EVALUATION OF FISH SILAGE AS A POTENTIAL PROTEIN SOURCE FOR REPLACEMENT OF SOYBEAN MEAL IN FEED OF COMMON CARP, *Cyprinus carpio* (L.) FINGERLINGS

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

MASTER OF FISHERIES SCIENCE
in
AQUACULTURE
(Minor Subject: Aquatic Environment Management)

By

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CERTIFICATE – I

This is to certify that the thesis entitled, **"EVALUATION OF FISH SILAGE AS A POTENTIAL PROTEIN SOURCE FOR REPLACEMENT OF SOYBEAN MEAL IN FEED OF COMMON CARP, *Cyprinus carpio* (L.) FINGERLINGS"** 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 **Mr. Khushwinderjit Singh (L-2015-F-02-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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This is to certify that the thesis entitled, **"EVALUATION OF FISH SILAGE AS A POTENTIAL PROTEIN SOURCE FOR REPLACEMENT OF SOYBEAN MEAL IN FEED OF COMMON CARP, *Cyprinus carpio* (L.) FINGERLINGS"** submitted by **Mr. Khushwinderjit Singh (L-2015-F-02-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 evaluate performance of fish silage as a potential feed ingredient source of animal origin to replace plant origin soybean protein at different levels in diets of Common carp (*Cyprinus carpio*, L.) fingerlings. The experiment was carried out in FRP tanks (1.5×1.0×0.75m) and five experimental diets ($D_1 – D_5$) were prepared to replace soybean meal with fish silage @ 15% (D_2), 30% (D_3), 45% (D_4) and 60% (D_5) against control (D_1) diet. No significant effect ($P \leq 0.05$) on water qualities recorded due to addition of fish silage to replace soybean protein at different levels. Significantly high specific growth rate (SGR) recorded in D_4 (0.77±0.03), protein efficiency ratio (PER) in D_3 (0.71±0.008) and low feed conversion ratio (FCR) in D3 (2.22±0.008). Addition of animal source protein, improved the FCR. Significantly high ($P \leq 0.05$) protein content in flash recorded (16.50±0.05%) in fish fed with diet containing 45% fish silage to replace soybean meal i.e. D_4. It can be concluded from the present study that fish acid silage can be used to replace soybean protein @ 45% in common carp diets with no effect on water quality. To maintain PER at optimum levels, diets should be prepared with desirable levels of protein, moreover it should be added in combination considering amino acids from different sources of plant and animal origin.

Keywords: Common carp (*Cyprinus carpio*), Fish silage, Soybean meal, survival and growth.

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Signature of Major Advisor **Signature of the Student**

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LIST OF ABBREVIATIONS

AOAC	:	Association of analytical communities
APHA	:	American public health association
Bv	:	Biological value
C	:	Centigrade
Ca	:	Calcium
$CaCO_3$	:	Calcium carbonate
CP	:	Crude protein
D	:	Diet
DAHDF	:	Department of animal husbandry, dairying and fisheries
DM	:	Dry matter
FAO	:	Food and agriculture organization
FCR	:	Feed conversion ratio
FFS	:	Formic acid preserved fish silage
FM	:	Fish meal
FRP	:	Fibre-reinforced plastic
FS	:	Fish silage
GOI	:	Government of India
HFM	:	Hydrolyzed feeder meal
Mg	:	Magnesium
mg^{-1}	:	Milligram per liter
MMT	:	Million metric tonne
NaOH	:	Sodium hydroxide
NBL	:	Net body length
NBLG	:	Net body length gain
NBW	:	Net body weight
NFDB	:	National fisheries development board
NFE	:	Nitrogen free extract
NH_3	:	Non-ionic form of ammonia
NH_4^+	:	Ionic form of ammonia
NWG	:	Net weight gain
OATA	:	Ornamental aquatic trade association
PER	:	Protein efficiency ratio
PUFA	:	Polyunsaturated fatty acid
SBM	:	Soybean meal
SE	:	Standard error
SGR	:	Specific growth rate
T	:	Treatment
TBLG	:	Total body length gain

CHAPTER I

INTRODUCTION

India is a major producer of fish in aquaculture sector and globally ranks second next to China. During 2015-16, total fish production of the country recorded 10.79 MMT, out of which inland sector contributed 7.21 MMT. In inland sector, fish production from culture segment contributes maximum, moreover share of this segment is increasing every year. Freshwater aquaculture has continued to hold a major share among the aquaculture production, with a contribution of over 95% in terms of quantity, out of which carp shares 87% (DAHDF, GOI 2017, Ayappan and Jena 2003, NFDB 2017). The three Indian major carp, namely catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*), contributes the bulk of production, followed by silver carp, grass carp and common carp (FAO 2005).

Common carp (*Cyprinus carpio L.*) is an important fresh water carp, cultured across the world. The common carp presently grown in India originate from two introductions to India, in 1939 'German strain' and 1957 'Bangkok strain' (Jhingran 1991). At present common carp is being grown in all parts of the country as a major fishery item. Seed is a principle component of any farming system. Production of quality seed is still a challenge due to varied reasons. Early stages of the life cycle of organism are very crucial for its survival and development and requires high amount of dietary proteinalong withhigh biological value. Waste from aquatic as well as terrestrial animals is considered as an excellent source of protein for fish growth, moreover rendered (recycled) by-products of animal origin are enriched with lysine, sulfur amino acids (methionine and cysteine), histidine, arginine as quality protein component (El Seyed 1998).

In fish farming, expenditure on feed is one of the major inputs among recurring cost.In feed based aquaculture, more than 60% of the operational cost goes to feed alone (Paul and Mohanty 2002). Feed helps in fast growth and better health of fish, even though in the absence of natural fish food organisms, which is preferential food for fish. Good production from culture system can be achieved by nutritionally balanced diet, given at right time; in optimum quantity. Nutritionally balanced feed is

measured in terms of its energy value w.r.t. essential amino acids, fatty acids, protein efficiency and their digestibility, which is decided by source of ingredients, level of feed stuff incorporated and it's processing.Protein and lipids are the primary sources of metabolic energy followed by carbohydrate in fish (Paul and Giri 2015); hence such nutrient sources must be considered before selecting feed ingredients. Oil cake and rice bran are the most common and cheapest feed ingredient used in carp feed, while fish meal is the preferred dietary animal protein source for many aqua farmed animals. As a plant protein source, soybean meal is the one of most commonly used feed ingredient due to its easy availability and richness in Glutamic acid, Aspartic acid, Arginine, Leucine in high quantity along with Histidine, Threonine, Serine, Proline, Glycine, Alanine, Valine, Isoleucine, Tyrosine, Phenylalanine (Kuiken and Lyman 1949). Soybean meal contains biologically valuable proteins and is very similar in the structureof amino acids to proteins of animal origin (Watanabe 2002). Fish meal is the most abundant animal protein source for the manufacture of rations for domestic animals. The world market has always been looking for an effective alternative to fish meal (Nogueira *et al* 1997), but ever increasing cost and demand along with quality are the major issues, which forces to explore an alternative source of protein.

Developing new resources and using the readily available ones in the best way possible is now one of the most prioritized concerns in all areas. However, the amount of fish meal manufactured does not completely meet the need by fish feed producers. At present, supply of quality fish meal w.r.t. quality is still a matter of concern; meanwhile, fish meal price is increasing day by day due to the diminishing fish stock from natural water resources and increasing demand as protein source of animal origin. On the other hand, in fish processing; nearly 60% of the processed fishery item turns out as waste material. As a result of this, in processing activity, a significant amount of unused waste piles up and being an animal waste it decomposes very fast and creates pollution and hygiene issues also. In this regard 'fish silage' may be considered as an economic alternative proteinsource, moreover re-processing this waste into fish silage and using this by-product as source of protein may help to use of waste for a good purpose on low costs (Gullu *et al* 2003).There are various

advantages in the production of silage, compared to fish meal, are: the process is virtually independent from the scale; the technology is simple; the investment is little, even at large-scale production; reduced effluents and odor problems. The silage process is fast in tropical climates and the product can be prepared at such place also. However, a disadvantage is the product's pasty nature, which implies an additional cost during its drying for its storage (Kompiang 1981, Beerli *et al* 2004).

Fish silage is defined as a liquid product produced from fish/fish waste to which acids, enzymes or lactic acid producing bacteria are added, with the liquefaction of the mass provoked by the action of enzymes from the fish (FAO 2007). The word silage is derived from silo; i.e., material stored in a silo as practiced on farms (Jangaard 1987). A.I. Virtanen was the first one to develop acid treated silage in 1920 from forage by using hydrochloric and sulphuric acid as preservative (Raa and Gildgerg 1982) and this method was further adopted by Edin in the 1930s to preserve and liquefy different types of fish and fish waste (Edin 1948). Experiments with fish silage began in Sweden in 1936; using hydrochloric acid and sugar as preservative (Tatterson and Windsor 1974). Fish silage as a fish feed ingredient started in 1980's for recycling of organic material (Hardy *et al* 1984). Fish silage can be produced by acid treatment (Petersen 1953), enzymatic action (Freeman and Hoogland 1956), lactic acid and proteolytic fungi and yeast fermentation (Raa *et al* 1983, Mackie *et al* 1971).

Success of rearing fish depends upon the feed given. The feed should be prepared based on the precise knowledge of their nutritional requirements; so that the optimum growth can be achieved in a reared time. The balanced diet to be given to these organisms should contain nutrients such as protein, carbohydrate, lipid, vitamins and minerals to meet structural nutrient requirement as well as basal energy requirements and also to ensure healthy growth. Among all nutritional components used for the formulated feed, protein plays an important role in the feed. It is also a costly component. The percentage of protein in the feed should be neither more nor less than the optimum required levels for the organisms (Ogmo and Saito 1970 and Mohanty *et al* 1990).

Development and performance of diet can be assessed only through field trials. The current study is designed to evaluate performance of different levels of fish silage, replacing soybean meal, as a source of protein in the diets of fingerlings of common carp (*C. carpio*).

In view of above discussion, the present study has been planned to evaluate the efficiency of fish silage for common carp fingerlings with the follow objectives

1. To evaluate survival and growth performance of common carp fingerlings with fish silage based diets.
2. To study biochemical changes in common carp flesh with fish silage incorporated diets.

CHAPTER II

REVIEW OF LITERATURE

Available information with respect to water quality, nutritional requirement, Silage development and performance of silage based diets is presented below:

2.1 Water quality requirement for common carp

2.2 Nutritional requirement and feed utilization in common carp

2.3 Development of fish silage

2.4 Development of fish silage in diets

2.5 Growth performance of common carp

2.6 Biochemical composition of common carp flesh

2.1 Water quality requirement for common carp

Fish, being an aquatic animal requires a suitable water quality for survival, growth and health. Water quality is the first most important limiting factor in pond fish production. According to Piper *et al* (1982) Water quality determines to a great extent the success or failure of a fish culture operation. The key water quality parameters for pond productivity are 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. Temperature is a variable factor w.r.t. diurnal, seasonal changes and longitude and latitude difference. Jhingran (1991) reported that carp thrive well in the temperature range of 18.3- 37.8ºC, while it can occur in temperature range of 3- 35ºC (Froese and Pauly 2011). FAO (2000) documented that common carp can tolerate high water temperature i.e. up to 32ºC without effect, but the optimum temperature for the growth is in between 20 – 25ºC (Froese and Pauly 2011) while fish is active in feeding, when the water temperature is over 18-20ºC. 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 -28ºC.

pH is an important limiting factor in fish culture. It indicates acid-base balance of water.The pH of most productive natural waters that are unaffected by pollution is normally in the range of 6.5 to 8.5 at sunrise, typically closer to 7 than 8. The controlling factor for pH in water bodies is the relationship between algal photosynthesis, carbon dioxide (CO_2), and the bicarbonate (HCO_3^-) buffering system. At night, respiration by bacteria, plants, and animals results in oxygen consumption and carbon dioxide production, which first produces carbonic acid (H_2C0_3), then bicarbonate $HC0_3^-$ and H^+ ions; the increase in H^+ causes the pH to drop. pH between 7.0 to 8.5 is more optimum and conducive to fish life and ideal for biological productivity , fishes can become stressed in water with a pH ranging from 4.0 to 6.5 and 9.0 to 11.0 and leads to death at a pH of less than 4.0 or greater than 11.0 (Ekubo and Abowei 2011). For fish culture, Jhingran (1991) suggested that pH in the range of 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 (Svobodova *et al* 1993). Zweig *et al* (1999) also suggested the optimum level of pH in-between 6.5 to 9.0 for fish culture, while 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). Fish require oxygen forrespiration, which physiologistsexpress as mgof oxygen consumed per kilogram. At a given temperature, small sized fish consumes more oxygen per unit of body weight than large sizedfish; or said in another way, for the same total weight of fish in a tank; smaller fish requiremore oxygen than larger fish. For optimum productivity of carp dissolved oxygen content >5.0 mg l^{-1} is required to be maintained in the ponds throughout the culture period (Swingle 1961, Boyd 1990). According to Banerjea (1967), D.O. between 3.0 to 5.0mg l^{-1} in pond water is considered as unproductive for fish production, while for good production, it is suggested that dissolved oxygen level should be at least 5.0 mg l^{-1}. 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 mg l^{-1} D.O. to sustain

fish for long period. Fish exposed to oxygen deficient water; stops feeding and accumulates near the water surface to engulf aerial oxygen (Svobodova *et al* 1993).

Alkalinity is the ability of water to resist the 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 $CaCO_3$mg $l^{-1}$is low productive, 20–40 $CaCO_3$mg l^{-1}low to medium, 40 – 90 $CaCO_3$mg l^{-1}medium to high and above 90 $CaCO_3$mg l^{-1}productive.Boyd and Lichtkoppler (1979) suggested that water with 20–150 $CaCO_3$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 in between 50 –300 $CaCO_3$mg l^{-1}. Stone and Thomforde (2004) suggested 50-150 $CaCO_3$mg l^{-1}as desirable range; with acceptable range of above 20mg $l^{-1}$and less than 400 mg l^{-1} for pond fish culture.

Hardness of water depends on the dissolved solids and its effect is pH dependent. 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 $CaCO_3$mg l^{-1}causes stress, 75 –150 $CaCO_3$mg $l^{-1}$is optimum for fish culture, while >300 $CaCO_3$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 $CaCO_3$mg l^{-1}, hardness > 20 $CaCO_3$mg l^{-1}as optimum range.

Ammonia in aquatic ecosystem including pond water is produced as by-product from protein metabolism excreted by fish, and microbial non-pretentious compounds (NPN) and decomposition of leftover feed and other organic matter. Ammonia in aquatic ecosystem sustain in two form, one is its ionic form (NH_4^+), which is extremely toxic, while another one is non-ionic form (NH_3), however sum of both forms called as total ammonia. Ammonia concentration > 0.1mgl^{-1} leads to gill damage, destroys mucous producing membranes, along with reduced growth, poor feed conversion, susceptible to disease, osmoregulatory imbalance, kidney failure.

Fish suffering from ammonia toxicity generally appear sluggish or often come to surface to take air (Bhatnagar and Devi 2013). Robinette (1976) reported toxic levels for unionized ammonia between 0.6 – 2.0 mg l^{-1} for short term exposure, however sub-lethal effect may occur at 0.1 – 0.3 mg l^{-1}. Meade (1985) reported maximum limit of ammonia concentration is 0.1mg l^{-1}. Stone and Thomforde (2004) suggested the desirable range of total ammonia (NH_3-N): 0-3mg l^{-1} and unionized ammonia (NH_3): 0mg l^{-1}, while acceptable range for total ammonia (NH_3-N) less than 4mg l^{-1} and unionized ammonia less than 0.4mg l^{-1}. Bhatnagar and Singh (2010) recommended the ammonia level <0mg l^{-1} for pond fish culture.

Nitrate (NO_2) is an intermediate product of the aerobic nitrification bacterial process, produced by the autotrophic *Nitrosomonas* bacteria combining oxygen and ammonia.Significance of nitrite in fish culture pond is more as it act as invisible killer of fish as it oxidizes hemoglobin to methemoglobin in the blood, turning the blood and gills brown and hindering respiration. It also damagesthe nervous system, liver, spleen and kidneys of the fish (Bhatnagar and Devi 2013). Stone and Thomforde (2004) suggested that the desirable range 0-1 mg $l^{-1}NO_2$ and acceptable range less than 4 mg $l^{-1}NO_2$. Santhosh and Singh (2007) recommended nitrite concentration in water should not exceed 0.5 mg l^{-1}. OATA (2008) recommended that it should not exceed 0.2 mg l^{-1}in freshwater.

In natural water bodies, total phosphorous is differentiated into soluble phosphate phosphorous (filterable or soluble orthophosphate), and organic soluble phosphate phosphorous and sestonic phosphorous. Orthophosphate is the most frequently measured form in fish culture and natural water bodies carrying fish. Concentrations of orthophosphate are usually low (0.001 – 0.05 mg l^{-1} as phosphorous) and represent only a fraction of the total phosphorous content (Boyd 1971). According to Stone and Thomforde (2004), 0.06 mg l^{-1} is desirable for fish culture. Bhatnagar *et al* (2004) suggested 0.05-0.7 mg l^{-1} is optimum and productive for fish culture.

2.2 Nutritional requirement and feed utilization in common carp

In fish farming, feed is the major input and accounts more than 60% of production cost. Feed helps in fast growth and better health of reared animal. Optimum production from fish culture system can be achieved by nutritionally

balanced diet, given at right time; in optimum quantity. The nutrient requirements of fish vary with species, genetics, life stages, sex, sexual maturity and stress. Poor nutritive feed affects growth rate, feed conversion and carcass composition; finally affecting fish yield and fish flesh quality.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. Fishes fulfill their nutritional requirement from natural food, but under commercial culture system, supplemental feed is provided as per their nutritional requirement.In fish nutrition, protein is the most important nutrient promoting growth and is the major component of body tissue as it acts as structural component and as most preferred energy source. Crude protein (CP) content in prepared feed is an important criterion of its nutritional value and the most expensive part of fish feed, it is important to accurately determine the protein requirements of cultured species.Protein play crucial role in survival and growth during early life stages as it requires high protein diet during early phase of life, while protein requirement of fish reduces with advancement of stage thereforediet needs to be adjusted accordingly. It is not only the quantity of protein but also the amino acid profile decides the nutritional value of feed. Significance of essential amino acid is more as it is not synthesized in body, therefore ingredients containing essential amino acids must be included in feed for their satisfactory development and growth.

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

Other than nutritional value, feeding frequency also play a substantial role in growth. Sultana *et al* (2001) observed the effect of feeding frequency on the growth of common carp. They conducted a feeding trial with 33.34% CP for 45 days on the fry of common carp reared in aquarium. Fishes were fed with ration at different feeding frequencies viz. 2, 3, 4, 5 and 6 times @ 5% fish body weight (BW) daily. 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 fry 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 $kcal^{-1}$.

Jaderand Sulevany (2012) evaluated the nutritional efficiency 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. Feed conversion ratio ranged between 2.27 (30% CP diet) to 3.01(25% CP diet), while least PER (0.79) was obtained, when fish fed with 35% CP diet.

Mohapatra and Patra (2014) reported maximum growth and survival in common carp fry fed at 35.2% protein as compared to diets with 27.1, 28, 30% and 35.2% protein. They recommended the use of animal protein for young ones of common carp.

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 (Teugels 1982), while 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 – 0.87Specific growth rate (SGR), 2.27 – 3.01FCR and 0.79 – 1.05 PER in juveniles of common carp, when fed with 25-35% CP diet.

2.3 Development of fish silage

Processing waste and spoiled fish due to improper handling leads to enormous amount of waste. The word silage is derived from silo; i.e., material stored in a silo as practiced on farms (Jangaard 1987). A.I. Virtanen was the first one to develop acid treated silage in 1920 from forage by using hydrochloric and sulphuric acid as

preservative (Raa and Gildberg 1982) and this method was further adopted by Edin in the 1930s to preserve and liquefy different types of fish and fish waste (Edin 1948). Experiments with fish silage began in Sweden in 1936; using hydrochloric acid and sugar as preservative (Tatterson and Windsor 1974). Fish silage as a fish feed ingredient started in 1980's for recycling of organic material (Hardy *et al* 1984). Fish silage can be produced by acid treatment (Petersen 1953), enzymatic action (Freeman and Hoogland 1956), lactic acid fermentation (Raa *et al* 1983, Mackie *et al* 1971) and fermentation by proteolytic fungi and yeast (Mackie *et al* 1971).

