GROWTH PERFORMANCE AND MEAT QUALITY OF PANGAS CATFISH (*PANGASIANODON HYPOPHTHALMUS*) FED ON FISH SILAGE AND LINSEED OIL SUPPLEMENTED DIETS

Dissertation

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

DOCTOR OF PHILOSOPHY
in
AQUACULTURE

(Minor Subject: Aquatic Environment Management)

By

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CERTIFICATE I

This is to certify that the dissertation entitled, **"GROWTH PERFORMANCE AND MEAT QUALITY OF PANGAS CATFISH (*PANGASIANODON HYPOPHTHALMUS*) FED ON FISH SILAGE AND LINSEED OIL SUPPLEMENTED DIETS"** submitted for the degree of Ph.D. in the subject of **AQUACULTURE** (Minor Subject: **AQUATIC ENVIRONMENT MANAGEMENT**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Ms. INJEELA KHAN (L-2016-F-01-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

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

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CERTIFICATE II

This is to certify that the dissertation entitled, **"GROWTH PERFORMANCE AND MEAT QUALITY OF PANGAS CATFISH (*PANGASIANODON HYPOPHTHALMUS*) FED ON FISH SILAGE AND LINSEED OIL SUPPLEMENTED DIETS"** submitted by **Ms. INJEELA KHAN (L-2016-F-01-D)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfilment of the requirements for the degree of **Ph.D.** 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 by undertaking two experiments [Experiment I (July – October 2018) and Experiment II (May - September, 2019)] in FRP pools (1.5×1×0.75m) and cemented tanks (80m^2) for pangas fry and fingerling rearing, respectively, by replacing fish meal in control diet (D1) @ 50 and 100% (D2 and D3) and a mixture of groundnut and soybean meal @ 25 and 50% (D4 and D5) with formic acid fermented fish silage. One more experimental diet (D6) was prepared without any animal protein source. During Experiment II, after 120 days of experimental period, all the 6 diets (D1-D6) were supplemented with linseed oil @ 5 % (95% formulated diet + 5 % linseed oil) as finishing diets and fish was fed for one month (September 2019). Both the experiments were conducted in triplicate to study the effect of formulated diets on water quality, fish survival, growth, health status and meat quality. Results revealed that all the water quality parameters remained in optimum range during both the experiments. Fish survival was 93.33 % in D1, 96.66 % in D2, 98.33 % in D3 and 100 % in D4 and D5 and 96.66 % in D6 respectively, during experiment I and 100 % in all the treatments and control during experiment II. Significant changes ($P \leq 0.05$) were observed w.r.to fish growth, haematological (Hb and Ht); biochemical (protein, albumin, globulin, Alb/Glb ratio); antioxidant (SOD, LPO) parameters along with improvement in serum transaminases (AST and ALT), lipid profile (cholesterol, triglycerides, LDL, HDL and VLDL), flesh composition and meat quality of fish products and fillet. Based on the results of present study, Diet D3 (100% replacement of fish meal with fish silage) and D5 (50 % replacement of plant protein sources with fish silage) can be recommended for rearing pangas fry to fingerling to fingerling to grow out, for improved growth and overall health stratus of fish. Further, product (fish fingers) and fillet prepared from fingerlings reared on diet D5 were highly acceptable based on meat quality (biochemical parameters), sensory evaluation and texture analysis.

Key Words: *Pangassius*, fish meal, fish silage, linseed oil, meat quality

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

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LIST OF ABBREVATIONS

%	:	Percentage
@	:	At the rate
A	:	Ash
ALA	:	Alpha Linolenic Acid
ALT	:	Alanine Aminotransferase
ANF	:	Anti- Nutritional Factors
ANOVA	:	Analysis of Variance
AST	:	Aspartate Aminotransferase
ATP	:	Adenosine Triphosphate
Av.	:	Average
BCG	:	Bromocresol Green
BW	:	Body weight
BHT	:	Butylated Hydroxy Toluene
BMR	:	Basal Metabolic Rate
Ca^{2+}	:	Calcium
$CaCO_3$	:	Calcium chloride
cm	:	Centimeter
CO_3^{2-}	:	Carbonate
CE	:	Cholesterol esterase
CP	:	Crude Protein
CVM	:	Chicken Viscera Meal
DHA	:	Docosahexaenoic acid
DIAS	:	Database on Introduction of Aquatic Species
D.O.	:	Dissolve Oxygen
dl	:	Deciliter
EE	:	Ether Extract

EPA	:	Eicosapentaenoic acid
FA	:	Fatty acid
FBW	:	Final Body Weight
FCR	:	Feed Conversion Ratio
FFA	:	Free Fatty acid
FM	:	Fish Meal
FO	:	Fish Oil
FSM	:	Flaxseed Meal
FWM	:	Fish Waste Meal
g	:	Gram
GHO	:	Glycerol Phosphate Oxidase
GO	:	Grape seed Oil
H_2SO_4	:	Sulphuric Acid
ha	:	Hectare
Hb	:	Haemoglobin
Hct	:	Hematocrit
HDL	:	High Density lipids
hr	:	Hour
HUFA	:	Highly Unsaturated Fatty Acid
kg	:	Kilogram
KH	:	Krill Hydrolysate
K-value	:	Condition Factor
l	:	Litre
LDL	:	Low Density lipids
LO	:	Linseed Oil
LPO	:	Lipid Peroxidation
m	:	Meters

MDA	:	Malonodialdehyde
MFW	:	Mean Final Weight
mg	:	Milligram
Mg^{2+}	:	Magnesium
min.	:	Minute
ml	:	Millilitre
mm	:	Millimetre
mmol	:	Millimole
mmt	:	Million metric ton
NADH	:	Nicotinamide Adenine Dinucleotide
NBT	:	Nitroblue Tetrazolium
NFE	:	Nitrogen Free Extract
NH_3-N	:	Ammonical – Nitrogen
NO_2-N	:	Nitrite Nitrogen
NO_3-N	:	Nitrate Nitrogen
nm	:	Nano metre
NWG	:	Net weight Gain
O-PO_4^{3-}	:	Orthophosphate
PBM	:	Poultry By-product Meal
PCV	:	Packed Cell Volume
PER	:	Protein Efficiency Ratio
PWG	:	Percent Weight Gain
pH	:	Power of Hydrogen
POD	:	Peroxidase
PUFA	:	Poly Unsaturated Fatty Acid
PV	:	Peroxide Value
ROS	:	Reactive Oxygen Species

RO	:	Rapeseed Oil
SBO	:	Soybean Oil
SGR	:	Specific Growth Rate
SH	:	Shrimp Hydrolysate
SOD	:	Super Oxide Dismutase
SO	:	Sunflower Oil
SPC	:	Soy protein Concentrate
SPSS	:	Statistical Package for the Social?
TA	:	Total Alkalinity
TBLG	:	Total Body Length Gain
TC	:	Total Cholestrol?
TG	:	Triglyceride
TH	:	Total Hardness
TLG	:	Total Length Gain
TP	:	Total Proteins
TH	:	Tilapia Hydrolysate
TVB-N	:	Total Volatile Base Nitrogen
U	:	Unit
VLDL	:	Very Low Density lipids
WG	:	Weight Gain

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Chapter I

INTRODUCTION

Freshwater catfish, *Pangasianodon hypophthalmus* (Asian catfish or pangas) belonging to family Pangasidae, is one of the fastest growing exotic species (native of Vietnam) in aquaculture. Over the last ten years, pangas catfish has emerged as a new aquaculture whitefish product in the world market (Singh and Lakra 2012). In India, it was introduced in West Bengal from Bangladesh during 1997 and eventually to other states viz. Andhra Pradesh, Kerala, Odisha, where it is now well established and cultured under monoculture system, with a production range of 15-20 t/ha/yr (Singh and Lakra 2012). Until 2012, the commercial production of pangas was only carried out in ponds, and farmers had started to switch back to the production of carps because of the consistent lower prices for pangasius. However in late 2012, with the initiatives of some private companies, experimental pangas culture was taken up in cages in states like Maharashtra, which has led to seed demand and thus few hatcheries came up in the States of West Bengal, Andhra Pradesh and Maharashtra, but with irregular supplies. The government of India started promoting the culture of pangas in cages in reservoirs and other water bodies a few years ago, and during 2016, National Fisheries Development Board (NFDB), Hyderabad finalized Guidelines for Pangasius Cage Culture along with government initiative through its "Blue Revolution" mission. Moreover, NFDB also funded and supported several cage farming projects, which gave a boost to the development of pangas culture in India (Mugaonkar *et al* 2017).

Recent data indicated that around 42,900 ha of area is under panags farming comprising 41,120 ha of earthen still water ponds and 1,780 ha of cage culture. Andhra Pradesh has the major cultivated area of about 24,000 ha followed by 8000 ha in Bihar and 6400 ha in West Bengal (Mohan *et al* 2019). Likewise, out of total pangas production of 8,55,500 metric tonnes (mt), Andhra Pradesh is leading with 5,00,000 mt followed by 1,50,000 mt from Bihar. In seed production, West Bengal is leading and has recently supplied more than 500 million seed to other states. In addition to West Bengal, commercial hatcheries have already come up in Andhra Pradesh, Chhattisgarh and Odisha. Continuous expansion in area and production under pangas culture along with increasing market demand in the form of various value added products and fillet, it has been estimated that exotic pangas catfish will

attain 1 million metric ton/annum production status by 2025, a position so far achieved only by Indian Major Carps.

In the recent past, pangas catfish has also been reared in Punjab and Haryana on experimental basis, as a potential candidate species for diversification in these states, due to huge demand of fish having less intramuscular spines. Pangas catfish has been introduced and cultured successfully in Punjab by Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, with productivity of 7.0 t/acre/6 months (3 times higher than carps) and the technology has been standardized to take up pangas catfish culture in Punjab from April to October (Datta *et al* 2016).

In commercial Pangas catfish culture, floating pellet feed is used, accounting more than 60 % of input cost. Pangas catfish is carnivorous and cannibalistic during early stages and becomes omnivorous in later stages of development (fingerling onwards). It readily accepts artificial feed under confined conditions. Most of the commercial feeds (crude protein 28-32 %) or farm made feeds are prepared with protein rich plant (soybean meal, ground nut meal) and /or animal (fish meal) sources. Plant protein sources suffer from low digestibility, high fiber content and anti-nutritional factors, which limit their use in aquafeed (Atanasoff 2014, Lall and Anderson 2005). Among animal protein sources, fishmeal and fish oil are still considered the most nutritious and most digestible ingredients for farmed fish feeds, but their inclusion in compound feeds for aquaculture have shown a decreasing trend, largely due to variations in supply and price. These are mostly used for specific stages of production, particularly for hatchery, brood stock and finishing diets and their incorporation in grower diets has decreased over time. In view of continued increase in price of both animal and plant protein sources and environmental concerns, it is vital to identify some alternative protein sources for inclusion in fish feeds (Haider *et al* 2015). Further, with increasing health concerns, demand for fortified meat products is also increasing, which also needs technological interventions with respect to supplementary feeding.

Fish processing generates solid wastes in the form of skin, fins, scales, body viscera, head and bones (Wang *et al.* 2011) and is as high as 50-80% of the original raw material. At present there are approximately 400 fish processing units in India with a daily capacity of 10,000 tonnes, and more than 2 metric million tonnes of waste (Ahmad and Bhuimbar 2019) is being generated annually. This waste, if used

properly, can be one of the excellent raw materials for the preparation of high value by-products like fishmeal, fish oil and organic fertilizer/fish silage including protein foods. Fish silage is a fermented brown liquid product produced from the whole fish or parts of it, to which acids, enzymes or lactic-acid-producing bacteria are added. Silage prepared by acid treatment is preferred over other methods due to its nutritional value and shelf life. It is an attractive alternative to fish meal due to various advantages such as simple technique, little investment, reduced effluents and odor problems and moreover the silage process is fast in tropical climate. However, a disadvantage is that the product is voluminous if consumed in its pasty form, implying in an additional drying cost (Beerli *et al* 2004). Further, fish silage, a rich source of protein hydrolysate, is one of the cost effective alternative to fishmeal and fish oil and a potential feed additive (Kim and Mendis 2006) in aquaculture and in the pet food industry. Obtained by preserving whole fish or fish by-products with an acid and letting enzymes from the fish hydrolyse the proteins, silage has potential to increase growth and reduce mortality of animals that receive it in their feed. There are several reports regarding usage of fish silage as sole ingredient or as one of the ingredient in formulated feeds at different levels for different fish species (Haider *et al* 2016). Moreover, using fish silage as an animal protein source can help in solving the environmental problems associated with disposal of waste and formulating cost effective livestock feed, including fish.

Fish (marine or freshwater) are the major dietary source of protein, vitamins, minerals and most importantly lipids in the form of n-3 long chain (LC) polyunsaturated fatty acids (PUFAs). Feed composition not only effects growth, health and reproductive potential of fish, but is also responsible for quality of fish meat. Further, food fish of high value can be fed with specially formulated finishing diets, which can maintain LC n-3 PUFA of fish and human as well. In addition to fish oil, number of vegetable oils like canola and linseed (flaxseed) oils, which are rich in alpha linolenic acid (18:3 n-3) can be utilized in fish feed, without affecting fish survival and growth. It is pertinent to mention here that the freshwater fish are capable of converting C-18 PUFAs to LC C-20 and C-22 PUFAs, the functional essential fatty acids in vertebrates.

Linseed or Flaxseed (*Linum usitatissimum*) commonly named as *Alsi, Atasi, Ulsi, Pesi, Phesi* or *Tisi* is a multi-purpose crop under family Linaceae and is abundant in many nutrients, such as PUFA, protein, and lignins (Wang *et al* 2007).

The important linseed growing countries are India, Russia, Canada, Argentina and the USA. India is the second largest (21.21%) linseed growing country in the world in terms of area of cultivation after Canada. It is the second most important rabi oilseed crop and stands next to rapeseed-mustard in area of cultivation and seed production in India (Dash *et al* 2017). It contains about 36-40 % oil and is richest (among crop plants) source of PUFAs, essential in the human diet. Linolenic (18:3), oleic (18:1) and linoleic (18:2) are the major unsaturated fatty acids (FA) present in linseed oil, which can be used for producing PUFA rich fish, for improving human health. Further, there is a small difference in using the terms flaxseed and linseed. Flaxseed is used to describe fax, when consumed as food by humans, while linseed is used to describe fax, when it is used in the industry and feed purpose (Morris 2008).

In the last two decades, there is growing popularity of linseed oil worldwide due to its health benefits in terms of reducing cardiovascular diseases, decreased risk of cancer, particularly of the mammary and prostate gland, anti-inflammatory activity, laxative effect, and alleviation of menopausal symptoms and osteoporosis (Goyal *et al* 2014, Dash *et al* 2017). Further, meat quality of farmed fish like pangas in terms of LC n-3 PUFA content can be increased by altering the nutritional composition of feed, not only to maximize fish production, but to improve human health. In this context, due to endogenous activity of herbivorous and omnivorous fish species to elongate α-linolenic acid (18:3 n-3 ALA) into LC n3 FA, there is possibility to increase the LC n-3 FA of fish by providing oil with high levels of ALA such as linseed (Watters *et al* 2012) in the form of finishing diets. Number of studies has been reported on the use of linseed oil in human and other animals, however very limited work has been done to evaluate efficacy of linseed oil in fish.

Objectives

In view of the above discussion, the present study has been designed with the following objectives

1. To assess the possibility of incorporating non-conventional animal protein source (fish silage) in nursery and grow out diets of pangas catfish for formulation of low cost feed.
2. To improve meat quality of pangas catfish with linseed oil supplemented ‘finishing diets’ for production of PUFA fortified health food.

Chapter II

REVIEW OF LITERATURE

2.1 Global Status of Pangas, *Pangasianodon hypophthalmus*

Culture of *P. hypophthalmus* (Iridescent shark/Pangas catfish) commonly known as sutchi catfish (Thailand) or Pla Sawai, Patin (Malaysia), tra or basa catfish (Vietnam) initiated during 1960, but it geared up only in 1996, when the technology for seed production was developed. Pangas catfish is one of world's fastest growing freshwater species (Jeyakumari *et al* 2016) and its global aquaculture production in key producer nations is estimated about 2.44 million metric tons (Mugaonkar *et al* 2017).Vietnam is the top producer and exporter of Pangas catfish, representing more than 75% of global production (FAO 2010 and Globelfish 2015). The United States used to be the major market for basa but that has changed over the past few years, as the United State's share of exported pangas has decreased from 80 to 4%. Over the last ten years, pangas has emerged as a new aquaculture whitefish product in the world market. While the market for pangas has expanded globally, however its production remained exclusively in Eastern Asia and centered principally in Vietnam. The major markets for this fish are the European Union, Russia, Southeast Asia and the United States. It has proven particularly adaptable for intensive production in several countries (Mukai 2011). The European Union is currently the major market for Pangasius (especially from Vietnam, which is the largest producer). Pangas is now traded well over to more than 100 countries globally, mainly in the form of skinless and boneless fillet and its value added products (Thi *et al* 2013). The growth in *Pangasianodon* aquaculture is driven, in large part, by the dramatic increased demand for the fish in the market. It is an excellent food fish with white fine grained sweet flesh thus having high consumer preference (Datta *et al* 2016). New markets such as Russia, the Middle East and some Asian countries have also demonstrated a growing demand for the fish (Josupeit 2009).

2.1.1 Introduction of *P. hypophthalmus* in Asia: *P. hypophthalmus* is a native of Mekong River in Vietnam and has been introduced to many Asian countries such as Singapore, Philippines, Taiwan, Malaysia, China, Myanmar, Bangladesh and Nepal including India. In view of its burgeoning trade, some of these countries have been culturing this fish to boost up aquaculture production (De Silva *et al* 2006, 2009).

Based on the information provided by FAO in the Database on Introduction of Aquatic Species (DIAS) and Fish Base, it has been found that this fish has established in wild in many countries wherever, it was introduced (Singh and Lakra 2010).

2.1.2 Aquaculture of *P. hypophthalmus* in India: In India, pangas was first introduced in the year 1997 in the state of West Bengal from Bangladesh (Mukai 2011). Initially its farming was carried in limited area in the state of West Bengal, but later on this was cultured on large scale in the state of Andhra Pradesh. Current status of the Pangasius farming in India was evaluated during the year 2018, which indicated that approximately 42,900 ha of area is under farming comprising 41,120 ha earthen still water ponds and cage culture in 1780 ha area. Andhra Pradesh has the major cultivated area of 24,000 ha followed by 8,000 ha in Bihar and 6,400 ha in West Bengal (Mohan *et al* 2019). Pangas is being farmed under monoculture or polyculture with carps and can grow upto 1 to 1.5 Kg in one year, with annual yields of around 10 to 15 tons per hectare (Mugaonkar *et al* 2017).

Culture of this species has grown over the years and has become popular among fish farmers in several states because of its acceptance in the market, fast growth and omnivorous feeding habits (Chheng *et al* 2004, Ali *et al* 2005 and Rohul Amin *et al* 2005). Today, farmers are over-whelmingly culturing pangas using improved management methods along with availability of improvised, supplementary feeds. Because of its remarkable growth rate (almost one kg in 90 days), this fish is being cultured in many states particularly the Andhra Pradesh, West Bengal, Kerala and Odisha and is famous for its fast growth rate, hardy nature, ability to consume different types of food and survive in low water quality environment. Commercial culture of this fish species is fast gaining importance as a result, there is continuous increase in its production volume. Current production of pangas is estimated at around 855,500 million tonnes (MT) with 500,000MT from Andhra Pradesh, followed by 150,000MT from Bihar (Mohan *et al* 2019). Pangas culture in a larger extent has paved way for demand for its seed and for establishment of more number of commercial scale hatcheries. Further, the cost of production of Thai-pangas utilizing conventional fish feed is not matching well with the farm gate price of the fish. Therefore, reduction of cost of feed for pangas with

special reference to replacement of traditional costly feed ingredients (plant/animal) is the need of the hour.

2.2 Replacement of Traditional Feed Ingredients in Fish Feed

Aquaculture is a fast growing sector with feed as the key input and fish meal is the main ingredient as a source of valuable animal protein in fish diets (Mukhopadhyay *et al* 1991). The availability, cost and environmental sustainability of fish feed are some of the main bottlenecks preventing the expansion of aquaculture industry (Worm *et al* 2006) including pangas culture. The current fish meal usage in aquafeeds is becoming unsustainable, as aquaculture production continues to expand with fluctuating supplies and increased prices of fish meal during the last two decades. This exacerbates pressures on wild fisheries which cannot be sustained to meet such demands (Hassan and Heath 2015) encouraging researchers to find appropriate alternatives for fish meal in aquafeeds (Slawski 2011). Traditionally, alternatives to protein meals have been sought from vegetable sources such as soybean meal (Soltan *et al* 2011), cottonseed meal (Soltan *et al* 2011), sunflower meal (Soltan *et al* 2015), linseed meal and canola seed meal (Soltan 2005) due to their wide spread availability, favourable amino acid profile, reduced cost and sustainable nature (Hardy 2010). However inclusion of plant based proteins in aquafeeds provides a number of problems, which include the occurrence of anti-nutritional factors (ANFs), reduced digestibility and palatability and limitations of certain essential amino acids (Oliva-Teles and Gonçalves 2001). In contrast, animal proteins had an adequate concentration of these amino acids, which are essential for normal growth. Animal protein also has the advantage of having low concentrations of anti-nutritional factors that might reduce the digestibility and assimilation of nutrients, as is the case, when fish are fed plant proteins (Abdel-Fattah and El-Sayed 1999).

Alternative protein resources such as meat and bone meal, hydrolyzed feather meal, flesh-meal and blood meal (Paul *et al* 1997 and Millamena 2002), dried fish / chicken viscera (Giri *et al* 2000), poultry silage (Middleton *et al* 2001), crayfish meal (Agouz and Tonsy 2003), shrimp meal (Al-Azab 2005) and fish silage (Najim *et al* 2014, Madage *et al* 2015) have been tried to replace fish meal either partially or fully, but even these pooled meals of various animal sources are not

sufficient to meet the growing demands of fish raising industry. Among alternative protein source, fish waste/fish silage can prove to be an protein ingredient due to number of advantages including easy availability, high nutrient value and low cost.

2.2.1 Fish Waste

Fish wastes is the non-edible portions of the fish body such as fish head, skin, bones and cartilage, fins, scales and viscera (gonads, intestine and liver) and are as high as 50-80% of the original raw material. A fish contain 45 % flesh, 24-27 % head, 12 % skeleton, 3 % skin and 12 % viscera including eggs, milt and liver of its total body weight (DOF 2013). The catching and processing of fish generates significant amount of waste, which remain mostly underutilized and have represented an environmental problem as they degrade rapidly in warm temperatures. Further, if it is not appropriately stored or managed, it creates aesthetic problems and strong odors due to bacterial decomposition. As per the recent estimate, global generation of fish industry waste stands in excess of 63 MMT (Jini *et al* 2011) while India alone generates >2 metric million tons of waste during fish processing of which 300,000 tons (Mahendrakar 2000). Due to its high organic content, fish waste is classified as certified waste which is even more costly to dispose off. This practice is coming under increased scrutiny due to environmental issues (Jesperson *et al* 2000) and is becoming an increasing concern and cost burden to the whole fishing industry. But on the other hand, these wastes also contain relatively high amount of nutrients such as protein, fat and minerals (Djazuli *et al* 2007) and a viable strategy would be to use the waste in form of fish silage and the process to convert fish waste to silage form is very simple, thereby eliminating the need for heavy and expensive processing equipment and maintenance.

2.2.2 Fish Silage

The word silage is derived from “silo” (Jangaard 1987), which refers to a structure, typically cylindrical, in which fodder or forage is kept. A.I. Virtanen (1920) was the first one to develop acid treated silage from forage using hydrochloric and sulphuric acid as preservative (Raa and Gilberg 1982) and this method was further adopted by Edin in 1930s to preserve and liquefy different types of fish and fish waste. Fish silage was produced for the first time

commercially in Denmark and Poland as a protein supplement in pig and poultry feed and was later incorporated in feeds for other domestic animals and fish (Arruda *et al* 2007). Use of fish silage as a fish feed ingredient started in 1980's for recycling organic material (Hardy *et al* 1984). Fish silage production for use in mink, pig and poultry diets is a common practice in countries like Poland, Norway and Denmark (Jackson *et al* 1984). Various experimental and commercial scale trials have been setup in various countries like Indonesia (Kompiang 1981), Iceland (Arason *et al* 1990), United Kingdom (Tatterson and Windsor 2001) and New Zealand (Gibbs 2012) for use of fish silage as protein supplement in livestock and various domestic animals.

Fish silage is a protein rich high biological value product for animal feeding, which involves dead fish, sub-utilized species, fishing by-products and industrial wastes. It is prepared by adding acid to fish waste which results in a rapid drop in pH, and the increasing concentrations of non-dissociated organic acids, which inhibit the growth of microorganisms and can represent a very good nutrient in the form of protein source for livestock including fish due to its high protein content and essential amino acid (Hafez *et al* 2002) profile. This procedure has the potential of reducing production cost of fish meal by approximately 15-20% (Yildirm *et al* 1999, Gullu and Guzel 2003, Turker and Buykhatipoglu 2006) and is considered safe, cost effective and eco-friendly (Hanafy and Ibrahim 2004). There is a better chance to produce huge amount of fish silage in tropical countries due to the availability of byproduct from capture, industrial wastes and also bycatch fish (Durairaj *et al* 1976; Gildberg and Raa 1977). In fish farming and animal rearing, 60% of the expenses are incurred in feed cost, which can be easily reduced by adding fish silage as a protein supplement in animal feed (Arruda 2007).

2.2.3 Types of Fish Silage

There are generally three types of fish silage depending on treatment method. Acid or chemical silage which can be prepared by direct acidification with addition of organic or inorganic acids (Ramasubburayan *et al* 2013); fermentative or biological fish silage (biosilage) - made by using bacterial inoculums and carbohydrate substrate as a sugar source (Dapkevicius 2002). A third type known as

the enzymatic fish silage is rarely applied using proteolytic enzyme preparations (Borghesi *et al* 2008).

2.2.3.1 Chemical Fish Silage

In the preparation of chemical silage, the choice of preservative reagent is made from inorganic acids, organic acids or the mixture of organic and inorganic acids. Sulphuric and hydrochloric acids are the mostly commonly used inorganic acids for the preparation of chemical fish silage, while formic and propionic acids are used as organic acids. Inorganic acids especially sulphuric acid is cheaper in comparison to organic acids, but the latter are used in less quantity (Abd El-Hakim *et al* 2007).

The raw material used for the preparation of the chemical silage, must preferably be presented in small pieces or should be in ground form. Afterwards, acid is added to allow for its action until liquefaction takes place. Normally, room temperature is used and the storage leads the desired biochemical modifications. It is essential that the mixture is stirred so that the raw material can remain in contact with the acid. After the initial mixing, the silage process naturally begins, but occasional stirring helps in obtaining the desired uniformity (Oetterer 2002). Liquefaction process in organic acids can be carried out within a pH range of 3.5-4.5, which might be considered beneficial due to the anti-bacterial properties present in them (Tanuja *et al* 2014). Acid silages if prepared with only inorganic acids, will have a very low pH (around 2), which requires neutralization before it can be used in feed (Vizcarra- Magana *et al* 1999). Formic acid (organic acid) is the best choice for the preparation of chemical silage, the silages prepared by using formic acid are not excessively acidic and therefore do not require neutralization before being used (Oetterer 2002). According to Strom *et al* (1980), use of propionic acid for silage preparation inhibits fungal growth.

Vidotti *et al* (2003) recommended mixture of 20 $mlkg^{-1}$ formic acid and 20 $mlkg^{-1}$ sulfuric acid due to the high cost of other organic acids. Abd El-Hakim *et al* (2007) used 1.5% formic acid with 1.5% sulphuric acid to ensilage nile tilapia wastes. The results revealed that the silage was of good nutritional quality and can be used as fish meal replacer in the feeds for nile tilapia, *Oreochromis niloticus*. Similarly, Ramasubburayan *et al* (2013) prepared acid silages using fish wastes

supplemented with three different concentrations (2, 2.5 and 3%) of formic acid, and fermented for 30 days with the conclusion that 2% formic acid was adequate to produce good quality silage as tested in feeds for the common carp fingerlings.

Table 1A: Acid sources and their levels (%) used for the chemical fish silage production

Reference	Acid source	Addition level
Balogun *et al* 1997	Sulfuric acid + Formic acid	2% + 0.75%
Vidotti *et al* 2003		1.5%
Abd El-Hakim *et al* 2007		2%
Arruda *et al* 2007		3%
Majumdar *et al* 2014		2%
Nwanna *et al* 2004	Formic + Ethanoic acids	2%
Mousavi *et al* 2013	Sulfuric acid	2.5%, 3.5% and 4.5%
Haider *et al* 2005	Formic acid	3%
Ramasubburayan *et al* 2013		2%, 2.5% and 3%
Gullu *et al* 2014		2.5%
Madage *et al* 2015		3%
Haider *et al* 2015		4%
Goosena *et al* 2016		4%
Tanuja *et al* 2014	Formic acid + Hydrochloric acid	1.5%
Goddard and Al- Yahyai 2001	Formic acid + Propionic acid	1.5%
Borghesi *et al* 2008		3%
Bhaskar *et al* 2008		0.75%
Raj *et al* 2018		3%
Barreto-Curiel *et al* 2016	Citric acid + Phosphoric acid	2.5%

2.2.3.2 Biological Fish Silage

Concentrated organic/inorganic acids used to prepare chemical silage are often hazardous and can impose various handling and storage risks especially in the developing countries. To overcome these problems, various raw materials and fermentation methods to produce biological or fermentative fish silage were evaluated (Moretro *et al* 2010). Fermentation is initiated by mixing fish waste with a fermentable sugar, which enables the growth of lactic acid bacteria. Lactic acid bacteria are usually added as a starter culture, which result in production of acid and antimicrobial substances which together control competing spoilage/pathogenic microflora (Raa and Gildberg 1982). Van wyk and Heydenrych (1985) evaluated production of naturally fermented fish silage with carbohydrate sources. Suitability of eight different *Lactobacillus* cultures for fish silage preparation was investigated with various industrial by-products such as whey powder, refined sugar and molasses were tested as fermentation substrates.

Faid *et al* (1996) studied biotransformation of fish waste into a stable feed ingredient. Fish waste of sardine (*Sardina pilchardus*) were mixed with molasses (25%) and inoculated with starter culture of *L. plantarum* and *Saccharomyces* species. Results indicated mixed fermentation by pure cultures of yeasts and lactic acid bacteria strains could be involved in preservation, transformation and the improvement of the organoleptic quality of the obtained product.

Biological silage has also been produced through spontaneous acidification resulted by using various carbohydrate substrates as shown in the following table:

Table 1B: Carbohydrate sources and their levels (%) used for the biological fish silage production

Reference	Carbohydrate source	Addition level
Jangaard 1987 Kompiang *et al* 1980	Molasses	2%
Raa and Gildberg 1982		10%
Lupin 1983		10-30%
Brown and Sumner 1985		12-15%
Twiddy *et al* 1987		9.5%

Giurca and Levin 1992		4%
Lassen 1995		7.5%
Faid *et al* 1996		30%
Collazos and Guio 2007 Dong *et al* 2013		5%
Van Wyk *et al* 1983 Van Wyk and Heydenrych 1985	Molasses + Whey	2.5+12.5%
Lamprecht *et al* 1982	Whey + Sucrose	11.5%+3.85%
Lupin 1983	Maize flour	13.5%
Lupin 1983 Batista *et al* 1987 Fagbenro and Bello-Olusoji 1997	Cassava flour	15- 20%
Lindgren and Pleje 1983	Pre-fermented Cereals + Molasses	10%+10%
Twiddy *et al* 1987	Cassava + Sugar	20%+2%
Twiddy *et al* 1987	Rice + Sugar	20%+2%
Palekar 2009	Curd	10%
Zahar *et al* 2002	Sugar beet molasses	5%
Mousavi *et al* 2013		15%
Ramírez 2016	Sugar cane molasses and *Lactobacillus sp.* strain B2	18%+5%

2.2.3.3 Enzymatic Fish Silage

Different proteolytic enzymes are used to prepare enzymatic fish silage. Borghesi *et al* (2008) used 1gkg^{-1} protease type II from *Aspergillus oryzae* to produce enzymatic silage from Nile tilapia processing (filleting) wastes and whole fish (trash). Results indicated that the enzymatic silage did not differ significantly from chemical and biological silages produced from the same raw materials.

Ghaly *et al* (2013) also reviewed the utilization of various enzymes such as alcalase, neutrase, carboxypeptidase, chymotrypsin, pepsin and trypsin in enzymatic fish silage production. It was also mentioned that such products are especially appropriate for extraction of valuable biological materials like amino acids, proteins

and oils from enzymatic fish silage. Similar observations were recorded by Ramakrishnan (2013) who prepared fish silage by using Alcalase enzyme at three enzyme concentrations (0.5, 1 or 2%) and four time intervals (1, 2, 3 and 4 h) to extract proteins and amino acids from the whole fish and fish wastes. Results concluded that enzymatic hydrolysis of fish tissues is cost effective method to extract many important biological materials with high quality.

2.3 Fish Silage and Aquaculture

2.3.1 Fish Silage as Protein Source in Aquafeeds as Growth Promoter

Fish silage can prove to be an effective protein source in aquafeeds due to the similarity of the protein source with the raw material and low cost, especially when compared to fish meal (Vidotti *et al* 2003). Further, according to Ramasubburayan *et al* (2013) silage production acquires increased importance compared to fish meal because of the advantages like the process is virtually independent from the scale, the technology is simple and the investment is little.

Plascencia-Jatomea *et al* (2002) evaluated feasibility of fishmeal replacement by shrimp head silage protein hydrolysate in Nile tilapia (*O. niloticus* L) diets. Six diets (28% protein, 12% lipid) were prepared, in which fishmeal protein was replaced at levels of 0 (control), 10, 15, 20, 25 and 30% with the hydrolysate. Results revealed that shrimp head hydrolysate is a promising alternative protein source for tilapia feeding, improving growth rate at dietary inclusion levels as high as 15%. In addition, the diets with added shrimp silage protein were well accepted by the fish, which avidly consumed the feed during the experiment. Similar observations were recorded by Najim *et al* (2014) who evaluated the effect of using fish biosilage as fish meal replacer on feeding, growth and gut histology in Common carp, *Cyprinus carpio* L. fingerlings. The produced biosilage was incorporated in feeds to replace 0, 25, 50 or 75% of fish meal protein and the study concluded that fish silage could replace fish meal without adverse effects on feeding, growth efficiency and gut histology.

Salah al-Din (1995) reported inclusion of fish silage improved growth performance ofcatfish (*C. lazera*) as compared to the control diet. Fagbenro and Jauncey (1994) also reported that fermented fish-silage co-dried with protein feedstuffs as suitable protein supplement in case of juvenile catfish (*C. gariepinus*), which can provide up to 50% of dietary protein without affecting feed efficiency, fish

growth or health. Better growth rate of fish fed with acid silage may be due to the presence of comparatively higher amount of free amino acids and active hydrolytic enzymes (Gallagher 1993). On the other hand, Wassef *et al* (2003) found that, partial or total replacement of fishmeal by fermented fish silage alone or mixed with soybean meal did not significantly affected FCR and PER. Soltan and Tharwat (2006) found that dried fish by-products silage can successfully replace upto 25 and 50% of fishmeal in tilapia and catfish diets without any negative effects in final body weight (BW), weight gain (WG) and SGR along with reduced feed cost, while the higher incorporation levels (50, 75 or 100%) significantly reduced the final BW in both the species. Similar observations were recorded by Fagbenro and Bello-Olusoji (1997) and Plascencia-Jatomea *et al* (2002), who reported that the best FCR and PER were recorded for nile tilapia fry fed diets contained 0, 10 and 15% shrimp head silage as a replacement to fish meal and higher FCR was obtained at higher replacement levels (20, 25 and 30%). Some other studies reported that FCR values varied from 1.4 to 8.7 (Lie *et al* 1988, Goncalves *et al* 1989, Stone *et al* 1989 and Gullu *et al* 2003). It is possible that the large range of differences in the studies is due to variation in raw material used for the feed ingredients, the ratios of feed ingredients, the differences between the species of fish that were fed, the environmental conditions and the quality of water.

Ramasubburayan *et al* (2013) studied characterization and nutritional quality of formic acid silage developed from marine fishery waste and their potential utilization as feedstuff for Common Carp, *C carpio* fingerlings. From the results, it was revealed that 2% acid silage diet had higher weight gain (2.38g), SGR (1.49%) and significant increase in biochemical constituents than other diets. Datta *et al* (2018) also reported fish silage to be equally effective as that of poultry waste, fish meal and soybean meal in striped catfish, *P. hypophthalmus* in terms of survival, growth and acquired immunity. Sotolu (2009) also evaluated performance of dietary fish waste meal (FWM) and imported fishmeal (FM) at 10 and 15% inclusion levels in catfish diets towards the utilization of fish wastes as a cheap alternative animal protein source for sustainable aquaculture and reported that Dietary FWM at 15% inclusion was better in terms of reduced overall diet cost as compared to imported FM diet. Similar observations were reported by Haider *et al* (2015) on nutritive evaluation of fish acid silage in *Labeo rohita* fingerlings feed and they reported that the fish

silage can be a cheaper and effective alternative to fishmeal in fish feeds, if carefully handled and properly processed. Kamei *et al* (2018) also studied use of fish silage based blended protein source for replacement of fish meal in Thai-Pangas diet and concluded that the cost effective diet for Thai pangas (*P. hypophthalmus*) may be formulated by replacing 75 % of the fish meal with blended protein source for better performance in terms of growth of the fish.

2.3.2 Fish Silage for Boosting Fish Immunity

Goosena *et al* (2016) studied effect of silage prepared from rainbow trout viscera as immune stimulant and feed ingredient in diets for Mozambique tilapia (*O. mossambicus*). It was concluded that rainbow trout viscera silage can stimulate the cellular non-specific immunity of *O. mossambicus* due to protein hydrolysis product in form of silage. Similar observations were reported by Sanaz *et al* (2015), who studied effects of protein hydrolysates supplementation in fish meal diets on growth performance, innate immunity and disease resistance of red sea bream *Pagrus major.* A fish meal based diet was used as a high fish meal diet (HFM) and a low fish meal (LFM) diet was prepared by replacing 50% of FM by soy protein concentrate. Three other diets were prepared by supplementing shrimp (SH), tilapia (TH) or krill hydrolysate (KH) to the LFM diet. The results of the study indicated that the inclusion of the tested protein hydrolysates, particularly SH, in a LFM diet can improve growth performance, feed utilization, digestibility, innate immunity and disease resistance of juvenile red sea bream.

Neill *et al* (2014) also evaluated effect of rainbow trout silage oil as immunity enhancing feed ingredient in formulated diets for South African abalone, *Haliotis midae* and concluded that dietary fish silage oil inclusion can improve cellular immune function but that the optimal inclusion level should be determined in order to negate the negative effects on production efficiency.

2.3.3 Fish Silage and Biochemical Composition of Fish

Biochemical parameters are frequently used to determine the influence of feed on overall fish health. Najim *et al* (2014) investigated the effects of replacing fish meal with locally produced fish biosilage on some haematological and biochemical parameters in common carp *C.carpio* fingerlings and reported a significant ($p \leq 0.05$) increase in total protein and albumin levels in fish, where fish meal was replaced with

fish bio silage at different levels (0, 25, 50 or 75%). Results concluded that fish silage could be a good replacer for fish meal without adverse effects on fish blood characteristics. Darsana and Sreekumar (2011) also studied the effect of processed fish wastes supplementation on blood biochemical and meat composition of broiler chicken. The serum total protein, albumin and globulin, the antioxidants (SOD, Catalase, GSH and LPO) and liver enzymes (ALT and AST) expressed a similar level in all the groups, but increased with increase of age. The results indicated that processed fish wastes could be used for complete replacement of animal protein requirements in broiler feed, as it adequately meets the nutritional requirements, alleviates stress, has no toxicity and also maintains the meat quality. Dean *et al* (1986) also evaluated effects of dietary protein quantity and protein quality on growth rate and enzyme activities in channel catfish (*Ictalurus punctatus*) and reported an increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with increase in dietary protein quantity in fingerling channel catfish (*I. punctatus*).

2.3.4 Fish Silage and Hematological Parameters

Blood indices are reflection of dietary treatment's effects on the fish health condition and its physiological, biochemical and metabolic necessities (Kamali-Sanzighi *et al* 2018), which help in assessing the health status of fish in culture conditions and for detecting disturbance if any has been introduced into the fish's habitat (Mulcany 1975). Datta *et al* (2018) reported improved haematological values in *P. hypophthalmus* young ones fed with diets containing different protein sources including poultry waste, fish meal, fish silage and soybean. Hedayati and Tarkhani (2014) reported that the average value of Hb (7.17) in *P. hypophthalmus* and the value is near to values recorded in experimental diets in this study.

2.3.5 Fish Silage and Antioxidant Parameters

Qi-you *et al* (2008) revealed enhanced non-specific immune parameters along with growth, when fishmeal was partially replaced with soy protein isolate and meat bone meal (30%, 20% and 10%) in the diet of rainbow trout (*O. mykiss*). Increased antioxidant activity (SOD) with increasing amounts of cricket meal (75% and 100%) in the diet of African catfish (*C. gariepinus*) was also observed by Taufek *et al* (2016). Similar observations were also reported by Dong *et al* (2013) with dietary

supplementation of maggot meal (390 g kg^{-1}) and soybean meal (450 g kg^{-1}) in gibel carp (*C. auratus gibelio*).

2.4 Fish Silage and Pelleted Feed Quality

The pellet quality of is not only important in determining its nutritional value in terms of higher feed intake and an improved nutritional status of fish (Aarseth and Prestlokken 2003, Salas-Bringas *et al* 2007), but also determine the physical quality in terms of hardness and durability, so as to withstand transportation stress. Hardness is the force necessary to crush a pellet or a series of pellets at a time; durability is the amount of fines returning from pellets after being subjected to mechanical or pneumatic agitation. Such quality parameters can also be used to evaluate the effects of diet formulation, conditioning, expander treatment, pellet binders, die selection and similar process parameters (Salas-Bringas 2011). In addition to the nutritional adequacy and digestibility, fish feeds especially pelleted feeds must meet certain other criteria to be successful and productive because of the peculiarity of the aquatic environment (Glencross *et al* 2009 and Sorensen *et al* 2011). Water stability and other related properties such as bulk density and floatability depend on several factors such as chemical composition and process parameters.

Fagbenro and Jauncey (1998) evaluated the physical and nutritional properties of moist fermented fish silage pellets as a protein supplement for tilapia (*O. niloticus*). In their study, wet silage (2:1, w:w) was mixed with poultry by-product meal, soybean-hydrolyzed feather meal blend or menhaden fish meal; and each mixture was pelleted by cold extrusion method. Results indicated that the moist pellets maintained a firm consistency during water immersion for 10 min and pellet stability has been similar among different diet formulations. Moreover, protein and lipid losses were low (<15%) on per gram diet recovered basis. Similar observations were recorded by Pantoja *et al* (2011), who prepared extruded feeds for red tilapia (*Oreochromis* spp.) in fattening stage by using fish biological silage and evaluated different physical characteristics of the prepared diets in relation to various extrusion parameters like temperature, screw speed and moisture. Their results indicated the significance of some factors and interactions. The design's mid-point formulated with 15% fish biological silage and processed with mid-levels of the three factors (125°C, 170 rpm

and 28% moisture) showed higher than 90% buoyancy and acceptable results in the rate of expansion, specific density, water absorption rate and water stability.

Wicki *et al* (2012) also used wet feed with high content of fish silage and investigated its effect on final growth of Pacu (*Piaractus mesopotamicus* Holmberg 1887) in Northeast Argentina. Results indicated that fish silage wet feed show good stability as fishmeal feed alternative with no adverse effects on feed quality.

2.5 Dietary Lipids and their Role in Fish Growth

Dietary lipids play an important role in commercial diets of fish as concentrated source of energy, essential fatty acids for growth and hence overall development of fish (Pei *et al* 2004). Lipids are also essential components of steroids and phospholipids, which are used as precursors in the synthesis of certain vitamins and hormones. In addition, they also provide transport of fat-soluble nutrients and are involved in the synthesis of metabolically active compounds (Weirich and Reigh 2001). Moreover, there is evidence that dietary lipids and their constituent fatty acids influence immune response and disease resistance in fish (El-Tawil *et al* 2014). The dietary lipids also spares dietary proteins as these are used as energy sources and also limit ammonia production through a process called protein sparing action (Gaylord and Gatlin 2000). Higher energy levels generally come from increased dietary lipid as lipid is an energy dense nutrient and readily metabolized by fish (NRC 1993). However, more than required lipid content in fish diet often results in oxidative stress and pathological conditions (Sakai *et al* 1998) along with decreased growth due to reduction in feed consumption (Daniels and Robinson 1986), increased lipid deposition and poor nutritional value of fishmeal (Scaife *et al* 2000).

Among various sources, fish oil, rich in high unsaturated fatty acid HUFA, is the main dietary lipid source for aquafeeds (Ng *et al* 2004). The price and production figures are dependent upon the wild catch of oil-yielding species. According to Tacon and Metian (2009), global fish oil production has reached a plateau and is not expected to rise beyond the current level of production. To sustain aquaculture development, many studies have been conducted to identify alternative lipid sources (Caballero *et al* 2002, Glencross *et al* 2003, Izquierdo *et al* 2005, Mourente and Bell 2006, Lin and Shiau 2007 and Piedecausa *et al* 2007). The sustainable alternatives to

fish oil are plant (vegetable) oils which are rich in C_{18} PUFA (Sargent *et al* 2002), but devoid of highly unsaturated fatty acids (HUFA). Inclusion of vegetable oils in fish diets also modifies body fatty acid profiles and may significantly affect flesh quality and sensory characteristics (Guillou *et al* 1995). Vegetable oils such as soybean, linseed and rapeseed oils are some of the promising alternatives for fish oil probably due to more stable prices and increased production volumes of plant oils (Bimbo 1990).

2.5.1 Introduction to Linseed

Linseed or flax (*Linum usitatissimum* L) belongs to the order Malpighiales, the family *Linaceae* and the tribe Lineae. It is the second most important rabi oilseed crop and stands next to rapeseed-mustard in area of cultivation and seed production in India (Dash *et al* 2017). Different varieties of linum have been developed for production of fibre oilseed. Varieties of Linum used as fibre are called flax, whereas the oilseed varieties are called linseed, oilseed flax or just flax (Popa *et al* 2012).

Linseed is popularly known as *Atasi*, *ulsi*, *Pesi*, *Phesi* or *Tisi*. It is a multi-purpose crop and is abundant in many nutrients, such as polyunsaturated fatty acid, protein, and lignins (Wang *et al* 2007). Its seeds contain about 36-40% oil and have long been used as a source of oil in human/ animal diets and industrial use (Simopoulos 2002). Linseed oil is derived from the seeds of flaxseed (*L. usitatissimum* L.), a plant widely cultivated for fiber or oil for industrial use (Bayrak *et al* 2010). The most important linseed producing countries are Canada, Argentina, USA, China, India and Europe (Lidefelt 2007). It has an important position in Indian economy due to its wide industrial utility. But the national average productivity of linseed is quite low as compared to other countries. In India, it is grown mostly under rainfed (63%), utera (25%), irrigated (17%) and in input starved conditions in major linseed producing states i.e. Madhya Pradesh, Chhattisgarh, Maharashtra, Jharkhand, Uttar Pradesh and Odisha (Srivastava 2009).

2.5.2 History and Origin of Linseed

Linseed is one of the oldest crops, having been cultivated since the beginning of civilization. The generic name "*Linum*" comes from Celtic word *lin* means thread and the species name "*usitatissimum*" given by Carl Linnaeus, means very useful. So it directly refers to its multiple applications and their importance in ancient times

(Dash *et al* 2017). It was first introduced in United States by colonists, primarily to produce fiber for clothing. The origin of flax, which is one of the oldest of cultivated plants, is uncertain. However, it is generally accepted that linseed has originated from "Fertile Crescent" an area east to Mediterranean Sea towards India (Zeven and Zhukovsky 1975) and was probably first domesticated there. Linseed is supposed to have originated in the four centres of origin, viz. Central Asiatic, Near Eastern, Mediterranean and Abyssinian Centre. It spread northward to Europe and other parts of Asia and southward to India.

In the last two decades, flaxseed has been the focus of increased interest in the field of diet and disease research due to the potential health benefits associated with some of its biologically active components (Dash *et al* 2017). The first scientist to identify the beneficial properties of linseed oils was a doctor of biological and natural sciences; Joanna Budwig (1909-2003) and was the first to introduce linseed oil in a patient's diet (Pitat and Zadernowski 2010).

Flaxseeds have various nutritional characteristics and are rich sources of ω-3 fatty acid: α-linolenic acid (ALA), short chain polyunsaturated fatty acids (PUFA), soluble and insoluble fibers, phytoestrogenic lignans (secoisolariciresinol diglycoside-SDG), proteins and an array of antioxidants (Oomah 2001, Alhassane and Xu 2010, Ivanova *et al* 2011 and Singh *et al* 2011). Its growing popularity is due to health promoting benefits in reducing cardiovascular diseases, decreased risk of cancer, particularly of the mammary and prostate gland, anti-inflammatory activity, laxative effect and alleviation of menopausal symptoms and osteoporosis. Today, linseed oil is being used worldwide due to its associated health benefits. However, little work has been done to evaluate its efficacy on fish, however number of studies have been reported in humans, mice etc.

2.5.3 Linseed as Source of Lipid

Mantzioris *et al* (1994) evaluated that the dietary substitution with an α-linolenic acid-rich vegetable oil increases eicosapentaenoic acid (EPA) concentrations in tissues. Thirty healthy male volunteers were randomly allocated into two dietary treatment groups - high α-linolenic acid (α-LA; 18:3n−3) and low linoleic acid (LA; 18:2n−6) formed by using a flaxseed oil. The control group (n = 15) was maintained on a diet high in α-LA and low in α-LA, typifying a Western diet. The flaxseed oil-

containing diet resulted in significant increases in α-LA concentrations in the plasma phospholipid, cholesteryl ester, and triglyceride fractions (eight fold increase) and neutrophil phospholipids (50% increase). EPA concentrations increased by 2.5-fold in the plasma lipid fractions and neutrophil phospholipids. The results indicated that α-LA-rich vegetable oils can be used (in conjunction with a background diet low in LA) to elevate EPA in tissues to concentrations comparable with those associated with fish-oil supplementation.

Similar observations were recorded by Mourente and Bell (2006), who partially replaced dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth studying effects on muscle and liver fatty acid composition. The diets differed only in the added oil and were 100% fish oil (FO; diet A), 40% FO/60% vegetable oil blend (VO; diet B), where the VO blend was rapeseed oil, linseed oil and palm oil in the ratio 10/35/15 by weight and 40% FO/60% VO blend (diet C), where the ratio was 24/24/12 by weight. Results indicated sea bass grown for most of the production cycle using diets containing 60% VO can contribute a significant quantity of healthy n-3 HUFA to the human consumer.

2.6 Linseed and its Health Promoting Properties

2.6.1 Linseed as Anti-Carcinogenic Agent

Flaxseed, a rich source of mammalian lignan precursor secoisolariciresinol-diglycoside (S.D.) and α-linolenic acid (ALA), has been shown to be protective at the early promotion stage of carcinogenesis. The cancer protective effects of flaxseed were first studied by Serriano and Thompson (1992), who studied the effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis in rats by feeding 5% flaxseed supplemented in a high-fat diet at the promotional stage of tumorigenesis, i.e., after 7,12-dimethyl-benz[a]anthracene administration. Results indicated that flaxseed significantly reduced the size of the tumors.

Thompson *et al* (1996) also evaluated effects of flaxseed and its lignan and oil components on mammary tumor growth at a late stage of carcinogenesis in rats. Results concluded that S.D. in linseed is beneficial in the promotional phase of carcinogenesis, whereas the oil component is more effective at the stage, when

tumors have already been established. Similar observations were reported by Caughney *et al* (1996) who studied the effect on human tumor necrosis factor a and interleukin 1 J3 production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Use of linseed oil in domestic food preparation for 4 week inhibited TNFa and IL-l production by 30%. Fish-oil supplementation (9 gd^{-1}) continued for a further 4 week; TNFa and IL-l synthesis were inhibited by 74% and 80%, respectively. The results indicated that vegetable oils rich in n-3 fatty acids inhibit TNFa and IL-l synthesis.

Lin *et al* (1998) investigated the effect of dietary supplementation of flaxseed on experimental metastasis of B16BL6 murine melanoma cells in C57BL/6 mice. Mice were fed a basal diet (without flaxseed) and the basal diet supplemented with 2.5, 5 or 10% linseed for 2 weeks before and after the intravenous injection of 0.75×105 melanoma cells. The median number of tumors in mice fed the 2.5, 5 and 10% linseed -supplemented diets was 32, 54 and 63% lower than that of the control, respectively. The addition of flaxseed to the diet also caused a dose-dependent decrease in the tumor cross-sectional area and the tumor volume. These results provided experimental evidence that linseed reduces metastasis and inhibits the growth of the metastatic secondary tumors in animals. It is concluded that linseed may be a useful nutritional adjuvant to prevent metastasis in cancer patients. Wang *et al* (2007) also investigated the inhibitory effect of linseed on the growth and metastasis of estrogen receptor and negative human breast cancer xenografts is attributed to both its lignan and oil components. Athymic nude mice were orthotopically injected with ER- breast cancer cells (MDA-MB-435) and 8 weeks later were fed either the basal diet (BD) or BD supplemented with 10% linseed, lignan secoisolariciresinol diglycoside (SDG), Fish oil (FO), or combined SDG and FO (SDG 1 FO) for 6 weeks. In conclusion, flaxseed reduced the growth and metastasis of established ER- human breast cancer in part due to its lignan and FO components.

Dwivedi *et al* (2005) also evaluated the effects of dietary linseed oil (containing α-linolenic acid, an ω-3 polyunsaturated fatty acid) on azoxymethane-induced colon tumor in rats. Male Fischer rats were separated into 2 groups of 30 and were assigned to the AIN-93M diet, which was supplemented with either 15% corn oil or 15% linseed oil. Carcinogenesis was initiated with subcutaneous injections of

azoxymethane (15mgkg^{-1}) once a week for three consecutive weeks. The results indicated that dietary linseed oil, containing high levels of ω-3 fatty acids, is effective in preventing colon tumor development, when compared with dietary corn oil containing ω-6 fatty acids in rats.

2.6.2 Linseed and Hypolipidemic Activity

Richard *et al* (2006) reported replacement of dietary fish oil by blend of vegetable oils (55 % rapeseed oil, 30 % palm oil, 15 % linseed oil) in rainbow trout (*O. mykiss*) induced a decrease in plasma cholesterol, Low density lipids (LDL) and expression of LDL receptor gene in the liver was down-regulated. Similar observations were recorded by Vijaimohan *et al* (2006) with respect to beneficial effects of α-linolenic acid rich flaxseed oil (FO) on growth performance and hepatic cholesterol metabolism in high fat diet fed rats. Results indicated that FO may be developed as a useful therapy for hyperlipidemia through reducing hepatic lipids.

2.6.3 Linseed and Meat Quality

Lopez-Ferrer *et al* (1999) evaluated n-3 enrichment of chicken meat using fish oil substituted with rapeseed and linseed oils. A diet enriched with 8.2% fish oil (FO) was fed to the birds throughout the 5-week growth period (T1), the same basal diet supplemented with 8.2% linseed oil (LO, Experiment 1) or rapeseed oil (RO, Experiment 2) in three different periods: the last week before slaughtering at 35 d (T2), the last 2 week (T3), and throughout the experiment (T4). Results indicated that meat quality was unacceptable from T1 in both experiments. Replacing 1 (T2) or 2 (T3) week FO with vegetable oil clearly resulted in the improved sensory quality of meat. Lopez-Ferrer *et al* (2001) also evaluated the effect of supplying linseed oil (LO) in the diet on performance, fatty acid (FA) composition, and quality objective parameters of broiler chicken meat diets enriched with 0, 2, or 4% LO. Results indicated LO increased the amount of polyunsaturated FA (PUFA), mainly because of the linolenic (LNA) and linoleic (LA) acid content present in LO.

Jeronimo *et al* (2009) studied effect of dietary replacement of sunflower oil (SO) with linseed oil (LO) on intramuscular fatty acids of lamb meat. Thirty-six lambs were fed one of four diets consisting of pellets of lucerne with oil (60 gkg^{-1}): the diet varied in the composition of added oil and were: 100% SO; 66.6% SO plus

33.3% LO; 33.3% SO plus 66.6% LO and 100% LO. The experimental period was 7 weeks and results indicated that the utilization of blends of SO and LO is a good approach for obtaining lamb meat enriched with both conjugated linolenic acid and n3 Long chain-PUFA.

Pieretti and Meineri (2010) evaluated the effects of diets with increasing levels (0, 8, or 16%) of golden flaxseed (GFS; *L. usitatissimum* L.) on carcass characteristics, meat quality and lipid traits of growing rabbits. Results indicated that GFS dietary supplementation has shown to be effective in improving the n-3 PUFA proportion (76% in the muscle and 77% in the fat, respectively), decreasing the n-6/n-3 ratio and reducing the saturation, atherogenic and thrombogenic indexes of the meat, with consequent benefits on the nutritional quality of rabbit meat for consumers. Similar observations were recorded by Kitessa *et al* (2012) who reported an enhanced level of EPA and DHA in lambs fed with echium oil and linseed oil (@ 25 ml and 50 ml). Anjum *et al* (2013), evaluated the impact of extruded flaxseed meal supplemented diet on growth performance, oxidative stability and quality of broiler meat and meat products.120 day old broiler chicks were randomly allotted to 12 experimental groups and fed on diets containing extruded flaxseed meal at 0, 5, 10 and 15%. Feeding extruded flaxseed to chicken through feed strongly inflated the quality and functional properties, fatty acid contents and reduced the oxidative stability of broiler meat and meat products. Panda *et al* (2015) too reported a significant increase in poly-unsaturated FA (PUFA), n-3 FA and a significant decrease in n-6:n-3 in broiler chicken due to dietary incorporation of LO in the diets at 33, 67 and 100% levels.

