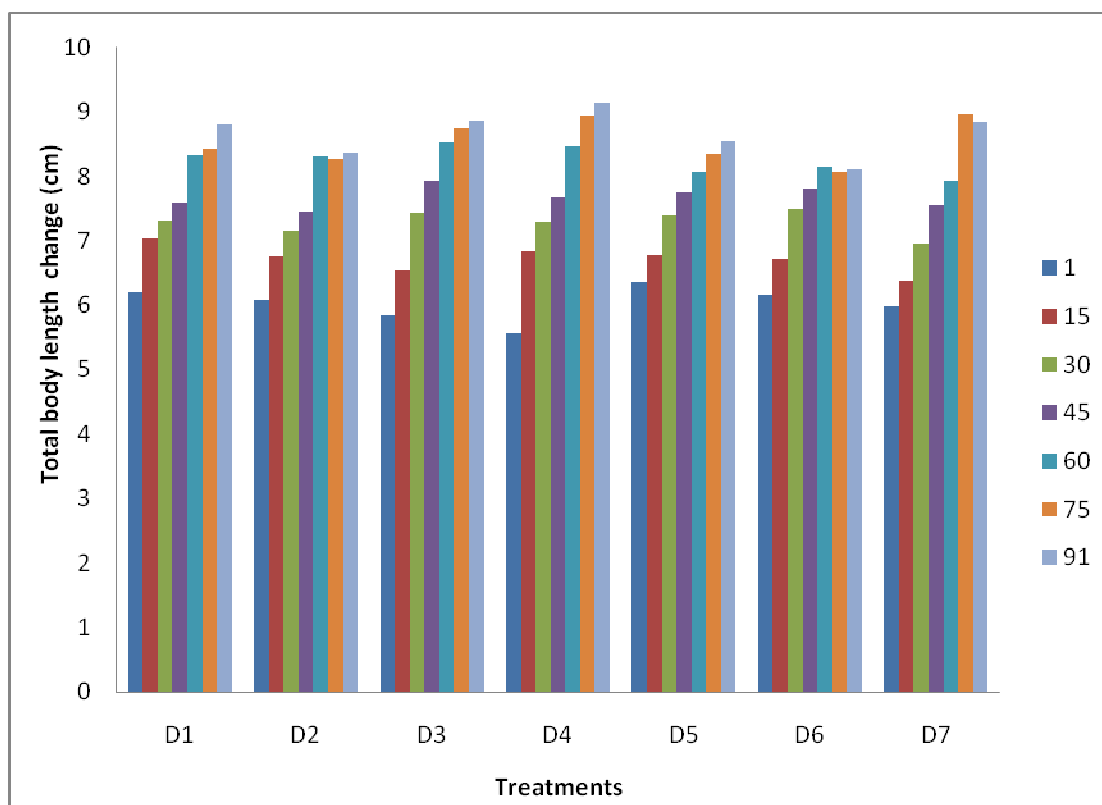


Fig 11: Changes in total body length (cm) of fish in different treatments during the experimental period

4.2.4 Body weight

At the end of experiment, NWG (g), in all treatments (D₁ to D₇) ranged between 2.19 to 4.01 during the experimental period. NWG was 2.85, 3.39, 3.13, 2.96, 2.19, 3.35 and 4.01 g in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively (Table 14 & Fig.12, 13) and the differences among the treatments were insignificant ($P \leq 0.05$).

4.2.5 Specific growth rate (SGR)

Specific growth rate in all the treatments (D₁ to D₇) ranged between 0.48 to 0.92. SGR was recorded 0.56, 0.81, 0.83, 0.72, 0.48, 0.69 and 0.92 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively (Table 14 & Fig. 14) and the differences among treatments were insignificant ($P \leq 0.05$).

4.2.6 Feed conversion ratio (FCR)

Feed conversion ratio in all the treatments (D₁ to D₇) ranged between 2.42 to 5.68. FCR was recorded 4.54, 3.27, 4.12, 5.68, 5.68, 3.71 and 2.42 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively (Table 14 & Fig. 14) and differences among treatments were insignificant ($P \leq 0.05$).

4.2.7 Protein efficiency ratio (PER)

Protein efficiency rate in all the treatments (D₁ to D₇) ranged between 0.56 to 1.08. PER was recorded 0.64, 0.93, 0.86, 0.71, 0.56, 0.81 and 1.08 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively (Table 14 & Fig. 14) and differences among treatments were insignificant ($P \leq 0.05$).

In present study, no significant difference in growth in terms of body weight gain recorded. It may be due to the almost same nutritional composition of feed. Satoh *et al* (1993) also reported no significant difference in growth of *O. mykiss* fed with and without calcium phosphate supplemented diets.

No significant change ($P \leq 0.05$) in SGR, FCR and PER value of common carp (*C. carpio*) was recorded between the treatments (D₁ to D₇), however FCR value (ratio) was recorded minimum in diet D₇, when Zn was added @ 40 mg kg⁻¹ along with 1% methionine, while it was toward higher side in diets containing TCP without adding methionine (D₄ and D₅). Such higher ratio in D₄ and D₅ even though in comparison to addition of Zn only (D₂ and D₃) indicates the negative effect of TCP in feed utilization. During experiment, difference in PER value in treatments were insignificant and was recorded maximum in D₇ when Zn was added @ 40 mg kg⁻¹ along with 1% methionine, while it was minimum in diets containing TCP without adding methionine (D₄ and D₅). The value recorded for PER in diets D₂ and D₃ was higher than the diets D₄ and D₅. The result indicates positive effect of methionine in protein utilization by the fish. Overall result indicates that addition of Zn @ 40 mg kg⁻¹ along with addition of 1% methionine, improves the protein assimilation in body. However in case of FCR and PER the values were insignificant but on average basis it clearly indicate the positive effect of adding 1% methionine in diets to mitigate negative effect of TCP in protein utilization.

Table 14: Body weight (BW) and growth parameters of fish in different treatments during the experimental period (Mean±SE)

Month	Days	Diets						
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
August	1	4.17 ^{a,3} ±0.48	3.08 ^{a,5} ±0.37	2.92 ^{a,4} ±0.34	3.81 ^{a,3} ±0.59	4.06 ^{a,3} ±0.45	3.53 ^{a,4} ±0.40	2.88 ^{a,5} ±0.24
	15	4.65 ^{a,3} ±0.47	3.30 ^{b,45} ±0.34	3.38 ^{ab,34} ±0.33	4.33 ^{ab,23} ±0.51	4.46 ^{ab,23} ±0.46	4.45 ^{ab,34} ±0.45	3.24 ^{b,45} ±0.15
September	30	4.65 ^{a,3} ±0.47	3.46 ^{bc,45} ±0.33	3.38 ^{b,34} ±0.33	4.33 ^{abc,23} ±0.51	4.46 ^{abc,23} ±0.46	5.17 ^{a,23} ±0.41	3.74 ^{bc,34} ±0.13
	45	5.26 ^{a,23} ±0.55	4.35 ^{a,34} ±0.41	4.22 ^{a,3} ±0.27	5.19 ^{a,123} ±0.51	5.31 ^{a,23} ±0.42	5.45 ^{a,23} ±0.42	4.24 ^{a,3} ±0.18
October	60	5.63 ^{a,23} ±0.58	5.08 ^{a,23} ±0.42	5.15 ^{a,2} ±0.19	5.66 ^{a,12} ±0.53	5.18 ^{a,123} ±0.24	5.91 ^{a,12} ±0.41	4.78 ^{a,2} ±0.16
	75	6.29 ^{a,12} ±0.60	5.70 ^{a,12} ±0.40	5.91 ^{a,12} ±0.22	6.56 ^{a,1} ±0.55	5.70 ^{a,1} ±0.18	6.20 ^{a,12} ±0.52	5.27 ^{a,2} ±0.17
November	91	6.64 ^{ab,1} ±0.30	6.45 ^{b,1} ±0.31	6.06 ^{b,1} ±0.25	6.78 ^{ab,1} ±0.44	6.26 ^{b,1} ±0.26	7.06 ^{a,1} ±0.52	7.08 ^{a,1} ±0.19
NWG		2.85 ^a ±0.69	3.39 ^a ±0.49	3.13 ^a ±0.67	2.96 ^a ±0.50	2.19 ^a ±0.24	3.35 ^a ±0.89	4.01 ^a ±0.42
Survival %		96.9%	93.9%	90.9%	90.9%	90.9%	100%	96.9%
SGR		0.56 ^a ±0.12	0.81 ^a ±0.14	0.83 ^a ±0.21	0.72 ^a ±0.21	0.48 ^a ±0.65	0.69 ^a ±0.20	0.92 ^a ±0.13
FCR		4.54 ^a ±0.90	3.27 ^a ±0.65	4.12 ^a ±1.59	5.68 ^a ±2.86	5.68 ^a ±1.78	3.71 ^a ±1.05	2.42 ^a ±0.30
PER		0.64 ^a ±0.13	0.93 ^a ±0.20	0.86 ^a ±0.24	0.71 ^a ±0.25	0.56 ^a ±0.13	0.81 ^a ±0.23	1.08 ^a ±0.12