Commercially, first time fish silage was produced in Poland and Denmark for its use in poultry and pig feed as a protein complement later on incorporated in feeds for domestic animals and fish (Arruda *et al* 2007). In Denmark, Poland and Norway, the production of fish silage for inclusion in pig, poultry and mink diets is a common practice (Jackson *et al* 1984). A pilot scale trial has been set up and functional in United Kingdom (Tatterson and Windsor 2001). In Indonesia, silage was produced at experimental scale and used in rations which substituted fish and soy meal in the feed of swine, fish and birds (Kompiang 1981). In Iceland, silage has been tested on experimental scale and produced by companies on a commercial scale in the 1980's (Arason *et al* 1990). In New Zealand, a project tested for the use of fish silage as protein supplement in livestock and concluded on a reduction of peak rumen methanogenesis, a reduction of faecal egg counts of internal parasites and an increase in omega fatty acids in milk of dairy cows (Gibbs 2012). Fish silage is mainly used as an animal feeding stuff but is also used in soil health improvement in agriculture and in horticulture.

In most fish species, heads represent the biggest by-product fraction amounting 10% in Atlantic salmon (Rustad 2007) and between 20% and 25% in carp (Bukovskaya and Blockhin 2004). Safe disposal of processing waste is a challenge and creates environmental issues when not handled properly. The most important factor in successful fish silage production is the freshness of the raw material. Whole fish or processing waste in which some spoilage or bacteria breakdown occurred is not suitable for silage-making, because the resulting product will be poor in quality, with a high bacteria content and unpleasant odour (Winter and Feltham 1983).

2.4 Application of fish silage in fish diet

The use of fish silage in the feeding of fish has been widely studied. Due to the similarity of this protein source with the commonly used raw materials and low cost, especially when compared to fish meal, silage has a high potential for its use in aquaculture as a protein supplement (Hussain and Offer 1987, Fagbenro 1994, Vidotti *et al* 2003, Goddard and Perret 2005). Fagbenro (1994) and Fagbenro and Jauncey (1998) studied the nutritional value of diets containing microbial fermented fish silage partially dehydrated by the addition of soy meal, poultry by-products, bone and meat powder, and found no significant differences in the performance and protein use when compared to diets based on fish meal.Fish silage is characterized by similar or even better proximate composition in comparison withfish meal. The high quality content of fish oil rich in PUFA fatty acids makes fishsilage an excellent source of essential fatty acids (Ghaly 2013, Goosen 2014).

Haard *et al* (1985) used processed cod fish (*Gadus morhua*) waste for silage preparation using 3.5% (v/w) formic acid. The silage obtained became homogenous liquid in 36 to 48 h at 20ºC. Honczaryk and Maeda (1998) studied the efficacy ofbiological-fish-ensilage-based diets in the feeding of arapaima (*Arapaima gigas*), an important carnivorous fish from the Amazon area, andconcluded that silage incorporated diets shows higher level ofingestion.

Commonly, the fishery by-products are discarded as waste all over the world that causes serious environmental problems and economic losses (Kjos *et al* 2000, Barroga *et al* 2001). It is estimated that fish waste production is between 17.9 and 39.5 million tons per year, representing an important loss of valuable nutrients. Fish waste can be transformed into concentrated protein source i.e. fishmeal. However, its production process is expensive and requires energy. The high prices of fishmeal and its periodic scarcity have encouraged researchers to look for alternative protein feedstuffs (Fagbenro and Jauncey 1998).

Several studies conducted on the use of fish silage as a fishmeal (FM) replacer in tilapia feeds showed varying, but promising, results. From such studies, it is evident that fish silage has significant potential as a protein source for tilapia (Lapie and Bigueras-Benitez 1992, Fagbenro *et al* 1994, Hakim *et al* 2007). Lapie and Bigueras-Benitez (1992) reported that the growth of Nile tilapia fed with formic acid

preserved fish silage (FFS) blended with fish meal at 1:1 ratio was similar to that of fish fed with fish meal (FM) based other diets. When fish silage : fish meal ratio was increased to 3:1, growth performance was significantly reduced, presumably due to acidity of the diet and high proportion of free amino acids in fish silage.

Fagbenro *et al* (1994) evaluated the nutritive value of diets containing dried lactic acid fermented fish silage (FS) and soybean meal (SBM) (1:1) for juvenile *Oreochromis niloticus* and *Clarias gariepinus* to replace fish meal protein @ 25, 50 and 75%. *Oreochromis niloticus* and *Clarias gariepinus* were @ 5 % body weight per day for 70 days. Diets containing Co-dried FS:SBM supported weight gains and growth rates similar to those in the control treatment without significantdifferences.

Acid fish silage prepared from a mixture of inedible parts from Nile tilapia with 1.5% concentrated sulphuric acid and 1.5% concentrated formic acid was used to formulate three experimental diets for tilapia in which fish meal was replaced by fish silages (50,75 and 100% silage). After 13 weeks of feeding, a significant difference in growth performance and protein productive value were noted in tilapia fed on 100% fish silage and other treatments; however feed conversion ratio and protein efficiency ratio showed no significant differences between all treatments (El-Hakim *et al* 2007). Feeding trials on juvenile tilapia showed that sardine silage can replace fish meal at levels up to 40% of total diet without significantly affecting the growth rate. Apparent digestibility of protein, dry matter and energy of acid silage (formic and propionic) from sardine was comparable to that of fish meal in tilapia (Goddard and Al-Yahyai 2001).

Many workers have evidenced the nutritional benefits and economic feasibility of using fish silage as good feed stuff for aqua feeds. For instance, it was found that acid fish silage based diets supplied to Nile tilapia *(Oreochromis niloticus*) and blue tilapia (*O. aureus*) had good growth performance equivalent to that of fish meal diet (Wassef *et al* 2003). Likewise, fermented fish silage based diets supplied to Nile tilapia *O. niloticus* and African cat fish *C. gariepinus* evidenced 25 to 50% replacement of fishmeal without any significant effect (Hanafy and Ibrahim 2004).

Fagbenro and Jauncey (1995b) evaluated the performances of different fermented tilapia silage blends on African catfish. Catfish fed on different fish-silage diets showed some differences in mean weight gain, specific growth rate and protein

productive value, but feed conversion and protein efficiency ratios were similar. Protein digestibility was reduced in catfish fed diets containing fish silage (FS): Hydrolysed feeder meal (HFM) while digestibility of energy content was lower in those fed diets containing FS: Soybean meal (SBM). He concluded that fermented fish silage co- dried with protein feedstuffs was a suitable protein supplement, which can provide up to 50% of dietary protein without affecting feed efficiency, fish growth or health.

Feeding digestibility and growth studies on pacu (*Piaractus mesapotamicus*) have shown that fish silage is highly digestible and recorded effective replacement for up to 75% of fish meal in aquafeeds; however diet containing defatted silage concentrate showed reduced feed acceptance by the fish (Vidotti *et al* 2002).

Ramasubburayan *et al* 2013 did the characterization and studied the nutritional quality of formic acid silage developed from marine fishery waste and their potential utilization as feed stuff for common carp *C. carpio* fingerlings. They concluded that pH, dry matter, ash, protein contents and aerobic plate count showed a declining trend during ensilaging day's interval. However, lipid and mineral contents were gradually increased up to 30th day. After 30 days of fermentation, the amino acids and vitamins were quantitatively as well as qualitatively ascertained in all silages. Further, the individual silage products were used as one of the major ingredients to prepare formulated diets (E1: 2%; E2: 2.5% and E3: 3%). The prepared diets were fed to *C. carpio* fingerlings for 45 days and the growth promoting efficacy of the diets was tested. Results clearly indicates that 2% acid silage diet gained higher weight (2.38g), SGR (1.49%) and significant increase in biochemical constituents than other diets fed fish.

2.5 Growth performance of common carp

Specific growth rate expresses the percent body weight gain per day.SGR '0' indicates animal is no longer gaining or losing weight and is referred as maintenance feeding (De Silva and Anderson 2009). 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 parameter to measure the efficiency or suitability of feed to animal as it expresses the accumulation of structural nutrient in animal; ultimately reflects the suitability of feed to animal (Teugels 1982, Kiaalvandi *et al* 2011), while PER

expresses the efficiency of protein utilization. Noor *et al* (2014) evaluate the partial replacement of fish meal by fish silage in feeds formulated for common carp (*C. carpio*) fry. They incorporated bio-silage in feed @ 0, 25, 50 or 75% to replace fish meal. Fish were fed for 10 weeks on iso-nitrogenous (42% protein) and iso-caloric (4600 Kcal/kg) feeds and feeding and growth parameters were close in the four feed groups. SGR ranged 3.178–3.220, FCR 2.101– 2.151, PER 1.179 – 1.466 and survival 84.4–96.7% and recommended that fish bio-silage can be used successfully as fish meal alternative in feeds for common carp fry without adverse effects on feeding and growth efficiency. Al-Faraje (2000) reported SGR 0.788 – 1.098 and FCR 4.5 – 7.8 when total animal protein is replaced by lactic acid fermented fish silage in diets of common carp fingerlings. Haider *et al* (2015) observed the SGR ranging 0.91 – 1.29, FCR 2.43 – 3.87 in *Labeo rohita*, when fed with diets containing 32% protein level prepared from 25, 50, 75 and 100% silage. Kaur (2016) reported the SGR 0.48 – 0.92, FCR 2.42–5.68, PER 0.56–1.08, in the fingerlings of common carp (*C. carpio*) when fed with diet containing 35 – 39% protein.

2.6 Biochemical composition of common carp flesh

Biochemical studies of fish tissue are of considerable interest for their specificity in relation to the food valuesof the fish and for the evaluation of their physiological needs at different periods of life. It is also necessary to have the data on the composition of fish in order to make the best use of it as food and also to develop the technology of processing fish and fish products. Generally changes in chemical composition of body have been known to reflect storage or depletion of energy reserves. The values of body composition in fishes vary considerably within and between species, with fish size, sexual condition, feeding, time of the year and activity (Weatherly and Gill 1987). Food composition, environment and genetic trait are also known to influence chemical composition of fish (Oni *et al* 1983).

In general, the biochemical composition of the whole body indicates the fish quality. The percentage composition of the four major constituents of fish viz. water, protein, lipid and ash (minerals) is referred to as proximate composition (it may be noted that the term does not indicate any degree of inaccuracy in the analysis). These four components account for about 96-98% of total tissue constituents in most cases. The principle constituents are water (66 – 84%), protein (15 – 24%), lipids (0.1 –

22%), minerals (0.8 – 2%) and carbohydrate in very minute quantity (0.3%) at maximum value in fishes (Jacquot 1961). According to Nair and Suseela (2000), the proximate composition of Indian fishes ranges between 65 – 90% water; 10 – 22% protein, 01 –20% lipid and 0.5–05% minerals.

Water is essential for all living systems. Body fluids act as medium of transport of nutrients, metabolites etc. and water is the major component in these fluids. It is required for the normal functioning of many biological molecules. Proteins, for example, can maintain its native form and normal functions only in presence of water. The proportion of water in the flesh varies widely, though in a majority of cases the variation is much narrower, between 70-80%. One of the examples of very high water content is Bombay duck *(Harpodon nehereus)* a species found abundantly along the north-west coast of India, in which case the muscle tissue contains about 90% water. Water is present in two forms in the tissues, bound to the proteins and in the free form. These forms have well defined biological roles. Water is lost from the tissue in many ways during processing and this may affect the quality, especially the texture of the processed products.

There exists an inverse relationship between the water content and lipid content of fish, such that the sum of the percentages of the two approximates 80 percent. The summation of oil and water, however, is not necessarily constant and frequently spans a range of 78 to 85 percent

An inverse relationship between water and protein in the tissues has been documented by many authors. Merayo (1996) reported that during maturation of the gonad in bib (*Trisopterus luscus* L.), the muscle water content increases to the highest levels and the muscle protein content decreases. Lipids are the primary energy storage material in fish (Love 1970, Adams 1999,Tocher 2003). Fish store the lipids invarious organs; particularly in muscles and liver. Lipid composition and distribution between and within tissues in fish vary from species to species and are influenced by seasonal and dietary variations (Ackman 1980, Henderson and Tocher 1987). Thus, the lipid content of a fish indicates the surplus energy available for future maintenance, growth and reproduction.

Kaur (2016) observed the moisture content 77.2 – 82.4, protein content 13.60 – 16.7, lipid content 1.75– 2.73 and ash content 1.40 – 2.61, when fed with diet containing 35 – 39% protein in fingerlings of common carp (*C. carpio*).

CHAPTER III

MATERIALS AND METHODS

3.1Site of the Experiment

The present experiment was to evaluate performance of fish silage as a potential source of digestible protein for the partial replacement of soybean meal in feed of common carp (*Cyprinus carpio*, L.) fingerlings. The experiment was conducted in the Department of Aquaculture, College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, from August to November 2016.

3.2 Setting of the experimental FRP pools

The experiment was carried out in the FRP tanks (1.5×1.0×0.75m) in triplicate. A total 15 FRP pools for 5 treatments, with 3 replicates, were set for the proposed study. FRP pools were filled with fresh underground water and each pool was connected with aeration facility.

3.3 Stocking of fish

After 2 days of water filling in the FRP tanks, each pool was stocked with pre-acclimatized fingerlings of common carp (*C. carpio*) @ 11 fingerlings per pool. Average weight of the fish ranged between 5.41 – 5.49 g, while length ranged 6.61 – 6.74 cm.

3.4 Preparation of fish silage

Fish silage was prepared by as per method described by Ramasubburayan *et al* (2013) using 3% formic acid

Fish waste
(carp fish, crushed and minced form)
↓
Addition of 85% formic acid @ 3% v/w
↓
Left for 10 days for proteolytic activity and semi dry
↓
Fish silage* (semi dried)

* Fish silage was neutralized with 1%NaOH before adding in fish diet.

3.5 Preparation of experimental diets

Following five experimental diets (D_1– D_5) were formulated replacing soybean meal with fish silage @ 15% (D_2), 30% (D_3), 45% (D_4) and 60% (D_5) to compare with control (D_1) (Table 1).

Table 1: Percent composition of diets

Ingredients	D_1 Control	D_2 (15%)	D_3 (30%)	D_4 (45%)	D_5 (60%)
Rice bran	38	38	38	38	38
Mustard meal (de-oiled)	30	30	30	30	30
Soybean meal	26	22.1	18.2	14.3	10.4
Fish silage	-	3.9	7.8	11.7	15.6
Carboxy methyl cellulose	2	2	2	2	2
Mineral mixture	2	2	2	2	2
Vitamin mixture	2	2	2	2	2

Table 2: Proximate composition of different feed ingredients used in experimental diets

Ingredients	Moisture	Protein	Lipid	Ash	NFE
Fish silage	30.70	36.92	15.24	14.02	2.60
Soybean meal	8.52	42.10	3.37	5.76	40.08
Mustard oil cake (de-oiled)	8.64	32.70	3.28	9.62	45.28
Rice bran	8.32	12.10	3.70	10.40	65.12

3.6 Proximate composition of experimental diets

The proximate analysis (moisture, crude protein, ether extract, ash and nitrogen free extract) of feed ingredients used in experimental diets (Table 2) was evaluated on dry matter (DM) basis by following the standard methods of AOAC (2000). Similarly, proximate analysis (moisture, crude protein, ether extract, ash, crude fibre and nitrogen free extract) of experimental diets was also performed. Details of the proximate composition of experimental diets are given in table 3.

3.6.1 Moisture

Approximately 10 g sample was taken in pre weighed petridish. Weight of petridish was further taken with sample and kept in hot air-oven maintained at 100 ± 2°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)

3.6.2 pH of fish silage

Fish silage samples were diluted in distilled water (1:10) and left for 25-30 minutes. pH was recorded by using digital pH meter (METLLER TOLEDO: MODEL FG2-1).

3.6.3 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

a) Concentrated sulphuric acid (H_2SO_4) – specific gravity1.84
b) Catalyst/digestion mixture – cupric sulphate ($CuSO_4.5H_2O$) 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 final volume was maintained to 1 liter by adding 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.

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 clear 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 (containing 15% NaOH and distilled water) was kept running for the absorption of acid fumes.

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.

Titration

i. Contents in the conical flask were titrated with 0.1 N H_2SO_4 till wine colour appears and considered as end point.

ii. Volume of H_2SO_4 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_2SO_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 acidused	-	$(X_2 – X_1) – Y = Z$ ml

Calculations

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

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

Where,

Z= ml of titrant used

W= weight of sample

3.6.4 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. Approximately 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. Beakers were loaded in the Soxhlet apparatus for fat extraction.
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
Weight of empty beaker	–	W_1
Weight of beaker + EE	–	W_2
Weight of EE or crude fat	–	$W_2 - W_1$

Calculations

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

3.6.5 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^{o}C for 3 hrs.

iii. Weight of crucible with ash was recorded after cooling it.

Calculations

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

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_2S0_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) and transferred to oven dried sintered glass crucible.

ii. Cruciblewas placed into the metal adapters of FIBRA PLUS hot extraction unit.

iii. Acid wash

- 150 ml of 1.25 % H_2S0_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 (100^oC) to make the samples moistures free.

vi. Hot crucible was cooled down to room temperature using a desiccator.

vii. Crucible with dry samplewas weighed (W_1).

viii. Crucible was further placed in the muffle furnace at 330 °C for ashing.

ix. Hot cruciblewas cooled down to room temperature after ashing using a desiccator and weighed (W_2).

Calculations

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

3.6.6 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 3: Proximate composition (%) of formulated diets (on DM basis)

Diets	**T1**	**T2**	**T3**	**T4**	**T5**
Moisture (%)	8.18	8.52	8.72	8.04	8.18
Ash (%)	14.18	14.74	15.14	15.86	16.68
Crude Protein (%)	29.32	31.12	33.42	34.98	37.04
Total lipids (%)	3.46	4.96	4.66	4.92	5.08
NFE (%)	39.54	35.44	33.02	32.14	29.92
Fiber (%)	4.34	4.12	4.88	4.02	3.08

3.7 Feeding of fish

Fish were fed daily with experimental diets @ 5% of fish body weight (BW) twice (9:00 am and 4:00 pm) for 90 days. Amount of feed was adjusted at every sampling according to increase in fish BW.

3.8 Observations recorded

- Physico-chemical parameters (temperature, pH, dissolved oxygen, total alkalinity, phenolphthalein alkalinity, methyl orange alkalinity, total hardness, ammonia, nitrite and orthophosphate) were recorded at weekly interval.
- Survival and growth (length and weight) of fish recorded at every fortnightly.
- Biochemical changes (moisture, proteins, lipids, ash and total carbohydrates) in fish flesh 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.

3.9Physico-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.

3.9.1 Temperature – Water temperature (°C) was recorded with the help of digital thermometer (METLLER TOLEDO: MODEL FG2-1). The working range of digital thermometer was 0-50°C with ± 0.1°C precision.

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

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

Reagents

1. Manganese sulphate: 480 g $MnSO_4.4H_2O$ 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 $Na_2S_2O_3.5H_2O$ was dissolved in distilled water and preserved by adding 0.4 g solid NaOH and volume is maintained to 1000ml by adding distilled water.
6. Standard sodium thiosulphate (0.025N): 250 ml stock $Na_2S_2O_3$ solution was dissolved to 1000 ml with freshly boiled and cooled distilled water.

Procedure

i. Water 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 3-4 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 $Na_2S_2O_3$ solution using starch solution (2 ml) as an indicator.

Calculations:

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

Where,

T = Volume of titrant ($Na_2S_2O_3$) used in ml

N = Normality of $Na_2S_2O_3$ solution

3.9.4 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 $MgSO_4.7H_2O$ 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 mix 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

i. 50ml of well mixed water 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 Tindicatior 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 I.