2.6.4 Linseed and Carcass Quality

Eastwood *et al* (2009) evaluated nutritional value of flaxseed meal (FSM) for swine and its effects on the fatty acid profile of the carcass in swine. Diets contained 0,100, 200 or 300 g of FSM Kg^{-1} at expense of wheat, barley and soybean meal. Results indicated that FSM can be included upto 150g Kg^{-1} in the diets of swine resulting in enrichment of carcass with n-3 fatty acids. Eiben *et al* (2010) also studied effect of different dietary ratios of sunflower (S) and linseed oils (L) [4% S to 0% L(diet 4% S), 3% S to 1% L (diet 3:1% SL), 2% S to 2% L (diet 2:2% SL) and 0% S to 4% L (diet 4% L)] on growth and carcass traits of rabbits. Results indicated a

significant difference in carcass traits with major effects on colour values (lightness, redness and yellowness) of meat and fat.

Similar observations were reported by Rosa *et al* (2013) who evaluated performance and carcass characteristics of Nellore young bulls fed different sources of oils (soybean and linseed oils). All diets were formulated with the same amount of protein and with a roughage: concentrate ratio of 40:60, with sugarcane as the only roughage source. Results indicated better yield and carcass quality in diet fed with oils as compared to control. Omar *et al* (2018) also reported significantly decreased breast and flank cuts and a significant increase in rib eye area while evaluating effect of ground flaxseed (0, 4 and 8%) on the carcass characteristics of Karadi male lambs. Fat thickness decreased which may be reflecting the decrease in fat percentages in the carcass that indicates positive effect of ground flaxseed in improvement of carcass traits.

2.6.5 Linseed and Antioxidant Properties

Febel *et al* (2008) evaluated the effect of dietary fatty acid pattern on growth, body fat composition and antioxidant parameters in broilers. The broilers were fed with diets having different energy sources: lard (L); sunflower oil (SFO); soybean oil (SBO); and linseed oil (LSO). The treatments did not modify significantly growth performance and feed intake of the broilers. LSO diet increased the level of C18:3, C20:5 and C22:6 in tissue lipids in relation to L, SFO and SBO diets. Significantly increased plasma radical scavenging capacity in consent with enhanced C20:5 and C22:6 proportion in liver and muscle during LSO feeding indicate metabolic changes to counteract the oxidative injury. This may be related to the compounds produced after different biochemical pathways of n-6 and n-3 FAs. Baron *et al* (2013) also reported improved oxidative stability of the fish fillets in rainbow trout (*O. mykiss*) while studying impact of a mixture of organic plant oils ingredients [rapeseed (RO), linseed/flaxseed (LO), grape seed (GO), or sunflower (SO)] replacing fish meal @ 40%.

2.6.6 Linseed and Finishing Diets

The rapidly increasing aquaculture industry has been fueled by the subsequent use of manufactured aqua feeds. Conventionally, fish oil (FO) is one of the key biological sources of feeding components for aquaculture (Baweja 2018) and in

finishing diet, it is of much importance due to its multiple health benefits. The demand for fish oils has increased over recent years although production has remained static compelling fish nutritionists to reduce utilization of fish oil in aquafeed formulations with readily available and more economical terrestrial alternatives (Turchini *et al* 2009) and the current trend is towards the complete or partial replacement of fish oils by alternative lipid sources such as vegetable oils and animal fats in feeds for farmed fish. The common vegetable oils used as alternative lipids in the feeds of farmed fish species include palm oil, soybean oil, canola oil, sunflower oil, cottonseed oil, groundnut oil, coconut oil, olive oil, corn oil, sesame oil, linseed oil etc, has been studied in many fish species such as rainbow trout (Trushenski *et al* 2011, Masiha *et al* 2013 and Yildiz *et al* 2013), brown trout (Arslan *et al* 2012), African catfish (Bababola *et al* 2011) and Japanese seabass (Xue *et al* 2006), etc.

Glencross *et al* (2009) studied restoration of the fatty acid composition of red seabream (*Pagrus auratus*) using a fish oil finishing diet after grow-out on plant oil based diets. Results indicated that using a fish oil finishing diet increases long-chain polyunsaturated fatty acids (lc-PUFA) supports the usefulness of a fish oil based finisher diet for fish raised predominantly on plant oil based diets. Ng *et al* (2013) evaluated the effects of dietary fish and vegetable oils [fish oil + crude palm oil (1:1), linseed oil + crude palm oil (1:1), crude palm oil or soybean oil] on the growth, tissue fatty acid composition, oxidative stability and vitamin E content of red hybrid tilapia (*Oreochromis* sp.) and efficacy of using fish oil finishing diets. Among the tested alternative oil based-diets, tilapia fed by the finishing diet having resulted in the highest EPA, DHA and n-3/n-6 ratios, which should decrease feeding costs while improving the fillet quality of tilapia.

Chapter III

MATERIALS AND METHODS

3.1 Site of the Experiment

i) Experiment I - "Growth performance and health status of Pangas catfish (*Pangasianodon hypophthalmus)* fry fed on fish silage supplemented formulated diets"

Experiment I was conducted in indoor FRP pools (1.5×1×0.75m) at the Fish Farm of College of Fisheries, GADVASU, Ludhiana from July to October 2018 (120 days).

ii) Experiment II - "Growth performance, health status and meat quality of Pangas catfish (*Pangasianodon hypophthalmus)* fingerling fed on fish silage and linseed oil supplemented formulated feeds for grow-out production"

Experiment II was conducted in outdoor cemented tanks ($80m^2$) at the Fish Farm of College of Fisheries, GADVASU, Ludhiana from May to September, 2019 (150 days).

iii) Proximate analysis of the feed ingredients, formulated feeds and fish flesh, water quality parameters, hematological, biochemical, antioxidant parameters and meat quality during both experiments was carried out in the Nutrition Lab, Water Quality Lab, Central Equipment Lab, Aquatic Ecology Lab and Harvest and Post-Harvest Technology Lab of College of Fisheries, GADVASU, Ludhiana.

3.1.1 Preparation and maintenance of experimental pools/tanks

- The experiment I and II were carried out in indoor FRP pools (1.5×1×0.75m) and outdoor cemented tanks ($80m^2$), respectively. At the bottom of each pool/tank, two-inch thick layer of soil was spread to provide natural conditions.

- During the experimental period, bore well water was used for filling, exchanging and maintaining the water level in the pools/tanks. Initial liming was done with limestone @ 300kg ha^{-1} for disinfection and as per requirement (pH balance) throughout the experiment.

- $1/4^{th}$ of water from experimental pools/tanks was exchanged with fresh water once a week.

3.1.2 Procurement and acclimatization of experimental fishes

- For experiment I, equal sized, active and healthy fry (n= 360 without any sign of disease or parasitic infestation) of Pangas catfish (*P. hypophthalmus)* (average total body length 5-6 cm, average body weight 1-2 g) were procured from West Bengal and acclimatized for 15 days in indoor cemented cisterns for conducting the experiment during July to September 2018.

- For experiment II, Equal sized, active and healthy fingerlings (overwintered stock of experiment I) of Pangas catfish, *P. hypophthalmus* (average body weight 55g, average total body length 18cm) were used for conducting the experiment during May and September 2019.

- Fish were transported in plastic tubs with well oxygenated water, covered with net from collection pond to experimental pools/tanks.

- For experiment I, fish were acclimatized in FRP pools for one week with stocking density of 20 fish $tank^{-1}$ (in triplicate) before initiating the experiment and fed twice daily @ 2% with control diet.

- For experiment II, The fish were acclimatized in experimental tanks ($80m^2$) for two weeks with stocking density of 40 fish $tank^{-1}$ (in triplicate) before initiating the experiment and fed twice daily @ 2 % with control diet during the acclimatization.

- The water quality parameters of all the experimental pools/tanks were analyzed before stocking the fish.

3.2 Experimental treatments

Experiment I - Six treatments (Table 2) including control (with 3 replicates of each) viz. D1 (control), D2, D3, D4, D5 and D6 with different incorporation levels of fish silage.

Experimental II – same as for Experiment I

a. Grow out diets – 6 diets (D1 to D6) - same as in experiment I (Table 1) – feeding for 4 months (120 days)

FRP experimental pools For Experiment-I

Cemented Tanks For Experiment-II

Pangas catfish fry for experiment-I

Pangas catfish fingerlings for experiment-II

Fish waste collected from fish market

Fish silage under preparation

Experimental floating pelleted feeds

Plate I – Experimental Setup and Experimental Fish

b. Finishing diets – All the 6 diets (D1-D6) were supplemented with linseed oil @ 5 % (95% formulated diet + 5 % linseed oil) – feeding for one month (30 days)

Table 2: Percent composition of experimental diets

Ingredients	Experimental Diets					
	D1 (Control)	**D2**	**D3**	**D4**	**D5**	**D6**
Rice bran	28	28	28	28	28	28
Groundnut Meal	30	30	30	26.25	17.5	35
Soybean Meal	30	30	30	26.25	17.5	35
Fish Meal (FM)	10	5	-	-	-	-
Fish Silage (FS)	-	5	10	17.5	35	-
Vitamin-mineral mixture	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5

3.3 Stocking of fish

a) For Experiment I stocking was done with Pangas catfish (*P. hypophthalmus)* fry @ 20 fry pool^{-1}, during the month of July 2018.

b) For Experiment II Fingerlings (acclimatized) of Pangas catfish (*P. hypophthalmus)* were stocked @ 40 fingerlings tank^{-1}, during the month of May 2019.

3.4 Feed Preparation

- For experiment I, five experimental supplemented floating pelleted diets were prepared by replacing FM from control diet (D1) @ 50 and 100% (D2 and D3) and a mixture of groundnut and soybean meal @ 25 and 50% (D4 and D5) with FS. One more experimental diet (D6) was prepared without any animal protein source (FM or FS).

- For preparation of Fish silage, fish waste (including viscera, scales, head, fins etc.) was procured from local fish market of Ludhiana. Waste was finely chopped and 4% formic acid (weight by volume) was added to lower the pH

up to 3.5 along with butylated hydroxyl toluene (BHT) as antioxidant @ 250 mgl^{-1}. The mixture was stored at room temperature for a period of 30 days (30-35^{o}C), with daily thorough mixing along with maintaining pH at 3.5 to avoid putrification (Oetterer 2002). The silage (after neutralization with 8% NaOH to pH 7.0) was added to finely grounded ingredients as per feed formulation (Table 2) and floating feed pellets were prepared with extruder (Unitech-DOLLY). Pelleted feeds were sun dried and stored in airtight plastic containers at room temperature. The proximate composition of feed ingredients and experimental diets (Table 3) was analyzed by following the methods of AOAC (2000).

- For experiment II, 6 Grow out diets were similar as in experiment I, but finishing diets were supplemented with linseed oil @ 5 % (95% formulated diet + 5 % linseed oil). Linseed was procured from market and oil was extracted from local oil expeller plant. The oil was added to the finely grounded ingredients as per feed formulation (Table 2) and floating feed pellets were prepared with extruder (Unitech-DOLLY). Pelleted feeds were sun dried and stored in airtight plastic containers at room temperature. The proximate composition of feed ingredients and experimental diets (Table 3) was analyzed by following the methods of AOAC (2000).

3.5 Proximate analysis of feed ingredients and formulated feeds

The proximate analysis of different feed ingredients and formulated feeds (experiment I and II) with respect to crude protein (CP), ether extract (EE), ash (A), moisture and nitrogen free extract (NFE) content was done on dry matter (DM) basis by following the methods of AOAC (2000) (Table 2 and 3). Gross energy (Kcal g^{-1}) of feed ingredients and diets, was calculated from their respective CP, NFE and EE content by using energy factor 5.65 for proteins, 9.45 for fats and 4.10 for carbohydrates (Hepher *et al* 1983, NRC 1993).

3.5.1 Crude protein (CP)

The nitrogen content of the sample was estimated quantitatively by following the Kjeldahl method. Sample digestion was done with digestion system (KEL PLUS) and distillation with automatic distillation system (KEL PLUS- Classic DX Model) of Pelican Equipment.

Principle: The sample is digested in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia. Then, distillation of ammonia into a trapping solution (boric acid) and trapped ammonia (ammonium borate) is quantified by titration with a standard acid solution. The crude protein percentage was obtained by multiplying the nitrogen percentage by a factor of 6.25.

Reagents for digestion

- Concentrated sulphuric acid (H_2SO_4)
- Catalyst/Digestion mixture [Cupric sulfate ($CuSO_4.5H_2O$) and potassium sulfate (K_2SO_4) in the ratio of 1:9].

Reagents for distillation

- 40% sodium hydroxide solution [40 g NaOH dissolved in distilled water to make a total volume of 100 ml].
- 15% sodium hydroxide solution [15 g NaOH dissolved in distilled water to make a total volume of 100 ml].
- 4% boric acid-indicator solution [40 g of boric acid in 900 ml of distilled water + 10 ml of mixed indicator solution* to make the final volume to 1000 ml]

*Mixed indicator solution [0.1 g methyl red and 0.5 g bromocresol green dissolved in 100 ml of 95% alcohol].

Reagents for titration

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

Procedure for digestion

- Digestion system is preheated to 320°C.
- 0.1 g of sample was added in the digestion tube, with 3 g of catalyst mixture and 10 ml of conc. H_2SO_4.
- The temperature of the digestion tubes was increased to 420°C after the contents started boiling, continue heating until the color of the digestion mixture turned blue and finally green.

- Digested samples were cooled by keeping in rack undisturbed overnight.

Procedure for distillation

- 10 ml of distilled water was added in cooled digestion tube having digested sample.
- Digestion tube and 250 ml conical flask were kept in distillation unit for pre-programmed automatic distillation.
- Ammonia was collected in a conical flask having boric acid indicator solution in the form of ammonium borate (blue colored solution).

Procedure for titration

- Titrate the conical flask content with 0.1 N H_2SO_4 till wine color end point and volume of H_2SO_4 used for titration was recorded.

Note: A blank was run simultaneously to detect nitrogen present in the reagents and absorbed from the atmosphere if any.

Observations

Weight of the sample	-	W g (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 acid used	-	(X_2-X_1) - Y = Z ml

Calculations

N_2 (%) in the sample = 0.014 x Z x 0.1 N / W

Crude protein (%) = N_2 (%) x 6.25

Table 3: Proximate composition (% DM basis) of different feed ingredients and experimental diets used for experiment I and II

Ingredient/Diet	Crude Protein	Ether extract	Ash	Crude fiber	NFE	Gross Energy ($Kcalg^{-1}$)
Rice bran	14.67	2.47	11.00	8.56	63.30	365.75
Groundnut meal	35.62	1.76	8.40	19.26	34.96	361.22
Soybean meal	41.67	2.18	4.53	6.77	44.85	439.92
Fishmeal	51.33	5.56	24.33	3.44	15.34	405.45
Fish silage (Expt-I)	35.67	14.53	12.00	3.70	34.10	478.65
Fish silage (Expt-II)	36.45	15.23	10.89	4.50	32.93	484.87
Experiment I						
D1	32.43	2.43	9.39	10.54	45.21	394.78
D2	31.64	2.88	8.78	10.65	46.05	397.95
D3	30.87	3.32	8.16	10.78	46.87	404.59
D4	30.64	4.26	8.58	9.88	46.64	418.42
D5	30.11	6.47	9.54	8.23	45.65	391.02
D6	31.16	2.07	7.61	11.50	47.66	365.75
Experiment II (Grow out diets)						
D1	32.41	2.45	9.36	10.57	45.21	391.63
D2	31.67	2.91	8.71	10.61	46.1	395.44
D3	30.93	3.39	8.05	10.69	46.94	399.24
D4	30.81	4.38	8.39	10.01	46.41	405.74
D5	30.32	6.71	9.15	8.51	45.31	420.48
D6	31.16	2.07	7.61	11.4	47.68	391.10
Experiment II (Finishing diets)						
D1	32.42	7.37	9.47	10.56	40.18	417.55
D2	31.65	8.11	8.72	10.62	40.90	423.15
D3	30.91	8.39	8.07	10.65	41.98	426.04
D4	30.83	8.65	8.41	10.05	42.06	428.37
D5	30.35	9.23	9.14	8.52	42.76	434.01
D6	31.18	5.50	7.61	11.45	44.26	409.60

3.5.3 Crude fiber (CF):

The crude fiber content in the sample was determined by fiber extraction system (FIBRA-PLUS, FES 4) of Pelican Equipment.

Principle: The samples were treated successively with boiling solutions of sulphuric acid and sodium hydroxide of specified concentrations. The residue is separated by filtration, dried, weighed and ashed. The loss of weight resulting after ashing corresponds to the crude fibre present in the sample.

Reagents

- 1.25 % H_2S0_4 - 0.7 ml conc. H_2SO_4 dissolved in distilled water to make final volume of 100 ml.
- 1.25 % NaOH - 1.25 g NaOH pellets dissolved in distilled water to make total volume of 100 ml.

Procedure

- 1 g of sample (W g) was taken to an oven dried sintered glass crucibles. Crucibles were placed into the metal adapters of FIBRA PLUS hot extraction unit.
- Acid wash - 150 ml of 1.25 % H_2S0_4 was poured into each extractor from the top. The instrument was switched on and the initial temperature was set at 500°C. When boiling started, the temperature was reduced to 400°C. The sample was allowed to boil for 40 minutes in acid and after this, acid was drained and the sample was washed twice or thrice with distilled water.
- Alkali wash- 150 ml of 1.25 % NaOH was poured into each extractor from the top. The instrument was switched on and the initial temperature was set at 500°C. When boiling started, the temperature was reduced to 400°C. The sample was allowed to boil for 40 minutes in alkali and after this, alkali was drained and the sample was washed twice or thrice with distilled water.
- After alkali wash, crucibles were taken out and dried in a hot air oven to make the samples moistures free. Hot crucibles were cooled down to room temperature using a desiccator. Crucibles were weighed (W_1). All the

crucibles were placed in the muffle furnace at 330 ±10 °C for 3 hours for ashing. Hot crucibles were cooled down to room temperature after ashing using a desiccator and weighed (W_2).

Calculations - CF (%) = $W_1 – W_2 / W \times 100$

3.5.4 Ash

Principle: Ash content is determined by ignition of known weight of sample at about 550^{o}C in a muffle furnace till all carbon has been removed. The residue represents the inorganic constituents of food (as total ash) while the loss in weight is taken the organic matter.

Procedure:

- Cleaned, dried and empty silica crucibles were weighed accurately.
- 1g of feed sample (W) was added in the crucible and heated till completely burned (smokeless).
- Crucibles were placed in a muffle furnace at 550^{o}C±10°C for 3 hours.
- Crucibles were cooled overnight and weighed.
- Ash % was calculated by following formula.

Observations

W_1 = Weight of empty crucible

W_2 = Weight of crucible and Ash

W = Weight of the sample

Calculations - Ash (%) = $W_2 – W_1 / W \times 100$

3.5.5 Nitrogen Free Extract (NFE)

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

Calculations

NFE (%) = 100 - (% CP + % EE + % CF + % ash)

3.5.6 Gross Energy

It was calculated on the basis of gross energy values of crude protein, soluble carbohydrates (nitrogen-free extract) and total lipids (ether extract) of respective diets in terms of K cal g^{-1} by using energy factor 5.65 for proteins, 9.45 for fats and 4.10 for carbohydrates (Hepher *et al* 1983).

Gross energy (kcal 100 g^{-1}) = Protein (%) x 5.65 + Lipid (%) x 9.45 + Carbohydrate (%) x 4.10

3.6 Feeding of fish

- The fishes were fed with floating pelleted feed @ 5-3% (I^{st} month- 5%, 2^{nd} and 3^{rd} month- 4%, 4^{th} month- 3%) twice daily during the experimental period of 120 days during experiment I.
- The fishes were fed @ 5-3% (I^{st} month- 5%, 2^{nd} and 3^{rd} month- 4%, 4^{th} and 5^{th} month- 3%) twice daily during the experimental period of 150 days during experiment II.
- Amount of feed was adjusted at every monthly sampling according to increase in fish weight.

3.7 Observations recorded during Experiment I and II

3.7.1 Fortnightly interval

- **Physico-chemical parameters of water**- temperature, dissolved oxygen (DO), pH, total alkalinity (TA), ammonical nitrogen (NH_3-N), nitrite nitrogen (NO_2^-N), nitrate nitrogen (NO_3^-N) and orthophosphate ($O\text{-}PO_4^{3-}$) - as per methods of APHA (2012)

3.7.2 Monthly interval

- Survival
- Growth (length and weight) of fish

3.7.3 At completion of the experiment

- **Survival of fish**

- **Fish growth performance** – Total body length gain (TBLG), Net weight gain (NWG), Specific growth rate (SGR), Feed conversion ratio (FCR), Protein efficiency ratio (PER) and Condition factor (K)

- **Haematological parameters** - Haemoglobin (Hb) and haematocrit (Ht)
- **Biochemical Parameters** - Total protein, albumins, globulins and albumin/globulin ration
- **Anti-oxidant status** - Superoxide dismutase (SOD) and lipid peroxide (LPO)
- **Serum Transaminases** - Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)
- **Lipid profile** - total cholesterol, triglyceride (TG), high density lipid (HDL), low density lipids (LDL) and very low density lipid (VLDL)

For experiment II – in addition to the parameters analyzed in experiment I, following parameters were also analyzed at the completion of the experiment:

- **Flesh quality** – total proteins, total lipids, total carbohydrates, moisture and ash

- **Meat Quality** of fish product (fish fingers) and fish fillet

    - **Biochemical Parameters** – pH, peroxide value (PV), free fatty acid (FFA), titratable acidity (TA) and total volatile base-nitrogen (TVB-N) of fish product (stored at 4°C) at day 0, 3 and 6 and of fish fillet (stored at -20°C) at day 0, 10, 20, 30, 35 and 40

- **Sensory evaluation** of fish product (stored at 4°C) at day 0, 3 and 6

- **Texture evaluation** of **fish product** (stored at 4°C) at day 0, 3 and 6 and of **fish fillet** (stored at -20°C) at day 0, 10, 20, 30, 35 and 40

- **Relative productivity and economics of treatments**

3.8 Physico-chemical parameters of water

Water samples were collected at fortnightly intervals in the morning hours for the analysis of various physico-chemical parameters mentioned below:

3.8.1 Temperature

Water temperature (°C) was recorded by using digital thermometer (0 to 50 °C).

3.8.2 pH

pH was recorded by using a digital pH meter (Metler Toledo - FE 20-1).

3.8.3 Dissolved oxygen (D.O.)

Dissolved oxygen of water was estimated by modified Winkler's method (APHA 2012).

Principle: It is based on the addition of divalent manganese solution, followed by a strong alkali to a glass-stoppered bottle. DO rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions in an acidic solution, the oxidized manganese reverse to the divalent state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated against standard sodium thiosulphate, using starch indicator.

Reagents

- N/40 sodium thiosulphate [6.205 g sodium thiosulphate in one liter water]
- Manganous sulphate [480 g $MnSO_4.4H_2O$ in one liter water]
- Alkaline iodide-azide solution [350 g KOH and 75 g KI dissolved separately in distilled water. Both were mixed and the volume was made up to 500 ml. 5 g sodium azide (NaN_3) was mixed in 20 ml distilled water separately. This azide solution was added to the alkaline-iodide reagent].
- Starch indicator: Dissolve 1g of starch (soluble) in 200 ml distilled water and few drops of toluene as preservative.
- Concentrated sulphuric acid

Procedure

Water sample was collected without bubbling in 250 ml BOD bottle. 2 ml each of manganous sulphate and alkaline iodide-azide solutions were added one after the other for the formation of brown coloured precipitates. The bottle was shaken upside down and the brown precipitates were allowed to settle down. The precipitates were dissolved by adding 2 ml concentrated sulphuric acid and by shaking the bottle.

50 ml of the sample was taken and titrated with N/40 sodium thiosulphate solution till colour changed to pale straw. Two drops of starch solution were added and titrated further till the colourless end point.

$$DO\ (mg\ l^{-1}) = \frac{8 \times 1000 \times N}{V} \times v$$

V = Volume of sample (ml)

v = Volume of titrant (sodium thiosulphate) used (ml)

N = Normality of titrant (sodium thiosulphate)

3.8.4 Total Alkalinity (TA)

Total Alkalinity of water was estimated by volumetric method (APHA 2012).

Principle: Alkalinity is determined by titrating the sample with a standard solution of the strong acid. Alkalinity due to hydroxide and carbonate is determined to first end point (pH 8.3) using phenolphthalein indicator and bicarbonate alkalinity is determined to the second end point (pH 4.5) using methyl orange indicator.

Reagents

- 0.02 N sulphuric acid (H_2SO_4)
- Phenolphthalein indicator
- Methyl orange indicator

Procedure

- 50 ml of water sample was taken in an Erlenmeyer flask and two drops of phenolphthalein indicator were added to it.
- The pink color developed and was titrated with 0.02N H_2SO_4 till disappearance of the pink color.
- The volume of H_2SO_4 (A) used was recorded to calculate phenolphthalein alkalinity (PA).

- After this, two drops of methyl orange indicator were added to the same flask, the yellow color appeared.
- The solution was further titrated till yellow color changed to orange.
- The volume of H_2SO_4 (B) used was recorded to calculate methyl orange alkalinity (MOA). The TA was calculated as the sum of PA and MOA.

Calculation:

$$\text{PA } (CaCO_3 \text{ mg } l^{-1}) = \frac{A}{\text{Volume of the sample (ml)}} \times 1000$$

$$\text{MOA } (CaCO_3 \text{ mg } l^{-1}) = \frac{B}{\text{Volume of the sample (ml)}} \times 1000$$

$$\text{TA } (CaCO_3 \text{ mg } l^{-1}) = \text{PA+ MOA}$$

3.8.5 Ammonical-Nitrogen (NH_3-N)

Ammonical nitrogen of water was estimated by following the method given by APHA (2012)

Principle: Ammonia reacts with phenol and alkaline hypochlorite to form indophenols blue. The reaction is catalyzed by the nitroprusside or ferric cyanide. The resulting absorbance is proportional to the concentration of ammonia and is measured spectrophotometrically at 635 nm.

Reagents

- Alkaline hypochlorite reagent: Dissolve 15g phenol in 500 ml water. To this add 1 ml of freshly prepared 1.5% (w/v) nitroprusside.
- Phenol-nitroprusside reagent
- Standard ammonium chloride solution

Procedure

- 20 ml of water sample was taken in 25 ml capacity amber colored volumetric flask.

- To this 2 ml of each; phenol-nitroprusside and alkaline hypochlorite solution was added one after another.
- Distilled water was added to make a total volume of 25 ml. It was incubated at 25°C for 1 hour. Absorbance (A) was read at 635 nm.

Result - The value of Ammonical-Nitrogen was calculated from standard Ammonium chloride solution.

3.8.6 Nitrite-Nitrogen (NO_2^--N)

NO_2 - N of water was estimated spectrophotometrically (APHA 2012).

Principle:- In acid solution the nitrite yields nitrous acid, which diazotizes the sulphanilamide. The diazonium salt when reacts with aromatic amine, N-1-napthylethylenediamine dihydrochloride, forms a red azo dye; that is determined spectrophotometrically at 543nm.

Reagents

- Sulphanilamide [1 g sulphanilamide was dissolved in 100 ml of 10% hydrochloric acid]
- Aromatic amine reagent [0.1 g N-1 naphthyl ethylenediamine dihydrochloride was dissolved in 100 ml distilled water]
- Standard sodium nitrite solution [2.46 g anhydrous sodium nitrite was dissolved in distilled water and dilutes upto 1 litre] 1ml of this solution contain 0.5 mg (500 *u*g) NO_2^--N.

Procedure

- 45 ml of water sample was taken in 50 ml capacity volumetric flask.
- To this 1 ml of sulphanilamide made up to 50 ml with distilled water.
- Absorbance was read at 543 nm.

Result: The value of nitrite-nitrogen was calculated from the sodium nitrite standard curve.

3.8.7 Nitrate-Nitrogen (NO_3^--N) – NO_3^--N was estimated spectrophotometrically by the phenol - disulphonic acid method as given by APHA (2012).

Reagents

- Phenoldisulphonic acid [25 g pure phenol was dissolved in 150 ml conc. H_2SO_4, 75 ml of fuming sulphuric acid was added, stirred well and heated in water bath for 2 hours].
- 12 N KOH [36.5 g KOH was dissolved in distilled water and diluted to 500 ml]
- Standard potassium nitrate solution [7.22 g of KNO_3 was dissolved in distilled water and diluted to 1 liter] 1 ml of this solution contains 1 mg NO_3^-- N.

Procedure

- 25 ml water sample was taken and evaporated to dryness on a hot plate.
- Residue dissolved with 0.5 ml phenoldisulphonic acid.
- Add 5ml distilled water and 1.5 ml KOH.
- Yellow color developed and absorbance was read spectrophotometerically at 410 nm.

Result: Concentration of nitrate-nitrogen was calculated from the potassium nitrate standard curve.

3.8.8 Orthophosphates (soluble phosphate) - Stannous chloride method (Rand *et al* 1975) was used for the spectrophotometric determination of water soluble phosphates.

Reagents

- Ammonium molybdate strong acid solution [5 g ammonium molybdate $(NH_4)_6 MO_7O_{24}.4H_2O$ was dissolved in 35 ml distilled water. 62 ml conc. H_2SO_4 acid was added to 80 ml distilled water and cooled. Ammonium molybdate solution was added to it and diluted to 200 ml]
- Stannous chloride solution [0.5 g fresh $SnCl_2.2H_2O$ was dissolved in 2 ml concentrated HCl and diluted to 20 ml with distilled water]

- Standard phosphate solution [0.1757 g KH_2PO_4 was dissolved in distilled water and diluted to one liter] 1 ml of this solution contains 40 ug ortho-phosphates.

Procedure

- 25 ml of water sample was taken in flask, to which 1ml of ammonium molybdate strong acid solution was added
- 3 drops of stannous chloride solution were added and mixed thoroughly
- Blue colour developed
- After 10 min, absorbance was read on spectrophotometer at 690 nm.

Result: The amount of phosphate was calculated from the standard curve prepared with standard phosphate solution

3.9 Parameters observed at monthly interval

3.9.1 Survival and growth of fish

3.9.1.1 Survival of fish

Survival (%) of fish in control and all the treatments was calculated by comparing the live fish recovered at the completion of experiment with that of total fish stocked at the time of the initiation of experiment.

3.9.1.2 Growth of fish

- Fish sampling was done at monthly intervals to record fish growth in terms of total body length and body weight.
- Growth parameters in terms of total length gain (TLG), net weight gain (NWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (k) of fish for each treatment were calculated by using following formulae (Halver 1976):

TLG = Av. final total body length (cm) – Av. initial total body length (cm)

NWG = Av. final body weight (g) – Av. initial body weight (g)

SGR (%weight gain day^{-1}) = ln final BW (g) – ln initial BW (g) /culture days x 100

Where, ln = natural logarithm

Feed conversion ratio (FCR) = Feed given (g) / Weight gain (g)

Condition factor (K-value) = Body weight (g) / Body Length (cm) ^{3}×100

Protein efficiency ratio (PER) = Weight gain (g) / Protein intake (g)

3.9.2 Hematological parameters

Blood collection

Blood was collected by the caudal vein puncture and pooled from a random sample of five fish from each replicate after anesthetized by clove oil @ 30-50 mg l^{-1} (1 part clove oil and 9 parts 94% ethanol) (Hajek *et al* 2006). The blood (heparinised 150 IU ml^{-1}) collected from each group was tested for hemoglobin (Hb) and haematocrit (Ht). Blood from experimental fish (5) were collected and replicates were pooled for each treatment for hematological analysis.

3.9.2.1 Hemoglobin (Hb) - Sahli (1962)

Hb is a reasonable index of the red cell population and was estimated by acid haematin method.

Principle: When Hb reacts with 0.1 N HCl, it forms acid hematin which is brown in colour.

Reagents

- 0.1 N Hydrochloric acid (HCl)

Procedure:

- 0.1 N HCl was taken up to the mark 20 in the graduated tube and a drop (0.1 ml) of blood was added.
- It was allowed to stand for 5 minutes until it changes to dark brown colour. The solution was diluted by adding distilled water drop by drop (each time mixing the solution with a stirring rod) until it matches standard colour.
- Then reading was taken from the scale on the graduated tube and the Hb concentration was expressed as gram percent (g %).

3.9.2.2 Hematocrit (Ht) or Packed cell volume (PCV) - Mukherjee (1988)

Ht (%) was estimated by micro-capillary method.

Principle: It is based on the principle of separation of blood by centrifugation.

Procedure:

- In the micro capillary method, filled and sealed capillaries are centrifuged at 10,000 rpm for 8 minutes and subsequently final observations are taken from micro-capillary scale.
- Results were expressed in %.

3.9.3 Biochemical parameters

Biochemical parameters of fish in terms of serum total proteins, globulin, albumin and albumin/globulin ratio were estimated/calculated after completion of the experiment from blood serum by using Erba Manhelm Kit.

Serum collection: For separation of serum, blood samples were withdrawn from the caudal vein and transferred to Eppendorf tubes without anticoagulant. The blood samples were centrifuged at 3000 rpm for 15 minutes and the supernatant serum was collected and stored at -20^0 C until used.

3.9.3.1 Total protein

Total proteins (TP) in blood serum was analysed by following the principle of Biuret reaction (Gornall *et al* 1949)

Principle: The peptide bonds of protein react with copper (Cu^{2+}) ions in alkaline solution to form a blue-violet ion complex, (biuret reaction); each copper ion complexing with 5 or 6 peptide bonds. Tartrate is added as a stabilizer, whilst iodide is used to prevent auto-reduction of the alkaline copper complex. The colour formed is proportional to the protein concentration and is measured at 546 nm (520-560 nm).

Reagents

- Copper II sulphate - 19 mmol l^{-1}
- Potassium sodium tartrate - 43 mmol l^{-1}
- Potassium iodide - 30.0 mmol l^{-1}
- Sodium hydroxide - 600 mmol l^{-1}

Procedure

Reagent blank, standard and test samples were prepared as follows

	Reagent blank	**Standard**	**Sample (Test)**
Reagent (R1)	1000 µl	1000 µl	1000 µl
Distilled water	20 µl	–	–
Standard Reagent (R2)	–	20 µl	–
Sample (Blood serum)	–	–	20 µl

- Reagent 1, Standard Reagent 2 and sample (blood serum) were mixed and incubated for 10 minutes at 37^{o} C.
- Absorbance of the standard and each sample was read at 546 nm (520-560 nm) against reagent blank

Calculations

TP (gdl^{-1}) = Absorbance of sample/Absorbance of standard x Concentration of standard

3.9.3.2 Albumin

Principle: Albumin binds with Bromocresol Green (BCG) at pH 4.2 causing a shift in absorbance of the yellow BCG dye. The blue-green colour formed is proportional to the concentration of albumin, when measured photometrically between 540-630 nm with maximum absorbance at 625 nm.

Reagents

- Bromocresol green - 0.08 mmol l^{-1}
- Succinate buffer - 50 mmol l^{-1}
- Sodium azide - 1.0 g l^{-1}

Procedure

Reagent blank, standard and test samples were prepared as follows

	Reagent blank	Standard	Sample (Test)
Reagent (R1)	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	–	–
Standard Reagent (R2)	–	10 µl	–
Sample (Blood serum)	–	–	10 l

Reagent 1, Standard Reagent 2 and Test Sample were mixed and the absorbance of the standard and each test sample was read at 630 nm (580-630 nm) against reagent blank, after one minute of incubation at 37°C.

Calculation

Albumin (gdl^{-1}) = Absorbance of test sample/Absorbance of Standard x Concentration of standard

3.9.3.3 Globulin and Albumin/Globulin ratio

These were calculated as

Globulin (gdl^{-1}) = Total protein (gdl^{-1}) – Albumin (gdl^{-1})

Albumin/Globulin ratio (gdl^{-1}) = Albumin (gdl^{-1}) / Globulin (gdl^{-1})

3.9.4 Aminotransferases

The aminotransferases including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are indicators of hepatocyte damage. These enzymes are present in hepatocyte cytosol and during episodes of altered plasma membrane permeability, they leak into the extracellular fluid.

3.9.4.1 Aspartate aminotransferase (AST)

AST was estimated with Erba Diagnostic Mannheim GmbH kits **(**International Federation of Clinical chemistry).

Principle: The principle involved in the estimation of AST involves the interactions of L-aspartate and 2-oxoglutarate in the presence of aspartate aminotransferase (AST) to form oxaloacetate. The oxaloacetate in the presence of malate dehydrogenase (MDH) forms malate. The pyruvate present in the sample reacts with lactate dehydrogenase (LDH) to form L-lactate.

AST

L-Aspartate+ 2-Oxoglutarate ----------► Oxaloacetate + L-Glutamate

MDH

Oxaloacetate + NADH ----------►Malate + NAD

LDH

Sample pyruvate + NADH ----------► L-lactate + NAD

Procedure

Pipette	**Sample**
Working reagent	1000 µl
Sample (Blood serum)	100 µl

The serum was mixed well with the working reagent and the absorbance of the sample was read at 340 nm after mixing.

3.9.4.2 Alanine aminotransferase (ALT)

ALT was estimated with Erba Diagnostic Mannheim GmbH kits **(**International Federation of Clinical chemistry).

Principle: The principle involved in the estimation of ALT involves the interactions of L-Alanine and 2-Oxoglutarate in the presence of alanine aminotransferase (ALT) to form pyruvate, which further interacts with lactate dehydrogenase (LDH) and forms L-lactate.

ALT

L-Alanine + 2-Oxoglutarate ----------► Pyruvate + L-Glutamate

LDH

Pyruvate + NADH ----------► L-Lactate + NAD

Pipette	**Sample**
Working reagent	1000 µl
Sample (Blood serum)	100 µl

Procedure

The serum was mixed well with the working reagent and the absorbance of the sample was read at 340 nm after mixing.

3.9.5 Anti- oxidant parameters

Antioxidant parameters were analyzed from blood hemolysate (RBC lysate).

Preparation of haemolysate: Blood haemolysate was prepared before proceeding for different markers to determine erythrocyte oxidative damage**.** Blood samples were centrifuged at 3000 rpm for 15 minutes and supernatant was separated out. The sedimented cells were washed thrice with chilled 0.85% NaCl solution. Washed erythrocytes were lysed with nine parts of distilled water to prepare 10% haemolysate. Haemolysate was stored in aliquots at -20°C for determination of oxidative stress markers.

3.9.5.1 Superoxide dismutase (SOD) - Nishikimi *et al* (1972)

Principle: - The assay is based on the principle that the nitroblue tetrazolium inhibits superoxide dismutase with reduced nicotinamide adenine dinucleotide (NADH) mediated by phenazonium methosulphate under aerobic conditions.

Reagents

- 0.017 M sodium phosphate buffer (pH 8.3)
- Solution 1: 2.052 g of sodium dihydrogen phosphate (NaH_2PO_4) l^{-1} distilled water.
- Solution 2: 2.413 g of disodium hydrogen phosphate (Na_2HPO_4) l^{-1} distilled water.
- Solution 1 (7.36 ml) and solution 2 (92.64 ml) were mixed and diluted to 200 ml with distilled water after adjusting the pH to 8.3.
- 1.5 mM Nitroblue tetrazolium chloride (NBT): 132.26 mg of nitroblue tetrazolium per 100 ml distilled water.
- 2.34 mM Nicotinamide adenine dinucleotide- disodium salt (NADH): 16.6 mg of NADH per 10 ml distilled water.
- 0.093 mM Phenazine methosulphate (PMS): 2.85 mg of phenazonium methosulphate per 100ml distilled water.

Procedure:

- To 2.6 ml of phosphate buffer at 20^0C in the cuvette, 100 µl each of PMS, NBT and haemolysate (1:100 v/v) were added.
- The reaction was initiated by adding 100 µl of NADH and increase in absorbance was recorded at 560 nm for 2 minutes at 30 seconds interval using UV/VIS spectrophotometer.
- Unit of superoxide dismutase was defined was defined as activity of enzyme concentration required to inhibit chromogen production by 50% in 1 min under assay conditions.
- All determinations were performed in triplicate.

Calculations:

$$\text{Erythrocytic SOD activity (U mgl}^{-1}\text{ Hb)} = \frac{\Delta T}{\Delta C/2} \times \frac{100}{Y}$$

ΔT : Change in optical density of test at 30 sec interval

ΔC : Change in optical density of control at 30 sec interval

Y: Haemoglobin concentration in haemolysate (in mg)

3.9.5.2 Lipid peroxidation (LPO) - Placer *et al* (1966)

Principle: The assay is based on the reaction of malondialdehyde (MDA), an end product of lipid peroxidation with thiobarbituric acid to yield a pink coloured trimethine complex exhibiting an absorption maximum at 548 nm wavelength.

Reagents:

- 0.2 M Tris-0.16 M Kcl buffer (pH-7.4): 2.422 g Tris and 1.192 g Kcl per 100 ml distilled water.
- 7% Perchloric acid
- 1N Sodium hydroxide (NaOH): 4g NaOH in 100 ml distilled water.
- Thiobarbituric acid reagent:

- TBA solution: 0.8 g TBA in 100 ml 1N NaOH
- TBA reagent: Two volumes of TBA solution and one volume of 7% perchloric acid.

- Pyridine –n-butanol reagent (3:1, v/v)

Procedure:

- 0.1ml RBC lysate was taken in a test tube and 1.4 ml tris buffer was added to it.
- The contents were incubated for 30 minutes followed by addition of 1.5 ml TBA reagent.
- Mixture was heated in boiling water bath for 10 minutes. 3 ml pyridine-n-butanol reagent and 1 ml NaOH was added to test tube after cooling and mixed properly by shaking.
- Control was neither incubated nor heated.
- Absorbance was recorded at 548 nm using UV/VIS spectrophotometer against distilled water blank. All determinations were performed in triplicate.

Calculation:

$$\text{Erythrocytic lipid peroxidation (nmol MDAg Hb}^{-1}) = \frac{(A_{test} - A_{control}) \times 46 \times 1000}{\gamma}$$

γ: Haemoglobin concentration in g 0.1^{-1} ml haemolysate

3.9.6 Lipid profile

3.9.6.1 Cholesterol

Total cholesterol was estimated by modified Roeschlau (1988) method.

Principle: The conversion of cholesterol ester to cholesterol and fatty acid in the presence of cholesterol esterase (CE). Cholesterol is oxidized to Cholest-4-en-3-one and hydrogen peroxide (H_2O_2) in the presence of cholesterol oxidase. The phenol and 4- aminoantipyrine (4-AAP) present in the cholesterol reagent kit interacts with the hydrogen peroxide in the presence of peroxidase (POD) forms a red dyestuff

Quinoneimine. The absorbance of Quinoneimine so formed is directly proportional to cholesterol concentration.

Cholesterol esterase

Cholesterol esters+H_2O ----------► Cholesterol + fatty acids

Cholesterol oxidase

Cholesterol + O_2 ----------► Cholest-4-en-3-one + H_2O_2

Peroxidase

$2H_2O_2$ + 4-AAP + phenol----------►H_2O + Quinoneimine dye

Procedure: Assay procedure for serum cholesterol estimation

	Blank	**Standard**	**Test**
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	20 µl	---	---
Standard	---	20 µl	---
Sample (Blood serum)	---	---	20 µl

All the reagents mixed well and incubated at 37°C for 10 minutes. Blank was aspirated followed by standard and tests.

3.9.6.2 Triglycerides

Triglyceride was estimated by the method of Fossati *et al* (1969).

Principle: The triglycerides are converted into glycerol and free fatty acids in the presence of lipase. The glycerol and adenosine triphosphate (ATP) in the presence of glycerol kinase forms glycerol-3-phosphate. The glycerol-3-phosphate is oxidized to dihydroxy acetone phosphate (DHAP) and H_2O_2 in the presence of glycerol phosphate oxidase (GPO). The H_2O_2, 4-aminoantipyrine (4-AAP) and 3, 5-Dichloro-2-hydroxybenzene sulfonate (DHBS) forms quinoneimine dye in the presence of peroxidase. The intensity of dye is proportional to the triglycerides concentration in the sample.

Lipoprotein lipase

Triglyceride + H_2O ----------► Glycerol + Fatty acid

Glycerol kinase, Mg^{++}

Glycerol + ATP ----------► Glycerol-3-phosphate + ADP

GPO

Glycerol-3-phosphate + O_2 ----------► DHAP + H_2O_2

Peroxidase

H_2O_2 + 4-AAP + 3, 5-DHBS ----------► Quinoneimine dye + $2H_20$

Procedure: Assay procedure for serum triglyceride estimation

	Blank	**Standard**	**Test**
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	----	----
Standard	----	10 µl	----
Sample (Blood serum)	----	----	10 µl

All the reagents mixed well and incubated at 37°C for 10 minutes. Blank was aspirated followed by standard and tests.

3.9.6.3 Low Density Lipids (LDL)

LDL was estimated with Erba Diagnostic Mannheim GmbH kits **(**International Federation of Clinical chemistry).

Principle: The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. LDL, VLDL, and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER), whereas HDL reacts with the enzymes. Addition of R2 containing a specific detergent releases LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes to produce H_2O_2 which is quantified by the Trinder reaction.

PVS

PEGME

HDL+LDL+VLDL+CM ----------► HDL+(LDL+VLDL+CM). PVS/PEGME

CHOD, CHER

HDL ----------► Fatty Acid + H_2O_2

Detergent

(LDL+VLDL+CM).PVS/PEGME ----------► LDL+(VLDL+CM).PVS/ PEGME

CHOD, CHER

LDL ----------► Fatty Acid + H_2O_2

Peroxidase

2 H_2O_2 + 4-AA + TODB ----------► Quinone + 5 H_2O

Reagent Preparation: Both Reagents R1 and R2 are liquid, ready to use.

Calculation : Results are calculated automatically by the instrument.

3.9.6.4 High Density Lipids (HDL)

HDL was estimated with Erba Diagnostic Mannheim GmbH kits **(**International Federation of Clinical chemistry).

Principle: The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. LDL, VLDL, and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER), whereas HDL reacts with the enzymes. Addition of R2 containing a specific detergent releases LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes to produce H_2O_2 which is quantified by the Trinder reaction.

PVS

PEGME

HDL+LDL+VLDL+CM ----------► HDL+(LDL+VLDL+CM). PVS/PEGME

CHOD, CHER

HDL ----------► Fatty Acid + H_2O_2

Peroxidase

2 H_2O_2 + 4-AA + TODB ----------► Quinone + 5 H_2O

Procedure: Assay procedure for HDL estimation

	Reagent blank	**Sample / Calibrator**
Reagent 1	375 µl	375 µl
Distilled water	5 µl	----
Sample / Calibrator	----	5 µl
Mix and incubate at 37°C for 5 minutes		
Add Reagent 2	125 µl	125 µl
Mix and incubate at 37°C for 5 minutes		

Read final absorbances at the specified wavelength against reagent blank

Calculation:

$$\text{HDL-C} = \frac{\text{(Abs. of Sample - Abs. of Sample blank)}}{\text{(Abs. of Cal. - Abs. of Cal. blank)}} \times \text{Concentration of Calibrator}$$

3.9.6.5 Very Low Density Lipids (VLDL)

VLDL is calculated by the formula = Triglycerides/5

3.9.7 Flesh quality

At the completion of experiment II, flesh samples of fish were collected from all the treatments (each replicate) and flesh quality in terms of total protein, total lipids, total carbohydrates, total ash and moisture content was estimated.

3.9.7.1 Total protein: Total protein of flesh was estimated by following Kjeldahl method as described into section 3.5.1 (followed for protein content in feed).

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

Principle: Lipids are soluble in organic solvents, but sparingly soluble or insoluble in water. Solubility of lipids is an important criterion for their extraction from source material and depends heavily on the type of lipid present, and the proportion of nonpolar (principally triacylglycerols) and polar lipids (mainly phospholipids and

glycolipids) in the sample; therefore, several solvent systems might be considered, depending on the type of sample and its components. The solvents of choice are usually chloroform/methanol or chloroform/methanol/water, in the case of the Folch Method.

Reagents

- Sodium sulphate
- Chloroform
- Methanol
- 0.9% Saline-0.9g NaCl in 100ml distilled water.

Procedure

Extraction

- 1.0 g of flesh was crushed 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 keep for thorough mixing on an electric shaker for 2 hours. The contents were then filtered through sintered funnel (G3) under vacuum.
- To remove water soluble impurities, 0.9% saline ($1/5^{th}$ of filtrate volume) was added in separating funnel, shake for 15 minutes and allowed to stand for 30 minutes for separation of two layers. The pure lipid fraction was collected from the lower chloroform layer.
- The upper layer containing soaps, glycerol, short chain fatty acids and 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 crucible, till the solvent was evaporated completely. Total lipid content was estimated by the following formula.

Observation

W_1 = Weight of empty crucible

W_2 = Weight of crucible+lipid

W = Weight of sample

Calculation

Total lipid (%) = W_2-W_1 / W ×100

3.9.7.3 Total carbohydrate: Total carbohydrate content in flesh was determined by method of Dubois *et al* (1956).

Principle: Simple sugars, oligosaccharides, polysaccharides and their derivatives are stained orange-yellow when treated with phenol and concentrated sulphuric acid. This quality can be utilized to perform quantitative analyses of sugars and their derivatives using colorimetric methods such as spectrophotometry.

Reagents

- 70% and 80% Ethanol
- 5% Phenol
- Concentrated sulphuric acid

Procedure

Extraction

- 100 mg of flesh was crushed using 5ml of 80% ethanol in pestle mortar, so as to extract the carbohydrate.
- The sample was centrifuged and the supernatant was collected in separate test tube. To the residue, 5 ml of 70% ethanol was added.
- The mixture was homogenized in homogenizer. The extraction procedure was repeated with 70% ethanol.
- The supernatant were pooled and volume was made to 25 ml with distilled water.

Estimation:

- 1ml of extract was taken in a test tube and to this 1ml of 5% phenol solution was added followed by the addition of 5 ml of concentrated sulphuric acid.
- The acid was added rapidly, so that the solution got heated up to 70°C for optimum reaction.
- After mixing, the tube was kept for 10 minutes and absorbance was read at 490 nm.
- The values were calculated from the standard curve prepared by using glucose solution.

3.9.7.4 Ash

Principle: Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter *in* a food sample.

Procedure

- Clean and dried crucibles were accurately weighted.
- 1g of sample was taken in the crucibles and kept over the heater till it completely burnt.
- Crucibles were kept in a muffle furnace at 550±10°C for 3 hours.

Observation

W_1 = Weight of empty crucible

W_2 = Weight of crucible + Ash

W = Weight of sample

Calculation:

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

3.9.7.5 Moisture

Principle: The principle of the thermo gravimetric method of moisture content determination is defined as the weight loss of mass that occurs, as the material is

heated. The sample weight is taken prior to heating and again after reaching a steady-state mass subsequent to drying.

Procedure

- Dried and clean crucibles were weighed.
- 1.0 g sample was taken in the crucible and dried in an oven at 100°C±5°C for overnight, till the constant weight was obtained.
- Moisture was calculated by the following formula.

Observation

W_1 = Weight of empty crucible

W_2 = Weight of crucible + dried sample

W = Weight of sample

Calculation

Moisture (%) = $100 - W_2-W_1 / W \times 100$

3.9.8 Meat Quality

Meat quality of the fresh and refrigerated (4^{o}C) product (0, 3, 6 day) and frozen (-20^{o}C) fillet (0, 20, 30, 35, 40 days) was estimated in terms of biochemical parameters after completion of experiment II in terms of following parameters.

3.9.8.1 pH

Principle: pH was recorded by using a digital pH meter (Metler Toledo- FE 20-1).

Procedure:

- Take 15g sample and blend it with 30 ml distilled water at 27 -30^{o}C.
- Take the reading by dipping the pH electrode in prepared sample solution.

3.9.8.2 Peroxide value (PV)

Principle:

The hydroperoxides have the oxidation potential to oxidize iodide to iodine, which is determined by titration against thiosulphate using starch as indicator. The PV is expressed as milli equivalents peroxide kg^{-1} of fat extracted from the fish. The PV

value should not be above 10-20 meq kg^{-1} of fish fat, provided PV has not been lower through extended storage or high temperature exposure.

Reagents:

- **Solvents:** Glacial acetic acid and chloroform.
- **10% Potassium iodide solution:** Dissolve 10 g of potassium iodide in 100 ml of distilled water.
- **Starch solution:** Dissolve 1.0 g of soluble starch in 100 ml of water, boil well before use. Prepare fresh.
- **0.02 N Sodium thiosulfate*:*** Dilute from 0.1 N sodium thiosulfate solution on the day of use.

Procedure:

- Homogenate 5 g of sample with 15 g of anhydrous sodium sulfate to remove the moisture.
- Extract the fat with 50 ml of chloroform and filter the chloroform extract.
- 15 ml of the chloroform filtrate was taken, to this, 15 ml of glacial acetic acid and 10 ml of 10% KI solution was added.
- Solution was kept in dark place for 10 min with occasional shaking and to this, 50 ml of distilled water and 1 ml of starch solution was added.
- Liberated iodine was titrated against 0.02 N sodium thiosulfate until disappearance of blue colour (end point).

Calculation:

$$\text{Peroxide value}\,(\text{meq}\,\text{kg}^{-1}) = \frac{\text{Titrant used (ml) X Normality of } Na_2S_2O_3 \text{ X } 1000}{\text{Wt.of sample (g)}} \times \text{Dilution factor}$$

3.9.8.3 Free fatty acids (FFA)

Principle: Free fatty acids are estimated by titrating it against standard alkali in the presence of meta cresol purple indicator. The acid number is defined as the mg of KOH or NaOH required neutralizing the free fatty acids present in 1g of the sample. However, the free fatty acid content is expressed as oleic acid equivalents.

Reagents:

- **0.05N NaOH:** 20 g NaOH in 500 ml water let it cool down to room temperature and fill it up to one litre.
- **Indicator**: 0.5% metacresol purple
- **Solvent mix:** Chloroform, Methanol and isopropanol (2:1:2)

Procedure:

- 10 g of sample was mixed with 15 g of anhydrous Na_2SO_4 and grinded to remove moisture. Contents were transferred to a conical flask and 50ml of chloroform was added. Contents were mixed well for 10-15 minutes and filtered to get chloroform extract of fat.
- To 10 ml of chloroform extract, 3 drops of 0.5% meta cresol purple was added as indicator.
- Free fatty acid in the extract was titrated to the purple end point using 0.05 N aqueous NaOH solutions.
- Amount of NaOH required for neutralizing free fatty acids present in 1g of fat (Acid number) was found out.
- 10ml of chloroform extract was taken in weighed beaker, evaporated to dryness over water bath and amount of free fatty acid present was calculated.

Calculation:

$$\text{Free fatty acid (\% as oleic acid)} = \frac{\text{Titre value x Normality of NaOH x 2.82}}{\text{Wt.of sample (g)}} \times \text{Dilution factor}$$

3.9.8.4 Titratable acidity (TA)

Principle: Titratable Acidity (TA) is a measurement of the amount of acid present in a solution. It is expressed as grams liter^{-1} (gl^{-1}) and is obtained by multiplying to percent TA by 10. So, a TA of 0.60% is expressed as $6gl^{-1}$. Titratable acidity is expressed as lactic or acetic acid may be used as an indirect measure of bacterial growth in brine or liquid food.

Reagents:

- **0.1N NaOH:** Dissolve 4g of NaOH in I liter distilled water.
- 1% Phenolphthalein Indicator.

Procedure:

- To 10 g of sample, 200 ml of distilled water was added and volume was made to 250 ml in volumetric flask.
- The mixture was filtered through filter paper.
- 25 ml of filtrate was collected, to which, 75 ml distilled water and 2-3 drops of phenolphthalein indicator were added.
- It was titrated with 0.1 NaOH Solution till light pink
- Volume of NaOH solution used was recorded.

Calculation:

Titratable Acidity (%)

$$= \frac{\text{Vol.of 0.1 N NaOH used (ml)x milli}-\text{equivalent weight of lactic acid}}{\text{Wt.of sample (g)}} \times 100$$

Milli-equivalent weight of lactic acid = 90/100 = 0.09

3.9.8.1.5 Total volatile base-nitrogen (TVB-N)

Principle: The volatile bases in most species of fish consist of ammonia together with appreciable amounts of amines. In meat, trymethylamine is only present in significant quantities and total volatile nitrogen consists almost entirely as ammonia. As ammonia production due to de-amination of protein increases during spoilage, its determination represents a simple method of following the course of determination of the quality lean meat. Meat extract is treated with relatively weak alkali and the volatile base is distilled or diffused over into standard acid or boric acid. TVBN values are expressed as mg%

Reagents:

- **7.5% TCA (trichloroacetic acid) solution:** Dissolve 7.5g of TCA in 100 ml of distilled water.