* Values with different alphabetical superscripts differ significantly within row ($P \leq 0.05$)

** Values with different numerical superscripts differ significantly within column ($P \leq 0.05$)

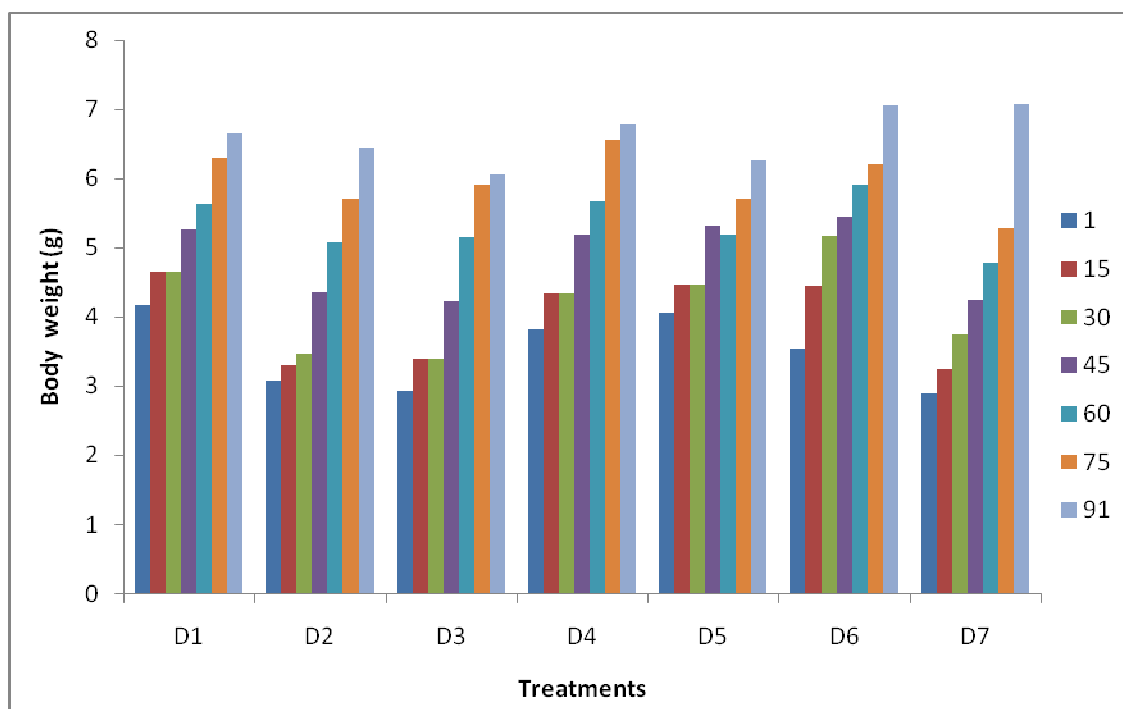


Fig 12: Changes in fish body weight (g) in different treatments during the experimental period

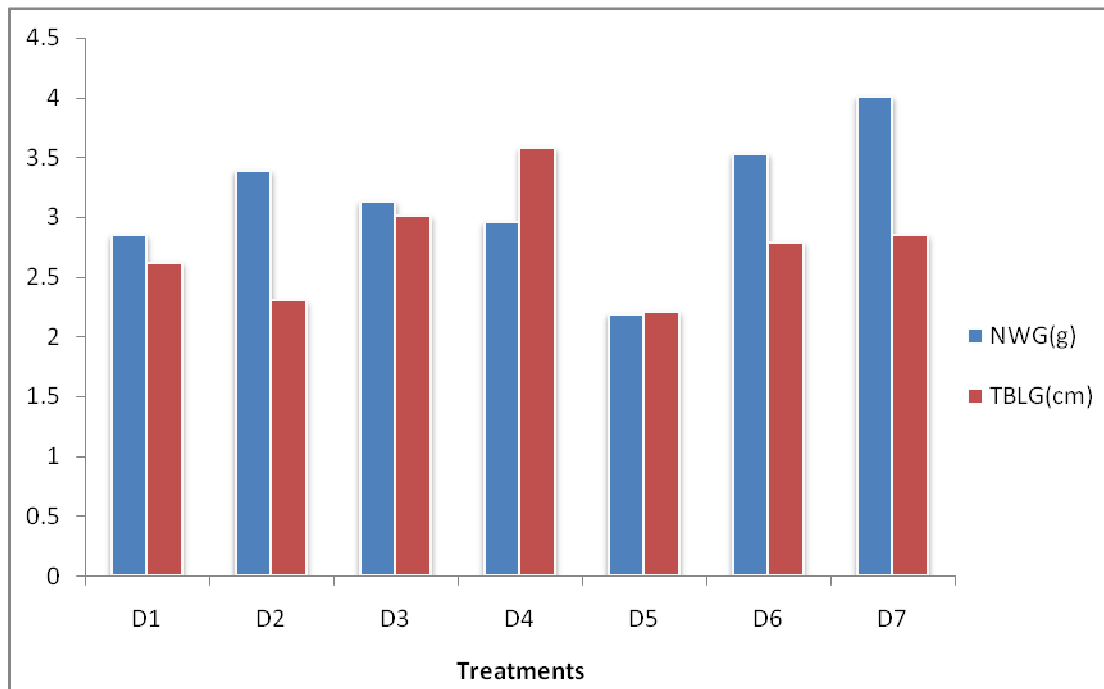


Fig 13: Changes in net weight gain (NWG) and total body length gain (TBLG) in different treatments during the experimental period

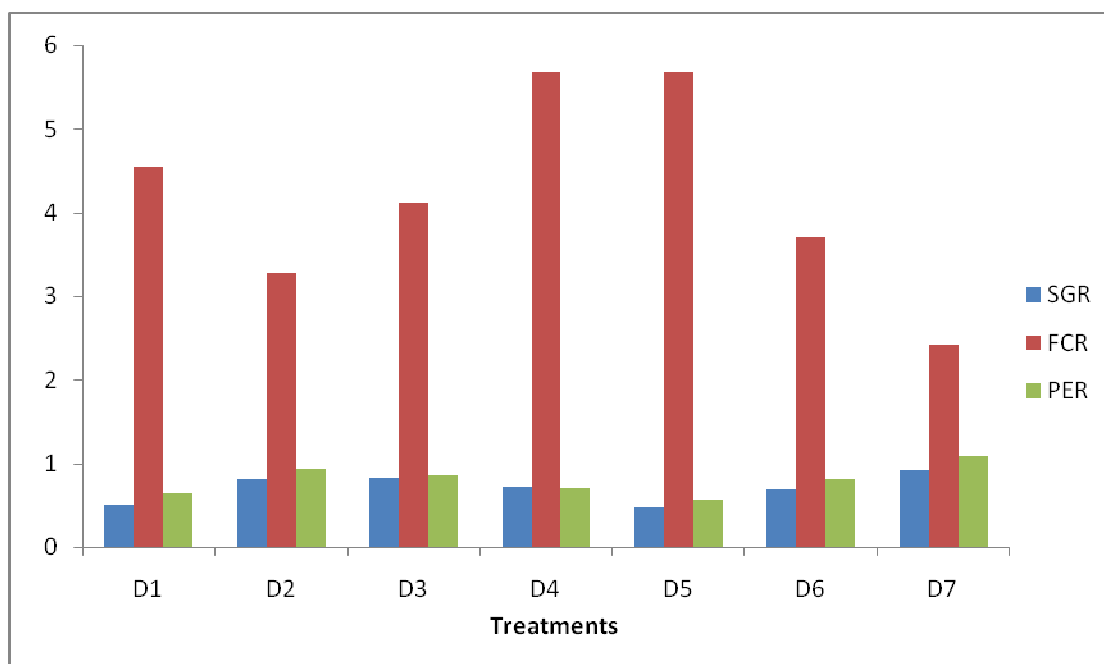


Fig 14: Changes in specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) in different treatments during the experimental period