Calculations

$$\text{Total hardness } (CaCO_3 \text{ mg } l^{-1}) = \frac{C \times 1000}{\text{Sample (ml)}}$$

3.9.5 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 diluted by adding 500 ml of distilled water. 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 mg $CaCO_3 l^{-1}$ = A x 1000 ml^{-1} sample = X

Methyl Orange alkalinity, as mg $CaCO_3$ l^{-1} = B x 1000 ml^{-1} sample = Y

Total alkalinity, as mg $CaCO_3l^{-1}$ = X + Y

3.9.6 Ammonia: Ammonical-Nitrogen (NH_4^+) of water was estimated by Nesslerisation method APHA (2005).

Reagents

1. Zinc sulphate: 10 g $ZnSO_4.7H_2O$ was dissolved in distilled water and diluted to 100 ml.
2. Sodium hydroxide (6N): 24 g NaOH was dissolved and diluted to 100 ml.
3. EDTA reagent: 50 g EDTA was dissolved in 60 ml water containing 10 g NaOH. It was cooled and diluted to 100 ml.
4. Rochelle salt solution: 50 g potassium sodium tartarate was dissolved in 100 ml and then 30 ml of this solution was boiled off to remove ammonia. It was then cooled and diluted to 100 ml.
5. Nessler reagent: 100 g $HgCl_2$ and 70 g Kl were well mixed and dissolved in small quantity of water. This mixture was added to a cooled solution of 160 g NaOH in 500 ml water and diluted to 1000 ml. It was kept overnight and supernatant was stored in coloured bottle.
6. Standard ammonium solution: 3.819 g NH_4Cl dried at 100°C was dissolved in distilled water and diluted to 1000 ml.

Procedure

i. A 100 ml of sample was taken in conical glass bottle and to it 1 ml $ZnSO_4$ solution and 0.4 ml NaOH solution was added to obtain the pH of 10.5.

ii. The contents were allowed to settle and the supernatant was filtered through Whatman no. 42 filter paper.

iii. Suitable aliquot of sample was taken.

iv. To this, 1 drop of EDTA was added and contents were mixed properly followed by which 3ml of Nessler reagent was added and volume was made up to 100 ml.

v. After mixing the contents very well, percent transmission was read at 410 nm after 10 minutes interval; using a blank prepared in the same way by taking distilled water instead of sample.

Calculation

The concentration was obtained directly from standard graph and expressed as mg l^{-1}.

3.9.7 Nitrite: Nitrite-Nitrogen of water was estimated by Colorimetric method APHA (2005).

Reagents

1. Sulphanilamide reagent: 5 g of sulphanilamide was dissolved in a mixture of 50 ml conc. HCl and about 300 ml water and diluted to 500 ml with water.
2. NED-dihydrochloride solution: 500 mg of N-(1-Naphthyl)-ethylene diaminedihydrochloride (NED) was dissolved in 500 ml water and stored in a dark bottle.
3. Sodium oxalate (0.05N): 3.35 g of $Na_2C_2O_4$was dissolved in water and diluted to 1000 ml.
4. Stock nitrite solution: 1.2320 sodium nitrite ($NaNO_2$) was dissolved in water and diluted to 1000 ml.

Procedure

i. 50 ml of water sample was neutralized to pH 7 and 1ml of sulphanilamide solution was added to it.
ii. The reagent was allowed to react for 2 to 8 min.
iii. A1 ml NED dihydrochloride solution was added and the contents were mixed immediately.
iv. The absorbance was measured after 10 min at 543 nm.
v. A blank of distilled water was run in the same way.

3.9.8 Orthophosphate: Phosphate-phosphorous of water was estimated by stannous chloride method described in APHA (2005).

Reagents

1. Stock phosphate solution: 219.5 mg of anhydrous $KH_2PO_4$was dissolved in distilled water and diluted to 1000 ml.
2. Phosphate working solution: 50 ml of stock solution was diluted to 1000 ml with distilled water.
3. Ammonium molybdate solution: 25 g was dissolved in about 175 ml distilled water. 280 ml of concentrated $H_2SO_4$was added carefully to 400 ml distilled

water. It was then cooled and molybdate solution was added and diluted to make volume 1000 ml.

4. Strong acid reagent: 300 ml of concentrated $H_2SO_4$was added to 600 ml distilled water. 4 ml of concentrated HNO_3 was added, cooled and diluted to 1000 ml.
5. Sodium hydroxide (6N): 24 g of NaOH was dissolved and diluted to 100 ml.
6. Stannous chloride reagent I: 2.5 gm of fresh $SnCl_2.H_2O$ was dissolved in 100 ml glycerol and heated on water bath to ensure complete dissolution.
7. Dilute stannous chloride reagent II: 8 ml of stannous chloride reagent I was added in 50 ml glycerol and mixed thoroughly.

Procedure

i. 25 ml of the sample was taken in a Nessler tube and distilled water blank was also prepared simultaneously followed by which 1 ml of ammonium molybdate solution and 3 drops of stannous chloride solution was added to it.
ii. Blue colour appeared gradually and reading was taken on spectrophotometer at 690 nm after 10 minutes against a blank.
iii. From the standard curve, the concentration of phosphate was computed in the volume of sample taken.
iv. It was expressed as mg l^{-1} PO_4^{3-}P.

3.10 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 pool to record total body length (TBL) and body weight (BW) of fish. Total body length gain (TBLG), net weight gain (NWG), specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER) were calculated according to the following formulae:

TBLG (cm) = Average final TBL (cm) – Average initial TBL (cm)

NWG (g) = Average final BW (g) – Average initial BW (g)

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

Where, ln is natural log

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

$$\textbf{PER} = \frac{\text{Wet weight gain in fish (g)}}{\text{Protein intake (g)}}$$

3.11 Proximate analysis of fish flesh

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

3.11.1 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 100ºC 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)

3.11.2 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 added in 100 ml of 0.1 N sodium hydroxidesolution.

c) Reagent B – 0.5 g copper sulphate ($CuSO_4.5H_2O$) 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.

3.11.3 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.

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 $1/5^{th}$ of volume filtrate) was added in separating funnel, and shaked 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

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)

3.11.4. 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 550º 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)

3.11.5 Crude Fiber: Crude fibre content of dry sample was determined by using AOAC 2005 method.

Reagents

1. Sulphuric acid (1.25%): 6.7 ml in 1 litre distilled water.
2. NaOH(1.25%): 12.5 g in 1 litre distilled water.

Procedure

1. 1 g of moisture and fat free sample was weighed and kept in the fibre bags.
2. The glass spacer was put in to the bags.
3. The bag in the sample carousel was loaded at the previewed positions (positions 1-12).
4. The sample carousel was put into the glass container carefully.
5. The glass container was placed axial on the previewed position of the hot plate.
6. After programming w.r.t. time and temperature, fibre containing sample started.
7. After completion of the programme, the fibre bags were removed.
8. The residue was transferred to weighed crucible (W_1) and drier over night at 80^oC- 100^oC and weighed (W_2).
9. The crucible was heated in muffle furnace at 600^oC for 2-3 hours.
10. Cooled in desiccator and weight of the crucible was taken after cooling (W_3).

Calculation

Weight of the sample (g) $= W_1$

Weight of the crucible + sample before heating (g) $= W_3$

Weight of the crude fibre (g) $= (W_2 - W_3)$

Crude fibre (g %) =100-(moisture + fat) X weight of fibre / Weight of the sample taken

3.11.6 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 – (%moisture + %CP + %EE + % Ash)

3.12 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.

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 Conclusion

4.1 Physico-chemical parameters of water

Physico-chemical parameters of water play key role in the survival, growth, reproductive biology, physiology and other metabolic activities of the fish. Water quality determines to a great extent the success or failure of a fish cultural operation (Piper *et al* 1982). 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 on interactions between abiotic and biotic factors along with the supplementary feed. Though the supplementary feeding improves fish growth but composition, digestibility and physical characteristics and their excretion 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, ammonia, nitrite and orthophosphate in different treatments is presented below.

4.1.2 Temperature

The water temperature (°C) in all treatments (T_1 to T_5) ranged in-between 19.26–29.63 during the experimental period (August to November) and the differences in temperature in between treatments were insignificant ($P \leq 0.05$). The reason behind no significant difference in water temperature may be due to equal exposure of environmental conditions to all treatments. Water temperature in between treatments ranged 19.63–29.36, 19.66–29.43, 19.40 – 29.26, 19.30 –29.26 and 19.26–29.63 °C in T_1 (control), T_2, T_3, $T_4$and T_5, respectively (Table 4 & Fig. 1), however the water temperature declined w.r.t. progress of season towards winter.

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

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	29.36^{a}±0.40	29.43^{a}±0.03	29.26^{a}±0.08	29.26^{a}±0.14	29.63^{a}±0.36
	7	29.16^{a}±0.40	29.13^{a}±0.03	29.16^{a}±0.08	29.16^{a}±0.14	29.23^{a}±0.36
	14	28.23^{a}±0.03	28.16^{a}±0.06	28.23^{a}±0.03	28.10^{a}±0.057	28.50^{a}±0.37
	21	28.36^{a}±0.08	28.56^{a}±0.08	28.43^{a}±0.18	28.43^{a}±0.17	29.13^{a}±0.41
September	28	28.40^{a}±0.05	28.43^{a}±0.17	28.40^{a}±0.11	28.46^{a}±0.03	28.40^{a}±0.001
	35	28.50^{a}±0.05	28.56^{a}±0.08	28.50^{a}±0.152	28.56^{a}±0.23	28.50^{a}±0.057
	42	28.53^{a}±0.03	28.50^{a}±0.05	28.53^{a}±0.033	28.53^{a}±0.066	28.50^{a}±0.057
	49	28.56^{a}±0.88	28.53^{a}±0.13	28.50^{a}±0.057	28.56^{a}±0.033	28.50^{a}±0.057
October	56	27.73^{a}±0.08	27.70^{a}±0.05	27.73^{a}±0.03	27.76^{a}±0.08	27.76^{a}±0.03
	63	23.73^{a}±0.52	23.76^{a}±0.08	23.76^{a}±0.16	23.73^{a}±0.13	23.76^{a}±0.08
	70	21.63^{a}±0.52	21.63^{a}±0.08	21.63^{a}±0.08	21.66^{a}±0.06	21.63^{a}±0.08
	77	20.86^{a}±0.08	20.80^{a}±0.05	20.83^{a}±0.08	20.83^{a}±0.03	20.80^{a}±0.05
November	84	19.83^{a}±0.08	19.96^{a}±0.06	19.40^{a}±0.05	19.30^{a}±0.11	19.26^{a}±0.03
	91	19.63^{a}±0.08	19.66^{a}±0.06	19.60^{a}±0.05	19.60^{a}±0.11	19.66^{a}±0.03
Mean		26.10^{a}±1.04	26.16^{a}±1.087	26.10^{a}±1.067	26.12^{a}±1.111	26.14^{a}±1.175
Range		19.63±0.08–29.36±0.40	19.66±0.06–29.43±0.03	19.40±0.05–29.26±0.08	19.30±0.11–29.26±0.14	19.26±0.03–29.63±0.36

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

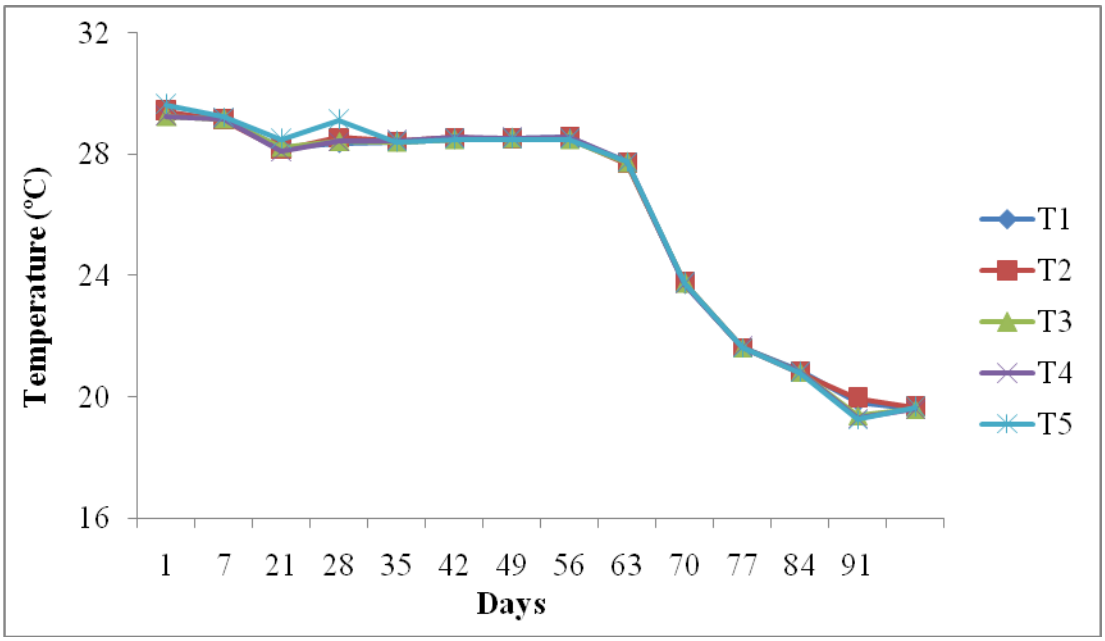

Fig 1: Changes in water temperature (°C) in different treatments during the experimental period.

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 (19.26 to 29.63^{o}C) during present study were well within the optimum range of 26-32^{o}C suggested 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 and reported maximal foraging and growth of common carp between 24 –28°C.

4.1.3 Water pH

The water pH in all treatments (T_1 to T_5) ranged in-between 8.23– 9.13 during the experimental period and the differences were insignificant ($P \leq 0.05$) except significant difference during the first week of October. The reason behind no significant difference in water pH may be due to use of water from single source for the initial filling and maintaining water level in FRP pools in all treatments. In October, significantly low ($P \leq 0.05$) pH (8.70) recoded during first week in the treatment T_5. The pH of water ranged between 8.43 – 9.13, 8.26 – 9.10, 8.40 – 9.06, 8.40 – 9.06 and 8.23 – 9.05 in treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 5& Fig.2).

Table 5: Water pH in different treatments during the experimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	8.73^{a}±0.060	8.70^{a}±0.050	8.80^{a}±0.050	8.80^{a}±0.050	8.63^{a}±0.030
	7	8.43^{a}±0.060	8.50^{a}±0.050	8.60^{a}±0.050	8.40^{a}±0.050	8.23^{a}±0.030
	14	9.13^{a}±0.003	9.04^{a}±0.005	8.90^{ab}±0.005	8.80^{ab}±0.250	8.90^{ab}±0.170
	21	8.86^{a}±0.030	8.96^{a}±0.060	8.80^{a}±0.050	8.90^{a}±0.100	8.86^{a}±0.030
September	28	8.80^{a}±0.050	8.86^{a}±0.140	8.80^{a}±0.050	8.86^{a}±0.120	8.83^{a}±0.030
	35	8.70^{a}±0.057	8.86^{a}±0.120	8.96^{a}±0.066	8.80^{a}±0.100	8.83^{a}±0.033
	42	8.70^{a}±0.057	8.73^{a}±0.120	8.76^{a}±0.088	8.83^{a}±0.180	8.76^{a}±0.066
	49	8.70^{a}±0.057	8.76^{a}±0.088	8.76^{a}±0.088	8.93^{a}±0.140	8.83^{a}±0.033
October	56	8.80^{a}±0.0001	8.76^{a}±0.030	8.80^{a}±0.0001	8.80^{a}±0.0001	8.70^{b}±0.0001
	63	8.53^{a}±0.120	8.43^{a}±0.080	8.40^{a}±0.100	8.53^{a}±0.330	8.53^{a}±0.330
	70	8.53^{a}±0.120	8.26^{ab}±0.080	8.40^{ab}±0.057	8.50^{ab}±0.057	8.66^{a}±0.330
	77	8.86^{a}±0.030	8.70^{a}±0.050	8.66^{ab}±0.030	8.60^{ab}±0.050	8.46^{ab}±0.120
November	84	8.66^{a}±0.060	8.99^{a}±0.010	8.96^{a}±0.080	8.88^{a}±0.080	8.95^{a}±0.080
	91	8.96^{a}±0.060	9.10^{a}±0.010	9.06^{a}±0.080	9.06^{a}±0.080	9.05^{a}±0.080
Mean		8.73^{a}±0.036	8.68^{a}±0.106	8.76^{a}±0.057	8.83^{a}±0.050	8.75^{a}±0.055
Range		8.43±0.060 – 9.13±0.003	8.26±0.080 – 9.10±0.010	8.40±0.057 – 9.06±0.080	8.40±0.050 – 9.06±0.080	8.23^{a}±0.030– 9.05±0.080

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

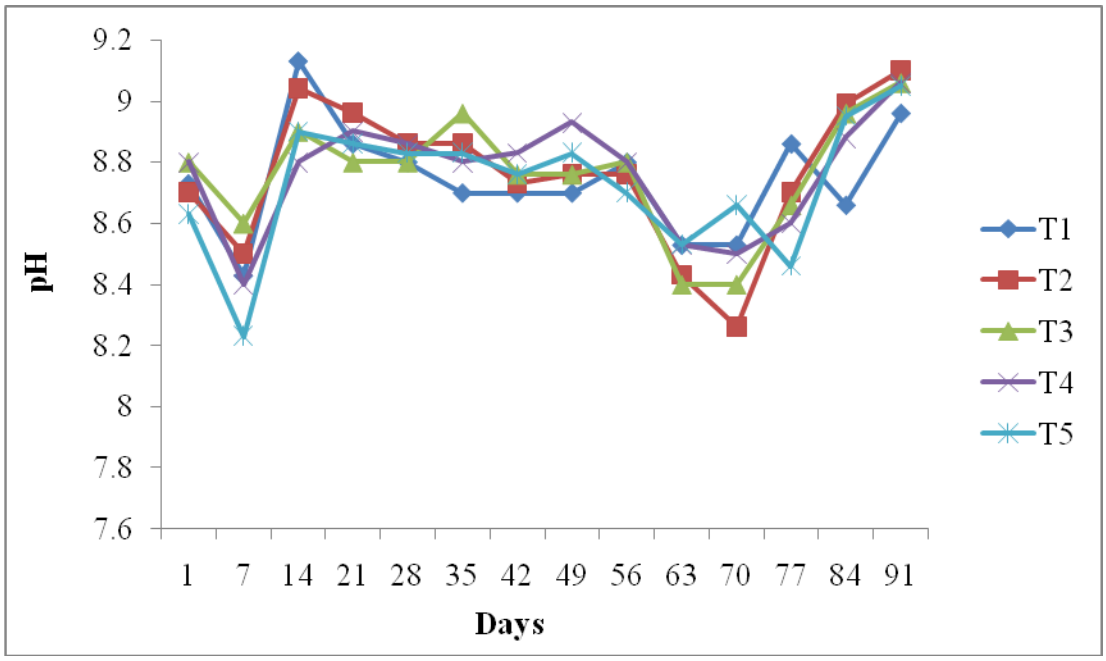

Fig 2: Changes in water pH in different treatments during the experimental period.

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). Change in pH also depends on the net concentration of electrolytes produced during different chemical reactions including its state of formation during carbonate, bicarbonate and hydroxide buffer.pH value also depends on hardness of the water as it increases with increase in alkaline elements such as calcium and magnesium. Excess load of organic matter (excretory and leftover feed) and their decomposition also tends to change in pH value.

In present study, water pH recorded in the different treatments remained well within the recommended valuessuggested by different workers and not varied significantly in-between treatments except during first week of October, when it was significantly low ($P \leq 0.05$) treatment T_5.

4.1.3 Dissolved oxygen (D.O.)

Dissolve oxygen (mg l^{-1}) in all treatments (T_1 to T_5) ranged in-between 7.60 – 9.65 during the experimental period and the differences were insignificant ($P \leq 0.05$).