- **10% NaOH:** Dissolve 10g of NaOH in 100 ml of distilled water.
- **4% boric acid:** Dissolve 4g of boric acid in 100 ml of distilled water.
- **0.1 N Sulphuric acid**

Procedure:

- 100 grams of sample was homogenized in 200 ml of 7.5% aqueous TCA (trichloroacetic acid) solution.
- The homogenate was centrifuged at 400 x g for 5 min and the supernatant liquid was filtered through a funnel using a Whatman No. 3 filter paper.
- Steam distillation was carried out using a Kjeldahl-type distillator.
- Twenty-five milliliters of filtrate were loaded into the distillation tube followed by 5 ml of 10% NaOH.
- A beaker containing 10 ml of a 4% boric acid solution and methyl red and bromocresol green indicator for titration of ammonia was placed at the end of the condenser.
- Distillation was continued until a final volume of 50 ml was obtained in the beaker (40 ml of distillate).
- The boric acid solution turned green when alkalinized by the distilled TVBN.
- This was titrated using a 0.01-ml graduated micro burette containing an aqueous 0.1 N sulfuric acid solution.
- Complete neutralization was obtained when the color turned pink on the addition of a further drop of sulfuric acid.

Calculation:

The quantity of TVBN in mg was determined from the volume of sulfuric acid (n ml) added as follows:

TVBN = n × 16.8 mg of nitrogen/100 g

3.9.9 Sensory Evaluation

The sensory evaluation of product (fish fingers) was estimated at 0, 3 and 6 day by 9 trained panelists, using a **nine point (1-9) hedonic scale** for product acceptability.

Hedonic Scale	Particular
9	**Like Extremely**
8	**Llike Very Much**
7	**Like Moderately**
6	**Like Slightly**
5	**Neither Like nor Dislike**
4	**Dislike Slightly**
3	**Dislike Moderately**
2	**Dislike Very Much**
1	**Dislike Extremely**

Panelists scored for sensory parameters including appearance, odour/smell, crispiness, juiciness, texture, flavour, taste and overall acceptability. During the evaluation sessions, the samples were coded by a number and presented in random order.

3.9.10 Texture Evaluation

Texture evaluation of fish product and fillet was done on Taxt-plus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK), attached with a 50 kg load cell. For fish product (fish fingers), a blade set (HDP/BSW) was used with a pre-test, test and post-test speed of 1 mm sec^{-1}, 1 mm sec^{-1} and 10 mm sec^{-1}, respectively. For fillet, blade set (HDP/BSW) was used with a pre-test, test and post-test speed of 1 mm sec^{-1}, 10 mm sec^{-1} and 10 mm sec^{-1}, respectively. Fish product and fillets were compressed to 20 mm at room temperature (30^{o}C) in auto force mode (10 g).

Three measurements were made for each treatment and the average value was reported for each parameter. Textural parameters i.e. Firmness (N) and work of shear (N.sec) were evaluated for product (fish fingers), whereas for fish fillet, cutting strength (N) and work of shear (N.sec) were calculated with the help of software provided along with the instrument.

3.9.11 Relative Productivity and Economics

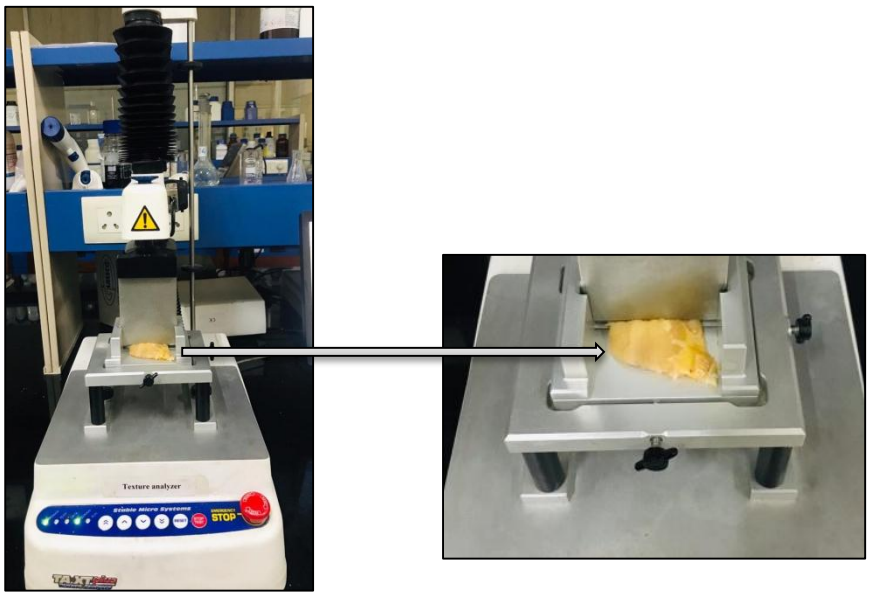
- Relative productivity and economics was worked out to know the efficiency of the fish silage and linseed oil supplemented diets for their application at farmer level.

3.9.12 Statistical analysis

Statistical analysis of the data was performed with a statistical package (SPSS 20.0 for Windows, SPSS Inc., Richmond, CA, USA). Significant ANOVA followed by Duncan's multiple comparison to determine difference between treatments (experiment I and II) was applied to work out the effect of experimental diets on water quality parameters, survival, growth, hematology, biochemical parameters, transaminases, antioxidant status, lipid profile, flesh quality and meat and sensory quality of fish product (fish fingers) and meat quality and texture analysis of fillet ($p \leq 0.5$).

Sensory Evaluation of Fish Fingers by Team of Panelists

Fish Fillet **Fish Product (Fish Fingers)**



Texture Analysis of Fillet on Texture Analyzer

Plate II – Sensory &Texture Evaluation of fish fingers & fish fillet

Chapter IV

RESULTS AND DISCUSSION

The results of the present study are covered under the following heads:

4.1 Experiment I – "Growth performance and health status of pangas catfish (*Pangasianodon hypophthalmus*) fry fed on fish silage supplemented formulated diets"

4.1.1 Physico-chemical parameters of water

4.1.2 Survival and growth parameters

4.1.3 Hematological parameters

4.1.4 Biochemical parameters

4.1.5 Antioxidant status

4.1.6 Serum Transaminases

4.1.7 Lipid profile

4.2 Experiment II – "Growth performance, health status and meat quality of Pangas catfish (*Pangasianodon hypophthalmus*) fingerling fed on fish silage and linseed oil supplemented formulated feeds for grow-out production"

In addition to parameters studied in experiment I, following parameters were studied after completion of experiment II.

4.2.1 - 4.2.7 (Similar as in Experiment I - 4.1.1 to 4.1.7)

4.2.8 Flesh quality

4.2.9 Meat quality

4.2.10 Sensory evaluation

4.2.11 Texture analysis

4.2.12 Relative productivity and economics of treatments

4.1.1 Physico-chemical parameters of water

Physico-chemical parameters of water in experimental FRP pools were analyzed every fortnight during 120 days of experimental period (July 2018 to October 2018).

4.1.1.1 Temperature

The water temperature (°C) fluctuated between 30.13 to 36.67 in different treatments during the experimental period (Table 4, Fig 1). Among different treatments, mean temperature (°C) was 33.48, 33.40, 33.30, 33.23, 33.26 and 33.24 in D1, D2, D3, D4, D5 and D6 respectively (Table 4) and the difference among treatments were insignificant.

Fish being cold blooded animal, therefore its growth, survival and overall production depends on water temperature. Moreover, each species has a preferred or optimum temperature range, where it can grow to maximum and above or below this optimum range, survival as well as growth is reduced. In the present study, temperature of experimental water ranged between 30.13 to 36.67°C with mean temperature remaining around 33°C. Abedin *et al* (2017) reported temperature range of 29.0 to 34°C as optimum for pangas culture under semi-intensive culture systems.

4.1.1.2 pH

The pH of water fluctuated between 7.24 to 8.00 in different treatments during the experimental period (Table 5, Fig 2). Among different treatments, mean pH was 7.11 in D1 and D4; 7.07 in D2, D5 and D6; 7.15 in D3 respectively (Table 5) and the differences among treatments were insignificant ($p \geq 0.05$).

Water pH plays an important role in optimum physiological activities of fish, decomposition of dead organic matter and release of nutrients from bottom soil and hence fish growth and productivity. The pH scale extends from 0 to 14 with 0 being the most acidic and 14 as the most alkaline. pH 7 is a condition of neutrality and for most of the aquaculture practices, pH should remain in the range 7.0 to 9.0 with optimum of 7.5 to 8.5.

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

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
July	**0**	36.67 ^{a} ±0.13	34.33^{ab}±0.12	34.30^{ab}±0.11	34.13^{b}±0.08	34.40^{ab}±0.10	34.63^{a}±0.09
July	**15**	34.66^{ab}±0.08	34.53^{ab}±0.08	34.83^{a}±0.03	34.33 ^{b}±0.24	34.56^{ab}±0.03	34.66^{ab}±0.12
July	**30**	34.70 ^{a} ±0.11	34.63 ^{a}±0.03	34.66^{a}±0.06	34.50 ^{a}±0.05	34.40 ^{a}±0.10	34.40 ^{a}±0.12
August	**45**	34.63^{b} ±0.14	34.33^{ab}±0.08	34.13^{b}±0.03	34.33^{ab}±0.06	34.33^{ab}±0.08	34.03^{b}±0.12
August	**60**	33.90 ^{a} ±0.05	33.83^{ab}±0.18	34.03^{a}±0.12	33.86^{ab}±0.03	33.5^{bc} ±0.03	33.50^{bc}±0.06
September	**75**	33.73 ^{a} ±0.12	33.73 ^{a}±0.06	33.56^{a}±0.06	33.66 ^{a}±0.14	33.70 ^{a}±0.10	33.75 ^{a}±0.12
September	**90**	32.20 ^{b} ±0.08	32.80 ^{a}±0.05	32.30^{b}±0.05	32.73 ^{a}±0.08	32.80 ^{a}±0.05	32.70 ^{a}±0.05
October	**105**	32.26 ^{a} ±0.18	31.63^{b} ±0.12	31.36^{ab}±0.08	31.36^{ab}±0.18	31.30^{ab}±0.17	31.06 ^{c}±0.06
October	**120**	30.56^{ab}±0.04	30.80^{a} ±0.05	30.50^{ab}±0.05	30.13 ^{b}±0.06	30.26^{b}±0.08	30.46^{ab}±0.03
Mean		**33.48 ^{a} ±0.28 (30.56-36.67)**	**33.40 ^{a}±0.26 (30.80-34.63)**	**33.30 ^{a}±0.29 (30.50-34.83)**	**33.23 ^{a}±0.29 (30.13-34.50)**	**33.26 ^{a}±0.28 (30.26-34.56)**	**33.24 ^{a}±0.29 (30.26-34.56)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly (p≤0.05)
Value in parenthesis depicts range

Table 5: Water pH in different treatments during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
July	**0**	7.81 ^{a} ±0.02	7.65 ^{a} ±0.07	7.90^{a}±0.06	8.00 ^{a}±0.10	7.92 ^{a}±0.05	7.67 ^{a}±0.08
July	**15**	7.62 ^{a} ±0.08	7.71 ^{a} ±0.07	7.86^{a}±0.08	7.69 ^{a}±0.35	7.57 ^{a}±0.09	7.45 ^{a}±0.23
July	**30**	7.80 ^{a} ±0.01	7.74 ^{ab}±0.05	7.88^{a}±0.07	7.24^{c}±0.05	7.47^{bc}±0.11	7.67^{ab}±0.14
August	**45**	7.58 ^{a} ±0.18	7.64 ^{a} ±0.03	7.72^{a}±0.06	7.58 ^{a}±0.18	7.64 ^{a}±0.03	7.72 ^{a}±0.06
August	**60**	7.64 ^{a} ±0.12	7.68 ^{a} ±0.14	7.77^{a}±0.12	7.64 ^{a}±0.11	7.42 ^{a}±0.53	7.74^{a}±0.56
September	**75**	7.92 ^{a} ±0.19	7.81 ^{a} ±0.06	7.37^{a}±0.18	7.50 ^{a}±0.35	7.89 ^{a}±0.25	7.47 ^{a}±0.23
September	**90**	7.25 ^{b} ±0.02	7.57^{ab} ±0.05	7.89^{a}±0.09	7.57^{ab}±0.04	7.68 ^{a}±0.04	7.84 ^{a}±0.08
October	**105**	7.31 ^{b} ±0.06	7.51^{ab} ±0.11	7.77^{a}±0.07	7.47^{ab}±0.10	7.82 ^{a}±0.11	7.66^{ab}±0.15
October	**120**	7.86 ^{a} ±0.33	7.90 ^{a} ±0.40	7.61^{a}±0.33	7.49 ^{a}±0.15	7.26 ^{a}±0.21	7.70 ^{a}±0.15
Mean		**7.11 ^{a} ±0.08 (7.31-7.92)**	**7.07 ^{a} ±0.05 (7.51-7.90)**	**7.15 ^{a} ±0.08 (7.37-7.90)**	**7.11 ^{a} ±0.06 (7.24-8.00)**	**7.07 ^{a} ±0.09 (7.26-7.92)**	**7.07 ^{a} ±0.05 (7.45-7.84)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
Value in parenthesis depicts range

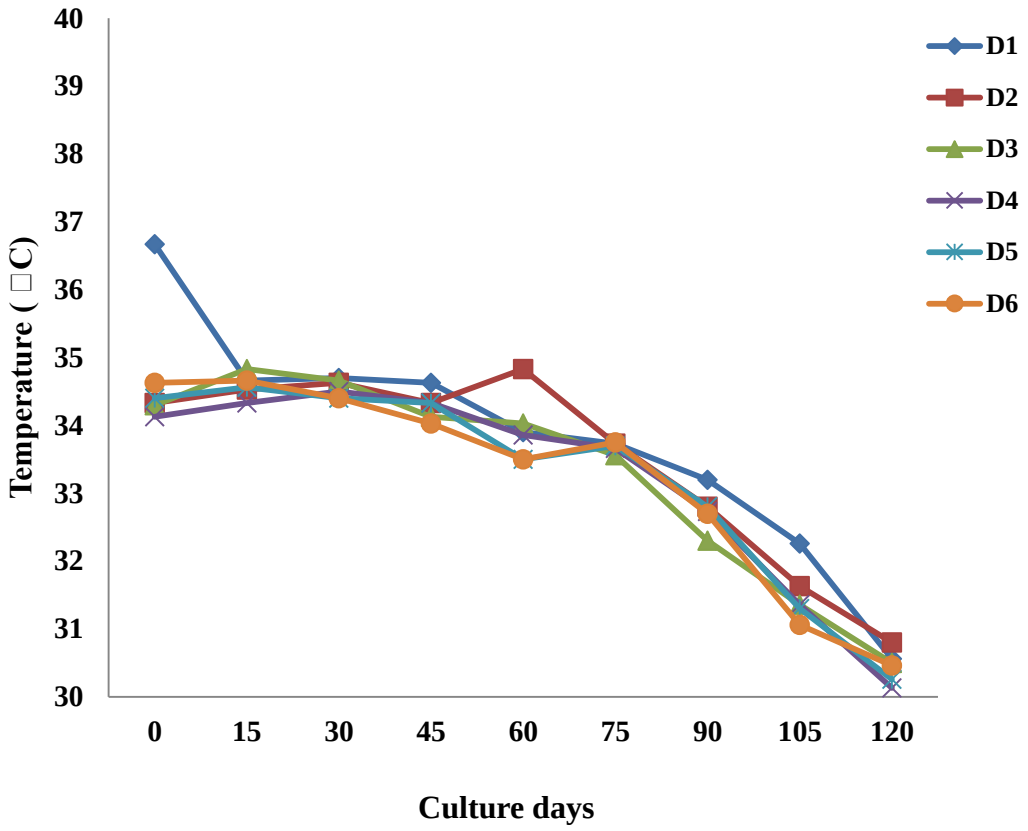


Fig 1: Changes in water temperature (ºC) in different treatments during the experimental period

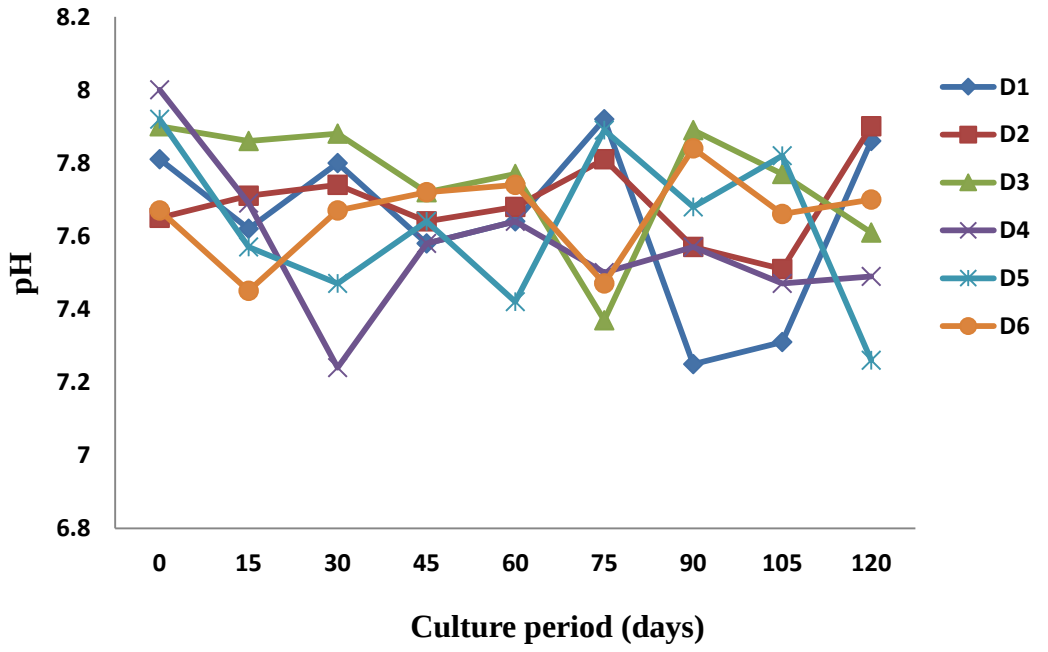


Fig 2: Changes in water pH in different treatments during the experimental period

During the present study, pH remained well within the range (7.24 to 8.00), depicting well buffered conditions of water in all the treatments. Consistency and uniformity w.r.t pH also revealed that fish silage supplemented diets did not have any detrimental effect on water quality (Santosh and Singh 2007). Sarker (2006) too reported optimum pH range of 6.8 to 7.4 with mean pH of 7.06 for satisfactory growth of *P. hypophthalmus* under monoculture and polyculture conditions.

4.1.1.3 Dissolved Oxygen (DO)

The Dissolved Oxygen (DO) of water fluctuated between 9.86 to 12.46 mg l^{-1} in different treatments during the experimental period (Table 6, Fig 3). Among different treatments, mean DO (mg l^{-1}) was 10.75, 10.66, 10.83, 10.71, 10.61 and 10.96 in D1, D2, D3, D4, D5 and D6, respectively (Table 6) and the differences among treatments were insignificant ($p \leq 0.05$).

Dissolved oxygen is probably the most critical water quality parameter affecting survival and growth of aquatic organisms. It regulates all the metabolic activities of aquatic organisms and is also a very good indicator of water health. It affects the growth, survival, distribution, behavior and physiology of aquatic organisms (Solis 1988). Oxygen depletion in water leads to poor feeding of fish, starvation, reduced growth and hence fish mortality, either directly or indirectly. The findings of the present study are in line with the reports of Bhatnagar and Singh (2010) and Bhatnagar *et al* (2004), according to which DO level >5 mg l^{-1} is essential to support good fish production. During the present study, mean DO level remained well above the optimum level of 5 mg l^{-1} in all the treatments. Bhatnagar *et al* (2004) also suggested that 1-3 mg l^{-1} of DO has sub-lethal effect on growth and feed utilization; 0.3-0.8 mg l^{-1} is lethal to fishes and >14 mg l^{-1} is lethal to fish fry resulting in gas bubble disease. DO less than 1 mg l^{-1} lead to death of fish, and at less than 5 mg l^{-1} fish survive, but grow slowly and will be sluggish, whereas DO of 5 mg l^{-1} and above is desirable. Ekubo and Abowei (2011) also recommended that fish can die if exposed to less than 0.3 mg l^{-1} of DO for a long period of time, minimum concentration of 1.0 mg l^{-1} DO is essential to sustain fish for long period and 5.0 mg l^{-1} are adequate in fish ponds. According to Santhosh and Singh (2007), catfishes and other air breathing fishes can survive in low oxygen concentration of 4 mg l^{-1}.

Table 6: Dissolved oxygen content (mg l^{-1}) of water during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
July	**0**	10.33^{ab}±0.19	10.13^{ab}±0.18	10.00^{b}±0.21	9.93^{b}±0.38	10.43^{ab}±0.24	10.80^{a}±0.10
July	**15**	9.86^{b} ±0.18	11.13^{a}±0.23	10.11^{b}±0.16	10.47^{ab}±0.15	10.28^{ab} ±0.13	10.67^{ab}±0.17
July	**30**	11.16^{a}±0.82	10.00^{a} ±0.30	11.06^{a}±0.67	10.49^{a}±0.52	10.60^{a} ±0.41	11.26^{a} ±0.67
August	**45**	11.76^{a}±0.64	11.93^{a} ±0.23	10.86^{a}±0.89	11.53^{a} ±0.83	11.26^{a} ±0.76	12.00^{a} ±0.87
August	**60**	11.06^{a}±0.81	11.20^{a} ±0.26	10.40^{a}±0.30	10.93^{a} ±0.88	10.20^{a} ±0.23	12.46^{a} ±0.17
September	**75**	11.13^{a}±0.63	11.33^{a}±0.85	12.20^{a}±0.72	11.00^{a}±0.83	10.06^{a}±0.37	11.00^{a}±0.61
September	**90**	10.06^{a}±0.26	9.86^{a}±0.17	11.06^{a}±0.67	11.26^{a}±0.76	11.06^{a}±0.67	9.90^{a}±0.55
October	**105**	11.20^{a}±0.60	10.00^{a}±0.30	11.26^{a}±0.63	10.20^{a}±0.23	11.33^{a}±0.64	10.20^{a}±0.23
October	**120**	10.18^{a}±0.14	10.40^{a}±0.23	10.53^{a}±0.20	10.63^{a}±0.14	10.34^{a}±0.27	10.13^{a}±0.17
Mean		**10.75^{a} ±0.19 (9.86-11.76)**	**10.66^{a} ±0.24 (9.86-11.93)**	**10.83^{a} ±0.21 (10.00-12.20)**	**10.71^{a} ±0.19 (9.93-11.53)**	**10.61^{a} ±0.16 (10.20-11.33)**	**10.96^{a} ±0.21 (9.90-12.46)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
Value in parenthesis depicts range

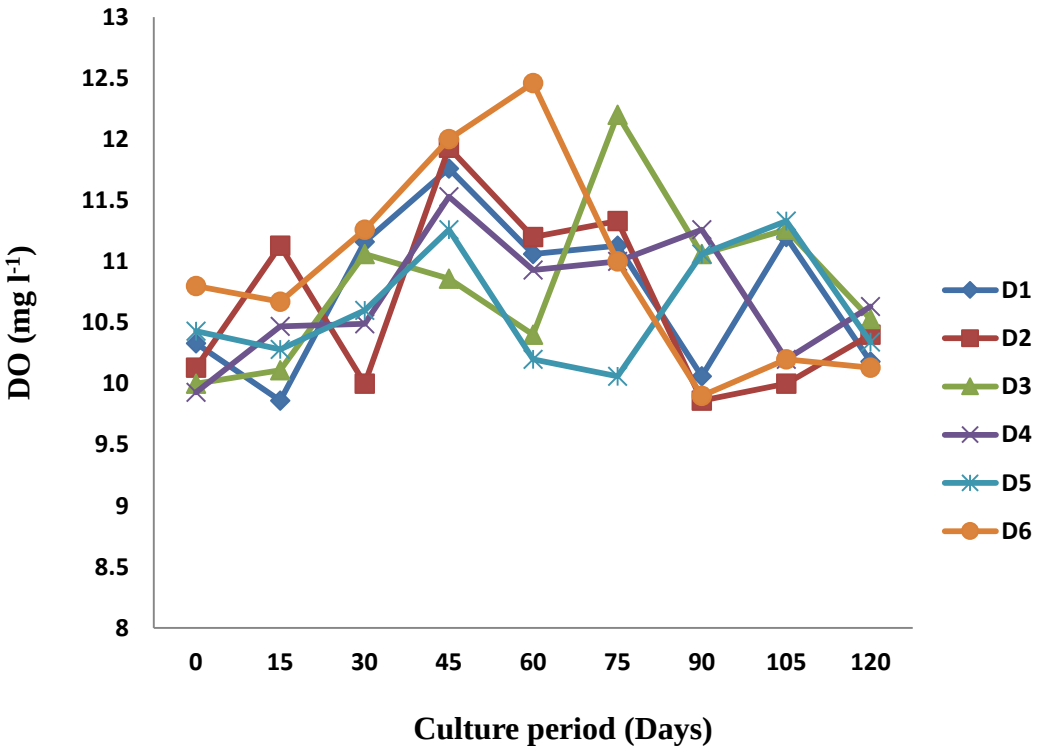


Fig 3: Changes in Dissolved oxygen (mg l^{-1}) of water in different treatments during the experimental period

4.1.1.4 Total alkalinity (TA)

The total alkalinity ($CaCO_3$ mg l^{-1}) of water fluctuated between 130 to 167 in different treatments during the experimental period (Table 7, Fig 4). Among different treatments, mean TA was 152 in D1; 154 in D2 and D3; 155, 153 and 151 in D4, D5 and D6, respectively (Table 7) and the differences among the treatment were insignificant ($p \leq 0.05$).

Alkalinity is the water's ability to resist changes in pH and is a measure of the total concentration of bases in pond water including carbonates, bicarbonates, hydroxides, phosphates and borates, dissolved calcium, magnesium, and other compounds in the water. If the alkalinity of water is less than 20 mg l^{-1}, it indicates that even a small amount of acid can cause a large change in pH of water.

Wurts and Durborow (1992) suggested alkalinity should range between 75 to 200 mg l^{-1}, but it should never be less than 20 mg l^{-1}. Swann (1997) too recommended total alkalinity values of at least 20 mg l^{-1} for aquaculture

Table 7: Total alkalinity ($CaCO_3$ mg l^{-1}) of water during the experimental period

Month	Culture Days	Treatments					
		D1	**D2**	**D3**	**D4**	**D5**	**D6**
July	**0**	144 ^{a} ±3.05	149 ^{a} ±2.40	142 ^{a} ±1.15	148 ^{a} ±3.46	140 ^{a} ±9.45	136 ^{a} ±5.03
July	**15**	150 ^{a} ±1.04	152 ^{a} ±3.05	149 ^{a} ±1.76	157 ^{a} ±3.00	160 ^{a} ±2.40	154 ^{a} ±3.05
July	**30**	142 ^{c} ±1.33	144 ^{c} ±1.15	154 ^{b} ±2.40	164 ^{a} ±0.66	156 ^{b} ±2.30	159 ^{b} ±0.66
August	**45**	163 ^{a} ±1.76	163 ^{a} ±3.71	154 ^{a} ±1.36	166 ^{a} ±1.33	154 ^{a} ±2.29	152 ^{a} ±3.21
August	**60**	166 ^{a} ±2.90	167 ^{a} ±1.00	159^{ab}±1.45	148 ^{b} ±2.66	163^{ab}±2.91	151^{ab}±3.54
September	**75**	151 ^{a} ±2.84	149 ^{a} ±2.44	160 ^{a} ±2.37	149 ^{a} ±1.56	162 ^{a} ±1.76	166 ^{a} ±2.30
September	**90**	148 ^{a} ±2.11	157 ^{a} ±1.76	146 ^{a} ±2.33	147 ^{a} ±1.33	130 ^{b} ±2.40	154 ^{a} ±3.52
October	**105**	154^{a} ±4.66	148^{ab} ±2.30	148^{ab}±3.28	152^{a}±2.33	159 ^{a} ±2.90	143^{ab}±3.33
October	**120**	145 ^{b} ±1.66	151 ^{b} ±2.40	165 ^{a} ±1.66	161 ^{a} ±2.88	150 ^{b} ±2.66	146 ^{b} ±3.75
Mean		**152 ^{a} ±2.53 (142-166)**	**154 ^{a} ±1.55 (144-167)**	**154 ^{a} ±1.81 (142-165)**	**155 ^{a} ±2.02 (148-166)**	**153 ^{a} ±2.40 (130-163)**	**151 ^{a} ±2.00 (136-159)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
Value in parenthesis depicts range

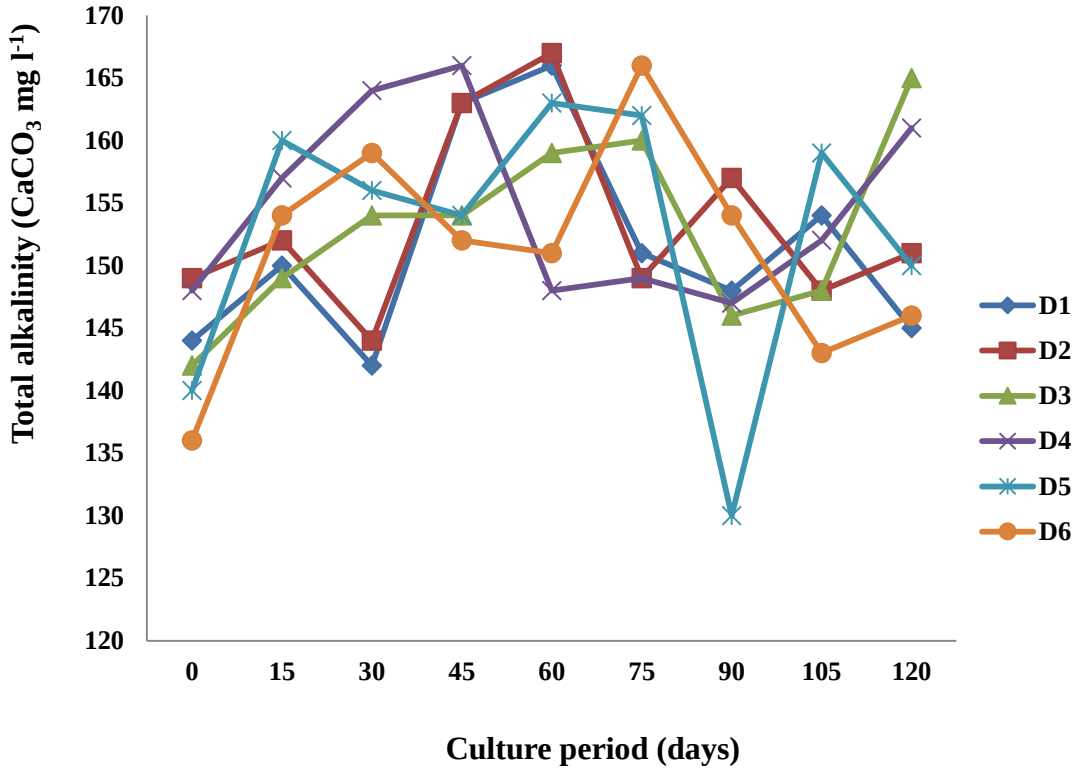


Fig 4: Changes in total alkalinity ($CaCO_3$ mg l^{-1}) of water in different treatments during the experimental period

production including catfish culture and for good pond productivity. Bhatnagar *et al* (2004) too suggested total alkalinity of <20 mg l^{-1} indicates poor status of water body, 20-50 mg l^{-1} shows low to medium and 80-200 mg l^{-1} as desirable for fish/prawn, whereas >300 mg l^{-1} is undesirable due to non-availability of CO_2. Stone and Thomforde (2004) also suggested 50-150 mg l^{-1} ($CaCO_3$) as desirable range of total alkalinity; an acceptable range of above 20 mg l^{-1} and less than 400 mg l^{-1} for ponds and above 10 mg l^{-1} for hatchery water. In the present study, total alkalinity remained in the desirable range throughout the experimental period.

4.1.1.5 Ammonical-nitrogen (NH_3-N)

The NH_3-N (mg l^{-1}) of water ranged between 0.01 to 0.07 in different treatments during the experimental period (Table 8, Fig 5). Among different treatments, mean NH_3-N was 0.05 in D1, D2, D4, D5, D6 and 0.06 in D3 respectively (Table 8) and the differences among treatments were insignificant ($p \leq 0.05$).

Table 8: Ammonical nitrogen (mg l^{-1}) of water during the experimental period

Month	Culture Days	Treatments					
		D_1	**D_2**	**D_3**	**D_4**	**D_5**	**D_6**
July	**0**	$0.10^{a}\pm0.06$	$0.06^{a}\pm0.04$	$0.07^{a}\pm0.03$	$0.05^{a}\pm0.02$	$0.03^{a}\pm0.009$	$0.04^{a}\pm0.02$
July	**15**	$0.04^{a}\pm0.01$	$0.03^{a}\pm0.006$	$0.01^{a}\pm0.006$	$0.02^{a}\pm0.01$	$0.03^{a}\pm0.01$	$0.04^{a}\pm0.008$
July	**30**	$0.01^{a}\pm0.003$	$0.03^{a}\pm0.01$	$0.07^{a}\pm0.04$	$0.04^{a}\pm0.01$	$0.02^{a}\pm0.005$	$0.06^{a}\pm0.008$
August	**45**	$0.05^{ab}\pm0.01$	$0.07^{a}\pm0.003$	$0.07^{a}\pm0.003$	$0.07^{a}\pm0.003$	$0.05^{ab}\pm0.01$	$0.04^{b}\pm0.01$
August	**60**	$0.04^{a}\pm0.008$	$0.04^{a}\pm0.01$	$0.03^{a}\pm0.003$	0.05 ± 0.02^{a}	$0.05^{a}\pm0.005$	$0.05^{a}\pm0.02$
September	**75**	$0.04^{ab}\pm0.01$	$0.04^{ab}\pm0.008$	$0.07^{a}\pm0.01$	$0.05^{ab}\pm0.004$	$0.04^{ab}\pm0.002$	$0.03^{b}\pm0.006$
September	**90**	$0.07^{a}\pm0.003$	$0.05^{a}\pm0.01$	$0.05^{a}\pm0.02$	$0.04^{a}\pm0.008$	$0.06^{a}\pm0.01$	$0.07^{a}\pm0.003$
October	**105**	$0.06^{ab}\pm0.005$	$0.03^{b}\pm0.014$	$0.05^{ab}\pm0.008$	$0.06^{ab}\pm0.01$	$0.07^{a}\pm0.003$	$0.06^{b}\pm0.01$
October	**120**	$0.05^{a}\pm0.01$	$0.04^{a}\pm0.01$	$0.06^{a}\pm0.008$	$0.04^{a}\pm0.01$	$0.05^{a}\pm0.01$	$0.06^{a}\pm0.008$
Mean		**$0.05^{a}\pm0.007$ (0.01-0.10)**	**$0.05^{a}\pm0.006$ (0.03-0.06)**	**$0.06^{a}\pm0.007$ (0.01-0.07)**	**$0.05^{a}\pm0.005$ (0.02-0.07)**	**$0.05^{a}\pm0.004$ (0.02-0.07)**	**$0.05^{a}\pm0.004$ (0.03-0.07)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p\leq0.05$)
Value in parenthesis depicts range

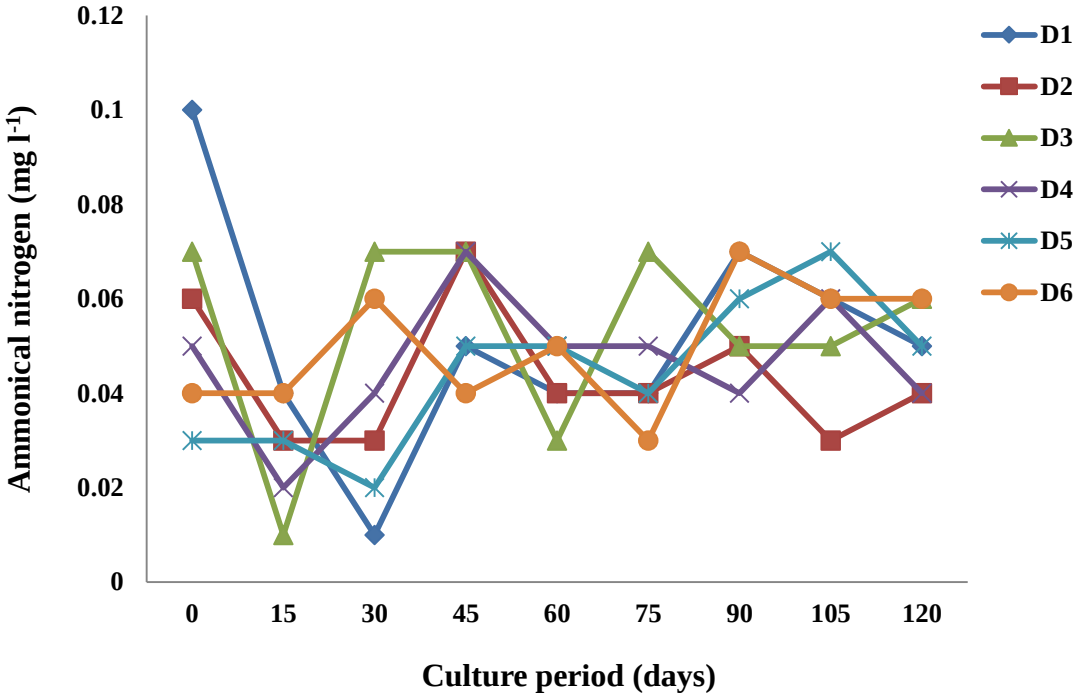


Fig 5: Changes in Ammonical nitrogen (mg l^{-1}) of water in different treatments during the experimental period

Ammonia is the principal nitrogenous waste product excreted by most fishes. High ammonia can be due to over feeding with protein rich feed, that ultimately can decay to liberate toxic ammonia gas. In water, ammonia is present as molecular (NH_3) and ionic (NH_4^+) form and the ratio between these two forms depends on the pH and water temperature. In an unpolluted water body, it concentration is very low (<0.1 mg l^{-1}) however, it may increase up to 12 mg l^{-1} or more in organic rich water, which may become lethal to fishes (Abedin *et al* 2017). Jhingran (1991) reported that fish is very sensitive to unionized ammonia (NH_3) and its acceptable range is 0.02 – 0.05 mg l^{-1}, with permissible upper limit of 0.1 mg l^{-1} (Boyd 1988). The mean ammonical nitrogen during the present experimental period ranged between 0.05 to 0.06 mg l^{-1}, well within the desirable range (0.05 to 0.096 mg l^{-1}) as reported by Alim (2009).

4.1.1.6 Nitrite-nitrogen (NO_2^--N)

The NO_2^--N of water (mg l^{-1}) ranged between 0.016 to 0.091 mg l^{-1} in different treatments during the experimental period (Table 9, Fig 6). Among different treatments, mean NO_2^--N was 0.04 in D1 and D6 and 0.06 in remaining treatments (D2, D3, D4 and D5) (Table 9) and the differences among treatments were insignificant ($p \leq 0.05$).

Table 9: Nitrite nitrogen (mg l^{-1}) of water during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
July	**0**	0.023^{d}±0.001	0.065^{b}±0.03	0.045^{c}±0.004	0.045^{c}±0.009	0.091^{a}±0.002	0.054^{a}±0.002
July	**15**	0.016^{e}±0.002	0.035^{d}±0.02	0.046^{c}±0.01	0.087^{a}±0.02	0.047^{c}±0.02	0.065^{b}±0.004
July	**30**	0.085^{a}±0.008	0.063^{b}±0.003	0.057^{c}±0.01	0.042^{d}±0.02	0.064^{b}±0.01	0.035^{e}±0.02
August	**45**	0.035^{d}±0.003	0.057^{c}±0.006	0.065^{b}±0.008	0.054^{c}±0.09	0.070^{a}±0.05	0.054^{c}±0.006
August	**60**	0.030^{d}±0.006	0.045^{c}±0.04	0.081^{a}±0.005	0.045^{c}±0.03	0.064^{b}±0.09	0.023^{e}±0.05
September	**75**	0.050^{c}±0.001	0.068^{b}±0.007	0.045^{d}±0.01	0.057^{c}±0.06	0.064^{b}±0.05	0.085^{a}±0.06
September	**90**	0.069^{b}±0.001	0.081^{a}±0.003	0.048^{c}±0.08	0.069^{b}±0.01	0.061^{b}±0.09	0.045^{c}±0.05
October	**105**	0.045^{c}±0.002	0.060^{b}±0.01	0.085^{a}±0.05	0.039^{d}±0.06	0.049^{c}±0.02	0.081^{a}±0.04
October	**120**	0.025^{e}±0.004	0.043^{c}±0.006	0.056^{b}±0.008	0.090^{a}±0.02	0.039^{d}±0.09	0.091^{a}±0.002
Mean		**0.04^{a} ±0.02 (0.016-0.085)**	**0.06^{a} ±0.04 (0.035-0.081)**	**0.06^{a} ±0.02 (0.045-0.085)**	**0.06^{a} ±0.01 (0.039-0.090)**	**0.06^{a} ±0.03 (0.039-0.091)**	**0.04^{a} ±0.02 (0.016-0.085)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
Value in parenthesis depicts range

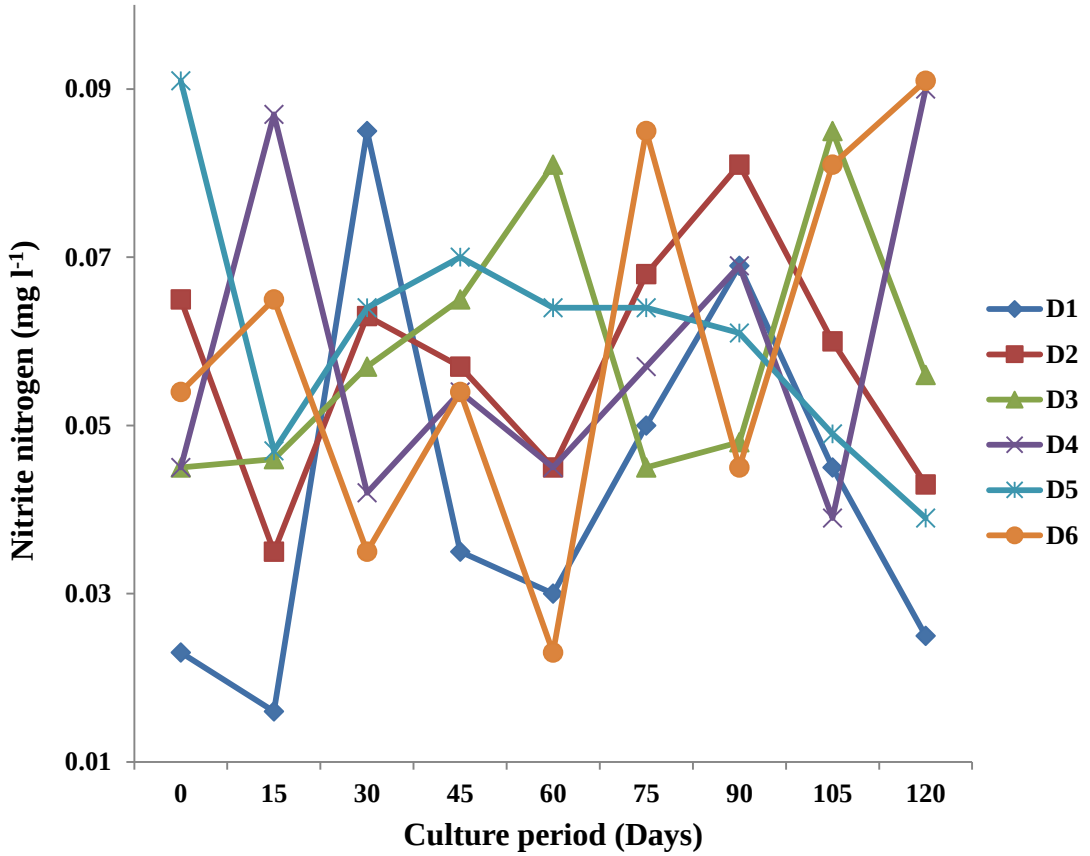


Fig 6: Changes in Nitrite nitrogen (mg l^{-1}) of water in different treatments during the experimental period

Nitrite concentration is directly associated with ammonia level under culture conditions. It is produced in first stage of ammonia decomposition with help of aerobic bacteria *Nitrosomonas*. The results with respect to NO_2^--N during the present study, were well within the permissible levels (<0.3 mg l^{-1}) as reported by Boyd (1988) and Ahsan (2009), which revealed that the water was well buffered in all the treatments.

4.1.1.7 Nitrate-nitrogen (NO_3^--N)

The nitrate nitrogen (mg l^{-1}) of water ranged between 0.09 to 0.70 (mg l^{-1}) in different treatments during the experimental period (Table 10, Fig 7). Among different treatments, mean NO_3^--N level was 0.53, 0.39, 0.48, 0.47, 0.45 and 0.53 in D1, D2, D3, D4, D5 and D6, respectively (Table 10) and the differences among treatments were insignificant ($p \leq 0.05$).

Table 10: Nitrate nitrogen (mg l^{-1}) of water during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
July	**0**	0.44^{a}±0.08	0.26^{ab}±0.14	0.09^{b}±0.06	0.18^{ab}±0.04	0.42^{a}±0.04	0.35^{ab}±0.09
July	**15**	0.38^{a} ±0.04	0.26^{a} ±0.05	0.34^{a} ±0.05	0.52^{a}±0.16	0.41^{a}±0.03	0.26^{a}±0.02
July	**30**	0.85^{a} ±0.05	0.54^{b} ±0.18	0.64^{ab} ±0.06	0.57^{ab}±0.04	0.36^{b} ±0.04	0.37^{b}±0.08
August	**45**	0.21^{ab} ±0.04	0.15^{b} ±0.04	0.25^{ab} ±0.10	0.47^{ab}±0.14	0.45^{ab}±0.15	0.59^{a}±0.20
August	**60**	0.56^{a} ±0.18	0.35^{a} ±0.05	0.70^{a} ±0.09	0.48^{a}±0.17	0.50^{a} ±0.09	0.52^{a}±0.09
September	**75**	0.41^{a} ±0.03	0.26^{a} ±0.02	0.60^{a} ±0.17	0.57^{a}±0.06	0.30^{a} ±0.09	0.35^{a}±0.15
September	**90**	0.58^{a} ±0.11	0.59^{a} ±0.07	0.47^{a} ±0.09	0.38^{a}±0.10	0.66^{a} ±0.12	0.54^{a}±0.12
October	**105**	0.48^{ab} ±0.11	0.62^{ab} ±0.06	0.47^{ab} ±0.11	0.48^{ab}±0.10	0.37^{b} ±0.03	0.34^{b}±0.06
October	**120**	0.68^{a} ±0.14	0.47^{a} ±0.15	0.70^{a} ±0.10	0.52^{a}±0.07	0.57^{a} ±0.10	0.67^{a}±0.11
Mean		**0.53^{a} ±0.05 (0.21-0.85)**	**0.39^{b}±0.04 (0.15-0.62)**	**0.48^{ab}±0.05 (0.09-0.70)**	**0.47^{ab}±0.04 (0.18-0.57)**	**0.45^{ab}±0.04 (0.30-0.66)**	**0.53^{a} ±0.05 (0.21-0.85)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
Value in parenthesis depicts range

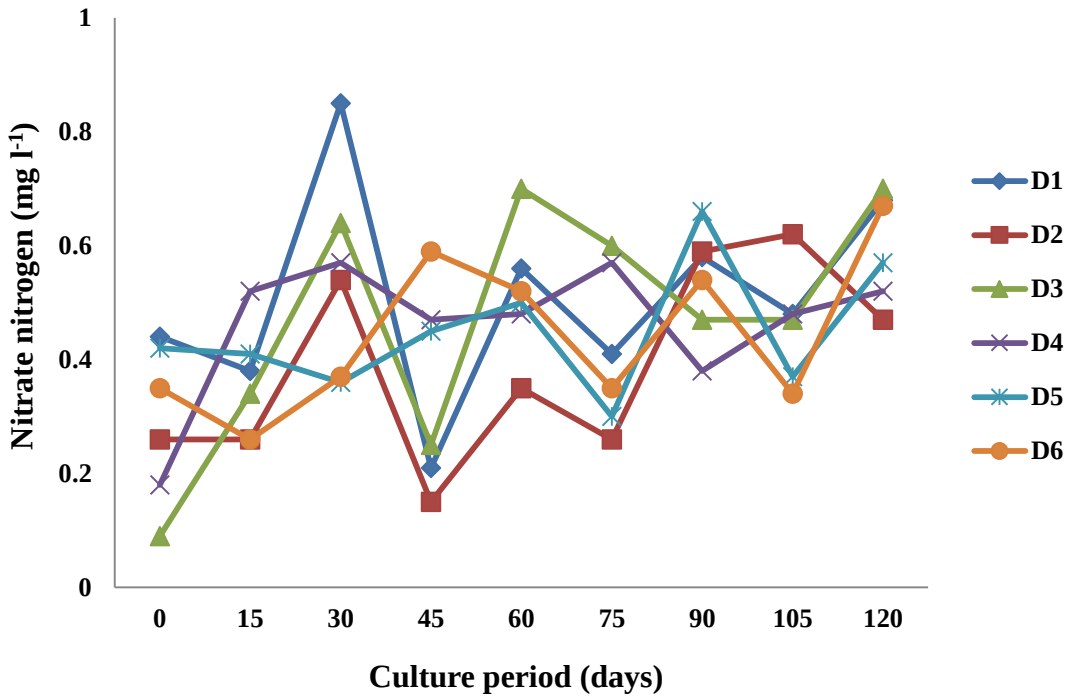


Fig 7: Changes in Nitrate nitrogen (mg l^{-1}) of water in different treatments during the experimental period

Nitrate nitrogen is the final product in the nitrogen cycle resulting from aerobic decomposition of toxic nitrogenous waste ammonia. It is the readily available form of nitrogen to the phytoplankton and other plants in fish culture pond. During the present experimental period, the mean NO_3^--N value remained towards lower limit of the permissible range (0.2-1.0 mgl^{-1}) as reported by Boyd (1988) and Jhingran (1991), which may be due to the no use of any organic manure or inorganic fertilizer.

4.1.1.8 Orthophosphates ($O\text{-}PO_4^{3-}$)

The Orthophosphates (mg l^{-1}) of water fluctuated between 0.01 to 0.15 in different treatments during the experimental period (Table 11, Fig 8). Among different treatments, mean $O\text{-}PO_4^{3-}$ was 0.05 in D1, D3 and D6; 0.06 in D2 and D5 and 0.07 in D4 respectively, and differences among treatments were insignificant ($p \leq 0.05$).

Phosphate is considered as a limiting factor in almost all water bodies because it is present in water in a very small amount (in most cases less than 0.1 mg l^{-1}). Desirable range of soluble phosphates (orthophosphates) for optimum productivity is 0.005-0.20 mg l^{-1} (Boyd 1988). Hasan (2003) reported $O\text{-}PO_4^{3-}$ levels from 0.05-3.00 mg l^{-1} for good productivity. In the present study, $O\text{-}PO_4^{3-}$ levels remained well within the desirable range indicating sufficient availability of phosphates.

Table 11: Orthophosphate (mg l^{-1}) of water in different treatments during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
July	**0**	0.02^{b}±0.004	0.04^{ab}±0.003	0.03^{b}±0.012	0.02^{b}±0.006	0.06^{a}±0.009	0.03^{ab}±0.005
July	**15**	0.03^{a} ±0.02	0.02^{a}±0.003	0.03^{a}±0.005	0.02^{a}±0.005	0.02^{a}±0.008	0.01^{a}±0.006
July	**30**	0.06^{ab} ±0.005	0.07^{a}±0.003	0.04^{b}±0.003	0.07^{a}±0.005	0.06^{a}±0.003	0.06^{ab}±0.008
August	**45**	0.07^{a} ±0.003	0.07^{a}±0.005	0.08^{a}±0.001	0.06^{a}±0.003	0.07^{a}±0.006	0.07^{a}±0.001
August	**60**	0.08^{a} ±0.003	0.07^{a}±0.006	0.08^{a}±0.001	0.15^{a} ±0.09	0.03^{a}±0.007	0.04^{a} ±0.01
September	**75**	0.04^{ab} ±0.01	0.07^{ab}±0.008	0.06^{b} ±0.01	0.08^{ab}±0.004	0.07^{ab}±0.002	0.07^{ab} ±0.30
September	**90**	0.07^{ab} ±0.003	0.08^{a}±0.006	0.06^{ab}±0.01	0.07^{ab}±0.008	0.05^{b}±0.007	0.07^{ab}±0.004
October	**105**	0.08^{a} ±0.001	0.07^{a}±0.006	0.07^{a}±0.003	0.08^{a}±0.004	0.07^{a}±0.003	0.07^{a}±0.004
October	**120**	0.08^{a} ±0.006	0.07^{a}±0.002	0.06^{a}±0.016	0.07^{a}±0.002	0.08^{a}±0.007	0.07^{a}±0.007
Mean		**0.05^{a} ±0.002 (0.02-0.08)**	**0.06^{a}±0.004 (0.02-0.08)**	**0.05^{a}±0.001 (0.03-0.08)**	**0.07^{a}±0.006 (0.02-0.15)**	**0.06^{a}±0.003 (0.02-0.08)**	**0.05^{a}±0.008 (0.01-0.07)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
Value in parenthesis depicts range

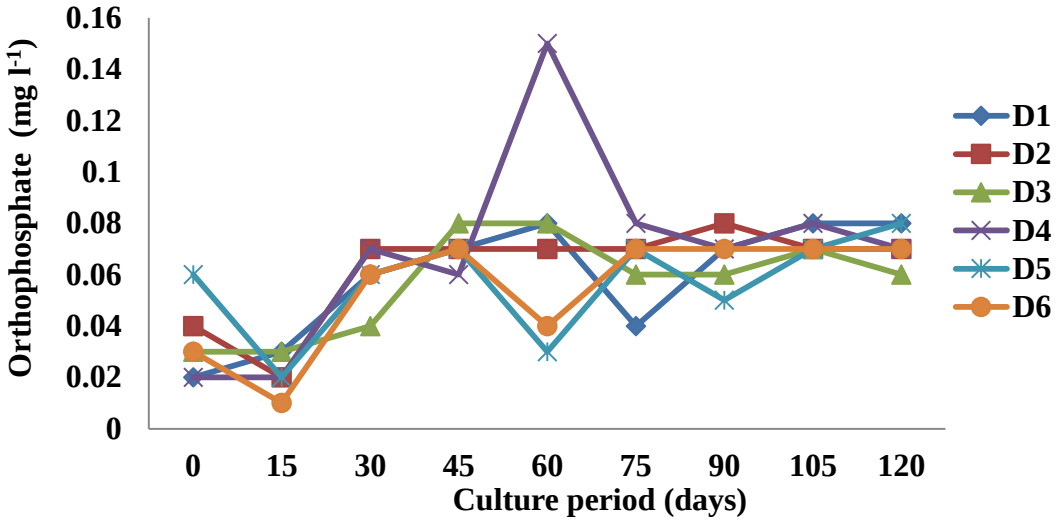


Fig 8: Changes in Orthophosphate (mg l^{-1}) of water in different treatments during the experimental period

Consistency and uniformity of physico-chemical parameters among different treatments during the experimental period of 120 days, revealed that the presence of silage in experimental diets did not have any detrimental effect on water quality.

4.1.2 Survival and growth of fish

4.1.2.1 Survival of fish

At the end of experiment, all the experimental pools were drained out completely to harvest all the fish for calculating the % survival of fish in different treatments. Average survival (%) of fish was 93.33, 96.66, 98.33, 100, 100 and 96.66 in D1, D2, D3, D4, D5 and D6 respectively (Table 12, Fig 9), which revealed that fish silage can be incorporated in feed without affecting the survival of fish. Survival (%) of fish was significantly higher in all the treatments as compared to control. It was 100% in D4 and D5 i.e diets having no fish meal, but having combination of fish silage and plant protein sources. Fish silage is considered as product of high biological value, presenting practically the same composition as the original raw material (Tacon 1993) and with strong fishy odour, which must have resulted in higher acceptibility of silage incorporated diet resulting in enhanced fish survival.

Kamei *et al* (2018) reported % survival of pangas varied from 93.33 to 96.19, when fed with diets, in which fish meal was replaced with blended protein source consisting of one third each of fish silage, groundnut oil cake and soybean meal @ 25%, 50%, 75% and 100% level. Maximum survival of 96.19% was observed in diet, where 75% of fish meal was replaced with blended protein source. Datta *et al* (2018)

revealed poultry waste, fish meal, fish silage and soybean meal equally effective in terms of pangas survival, which remained 100% after 120 days of experiment. In the present study too, feed formulated with fish silage and plant proteins; fish silage and fish meal proved better as compared to fish meal (control) diets in terms of fish survival.

4.1.2.2 Growth of fish

The growth performance of fish was assessed in terms of total body length (TBL) and body weight (BW) at monthly intervals during the experimental period. After completion of the experiment, growth (length and weight) parameters in terms of total body length gain (TBLG), net weight gain (NWG), specific growth rate (SGR), condition factor (K) and feeding efficiency in terms of feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish for each treatment were calculated.

4.1.2.2.1 Length parameters

Among different treatments, total body length (cm) increased from 6.06 to 10.27 in D1, 6.02 to 11.76 in D2, 6.04 to 11.40 in D3, 5.88 to 11.39 in D4, 6.00 to 12.03 in D5 and 5.96 to 11.49 in D6 respectively (Table 12, Fig 10). At the end of experimental period, final total body length (cm) and total body length gain was significantly higher ($p \leq 0.05$) in D5 (12.03, 6.03) and minimum in D1 (10.27 and 4.21) (Table 12; Fig 10 and 11).