Specific growth rate expresses the percent body weight gain per day. Feed conversion ratio is a numerical value used to measure the gross utilization of feed for growth in fish and other animals. It is also a measure of the efficiency or suitability of feed to animal (Teugels1982, Kiaalvandi *et al* 2011), while PER expresses the efficiency of protein utilization.

Values recorded for SGR, FCR and PER are well supported by the findings published by different scientists. Sultana *et al* (2001) reported the SGR in between 2.53 – 3.24, FCR 1.22 – 1.78 and PER 1.68 – 2.48 in common carp fry fed with 33.34% crude protein @ 5% BW for 45 days. Kiaalvandi *et al* (2011) reported the FCR in between 4.76 – 6.25 and PER 0.38 – 0.47 in common carp juveniles (8.6 g) fed with 26 – 28% crude protein @ 5% BW daily, while Jader and Sulevany (2012) recorded the SGR 0.71 – 0.87, FCR 2.27 – 3.01 and PER 0.79 – 1.05 in juveniles of common carp, when fed with 25-35% crude protein.

4.3 Biochemical composition of fish flesh

Flesh composition of fish was analyzed for moisture, protein, lipid, ash and total carbohydrates at the start of experiment and termination of experiment.

4.3.1 Moisture content

At the termination of experiment, moisture content (%) in fish flesh in treatments (D₁ to D₇) ranged between 77.2 to 82.1. Moisture content was recorded 78.9, 79.0, 78.2, 78.1, 78.0, 77.6 and 77.2 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively and flesh moisture content was 82.1 at the time of stocking (Table 15 & Fig. 15). The differences among the treatments with respect to moisture content were significant (Initial $\geq$ D₂=D₁ $\geq$ D₃ $\geq$ D₄=D₅ $\geq$ D₆ $\geq$ D₇) (P $\leq$ 0.05).

4.3.2 Total Proteins

Total protein content (%) in fish flesh in all treatments (D₁ to D₇) was ranged between 13.60 to 16.7. Protein content was recorded 14.8, 14.8, 15.7, 15.6, 15.6, 16.6 and 16.7 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively. Flesh protein content of fish was 13.60 at the time of stocking (Table 15 & Fig. 16). The differences among the treatments, with respect to protein content were significant (D₇=D₆ $\geq$ D₃=D₄=D₅=D₂ $\geq$ D₁ $\geq$ Initial) (P $\leq$ 0.05).

4.3.3 Total lipids

Total lipid content (%) in fish flesh ranged between 1.75 to 2.73. Lipid content was recorded 2.73, 2.49, 2.50, 2.27, 2.00, 2.08 and 1.75 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively. Flesh lipid content of fish was 1.97 at the time of stocking (Table 15 & Fig. 17). The differences among the treatments, with respect to lipid content were significant (D₁ $\geq$ D₃=D₂ $\geq$ D₄ $\geq$ D₆ $\geq$ D₅ $\geq$ Initial $\geq$ D₇) (P $\leq$ 0.05).

4.3.4 Ash

Ash content (%) in fish flesh ranged between 1.40 to 2.61. Ash content was 1.82, 2.41, 2.32, 2.52, 2.32, 2.48 and 2.61 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively. Flesh ash content of fish was 1.40 at the time of stocking (Table 15 & Fig. 18). The differences among the treatments, with respect to ash content were significant (D₇ $\geq$ D₄ $\geq$ D₆=D₂ $\geq$ D₃=D₅ $\geq$ D₁ $\geq$ Initial) (P $\leq$ 0.05).

4.3.4 Total carbohydrates

Total carbohydrates in fish flesh ranged between 1.92 to 0.48. Total carbohydrates content was 1.67, 0.48, 1.19, 1.41, 1.92, 1.13 and 1.70 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively. Total carbohydrates content of fish was 0.77 at the time of stocking (Table 15 & Fig. 19). The differences among the treatments, with respect to total carbohydrates content were significant (D₅ $\geq$ D₇ $\geq$ D₁=D₄ $\geq$ D₃ $\geq$ D₆ $\geq$ Initial $\geq$ D₂) (P $\leq$ 0.05).

Table 15: Biochemical composition (%) of common carp flesh (Mean±SE)

Parameters (%)	Initial	D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇
Moisture	82.10 ^a ±0.27	78.90 ^b ±0.24	79.00 ^b ±0.08	78.20 ^c ±0.18	78.10 ^{cd} ±0.35	78.00 ^{cd} ±0.49	77.60 ^{de} ±0.14	77.20 ^e ±0.02
Total proteins	13.60 ^d ±0.08	14.80 ^c ±0.06	15.50 ^b ±0.06	15.70 ^b ±0.14	15.60 ^b ±0.00	15.60 ^b ±0.03	16.60 ^a ±0.06	16.70 ^a ±0.15
Total lipids	1.97 ^{cd} ±0.21	2.73 ^a ±0.06	2.49 ^{ab} ±0.05	2.50 ^{ab} ±0.07	2.27 ^{bc} ±0.08	2.00 ^{cd} ±0.00	2.08 ^c ±0.03	1.75 ^d ±0.05
Ash	1.40 ^e ±0.05	1.82 ^d ±0.07	2.41 ^{bc} ±0.01	2.32 ^c ±0.04	2.52 ^{ab} ±0.01	2.32 ^c ±0.01	2.48 ^b ±0.00	2.61 ^a ±0.01
Total carbohydrates	0.77 ^{de} ±0.27	1.67 ^{abc} ±0.24	0.48 ^e ±0.23	1.19 ^{bcd} ±0.34	1.41 ^{abc} ±0.17	1.92 ^a ±0.07	1.13 ^{cd} ±0.21	1.70 ^{ab} ±0.31

* Values with different alphabetical superscripts differ significantly within row (P ≤ 0.05)