Table 6: Waterdissolved oxygen (mg l^{-1}) in different treatments during the experimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	8.80^{a}±0.61	8.66^{a}±0.53	8.66^{a}±0.66	8.66^{a}±0.66	7.80^{a}±0.20
	7	8.40^{a}±0.61	8.46^{a}±0.53	8.26^{a}±0.66	8.26^{a}±0.66	7.60^{a}±0.20
	14	8.06^{a}±0.11	8.66^{a}±0.13	8.26^{a}±0.17	8.24^{a}±0.17	8.66^{a}±0.17
	21	8.46^{a}±0.10	8.80^{a}±0.06	8.88^{a}±0.13	8.28^{a}±0.06	8.44^{a}±0.13
September	28	8.66^{a}±0.37	8.46^{a}±0.17	8.66^{a}±0.06	8.80^{a}±0.23	8.64^{a}±0.06
	35	8.86^{a}±0.17	8.40^{a}±0.23	8.42^{a}±0.35	8.60^{a}±0.11	8.60^{a}±0.11
	42	8.66^{a}±0.066	8.88^{a}±0.20	8.80^{a}±0.13	8.82^{a}±0.066	8.88^{a}±0.24
	49	8.80^{a}±0.23	8.60^{a}±0.11	8.88^{a}±0.20	8.63^{a}±0.13	8.86^{a}±0.17
October	56	8.86^{a}±0.17	8.43^{a}±0.24	8.46^{a}±0.48	8.60^{a}±0.11	8.86^{a}±0.17
	63	8.42^{a}±0.13	8.20^{a}±0.11	8.22^{a}±0.24	8.40^{a}±0.11	8.24^{a}±0.06
	70	8.06^{a}±0.17	8.06^{a}±0.29	8.22^{a}±0.24	8.66^{a}±0.17	8.00^{a}±0.11
	77	9.40^{a}±0.11	9.22^{a}±0.29	9.20^{a}±0.30	9.20^{a}±0.20	9.62^{a}±0.11
November	84	9.20^{a}±0.17	9.46^{a}±0.06	9.45^{a}±0.17	9.20^{a}±0.11	9.40^{a}±0.11
	91	9.20^{a}±0.17	9.26^{a}±0.06	9.65^{a}±0.17	9.40^{a}±0.11	9.40^{a}±0.11
Mean		8.69^{a}±0.116	8.64^{a}±0.106	8.69^{a}±0.124	8.67^{a}±0.091	8.67^{a}±0.151
Range		8.06±0.11 – 9.40±0.11	8.06±0.29 – 9.46±0.06	8.22±0.24 – 9.65±0.17	8.24±0.17 – 9.40±0.11	7.60±0.20 – 9.62±0.11

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

The reason behind no significant difference in D.O. concentrations may be due to, all treatmentswere aerated with the help of motorized aerator and pools were free from aquatic vegetation. In-between treatment, D.O. ranged between 8.06 – 9.40, 8.06 – 9.46, 8.22 – 9.65, 8.24 – 9.40 and 7.60 – 9.62 in treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 6 & Fig. 3). However the D.O. concentration in water showed the increasing trend w.r.t. progress of season towards winter.

In present study, D.O. concentration recorded in the different treatments remained well above than the minimum recommended concentration i.e. 5 mg l^{-1} suggested by different workers. For optimum productivity of carps, D.O. content >=5.0 mgl^{-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 may die if exposed to less than 0.3 mg l^{-1} dissolved oxygen for longer period, they also suggested that minimum concentration of D.O. should be 1.0 mg l^{-1} for survival, whileit should be atleat5.0 mg l^{-1}or more to sustain fish for long period.

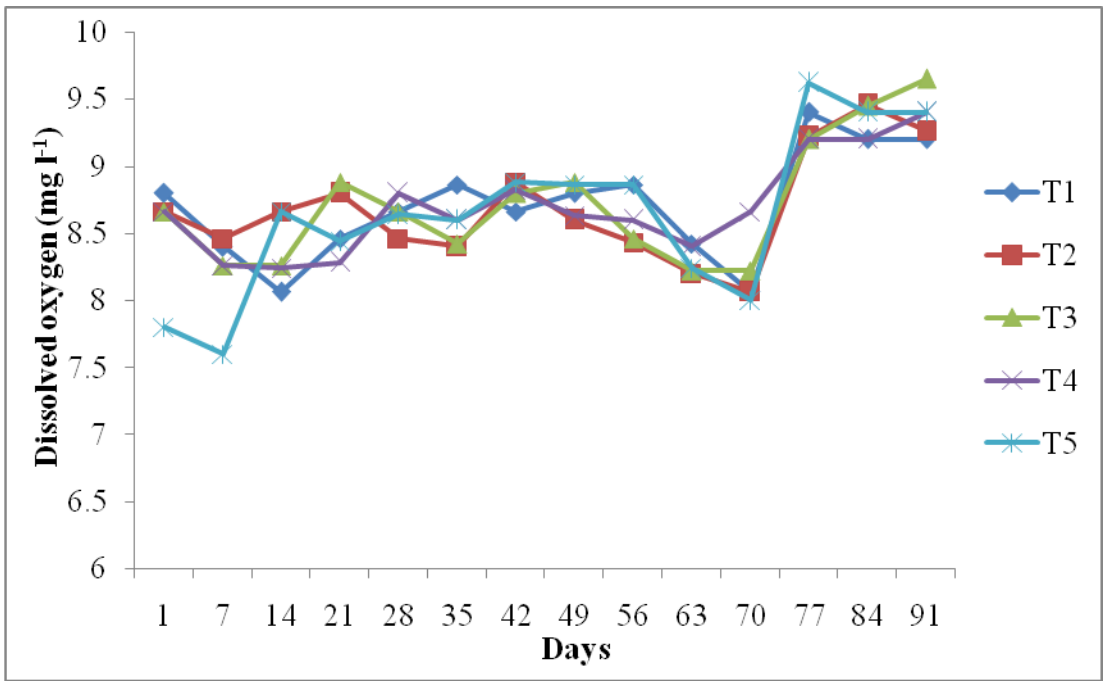

Fig 3: Changes in dissolved oxygen (mg l^{-1}) in different treatments during the experimental period.

4.1.4 Total alkalinity

Total alkalinity ($CaCO_3$mg l^{-1}) in all treatments (T_1 to T_5) ranged in-between 265.33 – 312.33during the experimental periodand the differences were non-

Table 7: Watertotal alkalinity ($CaCO_3$mg l^{-1}) in different treatments during the experimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	312.33^a±6.74	311.66^a±9.27	304.62^a±5.56	311.66^a±6.00	306.44^a±2.30
	7	298.13^a±6.74	295.66^a±9.27	291.62^a±5.56	296.66^a±6.00	293.44^a±2.30
	14	280.66^a±5.81	280.22^a±2.88	270.23^a±5.77	271.66^a±4.40	283.33^a±5.69
	21	284.66^a±4.37	281.33^a±5.81	290.66^a±2.90	283.36^a±4.05	282.66^a±2.90
September	28	287.33^a±4.66	288.66^a±4.05	295.33^a±2.90	290.66^a±6.66	287.33^a±1.76
	35	293.23^a±4.58	297.33^a±3.33	298.66^a±5.20	300.22^a±3.05	294.66^a±1.33
	42	296.33^a±4.80	284.21^a±4.00	284.23^a±5.70	291.33^a±3.71	295.33^a±3.71
	49	282.66^a±3.33	280.66^a±7.51	280.33^a±1.76	282.66^a±2.40	285.33^a±2.90
October	56	271.66^a±3.48	276.46^a±3.05	273.84^a±2.08	274.81^a±3.05	275.01^a±2.72
	63	274.66^a±2.40	274.66^a±2.02	277.34^a±2.02	277.20^a±0.57	272.44^a±1.45
	70	282.66^a±2.84	281.20^a±2.52	281.33^a±3.38	284.00^a±2.08	281.00^a±2.30
	77	273.66^a±2.33	273.66^a±1.45	265.33^a±6.06	275.66^a±1.76	268.20^a±4.04
November	84	275.66^a±1.76	273.21^a±2.08	271.22^a±2.51	271.41^a±4.16	279.25^a±1.15
	91	278.66^a±1.76	274.21^a±2.08	276.22^a±2.51	279.41^a±4.16	276.25^a±1.15
Mean		280.94^a±2.93	282.75^a±3.37	280.92^a±4.01	280.08^a±2.90	281^a.22±3.17
Range		271.66±3.48 – 312.33±6.74	273.21±2.08 – 311.66±9.27	265.33±6.06 – 304.62±5.56	271.41±4.16 – 311.66±6.00	268.20±4.04 – 306.44±2.30

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

significant (P≤0.05). Total alkalinity in water ranged between 271.66 – 312.33, 273.21 – 311.66, 265.33 – 304.62, 271.41 – 311.66 and 268.20 – 306.44 in treatments T_1, T_2, T_3, $T_4$and T_5, respectively (Table 7 & Fig. 4). Changes in total alkalinity may be due to changes in carbonate, bicarbonate concentration and hydroxides.

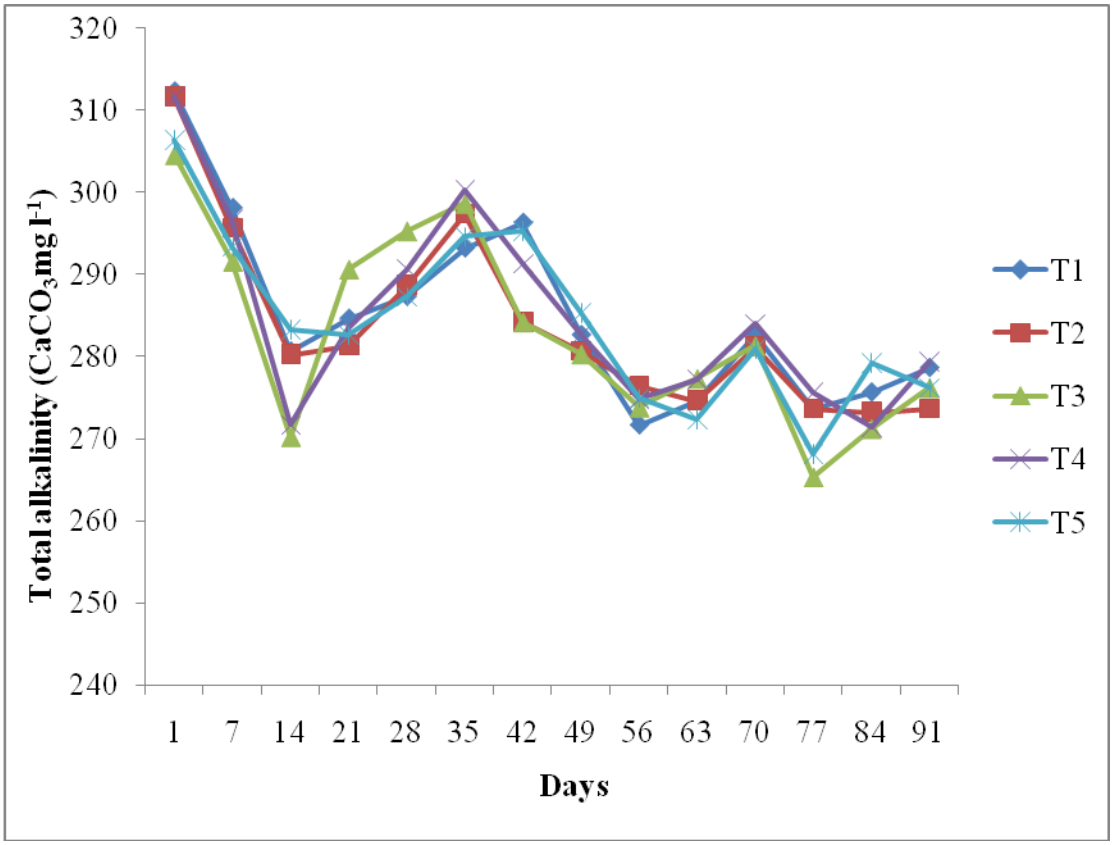

Fig 4: **Changes in total alkalinity ($CaCO_3$mg l^{-1}) in different treatments during the experimental period.**

Alkalinity is the measure of total concentration of bases in pond water including carbonates, bicarbonates, and hydroxides ions along with phosphate, calcium, magnesium and other elementsin the water,such elements provide a good buffering capacity to the diurnal pH changes.It is the sum of phenolphthalein and methyl orange alkalinity,one is due to carbonates and hydroxyl ions and called phenolphthalein alkalinity, while another one is due to bicarbonates and called methyl orange alkalinity.

Water having total alkalinity less than 50 $CaCO_3$ mg l^{-1} is poor in buffering capacity and less productive. Total alkalinity between 50- 300 $CaCO_3$ 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 $CaCO_3$ mg l^{-1}. Bhatnagar and Devi (2013) also suggested the total alkalinity 50 -200 $CaCO_3$ mg l^{-1}; as an acceptable range for fish culture. Stone and Thomforde (2004) suggested an acceptable range as above 20 mg l^{-1} and less than 400 mg l^{-1} for pond fish culture.In present study, total alkalinity in different treatments was recorded well within the recommended range.

4.1.5 Phenolphthalein alkalinity

Phenolphthalein alkalinity ($CaCO_3$ mg l^{-1}) in all treatments (T_1 to T_5) ranged in-between 13.36 – 20.96; during the experimental period and the differences were non-significant ($P \leq 0.05$). In between treatments, phenolphthalein alkalinity in water ranged between 13.36 – 20.72, 14.43 – 20.96, 13.43 – 20.93, 14.83 – 20.92 and 14.46 – 20.86 in treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 8 & Fig. 5).

4.1.6 Methyl orange alkalinity

Methyl orange alkalinity ($CaCO_3$mg l^{-1}), in all treatments (T_1 to T_5) ranged in-between 248.63 – 295.20 during the experimental periodand the differences were insignificant ($P \leq 0.05$). In between treatments, methyl orange alkalinity in water ranged between 250.93 – 295.20, 255.03 – 293.76, 248.63 – 285.80, 253.46– 293.40 and 251.21 – 288.83 in treatments T_1, T_2, T_3,T_4 and T_5, respectively (Table 9 & Fig. 6). Although, some significant($P \leq 0.05$) changes observed within the treatments during the experimental period, but differences with respect to mean methyl orange alkalinity values among different treatments were insignificant ($P \leq 0.05$). The values of methyl orange continuously declined w.r.t. progress of season towards winter season.

Table 8: Water phenolphthalin alkalinity ($CaCO_3$mg l^{-1}) in different treatments during the experimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	17.13^{a}±0.37	17.90^{a}±0.41	18.20^{a}±0.20	18.26^{a}±0.32	17.16^{a}±0.17
	7	15.13^{a}±0.37	15.90^{ab}±0.41	16.20^{a}±0.20	15.26^{a}±0.32	15.16^{a}±0.17
	14	13.36^{a}±0.13	14.43^{a}±0.24	13.43^{a}±0.20	14.83^{a}±0.40	14.60^{a}±0.40
	21	20.46^{a}±0.47	20.73^{a}±0.32	19.96^{a}±0.59	20.92^{a}±0.08	20.52^{a}±0.40
September	28	16.86^{a}±1.56	18.33^{a}±0.90	18.53^{a}±0.50	19.56^{a}±0.32	16.70^{a}±0.55
	35	18.22^{a}±0.20	16.86^{a}±0.34	17.50^{a}±0.34	16.80^{a}±0.37	17.26^{a}±0.89
	42	17.16^{a}±0.52	18.20^{a}±0.20	17.26^{a}±0.32	18.16^{a}±0.17	18.36^{a}±0.13
	49	14.43^{a}±0.24	15.43^{a}±0.20	14.83^{a}±0.40	15.60^{a}±0.40	14.46^{a}±0.47
October	56	20.72^{a}±0.32	20.96^{a}±0.59	20.93^{a}±0.08	20.53^{a}±0.40	20.86^{a}±1.56
	63	18.33^{a}±0.90	18.53^{a}±0.50	18.56^{a}±0.32	18.70^{a}±0.55	18.00^{a}±0.20
	70	16.86^{a}±0.34	17.50^{a}±0.34	16.80^{a}±0.37	16.60^{a}±0.40	17.16^{a}±0.52
	77	16.53^{a}±0.50	16.56^{a}±0.32	16.70^{a}±0.55	16.46^{a}±0.20	16.86^{a}±0.34
November	84	16.40^{a}±0.34	16.60^{a}±0.37	16.20^{a}±0.40	16.10^{a}±0.52	16.23^{a}±0.40
	91	16.50^{a}±0.34	16.80^{a}±0.37	16.60^{a}±0.40	16.16^{a}±0.52	16.83^{a}±0.40
Mean		17.94^{a}±0.92	17.68^{a}±0.74	17.52^{a}±0.67	17.84^{a}±0.62	17.23^{a}±0.62
Range		13.36±0.13 – 20.72±0.32	14.43±0.24 – 20.96±0.59	13.43±0.20 – 20.93±0.08	14.83±0.40 – 20.92±0.08	14.46±0.47 – 20.86±1.56

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

Table 9: Water methyl orange alkalinity ($CaCO_3$mg l^{-1}) in different treatments during the experimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	295.20^{a}±6.63	293.76^{a}±9.65	285.80^{ab}±5.55	293.40^{a}±10.26	288.83^{ab}±2.42
	7	285.20^{a}±6.63	283.76^{a}±9.65	285.10^{a}±5.55	283.40^{a}±10.26	282.83^{a}±2.42
	14	267.30^{a}±5.82	265.56^{a}±3.06	256.56^{b}±5.66	256.83^{b}±4.26	268.22^{a}±5.51
	21	264.20^{a}±4.15	260.60^{a}±5.87	270.70^{a}±2.85	262.40^{a}±4.14	261.13^{a}±2.73
September	28	270.46^{a}±5.21	270.33^{a}±4.71	276.80^{a}±2.43	271.10^{a}±6.90	270.63^{a}±2.29
	35	275.02^{a}±4.76	280.46^{a}±3.38	281.16^{a}±5.02	283.20^{a}±2.81	277.40^{a}±0.79
	42	278.16^{ab}±4.67	265.80^{ab}±4.20	266.73^{ab}±5.54	273.16^{ab}±3.60	276.96^{a}±3.58
	49	278.23^{a}±3.56	275.23^{a}±7.71	261.50^{b}±1.50	277.06^{a}±2.78	271.86^{a}±2.58
October	56	250.93^{a}±3.37	255.03^{a}±2.57	252.06^{a}±2.00	253.46^{a}±2.68	254.80^{a}±4.28
	63	256.33^{a}±3.23	256.13^{a}±1.90	258.78^{a}±2.25	258.50^{a}±1.09	254.44^{a}±1.43
	70	265.80^{a}±3.15	263.70^{a}±2.83	264.53^{a}±3.75	267.40^{a}±2.08	263.83^{a}±2.13
	77	255.13^{a}±2.65	257.10^{a}±1.72	248.63^{a}±6.07	259.66^{a}±1.59	251.21^{a}±3.81
November	84	262.76^{a}±1.68	257.81^{ab}±2.45	259.22^{ab}±2.73	263.95^{a}±4.30	259.76^{ab}±1.55
	91	262.16^{a}±1.68	257.41^{ab}±2.45	259.62^{ab}±2.73	263.25^{a}±4.30	259.16^{ab}±1.55
Mean		263.74^{a}±3.27	265.05^{a}±3.50	261.63^{a}±4.38	261.13^{a}±3.00	265.82^{a}±3.44
Range		250.93±3.37 – 295.20±6.63	255.03±2.57 - 293.76±9.65	248.63±6.07 – 285.80±5.55	253.46±2.68– 293.40±10.26	251.21±3.81– 288.83±2.42

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

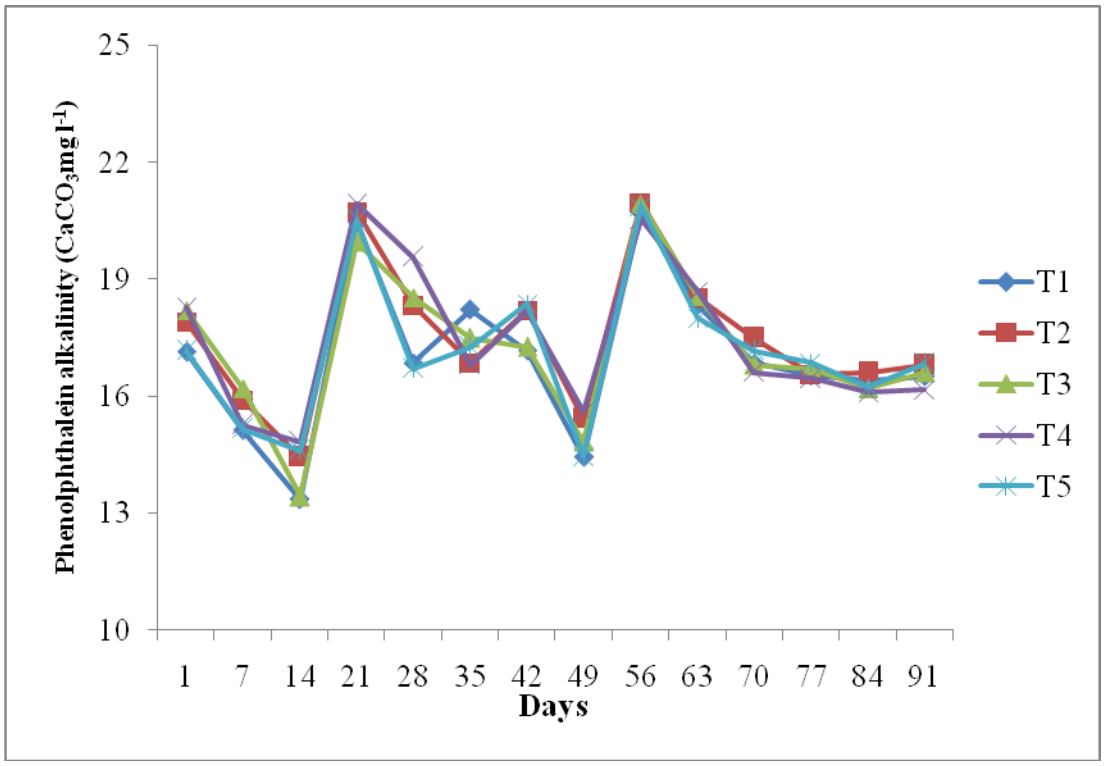

Fig 5: Changes in water phenolphthalein alkalinity ($CaCO_3$mg l^{-1}) in different treatments during the experimental period.