Table 12: Changes in length parameters of fish in different treatments during the experimental period (cm)

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
July	0	$6.06^{a}\pm0.02$	$6.02^{a}\pm0.04$	$6.04^{a}\pm0.06$	$5.88^{a}\pm0.04$	$6.00^{a}\pm0.15$	$5.96^{a}\pm0.04$
July	30	$7.90^{a}\pm0.01$	$7.94^{a}\pm0.45$	$8.02^{a}\pm0.26$	$8.03^{a}\pm0.27$	$8.05^{a}\pm0.45$	$7.91^{a}\pm0.01$
August	60	$9.52^{a}\pm0.49$	$10.43^{a}\pm0.21$	$9.16^{a}\pm0.15$	$9.58^{a}\pm0.53$	$9.78^{a}\pm0.61$	$9.26^{a}\pm0.25$
September	90	$9.96^{b}\pm0.39$	$11.24^{a}\pm0.36$	$10.99^{a}\pm0.24$	$11.42^{a}\pm0.16$	$11.58^{a}\pm0.17$	$11.04^{a}\pm0.02$
October	120	$10.27^{b}\pm0.15$	$11.76^{a}\pm0.30$	$11.40^{ab}\pm0.10$	$11.39^{ab}\pm0.63$	$12.03^{a}\pm0.29$	$11.49^{ab}\pm0.60$
TBLG		$4.21^{c}\pm0.06$	$5.74^{b}\pm0.08$	$5.36^{b}\pm0.05$	$5.51^{b}\pm0.02$	$6.03^{a}\pm0.08$	$5.53^{b}\pm0.04$
Survival (%)		$93.33^{c}\pm0.10$	$96.66^{b}\pm0.25$	$98.33^{ab}\pm0.15$	$100^{a}\pm0.21$	$100^{a}\pm0.53$	$96.66^{b}\pm0.45$

Values are Mean ± S.E., n= 10
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
TBLG= Total body length gain

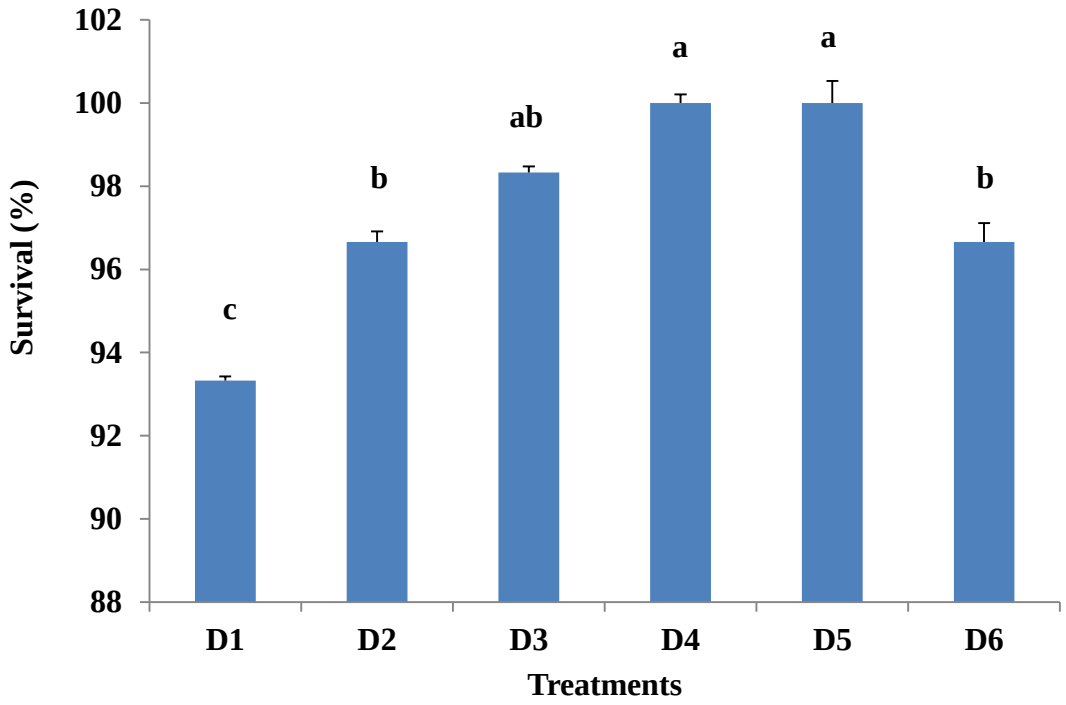


Fig 9: Changes in survival (%) of fish in different treatments after completion of the experimental period

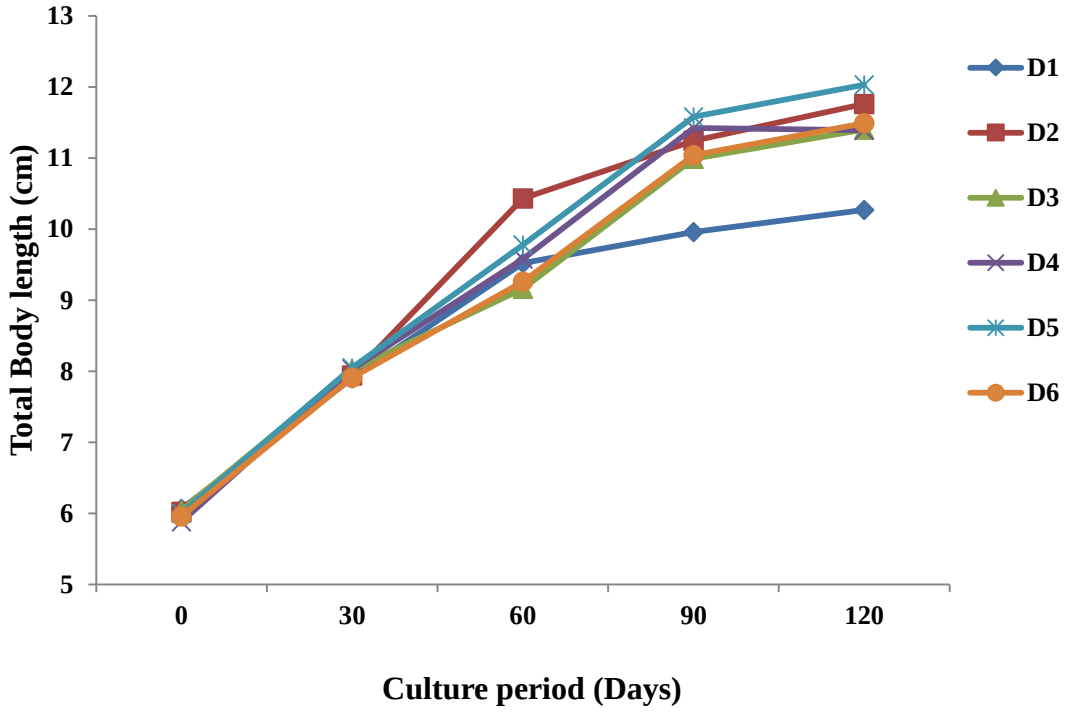


Fig 10: Change in total body length (cm) of fish in different treatments during the experimental period

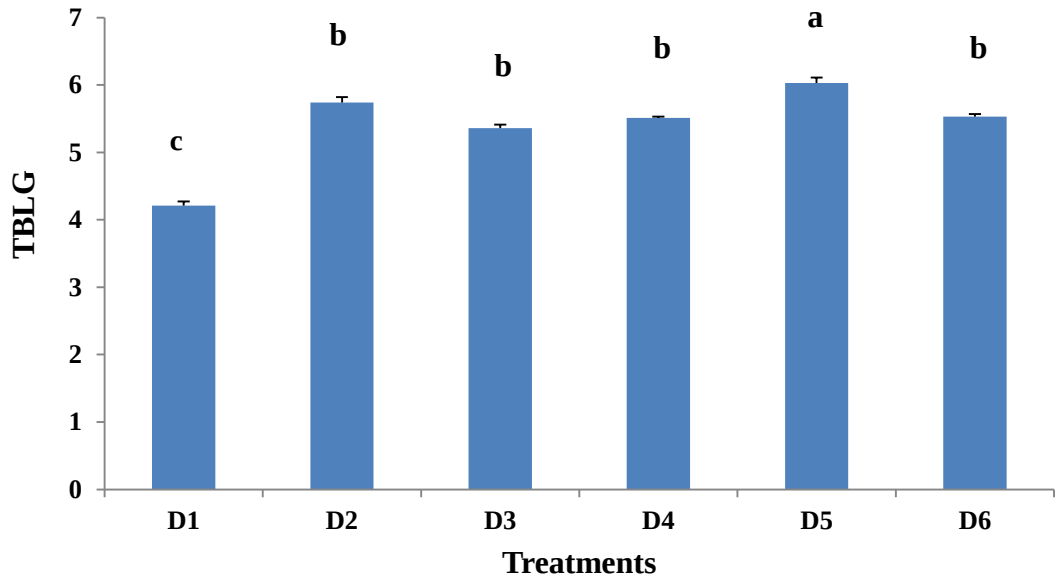


Fig 11: Comparative TBLG of fish in different treatments after completion of the experimental period

4.1.2.2.2 Body weight (BW)

Among different treatments, body weight (g) of fish increased from 1.97 to 15.10, 2.03 to 18.00, 1.94 to 21.33, 1.78 to 17.33, 1.85 to 17.90 and 1.52 to 14.48 in D1, D2, D3, D4, D5 and D6 respectively. At the end of experimental period, significantly higher ($p \leq 0.05$) fish growth in terms of final body weight (FBW), NWG and SGR was observed in D3 (21.33g, 19.00, 1.99), while minimum FBW and NWG in D6 (14.48 and 12.33) and SGR in D1 (1.69). Likewise, PER was significantly higher in D5 (1.35) and minimum in D1 (1.16). The differences for condition factor were insignificant for all the treatments and control, however improvement in FCR was observed in D3 (2.15) as compared to control (D1) with significantly higher value (2.63). The overall results of the present study revealed that fish growth improved in all silage fed fish as compared to control with significantly higher values for growth parameters in D3, in which fish meal was replaced with fish silage @ 100% level (Table 13, Fig 12 and 13). The results in terms of overall growth improvement in D3 clearly revealed acceptibility of fish silage supplemented diets. Moreover, during hydrolysis process of fish silage, good amount of free amino acids are produced, which must have acted as attractants (Madage *et al* 2015).

Table 13: Changes in weight of fish in different treatments during experiment and comparative weight parameters after completion of experiment

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
July	0	$1.97^{a}\pm0.06$	$2.03^{a}\pm0.06$	$1.94^{a}\pm0.04$	$1.78^{a}\pm0.03$	$1.85^{a}\pm0.16$	$1.52^{b}\pm0.04$
July	30	$3.95^{a}\pm0.12$	$4.48^{a}\pm0.25$	$4.29^{a}\pm0.53$	$4.30^{a}\pm0.55$	$4.29^{a}\pm0.44$	$4.04^{a}\pm0.16$
August	60	$7.84^{a}\pm0.68$	$8.89^{a}\pm0.51$	$7.58^{a}\pm0.63$	$7.69^{a}\pm0.89$	$8.2^{a}\pm1.09$	$6.82^{a}\pm0.31$
September	90	$9.93^{b}\pm0.99$	$12.10^{ab}\pm0.62$	$12.76^{a}\pm1.03$	$13.97^{a}\pm0.28$	$12.97^{a}\pm0.98$	$11.89^{ab}\pm0.35$
October	120	$15.10^{cd}\pm0.40$	$18.00^{b}\pm1.15$	$21.33^{a}\pm0.66$	$17.33^{bc}\pm0.66$	$17.90^{b}\pm1.24$	$14.48^{d}\pm0.58$
NWG		$12.67^{bc}\pm0.33$	$15.33^{bc}\pm1.45$	$19.00^{a}\pm0.57$	$15.33^{bc}\pm0.66$	$15.67^{b}\pm1.20$	$12.33^{c}\pm0.88$
SGR		$1.69^{c}\pm0.04$	$1.81^{b}\pm0.02$	$1.99^{a}\pm0.03$	$1.89^{a}\pm0.10$	$1.89^{a}\pm0.10$	$1.87^{ab}\pm0.02$
PER		$1.16^{c}\pm0.02$	$1.28^{b}\pm0.07$	$1.50^{a}\pm0.03$	$1.29^{b}\pm0.05$	$1.35^{ab}\pm0.05$	$1.22^{bc}\pm0.06$
FCR		$2.63^{a}\pm0.04$	$2.45^{b}\pm0.01$	$2.15^{c}\pm0.05$	$2.51^{b}\pm0.07$	$2.45^{b}\pm0.02$	$2.62^{a}\pm0.03$
K		$1.40^{a}\pm0.04$	$1.12^{a}\pm0.13$	$1.14^{a}\pm0.08$	$1.20^{a}\pm0.17$	$1.05^{a}\pm0.15$	$1.00^{a}\pm0.21$

Values are Mean ± S.E., n= 10

Values with same superscript (a, b,….d) in a row does not differ significantly ($p\leq0.05$)

[NWG= Net weight gain, SGR= Specific growth rate, PER= Protein efficiency ratio, FCR= Feed conversion ratio, K= Condition factor]

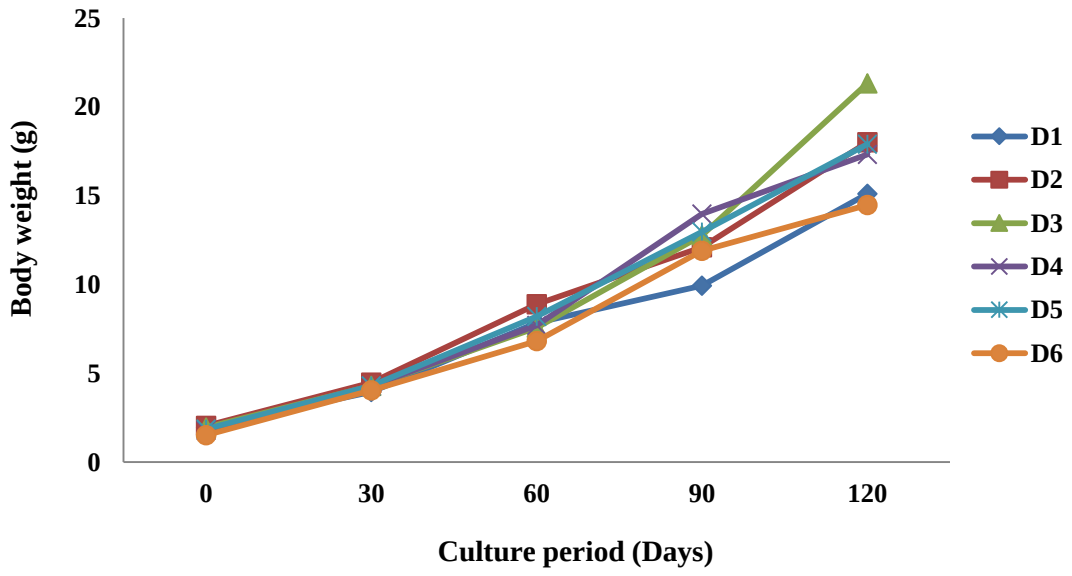


Fig 12: Change in body weight (g) of fish in different treatments during the experimental period

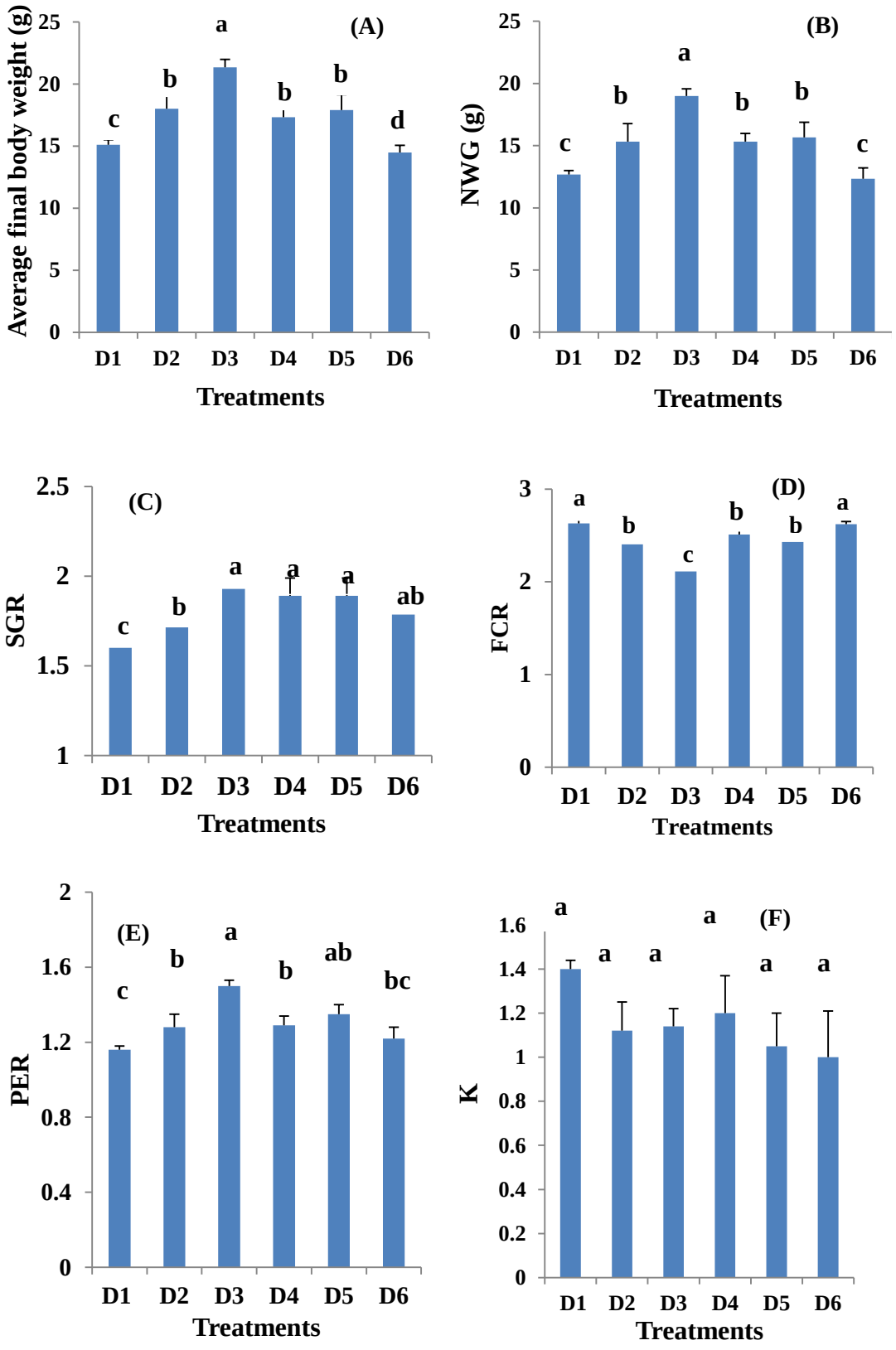


Fig 13: Comparative growth parameters of fish in different treatments after completion of experiment [A= Average final body weight (g), B= Net weight gain (g), C= Specific growth rate, D= Feed conversion ratio, E= Protein efficiency ratio and F= Condition factor (K)]

The findings of the present study are in line with results of previous study by Salah al-Din (1995), where inclusion of fish silage improved growth performance of catfish (*Clarias lazera*). Fagbenro and Jauncey (1998), also reported fermented fish-silage co-dried with protein feedstuffs as suitable protein supplement in case of juvenile catfish (*C. gariepinus*), which can provide up to 50% of dietary protein without affecting feed efficiency, fish growth or health. Improved growth of fish fed with acid silage may be due to the presence of comparatively higher amount of free amino acids and active hydrolytic enzymes (Gallagher 1993), released by the action of acid. Ramasubburayan *et al* (2013) too revealed that 2% acid silage supplemented experimental diet resulted in highest weight gain (2.38g) and SGR (1.49) as compared to fish meal control diet in *Cyprinus carpio* due to significant increase in levels of amino acids particularly glycine, threonine, arginine, serine and tyrosine. Similar observations were recorded by Vidotti *et al* (2003) and Geron *et al* (2007), in terms of higher levels of amino acids in acid fermented silage. Further, according to Flick (2007), silage blends with soybean meal found to be an excellent dietary protein supplement especially for Catfishes. Moreover, in the present study, along with fish silage and plant proteins, presence of fish meal had additional positive impact on overall growth of fish in diet D3.

In one of the study, by Soltan and Tharwat (2005), dried fish by-products silage successfully replaced 25 and 50% of fishmeal in tilapia and catfish diets without any negative effects on BW, WG and SGR, while the higher incorporation levels (75 or 100%) significantly reduced the final BW in both the species. Kamei *et al* (2018) too reported significantly higher weight gain (391.64%), SGR (1.77) and PER (1.41) of Thai Pangas, when fed with diet formulated by replacing 75% of fish meal with blended protein sources made up of fish silage and groundnut oil cake and soybean meal.

Fagbenro and Bello-Olusoji (1997) and Plascencia-Jatomea *et al* (2002) also reported best FCR and PER in Nile tilapia fry fed diets containing 0, 10 and 15% of shrimp head silage as a replacement of fish meal and value of FCR increased at higher replacement levels (20, 25 and 30%) depicting lower incorporation levels as beneficial for tilapia growth. However, in context to this, in the present study, 100% fish meal replacement with fish silage resulted in improved growth. Likewise, Wassef

et al (2003) too found that, partial or total replacement of fish meal by fermented fish silage alone or mixed with soybean meal did not significantly affected FCR and PER.

FCR values varied from 1.4 to 8.7 in various studies conducted previously on Atlantic salmon, *Salmo salar* (Lie *et al* 1988); Eel, *Anguilla Anguilla* (Goncalves *et al* 1989); Rainbow trout, *Salmo gairdneri* (Stone *et al* 1989) and Pearl mullet, *Chalcalburnus tarichi* (Gullu *et al* 2003). The possible reason for varied FCR values is due to the variety of silage materials, feed ingredients/composition, fish species tested, culture environment and water quality parameters etc. The results of growth parameters and feeding effeciency in the present study are in accordance with several previous studies, which indicated that fish biosilage is a potential alternative for fish meal in fish feed without negative impact on growth efficiency, if incorporated at proper level (Fagbenro 1994, Dapkebicius 2002, Soltan and El-Laithy 2008).

4.1.3 Haematological Parameters

Haematological parameters are important means of assessing the health status of fish in culture conditions and for detecting any stress or disturbance in fish's habitat (Mulcany 1975). Haematology of fish in terms of haemoglobin (Hb) and hematocrit (Ht) was studied at the completion of the experiment.

4.1.3.1 Haemoglobin content (Hb)

In different treatments, Hb (g%) in fish was 5.67, 6.00, 7.33, 8.00 in D1, D2, D3, D4 and 6.67 in D5 and D6, respectively and the differences among treatments were significant ($p \leq 0.05$) in all the treatments and control with maximum value in D4 (8.00) (Table 14, Fig 14). The results indicated that fish silage supplementation in pangas fish diet significantly improved Hb content in all the treatments with maximum value in D4.

4.1.3.2 Haematocrit (Ht) or Packed Cell Volume (PCV)

Among different treatments, Haematocrit (%) was 13.33, 16.67, 15.00, 20.00, 18.33 and 16.67 in D1, D2, D3, D4, D5 and D6, respectively and the differences were significant ($p \leq 0.05$) in all the treatments and control with maximum value in D4 (20.00) The result indicated that fish silage supplementation in pangas diet increased the haematocrit content significantly (Table 14, Fig 14).

Table 14: Comparative haematological parameters of fish in different treatments after completion of experiment

Haematological parameter	Treatments					
	D1	D2	D3	D4	D5	D6
Hb (g%)	$5.67^{c}\pm0.33$	$6.00^{c}\pm0.20$	$7.33^{b}\pm0.25$	$8.00^{a}\pm1.15$	$6.67^{bc}\pm0.58$	$6.67^{bc}\pm1.15$
Ht (%)	$13.33^{e}\pm1.66$	$16.67^{c}\pm1.66$	$15.00^{d}\pm2.10$	$20.00^{a}\pm1.45$	$18.33^{b}\pm2.88$	$16.67^{c}\pm2.88$

Values are Mean ± S.E., n= 3

Values with same superscript (a, b,….d) in a row does not differ significantly (p≤0.05)

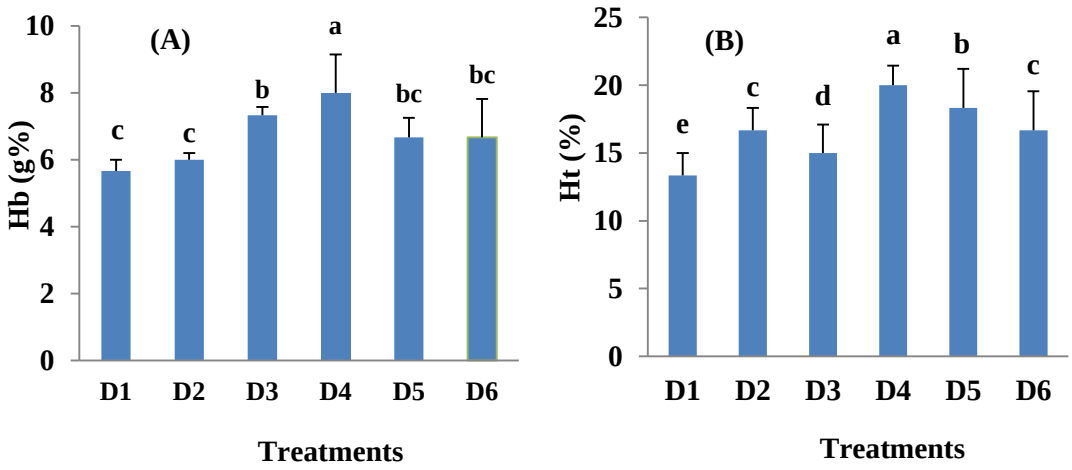


Fig 14: Comparative hematological parameters of fish in different treatments after completion of experiment [A= Haemoglobin (g%), B= Haematocrit (%)]

Hb and Ht are important indicators of fish health. Hb is the protein contained in the red blood cells and is responsible for supplying oxygen to the tissues and for continuous supply of oxygen, sufficient haemoglobin level should be maintained (Billett *et al* 1990). An appropriate quantity and quality of protein is to be maintained for keeping these parameters at a level required for optimized fish growth (Akinwande *et al* 2005, Datta *et al* 2018 and Khan *et al* 2018). Moreover, haematological parameters are closely related to the response of the animal to its environment including dietary factors (Gabriel *et al* 2004). The results of the present study depicted positive impact of fish silage supplementation on fish in terms of higher Hb and Ht, which may be due to its

ability to stimulate blood formation (Habte-Tsion *et al* 2013, Yones and Metwalli 2015).

4.1.4 Biochemical parameters

Biochemical parameters of fish in terms of total serum proteins, albumins, globulins and albumin/globulin ratio was studied at the completion of the experiment.

4.1.4.1 Total proteins

Among different treatments, blood serum total protein (gdl^{-1}) in blood serum was 2.94, 3.00, 3.09, 4.32, 4.16 and 2.47 in D1, D2, D3, D4, D5 and D6, respectively and the differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased the serum total protein levels significantly (Table 15, Fig 15) in D4 (4.32) and D5 (4.16).

4.1.4.2 Albumin

Among different treatments, albumin (gdl^{-1}) in blood serum was 0.72, 0.76, 1.14, 1.20, 0.82 and 0.68 in D1, D2, D3, D4, D5 and D6, respectively and the differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased the serum albumin levels significantly (Table 15, Fig 15) in D3 (1.14) and D4 (1.20).

4.1.4.3 Globulin

Among different treatments, Globulin (gdl^{-1}) in blood serum was 2.22, 2.24, 1.95, 3.33, 3.12 and 1.79 in D1, D2, D3, D4, D5 and D6, respectively and the differences among treatments were significant ($p \leq 0.05$) (Table 15, Fig 15). The result indicated that fish silage incorporation in fish diet increased the serum globulin levels significantly (Table 15, Fig 15) in D4 (3.33) and D5 (3.12).

4.1.4.4 Albumin/globulin (Alb/Glb) ratio

In different treatments, Alb/Glb ratio in blood serum was 0.34 in D1 and D2; 0.58, 0.36, 0.26 and 0.38 in D3, D4, D5 and D6, respectively and the differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased the serum Alb/Glb levels ratio significantly in D3 (0.58) (Table 15, Fig 15).

Table 15: Comparative blood serum biochemical parameters of fish in different treatments after completion of experiment

Biochemical parameter (gdl^{-1})	Treatments					
	D1	D2	D3	D4	D5	D6
Total protein	$2.94^{c}\pm0.49$	$3.00^{b}\pm0.12$	$3.09^{b}\pm0.37$	$4.32^{a}\pm0.51$	$4.16^{a}\pm0.12$	$2.47^{c}\pm0.26$
Albumin	$0.72^{b}\pm0.03$	$0.76^{b}\pm0.03$	$1.14^{a}\pm0.05$	$1.20^{a}\pm0.09$	$0.82^{b}\pm0.10$	$0.68^{c}\pm0.13$
Globulins	$2.22^{ab}\pm0.88$	$2.24^{ab}\pm0.18$	$1.95^{b}\pm0.55$	$3.33^{a}\pm0.98$	$3.12^{a}\pm0.17$	$1.79^{b}\pm0.23$
Alb/Glb ratio	$0.34^{b}\pm0.24$	$0.34^{b}\pm0.20$	$0.58^{a}\pm0.16$	$0.36^{b}\pm0.12$	$0.26^{c}\pm0.15$	$0.38^{b}\pm0.10$

Values are Mean ± S.E., n= 3

Values with same superscript (a, b,….d) in a row does not differ significantly ($p\leq0.05$)

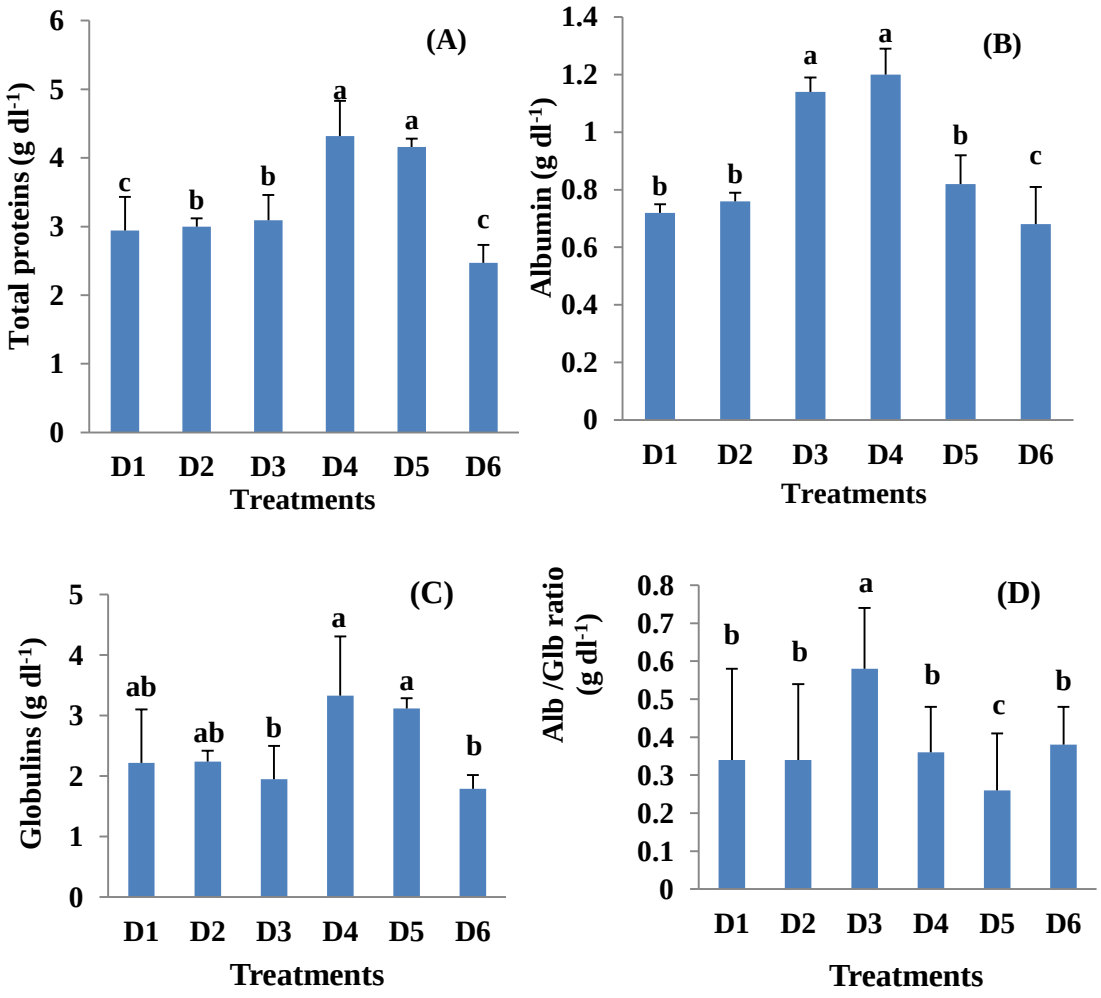


Fig 15: Comparative blood serum biochemical parameters (g dl^{-1}) of fish in different treatments after completion of experimental period [A= Total proteins, B= Albumin, C= Globulins and D= Albumin /Globulin ratio]

The results in terms of serum biochemical parameters i.e total protein, albumin, globulin and albumin/globulin ratio improved in all silage incorporated treatments (D2-D5) and remained at par or deteriorated as compared to control in D6. The results are in line with hematological parameters showing maximum values of all biochemical parameter in D4 (diet without fish meal and having fish silage @ 17.5%), in which fish silage replaced plant protein sources @ 25%. The increase in serum protein, albumin, globulin and alb/glb ratio are considered to be associated with a strong immune response of fish (Wiegertjes *et al* 1996) coupled with improved haematological parameters resulting from enhanced nutritional status of fish. Similar observations were recorded by Najim *et al* (2014) who reported a significant ($p \leq 0.05$) increase in total protein, albumin, globulin and alb/glb levels in common carp, *Cyprinus carpio*, when fish meal was replaced with fish bio silage at three different levels i.e. 25, 50 and 75%.

4.1.5 Antioxidant parameters

Antioxidant parameters in terms of Superoxide dismutase (SOD) (U mg^{-1} Hb) and Lipid peroxidation (LPO) (nmol MDA G Hb^{-1}) were analyzed at the completion of the experiment.

4.1.5.1 Superoxide dismutase (SOD)

Among different treatments, SOD (U mg^{-1} Hb) was 0.74, 0.59, 0.49, 0.46, 0.63 and 0.10 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet decreased SOD levels with maximum value in D1 (0.74) and minimum in D6 (0.10) (Table 16, Fig 16).

4.1.5.2 Lipid peroxidation (LPO)

Among different treatments, LPO (nmol MDAg Hb^{-1}) was 1.38, 0.44, 0.47, 0.93, 0.44 and 0.87 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet decreased LPO levels with maximum value in D1 (1.38) and minimum in D2 and D5 (0.44) (Table 16, Fig 16).

Table 16: Comparative antioxidant parameters in blood hemolysate of fish in different treatments after completion of experiment

Antioxidant parameters	Treatments					
	D1	D2	D3	D4	D5	D6
SOD (U mg^{-1} Hb)	$0.74^{a}\pm0.03$	$0.59^{b}\pm0.03$	$0.49^{c}\pm0.11$	$0.46^{c}\pm0.10$	$0.63^{ab}\pm0.50$	$0.10^{d}\pm0.16$
LPO (nmol MDAg $Hb^{-1)}$	$1.38^{a}\pm0.01$	$0.44^{d}\pm0.05$	$0.47^{d}\pm0.03$	$0.93^{b}\pm0.23$	$0.44^{d}\pm0.03$	$0.87^{c}\pm0.01$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly (p≤0.05)

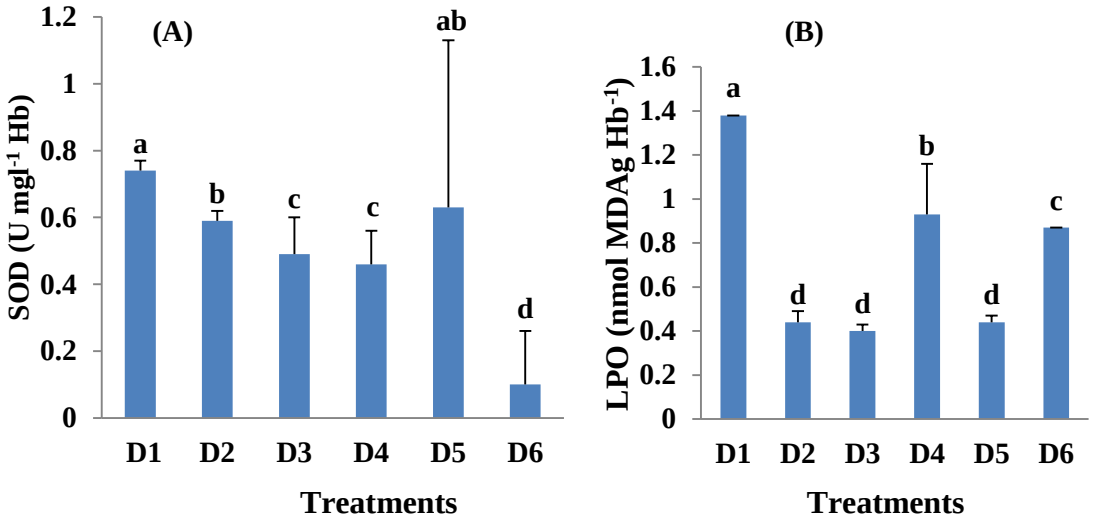


Fig 16: Comparative antioxidant parameters (SOD and LPO) in blood hemolysate of fish in different treatments after completion of experiment [A= SOD (U mgl^{-1} Hb) and B= LPO (nmol MDAg Hb^{-1})]

During acid fermentation of waste, proteins get hydrolysed and low molecular weight proteins are produced, some of which can have immunomodulatory effects leading to enhanced health parameters of fish (Kader *et al* 2012) and SOD, LPO and lysozyme activities are considered as indicators of immune status of any organism (Yoner 2012). In the present study, fish silage incorporation resulted in decreased antioxidant activities. Previous studies have

revealed decreased, increased or no change in antioxidant activities with variations in feed composition. Oncul *et al* (2018) reported no change in SOD and lysozyme activities of juvenile olive flounder (*Paralichthys olivaceus*), when fed with fermented tuna by-product meal supplemented diets. In contrast, Kader *et al* (2012) revealed that replacement of fish meal with fermented soybean meal and squid by-product meal increases the activities of non-specific immune responses in olive flounder. Qi-you *et al* (2008) too reported improvement in antioxidant defense mechanism of rainbow trout, *O. mykiss*, when fish meal was substituted with soy protein isolate and meat-bone meal @ 10%, 20% and 30%.

4.1.6 Serum Transaminases

Serum Transaminases (Ul^{-1}) in terms of alanine transaminase (ALT) and aspartate aminotransferase (AST) were studied at the completion of experiment.

4.1.6.1 Alanine transaminase (ALT)

Among different treatments, alanine transaminase (ALT) of fish was 7.00, 7.03, 18.33, 20.67, 20.00 and 6.00 (Ul^{-1}) in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased the ALT levels significantly with maximum value (20.67) in D4 (Table 17, Fig 17).

4.1.6.2 Aspartate aminotransferase (AST)

Among different treatments, aspartate aminotransferase (AST) of fish was 183.33, 199.33, 216.00, 232.00, 161.00 and 63.33 (Ul^{-1}) in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased the AST levels significantly with maximum value (232.00) in D4 (Table 17, Fig 17).

Significantly higher values of serum transaminases (ALT and AST) were found in all treatments as compared to control (D1) with maximum values in D4 (20.67 and 232) and minimum in D6 (6.00 and 63.33).

Table 17: Comparative serum transaminases in blood serum of fish in different treatments after completion of experiment

Parameters (Ul^{-1})	Treatments					
	D1	D2	D3	D4	D5	D6
ALT	7.00^{c} ±0.00	7.03^{c} ±1.01	18.33^{b}±1.33	20.67^{a}±0.88	20.00^{a} ±0.58	6.00^{d} ±3.00
AST	183.33^{bc}±6.06	199.33^{b}±6.45	216.00^{ab}±4.41	232.00^{a}±5.01	161.00^{b}±4.62	63.33^{c}±7.21

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly (p≤0.05)

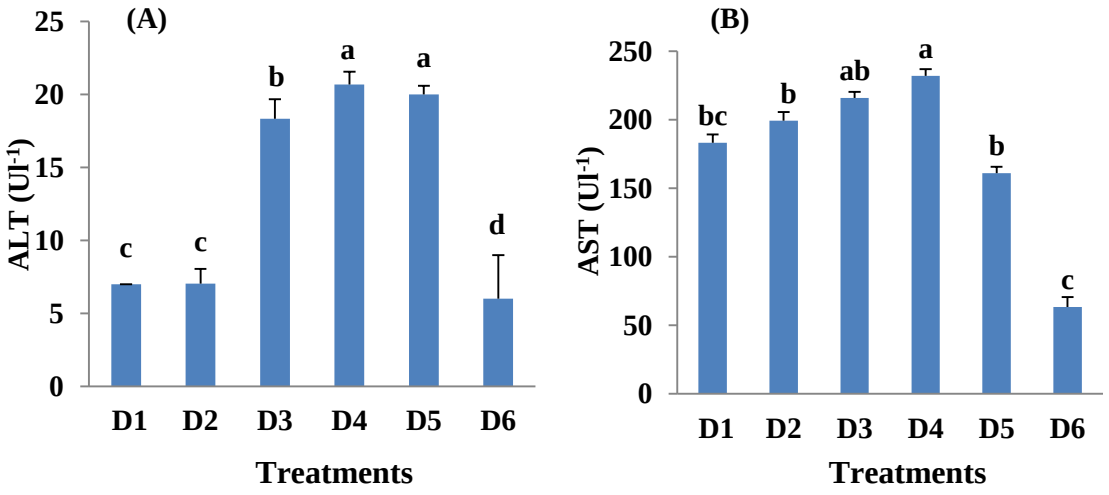


Fig 17: Comparative serum transaminases in blood serum of fish in different treatments after completion of experiment [A= Alanine transaminase (Ul^{-1}) and B= Aspartate aminotransferase (Ul^{-1})]

Liver function of any organism can be revealed by the levels of two enzymes i.e. ALT and AST. According to Abdel – Tawwab (2012), the protein quality and quantity can alter the physiological parameters like ALT and AST to a greater extent. Likewise Dean *et al* (1986) also reported an increased AST and ALT activity with increase in dietary protein quantity in fingerling channel catfish (*Ictalurus punctatus*). Not much work has been carried out particularly w.r.t effect of fish silage on AST/ALT level of fish, however it has been reported that fish silage play positive role in immunomodulation of fish (Samaddar 2018). In one of the study carried out by Panda *et al* (2017) in broiler Japanese quails, dietary supplementation of fermented fish filage (FFS) revealed no change in ALT activity, but AST activity was significantly higher at 15% FFS incorporation as compared to 5 and 10% FFS in the diet. 15% FFS might have increased the

metabolic activity of liver cell, which must have synthesized and degraded more of dispensable amino acids resulting in higher concentration of AST in serum.

4.1.7 Lipid profile

Lipid content in terms of triglycerides, cholesterol, high density lipids (HDL), low density lipids (LDL) and very low density lipids (VLDL) was studied at the completion of the experiment.

4.1.7.1 Triglycerides

Among different treatments, triglycerides (mg dl^{-1}) of fish was 16.00, 34.67, 56.00, 40.00, 33.33 and 24.00 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased triglyceride levels with maximum value in D3 (56.00) and minimum in D1 (16.00) (Table 18, Fig 18).

4.1.7.2 Cholesterol

Among different treatments, cholesterol (mg dl^{-1}) of fish was 247.60, 270.23, 366.53, 351.10, 223.73 and 193.20 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased cholesterol levels significantly ($p \leq 0.05$) with maximum value in D3 (366.53) and minimum in D6 (193.20) (Table 18, Fig 18).

Table 18: Comparative lipid profile of fish in different treatments after completion of the experiment

Parameter (mg dl^{-1})	Treatments					
	D1	D2	D3	D4	D5	D6
Triglycerides	$16.00^{e}\pm1.15$	$34.67^{bc}\pm0.33$	$56.00^{a}\pm0.66$	$40.00^{b}\pm1.00$	$33.33^{c}\pm1.85$	$24.00^{d}\pm0.57$
Cholesterol	$247.60^{c}\pm2.58$	$270.23^{b}\pm4.64$	$366.53^{a}\pm1.41$	$351.10^{ab}\pm3.38$	$223.73^{cd}\pm3.55$	$193.20^{d}\pm4.95$
HDL	$53.00^{a}\pm2.88$	$46.00^{ab}\pm0.00$	$41.67^{bc}\pm1.52$	$43.00^{b}\pm0.00$	$44.33^{b}\pm8.50$	$10.30^{c}\pm0.60$
LDL	$40.67^{e}\pm1.45$	$93.33^{b}\pm10.10$	$110.00^{a}\pm2.30$	$65.00^{c}\pm4.04$	$53.33^{d}\pm0.33$	$63.67^{c}\pm9.90$
VLDL	$3.31^{e}\pm0.22$	$7.06^{c}\pm0.06$	$11.32^{a}\pm1.71$	$8.12^{b}\pm0.23$	$6.73^{cd}\pm0.42$	$4.96^{d}\pm0.13$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)

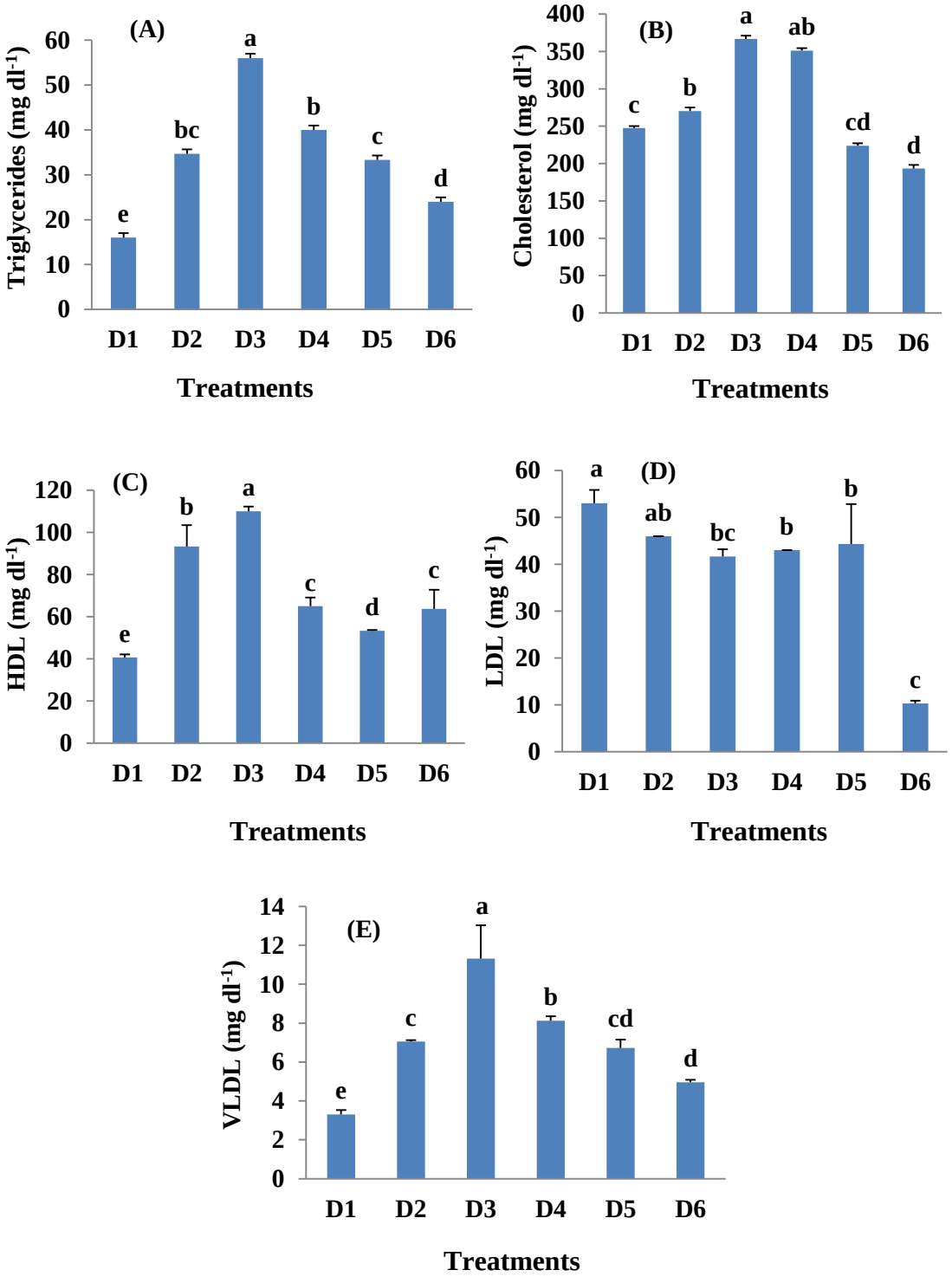


Fig 18: Comparative lipid profile (mg dl^{-1}) of fish in different treatments after completion of the experimental period [A= Triglycerides, B= Cholesterol, C= High Density Lipids, D= Low Density Lipids and E= Very Low Density Lipids]

4.1.7.3 High density lipids (HDL)

Among different treatments, HDL (mg dl^{-1}) of fish was 53.00, 46.00, 41.67, 43.00, 44.33 and 10.30 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet decreased HDL levels significantly ($p \leq 0.05$) with maximum value in D1 (53.00) and minimum in D6 (10.30) (Table 18, Fig 18).

4.1.7.4 Low density lipids (LDL)

Among different treatments, LDL (mg dl^{-1}) of fish was 40.67, 93.33, 110.00, 65.00, 53.33 and 63.67 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased LDL levels significantly ($p \leq 0.05$) with maximum value in D3 (110.00) and minimum in D1 (40.67) (Table 18, Fig 18)

4.1.7.5 Very low density lipids (VLDL)

Among different treatments, VLDL (mg dl^{-1}) of fish was 3.31, 7.06, 11.32, 8.12, 6.73 and 4.96 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased LDL levels significantly ($p \leq 0.05$) with maximum value in D3 (11.32) and minimum in D1 (3.31) (Table 18, Fig 18).

The concentration of triglycerides and cholesterol in the blood is influenced by the fat content of the experimental diet and thus directly indicate nutritional status of fish. Improved triglycerides and cholesterol can be co-related with better health status of fish resulting in improved survival (Maita *et al* 1998a,b). Low concentration of plasma triglycerides usually corresponds to poor lipid reserves (Wagner and Congleton 2004), while elevated triglycerides may be due to an imbalanced diet (Lemaire *et al* 1991). Similar results were observed by Goda *et al* (2007), who indicated higher body lipid content in African catfish, *C. gariepinus* fed diets containing either poultry by-product meal (75%) or soybean meal (100%). These results also agree with Gouveia (1992), who reported an increase of body lipid with the inclusion of poultry by-products in fish feed. In contrast to these studies, Najim *et al* (2014) reported significant decrease in total plasma cholestrol and triglycerides along with significant increase in HDL cholestrol and decreased LDL cholestrol, when fish meal was replaced @ 75% with fish silage in common carp.

Overall Results – Experiment I

Parameter	Results
Fish Survival	Improved in all the treatments with 100% survival in D4 and D5
Fish Growth	Significantly higher in all the treatments except D6 49.96 % higher growth in D3 as compared to D1
Haematological Parameters	Significant improvement in all the treatments
Biochemical Parameters	Significant improvement in all the treatments
Antioxidant Status	Significant decrease as compared to control (D1)
Liver Enzymes	Significant increase in AST and ALT in all treatments as compared to control (D1)
Lipid Profile	Cholesterol/TG – Significant increase
	HDL – Significant decrease
	LDL - Significant increase

Overall results of the present study (Experiment I) revealed that

Fish silage supplementation in diet of pangas fry revealed significant improvement in survival and growth in D3 (100% fish meal replacement with fish silage), whereas haematological and biochemical parameters showed improvement in D4 (25% replacement of plant protein sources with fish silage). Other parameters including antioxidant status, liver enzymes and lipid profile did not reveal any particular trend. Hence, based on overall results with special reference to growth, diet D3 can be recommended for rearing pangas fry to fingerling under indoor culture conditions. In view of variations in different parameters, field trials need to be conducted for better understanding of the effect of fish silage coupled with multiple factors under natural conditions.

4.2 Experiment II – "Growth performance, health status and meat quality of Pangas catfish (*Pangasianodon hypophthalmus*) fingerling fed on fish silage and linseed oil supplemented formulated feeds for grow-out production"

4.2.1 Physico-chemical parameters of water

Physico-chemical parameters of experimental tank water were analyzed every fortnight during 150 days of experimental period.

4.2.1.1 Temperature

The water temperature (°C) fluctuated between 30.26 to 34.86 in different treatments during the experimental period (Table 19, Fig 19). Among different treatments, mean temperature (°C) was 33.61, 32.97, 33.16, 33.19, 33.20 and 33.15 in D1, D2, D3, D4, D5 and D6 respectively and the difference among treatments were insignificant ($p \leq 0.05$).

Temperature plays significant role in metabolism and growth of fish (Schulte *et al* 2011), as basal metabolic rate (BMR) and feed intake increases with increase in temperature. Species suitable for culture under semi-intensive culture system might tolerate wide temperature range, but the temperature range for optimum growth remained narrow. Water temperature range of 30.26-34.86°C, with mean value of 33.21^{o}C, during the present study agreed well with the tolerant range of 28-32^{o}C with 34°C provided the best thermal conditions for Thai pangas culture as documented by De Silva and Phuong (2011). Pangas showed reduced appetite with increase in temperature to 36^{o}C, which may be due to stress caused by hypoxic conditions, as because the DO values were significantly declined at 36°C. During present experiment, the water temperature remained in optimum range providing comfortable rearing conditions for pangas.

Table 19: Water temperature (ºC) in different treatments during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
April	**0**	30.56^{a}±0.01	30.40^{a}±0.07	30.26^{a}±0.04	30.45^{a}±0.01	30.89^{a}±0.09	30.29^{a}±0.06
April	**15**	31.85^{a}±0.05	31.50^{a}±0.02	31.95^{a}±0.01	31.45^{a}±0.02	31.50^{a}±0.08	31.90^{a}±0.04
April	**30**	32.62^{a}±0.10	32.85^{a}±0.04	32.48^{a}±0.05	32.56^{a}±0.06	32.57^{a}±0.05	32.50^{a}±0.03
May	**45**	32.98^{b}±0.08	33.10^{a}±0.06	32.65^{b}±0.04	32.90^{b}±0.02	32.80^{b}±0.04	32.92^{b}±0.02
May	**60**	33.20^{a}±0.04	33.56^{a}±0.14	33.50^{a}±0.09	33.60^{a}±0.09	33.76^{a}±0.08	33.85^{a}±0.09
June	**75**	34.66^{a}±0.06	34.33^{a}±0.08	34.63^{a}±0.08	34.45^{a}±0.04	34.86^{a}±0.06	34.45^{a}±0.02
June	**90**	34.50^{a}±0.04	34.20^{a}±0.06	34.52^{a}±0.08	34.62^{a}±0.04	34.45^{a}±0.10	34.40^{a}±0.08
July	**105**	34.80^{a}±0.02	34.15^{a}±0.02	34.23^{a}±0.03	34.43^{a}±0.02	34.85^{a}±0.04	34.13^{a}±0.06
July	**120**	33.25^{b}±0.08	33.30^{b}±0.06	34.40^{a}±0.22	33.90^{ab}±0.13	33.45^{ab} ±0.08	33.64^{ab}±0.09
August	**135**	33.16^{a}±0.10	33.25^{a}±0.04	33.96^{a}±0.16	33.86^{a}±0.04	33.52^{a}±0.09	33.75^{a}±0.10
August	**150**	32.21^{a}±0.04	32.10^{a}±0.02	32.25^{a}±0.05	32.90^{a}±0.02	32.65^{a}±0.04	32.95^{a}±0.15
Mean		**33.61^{a}±0.02 (30.56-34.80)**	**32.97^{a}±0.08 (30.40-34.33)**	**33.16^{a}±0.01 (30.26-34.63)**	**33.19^{a}±0.09 (30.45-34.62)**	**33.20^{a} ±0.02 (30.89-34.86)**	**33.15^{a} ±0.10 (30.29-34.45)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
Values in parenthesis depicts range

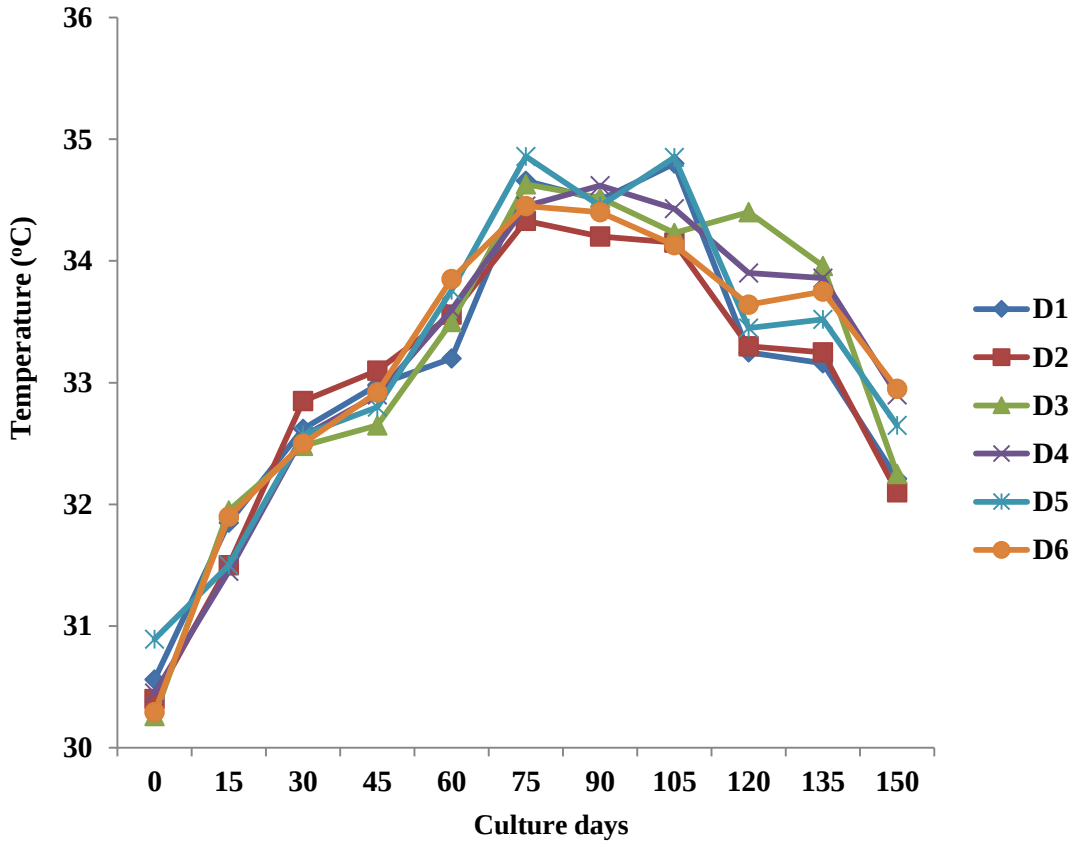


Fig 19: Changes in water temperature (°C) in different treatments during the experimental period

4.2.1.2 pH

The pH of water fluctuated between 7.26 to 7.95 in different treatments during the experimental period (Table 20, Fig 20). Among different treatments, mean pH was 7.60, 7.52, 7.51, 7.57, 7.63 and 7.61 in D1, D2, D3, D4, D5 and D6 respectively and the differences among treatments were insignificant ($p \leq 0.05$).