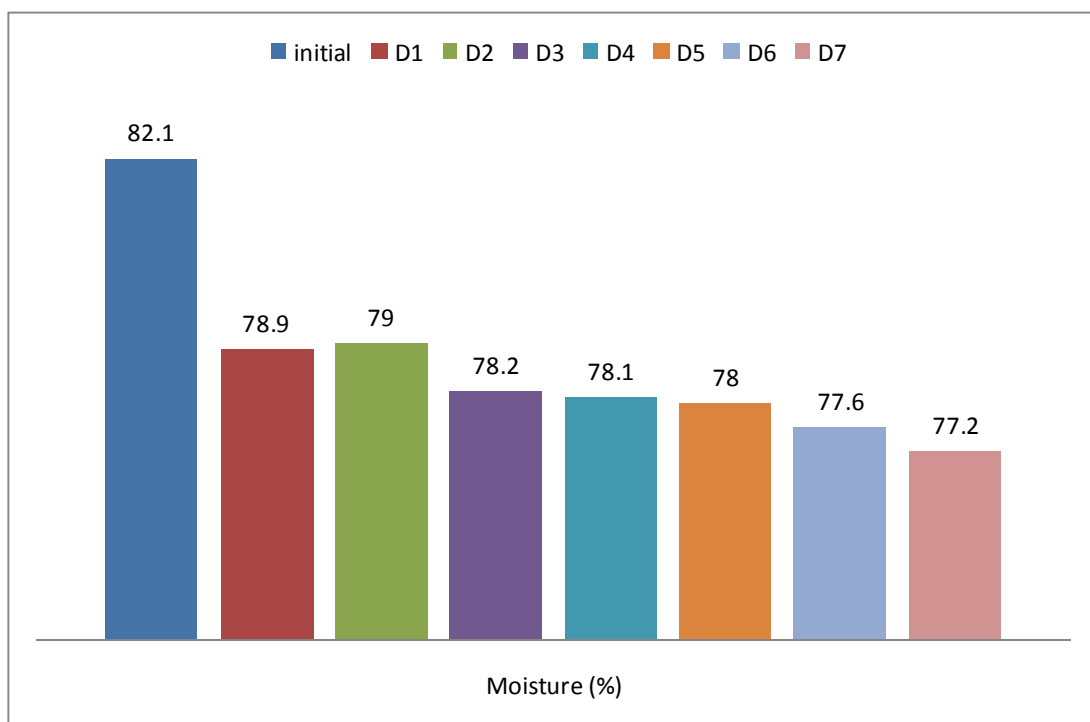


Fig 15: Flesh moisture content (%) of fish in different treatments during experimental period

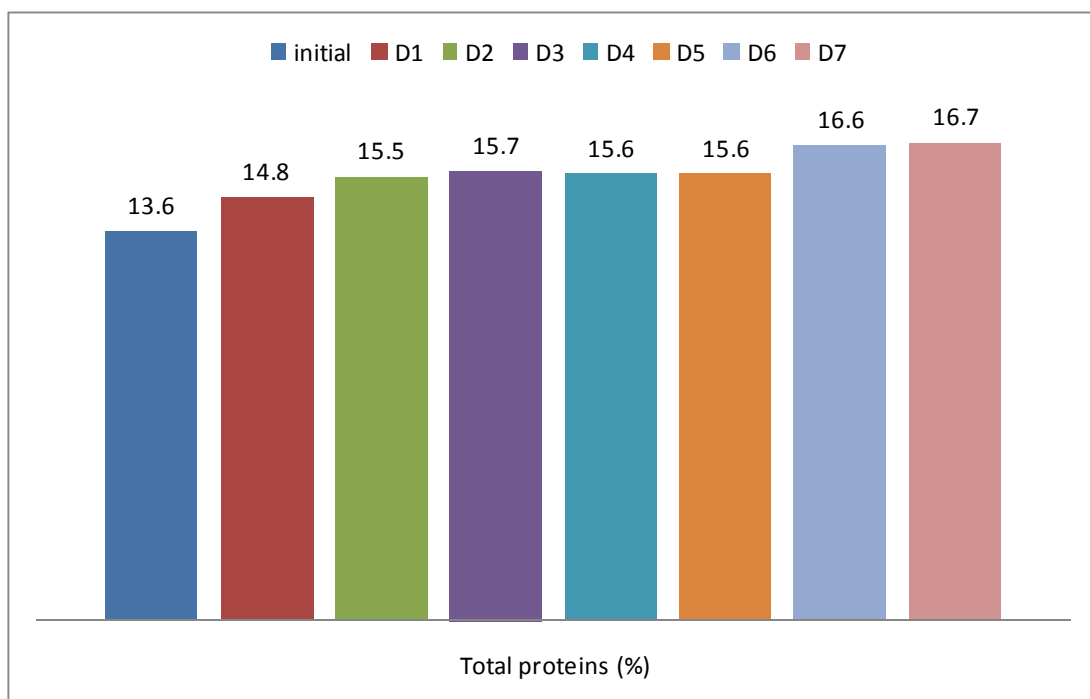


Fig 16: Flesh total protein (%) content (on dry wt. basis) of fish in different treatments during experimental period

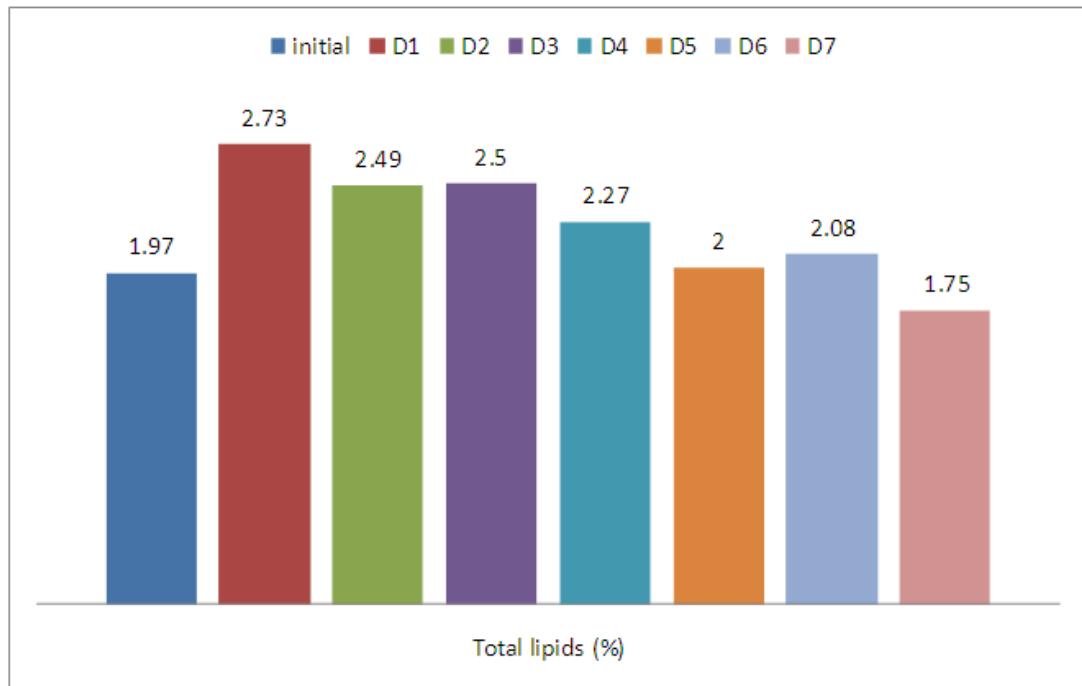


Fig 17: Flesh total lipid (%) content (on dry wt. basis) of fish in different treatments during experimental period

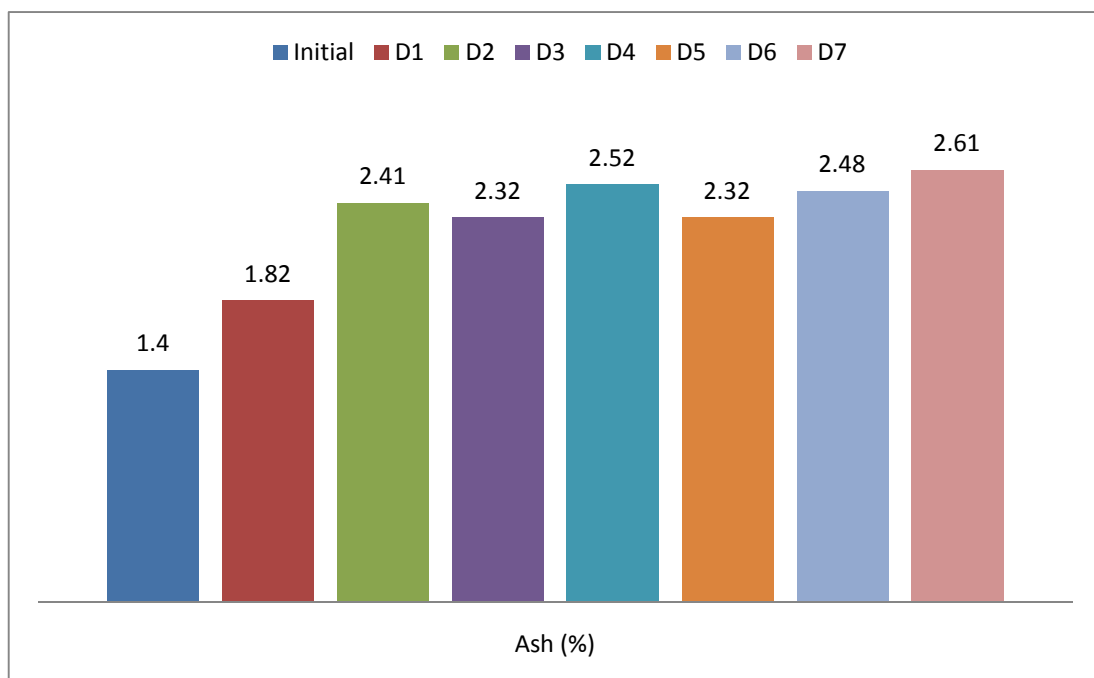


Fig 18: Flesh ash (%) content (on dry wt. basis) of fish in different treatments during experimental period