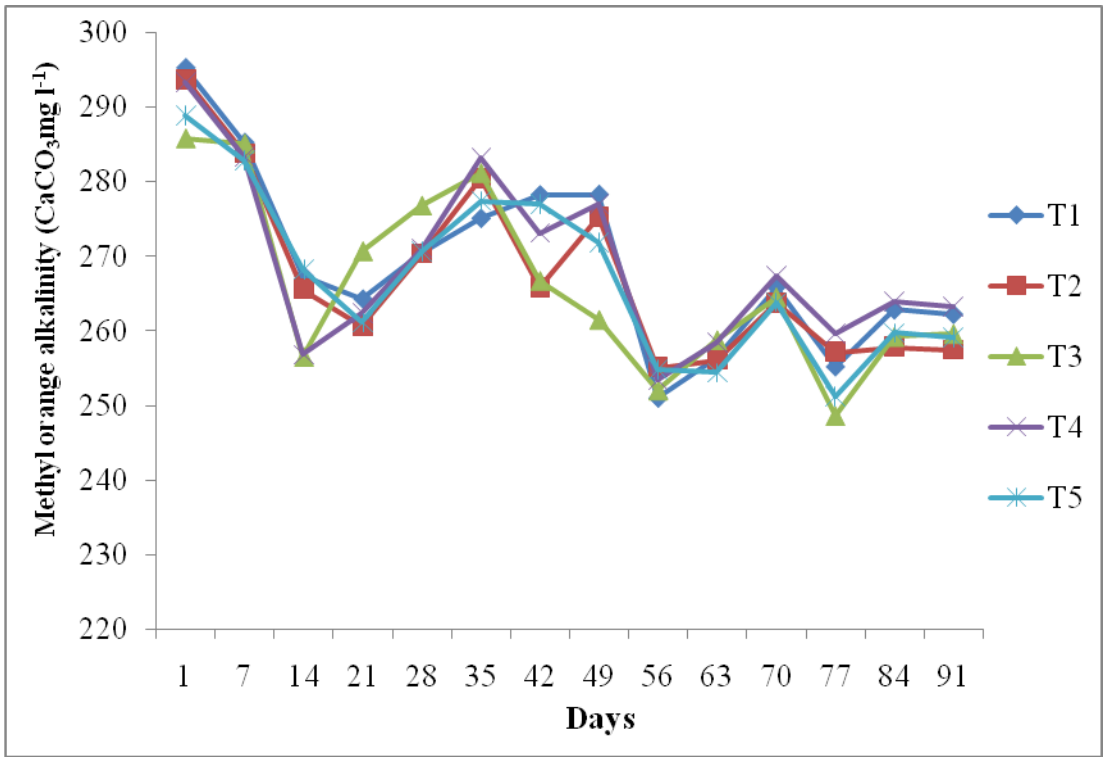

Fig 6: Changes in water methyl orange alkalinity ($CaCO_3$mg l^{-1}) in differenttreatments during the experimental period.

4.1.7 Total hardness ($CaCO_3$)

Total hardness ($CaCO_3$ mg l^{-1}) in all treatments (T_1 to T_5) ranged in-between 276.33 – 319.33 during the experimental periodand the differences were insignificant. The reason behind no significant difference in total hardness of water temperature may be due use of same underground water in all treatments, which might contain same level of dissolved solids particularly calcium and magnesium. In between treatments, total hardness in water ranged between 279.66 – 312.66, 276.33 – 319.33, 278.66 – 314.20, 280.04 – 315.33 and 285.04 – 317.66 in treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 10 & Fig. 7). The values of total hardness continuously declined w.r.t. progress of season towards winter season.

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 $CaCO_3$ mg l^{-1}, while Stone and Thomforde (2004) proposed the desirable range of hardness in between 50- 150 $CaCO_3$ mg l^{-1}. Bhatnagar and Devi (2013) suggested the hardness >300 $CaCO_3$ mg l^{-1} as stressed conditions for fish during culture. In present investigation, values estimated for the total hardness record sometimes slightly higher than the suggested values for fish rearing but most of the time period, values were within favorable range.No ill effect of high concentration of hardness on fish; in terms of behavioral change was recorded during the experimental period and fish survived well in such water during experiment period.

Table 10: Water total hardness ($CaCO_3$mg l^{-1}) in different treatments during theexperimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	312.66^{a}±3.48	319.33^{a}±2.96	314.20^{a}±7.0	315.33^{a}±6.0	317.66^{a}±7.21
	7	302.66^{a}±3.48	309.33^{a}±2.96	304.20^{a}±7.0	305.33^{a}±6.0	309.66^{a}±7.21
	14	305.33^{a}±2.90	310.02^{a}±5.29	306.02^{a}±2.88	308.06^{a}±3.05	307.02^{a}±4.04
	21	299.12^{ab}±4.04	292.33^{b}±3.52	288.04^{b}±6.00	288.04^{b}±4.05	308.02^{a}±1.15
September	28	306.33^{a}±2.40	302.02^{a}±4.16	302.02^{a}±6.35	308.02^{a}±1.15	304.02^{a}±3.05
	35	298.22^{a}±3.46	296.66^{a}±4.66	299.33^{a}±5.69	300.02^{a}±2.90	297.86^{a}±7.21
	42	279.66^{a}±16.47	276.33^{a}±3.48	284.66^{a}±0.66	280.04^{a}±2.30	291.02^{a}±6.02
	49	286.02^{a}±9.01	286.08^{a}±5.03	290.02^{a}±2.90	300.04^{a}±1.76	294.02^{a}±2.40
October	56	290.06^{a}±3.46	294.66^{a}±2.40	294.04^{a}±5.03	291.33^{a}±2.90	291.33^{a}±1.76
	63	280.33^{a}±3.84	287.02^{a}±1.15	278.66^{a}±1.76	282.33^{a}±2.84	287.02^{a}±3.05
	70	288.33^{ab}±0.88	289.33^{ab}±2.18	283.04^{b}±4.50	288.02^{ab}±1.52	294.33^{a}±1.76
	77	282.66^{a}±3.28	287.33^{a}±1.76	288.02^{a}±4.04	289.02^{a}±1.15	285.04^{a}±2.18
November	84	281.66^{a}±2.02	282.33^{a}±1.20	281.37^{a}±2.18	282.33^{a}±2.02	285.33^{a}±0.88
	91	283.66^{a}±2.02	285.33^{a}±1.20	286.37^{a}±2.18	284.33^{a}±2.02	286.33^{a}±0.88
Mean		291.08^{ab}±2.17	293.20^{a}±3.33	295.58^{a}±2.10	297.11^{a}±3.19	296.40^{a}±3.17
Range		279.66±16.47 – 312.66±3.48	276.33±3.48 – 319.33±2.96	278.66±1.76 – 314.20±7.0	280.04±2.30 – 315.33±6.0	285.04±2.18 – 317.66±7.21

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

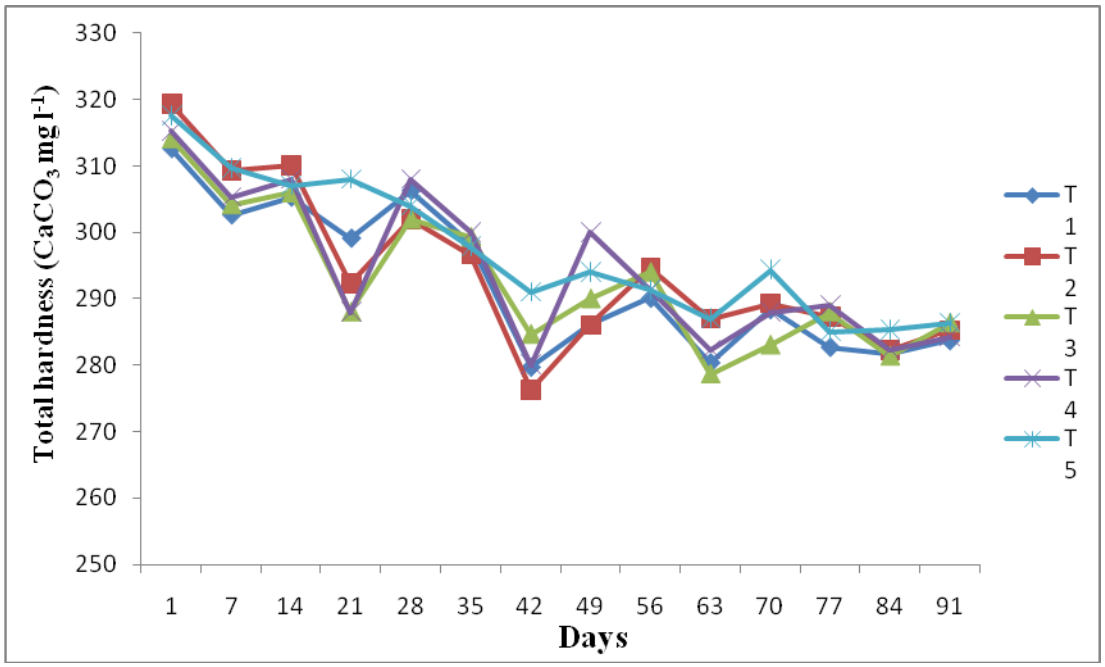

Fig 7: Changes in total hardness ($CaCO_3$mg l^{-1}) of water in different treatments during the experimental period.

4.1.8 Ammonia (NH_4^+)

Ammonia (NH_4^+) (mg l^{-1}) in all treatments (T_1 to T_5) ranged in-between 0.0040 – 0.0077 during the experimental periodand the differences were non-significant ($P \leq 0.05$). In between treatments, ammonia concentration in water ranged between 0.0040 – 0.0075, 0.0045 – 0.0074, 0.0041 – 0.0074, 0.0047 – 0.0077 and 0.0042 – 0.0072 in treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 11 & Fig. 8).

Ammonia (NH_3) is produced by fish respiration and by the decomposition of waste products (excessive organic matter and excessive feeding). It can be present as two forms: highly soluble toxic unionized ammonia (NH_3), or the less dangerous ammonium ion (NH_4^+). The levels of free ammonia can be influenced by pH, temperature and salinity. However, the pH of water is the most important factor that determines the ratio of NH_3 and NH_4^+. When the pH is high, more of the ammonia is in its toxic form. During present study, concentration of ammonia (NH_4^+) recorded very low than the toxic level, however concentration of ammonia (NH_4^+) recorded significantly ($P \leq 0.05$) higher in T4 and T1, which may be possibly due to either excess excretory matter (feed consumption) or decomposition of unutilized feed along with non-conversion of ammonia into nitrate and nitrite.

Table 11: Water ammonia (mg l^{-1}) in different treatments during the experimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	0.0055^{a}±0.002	0.0054^{a}±0.001	0.0054^{a}±0.0004	0.0057^{a}±0.0006	0.0056^{a}±0.017
	7	0.0055^{a}±0.002	0.0054^{a}±0.001	0.0064^{a}±0.0004	0.0067^{a}±0.0006	0.0066^{a}±0.017
	14	0.0051^{a}±0.001	0.0050^{b}±0.001	0.0043^{a}±0.0007	0.0056^{a}±0.0006	0.0053^{a}±0.0008
	21	0.0061^{a}±0.001	0.0046^{a}±0.0008	0.0058^{a}±0.0004	0.0061^{a}±0.0006	0.0047^{a}±0.0008
September	28	0.0064^{a}±0.001	0.0050^{a}±0.0008	0.0060^{a}±0.0003	0.0053^{a}±0.0005	0.0059^{a}±0.0004
	35	0.0067^{a}±0.001	0.0053^{a}±0.0007	0.0060^{a}±0.0004	0.0059^{a}±0.0005	0.0064^{a}±0.0006
	42	0.0066^{a}±0.001	0.0061^{a}±0.0006	0.0058^{a}±0.0007	0.0060^{a}±0.0002	0.0063^{a}±0.0006
	49	0.0075^{a}±0.0002	0.0074^{a}±0.0002	0.0074^{a}±0.0001	0.0077^{a}±0.0001	0.0072^{a}±0.0001
October	56	0.0052^{a}±0.0006	0.0046^{a}±0.0009	0.0052^{a}±0.0008	0.0052^{a}±0.0003	0.0052^{a}±0.0007
	63	0.0056^{a}±0.0009	0.0056^{a}±0.0006	0.0050^{a}±0.0008	0.0053^{a}±0.0007	0.0042^{a}±0.0006
	70	0.0069^{a}±0.0001	0.0061^{b}±0.0009	0.0071^{a}±0.0001	0.0064^{a}±0.0002	0.0065^{a}±0.0001
	77	0.0040^{a}±0.0009	0.0045^{a}±0.0002	0.0041^{a}±0.0002	0.0048^{a}±0.0001	0.0045^{a}±0.0002
November	84	0.0041^{a}±0.0001	0.0048^{a}±0.0002	0.0045^{a}±0.0001	0.0047^{a}±0.0002	0.0046^{a}±0.0001
	91	0.0049^{a}±0.0001	0.0048^{a}±0.0002	0.0043^{a}±0.0001	0.0047^{a}±0.0002	0.0046^{a}±0.0001
Mean		0.0056^{a}±0.0003	0.0054^{a}±0.0003	0.0057^{a}±0.0003	0.0053^{a}±0.0003	0.0055^{a}±0.0003
Range		0.0040±0.0009 – 0.0075±0.0002	0.0045±0.0002 – 0.0074±0.0002	0.0041±0.0002 - 0.0074±0.0001	0.0047±0.0002 – 0.0077±0.0001	0.0042±0.0006 – 0.0072±0.0001

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

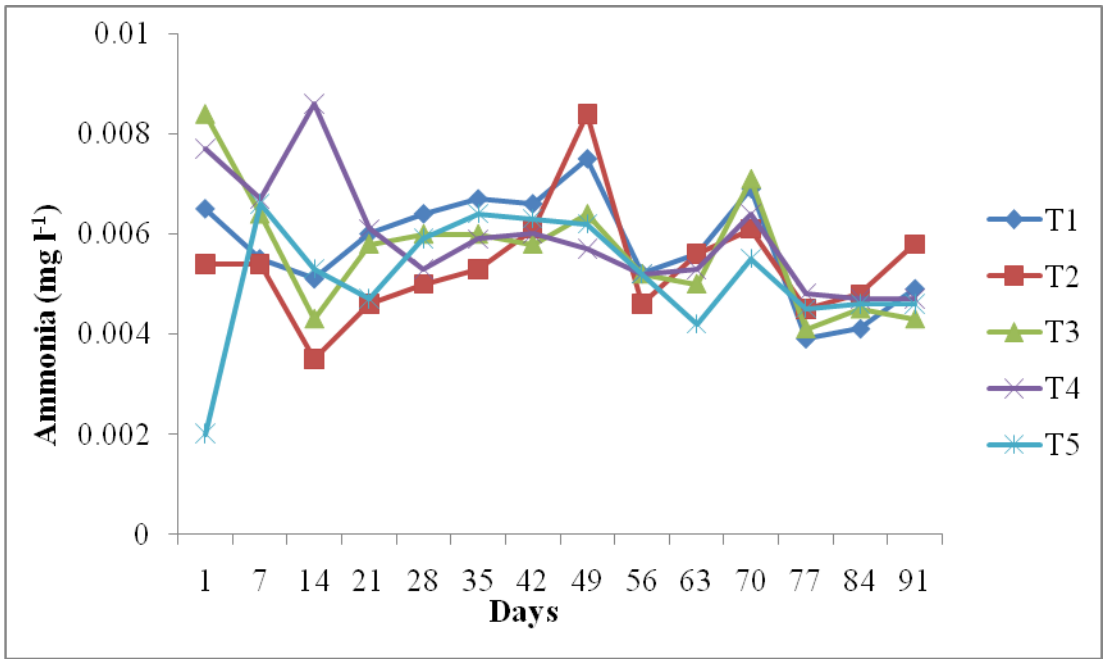

Fig 8: Changes in ammonia (mg l^{-1}) of water in different treatments during the experimental period.

4.1.9 Nitrite

Nitrite (mg l^{-1}) in all treatments (T_1 to T_5) ranged in-between 0.0232 – 0.0698 during the experimental periodand the differences were non-significant ($P \leq 0.05$). In between treatments, nitrite in water ranged between 0.0281 – 0.0682, 0.0243 – 0.0690, 0.0232 – 0.0698, 0.0241 – 0.0666 and 0.0285 – 0.0696 in treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 12 & Fig. 9).

Nitrite is a toxic compound produced from ammonia during the first step of nitrification. The toxicity of nitrite is dependent on chloride concentration and the acutely toxic concentration varies from less than 1 mg l^{-1} to 10 or more, depending on chloride. Under certain conditions it can be elevated even when ammonia is not, so it should be measured, as well. Nitrite is measured by a colorimetric assay that is altered by chloride. Stone and Thomforde (2004) suggested that the desirable range of nitrite 0-1 mg l^{-1} NO2 and acceptable range <4 mg l^{-1} NO2. Santhosh and Singh (2001) recommended nitrite concentration in water should not exceed 0.5 mg l^{-1}, while OATA (2008) proposed that its limit should not exceed 0.2 mg l^{-1} in fresh water. In present study, nitrite concentration recorded well below the toxic level as suggested by different workers, throughout experiment period.