Water pH plays an important role in optimum physiological activities of fish, decomposition of dead organic matter and release of nutrients from bottom soil and hence, fish growth and productivity. The most suitable pH range for optimum growth of fish is 6.5 to 9.6 as reported by Parameswaran *et al* (1971) and Jhingran (1991). The pH of the water (7.26-7.95) remained well within range during the entire experimental period, which indicates that supplementation of pangas feed with fish silage had no undesirable effect on the water quality with respect to pH with special reference to acidic nature of silage.

Table 20: Water pH in different treatments during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
April	**0**	7.50 ^{a} ±0.06	7.35^{a}±0.02	7.50^{a}±0.04	7.79^{a}±0.10	7.32^{a}±0.05	7.45^{a}±0.08
April	**15**	7.42 ^{a} ±0.03	7.45^{a}±0.07	7.53^{a}±0.15	7.49^{a}±0.35	7.47^{a}±0.09	7.48^{a}±0.23
May	**30**	7.45 ^{a} ±0.04	7.40^{ab}±0.08	7.58^{a}±0.06	7.32^{c}±0.05	7.87^{a}±0.11	7.60^{a}±0.14
May	**45**	7.68 ^{a} ±0.09	7.65 ^{a}±0.13	7.42^{a}±0.04	7.58^{a}±0.18	7.64^{a}±0.03	7.75^{a}±0.06
June	**60**	7.68 ^{a} ±0.10	7.80 ^{a}±0.09	7.50^{a}±0.12	7.54^{a}±0.11	7.52^{a}±0.53	7.74^{a}±0.56
June	**75**	7.75 ^{a} ±0.06	7.54 ^{a}±0.05	7.45^{a}±0.08	7.65^{a}±0.35	7.89^{a}±0.25	7.57^{a}±0.23
July	**90**	7.50 ^{a} ±0.02	7.45 ^{a}±0.04	7.39^{a}±0.06	7.85^{a}±0.04	7.88^{a}±0.04	7.84^{a}±0.08
July	**105**	7.45 ^{a} ±0.06	7.60 ^{a}±0.08	7.65^{a}±0.10	7.40^{a}±0.10	7.82^{a}±0.11	7.46^{a}±0.15
August	**120**	7.70^{a} ±0.03	7.65 ^{a}±0.08	7.30^{a}±0.02	7.35^{a}±0.15	7.26^{a}±0.21	7.30^{a}±0.07
August	**135**	7.85 ^{a} ±0.09	7.45 ^{a}±0.04	7.89^{a}±0.12	7.90^{a}±0.05	7.50^{a}±0.12	7.65^{a}±0.08
September	**150**	7.68 ^{a} ±0.05	7.40 ^{a}±0.10	7.45^{a}±0.21	7.50^{a}±0.04	7.86^{a}±0.09	7.95^{a}±0.06
Mean		**7.60 ^{a} ±0.15 (7.45-7.85)**	**7.52 ^{a}±0.10 (7.35-7.80)**	**7.51^{a}±0.02 (7.30-7.89)**	**7.57^{a}±0.08 (7.32-7.90)**	**7.63^{a}±0.12 (7.26-7.89)**	**7.61^{a}±0.09 (7.30-7.95)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly (p≤0.05)
Values in parenthesis depicts range

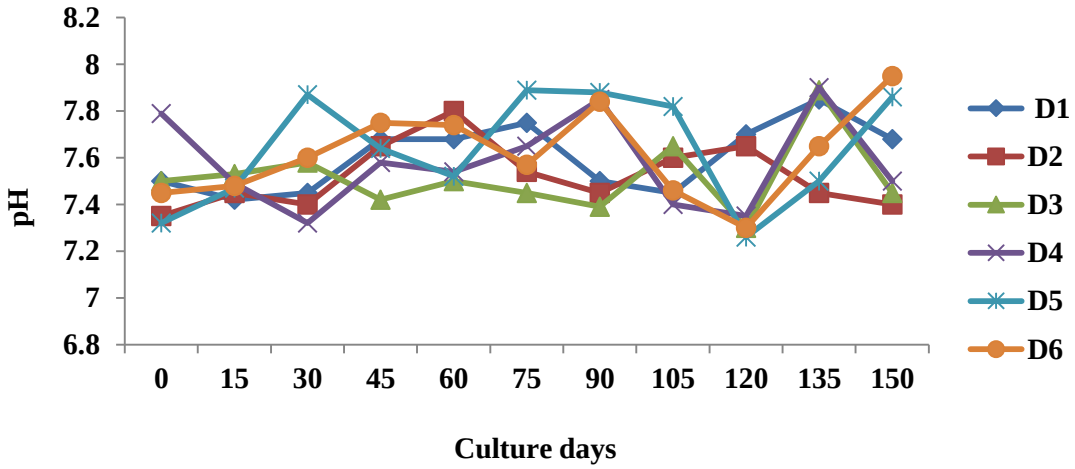


Fig 20: Changes in water pH in different treatments during the experimental period

4.2.1.3 Dissolved Oxygen (DO)

The Dissolved Oxygen (DO) of water fluctuated between 10.00 to 12.80 mg l^{-1}in different treatments during the experimental period (Table 21, Fig 21). Among different treatments, mean DO (mg l^{-1}) was 11.60, 11.25, 11.35, 11.83, 10.99 and 11.65 in D1, D2, D3, D4, D5 and D6 respectively and the differences among treatments were insignificant ($p \leq 0.05$).

The dissolved oxygen content of water ranged from 10.00 to 12.80 mg l^{-1}in the present study. DO is one of the most critical water quality parameter affecting survival and growth of aquatic organisms, as it regulates all the metabolic activities of aquatic organisms and is a very good indicator of water health. According to Summerfelt (1998), for aquaculture, minimum DO should be greater than 5 mgl^{-1} for growth of warm water fish and 6 mgl^{-1} for cold water fish at their optimum temperature. Sutchi Pangas is a continuous, obligatory air breather, whose air-breathing frequency declines from a mean of 21.0 breaths h^{-1} to 10.6 breaths h^{-1}, as oxygen increases from 0.0 $mg1^{-1}$ to 6.7 $mg1^{-1}$. For non-air breathing carps, >5.0 mg l^{-1} of DO of water is required to be maintained during culture (Swingle 1967), however, for air-breathing fish like pangas, low levels are also comfortable. In the present study, DO content remained well above the optimum limit of 5.0 mg l^{-1} for warm water fishes throughout the culture period in all the treatments, which reveals that fish silage supplementation in fish feed had no adverse effect on the water quality with respect to DO.

Table 21: Dissolved oxygen (mg l^{-1}) of water during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
April	**0**	$12.20^{a}\pm1.15$	$11.80^{ab}\pm0.81$	$12.60^{a}\pm1.04$	$12.60^{a}\pm0.17$	$11.20^{b}\pm0.17$	$12.20^{ab}\pm1.15$
April	**15**	$11.40^{b}\pm0.11$	$12.00^{ab}\pm0.17$	$11.40^{b}\pm0.46$	$12.40^{a}\pm0.20$	$11.48^{b}\pm0.30$	$12.40^{a}\pm0.11$
May	**30**	$10.60^{b}\pm0.29$	$11.50^{ab}\pm0.64$	$10.20^{b}\pm0.23$	$12.60^{a}\pm0.12$	$11.50^{ab}\pm0.40$	$10.10^{b}\pm0.29$
May	**45**	$12.40^{a}\pm0.87$	$11.40^{b}\pm0.12$	$11.20^{b}\pm0.58$	$11.40^{ab}\pm0.40$	$11.50^{ab}\pm0.52$	$12.10^{a}\pm0.87$
June	**60**	$11.76^{a}\pm0.64$	$11.93^{a}\pm0.23$	$10.86^{ab}\pm0.89$	$11.53^{a}\pm0.83$	$11.26^{a}\pm0.76$	$10.70^{b}\pm0.06$
June	**75**	$11.80^{a}\pm0.35$	$11.80^{a}\pm1.04$	$11.20^{a}\pm0.20$	$11.40^{a}\pm0.58$	$10.20^{ab}\pm0.35$	$11.80^{a}\pm0.35$
July	**90**	$11.10^{ab}\pm0.75$	$11.30^{ab}\pm0.06$	$12.80^{a,}\pm0.64$	$10.40^{b}\pm0.46$	$10.50^{b}\pm0.06$	$11.10^{ab}\pm0.75$
July	**105**	$12.20^{a}\pm1.15$	$10.80^{b}\pm0.81$	$12.60^{a}\pm1.04$	$12.90^{a}\pm0.17$	$10.10^{b}\pm0.17$	$12.20^{a}\pm0.15$
August	**120**	$10.70^{ab}\pm0.06$	$10.00^{b}\pm0.12$	$10.20^{ab}\pm0.23$	$11.50^{a}\pm0.52$	$11.10^{a}\pm0.75$	$10.70^{ab}\pm0.06$
August	**135**	$12.40^{a}\pm0.11$	$10.10^{b}\pm0.17$	$11.40^{ab}\pm0.46$	$12.40^{a}\pm0.00$	$10.90^{b}\pm0.30$	$12.40^{a}\pm0.11$
September	**150**	$11.06^{ab}\pm0.81$	$11.20^{ab}\pm0.26$	$10.40^{b}\pm0.30$	$10.93^{b}\pm0.88$	$11.20^{ab}\pm0.23$	$12.46^{a}\pm0.17$
Mean		**$11.60^{a}\pm0.21$ (10.60-12.40)**	**$11.25^{a}\pm0.16$ (10.00-12.00)**	**$11.35^{a}\pm0.12$ (10.20-12.80)**	**$11.83^{a}\pm0.58$ (10.40-12.90)**	**$10.99^{a}\pm0.35$ (10.10-11.50)**	**$11.65^{a}\pm0.26$ (10.10-12.46)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p\leq0.05$)
Values in parenthesis depicts range

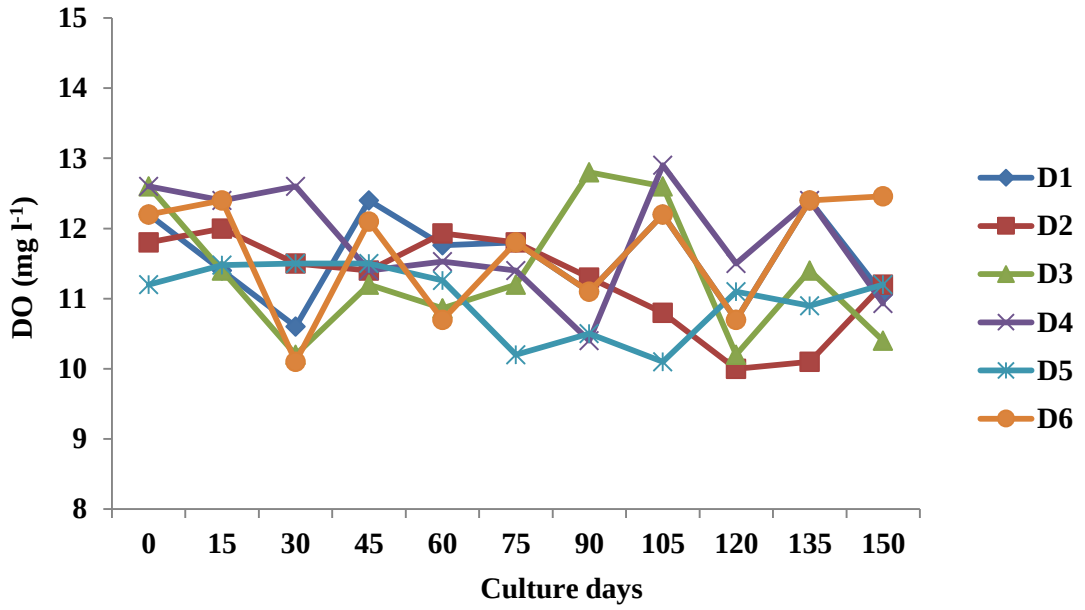


Fig 21: Changes in Dissolved oxygen (mg l^{-1}) of water in different treatments during the experimental period

4.2.1.4 Total alkalinity (TA)

The total alkalinity ($CaCO_3$ mg l^{-1}) of water fluctuated between 126 to 189 in different treatments during the experimental period (Table 22, Fig 22). Among different treatments, mean TA was 167, 169, 161, 162, 162 and 169 in D1, D2, D3, D4, D5 and D6 respectively and the differences among the treatment were insignificant ($p \leq 0.05$).

Alkalinity refers to amount of carbonates, bicarbonates and hydroxyl ions in the water, which provides a good buffering effect to the diurnal pH swings, that occur in aquaculture ponds due to respiration (continuous day-night process) of aquatic organisms (fauna and flora) and the photosynthetic activity occurring in the pond only during the day time. In case of poor buffering capacity of water, extreme pH fluctuations occur in the pond, which affect the health and growth of fish adversely and may cause mortality under severe conditions. Total alkalinity of > 50 mg $CaCO_3$ l^{-1} is reported to be ideal for fish culture (Jena and Das 2006). In the present study, TA of water in the different treatments remained well above the recommended value throughout the culture period, which reveals that fish silage supplementation in pangas feed had no adverse effect on the water quality with respect to TA of water.

Table 22: Total alkalinity ($CaCO_3$ mg l^{-1}) of water during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
April	**0**	156 ^{b} ±3.05	169 ^{ab} ±2.40	162 ^{ab} ±1.15	148 ^{b} ±3.46	180 ^{a} ±9.45	166 ^{ab} ±5.03
April	**15**	180 ^{a} ±1.04	142 ^{b} ±3.05	159 ^{b} ±1.76	167 ^{ab} ±3.00	160 ^{ab} ±2.40	184 ^{a} ±3.05
May	**30**	144 ^{c} ±1.33	154 ^{b} ±1.15	174 ^{ab} ±2.40	154 ^{b} ±0.66	146 ^{c} ±2.30	189 ^{a} ±0.66
May	**45**	183 ^{a} ±1.76	183 ^{a} ±3.71	155 ^{c} ±1.36	186 ^{a} ±1.33	158 ^{c} ±2.29	172 ^{ab} ±3.21
June	**60**	170 ^{a} ±2.90	167 ^{ab} ±1.00	165^{ab}±1.45	148 ^{b} ±2.66	166^{ab}±2.91	161^{ab}±3.54
June	**75**	165 ^{a} ±2.84	175 ^{ab} ±2.44	170 ^{ab} ±2.37	159 ^{b} ±1.56	142 ^{b} ±1.76	180 ^{a} ±2.30
July	**90**	188 ^{a} ±2.11	165 ^{b} ±1.76	126 ^{c} ±2.33	157 ^{bc} ±1.33	180 ^{a} ±2.40	154^{bc} ±3.52
July	**105**	174^{a} ±4.66	178^{a} ±2.30	168^{ab}±3.28	162^{ab}±2.33	159 ^{b} ±2.90	144^{b}±3.33
August	**120**	147 ^{c} ±1.66	161 ^{b} ±2.40	145 ^{c} ±1.66	181 ^{a} ±2.88	160 ^{b} ±2.66	156^{bc} ±3.75
August	**135**	160^{b}±0.66	182^{a}±0.31	186^{a}±0.48	144^{a}±0.63	160^{b}±0.26	182^{a}±0.31
September	**150**	167^{b}±0.58	180^{a}±1.15	166^{b}±8.94	182^{a}±2.72	167^{b}±0.58	180^{a}±1.15
Mean		**167 ^{a} ±0.30 (144-188)**	**169 ^{a} ±0.99 (142-182)**	**161 ^{a} ±1.25 (126-186)**	**162 ^{a} ±1.30 (144-186)**	**162 ^{a} ±0.85 (142-180)**	**169 ^{a} ±1.76 (144-189)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
Values in parenthesis depicts range

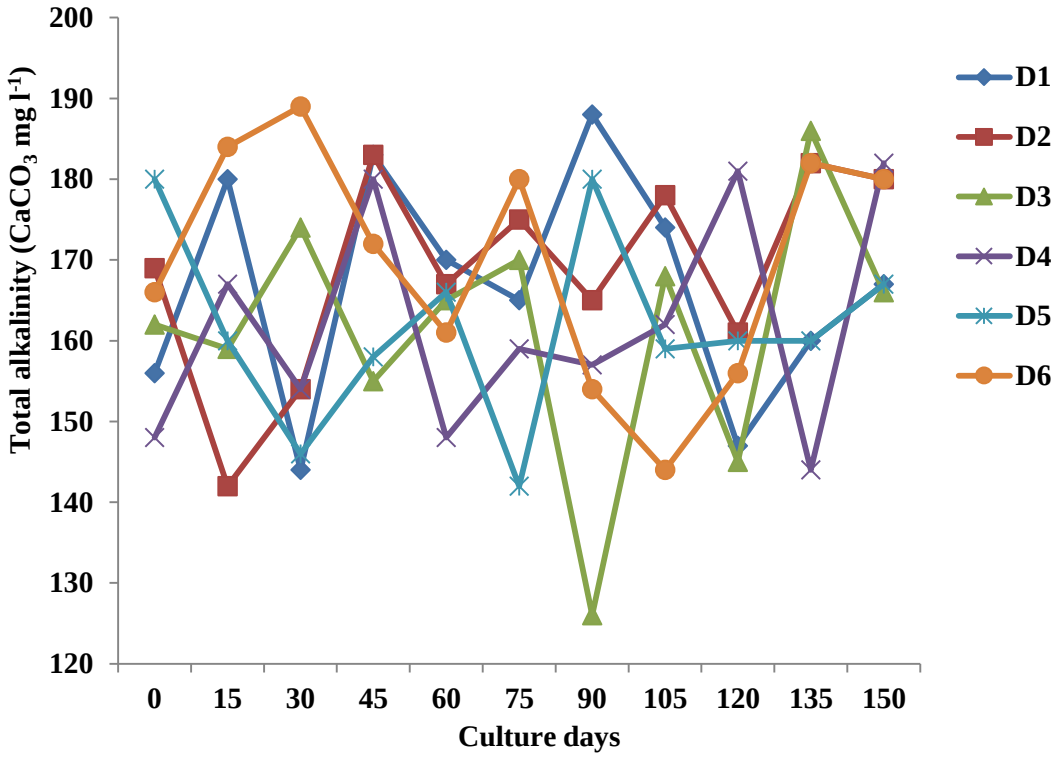


Fig 22. Changes in total alkalinity ($CaCO_3$ mg l^{-1}) of water in different treatments during the experimental period

4.2.1.5 Ammonical-nitrogen (NH_3-N)

The NH_3-N (mg l^{-1}) of water ranged between 0.01 to 0.09 in different treatments during the experimental period (Table 23, Fig 23). Among different treatments mean NH_3^+-N was 0.05 in D1, D4 and D6, 0.04 in D2, D3 and D5 respectively and the differences among treatments were insignificant ($p \leq 0.05$).

Ammonia is excreted as waste product by the fish and decomposition of dead organic matter, which include two components i.e. toxic unionized ammonia-N (NH_3-N) and comparatively safe ionized ammonical-N (NH_4^+-N). Fish is very sensitive to NH_3-N and its acceptable range for aquaculture is 0.02-0.05 mg l^{-1} (Jhingran 1991), with permissible upper limit of 0.1 mg l^{-1} (Boyd 1988). Mean NH_3-N content (0.04-0.05 mgl^{-1}) was within the permissible limit in all the treatments in all the experimental period. The toxicity of the NH_3-N also depends upon pH of water. During present study, the pH of 7.26 to 7.95 did not affect the ammonia in a toxic manner.

Table 23: Ammonical nitrogen (mg l^{-1}) of water during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
April	**0**	0.08^{a}±0.16	0.04^{a}±0.24	0.02^{a}±0.23	0.06^{a} ±0.32	0.04^{a}±0.49	0.02^{a} ±0.52
April	**15**	0.04^{a}±0.08	0.03^{a}±0.26	0.06^{a}±0.04	0.02^{a} ±0.12	0.03^{a} ±0.24	0.08^{a}±0.18
May	**30**	0.01^{a}±0.24	0.03^{a} ±0.11	0.05^{a}±0.64	0.04^{a} ±0.41	0.01^{a}±0.25	0.06^{a}±0.12
May	**45**	0.05^{a}±0.21	0.07^{a}±0.23	0.04^{a}±0.10	0.06^{a}±0.13	0.05^{ab}±0.09	0.04^{b} ±0.11
June	**60**	0.02^{a}±0.08	0.01^{a} ±0.07	0.03^{a}±0.23	0.05^{a}±0.12	0.04^{a}±0.05	0.06^{a}±0.02
June	**75**	0.04^{a} ±0.01	0.05^{a}±0.08	0.02^{a}±0.01	0.09^{a}±0.04	0.01^{a}±0.12	0.01^{a}±0.06
July	**90**	0.07^{a}±0.43	0.08^{a}±0.31	0.03^{a}±0.22	0.04^{a}±0.08	0.06^{a}±0.01	0.07^{a}±0.03
July	**105**	0.03^{a}±0.01	0.04^{a}±0.16	0.03^{a}±0.12	0.02^{a}±0.00	0.01^{a}±0.007	0.03^{a}±0.01
August	**120**	0.05^{a}±0.05	0.06^{a}±0.11	0.04^{a}±0.02	0.06^{a}±0.01	0.02^{a}±0.05	0.04^{a}±0.05
August	**135**	0.06^{a}±0.01	0.03^{a}±0.04	0.04^{a}±0.001	0.06^{a}±0.09	0.05^{a}±0.07	0.06^{a}±0.01
September	**150**	0.05^{a}±0.06	0.02^{a}±0.12	0.04^{a}±0.11	0.05^{a}±0.09	0.05^{a}±0.04	0.05^{a}±0.06
Mean		**0.05^{a}±0.22 (0.01-0.08)**	**0.04^{a}±0.11 (0.01-0.08)**	**0.04^{a}±0.15 (0.02-0.06)**	**0.05^{a}±0.21 (0.02-0.09)**	**0.04^{a}±0.3 (0.01-0.06)**	**0.05^{a}±0.20 (0.01-0.08)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
Values in parenthesis depicts range

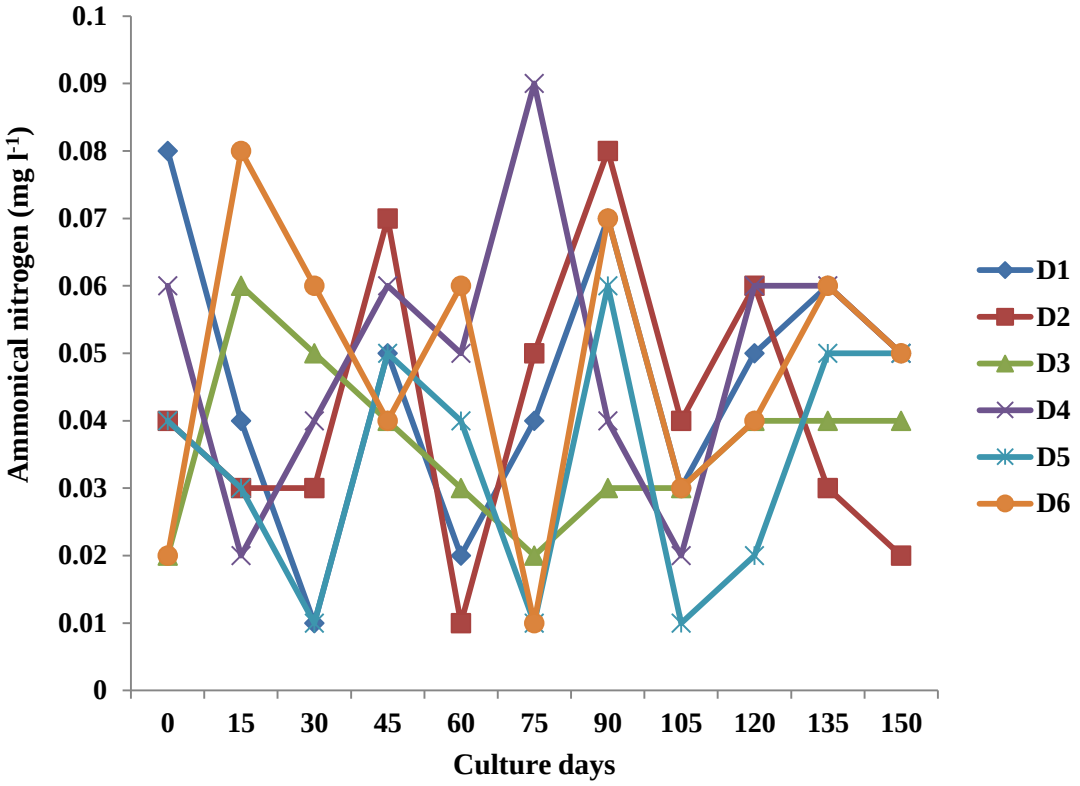


Fig 23: Changes in Ammonical nitrogen (mg l^{-1}) of water in different treatments during the experimental period

4.2.1.6 Nitrite-nitrogen (NO_2^--N)

The NO_2^--N (mg l^{-1}) of water ranged between 0.014 to 0.099 mg l^{-1} in different treatments during the experimental period (Table 24, Fig 24). Among different treatments, mean NO_2^--N was 0.04 in D1 and D6, 0.06 in D2, D3 and D5 and 0.05 in D4 respectively and the differences among treatments were insignificant ($p \leq 0.05$).

Nitrite is an intermediate product of the aerobic nitrification bacterial process, produced by the autotrophic *Nitrosomonas* bacteria combining oxygen and ammonia. According to Bhatnagar *et al* (2004), nitrite-nitrogen in the range of 0.02-1.0 mgl^{-1} is lethal to many fish species, >1.0 mg l^{-1} is lethal for many warm water fishes and <0.02 mg l^{-1} is acceptable. The mean NO_2^--N during the present study (0.04-0.05 mgl^{-1}) was in accordance with the tolerable value (not >0.5 mgl^{-1}) recommended by Santhosh and Singh (2007) for fish culture.

Table 24: Nitrite nitrogen (mg l^{-1}) of water during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
April	**0**	0.033^{d}±0.02	0.055^{c}±0.10	0.070^{b}±0.14	0.045^{c}±0.29	0.091^{a}±0.22	0.033^{d}±0.32
April	**15**	0.014^{d}±0.12	0.065^{a}±0.16	0.041^{c}±0.81	0.057^{b}±0.52	0.055^{b}±0.34	0.016^{d}±0.45
May	**30**	0.063^{b}±0.80	0.083^{a}±0.63	0.067^{b}±0.36	0.072^{b}±0.45	0.058^{c}±0.21	0.063^{b}±0.18
May	**45**	0.040^{c}±0.03	0.078^{a}±0.06	0.055^{b}±0.08	0.054^{b}±0.19	0.070^{a}±0.25	0.041^{c}±0.03
June	**60**	0.036^{c}±0.15	0.045^{c}±0.10	0.081^{a}±0.17	0.045^{c}±0.14	0.064^{b}±0.29	0.030^{c}±0.35
June	**75**	0.078^{a}±0.11	0.068^{a}±0.17	0.043^{c}±0.10	0.057^{b}±0.06	0.034^{c}±0.05	0.070^{a}±0.11
July	**90**	0.059^{b}±0.01	0.081^{a}±0.03	0.030^{c}±0.18	0.069^{b}±0.13	0.061^{b}±0.19	0.059^{b}±0.01
July	**105**	0.034^{b}±0.02	0.016^{c}±0.01	0.090^{a}±0.05	0.019^{b}±0.01	0.099^{a}±0.02	0.035^{c}±0.02
August	**120**	0.022^{c}±0.04	0.063^{b}±0.06	0.056^{b}±0.08	0.090^{a}±0.12	0.039^{c}±0.19	0.025^{c}±0.04
August	**135**	0.040^{b}±0.04	0.070^{a}±0.24	0.065^{a}±0.14	0.054^{ab}±0.53	0.066^{a}±0.05	0.055^{ab}±0.21
September	**150**	0.053^{b}±0.12	0.055^{b}±0.03	0.070^{ab}±0.04	0.045^{bc}±0.19	0.091^{a}±0.02	0.033^{c}±0.14
Mean		**0.04^{a}±0.25 (0.014-0.078)**	**0.06^{a}±0.16 (0.016-0.083)**	**0.06^{a}±0.15 (0.030-0.090)**	**0.05^{a}±0.41 (0.019-0.090)**	**0.06^{a}±0.25 (0.034-0.099)**	**0.04^{a}±0.30 (0.016-0.070)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)
Values in parenthesis depicts range

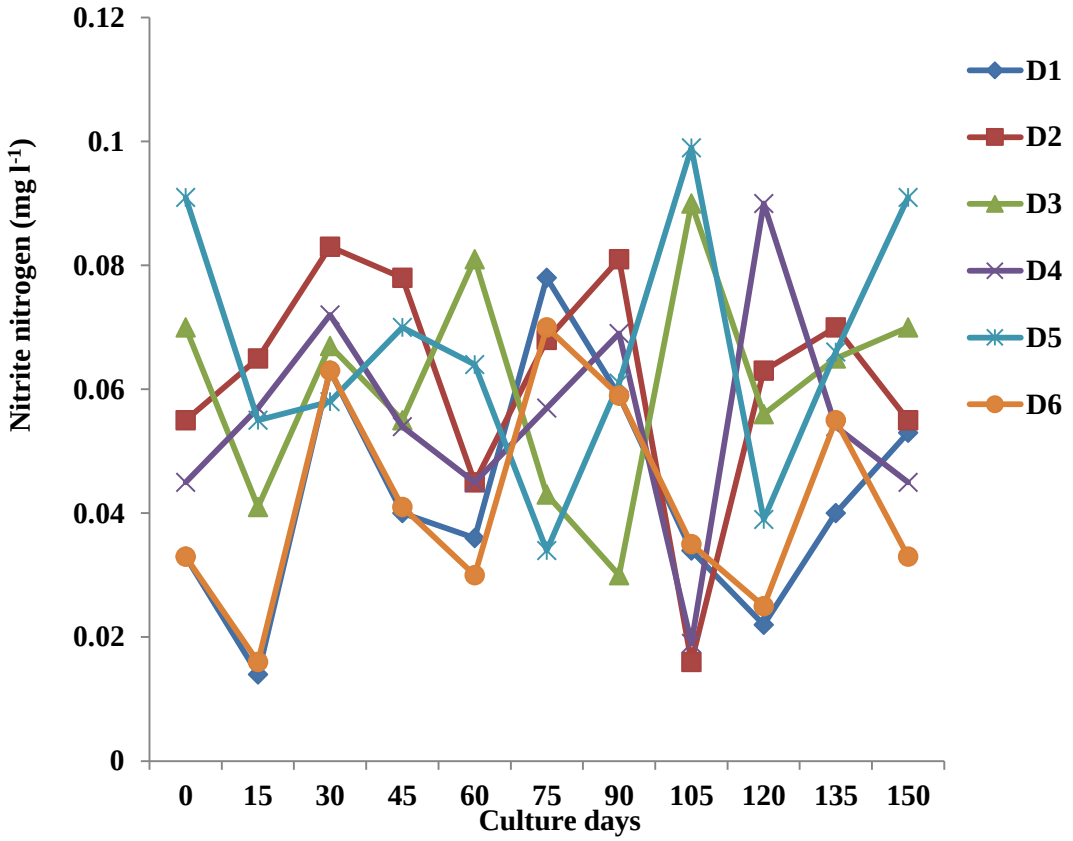


Fig 24: Changes in Nitrite nitrogen (mg l^{-1}) of water in different treatments during the experimental period

4.2.1.7 Nitrate-nitrogen (NO_3^--N)

The NO_3^--N (mg l^{-1}) of water fluctuated between 0.13 to 0.89 (mg l^{-1}) in different treatments during the experimental period (Table 25, Fig 25). Among different treatments, mean NO_3^--N was 0.44, 0.57, 0.48, 0.59, 0.42 and 0.46 in D1, D2, D3, D4, D5 and D6 respectively and the differences among treatments were insignificant ($p \leq 0.05$).

Under optimum culture conditions, total NH_3-N is converted into toxic nitrite (NO_2^-) and then into non-toxic nitrate (NO_3^-) through N_2 cycle. Mean NO_3^--N concentration remained near optimum (< 0.5 mg l^{-1}) in all the treatments (Boyd and Tucker 1998), indicating favorable conditions for conversion of NO_2^--N into non-toxic nitrate, which indicate that fish silage had no adverse effect on the water quality with respect to NO_3^--N concentration.

Table 25: Nitrate nitrogen (mg l^{-1}) of water during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
April	**0**	0.31^{c}±0.01	0.38^{c}±0.05	0.47^{b}±0.04	0.76^{a}±0.02	0.41^{b}±0.06	0.53^{b}±0.01
April	**15**	0.44^{b}±0.01	0.80^{a}±0.15	0.28^{c}±0.45	0.69^{a}±0.09	0.15^{c}±0.27	0.34^{b}±0.11
May	**30**	0.58^{b}±0.08	0.81^{a}±0.03	0.20^{c}±0.06	0.15^{c}±0.05	0.13^{c}±0.04	0.18^{c}±0.08
May	**45**	0.47^{a}±0.02	0.53^{a}±0.11	0.45^{a}±0.06	0.54^{a}±0.16	0.58^{a}±0.28	0.47^{a}±0.02
June	**60**	0.38^{b}±0.14	0.89^{a}±0.20	0.20^{c}±0.15	0.75^{a}±0.14	0.54^{ab}±0.14	0.48^{b}±0.24
June	**75**	0.41^{b} ±0.03	0.56^{ab} ±0.02	0.68^{a} ±0.17	0.77^{a}±0.06	0.38^{b}±0.19	0.55^{ab}±0.15
July	**90**	0.53^{b}±0.01	0.48^{b}±0.05	0.67^{a}±0.04	0.76^{a}±0.02	0.41^{b}±0.06	0.43^{b}±0.01
July	**105**	0.44^{b}±0.11	0.75^{a}±0.08	0.57^{ab}±0.45	0.79^{a}±0.29	0.45^{b}±0.07	0.84^{a}±0.11
August	**120**	0.18^{a}±0.08	0.16^{a}±0.03	0.20^{a}±0.06	0.15^{a}±0.05	0.33^{a}±0.04	0.18^{a}±0.08
August	**135**	0.56^{a}±0.02	0.43^{a}±0.01	0.55^{a}±0.16	0.64^{a}±0.06	0.62^{a}±0.18	0.47^{a}±0.10
September	**150**	0.54^{a} ±0.12	0.44^{a} ±0.45	0.65^{a} ±0.32	0.48^{a} ±0.22	0.60^{a} ±0.40	0.55^{a} ±0.52
Mean		**0.44^{a} ±0.22 (0.18-0.58)**	**0.57^{a} ±0.35 (0.16-0.89)**	**0.48^{a} ±0.14 (0.20-0.68)**	**0.59^{a} ±0.21 (0.15-0.79)**	**0.42^{a} ±0.12 (0.13-0.62)**	**0.46^{a} ±0.22 (0.18-0.84)**

Values are Mean ± S.E., n= 3
Values with same superscript (a,b,….d) in a row does not differ significantly ($p \leq 0.05$)
Values in parenthesis depicts range

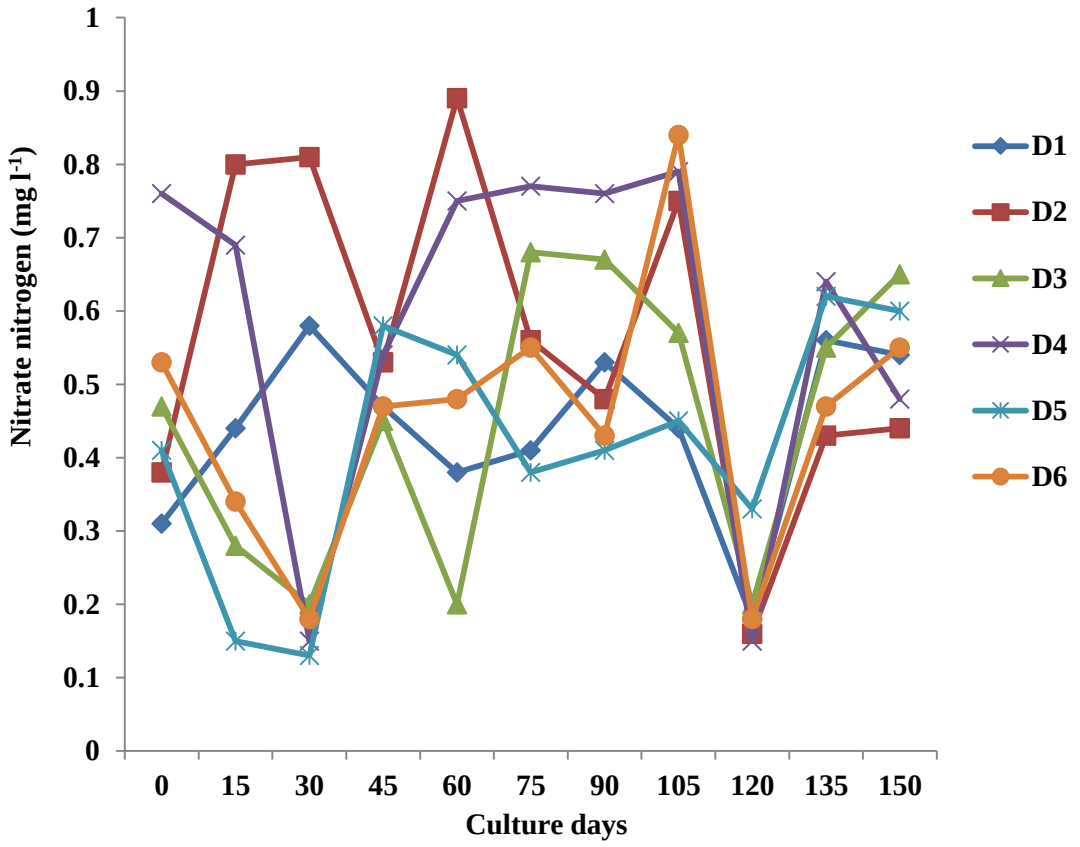


Fig 25: Changes in Nitrate nitrogen (mg l^{-1}) of water in different treatments during the experimental period

4.2.1.8 Orthophosphates (O-PO_4^{3-})

The Orthophosphates (mg l^{-1}) of water fluctuated between 0.01 to 0.09 in different treatments during the experimental period (Table 26, Fig 26). Among different treatments, mean O-PO_4^{3-} level was 0.06 in D1, 0.04 in D2 and D6 and 0.05 in D3, D4 and D5 respectively and the differences among treatments were insignificant ($p \leq 0.05$).

Phosphorus is present in natural waters as orthophosphate and undifferentiated organic phosphates. According to Stone and Thomforde (2004) the phosphate level of 0.06 mgl^{-1} is desirable for fish culture and Bhatnagar *et al* (2004) too suggested 0.05-0.07 mgl^{-1} as optimum range for orthophosphates for productive water. During the present study, mean O-PO_4^{3-} (0.04-06 mgl^{-1}) remained well within the optimum range.

Table 26: Orthophosphate (mg l^{-1}) of water in different treatments during the experimental period

Month	Culture Days	Treatments					
		D1	D2	D3	D4	D5	D6
April	**0**	$0.01^{a}\pm0.024$	$0.03^{a}\pm0.013$	$0.05^{a}\pm0.012$	$0.04^{a}\pm0.006$	$0.04^{a}\pm0.009$	$0.01^{a}\pm0.005$
April	**15**	$0.02^{a}\pm0.02$	$0.05^{a}\pm0.003$	$0.03^{a}\pm0.004$	$0.01^{a}\pm0.005$	$0.06^{a}\pm0.006$	$0.08^{a}\pm0.002$
May	**30**	$0.04^{a}\pm0.005$	$0.03^{a}\pm0.003$	$0.02^{a}\pm0.003$	$0.07^{a}\pm0.005$	$0.08^{a}\pm0.003$	$0.06^{a}\pm0.008$
May	**45**	$0.09^{a}\pm0.13$	$0.05^{a}\pm0.002$	$0.06^{a}\pm0.001$	$0.02^{a}\pm0.23$	$0.04^{a}\pm0.006$	$0.01^{a}\pm0.001$
June	**60**	$0.08^{a}\pm0.003$	$0.02^{a}\pm0.006$	$0.04^{a}\pm0.11$	$0.03^{a}\pm0.09$	$0.01^{a}\pm0.17$	$0.04^{a}\pm0.01$
June	**75**	$0.04^{a}\pm0.11$	$0.07^{a}\pm0.18$	$0.06^{a}\pm0.01$	$0.08^{a}\pm0.004$	$0.07^{a}\pm0.002$	$0.02^{a}\pm0.30$
July	**90**	$0.07^{a}\pm0.003$	$0.01^{a}\pm0.006$	$0.03^{a}\pm0.015$	$0.01^{a}\pm0.18$	$0.05^{a}\pm0.007$	$0.08^{a}\pm0.004$
July	**105**	$0.01^{a}\pm0.001$	$0.04^{a}\pm0.006$	$0.05^{a}\pm0.023$	$0.08^{a}\pm0.004$	$0.06^{a}\pm0.003$	$0.02^{a}\pm0.004$
August	**120**	$0.08^{a}\pm0.006$	$0.07^{a}\pm0.002$	$0.06^{a}\pm0.016$	$0.07^{a}\pm0.002$	$0.08^{a}\pm0.017$	$0.07^{a}\pm0.007$
August	**135**	$0.03^{a}\pm0.01$	$0.05^{a}\pm0.01$	$0.02^{a}\pm0.21$	$0.04^{a}\pm0.14$	$0.08^{a}\pm0.05$	$0.05^{a}\pm0.01$
September	**150**	$0.06^{a}\pm0.005$	$0.03^{a}\pm0.004$	$0.08^{a}\pm0.05$	$0.05^{a}\pm0.002$	$0.06^{a}\pm0.004$	$0.04^{a}\pm0.005$
Mean		**$0.06^{a}\pm0.023$ (0.01-0.09)**	**$0.04^{a}\pm0.045$ (0.01-0.07)**	**$0.05^{a}\pm0.045$ (0.02-0.08)**	**$0.05^{a}\pm0.030$ (0.01-0.08)**	**$0.05^{a}\pm0.015$ (0.01-0.08)**	**$0.04^{a}\pm0.022$ (0.01-0.08)**

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p\leq0.05$)
Values in parenthesis depicts range

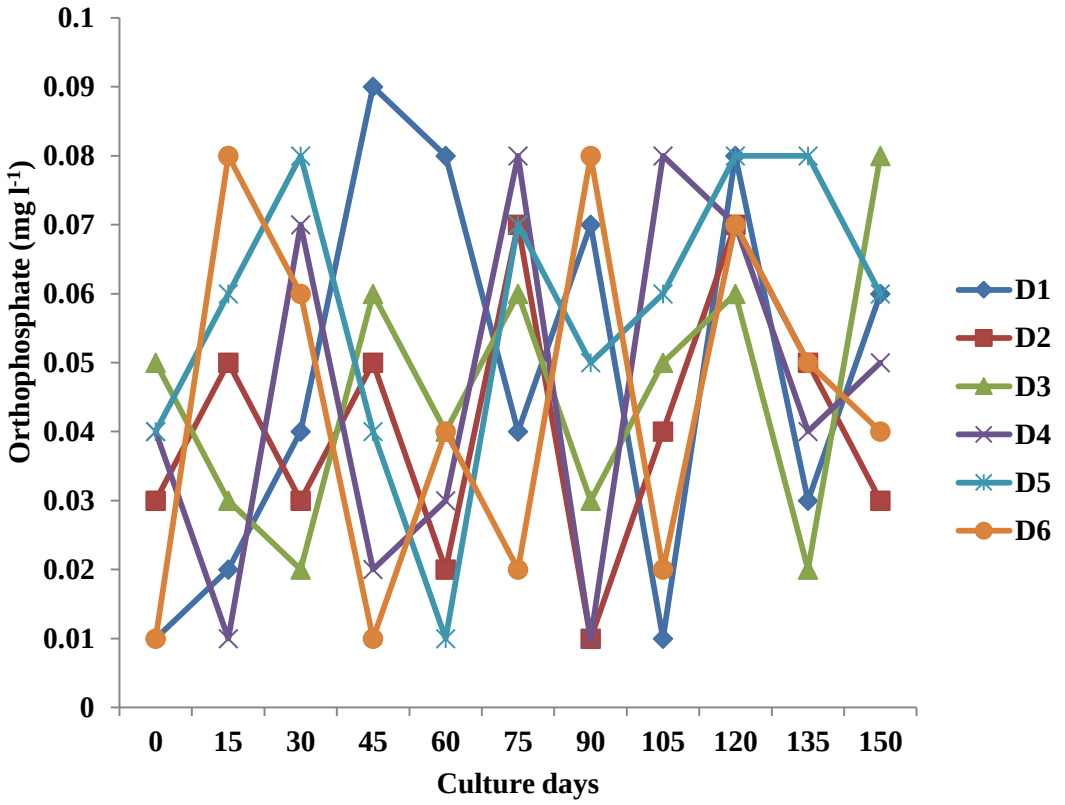


Fig 26: Changes in Orthophosphate (mg l^{-1}) of water in different treatments during the experimental period

The overall results in terms of physico-chemical characteristic during the present study (Experiment II for 150 days) revealed that all the water quality parameters remained well within the optimum range for pangas culture and differences among different treatments were insignificant throughout the culture period. Fish silage supplementation in pangas feed did not showed any negative effect on water quality parameters, thus it can be concluded that it is safe to use fish silage as one of the protein ingredient in supplementation with traditionally used animal/plant protein sources for pangas culture.

4.2.2 Survival and growth of fish

4.2.2.1 Survival of fish

At the end of experiment, all the experimental tanks were drained out completely to harvest all the fish for calculating the % survival of fish in different treatments. Fish survival was 100% in control and all the treatments at the completion of experiment, which revealed that the diets having only fish meal, fish

silage, fish meal and fish silage, plant protein and fish silage together or only plant protein are equally acceptable by pangas. According to Yulianto (2006), both internal and external factors affect the survival of fish. Internal factors are related to fish body and external factors include feed quality and the environmental variations including appropriate water quality (Amanlia 2013). 100% survival during present experimental study clearly indicated both external and internal factors were in optimum range for pangas. The findings of the present study are in line with results of previous study of Datta *et al* (2018) who reported that poultry waste, fish meal, fish silage and soybean meal were equally effective in terms of pangas survival, which remained 100% after 120 days of experiment. Similar observations were also recorded by Ogunji *et al* (2008) who reported 100% survival in Nile Tilapia (*O. niloticus*), when fed with housefly maggot meal (Magmeal) supplemented, isoenergetic diets containing 31.2, 34.0 and 36.1% of crude protein. Bekibele *et al* (2010) reported 100% survival in tilapia (*O. niloticus*), when fed with fish and blood meal based diet @ 10% level. Aladetohun and Sogbesan (2013), too recorded 100% survival in *O. niloticus* fingerlings fed with blood meal (@ 0, 50 and 100%) as a protein ingredient from animal waste product (blood meal).

4.2.2.2 Growth of fish

The growth performance of fish was assessed in terms of total body length (TBL) and body weight (BW) at monthly intervals during the 150 days experimental period. At the end of the experiment, growth (length and weight) parameters in terms of total body length gain (TBLG), net weight gain (NWG), specific growth rate (SGR), condition factor (K) and feeding efficiency in terms of feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish for each treatment were calculated.

4.2.2.2.1 Length parameters

Among different treatments, total body length (cm) increased from 18.77 to 28.91 in D1, 18.84 to 33.78 in D2, 18.73 to 33.85 in D3, 18.24 to 33.69 in D4, 18.01 to 33.68 in D5 and 18.24 to 30.92 in D6 respectively (Table 27, Fig 27). At the end of experimental period, final total body length and TBLG (cm) was significantly higher ($p \leq 0.05$) in D3 (33.85) and D5 (15.68) and minimum in D1 (28.91 and 10.14) (Table 27, Fig 27).

Table 27: Changes in length parameters of fish in different treatments during the experimental period

Month	Days	Treatments					
		D1	D2	D3	D4	D5	D6
April	0	18.77 ^{a}±0.07	18.84 ^{a}±0.03	18.73 ^{a}±0.02	18.24 ^{a} ±0.04	18.01 ^{a}±0.03	18.24 ^{a}±0.03
April	30	20.45^{bc}±0.52	21.65^{a}±0.15	21.03 ^{ab}±0.38	20.18^{bc}±0.07	19.69^{c}±0.41	20.27^{bc}±0.21
May	60	23.19^{b}±0.65	24.09 ^{a}±0.49	22.92 ^{c}±0.25	22.55 ^{c} ±0.07	22.91^{c} ±0.46	22.39 ^{c}±0.48
June	90	25.97 ^{b}±0.40	27.71 ^{a}±0.38	26.78^{ab}±0.14	26.48^{ab}±0.62	25.78 ^{b}±0.72	25.37 ^{b}±0.30
July	120	27.69 ^{b}±0.32	30.99 ^{a}±0.50	28.25 ^{b} ±0.29	27.75^{b}±0.53	27.66 ^{b}±0.21	25.44 ^{c} ±0.73
August	150	28.91^{c}±0.46	33.78 ^{a}±0.17	33.85 ^{a}±0.33	33.69^{ab}±0.50	33.68^{ab}±0.66	30.92 ^{b} ±0.18
TBLG		10.14^{c}±0.39	14.94 ^{b}±0.17	15.12^{ab}±0.35	15.46^{ab}±0.15	15.68^{a}±0.84	12.68 ^{c}±0.15
Survival (%)		100 ^{a} ±0.02	100 ^{a} ±0.01	100 ^{a} ±0.03	100 ^{a} ±0.01	100 ^{a} ±0.02	100 ^{a} ±0.04

Values are Mean ± S.E., n= 10
Values with same superscript (a, b,….d) in a row does not differ significantly (p≤0.05)
TBLG= Total body length gain

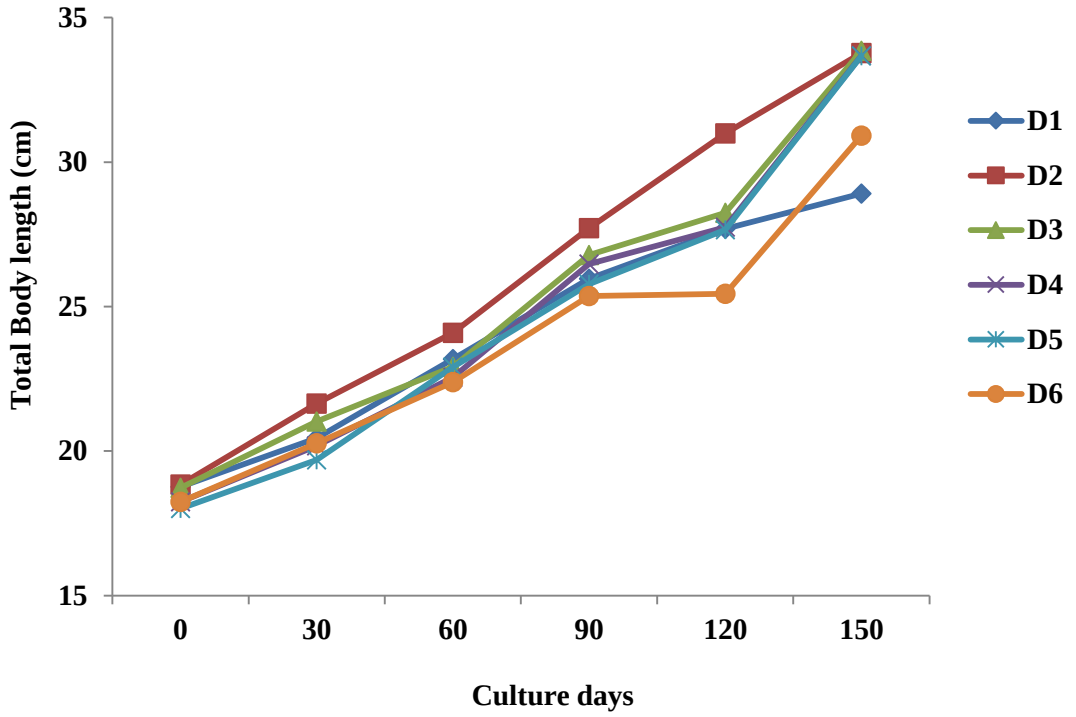


Fig 27: Change in total body length (cm) of fish in different treatments during the experimental period

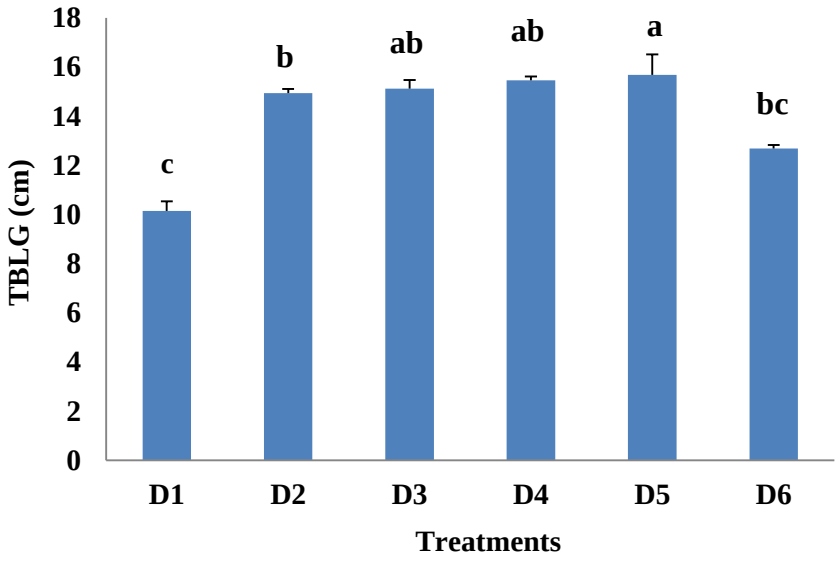


Fig 28: Comparative TBLG of fish in different treatments after completion of the experimental period

4.2.2.2.2 Body Weight Parameters

Among different treatments, body weight (g) of fish increased from 58.59 to 194.57, 57.30 to 237.98, 57.35 to 249.82, 57.64 to 271.57, 57.22 to 274.36 and 57.53 to 195.29 in D1, D2, D3, D4, D5 and D6 respectively (Table 28, Fig 29).

At the end of experimental period, significantly higher ($p \leq 0.05$) fish growth in terms of final body weight (FBW), NWG and SGR was observed in D5 (274.36g, 217.14 and 1.00), while minimum FBW and NWG in D1 (194.57g and 135.98) and SGR in D6 (0.74). The differences for condition factor were insignificant ($p \leq 0.05$) for all the treatments and control, with maximum value in D5 (0.99). Significantly lower ($p \leq 0.05$) value for FCR was observed in D4 and D5 (2.21 and 2.37) and maximum (3.63) in D1 (Table 28). Likewise, PER was also significantly higher in D5 (3.08) and minimum in D1 and D2 (1.99). The overall results of the present study revealed that fish growth improved in all silage fed groups as compared to control and D6 (diet having only plant protein sources) with significantly higher values for all growth parameters (Table 28, Fig 29-30) in D5 (without fish meal, but having fish silage and plant ingredients).

Number of studies revealed positive effect of animal protein sources (waste / byproducts) on number of omnivorous and carnivorous fish species. The higher growth performance in D5 in the present study can be explained by the synergistic effect of combining plant proteins with animal protein in the form of fish silage. These findings are in agreement with the results of number of studies conducted with combined protein sources showing better results in terms of fish growth as compared to single protein source (Ugwumba *et al* 2001, Sogbesan *et al* 2005, Sogbesan and Ugwumba 2008). Ability of fish to convert nutrient especially protein can positively influence growth performance. This is justified in terms of best protein efficiency ratio and overall improved growth performance of fish in D5 in the present study. Further, lowest feed conversion ratio indicates better utilization of feed by the fish along with fulfillment of amino acid requirement of fish from mixed protein sources (De Silva and Anderson 1995).