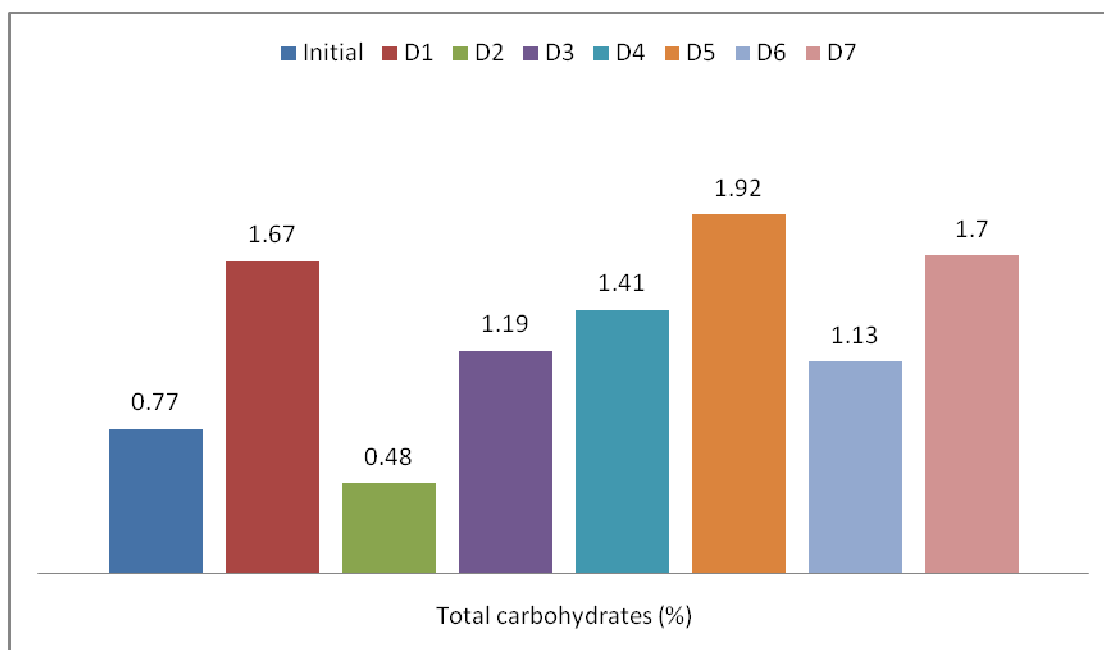


Fig 19: Flesh total carbohydrates (%) content (on dry wt. basis) of fish in different treatments during experimental period

In the present study, significantly high ($P \leq 0.05$) protein content in the flesh of common carp (*C. carpio*) was recorded in treatment D₆ and D₇ (1% methionine addition). Significant increase ($P \leq 0.05$) in protein content with respect to progress of experiment was also recorded. Flesh ash content in fish recorded highest in diets containing Zn @ 40 mg kg⁻¹ along with 1% methionine (D₇), while it was lowest in feed containing Zn @ 20 mg kg⁻¹ (D₂), with respect to progress of experiment assimilation of ash content in flesh was improved significantly.

4.4 Zinc (Zn) and copper (Cu) concentration in flesh, liver and bone of fish

Zinc and Copper concentration in different tissue (flesh, liver and bone) was analyzed at the start of experiment and after termination of the experiment.

4.4.1 Zinc concentration in flesh

Zinc concentration (mg kg⁻¹) in fish flesh ranged between 18.60 to 36.90. Zinc concentrations were 21.20, 29.63, 33.60, 20.10, 25.40, 35.70 and 36.90 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively. However, at the time of stocking Zn concentration in the fish flesh was 18.60 (Table 16 & Fig. 20). The differences among the treatments, with respect to Zn concentration were significant ($D_7 \geq D_6 \geq D_3 \geq D_2 \geq D_5 \geq D_1 \geq D_4 \geq \text{Initial}$) ($P \leq 0.05$). Significantly low concentration of Zn in flesh clearly indicates the inhibitory effect of TCP in zinc uptake in flesh among the treatments.

4.4.2 Zinc concentration in liver

Zinc concentration (mg kg^{-1}) in fish liver ranged between 17.40 to 62.20. Zinc concentrations were 20.10, 40.60, 41.20, 35.60, 36.40, 62.20 and 60.40 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively. However at the time of stocking, Zn concentration in the fish liver was 17.40 (Table 16 & Fig. 20). The differences among the treatments, with respect to zinc concentration in liver were significant ($D_6 \geq D_7 \geq D_3 \geq D_2 \geq D_5 \geq D_4 \geq D_1 \geq \text{Initial}$) ($P \leq 0.05$). Significantly low concentration of Zn in liver indicates the inhibitory effect of TCP in Zn uptake in liver among the treatments.

4.4.3 Zinc concentration in bone

Zinc concentration (mg kg^{-1}) in fish bone ranged between 84.70 to 109.56. Zinc concentrations were 89.70, 98.60, 102.40, 96.42, 94.92, 106.70 and 109.56 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively. However at the time of stocking, Zn concentration in fish bone was 84.70 (Table 16 & Fig. 20). The differences among the treatments, with respect to zinc concentration in bone were significant ($D_7 \geq D_6 \geq D_3 \geq D_2 \geq D_4 \geq D_5 \geq D_1 \geq \text{Initial}$) ($P \leq 0.05$). Significantly low concentration of Zn in bone indicates the inhibitory effect of TCP in Zn uptake in bone among the treatments.

In the present study, significantly higher ($P \leq 0.05$) concentration of Zn in flesh of fish fed with diet containing Zn @ 40 mg kg^{-1} along with 1% methionine (D₇) was recorded. In the presence of TCP, Zn concentration was improved in diets containing 1% methionine, while it was lowest in diets containing no methionine (D₄ and D₅) even though Zn was added exogenously. Moreover Zn concentration was significantly higher in diets containing only added Zn (D₂ and D₃) among variables. Similar pattern was also observed in liver and bone except Zn concentration in liver where it was recorded significantly high in diets containing Zn @ 20 mg kg^{-1} along with 1% methionine (D₆).

The present investigation reveals that low Zn uptake in diets D₁ and D₂ might be due to inhibitory effect of TCP while methionine helped in uptake of Zn from inorganic source. The present analysis matches with the findings reported by Satoh *et al* (1992) that TCP acts as inhibitory factor in the uptake of Zn in common carp. Carp lack an acid – secreting stomach essential for digesting and solubilizing both calcium and phosphorous thus TCP act as inhibitory factor for Zn uptake (Takeuchi 2002). Apines *et al* (2003) concluded that chelated amino acids as a feed supplement in rainbow trout, *O. mykiss* has more advantage as compared to inorganic source of minerals. The amino

acid - chelate showed a higher availability than inorganic compound, even in the presence of TCP and phytate which are normal components of plant protein sources in practical diets. Paripatananont and Lovell (1995) reported that Zn availability from Zn - methionine (ZnMet) is more than ZnSO₄ in catfish, *I.punctatus*. Nguyen and Devis (2009) also observed significant weight gain in Nile Tilapia (*O.niloticus*), when fed with diet containing 0.33 to 0.51% methionine.