Table 12: Water Nitrite (mg l^{-1}) in different treatments during the experimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	0.0291^{a}±0.009	0.0263^{a}±0.052	0.0315^{a}±0.059	0.0271^{a}±0.010	0.0365^{a}±0.066
	7	0.0281^{a}±0.009	0.0243^{a}±0.052	0.0305^{a}±0.059	0.0241^{a}±0.010	0.0315^{a}±0.066
	14	0.0343^{a}±0.005	0.037^{a}±0.008	0.0232^{a}±0.002	0.0282^{a}±0.009	0.0285^{a}±0.0004
	21	0.0369^{a}±0.003	0.0382^{a}±0.003	0.0332^{a}±0.006	0.0396^{a}±0.003	0.0430^{a}±0.003
September	28	0.0379^{a}±0.002	0.0463^{a}±0.002	0.0464^{a}±0.002	0.0404^{a}±0.0008	0.0403^{a}±0.005
	35	0.0414^{ab}±0.002	0.0485^{a}±0.002	0.0463^{a}±0.002	0.0445^{ab}±0.002	0.0472^{ab}±0.002
	42	0.0527^{a}±0.003	0.0553^{a}±0.005	0.0408^{a}±0.010	0.0477^{a}±0.003	0.0471^{a}±0.001
	49	0.0679±0.004	0.0654^{a}±0.001	0.0698^{a}±0.0005	0.0666^{a}±0.002	0.0696^{a}±0.0005
October	56	0.0682^{a}±0.012	0.0690^{a}±0.003	0.0512^{ab}±0.003	0.0552^{ab}±0.006	0.0664^{a}±0.001
	63	0.0557^{a}±0.0003	0.0587^{a}±0.0003	0.0577^{a}±0.001	0.0587^{a}±0.001	0.0570^{a}±0.002
	70	0.0453^{a}±0.0008	0.0453^{a}±0.002	0.0433^{a}±0.002	0.0423^{a}±0.0008	0.0453^{a}±0.001
	77	0.0450^{a}±0.003	0.0410^{a}±0.001	0.0457^{a}±0.002	0.0430^{a}±0.002	0.0393^{a}±0.001
November	84	0.0453^{a}±0.0008	0.0450^{a}±0.001	0.0458^{a}±0.002	0.0442^{a}±0.002	0.0439^{a}±0.003
	91	0.0423^{a}±0.0008	0.0430^{a}±0.001	0.0460^{a}±0.002	0.0440^{a}±0.002	0.0440^{a}±0.003
Mean		0.045^{a}±0.002	0.045^{a}±0.004	0.047^{a}±0.004	0.046^{a}±0.003	0.044^{a}±0.002
Range		0.0281±0.009 – 0.0682±0.012	0.0243±0.05- 0.0690±0.003	0.0232±0.002 – 0.0698±0.0005	0.0241±0.010 – 0.0666±0.002	0.0285±0.0004– 0.0696±0.0005

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

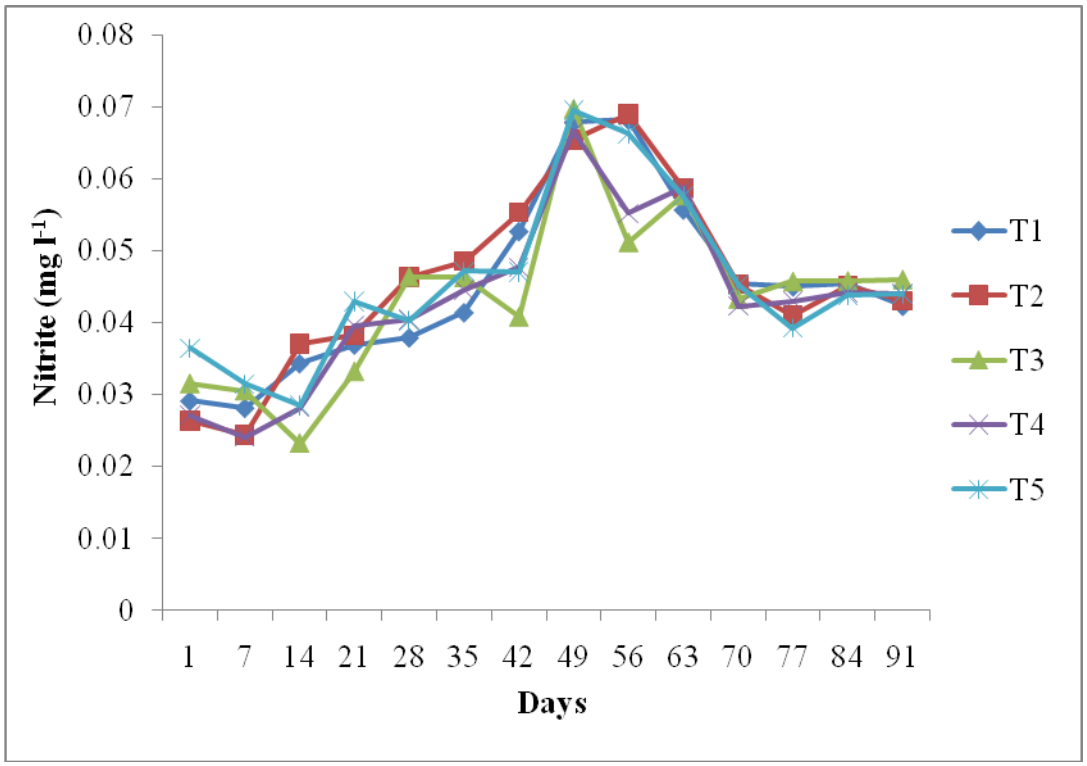

Fig 9: Changes in nitrite (mg l^{-1}) of water in different treatments during the experimental period.

4.1.10 Orthophosphate

Orthophosphate (mg l^{-1}) in all treatments (T1 to T5) ranged in-between 0.0301 to 0.0597 during the experimental periodand the differenceswere in-significant ($P \leq 0.05$). In between treatments, orthophosphatein water ranged between0.0301 – 0.0597,0.0307 – 0.0597,0.0302 – 0.0593, 0.0308 – 0.0593 and 0.0309 –0.0597 in treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 13 & Fig. 10). Orthophosphate concentration slightly declined and then its level increased and became stagnant at ≅ 0.059mg l^{-1} at the time of termination.The concentration of orthophosphate recorded continuously increasing with the progress of experiment but nosignificant ($P \leq 0.05$) effect on water quality recorded due to incorporation of fish silage to replace soybean protein.

Stone and Thomforde (2004) suggested that 0.06 mg l^{-1} orthophosphate is desirable for fish culture, while Bhatngar *et al* (2004) recommended 0.05-0.7 mg l^{-1} as optimum and productive for fish culture.In present study, orthophosphate concentration recorded well below the toxic level as suggested by different workers, throughout experiment period.

Table 13: Water orthophosphate (mg l^{-1}) in different treatments during the experimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	0.0386^{a}±0.006	0.0391^{a}±0.008	0.0382^{a}±0.008	0.0398^{a}±0.007	0.0388^{a}±0.009
	7	0.0326^{a}±0.006	0.0321^{a}±0.008	0.0322^{a}±0.008	0.0328^{a}±0.007	0.0328^{a}±0.009
	14	0.0301^{a}±0.004	0.0307^{a}±0.006	0.0302^{a}±0.01	0.0308^{a}±0.01	0.0309^{a}±0.0009
	21	0.0418^{a}±0.005	0.0416^{a}±0.003	0.0417^{a}±0.012	0.0411^{a}±0.011	0.0417^{a}±0.002
September	28	0.0461^{a}±0.006	0.0443^{a}±4.001	0.0449^{a}±0.003	0.0451^{a}±0.001	0.0455^{a}±0.004
	35	0.0504^{a}±0.003	0.0505^{a}±0.004	0.0508^{a}±0.005	0.0504^{a}±0.003	0.0505^{a}±0.005
	42	0.0510^{a}±0.002	0.0518^{a}±0.004	0.0513^{a}±0.005	0.0510^{a}±0.005	0.0513^{a}±0.003
	49	0.0564^{a}±0.007	0.0563^{a}±0.008	0.0566^{a}±0.003	0.0567^{a}±0.002	0.0565^{a}±0.0006
October	56	0.0581^{a}±0.008	0.0587^{a}±0.007	0.0574^{a}±0.002	0.0572^{a}±0.003	0.0584^{a}±0.004
	63	0.0593^{a}±0.005	0.0597^{a}±0.002	0.0597^{a}±0.001	0.0593^{a}±0.005	0.0597^{a}±0.003
	70	0.0583^{a}±0.006	0.0583^{a}±0.001	0.0583^{a}±0.001	0.0580^{a}±0.003	0.0584^{a}±0.0005
	77	0.0590^{a}±0.008	0.0593^{a}±0.001	0.0593^{a}±0.002	0.0590^{a}±0.001	0.0594^{a}±0.002
November	84	0.0590^{a}±0.008	0.0591^{a}±0.001	0.0593^{a}±0.001	0.0593^{a}±0.001	0.0590^{a}±0.002
	91	0.0597^{a}±0.008	0.0595^{a}±0.001	0.0593^{a}±0.001	0.0593^{a}±0.001	0.0590^{a}±0.002
Mean		0.051^{ab}±0.003	0.052^{ab}±0.001	0.059^{a}±0.001	0.058^{a}±0.001	0.059^{a}±0.001
Range		0.0301±0.004 – 0.0597±0.008	0.0307±0.006– 0.0597±0.002	0.0302±0.01 – 0.0593±0.001	0.0308±0.01 – 0.0593±0.001	0.0309±0.0009 – 0.0597±0.003

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

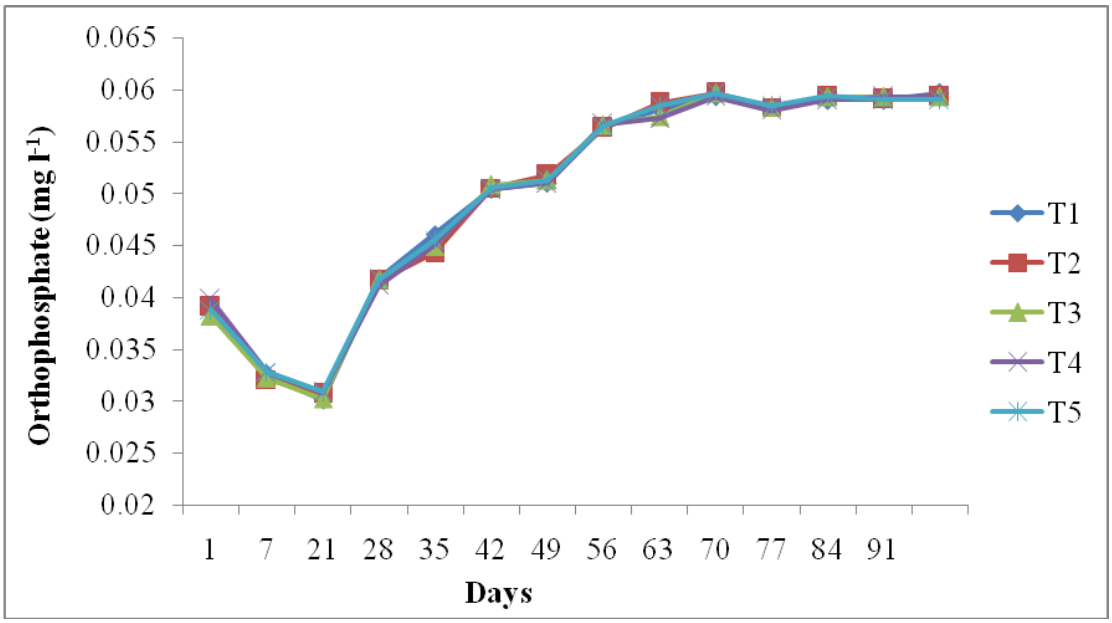

Fig 10: Changes in orthophosphate (mg l^{-1}) of water in different treatments during the experimental period.

4.2 Survival and growth of fish

4.2.1 Fish survival

During the experimental period, survival (%) of fish recorded 100% in all treatments (T_1 to T_5) (Table 14) at the completion of experiment. Survival rate is one of the most important indicators of favorable conditions for organism w.r.t. environmental condition and food availability.

4.2.2 Growth

The growth in fish stocked during experimental period was assessed in terms of gain innet body length(NBL, cm) and net body weight (NBW, g). At the end of experiment, net body length gain (NBLG), net weight gain (NWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish for each treatment was calculated to evaluate performance of silage incorporated diets mixed in different ratio.

4.2.3 Body length

At the end of experiment, NBLG (cm) in all treatments (T_1 to T_5) ranged between 2.77 to 5.20 during the experimental period. Total body length gain recorded 2.77, 3.89, 4.26, 5.20 and 4.98in treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 14 & Fig.11) and differences among the treatments were significant ($T_4=T_5>T_3=T_2>T_1$) ($P \leq 0.05$). Maximum gain in net length recorded in T_4, while minimum in T_1.

Table 14: Net body length gain (cm) in fish during the experimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August, 2016	1	6.74^{a}±0.24	6.61^{a}±0.18	6.63^{a}±0.10	6.64^{a}±0.06	6.63^{a}±0.26
	15	6.92^{a}±0.14	6.88^{a}±0.11	6.91^{a}±0.13	7.18^{a}±0.12	6.99^{a}±0.11
September	30	7.21^{b}±0.17	7.27^{b}±0.10	7.15^{b}±0.12	7.77^{a}±0.15	7.52^{a}±0.08
	45	7.48^{b}±0.04	7.57^{b}±0.12	7.34^{b}±0.16	8.58^{a}±0.19	8.77^{a}±0.14
October	60	7.71^{c}±0.17	8.20^{b}±0.19	8.57^{b}±0.17	9.64^{a}±0.08	9.28^{a}±0.26
	75	7.98^{c}±0.13	9.04^{b}±0.17	9.59^{b}±0.16	10.70^{a}±0.10	10.20^{a}±0.29
November	90	9.80^{c}±0.20	10.50^{b}±0.11	10.89^{b}±0.04	11.84^{a}±0.05	11.61^{a}±0.05
TBLG*		2.77^{c}±0.05	3.89^{b}±0.08	4.26^{b}±0.06	5.20^{a}±0.09	4.98^{a}±0.13

*NBLG= Net body length gain

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

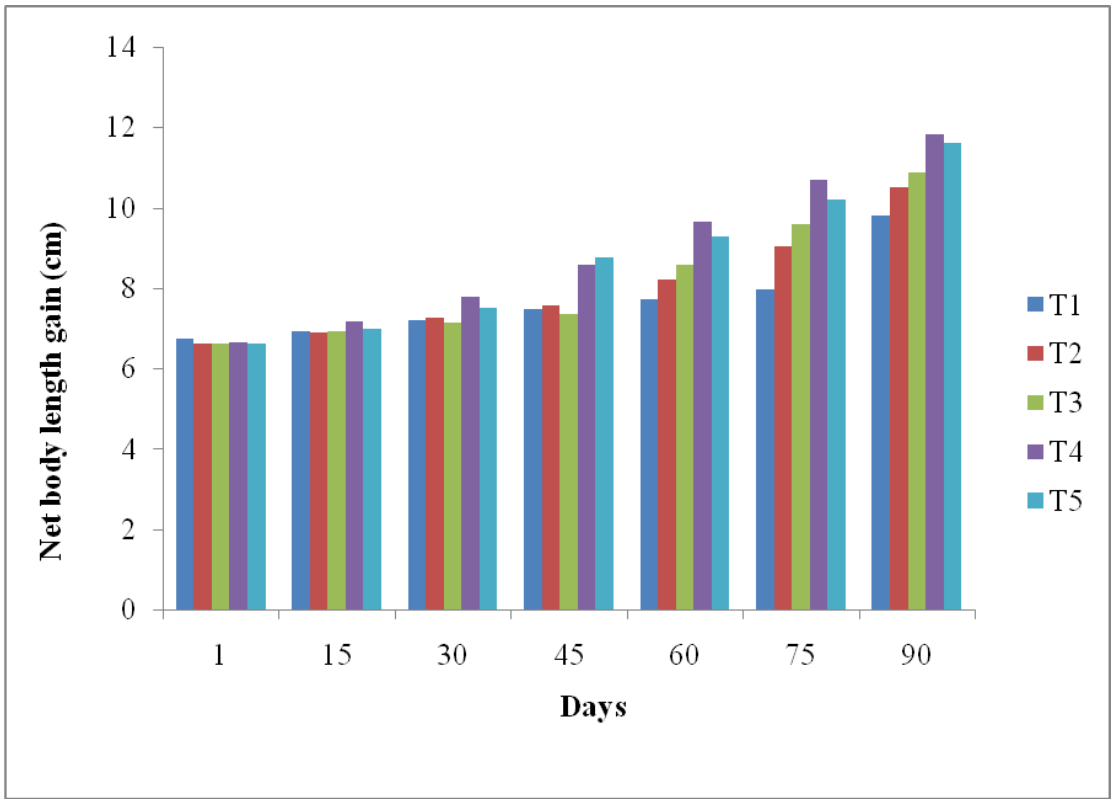

Fig 11: Changes in body length gain (cm) in fish in different treatments during the experimental period.

4.2.4 Body weight

At the end of experiment, net weight gain (g), in all treatments (T_1 to T_5) ranged in-between 3.06 to 5.54 during the experimental period. Net weight gainrecorded 3.06, 4.09, 4.65, 5.54 and 5.28 g in treatments T_1, T_2, T_3, $T_4$and T_5, respectively (Table 15 & Fig.12,13) and the differences among the treatments were significant ($T_4=T_5>T_3=T_2>T_1$) ($P \leq 0.05$). Changes in body weight showed the same pattern as changes recorded in net body length gain.

4.2.5 Specific growth rate (SGR)

Specific growth rate in all the treatments (T_1 to T_5) ranged in-between 0.56 to 0.77. Specific growth rate recorded 0.56, 0.61, 0.68, 0.77 and 0.73 in treatments T_1, T_2, T_3, T_4 and T_5,respectively (Table 15 & Fig. 14) and the differences among treatments were significant ($T_4=T_5\geq T_3>T_2\geq T_1$) ($P \leq 0.05$).

Table 15: Body weight (g) and growth parameters of fish in different treatments during the experimental period (Mean±SE)

Month	Days	Treatments				
		T_1	T_2	T_3	T_4	T_5
August	1	5.36^{a}±0.08	5.49^{a}±0.17	5.45^{a}±0.17	5.44^{a}±0.25	5.41^{a}±0.03
	15	5.58^{b}±0.09	5.83^{a}±0.12	5.90^{a}±0.14	5.88^{a}±0.29	5.98^{a}±0.21
September	30	6.04^{b}±0.12	6.17^{b}±0.05	6.39^{ab}±0.22	6.99^{a}±0.49	6.74^{a}±0.14
	45	6.30^{b}±0.11	6.80^{ab}±0.08	7.33^{a}±0.33	7.54^{a}±0.58	7.42^{a}±0.25
October	60	6.57^{b}±0.56	7.55^{ab}±0.22	8.14^{a}±0.55	8.43^{a}±0.33	8.24^{a}±0.32
	75	7.26^{b}±0.72	8.49^{b}±0.27	9.23^{ab}±0.23	9.92^{a}±0.41	9.71^{a}±0.42
November	90	8.42^{b}±0.13	9.58^{b}±0.09	10.10^{a}±0.16	10.98^{a}±0.04	10.30^{a}±0.15
NWG		3.06^{c}±0.07	4.09^{b}±0.04	4.65^{b}±0.18	5.54^{a}±0.21	5.28^{a}±0.15
Survival %		100%	100%	100%	100%	100%
SGR		0.56^{bc}±0.01	0.61^{b}±0.01	0.68^{ab}±0.03	0.77^{a}±0.03	0.73^{a}±0.01
FCR		2.70^{a}±0.02	2.57^{a}±0.03	2.42^{ab}±0.06	2.22^{b}±0.08	2.27^{b}±0.03
PER		0.60^{b}±0.01	0.67^{a}±0.006	0.71^{a}±0.008	0.66^{a}±0.005	0.64^{ab}±0.003

*Values with different alphabetical superscripts differ significantly within a row ($P\leq0.05$)

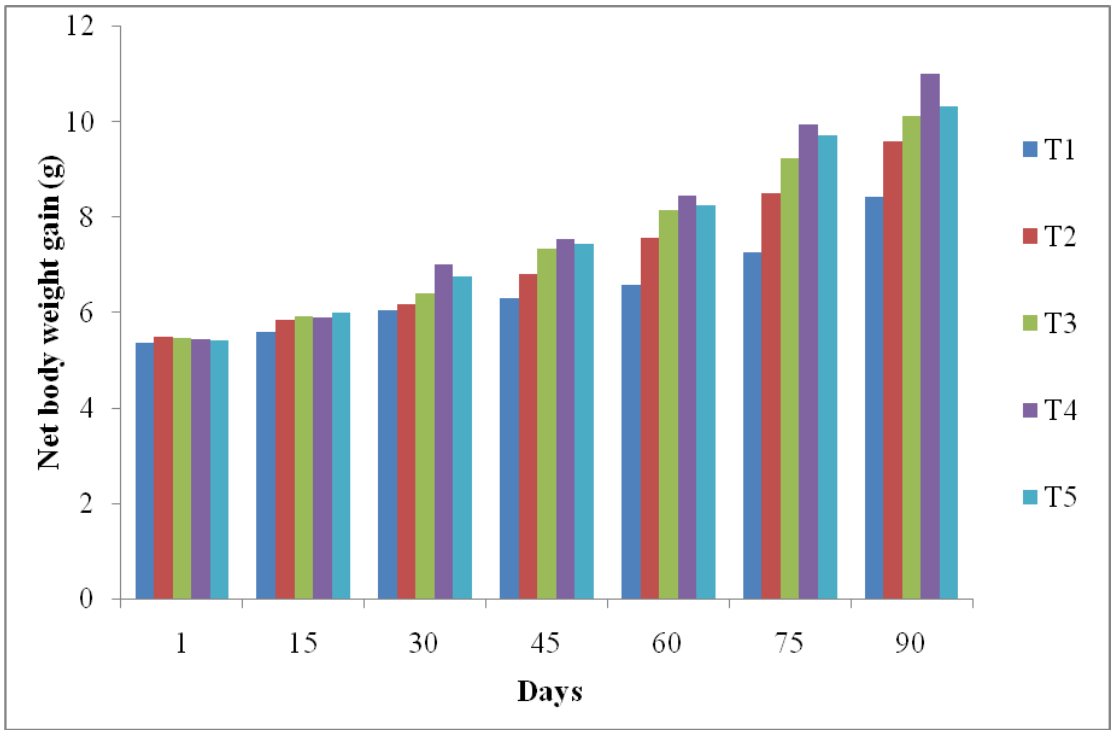

Fig 12: Changes in fish net body weight gain (g) in different treatments during the experimental period.