Table 28: Changes in weight parameters of fish in different treatments during and after completion of experiment

Month	Days	Treatments					
		D1	D2	D3	D4	D5	D6
April	0	$58.59^{a}\pm0.13$	$57.30^{a}\pm0.89$	$57.35^{a}\pm0.78$	$57.64^{a}\pm0.15$	$57.22^{a}\pm0.66$	$57.53^{a}\pm0.63$
April	30	$67.28^{b}\pm0.69$	$88.16^{a}\pm0.25$	$79.00^{ab}\pm0.19$	$66.63^{b}\pm0.20$	$74.14^{b}\pm0.47$	$74.07^{b}\pm0.42$
May	60	$111.23^{c}\pm0.48$	$149.94^{a}\pm0.90$	$122.7^{b}\pm0.70$	$113.85^{c}\pm0.55$	$121.20^{b}\pm0.47$	$103.94^{d}\pm0.42$
June	90	$148.33^{b}\pm0.49$	$183.33^{a}\pm0.12$	$156.33^{b}\pm0.14$	$142.66^{b}\pm0.11$	$152.33^{b}\pm0.56$	$131.53^{b}\pm0.65$
July	120	$162.55^{b}\pm0.74$	$197.13^{a}\pm0.12$	$178.99^{b}\pm0.33$	$172.71^{b}\pm0.93$	$175.56^{b}\pm0.52$	$132.72^{c}\pm0.99$
August	150	$194.57^{c}\pm0.11$	$237.98^{b}\pm0.19$	$249.82^{b}\pm0.28$	$271.57^{a}\pm0.17$	$274.36^{a}\pm0.83$	$195.29^{c}\pm0.79$
NWG		$135.98^{c}\pm0.10$	$180.68^{b}\pm0.25$	$192.47^{b}\pm0.28$	$214.11^{a}\pm0.37$	$217.14^{a}\pm0.85$	$137.76^{c}\pm0.86$
SGR		$0.76^{d}\pm0.31$	$0.92^{b}\pm0.20$	$0.88^{c}\pm0.15$	$0.96^{b}\pm0.11$	$1.00^{a}\pm0.35$	$0.74^{d}\pm0.42$
FCR		$3.63^{a}\pm0.20$	$3.50^{a}\pm0.30$	$2.79^{b}\pm0.10$	$2.21^{c}\pm0.15$	$2.37^{c}\pm0.09$	$3.42^{a}\pm0.35$
PER		$1.99^{d}\pm0.07$	$1.99^{d}\pm0.05$	$2.49^{c}\pm0.02$	$2.74^{b}\pm0.03$	$3.08^{a}\pm0.05$	$2.21^{cd}\pm0.08$
K		$0.80^{a}\pm0.25$	$0.81^{a}\pm0.29$	$0.81^{a}\pm0.35$	$0.94^{a}\pm0.45$	$0.99^{a}\pm0.58$	$0.84^{a}\pm0.66$

Values are Mean ± S.E., n= 10
Values with same superscript (a, b,….d) in a row does not differ significantly ($p\leq0.05$)
NWG= Net weight gain, SGR= Specific growth rate, PER= Protein efficiency ratio, FCR= Feed conversion ratio, K= Condition factor

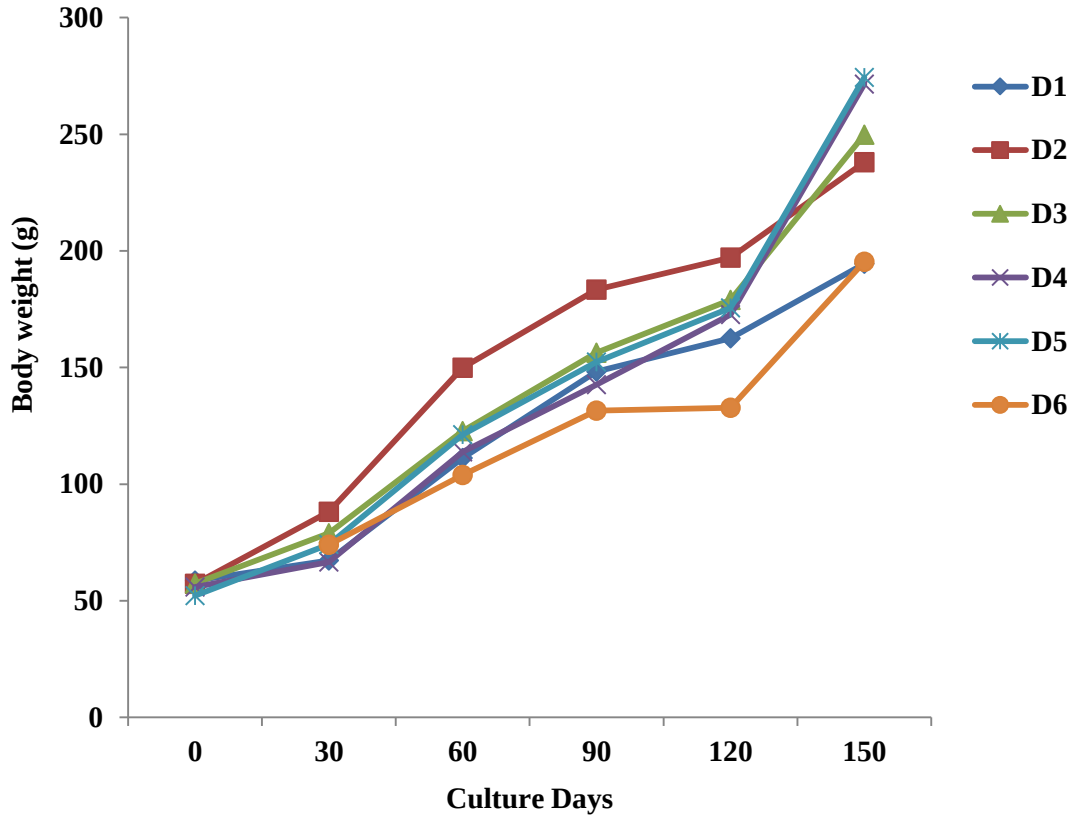


Fig 29: Change in body weight (g) of fish in different treatments during the experimental period

The findings of the present study are in line with results of study by Millamena (2002), who reported improved growth in juvenile grouper (*Epinephelus coioides*), when fed with animal by-product meal in the form of meat meal and blood meal (4:1 ratio). Better growth in terms of mean final weight (MFW), percent weight gain (PWG) and amount of diet fed was also reported by Thompson *et al* (2012) in Nile tilapia *O. niloticus* fry with a diet containing 20% soybean protein concentrate (SPC) and poultry by-product meal (PBM). Balogun *et al* (1997) reported improved growth performance of catfish, *C. gariepinus*, when fed on formic acid ensiled product consisting of 60% minced fish+ 40 % soybean meal. According to Fagbenro and Jauncey (1998), diet made up of silage and soymeal can be used to feed tilapia, *O. niloticus* (omnivorous) and African catfish, *C. gariepinus* (carnivorous) with no change in growth performance and carcass composition. Similar observations were also reported by Tabinda and Butt (2012) who reported significantly ($p \leq 0.05$) higher growth along with lower FCR in grass carp fry fed with chicken intestine @ 30%. Jamil *et al* (2007) also evaluated effects of mixture of animal by-products meal

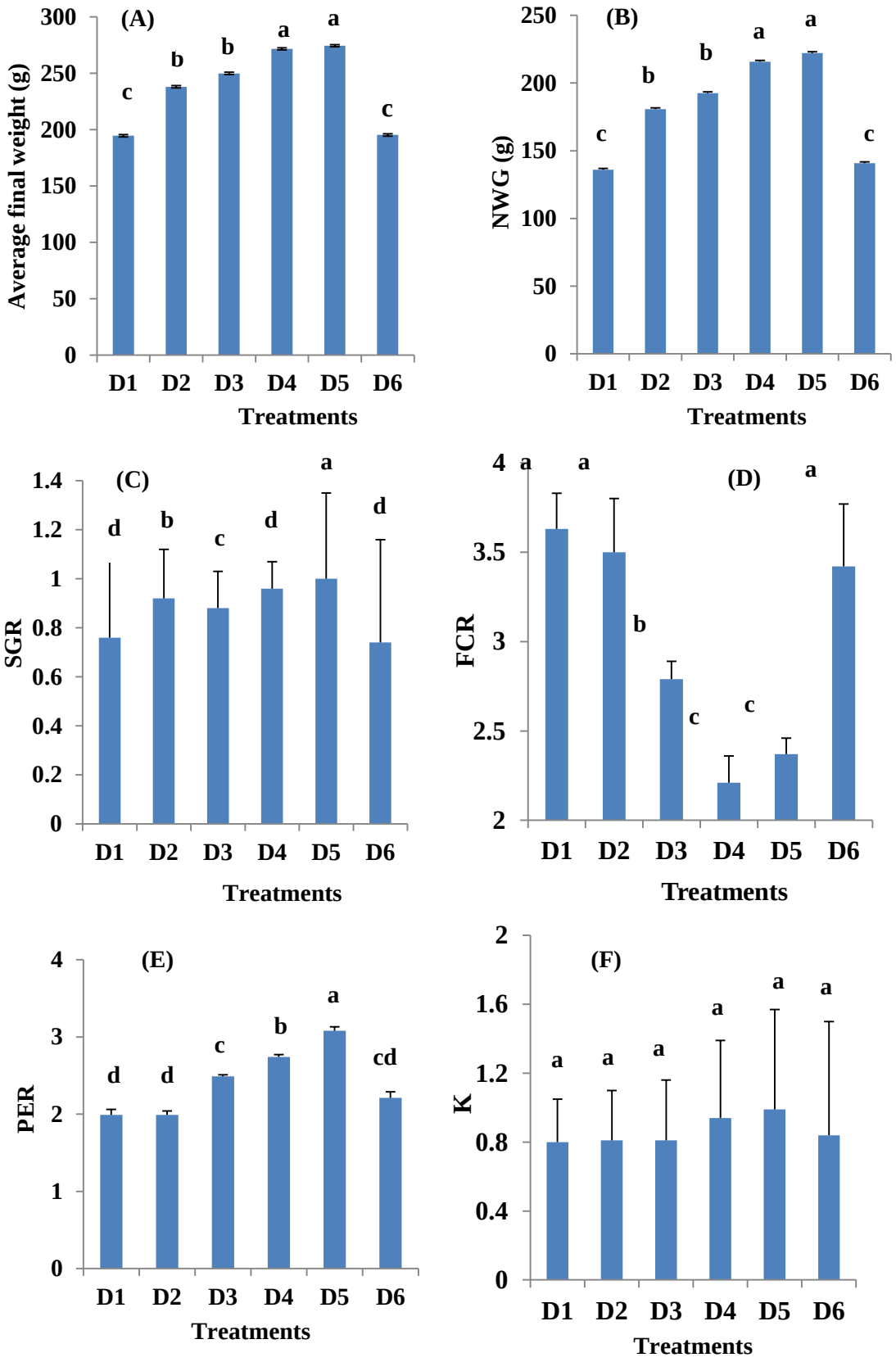


Fig 30: Comparative growth parameters of fish in different treatments after completion of experiment [A= Average final weight (g), B= Net weight gain (g), C= Specific growth rate, D= Feed conversion ratio, E= Protein efficiency ratio and F= Condition factor (K)]

supplementation (@ 0%, 25%, 50% and 100%) in diets of mangrove red snapper (*Lutjanus argentimaculatus).* Results indicated that approximately 23 % of fish meal supplementation with mixture of animal by-products meal supplementation improved NWG, SGR and feed conversion efficiency significantly ($p \leq 0.05$).

Further, in addition to silage supplementation, use of linseed oil showed positive effect on fish growth in all the treatments for 30 days feeding. Growth rate of pangas was significantly faster in D4 and D5 as compared to all other treatments and control after linseed supplementation. This may be due to the positive impact of linseed oil on fish growth due to improved lipid content of diets leading to improved fish growth as observed by Pei *et al* (2004), El-Marakby (2006) and Zupan *et al* (2016). Further, vegetable oils are less prone to oxidation, hence supporting higher growth and effecient feed conversion (Guillou *et al* 1995).

4.2.3 Haematological Parameters

Haematology of fish in terms of haemoglobin (Hb) and Hematocrit (Ht) was studied at the completion of the experiment.

4.2.3.1 Haemoglobin content (Hb)

In different treatments, Hb (g%) in fish was 7.33 in D1; 10.00 in D2 and D3; 10.23, 10.30 and 8.00 in D4, D5 and D6, respectively and the differences among treatments were significant ($p \leq 0.05$) with improved Hb level in all the treatments as compared to control with maximum value in D5 (10.30) (Table 29, Fig 31). The result indicated that fish silage supplementation in pangas fish diet significantly improved Hb content in all the treatments with maximum value in D5.

4.2.3.2 Haematocrit (Ht) or Packed Cell Volume (PCV)

Among different treatments, Ht (%) was 20.00, 25.00, 26.66, 30.00, 33.33 and 21.66 in D1, D2, D3, D4, D5 and D6, respectively and the values were significantly higher ($p \leq 0.05$) in all treatments except D6 as compared to control with maximum value in D5 (33.33) and minimum in D6 (21.66) and D1 (20.00). The result indicated that fish silage incorporation in fish diet increased the haematocrit content significantly with maximum value in D5.

Table 29: Comparative hematological parameters of fish in different treatments after completion of experiment

Hematological parameter	Treatments					
	D1	D2	D3	D4	D5	D6
Hb (g%)	$7.33^{c}\pm0.33$	$10.00^{a}\pm0.02$	$10.00^{a}\pm0.04$	$10.23^{a}\pm0.14$	$10.30^{a}\pm0.10$	$8.00^{b}\pm0.03$
Ht (%)	$20.00^{d}\pm0.04$	$25.00^{c}\pm0.06$	$26.66^{c}\pm0.08$	$30.00^{b}\pm0.09$	$33.33^{a}\pm0.16$	$21.66^{d}\pm0.07$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly (p≤0.05)

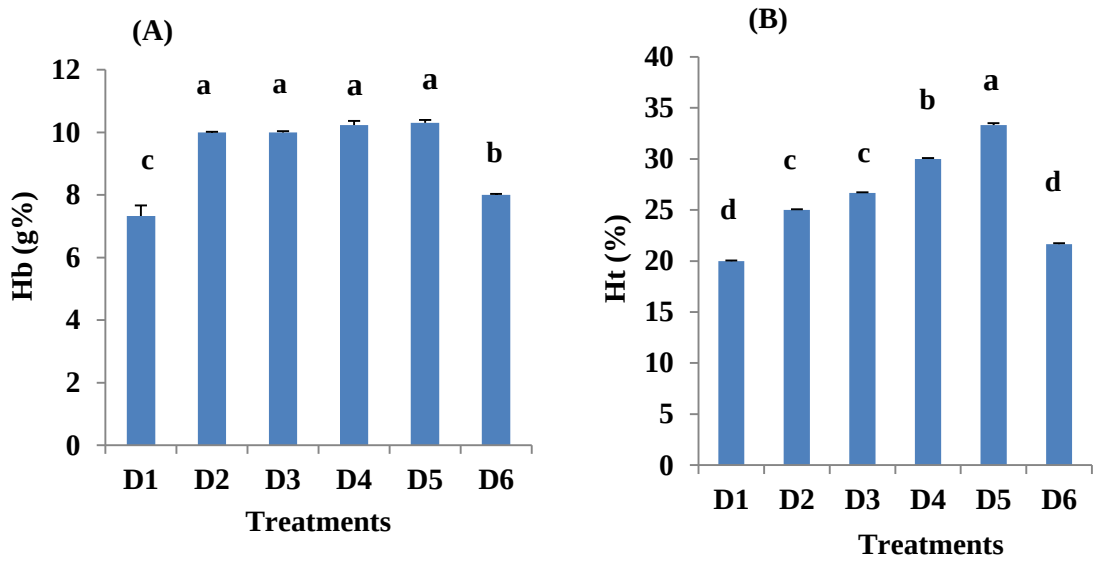


Fig 31: Comparative hematological parameters of fish in different treatments after completion of experiment [A= Haemoglobin (g%), B= Haematocrit (%)]

Haematological indices are essential indicators of physiological and physio pathological changes in fish (Hrubec *et al* 2000, Rainza-Paiva *et al* 2000), which in turn are affected by number of environmental factors including feeding habits. In most teleost, hematocrit level ranged between 20-40% of blood (Wells and Baldwin 1990) and reduced hematocrit value is indication of stressed condition. In the present study, Hb and Ht showed improvement in all the diets as compared to control, which indicates the positive effect of dietary manipulation i.e incorporation of fish silage along with linseed oil in pangas feed. Further, linseed oil (rich source of omega-3 fatty acid especially α-linolenic acid) incorporation in pangas diet for 30 days may have resulted in improved fish growth due to improved dietary lipid content, as

observed by Zupan *et al* (2016) in common carp along with improved health status (Watters *et al* 2012). Further health promoting effects of fish silage (Madage *et al* 2015) in terms of free amino acid and linseed oil (Gogus and Smith 2010, Goyal *et al* 2014) in terms of high content of n-3 linolenic acid, at appropriate level must have improved the haematological parameters of pangas. Moreover, Aderolu and Akinremi (2009) too reported positive effect of plant oil inclusion on haematological (Hb & Ht) parameters of *Clarias gariepinus*.

4.2.4 Biochemical parameters

Biochemical parameters of fish in terms of total serum proteins, albumins, globulins and albumin/globulin ratio was studied at the completion of the experiment.

4.2.4.1 Total proteins

Among different treatments, blood serum total protein (gdl^{-1}) of fish was 5.30, 5.47, 6.34, 6.63, 6.96 and 5.43 in D1, D2, D3, D4, D5 and D6, respectively and the differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet, increased the serum total protein levels significantly (Table 30, Fig 32) in all the treatments as compared to control with maximum value in D5 (6.96) and minimum in D1 (5.30).

Table 30: Comparative biochemical parameters in blood serum of fish in different treatments after completion of experiment

Biochemical parameter (gdl^{-1})	Treatments					
	D1	D2	D3	D4	D5	D6
Total protein	$5.30^{c}\pm0.80$	$5.47^{b}\pm0.10$	$6.34^{ab}\pm0.25$	$6.63^{a}\pm0.14$	$6.96^{a}\pm0.33$	$5.43^{b}\pm0.15$
Albumin	$0.68^{d}\pm0.19$	$0.75^{c}\pm0.09$	$0.86^{b}\pm0.19$	$0.89^{b}\pm0.24$	$0.96^{a}\pm0.05$	$0.74^{c}\pm0.07$
Globulins	$4.62^{d}\pm0.67$	$4.72^{c}\pm0.10$	$5.48^{b}\pm0.32$	$5.74^{b}\pm0.16$	$6.00^{a}\pm0.38$	$4.69^{d}\pm0.11$
Alb/Glb ratio	$0.14^{a}\pm0.17$	$0.15^{a}\pm0.23$	$0.15^{a}\pm0.45$	$0.16^{a}\pm0.27$	$0.16^{a}\pm0.35$	$0.15^{a}\pm0.62$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)

4.2.4.2 Albumin

Among different treatments, albumin (gdl^{-1}) in fish was 0.68, 0.75, 0.86, 0.89, 0.96 and 0.74 in D1, D2, D3, D4, D5 and D6, respectively and the differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased the serum albumin levels significantly (Table 30, Fig 32) with maximum value in D5 (0.96) and minimum in D1 (0.68).

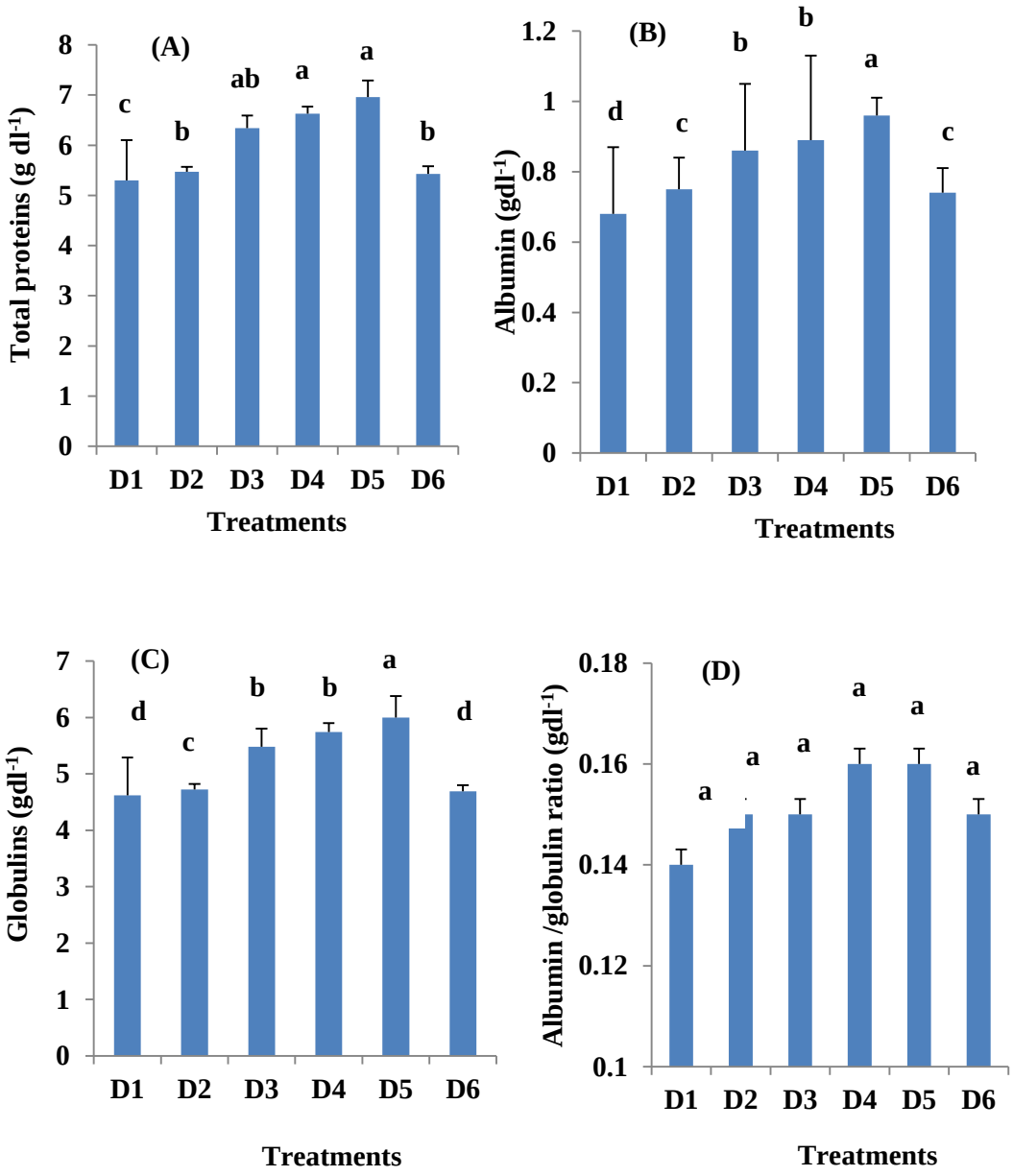


Fig 32: Comparative biochemical parameters (g dl^{-1}) in blood serum of fish in different treatments after completion of experimental period [A= Total proteins, B= Albumin, C= Globulins and D= Albumin /globulin ratio]

4.2.4.3 Globulin

Among different treatments, globulin (gdl^{-1}) of fish was 4.62, 4.72, 5.48, 5.74, 6.00 and 4.69 in D1, D2, D3, D4, D5 and D6, respectively and the differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased the serum globulin levels significantly (Table 30, Fig 32) with maximum value in D5 (6.00) and minimum in D1 (4.62).

4.2.4.3.4 Albumin/globulin ratio (Alb/Glb)

In different treatments, Alb/Glb ratio in fish was 0.14 in D1, 0.15 in D2, D3 and D6 and 0.16 in D4 and D5, respectively and the differences among treatments were insignificant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet had no significant effect on the Alb/Glb ratio (Table 30, Fig 32).

The results in terms of serum biochemical parameters i.e total proteins, albumins and globulins improved in all silage incorporated treatments as compared to control. The results in terms of serum biochemical parameters showed improvement in all silage treatments as compared to control are in accordance with hematological parameters showing maximum values in D5. The improved status of biochemical parameters is associated with a strong immune response of fish (Wiegertjes *et al* 1996) coupled with improved haematological parameters resulting from enhanced nutritional status of fish. According to Wedemeyer *et al* (1996), biochemical parameters are indicators of disease and stress in fish, and which in turn are affected by nutritional status, culture conditions, age, hormones and seasons etc. Significant increase in total proteins, albumins and globulins in D5 revealed that dietary manipulation in terms of fish silage and linseed oil found to be favourable for pangas. Najim *et al* (2014) reported significant ($p \leq 0.05$) increase in total protein and albumin levels in common carp, *C. carpio,*when fish meal was replaced with fish bio silage at different levels (25, 50 or 75%).

4.2.5 Antioxidant parameters

Antioxidant parameters in terms of Superoxide dismutase (SOD) (U mg^{-1} Hb) and Lipid peroxidation (LPO) (nmol MDAg Hb $^{-1}$) were analyzed at the completion of the experiment.

4.2.5.1 Superoxide dismutase (SOD)

Among different treatments, SOD (U mg^{-1} Hb) was 0.36, 0.40, 0.47, 0.52, 0.54 and 0.51 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased SOD levels in all the treatments with maximum value in D5 (0.54) and minimum in D1 (0.36) (Table 31, Fig 33).

4.2.5.2 Lipid peroxidation (LPO)

Among different treatments, LPO (nmol MDAg Hb $^{-1}$) was 1.84, 1.27, 0.91, 0.82, 0.78 and 0.80 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet decreased LPO levels with maximum value in D1 (1.84) and minimum in D5 (0.78) (Table 31, Fig 33).

Although, values for SOD and LPO increased and decreased with silage incorporation, however difference were insignificant for SOD in D4, D5 and D6 and for LPO in D3, D4, D5 and D6.

Table 31: Comparative antioxidant parameters in blood hemolysate of fish in different treatments after completion of experiment

Antioxidant parameter	Treatments					
	D1	D2	D3	D4	D5	D6
SOD	0.36^{c}±+0.04	0.40^{b}±0.01	0.47^{b}±0.01	0.52^{a}±0.03	0.54^{a}±0.03	0.51^{a}±0.04
LPO	1.84^{a}±0.20	1.27^{b}±0.15	0.91^{c}±0.06	0.82^{c}±0.02	0.78^{c}±0.01	0.80^{c}±0.03

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)

Reactive oxygen species (ROS) affects all aerobic organisms including fish and are considered as major indicators of oxygen cytotoxicity (Buetler *et al* 2004). To fight with the action of ROS, animals have developed an antioxidant defence mechanism in the form of enzymes like SOD, catalase, glutathione-s-transferase (GST) and lipid peroxidase (LPO) etc. The activities of these enzymes may differ in different organs (Wdzieczak *et al* 1982) of fish species due to varied feeding behavior and environmental conditions (Winston and Di Ginlio 1991, Roche and Boge 1996).

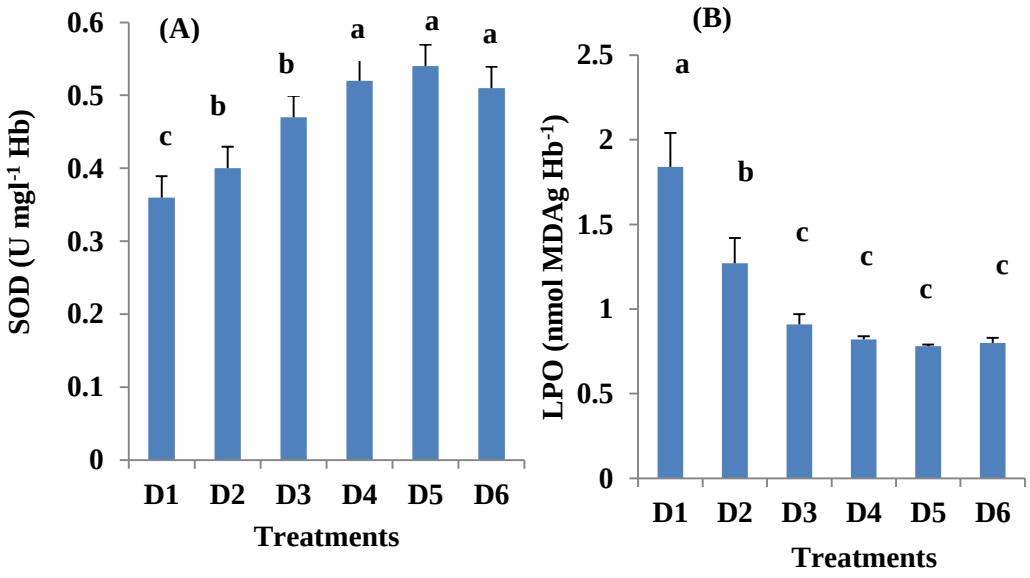


Fig 33: Comparative antioxidant parameters (SOD and LPO) in blood hemolysate of fish in different treatments after completion of experiment [A= SOD (U mgl^{-1} Hb) and B= LPO (nmol MDAg Hb^{-1})]

Further, the response of antioxidant enzymes could differ between fish size being lower activities for small fish as compared to larger ones. In the present study, SOD and LPO activity showed an increase and decrease with fish silage incorporation. The increased activity of SOD is consistent with the first line of defence against ROS, along with decreased lipid peroxidation as an indicative of reduced oxidative stress (Taufek *et al* 2016). Increased antioxidant activities were observed by Dong *et al* (2013) in gibel carp (*C. auratus gibelio*) with dietary supplementation of maggot meal and soybean meal (390 g kg^{-1} MGM or 450 g kg^{-1}). Results indicated a significantly increased ($p \leq 0.05$) antioxidant capacity compared with the control group with supplementation of maggot meal and soybean meal. Moreover, it has also been reported that low level of linseed oil inclusion in fish diet results in increased SOD activity along with total antioxidant status, which indicates improved total oxidation response of fish (Yu *et al* 2019).

4.2.6 Serum Transaminases

Serum Transaminases (Ul^{-1}) in terms of in terms of alanine transaminase (ALT) and aspartate aminotransferase (AST) were studied at the completion of experiment.

4.2.6.1 Alanine transaminase (ALT)

Among different treatments, ALT (Ul^{-1}) was 24.50, 21.25, 19.45, 19.23, 16.00 and 17.45 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet decreased the ALT levels significantly with minimum value in D5 (16.00) and maximum in D1 (24.50) (Table 32, Fig 34).

4.2.6.2 Aspartate amino transaminase (AST)

Among different treatments, AST (Ul^{-1}) was 196.45, 190.89, 188.20, 165.54, 135.25 and 141.68 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet decreased the AST levels significantly with minimum value in D5 (135.25) and maximum in D1 (196.45) (Table 32, Fig 34).

Table 32: Comparative serum transaminases of fish in different treatments after completion of experiment

Stress parameter	Treatments					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
ALT (Ul^{-1})	24.50^{a} ±0.90	21.25^{b} ±1.01	19.45^{c}±1.33	19.23^{c}±0.88	16.00^{e} ±0.58	17.45^{d} ±0.75
AST (Ul^{-1})	196.45^{a}±3.02	190.89^{ab}±4.23	188.20^{b}±2.41	165.54^{c}±5.01	135.25^{e}±3.35	141.68^{d}±2.51

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)

Nutrition of fish should target towards formulation of feed that can boost the immune system of fish. Use of alternate protein sources should be balanced with lipid source supplying essential fatty acids for proper growth and well-being of the fish. The decrease in serum transaminases i.e ALT and AST during the present study revealed the positive effect of mixed protein source (plant and animal) along with n-3 rich linseed oil. AST and ALT are one of the most sensitive parameters of liver function affected by number of factors including diet. According to Kim *et al* (2002) elevated AST and ALT activity can be associated with the release of transaminases from cytoplasm due to hepatic cellular damage.

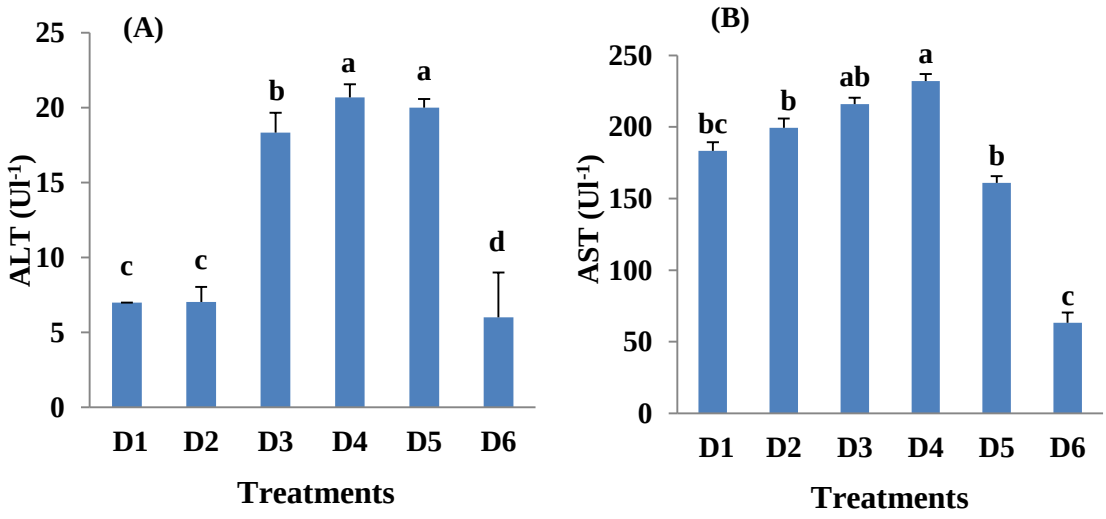


Fig 34: Comparative serum transaminases (Ul^{-1}) of fish in different treatments after completion of experimental period [A= Alanine transaminase and B= Aspartate aminotransferase]

The decrease in activities of AST and ALT in the present study indicated that the liver of fish is in healthy state and transaminases were not released from the cytoplasm (Chen *et al* 2002, Babalola *et al* 2016).

4.2.7 Lipid profile

Lipid profile in terms of triglycerides, cholesterol, high density lipids (HDL), low density lipids (LDL) and very low density lipids (VLDL) was studied at the completion of the experiment

4.2.7.1 Triglycerides

Among different treatments, triglycerides (mg dl^{-1}) of fish was 238.56, 352.53, 385.10, 397.83, 442.63 and 341.50 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased triglyceride levels with maximum value in D5 (442.63) and minimum in D1 (238.56) (Table 33, Fig 35).

4.2.7.2 Cholesterol

Among different treatments, cholesterol (mg dl^{-1}) of fish was 119.03, 192.66, 251.76, 261.93, 268.06 and 193.20 in D1, D2, D3, D4, D5 and D6,

respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased cholesterol levels with maximum value in D5 (268.06) and minimum in D1 (119.03) (Table 33, Fig 35).

4.2.7.3 High density lipids (HDL)

Among different treatments, HDL (mg dl^{-1}) of fish was 32.52, 38.05, 51.53, 61.73, 71.75 and 63.80 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased HDL levels significantly with maximum value in D5 (71.75) and minimum in D1 (32.52) (Table 33, Fig 35).

Table 33: Comparative lipid profile of fish in different treatments after completion of the experimental period

Parameter (mg dl^{-1})	Treatments					
	D1	D2	D3	D4	D5	D6
Triglycerides	238.56^{e}±0.27	352.53^{d}±0.22	385.10^{c}±0.55	397.83^{b}±0.72	442.63^{a}±0.69	341.50^{d}±0.13
Cholesterol	119.03^{d}±0.22	192.66^{c}±0.14	251.76^{b}±0.74	261.93^{a}±0.23	268.06^{a}±0.23	193.20^{c}±0.36
HDL	32.52^{e}±0.34	38.05^{d}±0.76	51.53^{c}±0.27	61.73^{b}±0.10	71.75^{a}±0.75	63.80^{b}±0.65
LDL	54.67^{a}±0.24	51.35^{b}±0.65	49.83^{c}±0.16	47.06^{c}±0.62	43.52^{d}±0.39	47.37^{c}±0.16
VLDL	47.71^{b}±0.54	70.50^{ab}±0.44	77.02^{ab}±0.11	79.56^{ab}±0.14	88.53^{a}±0.13	68.30^{ab}±0.27

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)

4.2.7.4 Low density lipids (LDL)

Among different treatments, LDL (mg dl^{-1}) of fish was 54.67, 51.35, 49.83, 47.06, 43.52 and 47.37 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet decreased LDL levels significantly with minimum value in D5 (43.52) and maximum in D1 (54.67) (Table 33, Fig 35).

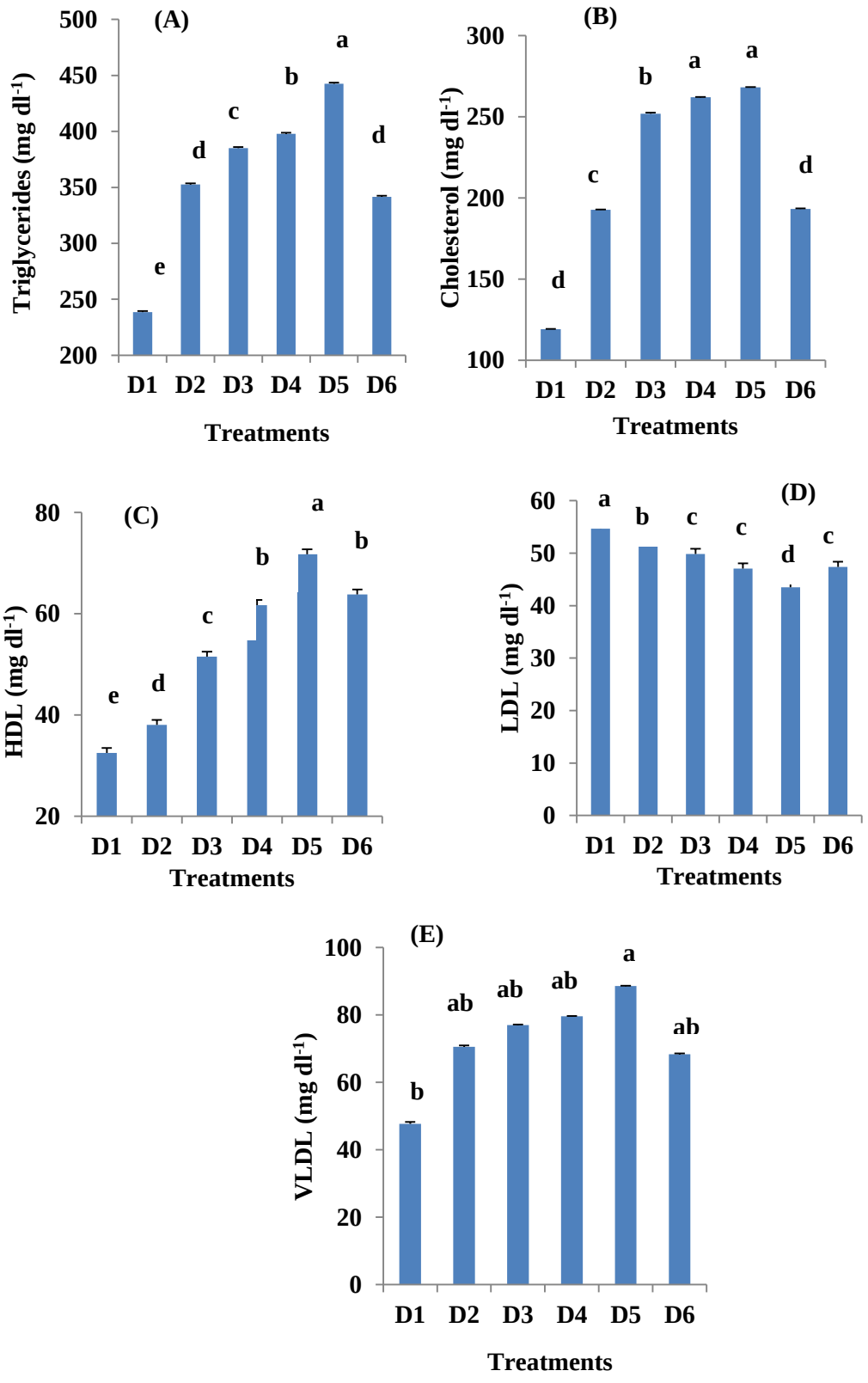


Fig 35: Comparative lipid profile (mg dl^{-1}) of fish in different treatments after completion of the experimental period [A= Triglycerides, B= Cholesterol, C= High density lipids, D= Low density lipids and E= Very low density lipids]

4.2.7.5 Very low density lipids (VLDL)

Among different treatments, VLDL (mg dl^{-1}) of fish was 47.71, 70.50, 77.02, 79.56, 88.53 and 68.30 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The result indicated that fish silage incorporation in fish diet increased VLDL levels significantly in D5 (88.53) with insignificant differences among control and other treatments (Table 33, Fig 35).

The total cholesterol and triglycerides (TG) were significantly higher in the fish silage fed treatments as compared to control in the present study. It may be due to the influence of combination of plant and animal protein and linseed oil on the lipids metabolism. The increase in total cholestrol appeared to be due to the increase in plasma HDL cholestrol which corresponds to higher body lipid content of fish (Robaina *et al* 1997, Goda *et al* 2007). Further, the nature and amount of dietary lipids and fatty acids also known to affect plasma cholestrol and its constituents i.e LDL, HDL, VLDL and triglycerides (Richard *et al* 2006). Babalola *et al* (2016) reported significant increase in serum triglycerides, HDL and LDL in *Heterobranchus longifilis* with incorporation of vegetable oil in the diet.

4.2.8 Flesh quality

Flesh quality in terms of total proteins, total lipids, total carbohydrates, moisture and ash content was studied at the completion of the experiment (Table 34, Fig 36).

4.2.8.1 Total proteins

Among different treatments, total proteins (g $100g^{-1}$) of fish flesh was 12.51, 13.04, 13.56, 13.21, 13.85 and 12.65 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The results indicated that fish silage incorporation in fish diet significantly increased ($p \leq 0.05$) total protein level with maximum value in D5 (13.85) and minimum in D1 (12.51) and D6 (12.65).

4.2.8.2 Total lipids

Among different treatments, total lipids (g $100g^{-1}$) of fish flesh was 3.63, 7.69, 8.01, 8.08, 8.12 and 3.43 in D1, D2, D3, D4, D5 and D6, respectively and differences

among treatments were significant ($p \leq 0.05$). The results indicated that fish silage incorporation in fish diet significantly increased ($p \leq 0.05$) total lipid level with maximum value in D5 (8.12) and minimum in D6 (3.43) and D1 (3.63).

4.2.8.3 Total carbohydrates

Among different treatments, total carbohydrates (g $100g^{-1}$) of fish flesh was 6.20, 1.45, 0.71, 0.43, 0.43 and 6.18 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The results indicated that fish silage incorporation in fish diet decreased total carbohydrate level with maximum value in D1 (6.20) and D6 (6.18) and minimum in D4 and D5 (0.43).

4.2.8.4 Ash

Among different treatments, ash (g $100g^{-1}$) of fish flesh was 1.19, 1.24, 1.20, 1.30, 1.32 and 1.24 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were significant ($p \leq 0.05$). The results indicated that fish silage incorporation in fish diet increased ash content significantly ($p \leq 0.05$) with maximum value in D5 (1.32) and minimum in D1 (1.19).

Table 34: Comparative flesh quality of fish in different treatments after completion of the experimental period

Parameter	Treatments					
	D1	D2	D3	D4	D5	D6
Total proteins (g $100g^{-1}$)	$12.51^{c}\pm0.17$	$13.04^{bc}\pm0.22$	$13.56^{ab}\pm0.15$	$13.21^{b}\pm0.12$	$13.85^{a}\pm0.29$	$12.65^{c}\pm0.13$
Total lipids (g $100g^{-1}$)	$3.63^{d}\pm0.22$	$7.69^{c}\pm0.14$	$8.01^{b}\pm0.74$	$8.08^{b}\pm0.23$	$8.12^{a}\pm0.21$	$3.43^{d}\pm0.36$
Total carbohydrates (g $100g^{-1}$)	$6.20^{a}\pm0.38$	$1.45^{b}\pm0.76$	$0.71^{c}\pm0.37$	$0.43^{d}\pm0.18$	$0.43^{d}\pm0.42$	$6.18^{a}\pm0.35$
Ash (g $100g^{-1}$)	$1.19^{d}\pm0.54$	$1.24^{b}\pm0.24$	$1.20^{c}\pm0.11$	$1.30^{ab}\pm0.14$	$1.32^{a}\pm0.23$	$1.24^{b}\pm0.27$
Moisture (%)	$76.46^{a}\pm0.14$	$76.58^{a}\pm0.25$	$76.52^{a}\pm0.16$	$76.98^{a}\pm0.22$	$76.28^{a}\pm0.29$	$76.50^{a}\pm0.16$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,….d) in a row does not differ significantly ($p \leq 0.05$)

4.2.8.5 Moisture

Among different treatments, moisture (%) of fish flesh was 76.46, 76.58, 76.52, 76.98, 76.28 and 76.50 in D1, D2, D3, D4, D5 and D6, respectively and differences among treatments were insignificant ($p \leq 0.05$). The results indicated that fish silage incorporation in fish diet had no significant effect on moisture in fish flesh.

The results of present study in terms of flesh quality revealed significant improvement in protein, lipid and ash content of fish flesh in D5, along with decreased carbohydrates without affecting the moisture content. This may be due to the supplementation of fish silage (having higher values of free amino acids) in diet along with linseed oil. Majumdar *et al* (2014) and Haider *et al* (2015) too reported increased flesh protein and fat content of *Labeo rohita*, when fed with fish silage and plant protein blend in place of fish meal. The findings of the present study in terms of increased fat content are in line with previous study of Giri *et al* (2000), who reported significant increase ($p \leq 0.05$) in fat (18.3%) in the body carcass in *C. batrachus* (Linn.) fingerlings, when fed with fish viscera (@ 4% of body weight). Muzinic *et al* (2006) also recorded increased lipid content in sunshine bass (*Morone chrysops*× *Morone saxatilis*) fillet fed with turkey meal (0, 97, 175 and 264 gkg^{-1}). El- Sayed (2008) too reported higher carcass lipid than other diets in Nile tilapia (*O. niloticus*) when fed with poultry by-product meal (PBM) in six isonitrogenous (30% crude protein), isocaloric (400 kcal GE 100 g^{-1}) diets. Giri *et al* (2010) also reported increasing quantities of lipids in carcass of *C. batrachus* (Linn.) fingerlings but with no effect on protein content, when fed with dried chicken viscera meal (CVM) (@ 400 and 500 g kg^{-1}). The increase in carcass lipid content may be in response to increased level of dietary CVM having higher values of fat content, resulting in increased digestible energy content of experimental diets. Likewise, in the present study, higher incorporation level of fish silage along with linseed oil resulted in higher lipid levels leading to enhanced carcass lipid content.

In the present study, fish was fed with finishing diets for 30 days by incorporating linseed oil @ 5%. The variations in carcass composition in terms of protein, fat and ash may be due to the effect of linseed oil along with multiple protein sources i.e plant proteins and fish silage.

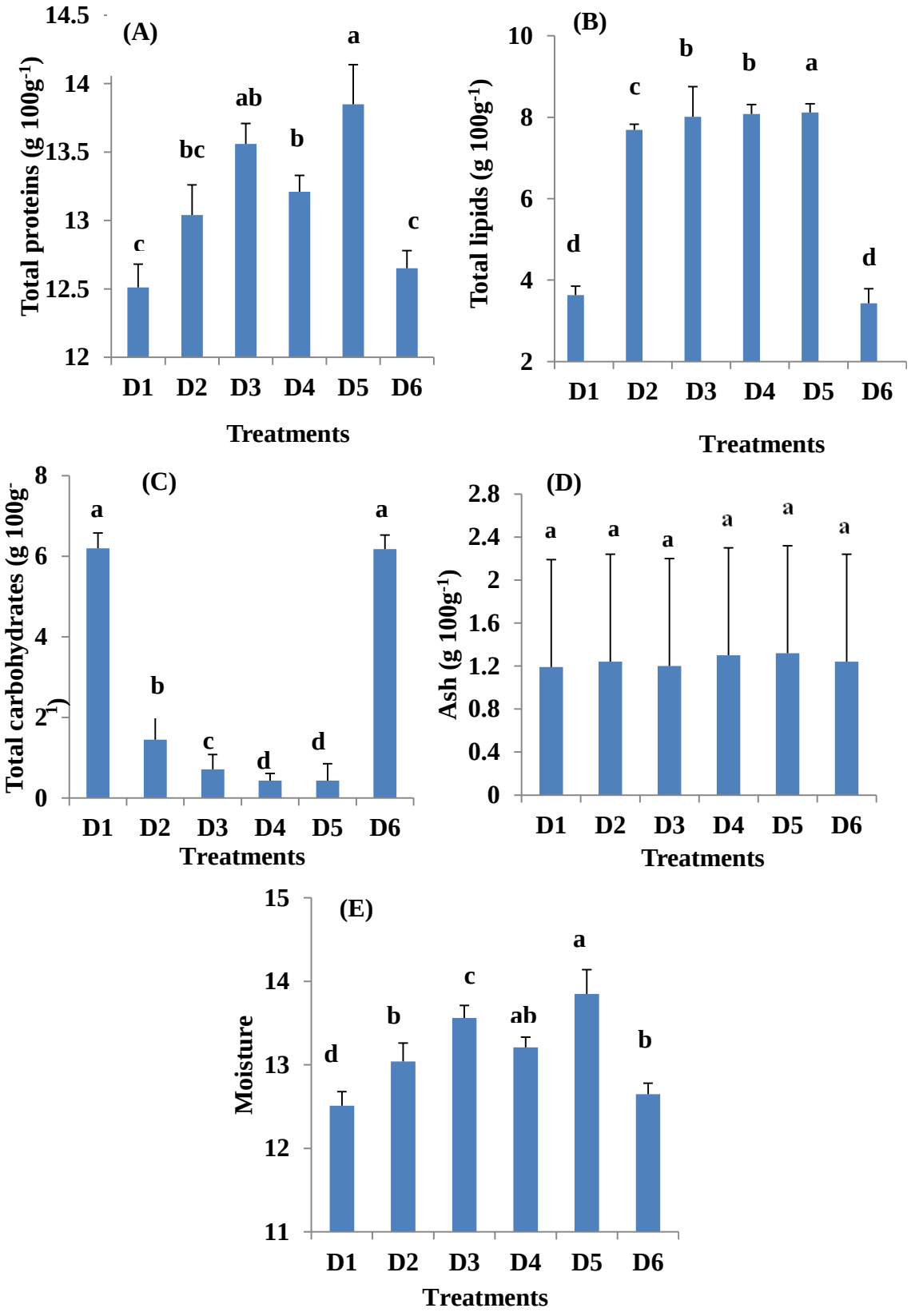


Fig 36: Comparative flesh quality of fish in different treatments after completion of the experimental period [A= Total proteins (g 100g^{-1}), B= Total lipids (g 100g^{-1}), C= Total carbohydrates (g 100g^{-1}), D= Ash (g 100g^{-1}) and E= Moisture (%)]

Further, the addition of linseed oil is positively co-related with flesh lipid content. Although, linseed oil was added in all the treatments at same level (@5%), however the silage supplemented diets (D2, D3, D4 and D5) showed significant improvement in flesh lipid content as compared to D1 (fish meal diet) and D6 (plant protein diet), which clearly indicated the positive combined effect of silage and linseed oil. A positive correlation between dietary lipid levels and total lipid levels in muscle tissue was also observed in previous studies in other species (Chou and Shiav 1996, Pei *et al* 2004). Moisture content of fish flesh may be negatively correlated with lipid content in the diet (Zupan *et al* 2016) or did not revealed any negative effect (Steffenns and Rennert 1995, Zakes *et al* 2010) as observed in present study. According to Karalazos *et al* (2014) incorporation of rapeseed oil resulted in increase in whole body protein as well as protein retention in Atlantic salmon due to presence of mid-chain fatty acids. On the contrary, number of studies revealed no variation in whole body protein, lipid and ash contents in different fish species (Benedito *et al* 2007, Peng *et al* 2008, Peng *et al* 2014, Li *et al* 2015 and Nayak *et al* 2017), with incorporation of linseed oil. These variations could be attributed to the culture period, lipid source, lipid level, fish species and size of fish along with type and composition of feed.

4.2.9 Meat quality

Meat quality in terms of pH, peroxide value (PV), free fatty acid (FFA), titratable acidity (TA), total volatile base-nitrogen (TVB-N) of fresh (0 day), refrigerated (4^{o}C) product (3, 6 day) and frozen (-20^{o}C) fillet (0, 20, 30, 37, 40 days) was estimated after feeding with grow-out diets (120 days) and finishing diets having linseed oil @ 5% for further 30 days.

4.2.9.1 pH

Among different treatments, pH of product (fish fingers) was 6.34, 6.48, 6.50, 6.40, 6.50 and 6.48 at day 0; 6.45, 6.68, 6.65, 6.53, 6.56 and 6.64 at day 3; 6.98, 6.80, 6.85, 6.65, 6.60 and 6.84 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 35, Fig 37). The results indicated that pH of product increased significantly ($p \leq 0.05$) on 3rd day in D3, D4 and D5 and on 6th day in D1, D2 and D6 with maximum value in D1 (6.98) and minimum in D4 (6.64) and D5 (6.60).

Among different treatments, pH of frozen fillet was 6.80, 6.73, 6.76, 6.89, 6.79 and 6.87 at day 0; 6.92, 6.85, 6.80, 6.94, 6.86 and 6.64 at day 20; 7.05, 6.98, 6.93, 7.09, 7.06 and 6.84 at day 30; 7.21, 7.15, 7.20, 7.18, 7.16 and 7.14 at day 35; 7.23, 7.18, 7.24, 7.20, 7.19 and 7.16 at day 40 in D1, D2, D3, D4, D5 and D6, respectively (Table 36, Fig 38). The results indicated that pH of frozen fillet increased significantly ($p \leq 0.05$) on 35th day in D4 and D6 and 40th day in D1, D2, D3 and D5 with maximum value in D1 (7.23) and D3 (7.24) and minimum in D6 (7.16).