4.4.4 Copper concentration in flesh

Copper concentration (mg kg⁻¹) in fish flesh ranged between 1.13 ± 0.00 to 2.14. Cu concentrations were 1.39, 1.75, 2.14, 1.75, 1.63, 2.06 and 1.94 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively. However at the time of stocking, Cu concentration in fish flesh was 1.13 (Table 16 & Fig. 21). The differences among the treatment, with respect to Cu concentrations were significant (D₃ ≥ D₆ ≥ D₇ ≥ D₄ = D₂ ≥ D₅ ≥ D₁ ≥ Initial) (P ≤ 0.05). Copper uptake in flesh not showed any negative effect of TCP.

4.4.5 Copper concentration in liver

Copper concentration (mg kg⁻¹) in fish liver ranged between 0.75 to 4.62. Cu concentrations were 3.26, 3.17, 4.44, 4.45, 4.62, 4.57 and 4.33 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively. However at the time of stocking, Cu concentration in fish liver was 0.75 (Table 16 & Fig. 21). The differences among the treatments, with respect to Cu concentration in liver were significant (D₅ ≥ D₆ ≥ D₄ = D₃ ≥ D₇ ≥ D₁ = D₂ ≥ Initial) (P ≤ 0.05). Copper uptake in liver not showed any negative effect of TCP.

4.4.6 Copper concentration in bone

Copper concentration (mg kg⁻¹) in fish bone ranged 1.96 to 2.62. Cu concentrations were 1.96, 2.16, 2.06, 2.62, 2.41, 2.06 and 2.33 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively. However at the time of stocking, Cu concentration in fish bone was 1.96 (Table 16 & Fig. 21). The differences among the treatments, with respect to Cu concentration in bone were significant (D₄ ≥ D₅ ≥ D₇ ≥ D₂ ≥ D₃ = D₆ ≥ D₁ ≥ Initial) (P ≤ 0.05). Copper uptake in bone not showed any negative effect of TCP.

In the present investigation, significant difference (P ≤ 0.05) in Cu concentration was recorded in different treatments within same kind of tissue. However no specific relationship of Cu concentration in different tissue with respect to addition of Zn, TCP and methionine was recorded. During the experimental period liver showed higher

affinity with Cu compared to bone and flesh. Based on present study, it is difficult to correlate Cu concentration in different tissue among different treatments. Copper was evaluated because it was added in the form of Cu SO₄ as a part of Zn free mineral in all the diets at equal level. Copper is itself an essential micronutrient which turns to toxic at higher concentration and it also shows bioaccumulation phenomenon in tissue.

Copper is one of the most toxic heavy metal to fish (Mance 1987) as well as Cu is required in trace amount for carrying out vital function in living organisms (Cousins 1985). As a part of the enzyme copper – zinc superoxide dismutase (Cu/Zn SOD) help in protecting the cells and some compounds from the harmful effects of free radicals. Excess quantity of Cu causes toxicity to fish (FAO/WHO 1983). Peyghan *et al* (2003) reported the Cu in liver (17.92 mg 100g⁻¹) in muscles (2.2 mg 100g⁻¹) in common carp when exposed to copper sulphate @10 mg l⁻¹ for 30 days without effect.

In present study copper concentration recorded in flesh, liver and bone was within the acceptable limit as per international standards for maximum permissible limit (MPL) decided by FAO/WHO (1983).

Table 16: Change in zinc and copper concentration (mg kg⁻¹) in different tissues of common carp (Mean± SE)

Fish organ		Initial	D₁	D₂	D₃	D₄	D₅	D₆	D₇
Flesh	Zn	18.60 ^h ±0.14	21.20 ^f ±0.01	29.63 ^d ±0.03	33.60 ^c ±0.02	20.10 ^g ±0.02	25.40 ^e ±0.02	35.70 ^b ±0.02	36.90 ^a ±0.03
	Cu	1.13 ^g ±0.005	1.39 ^f ±0.11	1.75 ^d ±0.02	2.14 ^a ±0.01	1.75 ^d ±0.02	1.63 ^e ±0.01	2.06 ^b ±0.01	1.94 ^c ±0.01
Liver	Zn	17.40 ^h ±0.01	20.10 ^a ±0.01	40.60 ^d ±0.02	41.20 ^c ±0.01	35.60 ^f ±0.01	36.40 ^e ±0.01	62.20 ^a ±0.00	60.40 ^b ±0.01
	Cu	0.75 ^e ±0.00	3.26 ^d ±0.12	3.17 ^d ±0.01	4.44 ^{bc} ±0.01	4.45 ^{bc} ±0.01	4.62 ^a ±0.01	4.57 ^{ab} ±0.01	4.33 ^c ±0.01
Bone	Zn	84.70 ^h ±0.005	89.70 ^g ±0.05	98.60 ^d ±0.01	102.40 ^c ±0.00	96.42 ^e ±0.01	94.92 ^f ±0.02	106.70 ^b ±0.01	109.56 ^a ±0.01
	Cu	1.96 ^g ±0.01	1.96 ^f ±0.00	2.16 ^d ±0.01	2.06 ^e ±0.01	2.62 ^a ±0.01	2.41 ^b ±0.00	2.06 ^e ±0.01	2.33 ^c ±0.01

* Values with different alphabetical superscripts differ significantly within row (P ≤ 0.05)

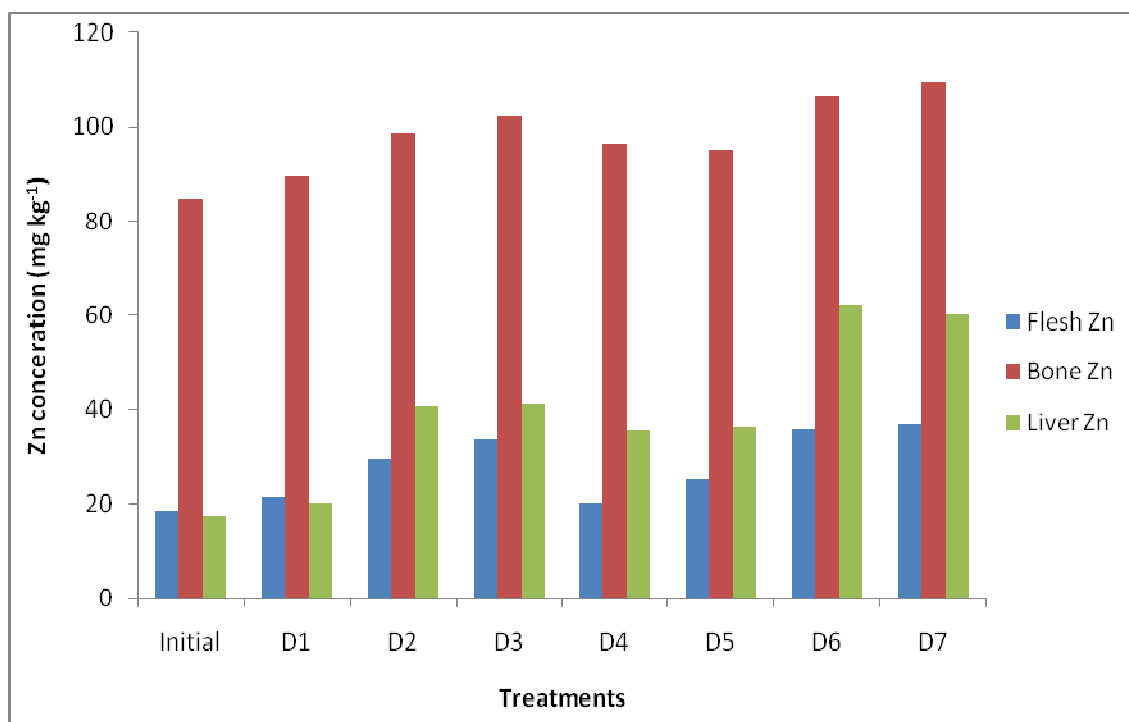


Fig 20: Zinc (Zn) concentration (mg kg⁻¹) in different tissues of common carp at initiation and termination of experiment