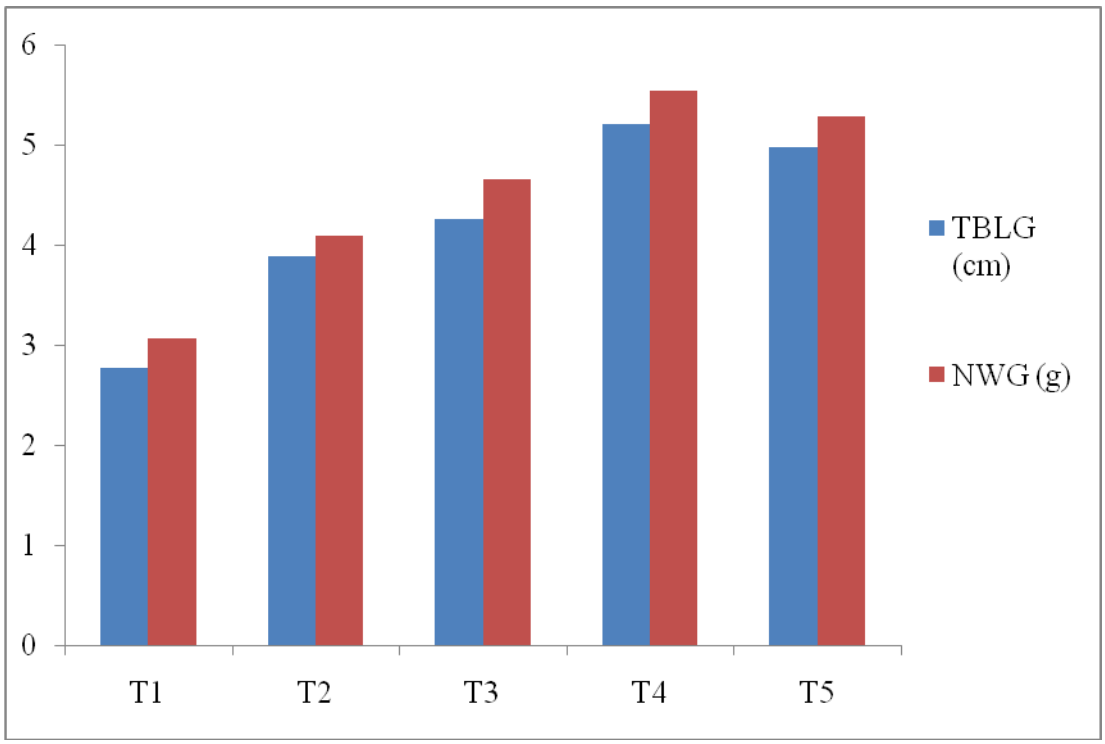

Fig 13: Changes in net weight gain (NWG) and net body length gain (TBLG)in different treatments during the experimental period.

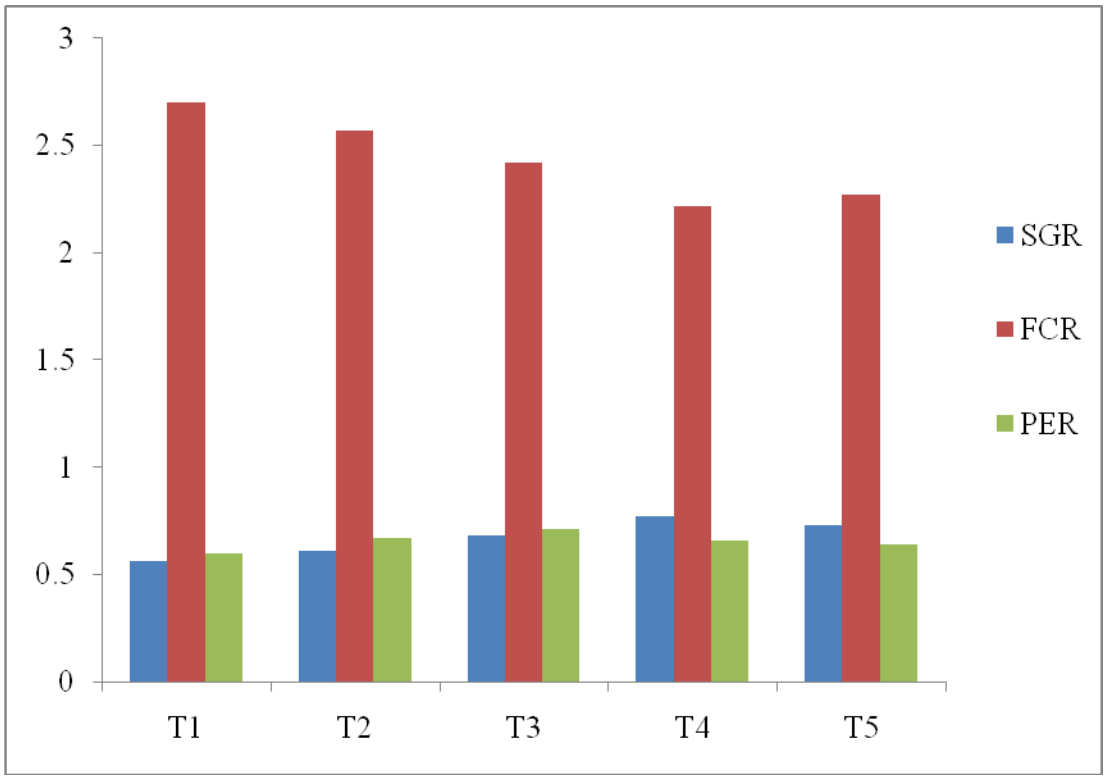

Fig 14: Changes in specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) in different treatments during the experimental period.

4.2.6 Feed conversion ratio (FCR)

Feed conversion ratio in all the treatments (T_1 to T_5) ranged between 2.22 to 2.70. Feed conversion ration recorded 2.70, 2.57, 2.42, 2.22 and 2.27 in treatments T_1, T_2, T_3, T_4 and T_5, respectively (Table 15 & Fig. 14) and differences among treatments were significant ($T_1=T_2\geq T_3>T_5\geq T_4$) ($P \leq 0.05$).

4.2.7 Protein efficiency ratio (PER)

Protein efficiency rate in all the treatments (T_1 to T_5) ranged in-between 0.60 to 0.71. Protein efficiency ratio recorded 0.60, 0.67, 0.71, 0.66 and 0.64 in treatments T_1, T_2, T_3, $T_4$and T_5,respectively (Table 15 & Fig. 14) and differences among treatments were significant ($T_3=T_2=T_4\geq T_5>T_1$) (($P \leq 0.05$).

Specific growth rate expresses the percent body weight gain per day, SGR '0' indicates animal is no longer gaining or losing weight and is referred as maintenance feeding (De Silva and Anderson 2009). 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 (Teugels 1982, Kiaalvandi *et al* 2011), while PER expresses the efficiency of protein utilization.

In present study, maximum value for NBLG, NWG and SGR recorded in T_4, while the values recorded minimum in T_1, which gives an indication that the replacement of soy protein with 45% silage protein can be done easily. Feed conversion ratio recorded best in T_4as ratiorecorded minimum, while poorest conversion ratio recorded in T_1, it gives an indication that it may be best combination of different w.r.t. nutrition and digestibility, Protein efficiency ratio recorded best in T_3, followed by T_2 and minimum in T_1, it may be possibly due to optimum protein requirement is 31 – 33%, more than this level is not as efficiently assimilates in body as in T_3 and T_2, however is difficult say that excess protein than required is the only reason for this. In contrast to PER, maximum net weight gain in T_4 followed by T_5 gives an indication that protein is not only factor for growth but lipid and carbohydrate level in diet also play significant role in growth. Paulraj (1997) suggested that in common carp temperature and metabolizable energy affects the protein requirement for promoting growth. Paul and Giri (2015) suggested that excess protein in diet w.r.t. desirable levels does not absorb and accumulate efficiently hence goes as waste and also causes stress to fish, similarly excess energy is known to induce lipogenesis thus necessitating a balance between protein and energy in diet formulation. Addition of animal source of protein improves the FCR due to high biological value (BV) and digestibility, incorporation of protein content in diets more than desirable levels reduces its efficacy hence declines the PER value. In present investigation low PER in T_4 and T_5 as compared to T_3 and T_2 may be due to higher levels of protein than required level.

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 – 6.25FCR 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 – 0.87 SGR, 2.27 – 3.01 FCR and 0.79 – 1.05 PER in juveniles of common carp, when fed with 25-35% CP diet. AL-Faraje (2000) reported SGR values of 0.788-1.098 and FCR 4.5-7.5 with total replacement of animal protein with lactic acid

fermented fish silage in feed of common carp fingerling. Ramasubburayn *et al* (2017) reported SGR values between 1.06-1.49 for common carp fingerlings, when fed with diets with silage incorporation @ 2, 2.5 and 3%, containing 40%CP.

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. The main objective of biochemical composition evaluation in fish flesh was to observe the effect of varied levels of protein and other nutrients on fish flesh composition.

4.3.1 Moisture content

At the termination of experiment, moisture content (%) in fish flesh among treatments (T_1 to T_5) ranged in-between 78.20 to 81.43. Moisture content recorded 81.32, 81.43, 80.44, 78.20 and 78.40 in treatments T_1, T_2, T_3, T_4 and T_5, respectively, while moisture content in flesh recorded 83.43, at the time of stocking (Table 16& Fig. 15). The differences among the treatments with respect to moisture content were significant (Initial=T_2=T_1> T_3=T_5=T_4) ($P \leq 0.05$). Moisture content in fish flesh declined with the progress of experiment.

4.3.2 Proteins

Protein content (%) in fish flesh in all treatments (T_1 to T_5) ranged in-between 13.90 to 16.50. Protein content recorded 13.90, 14.10, 14.20, 16.50 and 16.01 in treatments T_1, T_2, T_3, T_4 and T_5, respectively. Protein content in fish flesh recorded 12.66 at the time of stocking (Table 16& Fig. 16). The differences among the treatments, with respect to protein content were significant (T_4=T_5>T_3=T_2=T_1>Initial) ($P \leq 0.05$).

4.3.3 Total lipids

Total lipid content (%) in fish flesh in all treatments (T_1 to T_5) ranged in-between 1.60 to 2.50. Lipid content in fish flesh recorded 1.60, 1.83, 1.90, 2.10 and 2.50 in treatments T_1, T_2, T_3, T_4 and T_5, respectively. Lipid content in fleshrecorded 1.33 at the time of stocking (Table 16 & Fig. 17). The differences among the treatments, with respect to lipid content were significant (T_5=T_4>T_3=T_2=T_1=Initial) ($P \leq 0.05$).

4.3.4 Ash

Ash content (%) in fish flesh in all treatments (T_1 to T_5) ranged in-between 1.06 to 1.60. Ash content recorded 1.06, 1.20, 1.20, 1.60 and 1.23 in treatments T_1, T_2,

T_3, T_4 and T_5, respectively.Ash content in fish fleshrecorded 1.06 at the time of stocking (Table 16 & Fig. 18). The differences among the treatments, with respect to ash content were significant ($T_4>T_5=T_2=T_3>T_1$=Initial) ($P \leq 0.05$).

4.3.4 Crude Fiber

Crude fiber (%) in fish flesh in all treatments (T_1 to T_5) ranged in-between 0.30 to 0.36. Crude fiber content recorded 0.30, 0.33, 0.32, 0.36 and 0.30 in treatments T_1, T_2, T_3, T_4 and T_5, respectively. Crude fiber content in fish flesh recorded 0.38 at the time of stocking (Table 16 & Fig. 19). The differences among the treatments, with respect to crude fiber content were significant (Initial=$T_4>T_2=T_3=T_1=T_5$) ($P \leq 0.05$).

4.3.5 Total carbohydrate as NFE

Total carbohydrate as NFE in fish flesh in all treatments (T_1 to T_5) ranged in-between 1.10 to 1.50. Total carbohydrate as NFE content recorded 1.20, 1.10, 1.50, 1.20 and 1.40 in treatments T_1, T_2, T_3, T_4 and T_5, respectively. Total carbohydrate as NFE content in fish fleshrecorded 1.16 at the time of stocking (Table 16 & Fig. 20). The differences among the treatments, with respect to Total carbohydrate as NFE content were significant ($T_3=T_5>T_1=T_4$=Initial=T_2) ($P \leq 0.05$).

Table 16: Biochemical composition (%) of common carp flesh (Mean±SE)

Parameters (%)	Initial	T_1	T_2	T_3	T_4	T_5
Moisture	83.43^{a}±0.68	81.32^{a}±0.28	81.43^{a}±0.12	80.44^{b}±0.05	78.20^{b}±0.05	78.40^{b}±0.05
Proteins	12.66^{c}±0.08	13.90^{b}±0.05	14.10^{b}±0.05	14.20^{b}±0.05	16.50^{a}±0.05	16.01^{a}±0.05
Lipids	1.33^{b}±0.23	1.60^{b}±0.05	1.83^{b}±0.06	1.90^{b}±0.05	2.10^{a}±0.05	2.50^{a}±0.05
Ash	1.06^{c}±0.03	1.06^{c}±0.08	1.20^{b}±0.05	1.20^{b}±0.05	1.60^{a}±0.05	1.23^{b}±0.03
Crude fiber	0.38^{a}±0.03	0.30^{b}±0.05	0.33^{b}±0.05	0.32^{b}±0.05	0.36^{a}±0.05	0.30^{b}±0.05
NFE	1.16^{b}±0.09	1.20^{b}±0.05	1.10^{b}±0.05	1.50^{a}±0.05	1.20^{b}±0.05	1.40^{a}±0.05

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

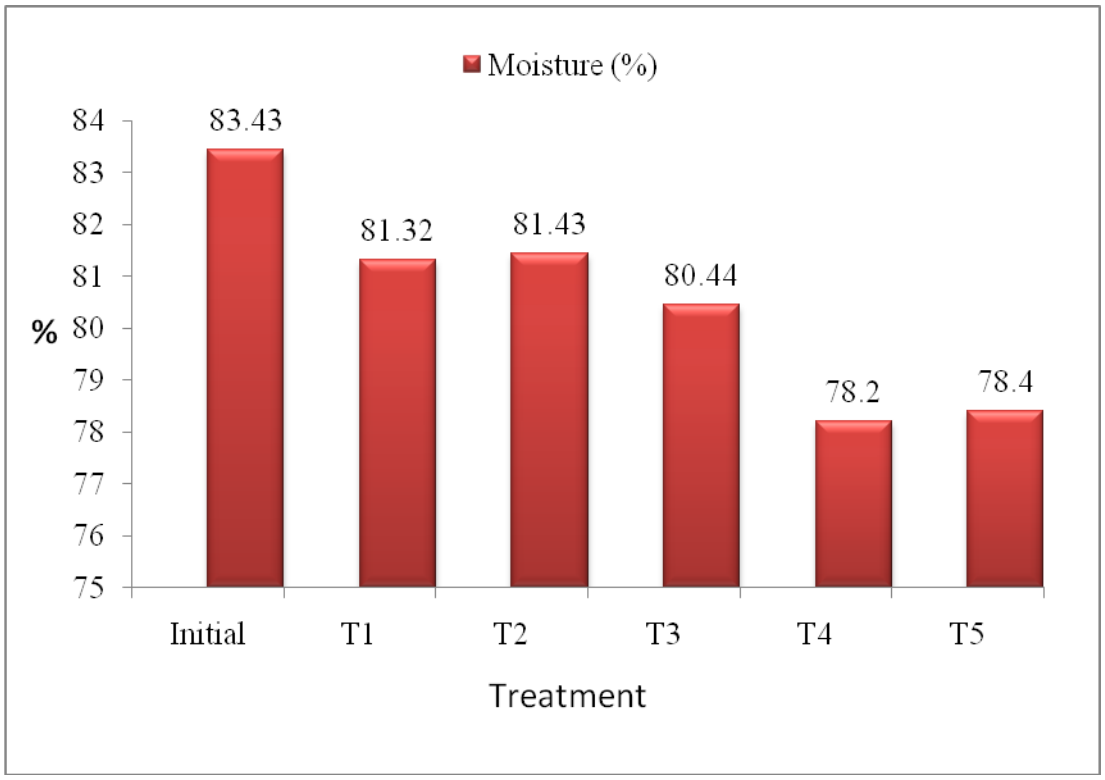

Fig 15: Flesh moisture content (%) of fish in different treatments during experimental period.

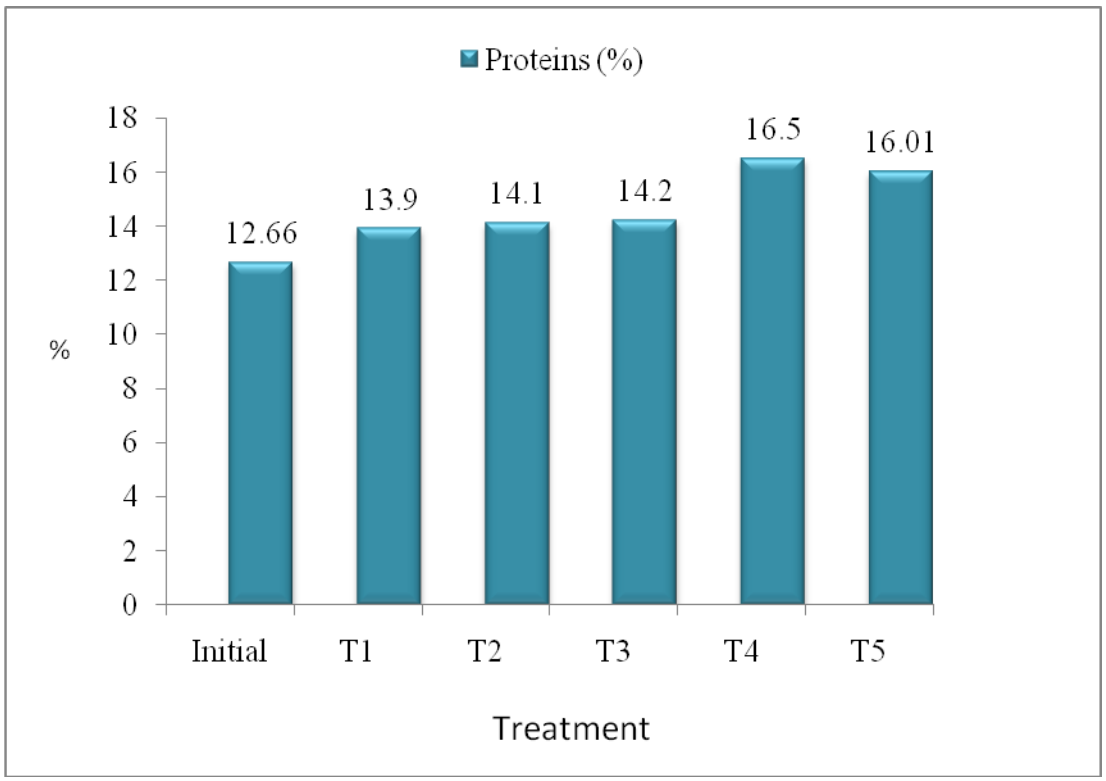

Fig 16: Flesh total protein (%) content (on dry wt. basis) of fish in different treatments during experimental period.