Table 35: Changes in pH of fish fingers in different treatments

Days	Treatments					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
0	$6.34^{b,3}\pm0.02$	$6.48^{a,3}\pm0.08$	$6.50^{a,2}\pm0.01$	$6.40^{a,2}\pm0.09$	$6.50^{a,2}\pm0.02$	$6.48^{a,3}\pm0.05$
3	$6.45^{b,2}\pm0.04$	$6.68^{a,2}\pm0.05$	$6.65^{a,1}\pm0.23$	$6.53^{a,1}\pm0.02$	$6.56^{a,1}\pm0.08$	$6.64^{a,2}\pm0.02$
6	$6.98^{a,1}\pm0.06$	$6.80^{b,1}\pm0.04$	$6.85^{b,1}\pm0.06$	$6.65^{c,1}\pm0.03$	$6.60^{c,1}\pm0.10$	$6.84^{b,1}\pm0.04$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

Table 36: Changes in pH of frozen fillet in different treatments

Days	Treatments					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
0	$6.80^{a,4}\pm0.08$	$6.73^{b,4}\pm0.04$	$6.76^{b,5}\pm0.01$	$6.89^{a,3}\pm0.02$	$6.79^{a,4}\pm0.01$	$6.87^{a,4}\pm0.04$
20	$6.92^{a,3}\pm0.01$	$6.85^{b,3}\pm0.03$	$6.80^{b,4}\pm0.06$	$6.94^{a,3}\pm0.04$	$6.86^{b,3}\pm0.02$	$6.64^{c,3}\pm0.10$
30	$7.05^{a,2}\pm0.05$	$6.98^{b,2}\pm0.01$	$6.93^{b,3}\pm0.04$	$7.09^{a,2}\pm0.06$	$7.06^{a,2}\pm0.01$	$6.84^{c,2}\pm0.02$
35	$7.21^{a,12}\pm0.01$	$7.15^{b,12}\pm0.01$	$7.20^{a,2}\pm0.02$	$7.18^{a,1}\pm0.02$	$7.16^{b,12}\pm0.01$	$7.14^{b,1}\pm0.02$
40	$7.23^{a,1,}\pm0.01$	$7.18^{b.1}\pm0.02$	$7.24^{a,1}\pm0.01$	$7.20^{ab,1}\pm0.02$	$7.19^{b,1}\pm0.01$	$7.16^{c,1}\pm0.01$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

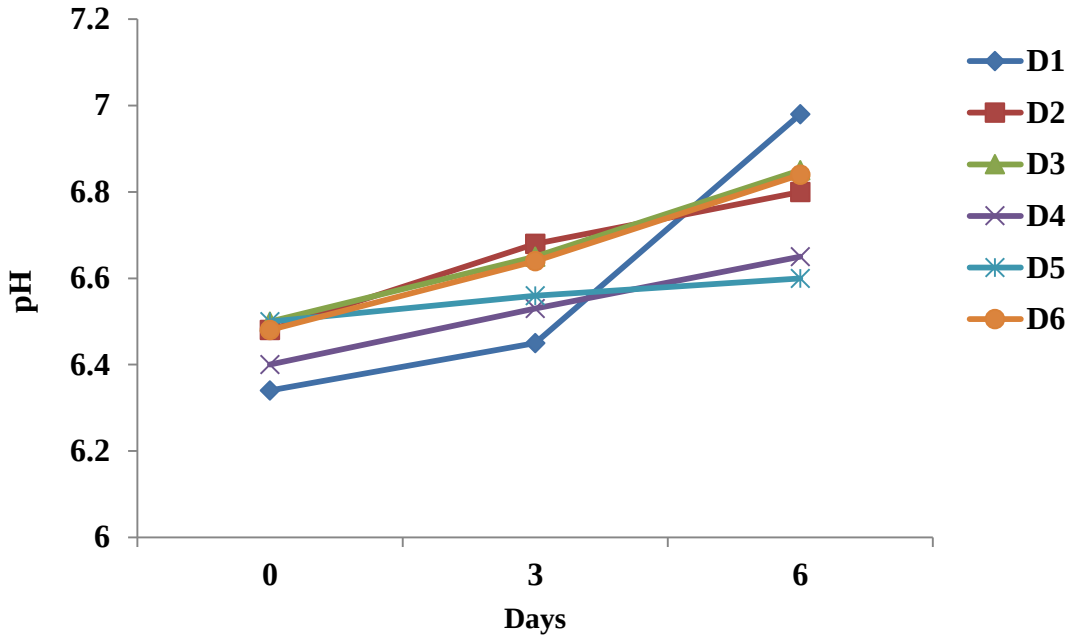


Fig 37: Changes in pH of fish fingers in different treatments

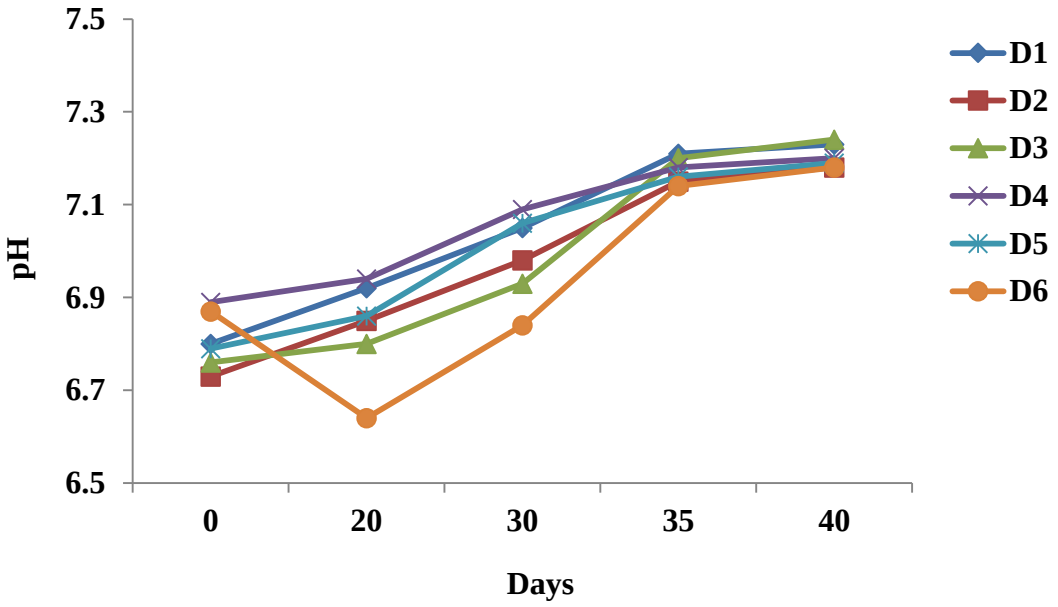


Fig 38: Changes in pH of frozen fillet in different treatments

In present study, pH of both product and fillet increased significantly with storage leading to decreased overall acceptability. These results are in line with results of study by Pawar (2011) who reported an increasing trend of pH (6.50 to 6.79) in fish cutlets made from Catla, when stored at temperature of 2-4^{o}C. During storage, the increase in pH is caused by the enzymatic degradation of fish muscle (Love 1992 and Vareltzis *et al* 1997) and production of basic

components triggered by the bacterial growth (Manthey *et al* 1988), leading to loss of quality of product leading to spoilage. Kyrana *et al* (1997) too reported similar results during shelf-life study of ice stored gilthead seabream and recorded final pH value of 6.60. Sallam *et al* (2007) reported significant increase ($p \leq 0.05$) in pH values with increase in storage time of brined and marinated fillets of Pacific saury (*Cololabis saira*) stored at 4ºC for 90 days. A significant increase in the pH values with the increasing storage time has also been recognized in brined chub mackerel (Goulas and Kontominas 2005), marinated anchovies (Poligne and Collignan 2000) and marinated sardine (Kilinc and Cakli 2005) during their storage at the refrigerated temperature. Chatli *et al* (2012) too reported increase in pH with advancement of storage period in *Cyrinus carpio*. Rathod and Pagarkar (2013) also reported increased pH (6.30 to 6.60) of pangas (*P. hypophthalmus*) cutlets during storage period of 18 days, under refrigerated conditions (-15 to -18°C). Although, pH of the product during present study remained near neutral till 6th day, however the product was unacceptable based on sensory evaluation. Further, pH of pangas fillet was near neutral till day 30th and after that there was significant increase in pH, depicting deteriorated quality of fillet.

4.2.9.2 Peroxide value (PV)

Among different treatments, PV (meq kg fat^{-1}) of fish product (fish fingers) was 0.89, 0.65, 0.75, 0.80, 0.85 and 0.80 at day 0; 3.50, 2.99, 2.81, 2.78, 2.56 and 2.66 at day 3; 4.88, 3.53, 3.04, 2.96, 2.72 and 2.84 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 37, Fig 39). The results indicated that PV of product increased significantly ($p \leq 0.05$) on 3rd day in D3 and on 6th day in rest of the treatments and control with maximum value in D1 (4.88) and minimum in D5 (2.72).

Among different treatments, PV (meq kg fat^{-1}) of frozen fillet was 2.0, 1.2, 1.6, 1.4, 1.4 and 1.8 at day 0; 2.4, 1.4, 1.8, 1.8, 1.6 and 2.0 at day 20; 4.5, 3.0, 2.8, 3.2, 3.0 and 3.6 at day 30; 4.8, 3.6, 3.6, 3.4, 3.2 and 4.0 at day 35 and 5.2, 4.2, 4.0, 3.8, 3.8 and 4.2 at day 40 in D1, D2, D3, D4, D5 and D6, respectively (Table 38, Fig 40). The results indicated that PV of frozen fillet increased significantly ($p \leq 0.05$) on 40th day in all the treatments with maximum value in D1 (5.2) and minimum in D4 and D5 (3.8).

Table 37: Changes in Peroxide Value (meq kg fat^{-1}) of fish fingers in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$0.89^{a,3}\pm0.02$	$0.65^{d,3}\pm0.08$	$0.75^{c,2}\pm0.02$	$0.80^{b,3}\pm0.01$	$0.85^{a,3}\pm0.09$	$0.80^{b,3}\pm0.02$
3	$3.50^{a,2}\pm0.26$	$2.99^{b,2}\pm0.01$	$2.81^{c,1}\pm0.09$	$2.78^{c,2}\pm0.05$	$2.56^{d,2}\pm0.04$	$2.66^{d,2}\pm0.02$
6	$4.88^{a,1}\pm0.10$	$3.53^{b,1}\pm0.12$	$3.04^{c,1}\pm0.24$	$2.96^{c,1}\pm0.08$	$2.72^{d,1}\pm0.06$	$2.84^{c,1}\pm0.04$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly (p≤0.05)

Table 38: Changes in Peroxide Value (meq kg fat^{-1}) of frozen fillet in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$2.0^{a,4}\pm0.04$	$1.2^{d,4}\pm0.15$	$1.6^{c,4}\pm0.10$	$1.4^{d,4}\pm0.02$	$1.4^{d,5}\pm0.09$	$1.8^{b,4}\pm0.02$
20	$2.4^{a,3}\pm0.09$	$1.4^{e,3}\pm0.08$	$1.8^{c,4}\pm0.07$	$1.8^{c,3}\pm0.06$	$1.6^{d,4}\pm0.05$	$2.0^{b,3}\pm0.16$
30	$4.5^{a,23}\pm0.12$	$3.0^{c,23}\pm0.14$	$2.8^{d,3}\pm0.06$	$3.2^{c,2}\pm0.09$	$3.0^{c,3}\pm0.10$	$3.6^{b,2}\pm0.14$
35	$4.8^{a,2}\pm0.08$	$3.6^{c,2}\pm0.06$	$3.6^{c,2}\pm0.12$	$3.4^{c,2}\pm0.16$	$3.2^{d,2}\pm0.04$	$4.0^{b,12}\pm0.10$
40	$5.2^{a,1}\pm0.02$	$4.2^{b,1}\pm0.04$	$4.0^{c,1}\pm0.01$	$3.8^{d,1}\pm0.06$	$3.8^{d,1}\pm0.03$	$4.2^{b,1}\pm0.01$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly (p≤0.05)

PV is a measure of first stage of rancidity and it depends on degree of oxidation in the fat (Govindan 1985, Gopakumar 2002) and is used for determining the formation of primary oxidation products during the storage period. The PV value of fish fingers and fillets ranged from 0.65-0.89 meq kg fat^{-1} and 1.2-2.0 meq kg fat^{-1} on day 1, which increased to 2.72-4.88 meq kg fat^{-1} and 3.82-5.2 meq kg fat^{-1} at day 6 and day 40, respectively. The PV value of both product and fillet increased with storage time, however, the values remained below the preferred value of 10 meq kg fat^{-1} (Connell 1975), indicating that fats were still not fully oxidized. The result of the present study in terms of increased PV with increasing storage period, are in line with the results of study by Tokur *et al* (2006), who reported similar

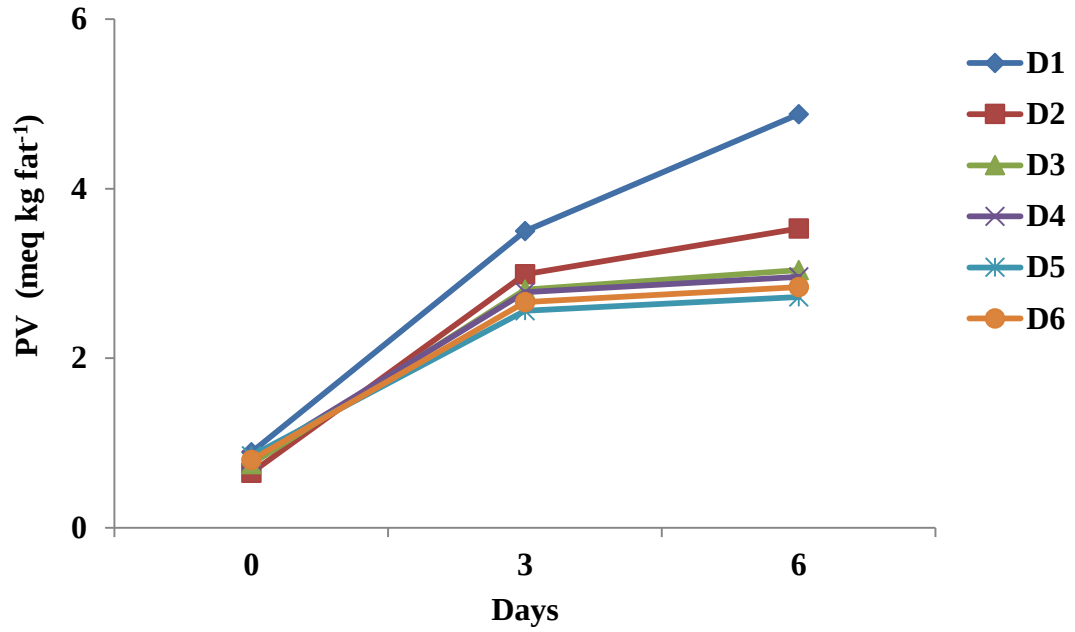


Fig 39: Changes in Peroxide Value (meq kg fat^{-1}) of fish fingers in different treatments

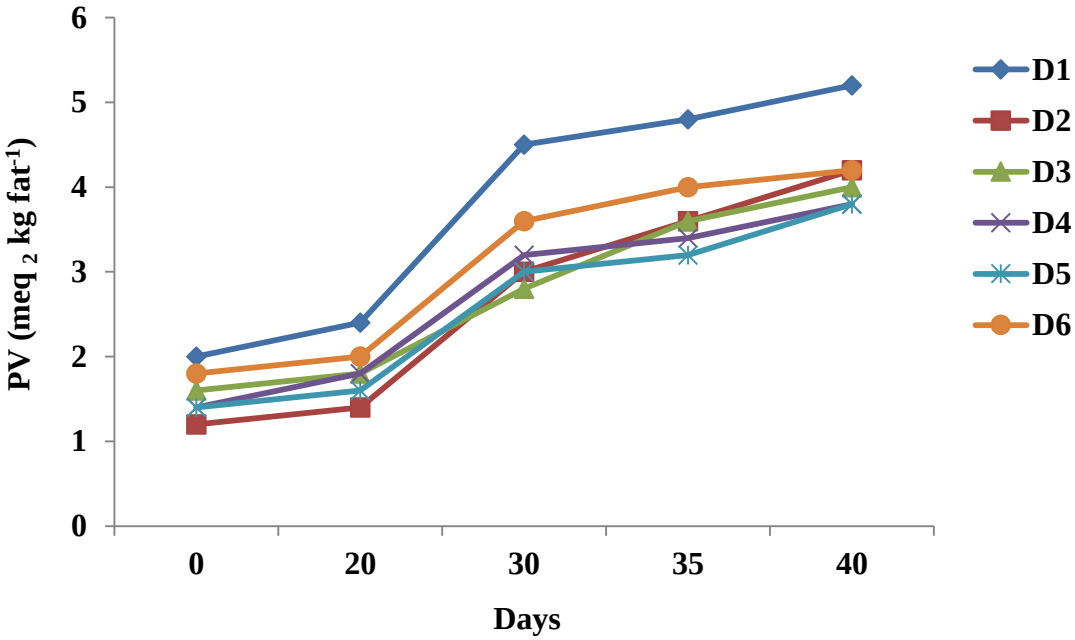


Fig 40: Changes in Peroxide Value (meq kg fat^{-1}) of frozen fillet in different treatments

changes in PV of fish burger produced from tilapia (*O. niloticus*) during frozen storage. Ninan *et al* (2008) too reported gradual increase in PV of tilapia (*O. mossambicus*) fish cutlet upto 12-15 weeks under frozen storage conditions. Further

the treatment of fillet before storage like chilling, vacuum treatment or polyphosphate treatment can enhance the shelf life of pangas fillet in terms of lesser increase in PV values due to positive effect of treatment in arresting fat oxidation process (Rao *et al* 2013). Chatli *et al* (2012), reported increasing trend of PV in *C. carpio* during storage period irrespective of packaging conditions, while evaluating shelf life of fish mince under modified atmosphere packaging at refrigerated temperature (4±1^{o}C). Rathod and Pagarkar (2013) too recorded an increased PV in pangasius cutlet stored under refrigerated conditions (-15 to -18°C). Rao *et al* (2013) revealed similar observations in pangasius fish fillets stored at chilled condition (<4°C), in which PV increased throughout the 12 days storage period.

4.2.9.3 Free fatty acid (FFA)

Among different treatments, FFA (mg 100g^{-1}) of fish product (fish fingers) was 0.90, 0.81, 0.71, 0.70, 0.66 and 0.61 at day 0; 1.90, 1.80, 1.77, 1.68, 1.56 and 1.62 at day 3; 3.34, 2.98, 2.83, 2.67, 2.54 and 2.61 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 39, Fig 41). The results indicated that FFA of product increased significantly ($p \leq 0.05$) on 6th day in all the treatments with maximum value in D1 (3.34) and minimum in D5 (2.54).

Table 39: Changes in free fatty acid (mg 100g^{-1}) of fish fingers in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$0.90^{a,3}$±0.08	$0.81^{b,3}$±0.02	$0.71^{c,3}$±0.02	$0.70^{c,3}$±0.03	$0.66^{d,3}$±0.01	$0.61^{d,3}$±0.03
3	$1.90^{a,2}$±0.04	$1.80^{a,2}$±0.16	$1.77^{b,2}$±0.01	$1.68^{b,2}$±0.12	$1.56^{b,2}$±0.06	$1.62^{b,2}$±0.01
6	$3.34^{a,1}$±0.24	$2.98^{b,1}$±0.01	$2.83^{b,1}$±0.09	$2.67^{c,1}$±0.06	$2.54^{d,1}$±0.02	$2.61^{c,1}$±0.04

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

Among different treatments, FFA (mg 100g^{-1}) of frozen fillet was 0.98, 0.80, 0.78, 0.65, 0.56 and 0.50 at day 0; 1.14, 0.98, 0.84, 0.74, 0.78 and 0.82 at day 20; 4.24, 4.32, 3.30, 3.20, 3.26 and 3.84 at day 30; 4.65, 4.62, 3.69, 3.89, 3.58, 4.00 at day

35 and 4.70, 4.65, 3.72, 3.90, 3.68 and 4.22 at day 40 in D1, D2, D3, D4, D5 and D6, respectively (Table 40, Fig 42). The results indicated that FFA of frozen fillet increased significantly ($p \leq 0.05$) on 35th day in D2 and D6 and on 40th day in D1, D3, D4 and D5 with maximum value in D1 (4.70) and minimum in D5 (3.68).

Table 40: Changes in free fatty acid (mg 100g^{-1}) of frozen fillet in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$0.98^{a,3}\pm0.14$	$0.80^{b,3}\pm0.09$	$0.78^{b,4}\pm0.06$	$0.65^{bc,4}\pm0.08$	$0.56^{c,5}\pm0.01$	$0.50^{c,3}\pm0.03$
20	$1.14^{a,3}\pm0.12$	$0.98^{a,2}\pm0.03$	$0.84^{c,3}\pm0.13$	$0.74^{d,3}\pm0.05$	$0.78^{d,4}\pm0.06$	$0.82^{c,2}\pm0.10$
30	$4.24^{a,2}\pm0.28$	$4.32^{a,12}\pm0.24$	$3.30^{c,2}\pm0.14$	$3.20^{c,2}\pm0.16$	$3.26^{c,3}\pm0.12$	$3.84^{b,12}\pm0.12$
37	$4.65^{a,12}\pm0.26$	$4.62^{a,1}\pm0.18$	$3.69^{c,12}\pm0.22$	$3.89^{c,12}\pm0.14$	$3.58^{c,2}\pm0.10$	$4.00^{b,1}\pm0.20$
40	$4.70^{a,1}\pm0.26$	$4.65^{a,1}\pm0.16$	$3.72^{b,1}\pm0.12$	$3.90^{b,1}\pm0.10$	$3.68^{c,1}\pm0.02$	$4.22^{a,1}\pm0.23$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

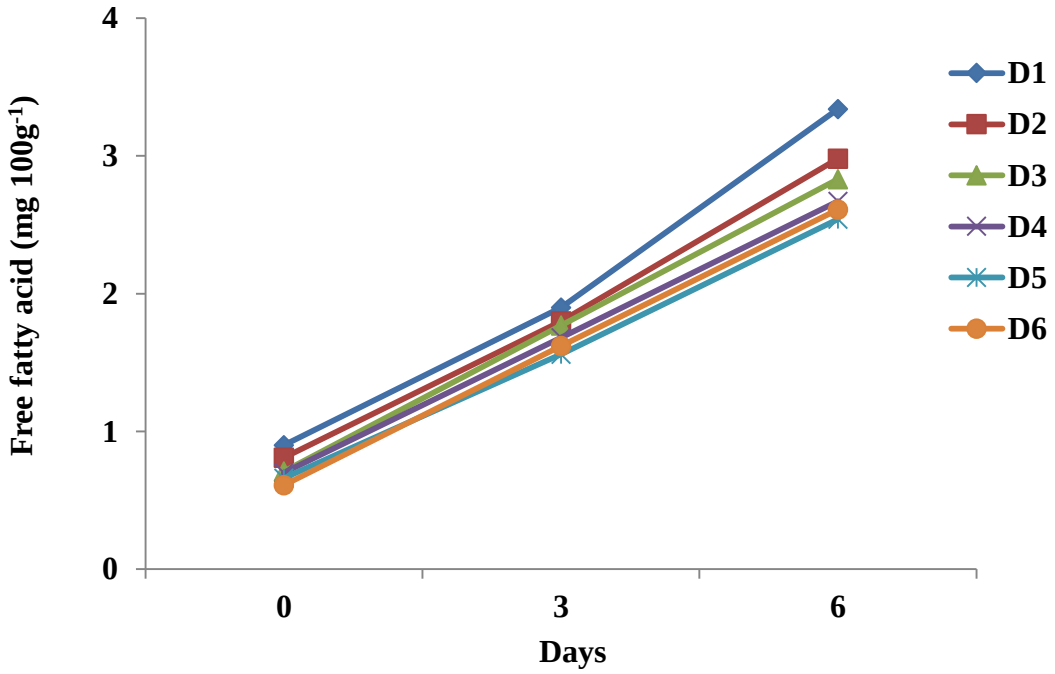


Fig 41: Changes in free fatty acid (mg 100g^{-1}) of fish fingers in different treatments

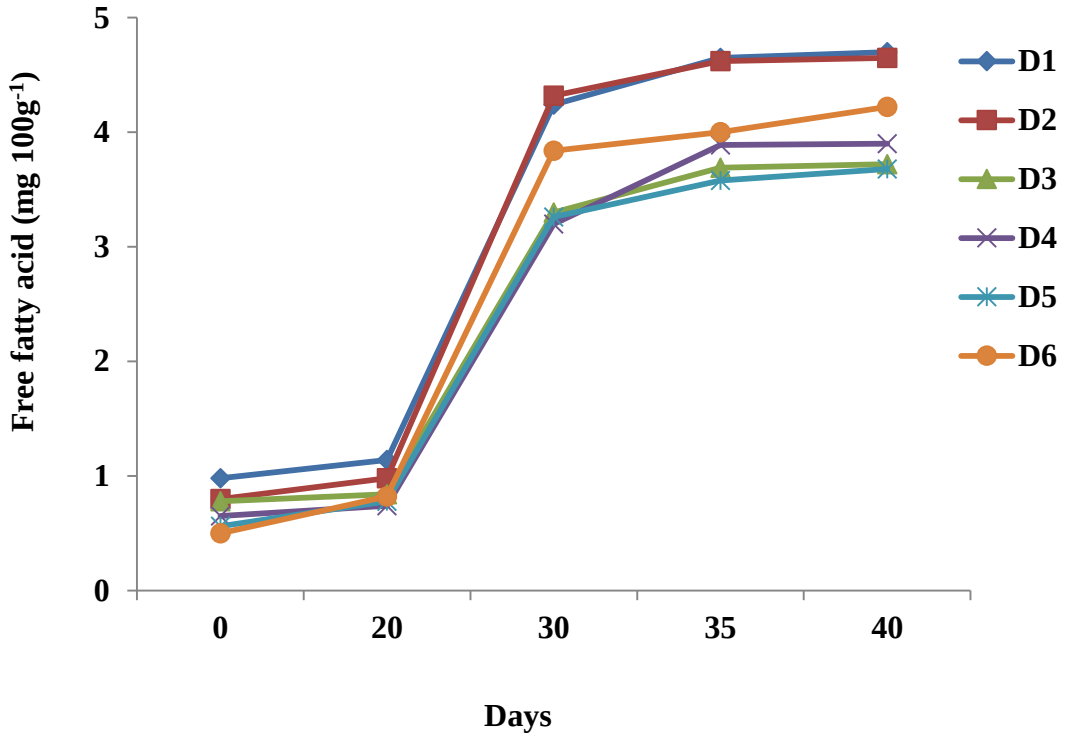


Fig 42: Changes in free fatty acid (mg 100g^{-1}) of frozen fillet in different treatments

The FFA is a result of enzymatic decomposition of lipids during storage (Tokur *et al* 2006) and is an indication of lipid hydrolysis. As the freshness quality of fish gets reduced, the FFA content in the lipids of fish increases due to the action of lipases (Reddy *et al* 2012). Further, fish lipid are not stable during storage at any temperature, either refrigerated or chilled and frozen conditions. Lipid degradation continued at lower rate even during chilling, leading to increased concentration of FFA which is used as one of the meat quality indicator (Sandor *et al* 2011).

The results of present study too revealed continuous increase in FFA in both fish products and fish fillet stored under refrigerated (4°C) and in chilled / frozen conditions (-20°C). The findings of present study are in line with results of Joseph *et al* (1984), who reported increased FFA content in flashed fried and raw cutlet (from 0.98 to 1.49 and 2.03 to 2.82 mg100g^{-1}) respectively, at 4°C. Reddy *et al* (2012) too reported increased FFA in fish finger developed from croaker and pink perch meat upto 6th week and 10th week respectively at -20°C. Tokur *et al* (2006) also reported increased FFA from the beginning of the storage up to 8th month at -18°C in fish

fingers made from mirror carp (*C. carpio*). Although, there was increase in FFA values of product and fillet with storage, however, among treatments lowest values of FFA was observed in D5, which may be due to the combined effect of best grow out diet with linseed oil on fish meat in terms of lipid quality.

4.2.9.4 Titratable acidity (TA)

Among different treatments, TA (gl^{-1}) of product (fish fingers) was 0.73, 0.72, 0.64, 0.54, 0.52 and 0.51 at day 0; 0.96, 0.87, 0.79, 0.73, 0.64 and 0.72 at day 3; 1.80, 1.65, 1.62, 1.60, 1.54 and 1.65 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 41, Fig 43). The result indicated that TA of product increased significantly ($p \leq 0.05$) on 6^{th} day in all the treatments with maximum value in D1 (1.80) and minimum in D5 (1.54).

Among different treatments, TA (gl^{-1}) of frozen fillet was 0.66, 0.60, 0.60, 0.75, 0.66 and 0.63 at day 0; 0.75, 0.70, 0.68, 0.79, 0.70 and 0.72 at day 20; 0.90, 0.74, 0.72, 0.88, 0.85 and 0.90 at day 30; 1.30, 1.10, 1.08, 1.09, 1.10 and 1.12 at day 35; 1.34, 1.14, 1.10, 1.10, 1.12 and 1.18 at day 40 in D1, D2, D3, D4, D5 and D6, respectively (Table 42, Fig 43). The result indicated that TA of frozen fillet increased significantly ($p \leq 0.05$) on 35^{th} day in D4, D5 and D6 and on 40^{th} day in D1, D2 and D3 with maximum value in D1 (1.34) and minimum in D3 and D4 (1.10) and D5 (1.12).

Table 41: Changes in titratable acidity (gl^{-1}) of fish fingers in different treatments

Days	Treatments					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
0	$0.73^{a,3}\pm0.06$	$0.72^{a,3}\pm0.04$	$0.64^{b,3}\pm0.02$	$0.54^{c,3}\pm0.01$	$0.52^{c,3}\pm0.02$	$0.51^{c,3}\pm0.09$
3	$0.96^{a,2}\pm0.14$	$0.87^{a,2}\pm0.08$	$0.79^{b,2}\pm0.01$	$0.73^{b,2}\pm0.02$	$0.64^{c,2}\pm0.03$	$0.72^{b,2}\pm0.01$
6	$1.80^{a,1}\pm0.04$	$1.65^{b,1}\pm0.02$	$1.62^{b,1}\pm0.09$	$1.60^{b,1}\pm0.06$	$1.54^{c,1}\pm0.01$	$1.65^{b,1}\pm0.04$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

Table 42: Changes in titratable acidity (gl^{-1}) of frozen fillet in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$0.66^{b,3}\pm0.11$	$0.60^{c,3}\pm0.02$	$0.60^{c,3}\pm0.04$	$0.75^{a,3}\pm0.05$	$0.66^{b,4}\pm0.02$	$0.63^{b,4}\pm0.24$
20	$0.75^{a,3}\pm0.03$	$0.70^{a,2}\pm0.14$	$0.68^{b,23}\pm0.03$	$0.79^{a,3}\pm0.08$	$0.70^{a,3}\pm0.02$	$0.72^{a,3}\pm0.16$
30	$0.90^{a,2}\pm0.02$	$0.74^{c,2}\pm0.01$	$0.72^{c,2}\pm0.04$	$0.88^{ab,2}\pm0.01$	$0.85^{b,2}\pm0.02$	$0.90^{a,2}\pm0.01$
35	$1.30^{a,12}\pm0.10$	$1.10^{b,12}\pm0.05$	$1.08^{b,12}\pm0.08$	$1.09^{b,1}\pm0.02$	$1.10^{b,1}\pm0.08$	$1.12^{a,1}\pm0.20$
40	$1.34^{a,1}\pm0.04$	$1.14^{b,1}\pm0.02$	$1.10^{c,1}\pm0.01$	$1.10^{c,1}\pm0.03$	$1.12^{c,1}\pm0.03$	$1.18^{b,1}\pm0.07$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p\leq0.05$)

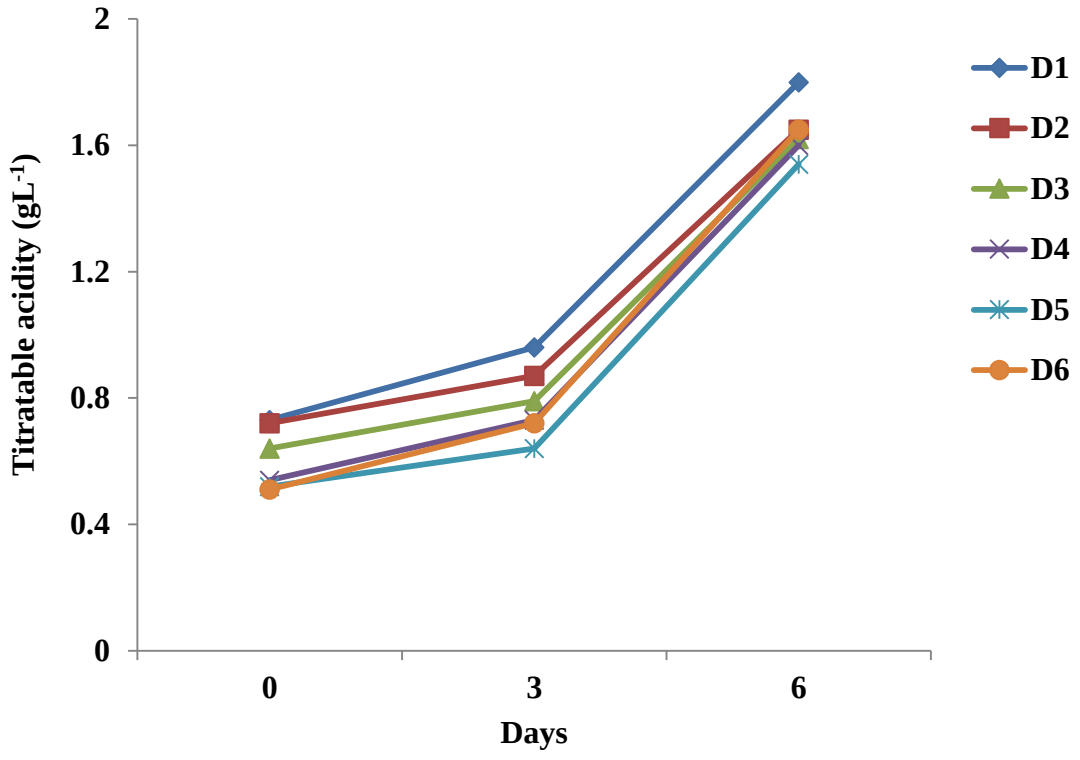


Fig 43: Changes in titratable acidity (gl^{-1}) of fish fingers in different treatments

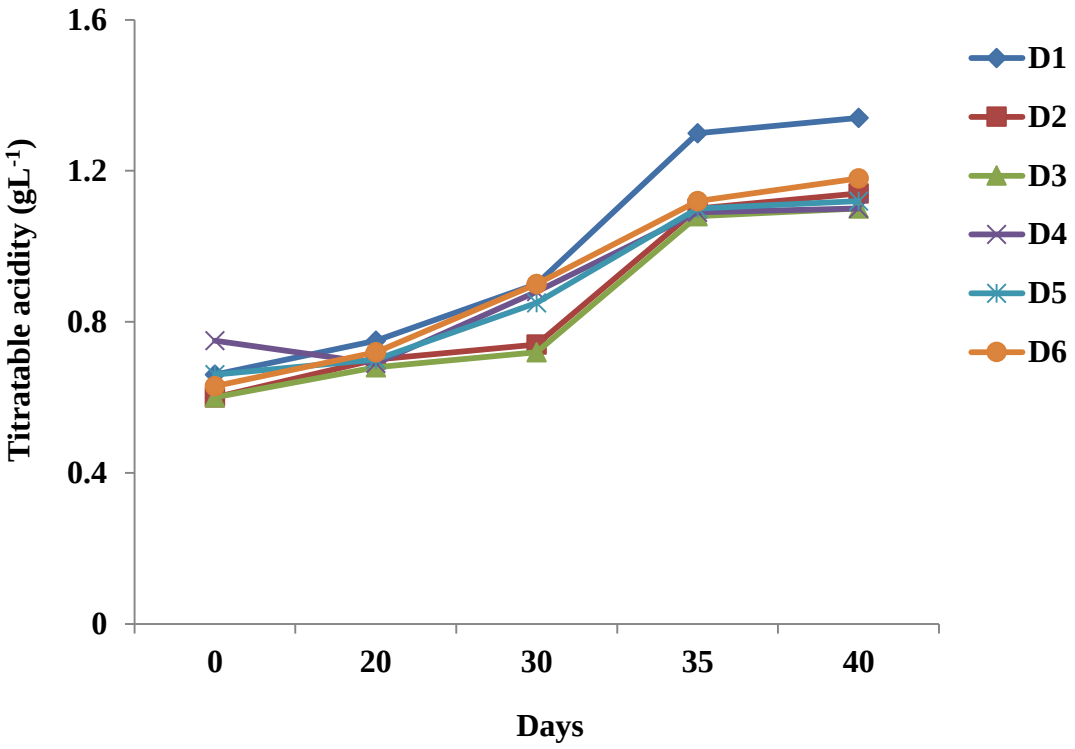


Fig 44: Changes in titratable acidity (gl^{-1}) of frozen fillet in different treatments

Titratable acidity of any food item is the total acid concentration contained within a food. It is also one of the important indicator, which determine the quality of any meat/ meat product along with pH during storage. The findings of present study are in line with results of previous study of Achinewhu and Oboh (2008), who reported increase in TA in *Sardinella* sp. during fermentation of the products. Xu *et al* (2010) too reported a progressive increase in TA, while investigating physical and chemical changes of silver carp sausages during fermentation with *Pediococcus pentosaceus*. Chatli *et al* (2012) also reported increasing trend of TA in fish mince made from *C. carpio* under refrigerated temperature (4±1^{o}C) throughout the storage period (12 to 15 days).

4.2.9.5 Total volatile base-nitrogen (TVB-N)

Among different treatments, TVB-N (mg $100g^{-1}$) of product (fish fingers) was 2.92, 2.85, 2.75, 2.66, 2.60 and 2.46 at day 0; 4.25, 3.09, 3.01, 2.96, 2.78 and 2.80 at day 3; 5.33, 4.24, 3.94, 3.45, 3.20 and 3.35 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 43, Fig 45). The result indicated TVB-N

of product increased significantly ($p \leq 0.05$) on 6th day in all the treatments with maximum value in D1 (5.33) and minimum in D5 (3.20).

Table 43: Changes in TVB-N (mg $100g^{-1}$) of fish fingers in different treatments

Days	Treatments					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
0	$2.92^{a,3}\pm0.01$	$2.85^{b,3}\pm0.02$	$2.75^{c,3}\pm0.01$	$2.66^{d,3}\pm0.03$	$2.60^{d,3}\pm0.06$	$2.46^{e,3}\pm0.04$
3	$4.25^{a,2}\pm0.12$	$3.09^{b,2}\pm0.14$	$3.01^{b,2}\pm0.19$	$2.96^{c,2}\pm0.01$	$2.78^{c,2}\pm0.20$	$2.80^{c,2}\pm0.08$
6	$5.33^{a,1}\pm0.22$	$4.24^{b,1}\pm0.25$	$3.94^{b,1}\pm0.11$	$3.45^{c,1}\pm0.09$	$3.20^{c,1}\pm0.10$	$3.35^{c,1}\pm0.15$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

Among different treatments, TVB-N (mg $100g^{-1}$) of frozen fillet was 2.62, 2.75, 2.68, 2.54, 2.60 and 2.58 at day 0; 6.45, 6.32, 5.85, 5.50, 4.32 and 4.35 at day 20; 10.45, 10.24, 9.89, 10.20, 9.79 and 8.48 at day 30; 15.12, 14.20, 14.25, 13.45, 12.50 and 12.85 at day 35 and 17.45, 15.32, 16.34, 14.48, 13.62 and 13.89 at day 40 in D1, D2, D3, D4, D5 and D6, respectively (Table 44, Fig 46). The result indicated TVB-N of frozen fillet increased significantly ($p \leq 0.05$) on 40th day with maximum value in D1 (17.45) and minimum in D5 (13.62).

Table 44: Changes in TVB-N (mg $100g^{-1}$) of frozen fillet in different treatments

Days	Treatments					
	D1(Control)	**D2**	**D3**	**D4**	**D5**	**D6**
0	$2.62^{b,5}\pm0.01$	$2.75^{a,4}\pm0.05$	$2.68^{b,5}\pm0.02$	$2.54^{c,4}\pm0.10$	$2.60^{c,5}\pm0.02$	$2.58^{c,5}\pm0.09$
20	$6.45^{a,4}\pm0.04$	$6.32^{a,3}\pm0.28$	$5.85^{b,4}\pm0.14$	$5.50^{c,3}\pm0.05$	$4.32^{d,4}\pm0.18$	$4.35^{d,4}\pm0.17$
30	$10.45^{a,3}\pm0.05$	$10.24^{a,2}\pm0.03$	$9.89^{b,3}\pm0.05$	$10.20^{a,2}\pm0.09$	$9.79^{b,3}\pm0.07$	$8.48^{c,3}\pm0.05$
35	$15.12^{a,2}\pm0.01$	$14.20^{b,12}\pm0.18$	$14.25^{b,2}\pm0.12$	$13.45^{c,12}\pm0.17$	$12.50^{d,2}\pm0.05$	$12.85^{d,2}\pm0.08$
40	$17.45^{a,1}\pm0.06$	$15.32^{c,1}\pm0.24$	$16.34^{b,1}\pm0.11$	$14.48^{d,1}\pm0.30$	$13.62^{e,1}\pm0.23$	$13.89^{e,1}\pm0.14$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

TVB-N is a commonly used chemical method to determine spoilage of fish. Increase in TVB-N values indicate increased bacterial activity and total volatile bases (DOF 2013). Increased bacterial activity in fish muscles during storage results in ammonia and volatile bases accumulation in fish flesh. Further, TVB-N values may vary according to species, season, age, activity of spoilage flora and analysis method as well (Dalin *et al* 2013 and Nasopoulou *et al* 2012). There was a gradual increase in TVB-N level of fish fingers and fish fillet with increasing storage time during the present study. According to the Codex Alimentarius Hungaricus (art. 3-1-95/194), MRL levels of TVB-N in marine fish is in between 25-35 mg $100g^{-1}$, but around 50 mg $100g^{-1}$ should be acceptable. However, for freshwater fish species, 12 mg $100g^{-1}$ TVB-N is determined as safe level (Lengyel *et al* 2000). In the present study, for fish products, the value of TVB-N remained well below the upper limit, but for frozen fillet, the values of TVB-N exceeds permissible limit after 30 days of storage.

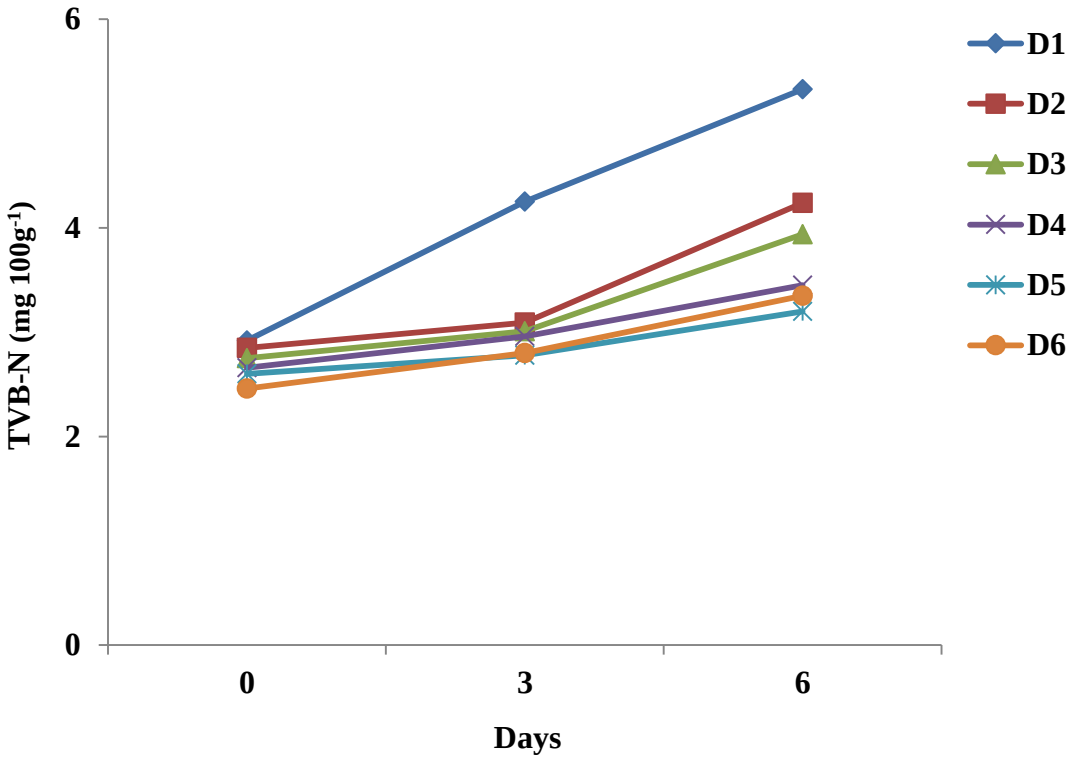


Fig 45: Changes in total volatile base-nitrogen (TVB-N) (mg $100g^{-1}$) of fish fingers in different treatments

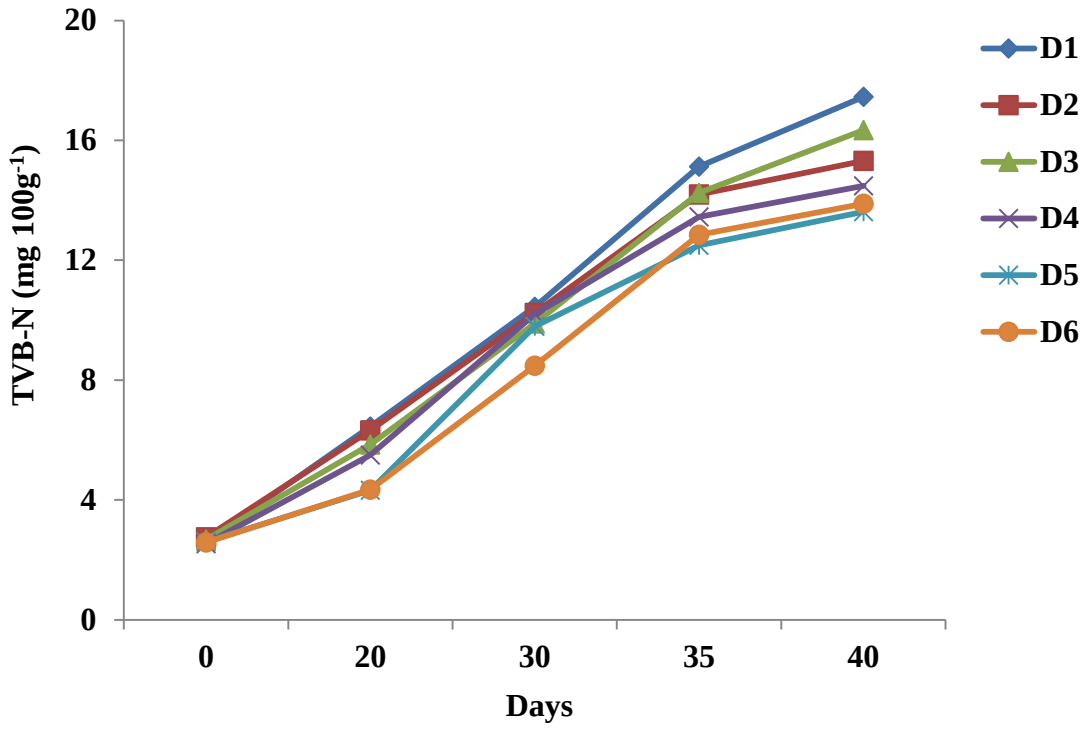


Fig 46: Changes in total volatile base-nitrogen (TVB-N) (mg 100g^{-1}) of frozen fillet in different treatments

Further, in the present study, the maximum increase in TVB-N in both fish fingers and fish fillets was in D1 i.e control diet and least was in D5, which may be due to the effect of grow out diet along with linseed oil on fish flesh. The findings of present study are in line with results of Tokur *et al* (2006), who reported increase in TVB-N value of fish burger produced from tilapia (*O. niloticus*) during frozen storage for 8 months. Bao *et al* (2007) also reported an increasing trend of TVB-N in Arctic charr (*Salvelinus alpines*) fillets at super chilling (-2°C) and chilling (3°C) storage temperature.

Similar observations were recorded by Ninan *et al* (2008) in terms of increased TVB-N value in tilapia (*O. mossambicus*) fish cutlet. Rathod and Pagarkar (2013) too reported an increased TVB-N of pangasius fish cutlet stored in refrigerated display unit (-15 to -18ºC). Rao *et al* (2013) also revealed gradual increase in TVB-N values of pangas fillets during chilled storage conditions (<4°C). Most of the previous studies reported increase in TVB-N with storage time, regardless of storage conditions, over the limit of acceptance after 20 days (Kyrana *et al* 1997, Tejada and Huidobro 2002, Cakli *et al* 2007, O¨zogul *et al* 2007).

4.2.10 Sensory evaluation

Sensory evaluation of fish product (fingers) in terms of appearance, odour/smell, crispiness, juiciness, texture, flavour, taste and hence, overall acceptability were studied at day 0, 3 and 6 (under refrigerated conditions at 4ºC) on hedonic scale of 1-9.

4.2.10.1 Appearance

Among different treatments, appearance of product (fish fingers) was 7.68, 8.22, 8.11, 8.00, 8.78 and 7.87 at day 0; 7.44, 7.33, 7.44, 7.44 8.22 and 7.22 at day 3 and 5.88, 6.33, 6.11, 7.33, 8.11 and 7.11 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 45, Fig 47). The result indicated that appearance of product decreased significantly ($p \leq 0.05$) on 6^{th} day in all the treatments except D4, with minimum decrease in D5 (8.11) and maximum decrease in D1 (5.88). Decrease in appearance of any product during storage is a general phenomenon due to biochemical changes. Similar observations were recorded during the present study, however product made from fish fed on diet D5 appeared best even at day 6, which may be due to the quality of feed (fish silage, plant protein diet in combination with linseed oil) provided to fish during 150 day experiment.

Table 45: Changes in appearance of fish fingers in different treatments

Days	Treatments					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
0	$7.68^{c,1}\pm0.24$	$8.22^{b,1}\pm0.02$	$8.11^{b,1}\pm0.05$	$8.00^{b,1}\pm0.22$	$8.78^{a,1}\pm0.33$	$7.87^{c,1}\pm0.15$
3	$7.44^{b,1}\pm0.32$	$7.33^{b,2}\pm0.23$	$7.44^{b,1}\pm0.72$	$7.44^{b,1}\pm0.83$	$8.22^{a,2}\pm0.06$	$7.22^{b,2}\pm0.24$
6	$5.88^{c,2}\pm0.38$	$6.33^{c,3}\pm0.18$	$6.11^{c,2}\pm0.15$	$7.33^{b,1}\pm0.28$	$8.11^{a,2}\pm0.24$	$7.11^{b,2}\pm0.20$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

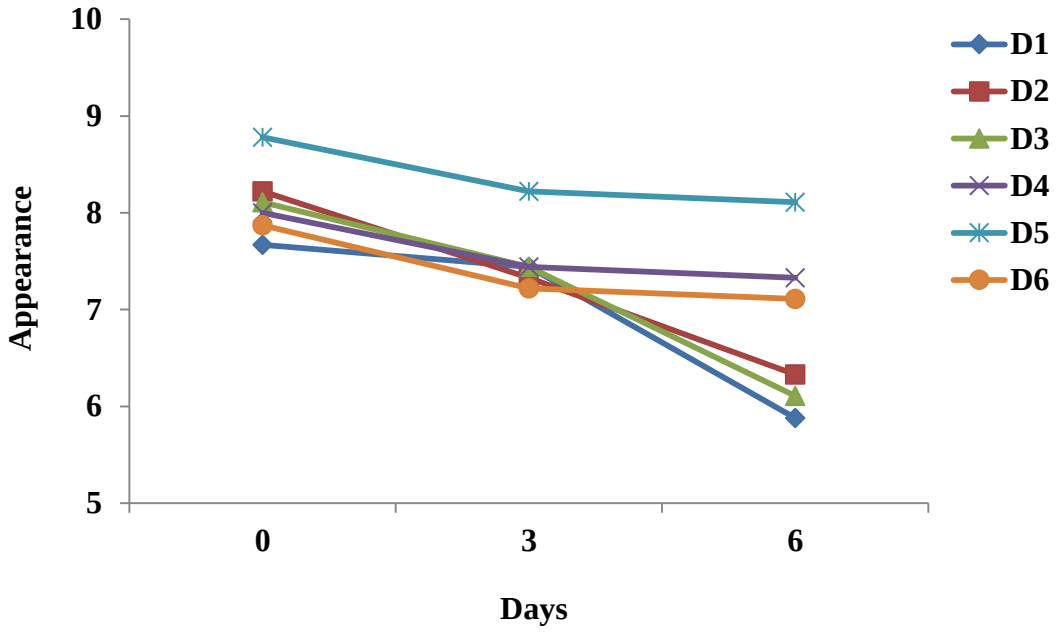


Fig 47: Changes in appearance of fish fingers in different treatments

The findings of present study are in line with results of previous study of Chang *et al* (1998), who reported a general trend of decreasing appearance and sensory score with increased storage time, while evaluating biochemical, microbiological and sensory changes of seabass (*Lateolabrax japonicus*) under partial freezing (-3°C) and refrigerated storage (5°C) for 4 weeks.

4.2.10.2 Odour

Among different treatments, odour of product (fish fingers) was 7.89, 8.11, 8.44, 8.22, 8.89 and 7.56 at day 0; 7.67, 7.22, 7.44, 7.67, 8.00 and 6.78 at day 3 and 6.00, 6.33, 6.11, 6.78, 6.80 and 6.22 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 46, Fig 48). The result indicated that odour of product decreased significantly ($p \leq 0.05$) on 6^{th} day in all the treatments. The value for odour on hedonic scale was significantly higher ($p \leq 0.05$) in D5 (6.80) and D4 (6.78) as compared to all other treatments and control which did not differ significantly.

Odour/Aroma of product is an important factor determining consumer acceptance, in addition to its contribution in flavour of the product. In agreement with appearence of product during storage, odour of product also showed significant decrease in product prepared from all treatments at day 6. Hence, decrease in odour lead to non-acceptibility of product from all treatments.

Table 46: Changes in odour of fish fingers in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$7.89^{c,1}\pm0.05$	$8.11^{b,1}\pm0.31$	$8.44^{ab,1}\pm0.24$	$8.22^{b,1}\pm0.28$	$8.89^{a,1}\pm0.11$	$7.56^{c,1}\pm0.04$
3	$7.67^{ab,1}\pm0.32$	$7.22^{ab,2}\pm0.21$	$7.44^{ab,1}\pm0.72$	$7.67^{ab,1}\pm0.83$	$8.00^{a,2}\pm0.06$	$6.78^{b,2}\pm0.14$
6	$6.00^{b,2}\pm0.17$	$6.33^{b,3}\pm0.28$	$6.11^{b,2}\pm0.26$	$6.78^{a,2}\pm0.35$	$6.80^{a,3}\pm0.01$	$6.22^{b,3}\pm0.03$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p\leq0.05$)

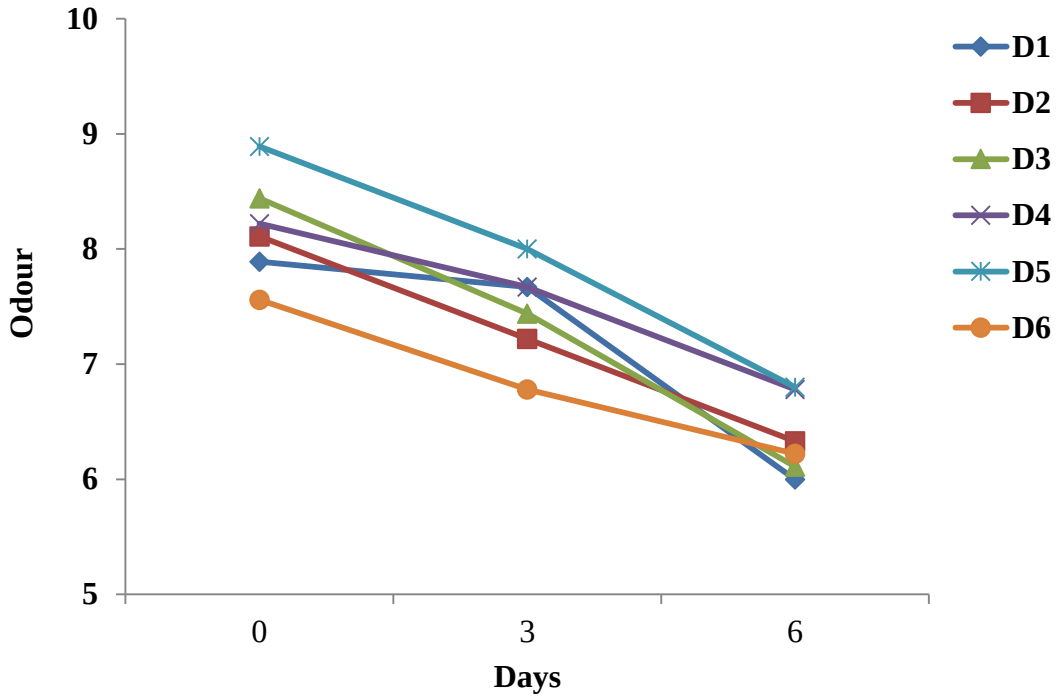


Fig 48: Changes in odour of fish fingers in different treatments

The findings of present study are in line with results of previous study of Arannilewa *et al* (2005) who reported a decreasing trend of odour (2.67 to 1.67) in frozen nile tilapia (*Sarotherudun galiaenus*) subjected to different freezing periods (0, 10, 20, 30, 40, 50 and 60 days). Tokur *et al* (2005) too reported similar observations in fish fingers, made from mirror carp (*C. carpio* L) during frozen storage (-18°C). Kamat (1999) also recorded gradual decrease in odour in fish cutlets made from mackerel mince meat during storage at -14°C.

4.2.10.3 Crispiness

Among different treatments, crispiness of product (fish fingers) was 7.22, 8.44, 8.78, 7.78, 8.89 and 6.89 at day 0; 7.00, 7.22, 7.00, 7.11, 8.11 and 6.78 at day 3 and 6.22, 6.22, 6.44, 6.88, 7.45 and 6.77 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 47, Fig 49). The result indicated that crispiness of product decreased significantly ($p \leq 0.05$) on 6th day in all the treatments except D3 (significant decrease at day 3) with significantly higher ($p \leq 0.05$) value in D5 (7.45) as compared to all other treatments and control, which did not differ significantly.