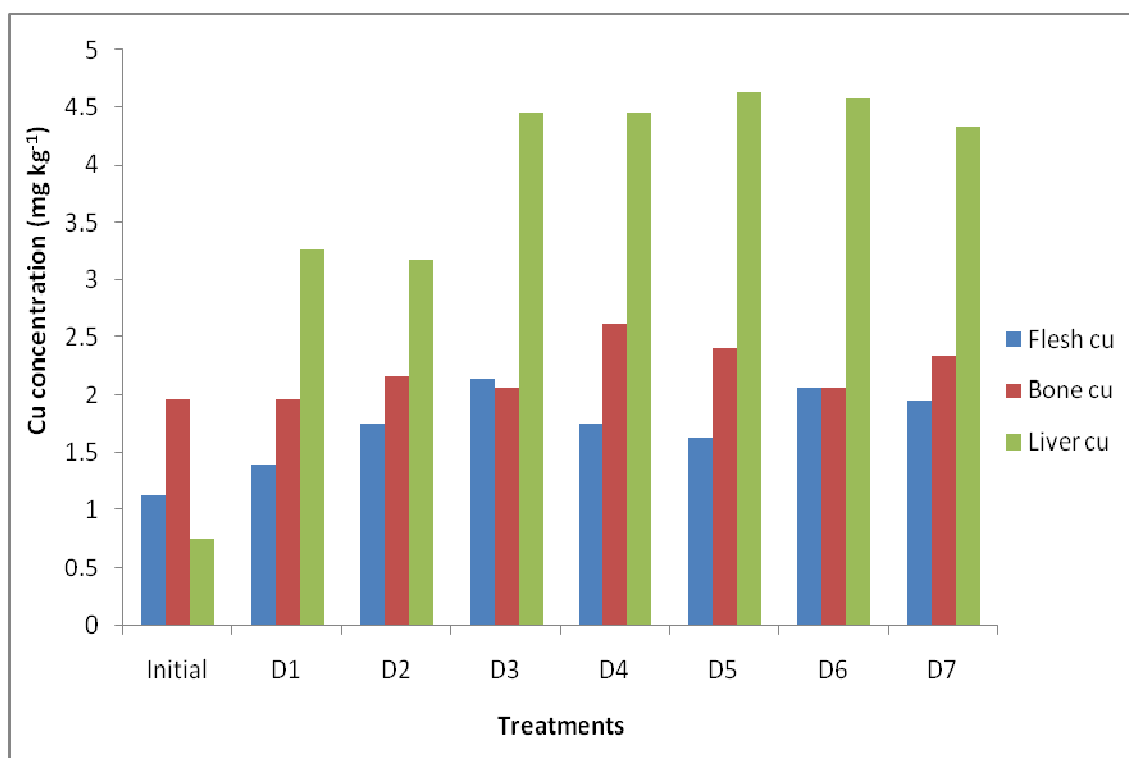


Fig 21: Copper (Cu) concentration (mg kg⁻¹) in different tissues of common carp at initiation and termination of experiment

4.5 Conclusion

1. Zinc is an essential micro mineral and addition of it in formulated diets for common carp especially in fingerlings improves the feed conversion ratio (FCR) and protein efficiency ratio (PER).
2. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ as inorganic zinc source can be added up to 40 mg kg^{-1} in the formulated diets of common carp fingerlings without negative effect on the survival and growth.
3. Tricalcium phosphate acts as inhibitory factor in the uptake of zinc from its inorganic sources in fingerlings of common carp.
4. Addition of 1% Methionine helps to mitigate the inhibitory effect of TCP and improves the uptake of Zn in fingerlings of common carp.

CHAPTER V

SUMMARY

The present experiment was conducted to study the methionine modulated bioavailability of inorganic zinc (Zn) in common carp (*Cyprinus. carpio*) fed with diets containing tricalcium phosphate (TCP). The experiment was carried out in the indoor glass aquaria (60× 30 ×30cm) in three tier system. A total 21 aquaria, for 7 treatments with 3 replicates, were set for the proposed study. Aquaria were filled with fresh underground water and each aquarium was connected with aeration facility. Each aquarium was stocked with pre-acclimatized 11 fingerlings (average length 6.02 cm and average weight 3.49 g) @ 33 m⁻³) of common carp (*C. carpio*). Experiment was carried out with seven diets including one control (D₁) and six experimental diets (D₂ to D₇). All seven experimental diets were prepared by mixing different ingredients such as casein, dextrin, gelatin, fish meal, sodium alginate, soybean meal, oil, carboxy methyl cellulose, Zn free mineral, vitamin mixture, zinc sulphate (H₂SO₄. 7H₂O), TCP and DL-methionine. Percentage of casein, dextrin, gelatin, fish meal, sodium alginate, soybean meal, oil, carboxy methyl cellulose, Zn free mineral, vitamin mixture were 20%, 30%, 8%, 10%, 12%, 8%, 2%, 5%, 3% and 2%, respectively. D₁ i.e. control diet was prepared without adding without adding inorganic source of Zn i.e. ZnSO₄.7H₂O, TCP and methionine. D₂ and D₃ contained 88.4 and 176.8 mg kg⁻¹ ZnSO₄.H₂O without TCP and methionine. D₄ and D₅ contained 88.4 and 176.8 mg kg⁻¹ ZnSO₄.H₂O and 2 % TCP without methionine. D₆ and D₇ contained 88.4 and 176.8 mg kg⁻¹ ZnSO₄.H₂O, 2 % TCP and 1% methionine.

5.1 Physico-chemical parameters of water

Water quality parameters were recorded at weekly intervals. The water quality parameters viz. temperature, pH, dissolved oxygen, total alkalinity, phenolphthalein alkalinity, methyl orange alkalinity and total hardness contents varied from 27.1 to 31.6°C; 8.04 to 8.84; 6.40 to 10.60 mg l⁻¹; 171.33 to 320 CaCO₃ mg l⁻¹; 9.33 to 33.3 CaCO₃ mg l⁻¹; 143.33 to 296.00 CaCO₃ mg l⁻¹ and 214.67 to 370.67 CaCO₃ mg l⁻¹ in the different treatments (D₁-D₇). Some water parameters such as calcium, magnesium and sodium were also recorded at fortnightly interval. Calcium, magnesium and sodium concentration in water varied from 8.45 to 19.6 mg l⁻¹, 50.0 to 86.3 mg l⁻¹ and 11.01 to 11.18 mg l⁻¹ in different treatments (D₁-D₇). Water quality parameters recorded during

the experimental period fluctuated within the favorable range recommended for ideal the growth of common carp. No significant difference in physico-chemical parameters except pH and calcium was observed during the experimental period in between different treatments. Supplementation of Zn in D₂ and D₃, with TCP @ 2% in D₄ and D₅ and with amino acid methionine @ 1% in D₆ and D₇ did not show any undesirable effect on the water quality.

5.2 Survival and growth of fish

During the experimental period survival (%) of fish ranged between 90.9 to 100% in all treatments (D₁ to D₇). At the end of experiment lowest survival i.e. 90.9 was recorded in D₃, D₄ and D₅ followed by 93.9 in D₂, 96.9 in D₁, D₇ and 100 in D₆ respectively.

In different treatments with Zn, TCP and methionine supplemented diets with respect to control, the gain in body length (cm) varied between 2.21 to 3.58 during the experimental period. In between treatments, net gain in body length was 2.62, 2.30, 3.01, 3.58, 2.21, 2.79 and 2.85 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively and differences among the treatments were significant ($D_4 \geq D_3 = D_7 = D_6 \geq D_1 \geq D_2 = D_5$) ($P \leq 0.05$). NWG (g) in all treatments (D₁ to D₇) ranged between 2.19 to 4.01 during the experimental period. NWG was 2.85, 3.39, 3.13, 2.96, 2.19, 3.35 and 4.01 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively and the differences among the treatments were insignificant ($P \leq 0.05$).