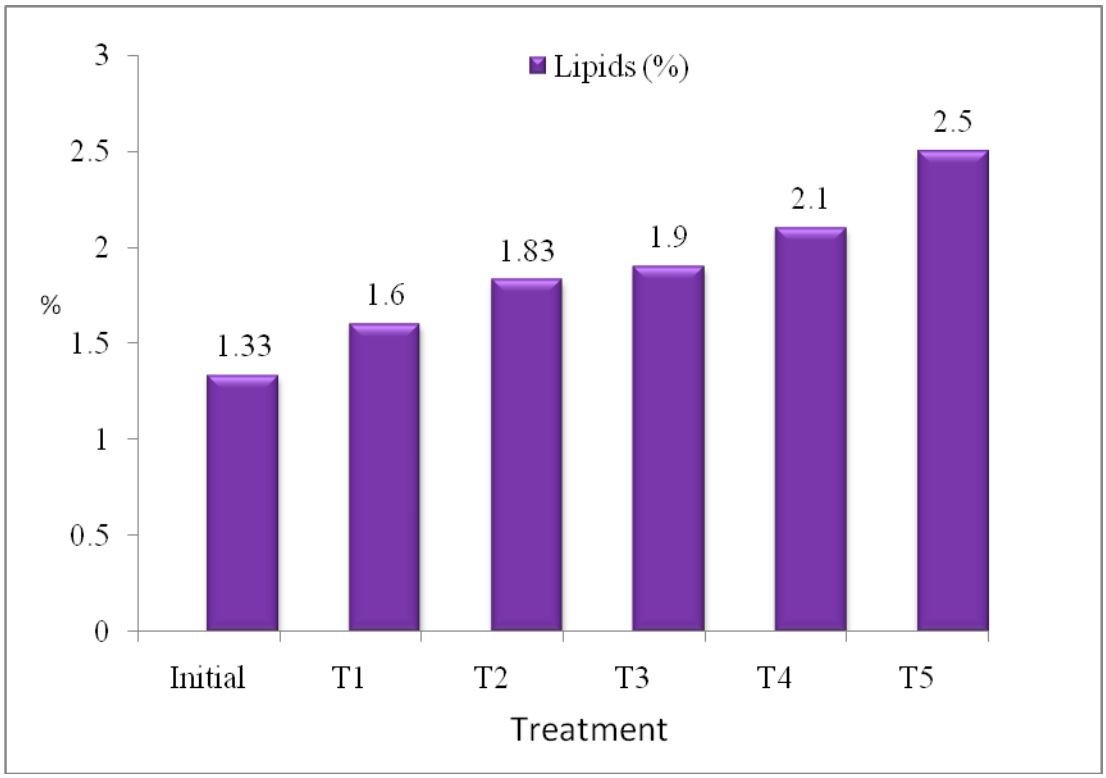

Fig 17: Flesh total lipid (%) content (on dry wt. basis) of fish in different treatments during experimental period.

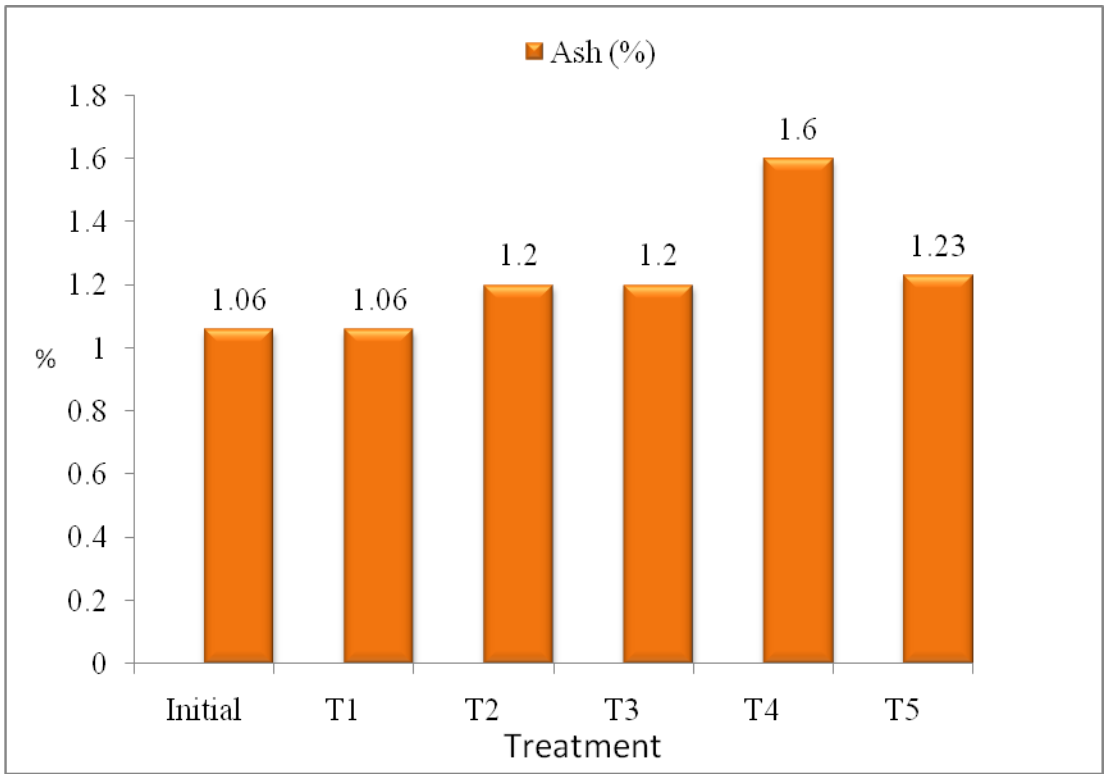

Fig 18: Flesh ash (%) content (on dry wt. basis) of fish in different treatments during experimental period.

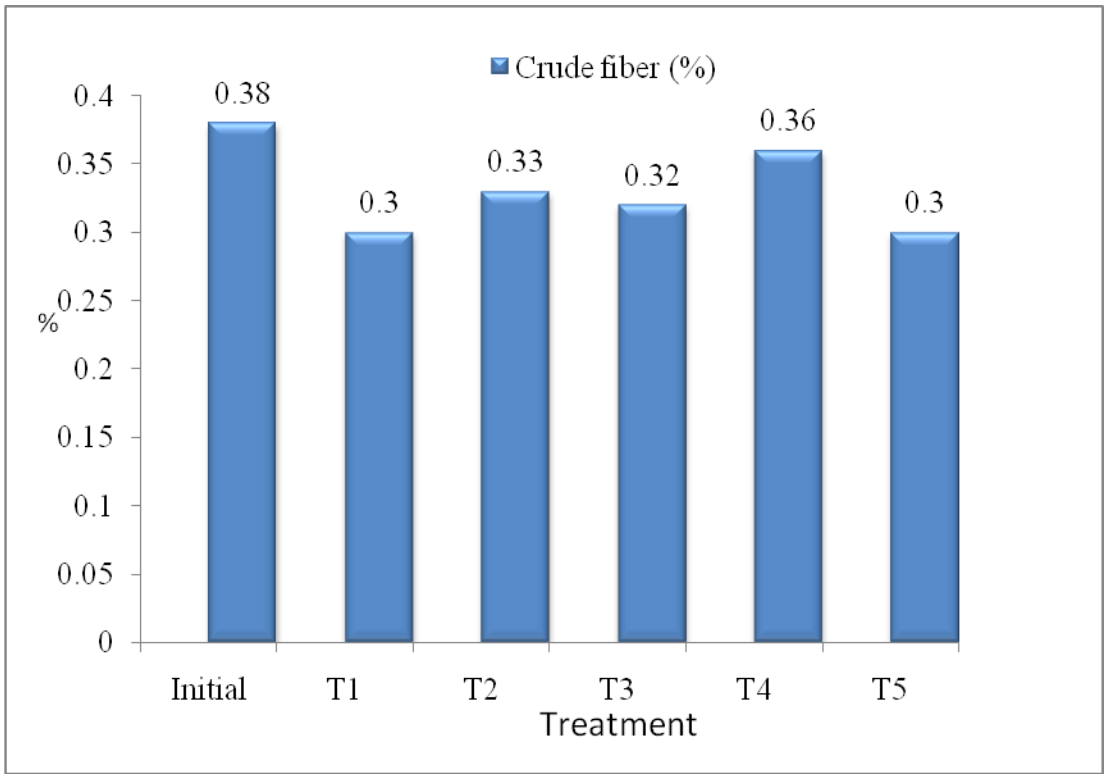

Fig 19: Flesh crude fiber (%) content (on dry wt. basis) of fish in different treatments during experimental period.

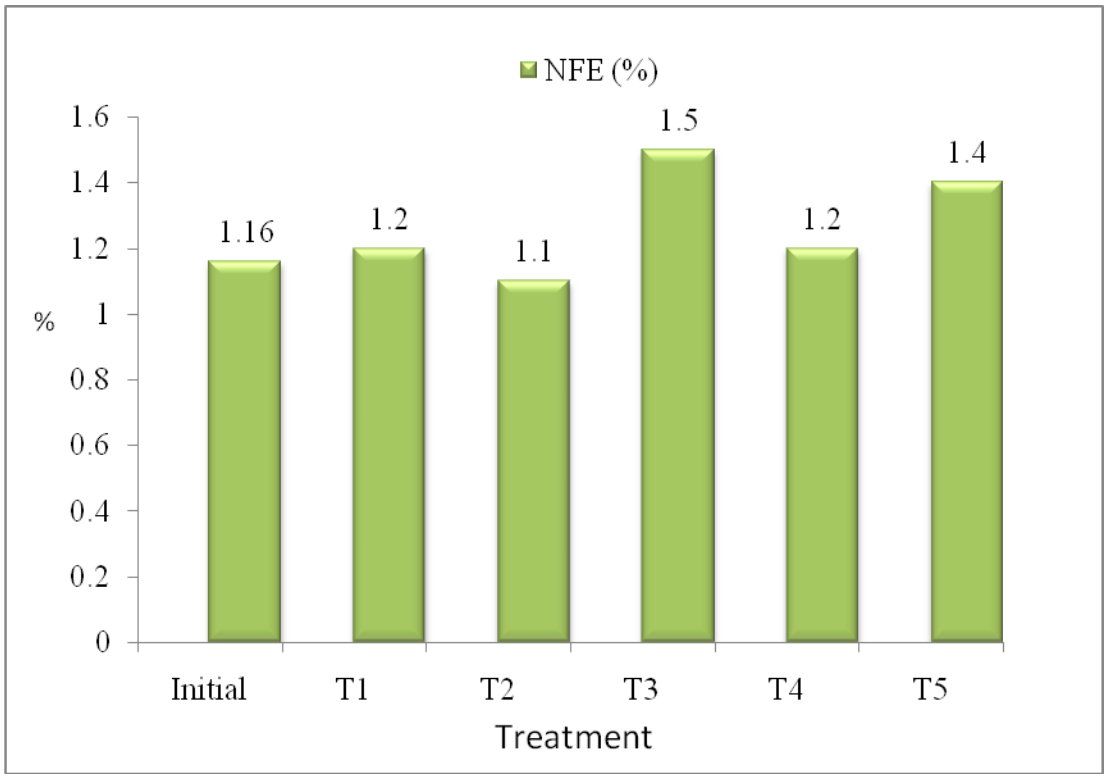

Fig 20: Flesh total carbohydrates (%) content (on dry wt. basis) of fish in different treatments during experimental period.

In general, the biochemical composition of the whole body indicates the nutritive value of animal; including fish. The percentage composition of the four major constituents of fish viz. water, protein, lipid and ash (minerals) is referred as proximate composition. These four components account for about 96-98% of total tissue constituents in most cases. The principle constituents are water (66 – 84%), protein (15 – 24%), lipids (0.1 –22%), minerals (0.8 – 2%) and carbohydrate in very minute quantity (0.3%) in most of the fishes (Jacquot 1961).

Kaur (2016) observed the moisture content 77.2 – 82.4, protein content 13.60 – 16.7, lipid content 1.75– 2.73 and ash content 1.40 – 2.61, when fed with diet containing 35 – 39% protein in fingerlings of common carp (*C. carpio*).

In present study, protein content was significantly improved when fed with diets replacing 45% (T_4) soybean meal with fish silage protein.

Conclusion

Partial replacement of plant origin soybean with animal protein in the form of fish silage; prepared from waste portion have not bring any significant change in water quality, hence if such silage incorporated diets will used to feed the fishes, no additional/ extra efforts will be required, other than existing pond water management practices to manage water quality. Partial replacement of soybean with silage protein improved the FCR, hence such fish silage incorporated feed will bring reduction in the quantum of feed required. To maintain PER at optimum levels, diets should be prepared with optimum levels of protein, moreover combination from different sources of plant and animal origin. It will help to improve the nutritional value, balance of amino acids. In present case, replacement of soybean source of protein with fish silage @ 45% is found optimum from FCR point of view, however PER value declined but it was non-significant.

Feeding common carp with artificial diets containing high levels of protein will improve the functional property as food. Addition of silage prepared from fish waste will help to mitigate the problem of waste disposal, reduction in cost of feed and will help to maintain environment clean.

CHAPTER V

SUMMARY

The study was conducted for the evaluation of fish silage as a potential source for the replacement of soybean meal in feed of Common carp (*Cyprinus carpio*, L.) fingerlings. The experiment was carried out in FRP tanks (1.5×1.0×0.75m) with five diets including one control (T_1) and four experimental diets (T_2 to T_7). All experimental diets were prepared by mixing fish silage, soybean meal, rice bran (38%), mustard oil cake (30%), carboxy methyl cellulose (2%), mineral mixture (2%) and vitamin mixture (2%). T_1 i.e. control diet was prepared without adding fish silage. T_2having 3.9 and 22.1 mg kg^{-1}, T_3having7.8 and 18.2 mg kg^{-1}, T_4 having 11.7 and 14.3 mg kg^{-1} and D_5 having 15.6 and 10.4 mg kg^{-1} of fish silage and soybean respectively.

5.1Physico-chemical parameters of water

Water quality parameters recorded at weekly intervals. The water quality parameters viz. temperature, pH, dissolved oxygen, total alkalinity, phenolphthalein alkalinity, methyl orange alkalinity, total hardness, ammonia, nitrite and orthophosphate contents varied in the range 19.26– 29.63 ^{0}C, 8.23– 9.13, 7.60 – 9.65 mg l^{-1}, 265.33 – 312.33 $CaCO_3$ mg l^{-1},13.36– 20.96 $CaCO_3$ mg l^{-1}, 248.63 – 295.20 $CaCO_3$ mg l^{-1}, 276.33 – 319.33 $CaCO_3$ mg l^{-1}, 0.0040 – 0.0077 mg l^{-1}, 0.0232– 0.0698 mg $l^{-1}$and 0.0301 mg l^{-1} – 0.0597 mg l^{-1} in the different treatments (T_1–T_5), during experimental period. Water quality parameters recorded during the experimental period fluctuated within the favorable range recommended for ideal the growth of common carp. No significant difference among different physico-chemical parameters observed during the experimental period in between different treatments.

5.2 Survival and growth of fish

During the experimental period survival (%) of fish is 100% in all treatments (T_1 to T_5). In different treatments with fish silage supplemented diets with respect to control, the net gain in body length (cm) varied between 2.77 to 5.20during the experimental period. In between treatments, net gain in body length were 2.77, 3.89, 4.26, 5.20 and 4.98 in treatments T_1, T_2, T_3, $T_4$and T_5,respectively and differences among the treatments were significant (T_4=T_5>T_3=T_2> T_1) ($P \leq 0.05$). Net weight gain

(g), in all treatments (T_1 to T_5) ranged in-between 3.06 to 5.54 during the experimental period. NWG recorded 3.06, 4.09, 4.65, 5.54 and 5.28 g in treatments T_1, T_2, T_3, T_4 and T_5, respectively and the differences among the treatments were significant ($T_4=T_5>T_3=T_2>T_1$) ($P \leq 0.05$).

Specific growth rate in all the treatments (T_1 to T_5) ranged in-between 0.56 to 0.77. Specific growth rate recorded 0.56, 0.61, 0.68, 0.77 and 0.73 in treatments T_1, T_2, T_3, T_4 and T_5, respectively and the differences among treatments were significant ($T_4=T_5 \geq T_3>T_2 \geq T_1$) ($P \leq 0.05$). FCR in all the treatments (T_1 to T_5) ranged in-between 2.22 to 2.70. Feed conversion ration recorded 2.70, 2.57, 2.42, 2.22 and 2.27 in treatments T_1, T_2, T_3, T_4 and T_5, respectively and differences among treatments were significant ($T_1=T_2 \geq T_3>T_5 \geq T_4$) ($P \leq 0.05$). PER in all the treatments (T_1 to T_5) ranged in between 0.60 to 0.71. Protein efficiency ratio recorded 0.60, 0.67, 0.71, 0.66 and 0.64 in treatments T_1, T_2, T_3, T_4 and T_5, respectively and differences among treatments were significant ($T_3=T_2=T_4 \geq T_5>T_1$) (($P \leq 0.05$).

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 in treatments (T_1 to T_5) ranged in-between 78.20 to 81.43. Moisture content recorded 81.32, 81.43, 80.44, 78.20 and 78.40 in treatments T_1, T_2, T_3, T_4 and T_5, respectively, while moisture content in flesh recorded 83.43 at the time of stocking. The differences among the treatments with respect to moisture content were significant (Initial=$T_2=T_1>T_3=T_5=T_4$) ($P \leq 0.05$). Protein (%) in fish flesh in all treatments (T_1 to T_5) was ranged in-between 13.90 to 16.50. Protein content recorded 13.90, 14.10, 14.20, 16.50 and 16.01 in treatments T_1, T_2, T_3, T_4 and T_5, respectively. Flesh protein content in flesh recorded 12.66 at the time of stocking. The differences among the treatments, with respect to protein content were significant ($T_4=T_5>T_3=T_2=T_1>$Initial) ($P \leq 0.05$). Total lipid content (%) in fish flesh in all treatments (T_1 to T_5) ranged in-between 1.60 to 2.50. Lipid content recorded 1.60, 1.83, 1.90, 2.10 and 2.50 in treatments T_1, T_2, T_3, T_4 and T_5, respectively. Flesh lipid content of fish recorded 1.33 at the time of stocking. The differences among the treatments, with respect to lipid content were significant ($T_5=T_4>T_3=T_2=T_1=$Initial) ($P \leq 0.05$). Ash content (%) in fish flesh in all treatments (T_1 to T_5) ranged in-between

1.06 to 1.60. Ash content recorded 1.06, 1.20, 1.20, 1.60 and 1.23 in treatments T_1, T_2, T_3, T_4 and T_5, respectively. Flesh ash content of fish recorded 1.06 at the time of stocking. The differences among the treatments, with respect to ash content were significant ($T_4>T_5=T_2=T_3>T_1$=Initial) ($P \leq 0.05$). Crude fiber in fish flesh in all treatments (T_1 to T_5) ranged in-between 0.30 to 0.36. Crude fiber content recorded 0.30, 0.33, 0.32, 0.36 and 0.30 in treatments T_1, T_2, T_3, T_4 and T_5, respectively. Crude fiber content of fish recorded 0.38 at the time of stocking. The differences among the treatments, with respect to crude fiber content were significant (Initial=$T_4>T_2=T_3=T_1=T_5$) ($P \leq 0.05$). Total carbohydrate as NFE in fish flesh in all treatments (T_1 to T_5) ranged in-between 1.10 to 1.50. Total carbohydrate as NFE content recorded 1.20, 1.10, 1.50, 1.20 and 1.40 in treatments T_1, T_2, T_3, T_4 and T_5, respectively. Total carbohydrate as NFE content of fish recorded 1.16 at the time of stocking. The differences among the treatments, with respect to Total carbohydrate as NFE content were significant ($T_3=T_5>T_1=T_4$=Initial=T_2) ($P \leq 0.05$).

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