Specific growth rate in all the treatments (D₁ to D₇) ranged between 0.48 to 0.92. SGR was recorded 0.56, 0.81, 0.83, 0.72, 0.48, 0.69 and 0.92 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively and the differences among treatments were insignificant ($P \leq 0.05$). FCR in all the treatments (D₁ to D₇) ranged between 2.42 to 5.68. FCR was recorded 4.54, 3.27, 4.12, 5.68, 5.68, 3.71 and 2.42 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively and differences among treatments were insignificant ($P \leq 0.05$). PER in all the treatments (D₁ to D₇) ranged between 0.56 to 1.08. PER was recorded 0.64, 0.93, 0.86, 0.71, 0.56, 0.81 and 1.08 in treatments D₁, D₂, D₃, D₄, D₅, D₆ and D₇, respectively and differences among treatments were insignificant ($P \leq 0.05$). Supplementation with 1% amino acid i.e. methionine in diet showed the positive improvement in FCR.

5.3 Biochemical composition in fish flesh

During experiment period, changes in fish flesh quality were observed at the time of start (initial) and at termination of the experiment. Moisture content (%) in fish flesh at termination of experiment period, ranged between 77.2 to 82.4 in all treatments (D_1 to D_7) and moisture content was recorded 78.9, 79.0, 78.2, 78.1, 78.0, 77.6 and 77.2 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. Flesh moisture content of fish was 82.1 at the time of stocking. The differences among the treatments with respect to moisture content were significant ($\text{Initial} \geq D_2 = D_1 \geq D_3 \geq D_4 = D_5 \geq D_6 \geq D_7$) ($P \leq 0.05$). Total protein content (%) in fish flesh at termination of experiment period, were ranged between 13.60 to 16.7 in all treatments (D_1 to D_7). Total protein content was recorded 14.8, 14.8, 15.7, 15.6, 15.6, 16.6 and 16.7 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. Flesh protein content of fish was 13.60 at the time of stocking. The differences among the treatments, with respect to total protein content were significant ($D_7 = D_6 \geq D_3 = D_4 = D_5 = D_2 \geq D_1 \geq \text{Initial}$) ($P \leq 0.05$). Total lipid content (%) in fish flesh at termination of experiment period, were ranged between 1.75 to 2.73 in all treatments (D_1 to D_7) and was recorded 2.73, 2.49, 2.50, 2.27, 2.00, 2.08 and 1.75 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. Flesh lipid content of fish was 1.97 at the time of stocking. The differences among the treatments, with respect to total lipid content were significant ($D_1 \geq D_3 = D_2 \geq D_4 \geq D_6 \geq D_5 \geq \text{Initial} \geq D_7$) ($P \leq 0.05$). Ash content (%) in fish flesh ranged between 1.40 to 2.61 and was 1.82, 2.41, 2.32, 2.52, 2.32, 2.48 and 2.61 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. Flesh ash content of fish was 1.40 at the time of stocking. The differences among the treatments, with respect to ash content were significant ($D_7 \geq D_4 \geq D_6 = D_2 \geq D_3 = D_5 \geq D_1 \geq \text{Initial}$) ($P \leq 0.05$). Total carbohydrates as NFE in fish flesh ranged between 1.92 to 0.48 and were 1.67, 0.48, 1.19, 1.41, 1.92, 1.13 and 1.70 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. Total carbohydrates content of fish was 0.77 at the time of stocking. The differences among the treatments, with respect to total carbohydrates content were significant ($D_5 \geq D_7 \geq D_1 = D_4 \geq D_3 \geq D_6 \geq \text{Initial} \geq D_2$) ($P \leq 0.05$). Significant changes in flesh quality during the experimental period were recorded. The changes may be due to addition or effect of tricalcium phosphate and methionine added or not present in diets.

5.4 Zinc and Copper concentration in different organs such as flesh, liver and bone

Zinc concentration in different tissues (flesh, liver and bone) was analyzed at the start of experiment and after termination of the experiment. Zinc concentration (mg kg^{-1}) in fish flesh ranged between 18.60 to 36.90. Zinc concentrations were 21.20, 29.63, 33.60, 20.10, 25.40, 35.70 and 36.90 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. However, at the time of stocking, Zn concentration in the fish flesh was 18.60. The differences among the treatments, with respect to Zn concentration were significant ($D_7 \geq D_6 \geq D_3 \geq D_2 \geq D_5 \geq D_1 \geq D_4 \geq \text{Initial}$) ($P \leq 0.05$). Zinc concentration (mg kg^{-1}) in fish liver ranged between 17.40 to 62.20. Zinc concentrations were 20.10, 40.60, 41.20, 35.60, 36.40, 62.20 and 60.40 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. However, at the time of stocking, Zn concentration in the fish liver was 17.40. The differences among the treatments, with respect to zinc concentration in liver were significant ($D_6 \geq D_7 \geq D_3 \geq D_2 \geq D_5 \geq D_4 \geq D_1 \geq \text{Initial}$) ($P \leq 0.05$). Zinc concentration (mg kg^{-1}) in fish bone ranged between 84.70 to 109.56. Zinc concentrations were 89.70, 98.60, 102.40, 96.42, 94.92, 106.70 and 109.56 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. However, at the time of stocking, Zn concentration in fish bone was 84.70. The differences among the treatments, with respect to zinc concentration in bone were significant ($D_7 \geq D_6 \geq D_3 \geq D_2 \geq D_4 \geq D_5 \geq D_1 \geq \text{Initial}$) ($P \leq 0.05$). Copper concentration (mg kg^{-1}) in fish flesh ranged between 1.13 to 2.14. Copper concentrations were 1.39, 1.75, 2.14, 1.75, 1.63, 2.06 and 1.94 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. However, at the time of stocking, Cu concentration in fish flesh was 1.13. The differences among the treatment, with respect to copper concentrations were significant ($D_3 \geq D_6 \geq D_7 \geq D_4 = D_2 \geq D_5 \geq D_1 \geq \text{Initial}$) ($P \leq 0.05$). Copper concentration (mg kg^{-1}) in fish liver ranged between 0.75 to 4.62. Copper concentrations were 3.26, 3.17, 4.44, 4.45, 4.62, 4.57 and 4.33 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. However, at the time of stocking, Cu concentration in fish liver was 0.75. The differences among the treatments, with respect to copper concentration in liver were significant ($D_5 \geq D_6 \geq D_4 = D_3 \geq D_7 \geq D_1 = D_2 \geq \text{Initial}$) ($P \leq 0.05$). Copper concentration (mg kg^{-1}) in fish bone ranged 1.96 to 2.62. Copper concentrations were 1.96, 2.16, 2.06, 2.62, 2.41, 2.06 and 2.33 in treatments D_1 , D_2 , D_3 , D_4 , D_5 , D_6 and D_7 , respectively. However, at the time of stocking, Cu concentration in fish bone was 1.96. The differences among the treatments, with respect to Cu concentration in bone were significant ($D_4 \geq D_5 \geq D_7 \geq D_2 \geq D_3 = D_6 \geq D_1 \geq \text{Initial}$) ($P \leq 0.05$).

The results revealed improved Zn uptake from inorganic source as compare to control moreover TCP had significant inhibitory effect on the Zn uptake but addition of 1% methionine improved the Zn uptake, in different tissue viz. flesh, liver and bone. During the experimental period concentration of Cu in different tissues did not showed any specific correlation more over its concentration was within maximum permissible limit as per international standards.

5.4 Conclusion

Zinc is an essential micro mineral and addition of it in formulated diets for common carp, especially in fingerlings improves the feed conversion ratio (FCR) and protein efficiency ratio (PER). Inorganic source of Zn in the form of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ showed no negative effect upto addition of 40 mg kg^{-1} in formulated diets of common carp fingerlings. At added 40 mg kg^{-1} level no negative effect on the survival and growth was recorded. However, still it is required to standardize optimum level of Zn concentration in formulated diets with respect to different life stages in common carp. Tricalcium phosphate acts showed its inhibitory effect reflected in terms of high FCR and low PER and moreover reduces Zn uptake from its inorganic sources. Methionine helps to mitigate the inhibitory effect of TCP and improves the Zn uptake in common carp fingerlings. Hence, it is concluded that to improve Zn uptake and to mitigate the inhibitory effect of TCP, methionine can be added @ 1% or more in the formulated diets for fingerlings of common carp.

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