Table 47: Changes in crispiness of fish fingers in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$7.22^{b,1}\pm0.52$	$8.44^{a,1}\pm0.24$	$8.78^{a,1}\pm0.15$	$7.78^{b,1}\pm0.28$	$8.89^{a,1}\pm0.21$	$6.89^{c,1}\pm0.04$
3	$7.00^{b,1}\pm0.12$	$7.22^{b,1}\pm1.00$	$7.00^{b,2}\pm0.72$	$7.11^{b,1}\pm0.13$	$8.11^{a,2}\pm0.26$	$6.78^{c,2}\pm0.01$
6	$6.22^{b,2}\pm0.15$	$6.22^{b,2}\pm0.10$	$6.44^{b,2}\pm0.12$	$6.88^{b,2}\pm0.16$	$7.45^{a,3}\pm0.18$	$6.77^{b,3}\pm0.04$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

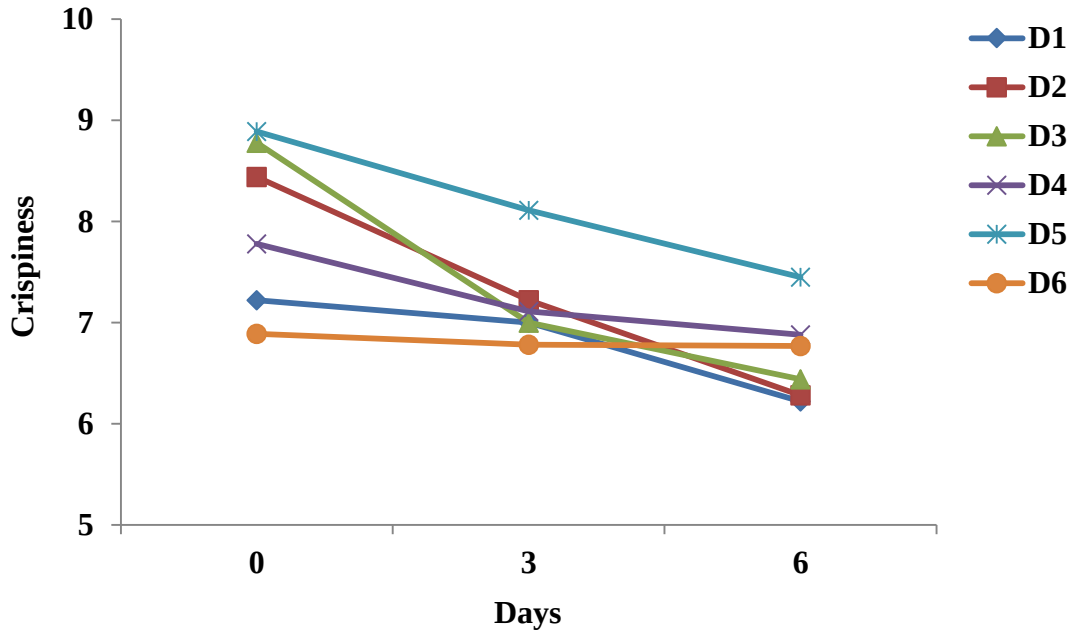


Fig 49: Changes in crispiness of fish fingers in different treatments

4.2.10.4 Juiciness

Among different treatments, juiciness of product (fish fingers) was 6.89, 8.00, 8.56, 7.56, 8.67 and 6.89 at day 0; 7.00, 7.11, 7.24, 7.11, 7.89 and 6.56 at day 3 and 6.00, 6.50, 6.44, 7.02, 7.45 and 6.10 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 48, Fig 50). The result indicated that juiciness of product decreased significantly ($p \leq 0.05$) on 6th day in all the treatments. Among treatments, significantly higher ($p \leq 0.05$) values for juiciness was in D5 (7.45) and minimum in D1 (6.00) and D6 (6.10).

Table 48: Changes in juiciness of fish fingers in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$6.89^{c,2}\pm0.03$	$8.00^{a,1}\pm0.07$	$8.56^{a,1}\pm0.17$	$7.56^{b,1}\pm0.18$	$8.67^{a,1}\pm0.24$	$6.89^{c,1}\pm0.01$
3	$7.00^{a,1}\pm0.12$	$7.11^{a,1}\pm1.00$	$7.24^{a,2}\pm0.72$	$7.11^{a,1}\pm0.83$	$7.89^{a,1}\pm0.26$	$6.56^{b,1}\pm0.24$
6	$6.00^{c,3}\pm0.28$	$6.50^{b,2s}\pm0.18$	$6.44^{b,3}\pm0.04$	$7.02^{b,2}\pm0.01$	$7.45^{a,2}\pm0.08$	$6.10^{c,2}\pm0.04$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

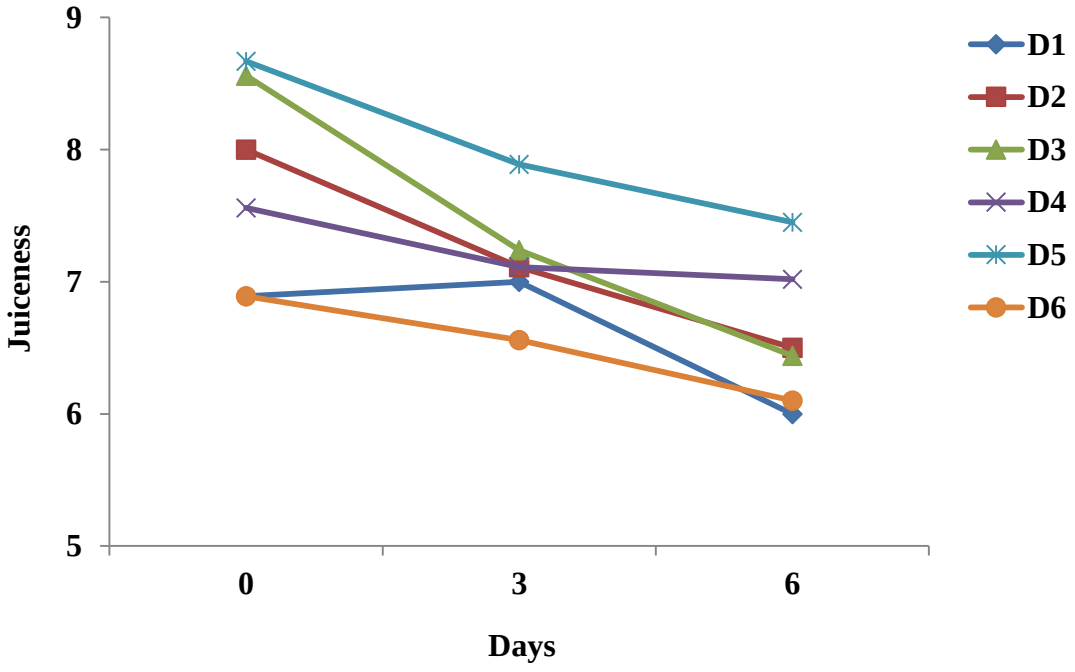


Fig 50: Changes in juiciness of fish fingers in different treatments

Ready to cook products are battered and breaded to provide appropriate crispiness and juiciness for overall improved acceptability. Battering makes the inside of the product juicy, while outside looks crispy (Fiszman and Salvador 2003). In the present study also, fish fingers in all the treatments were battered and breaded before frying and storage. On day 0, values of crispiness of fish singers on hedonic scale ranged from 6.89 to 8.89 with maximum value in D5 (8.89) and juiciness values ranged from 6.89 to 8.67 with maximum value in D5. Although, with increase in storage time (0-6 day) along with other sensory parameters, these two parameters too revealed significant decrease, however fish fingers prepared from fish reared on diet D5 showed maximum values for crispiness and juiciness. Decreasing values of sensory properties can be co-related with changing trend of lipid oxidation (PV, FFA etc). FFA affects protein stability and decomposes texture through reduction reaction with protein (Kolakowska *et al* 2006, Rodriguez *et al* 2008). Alam *et al* (2012) and Khanipour *et al* (2014) too revealed decreased juiciness and crispiness in rohu, *Labeo rohita* flesh and breaded Kika, *Clupeonella cultriventris* with increase in storage time.

4.2.10.5 Texture

Among different treatments, texture of product (fish fingers) was 6.68, 8.11, 8.44, 7.78, 8.67 and 6.78 at day 0; 6.77, 6.89, 7.33, 7.33, 8.11 and 6.56 at day 3 and 6.14, 6.22, 6.33, 6.88, 7.80 and 6.36 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 49, Fig 51). The result indicated that texture of product decreased significantly ($p \leq 0.05$) on 6^{th} day with maximum value in D5 (7.80) and minimum in D1 (6.14).

Table 49: Changes in texture of fish fingers in different treatments

Days	Treatments					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
0	$6.68^{b,1}\pm0.60$	$8.11^{a,1}\pm0.35$	$8.44^{a,1}\pm0.12$	$7.78^{b,1}\pm0.02$	$8.67^{a,1}\pm0.16$	$6.78^{b,1}\pm0.15$
3	$6.77^{b,1}\pm0.02$	$6.89^{ab,2}\pm0.10$	$7.33^{b,2}\pm0.02$	$7.33^{b,2}\pm0.13$	$8.11^{a,2}\pm0.06$	$6.56^{c,1}\pm0.14$
6	$6.14^{d,2}\pm0.15$	$6.22^{d,3}\pm0.01$	$6.33^{c,3}\pm0.13$	$6.88^{b,3}\pm0.17$	$7.80^{a,3}\pm0.11$	$6.36^{c,2}\pm0.04$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

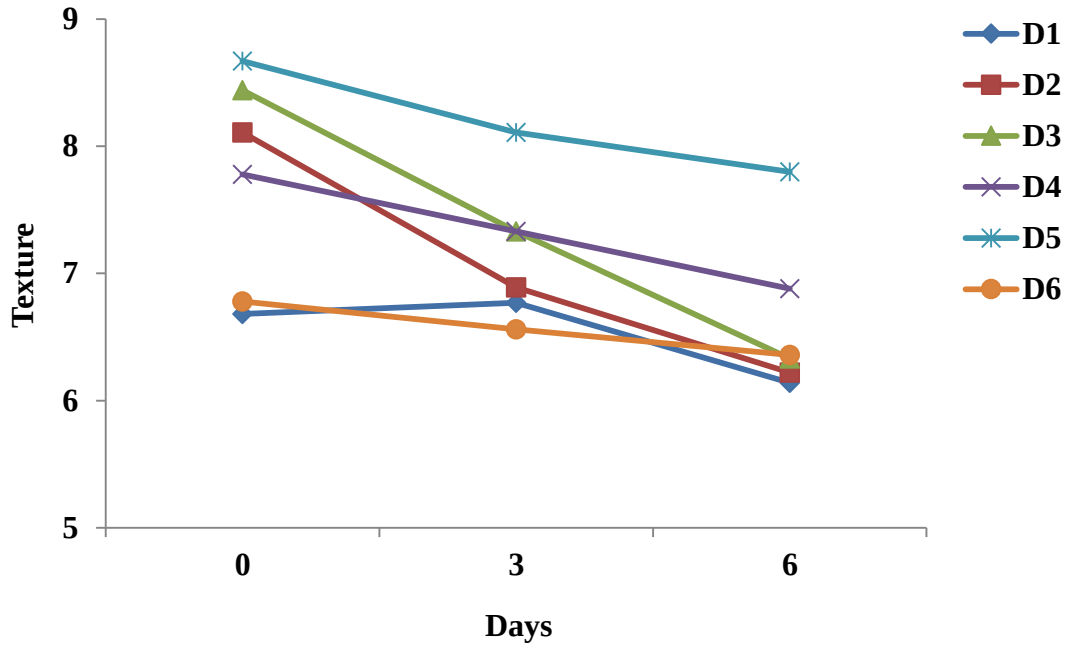


Fig 51: Changes in texture of fish fingers in different treatments

Texture is an important tool to check the acceptibility of meat products during its storage period. Arannilewa *et al* (2005) reported a decreasing trend of texture (3.00 to 1.67) in frozen nile tilapia (*Sarotherudun galiaenus*) subjected to different freezing periods (0, 10, 20, 30, 40, 50 and 60 days). Ninan *et al* (2008) too reported similar observations in fish cutlets prepared from tilapia (*O. mossambicus*) which had initial sensory score above 7 (rated as good to excellent) after which loss in texture was noticed.

4.2.10.6 Taste

Among different treatments, taste of product (fish fingers) was 7.11, 7.78, 8.56, 7.57, 8.56 and 7.10 at day 0; 7.56, 7.22, 7.44, 7.67, 7.89 and 7.00 at day 3 and 6.11, 6.00, 6.44, 7.00, 7.45 and 6.33 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 50, Fig 52). The result indicated that taste of product decreased significantly ($p \leq 0.05$) on 6^{th} day with maximum value in D5 (7.45) and minimum in D1 (6.11).

In sensory evaluation, taste is very important attribute for the acceptance of any product. The decreasing trend of taste of fish fingers may be associated with the peroxidation of polyunsaturated fatty acid (Anjum *et al* 2013).

Table 50: Changes in taste of fish fingers in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$7.11^{c,2}\pm0.06$	$7.78^{b,1}\pm0.12$	$8.56^{a,1}\pm0.17$	$7.57^{b,2}\pm0.09$	$8.56^{a,1}\pm0.24$	$7.10^{c,1}\pm0.04$
3	$7.56^{a,1}\pm0.32$	$7.22^{b,2}\pm0.02$	$7.44^{a,2}\pm0.12$	$7.67^{a,1}\pm0.23$	$7.89^{a,2}\pm0.06$	$7.00^{c,2}\pm0.03$
6	$6.11^{c,3}\pm0.05$	$6.00^{c,3}\pm0.16$	$6.44^{b,3}\pm0.04$	$7.00^{b,3}\pm0.38$	$7.45^{a,3}\pm0.14$	$6.33^{c,3}\pm0.01$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly (p≤0.05)

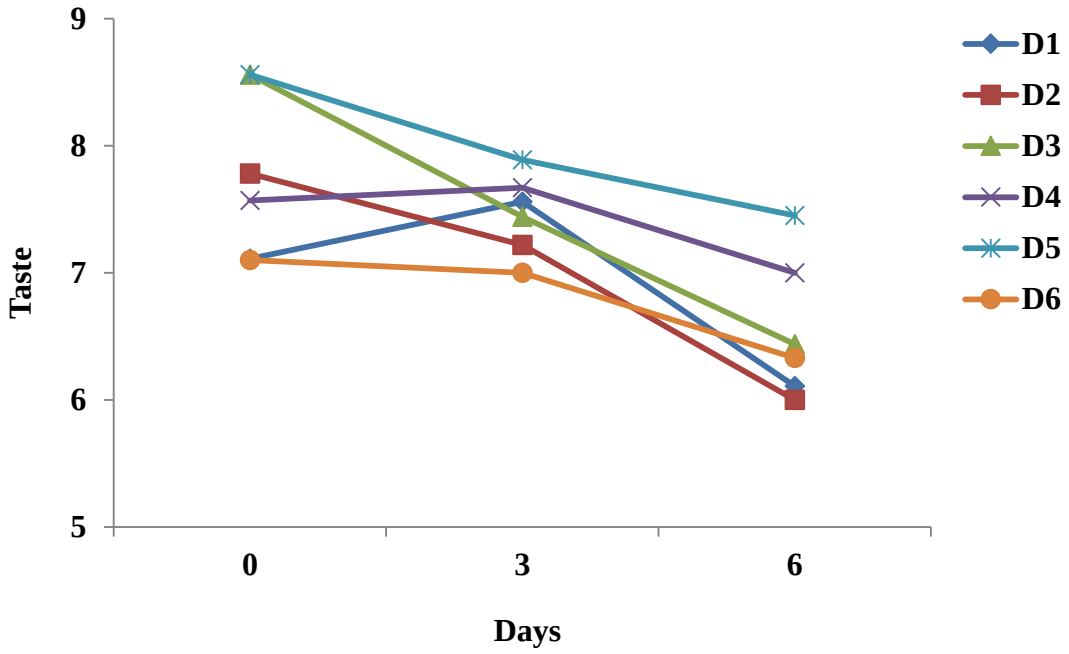


Fig 52: Changes in taste of fish fingers in different treatments

The mean values on 9 point hedonic scale for taste of fish fingers revealed that product prepared from treatment D5 were much liked by all the panelists and got highest score (above 7) at these time intervals (0, 3 and 6 day). Products were not tasted on 6th day due to deteriorative biochemical changes observed in terms of important parameter. However, the points for taste were given to product depending upon its flavour, texture and appearance. In sensory evaluation, taste is very important attribute for the acceptance of any product. The decreasing trend of taste of fish fingers may be associated with the peroxidation of polyunsaturated fatty acid (Anjum *et al* 2013).

4.2.10.7 Flavour

Among different treatments, flavour of product (fish fingers) was 7.56, 7.67, 8.33, 8.00, 8.67 and 7.00 at day 0; 6.89, 7.11, 7.44, 7.33, 8.00 and 6.89 at day 3 and 6.00, 6.11, 5.88, 7.22, 7.90 and 6.22 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 51, Fig 53). The result indicated that flavour of product decreased significantly ($p \leq 0.05$) on 6th day with maximum value in D5 (7.90) and minimum in D1 (6.00).

Table 51: Changes in flavour of fish fingers in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$7.56^{b,1}\pm0.02$	$7.67^{b,1}\pm0.07$	$8.33^{a,1}\pm0.17$	$8.00^{ab,1}\pm0.14$	$8.67^{a,1}\pm0.14$	$7.00^{c,1}\pm0.09$
3	$6.89^{b,2}\pm0.12$	$7.11^{b,2}\pm0.09$	$7.44^{ab,2}\pm0.22$	$7.33^{ab,1}\pm0.83$	$8.00^{a,2}\pm0.02$	$6.89^{b,1}\pm0.24$
6	$6.00^{c,3}\pm0.28$	$6.11^{c,3}\pm0.02$	$5.88^{c,3}\pm0.40$	$7.22^{b,2}\pm0.02$	$7.90^{a,3}\pm0.06$	$6.22^{c,2}\pm0.27$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

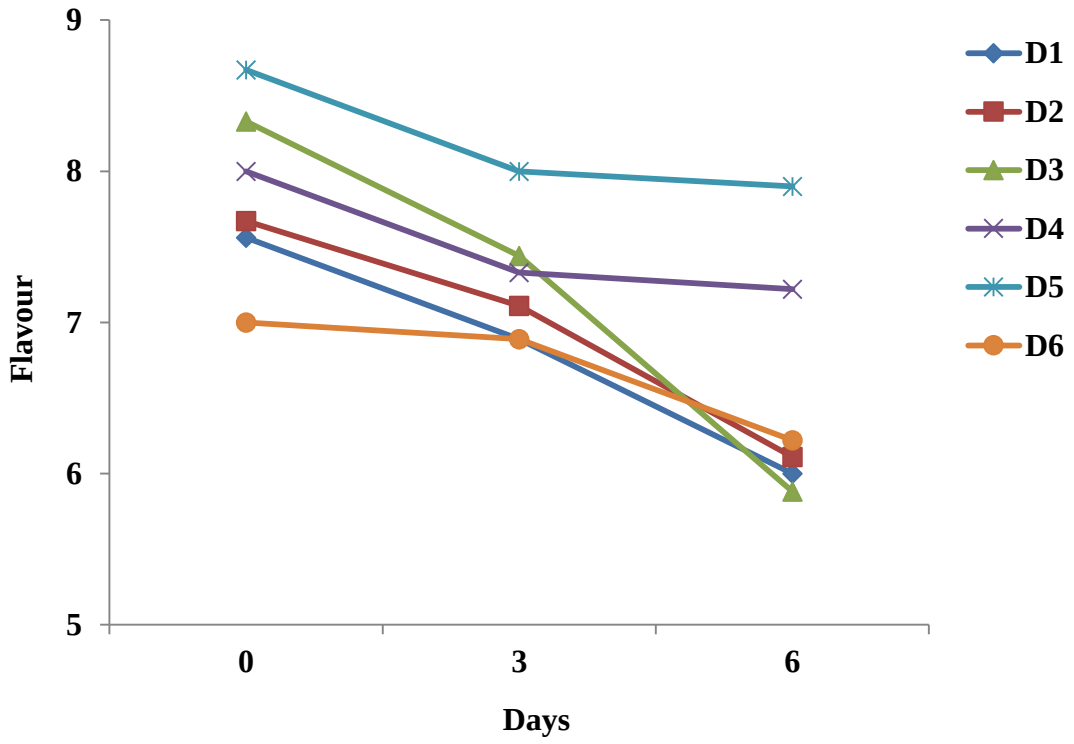


Fig 53: Changes in flavour of fish fingers in different treatments

Flavour of any product is directly related to its meat quality, which is affected by the biochemical changes observed in terms of pH, PV, FFA, TA, TVB-N etc. Both meat quality and flavour of products prepared in present study clearly revealed its deterioration on day 3 onwards, stored under refrigeration (4°C). Except D4 and D5, flavour of product in all other treatments showed score of less than 7, below which the product is rated as less then good. The findings of present study are in line with results of previous study of Ninan *et al* (2008), who reported similar observations in fish cutlets prepared from tilapia (*O. mossambicus*) which had initial sensory score above 7 (rated as good to excellent), after which loss in flavour was noticed. Alam *et al* (2012) reported decreased flavour in (*L. rohita*) fish flesh (at 1st, 15th and 30th day) at frozen storage of -18°C. Tokur *et al* (2005) also reported a significant ($p \leq 0.05$) decrease in flavour of fish fingers, made from mirror carp (*C. carpio* L) during frozen storage (-18°C).

4.2.10.8 Overall acceptability

Among different treatments, overall acceptability of product (fish fingers) was 8.00, 8.50, 8.44, 8.22, 9.00 and 7.44 at day 0; 6.55, 7.00, 7.44, 7.11, 8.95 and 7.10 at day 3 and 5.88, 6.11, 6.00, 6.88, 7.45 and 6.13 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 52, Fig 54). The result indicated that overall acceptability of product decreased significantly ($p \leq 0.05$) on 6th day with maximum acceptibility value in D5 (7.45) and minimum in D1 (5.88).

Table 52: Changes in overall acceptability of fish fingers in different treatments

Days	Treatments					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
0	$8.00^{c,1}\pm0.02$	$8.50^{b,1}\pm0.20$	$8.44^{b,1}\pm0.22$	$8.22^{b,1}\pm0.13$	$9.00^{a,1}\pm0.06$	$7.44^{c,1}\pm0.58$
3	$6.55^{c,2}\pm0.07$	$7.00^{b,2}\pm0.42$	$7.44^{b,2}\pm0.19$	$7.11^{b,2}\pm0.76$	$8.95^{a,1}\pm0.18$	$7.10^{b,1}\pm0.21$
6	$5.88^{d,3}\pm0.15$	$6.11^{c,3}\pm0.04$	$6.00^{c,3}\pm0.06$	$6.88^{b,2}\pm0.16$	$7.45^{a,2}\pm0.08$	$6.13^{c,2}\pm0.04$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

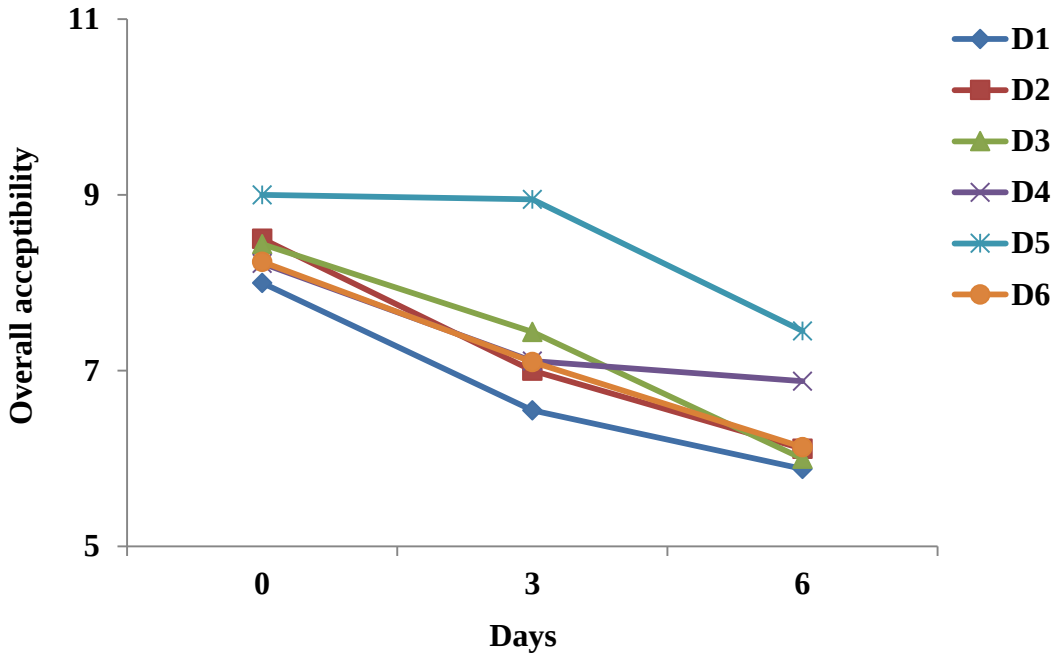


Fig 54: Changes in overall acceptability of fish fingers in different treatments

Overall acceptability of any product is based upon the individual sensory evaluation parameters including appearance, odour, crispiness, juiceness, texture, taste and flavour. The individual parameters of sensory evaluation during present study clearly revealed decreased overall acceptibility with storage, however the product prepared from fish harvested from treatment D5 was best among all other treatments and control. Arannilewa *et al* (2005) reported a decreasing trend in overall acceptability (3.00 to 2.00) in nile tilapia (*Sarotherudun galiaenus*) subjected to different freezing periods (0, 10, 20, 30, 40, 50 and 60 days). Tokur *et al* (2005) also revealed significant ($p \leq 0.05$) decline in overall acceptability in fish fingers, made from mirror carp (*C. carpio* L) during frozen storage (-18°C). Alam *et al* (2012) too observed decreased overall acceptability of rohu flesh with significantly higher ($p \leq 0.05$) score for overall quality at 1st day followed by 15th day and 30th day respectively, at frozen storage of -18°C. Yin *et al* (2014) also recorded decline in overall sensory score in grass carp (*Ctenopharyngodon idellus*) fillets during short-term chilled storage 4°C for 6 days, 40°C for 12 h and then at -20°C for next 5 days. Likewise, Kamat (1999) also recorded decrease in overall acceptability in fish cutlets made from mackerel mince meat during storage at -14°C.

During present study, there was significant decrease in the sensory parameters throughout the storage period (0-6 day) for fish product. Fish product developed

strong fishy, rancid and putrid odour at day 6 and was rejected for consumption by all the panelists. Sensory parameters are also positively co-related with the biochemical indicator like pH, TVB-N, FFA etc (Viji *et al* 2014). Among all the treatments, product prepared from treatment D5, fish showed score above 7 for all the sensory evaluation parameter except odour which clearly revealed highest overall acceptibility of the product, however product prepared from all the treatments were considered fit for human consumption before day 6, in relation to meat quality characteristics.

4.2.11 Texture analysis

Texture analysis in terms of firmness (N) and work of shear (N.sec) for fish product (fingers) was studied at day 0, 3 and 6; whereas in terms of work of shear (N.sec) and cutting strength (N) for fish fillets at day 0, 20, 30, 35 and 40 on texture analyzer with pre and post test speed fixed for product and fillet.

4.2.11.1 Firmness

Among different treatments, firmness (N) of product (fish fingers) was 20.09, 21.21, 55.72, 54.55, 57.11 and 56.50 at day 0; 19.85, 20.19, 43.77, 48.08, 51.76 and 54.13 at day 3 and 15.81, 19.65, 18.33, 34.07, 40.04 and 33.82 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 53, Fig 55). The result indicated that firmness of product was significantly different in all the treatments and control at all time intervals (0, 3 and 6 day) and it further decreased significantly ($p \leq 0.05$) on 6th day in all treatments with maximum firmness retention in D5 (40.04) and minimum in D1 (15.81).

Table 53: Changes in firmness (N) of fish fingers in different treatments

Days	Treatments					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
0	$20.09^{b,1}\pm1.83$	$21.21^{b,1}\pm0.86$	$55.72^{a,1}\pm3.95$	$54.55^{a,1}\pm6.22$	$57.11^{a,1}\pm1.46$	$56.50^{a,1}\pm1.24$
3	$19.85^{b,1}\pm0.77$	$20.19^{b,1}\pm0.42$	$43.77^{a,2}\pm3.79$	$48.08^{a,1}\pm2.76$	$51.76^{a,2}\pm1.78$	$54.13^{a,2}\pm0.21$
6	$15.81^{d,2}\pm0.15$	$19.65^{c,2}\pm0.08$	$18.33^{c,3}\pm1.12$	$34.07^{b,2}\pm1.16$	$40.04^{a,3}\pm2.38$	$33.82^{b,3}\pm2.04$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

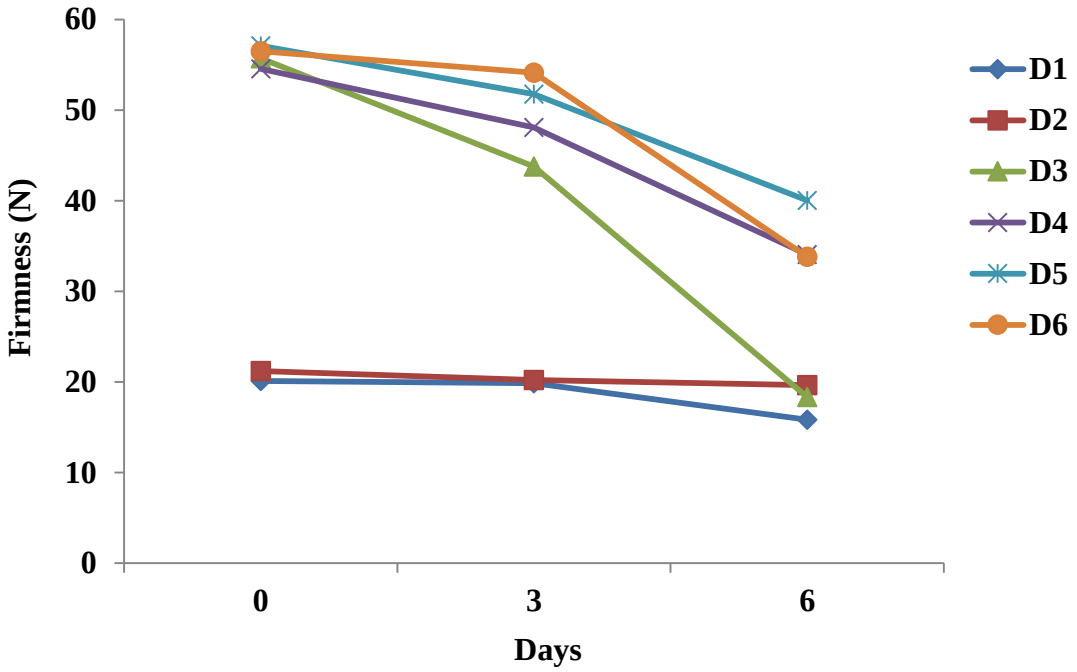


Fig 55: Changes in firmness (N) of fish fingers in different treatments

Firmness or hardness refers to the peak force during the compressive part of the texture analysis test. Significant decrease ($p \leq 0.05$) in the firmness values of fish product could be due to the weakening of connective tissues of fish muscle during storage by proteolysis caused by the endogenous and microbial enzymes.

Azam *et al* (1989) reported significant softening of both raw and cooked fillet of rainbow trout (*Salmo gairdneri*) during 15 days of ice storage using instrumental measurements. Similar observations were recorded by Hatae *et al* (1985) in terms of softening of texture in several fish species stored at 4°C upto 14 days. Likewise Aussanasuwannakul *et al* (2012) also reported decreased firmness in rainbow trout (*O. mykiss*) fillet during 14 day storage at refrigerated temperature (4°C), while studying comparison of Variable-Blade to Allo-Kramer shear method. Nima *et al* (2018) too reported decreased firmness of fillets of common carp (*C. carpio* L.), while evaluating the quality at frozen storage (–20°C) conditions. Decreased hardness was also recorded by Cordoso *et al* (2010) in ready-to-eat minced fish products developed from hake during 3.5 months under refrigeration and at 10±1°C. Further, diverse storage temperature also affect the firmness of fish muscle differently as observed by Suraez *et al* (2011) in terms of effect of 1°C and 4°C storage on seabream muscles and concluded that storage at 1°C induced a prolongation of firmness as compared with 4°C. However, the rate of degradation of muscle collagen was faster at 4°C

compared with 1°C, affecting the cross-linkage of connective tissue and becoming a major contributing factor to firmness loss of fish texture.

4.2.11.2 Work of shear

Among different treatments, work of shear (N.sec) of product (fish fingers) was 207.92, 206.24, 283.83, 256.99, 318.72 and 287.43 at day 0; 141.22, 155.74, 170.23, 189.83, 213.91 and 252.02 at day 3 and 120.35, 125.38, 122.35, 124.65, 144.49 and 138.54 at day 6 in D1, D2, D3, D4, D5 and D6, respectively (Table 54, Fig 56). The result indicated that work of shear of product decreased significantly ($p \leq 0.05$) on 6^{th} day in all treatments with maximum value in D5 (144.49) and minimum in D1 (120.35).

Table 54: Changes in work of shear (N.sec) of fish fingers in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$207.92^{c,1}\pm1.18$	$206.24^{c,1}\pm1.40$	$283.83^{b,1}\pm4.58$	$256.99^{b,1}\pm1.79$	$318.72^{a,1}\pm2.90$	$287.43^{b,1}\pm2.34$
3	$141.22^{d,2}\pm5.08$	$155.74^{c,2}\pm6.72$	$170.23^{d,2}\pm1.09$	$189.83^{c,2}\pm1.72$	$213.91^{b,2}\pm1.08$	$252.02^{a,2}\pm2.43$
6	$120.35^{c,3}\pm0.54$	$125.38^{c,3}\pm2.38$	$122.35^{c,3}\pm2.93$	$124.65^{c,3}\pm2.19$	$144.49^{a,3}\pm0.15$	$138.54^{b,3}\pm0.10$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly ($p \leq 0.05$)

Among different treatments, work of shear (N.sec) of frozen fillet was 157.08, 130.01, 162.58, 165.72, 156.63 and 131.02 at day 0; 125.50, 119.02, 150.50, 134.05, 139.26 and 118.01 at day 20; 95.23, 88.64, 115.98, 98.35, 118.50 and 54.12 at day 30; 54.29, 42.25, 48.95, 40.01, 56.98 and 38.54 at day 35 and 15.61, 16.62, 25.29, 21.19, 28.36 and 13.65 at day 40 in D1, D2, D3, D4, D5 and D6, respectively (Table 55, Fig 57). The result indicated that work of shear of fillet decreased significantly ($p \leq 0.05$) on 40^{th} day in all treatments with maximum value in D5 (28.36) and minimum in D6 (13.65).

Among texture attributes, shear force is one of the important properties for the consumer, because they determine the texture acceptability of a product (Chambers and Bowers 1993). Aussanasuwannakul *et al* (2012), reported decreased shear force in rainbow trout (*O. mykiss*) fillet on 3^{rd} day at frozen

storage (–25 °C). Dincer and Cakli (2010) also reported decreased work of shear for sausages prepared from refrigerated and frozen rainbow trout (*O. mykiss*) stored for 14 days at 0−4˚C.

Table 55: Changes in work of shear (N.sec) of frozen fillet in different treatments

Days	Treatments					
	D1	D2	D3	D4	D5	D6
0	$157.08^{b,1}\pm2.79$	$130.01^{c,1}\pm1.19$	$162.58^{a,1}\pm1.85$	$165.72^{a,1}\pm1.23$	$156.63^{b,1}\pm1.32$	$131.02^{c,1}\pm1.93$
20	$125.50^{c,12}\pm1.14$	$119.02^{d,2}\pm1.61$	$150.50^{a,2}\pm2.25$	$134.05^{b,2}\pm1.16$	$139.26^{b,2}\pm2.25$	$118.01^{d,2}\pm1.74$
30	$95.23^{b,2}\pm2.51$	$88.64^{c,3}\pm1.23$	$115.98^{a,3}\pm0.15$	$98.35^{b,3}\pm1.05$	$118.50^{a,3}\pm1.18$	$54.12^{d,3}\pm2.21$
35	$54.29^{a,3}\pm1.08$	$42.25^{b,4}\pm2.05$	$48.95^{b,4}\pm2.35$	$40.01^{b,4}\pm0.98$	$56.98^{a,4}\pm1.08$	$38.54^{c,4}\pm0.09$
40	$15.61^{c,4}\pm2.74$	$16.62^{c,5}\pm2.29$	$25.29^{a,5}\pm3.17$	$21.19^{b,5}\pm1.50$	$28.36^{a,5}\pm0.35$	$13.65^{c,5}\pm0.22$

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly (p≤0.05)

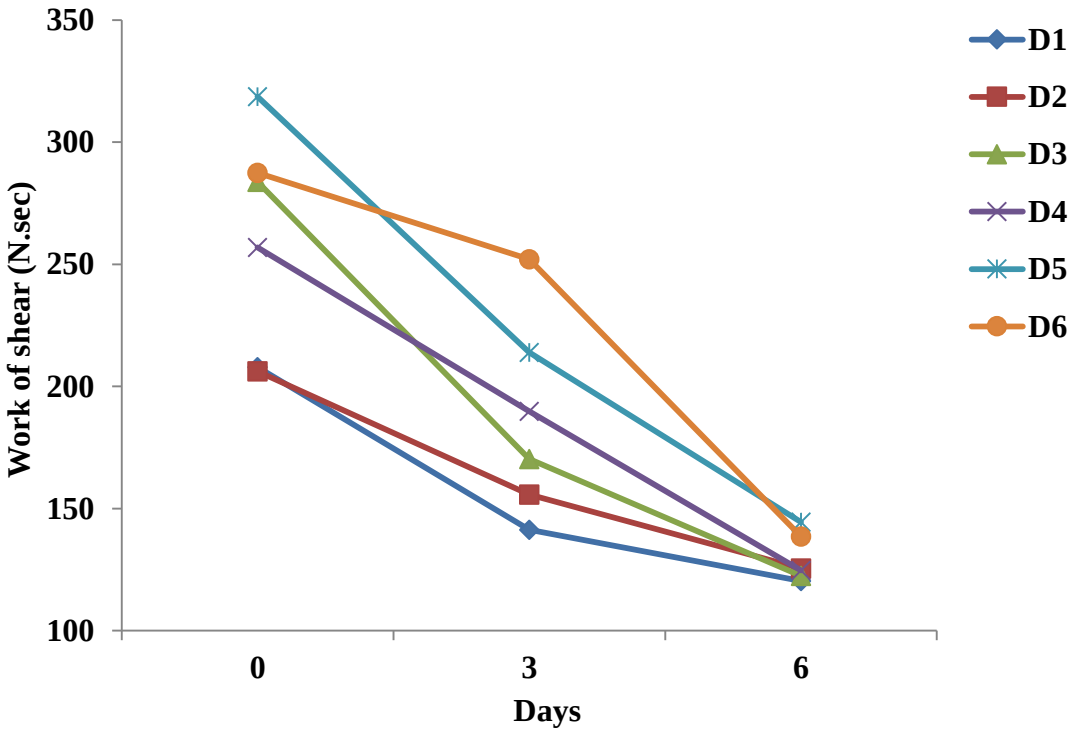


Fig 56: Changes in work of shear (N.sec) of fish fingers in different treatments

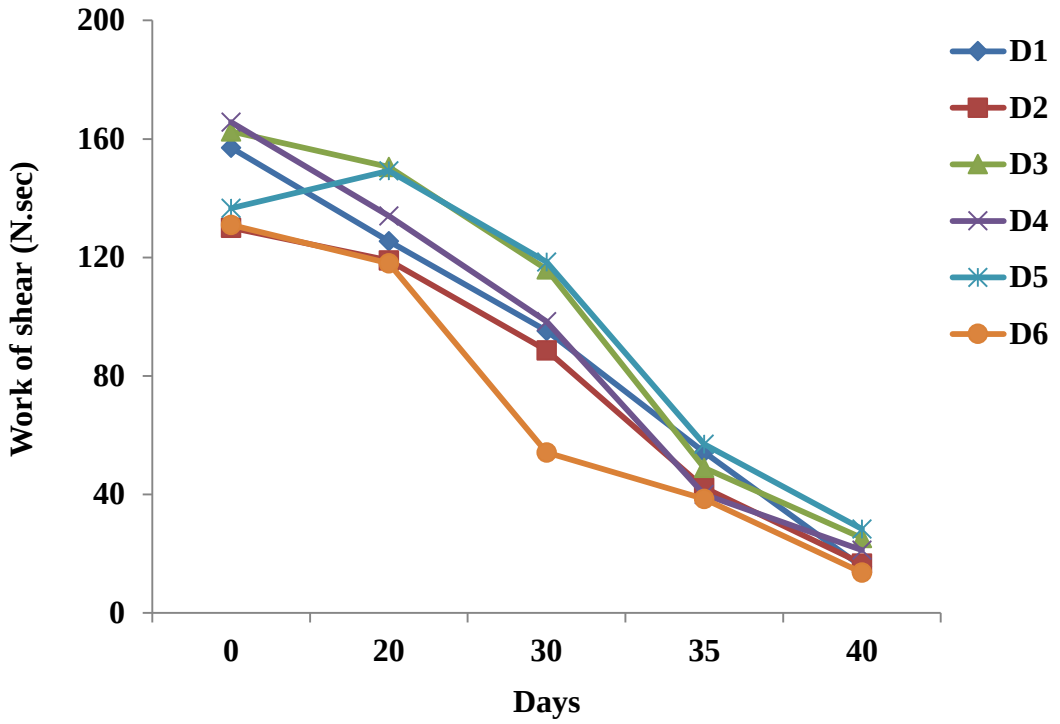


Fig 57: Changes in work of shear (N.sec) of fish fillet in different treatments

Dincer *et al* (2017) too revealed similar observations in fish sausage produced from saithe (*Pollachius virens*) after 15 days during cold storage at 0±4°C. The significant decrease in the work of shear of the fish product as well as fish fillet with increasing storage time may be explained in terms of decrease in toughness and loosing of muscle bundles resulting in increased gap and loosing of muscles also observed in terms of decreased firmness of the product/ fillet. Rawdkuen *et al* (2010), while studying the textural properties of giant catfish muscle revealed continuous decrease in shear force for both dorsal and ventral parts.

4.2.11.3 Cutting strength (N)

Among different treatments, cutting strength (N) of frozen fillet was 65.45, 61.21, 60.93, 59.19, 60.00 and 42.69 at day 0; 64.51, 58.81, 58.40, 53.96, 58.54 and 38.67 at day 20; 52.24, 46.25, 55.65, 55.32, 54.26 and 36.23 at day 30; 48.15, 42.12, 51.18, 53.28, 50.23 and 33.85 at day 35 and 36.21, 38.76, 47.73, 50.36, 49.27 and 32.45 at day 40 in D1, D2, D3, D4, D5 and D6, respectively (Table 56, Fig 58). The result indicated that cutting strength (N) of frozen fillet decreased significantly ($p \leq 0.05$) on 30^{th} day onwards in all treatments with maximum value in D4 (50.36) and minimum in D6 (32.45).

Along with firmness and work of shear, decrease in cutting strength is also one of the indicator depicting overall texture of product / fillet. Like other parameters, decrease in cutting strength with storage clearly revealed quality deterioration from day 30 onwards, which also coincides with meat quality of fillet. The results were in agreement with the findings of Ochieng *et al* (2015), who reported decreased hardness of vacuum-packaged solar rack dried sardines during chill storage (0-2°C) for 90 days. Sato *et al* (1997) too demonstrated sardine pericellular tissue weakening was in direct relation with muscle softening. Azam *et al* (1989) studied the killing method effect on rainbow trout quality during storage in ice in terms of significant softening of both raw and cooked fillets during storage. This was further confirmed by Ando *et al* (1991) and Hatae *et al* (1985) who reported softening of the texture in several fish species stored at 4°C. Dincer *et al* (2017) also recorded decreased cutting strength with increase in storage time in fish sausage produced from saithe (*Pollachius virens*) after 15 days during cold storage at 0±4°C.

Table 56: Changes in cutting strength (N) of fish fingers in different treatments

Days	Treatments					
	D1	**D2**	**D3**	**D4**	**D5**	**D6**
0	$65.45^{a,1}$±0.75	$61.21^{b,1}$±0.68	$60.93^{b,1}$±4.65	$59.19^{bc,1}$±4.64	$60.00^{b,1}$±2.23	$42.69^{c,1}$±0.68
20	$64.51^{a,1}$±2.10	$58.81^{b,1}$±2.72	$58.40^{b,1}$±5.73	$53.96^{b,1}$±1.07	$58.54^{b,12}$±2.81	$38.67^{c,2}$±0.34
30	$52.24^{c,2}$±1.07	$46.25^{d,2}$±1.52	$55.65^{a,21}$±2.25	$55.32^{a,1}$±1.21	$54.26^{a,2}$±1.98	$36.23^{e,23}$±1.42
35	$48.15^{b,3}$±1.25	$42.12^{c,23}$±1.62	$51.18^{a,2}$±0.98	$53.28^{a,12}$±0.52	$50.23^{a,3}$±1.65	$33.85^{d,3}$±1.84
40	$36.21^{b,4}$±1.61	$38.76^{b,3}$±2.78	$47.73^{a,3}$±5.84	$50.36^{a,2}$±1.83	$49.27^{a,3}$±2.56	$32.45^{c,3}$±1.46

Values are Mean ± S.E., n= 3
Values with same superscript (a, b,…..d in a row and 1,2…3 in a column) do not differ significantly (p≤0.05)

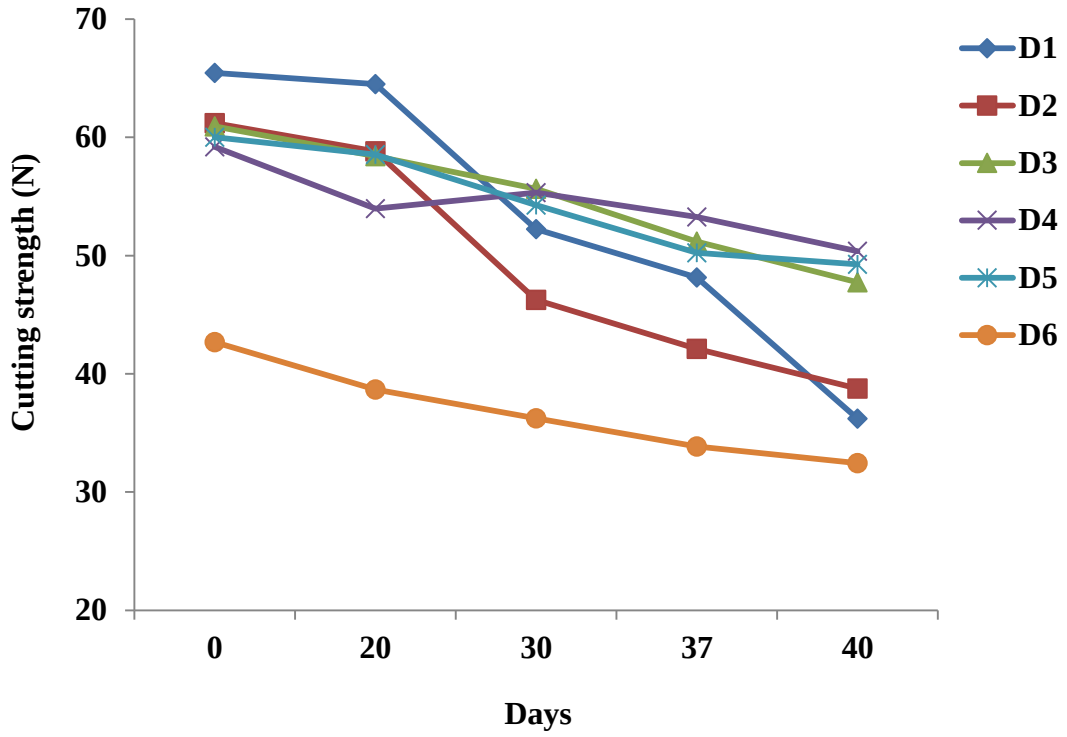


Fig 58: Changes in cutting strength (N) of fish fingers in different treatments

Texture of fish is one of the main features used to indicate the freshness of fish product/ fillet. It has also been confirmed that storage temperature during handling and operating process has their effect on textural properties (Pearce *et al* 2011). The most important change is in the form of muscle softening and gaping, which is clearly revealed in terms of decrease in firmness, work of shear as well as cutting strength, as clearly depicted in the results of present study. Although freshness of any product or raw fillet can be measured and evaluated through sensory, biochemical and instrument methods, but it is difficult to come to conclusion based on single method, as no single method is universally accepted and applied in fish and fish product industry (Cheng *et al* 2014). Moreover, no standard values are available for fish product of different fish species. Therefore, based on the collective results of present study, it can be concluded that fish fingers prepared from pangas fish were acceptable before 6 days, stored at 4°C with maximum acceptability of fingers prepared from fish fed on diet D5. Likewise, pangas fillet were acceptable till day 30, stored at -20°C in all treatments with significantly higher ($p \leq 0.05$) quality in terms of biochemical and texture parameter in D5.

4.2.12 Relative productivity and economics of treatments

Relative productivity and economics was calculated by considering the total feed cost, fish production (from 80m^2 tank), cost of production for 1 kg fish and total return from fish sale. Among all the treatments, the total feed cost and cost of production for 1 kg fish was minimum in D5 (22.79 % and 49.54 % lower than control) as compared to control, with maximum return from fish sale (62.18 % higher than control). Further, same treatment i.e. D5 showed improved fish growth and health status with special reference to blood metabolic profile (haematological, biochemical, antioxidant and liver transaminase) and flesh quality along with higher overall acceptability of product (fish fingers) prepared from the fish reared on diet D5.

S. No.	Particulars	Treatments					
		D1	**D2**	**D3**	**D4**	**D5**	**D6**
A	**Total Biomass Harvested kg $tank^{-1}$ (80 m^2)**	11.00	14.45	15.39	17.73	17.84	11.02
B	**Total Feed fed (Kg)**	40.00	50.64	42.97	39.29	41.29	37.77
C	**Feed Cost kg^{-1}**	32.24	30.49 (-5.40)	28.74 (-10.85)	27.56 (-14.51)	24.89 **(-22.79)**	30.29 (-6.05)
D	**Total feed cost/tank (Rs.)**	1289.40	1544.01 (+19.73)	1234.95 (-4.24)	1082.83 (-16.03)	1027.71 (-20.30)	1144.05 (-11.29)
E	**Cost of production (Rs. Kg^{-1} fish)**	117.21	106.53 (-8.86)	80.24 (-31.56)	60.98 (-47.91)	59.09 **(-49.54)**	103.82 (-11.45)
F	**Total return from fish sale @ Rs. 120 kg^{-1}**	1320.0	1734.0 (+31.36)	1846.8 (+39.91)	2127.6 (+61.18)	2140.8 **(+62.18)**	1322.4 (+0.18)

Values in parenthesis represent % change over control

Overall Results – Experiment II

Parameter	Results	Best Diet
Fish Survival	100 % in control & all treatments	--
Fish Growth	Significantly higher in all the treatments except D6	D5
Haematological Parameters	Significant improvement in all the treatments	D5
Biochemical Parameters	Significant improvement in all the treatments	D5
Antioxidant Status	Significant decrease in SOD & LPO	D5
Liver Enzymes	Significant decrease in AST & ALT	D5
Lipid Profile	Cholesterol/TG/HDL – Significant increase LDL - Significant decrease	D5
Flesh quality	Improved in terms of protein, Fat and Ash	D5
Quality of fish product (preserved at 4°C)		
Meat Quality	**Biochemical parameters & Sensory evaluation of fish product** Significant decrease in all parameters with storage (0-6 days) Product was acceptable before 6 days (4°C)	D5
Texture analysis	Significant decrease in Firmness and Work of Shear with storage (0-6 days)	D5
Quality of fish fillet (preserved at -20°C)		
Meat Quality	**Biochemical parameters** Significant decrease in all parameters (Shelf life upto 30 days)	D5
Texture analysis	Significant decrease in Work of Shear and Cutting strength (upto 30 days)	D5

Overall results of the present study (Experiment II) revealed that

- Fish silage supplementation in diet of pangas fingerlings revealed significant improvement in survival, fish growth and health status with special reference to blood metabolic profile (haematological, biochemical, antioxidant and liver transaminase) and flesh quality.

- Further, product (fish fingers) prepared from pangas fish were acceptable upto 6 days (should be consumed before 6 days), stored at 4°C with maximum acceptability of fingers prepared from fish fed on diet D5. Likewise, pangas fillet prepared from fish fed on diet D5 were acceptable till day 30, stored at -20°C in all treatments with significantly higher ($p \leq 0.05$) quality in terms of biochemical and texture parameter.

- In terms of relative productivity and economics of all the treatments, all the treatments, the total feed cost and cost of production for 1 kg fish was minimum in D5 (22.79 % and 49.54 % lower than control) as compared to control, with maximum return from fish sale (62.18 % higher than control).

Hence, based on the results of present study, diet D5 (plant protein sources replaced with fish silage @ 50%) along with linseed oil @ 5% can be recommended for rearing pangas fingerling to grow out, for improved growth, overall health stratus and meat quality of fish product and fillet.

Chapter V

SUMMARY

In view of expanding commercial Pangas catfish (*Pangasianodon hypophthalmus)* culture throughout India including non-coastal states like Punjab, Haryana and Uttar Pradesh, the study was conducted to standardise cost effective feed for pangas by incorporating fish silage as protein source as an alternative to traditionally used animal (fish meal) and plant protein sources (groundnut meal and soybean meal). Further, with regard to increasing market demand of pangas in the form of various value added products and fillet, there is a need to formulate finishing diets, which can maintain Long Chain n-3 PUFA for fish and human as well. Linseed oil contains about 36-40 % oil and is richest (among crop plants) source of PUFAs, essential for the human diet, which is now being used worldwide due to its health benefits. In view of the above facts, the present study **"Growth performance and meat quality of pangas catfish (*Pangasianodon hypophthalmus*) fed on fish silage and linseed oil supplemented diets"** was designed by undertaking following two experiments to explore the possibility of incorporating non-conventional animal protein source i.e. fish silage for fry and fingerling (grow out diets) rearing of Pangas catfish along with improving its meat quality with linseed oil supplemented 'finishing diets' for production of PUFA fortified health food.

- Growth performance and health status of Pangas catfish (*Pangasianodon hypophthalmus*) fry fed on fish silage supplemented formulated diets – **Experiment I**

- Growth performance, health status and meat quality of Pangas catfish (*Pangasianodon hypophthalmus*) fingerling fed on fish silage and linseed oil supplemented formulated feeds for grow-out production – **Experiment II**

5.1 Growth performance and health status of Pangas catfish (*Pangasianodon hypophthalmus*) fry fed on fish silage supplemented formulated diets – Experiment I

The experimental fish, Pangas (*P. hypophthalmus*) were procured from West Bengal and acclimatized in cemented tanks at College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana for one month.

For experimental study, 20 uniformly sized (average body length 5-6 cm, average body weight 1-2 g) fishes were stocked in each FRP pool (1.5×1×0.75m) with continuous oxygen supply (in triplicate). 1-2 inch thick soil was spread at the bottom of FRP pools before filling of water. Borewell water was used for initial filling and exchange of water during the experimental period and fed with five experimental supplemented pelleted diets which were prepared by replacing fish meal (FM) @ 50 and 100% (D2 and D3) and a mixture of groundnut and soybean meal @ 25 and 50% (D4 and D5) with fish silage (FS). Experimental diet (D6) was prepared without any animal protein source (FM or FS), while D1 served as control having FM. For preparation of fish silage, fish waste (including viscera, scales, head, fins etc.) was procured from local fish market of Ludhiana. Waste was finely chopped and 4% formic acid (weight by volume) was added to lower the pH up to 3.5 along with butylated hydroxyl toluene (BHT) as antioxidant @ 250 mgl^{-1}. The mixture was stored at room temperature for a period of 30 days (30-35^{o}C), with daily thorough mixing along with maintaining pH at 3.5 to avoid putrification. The silage (after neutralization with 8% NaOH to pH 7.0) was added to finely grounded ingredients as per feed formulation and floating feed pellets were prepared with extruder (Unitech-DOLLY). Pelleted feeds were sun dried and stored in airtight plastic containers at room temperature. The experiment I was conducted for 120 days (July – October 2018), during which the fishes were fed with floating pelleted feed @ 5-3% (I^{st} month- 5%, 2^{nd} and 3^{rd} month- 4%, 4^{th} month- 3%) twice daily for whole experimental period. Observations were recorded w.r.t physico-chemical parameters - temperature, pH, dissolved oxygen (DO), total alkalinity (TA), ammonical nitrogen (NH_3-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N) and orthophosphate ($O\text{-}PO_4^{3-}$) at fortnightly interval; survival and fish growth at 30 day interval and hematological parameters, biochemical parameters, antioxidant status, serum transaminases and lipid profile at the completion of the experiment (120 days).

5.1.1 Physico-chemical parameters of water

The water quality parameters viz. temperature, pH, DO, TA, NH_3-N, NO_2^--N, NO_3^--N) and $O\text{-}PO_4^{3-}$ varied from 30.13-36.67°C, 7.24-8.00, 9.86-12.46 mgl^{-1}, 130-167 $CaCO_3$ mgl^{-1}, 0.01-0.07 mgl^{-1}, 0.016-0.091 mgl^{-1}, 0.09-0.70 mgl^{-1} and 0.01-0.15 mgl^{-1}, respectively in all the treatments (D1-D6). All the water quality parameters

remained in optimum range for fish culture throughout the experimental study in all the treatments.

5.1.2 Survival and growth parameters

Fish survival (%) was 93.33, 96.66, 98.33, 100, 100 and 96.66 in D1, D2, D3, D4, D5 and D6, respectively. At the end of the experimental period (120 days), final total body length (cm) and total body length gain was significantly higher ($p \leq 0.05$) in D5 (12.03, 6.03) and lowest in D1 (10.27 and 4.21). Significantly higher ($p \leq 0.05$) fish growth in terms of final body weight (FBW), NWG and SGR was observed in D3 (21.33g, 19.00, 1.99), while minimum FBW and NWG in D6 (14.48 and 12.33) and SGR in D1 (1.69). Likewise, PER was significantly higher in D5 (1.35) and minimum in D1 (1.16). The differences for condition factor were insignificant for all the treatments and control, however significant improvement in FCR was observed with minimum value in D3 (2.15) as compared to other treatments and control.

5.1.3 Haematological Parameters

Haematological parameters including Hb (g%) and Ht (%) enhanced significantly with fish silage supplementation in all the treatments, however, significant improvement was observed in D4 (8.00 and 20.00) i.e. diet replacing plant protein sources with fish silage @ 25%.

5.1.4 Biochemical parameters

All biochemical parameters including total serum proteins (gdl^{-1}), albumins (gdl^{-1}), globulins (gdl^{-1}) and albumin/globulin ratio (gdl^{-1}) showed significant improvement in all silage treatments as compared to control. The values for all these parameters were significantly higher in D4 (4.32, 1.20 and 3.33) except albumin/globulin ratio, which was significantly higher in D3.

5.1.5 Antioxidant parameters

Antioxidant parameters in terms of Superoxide dismutase (SOD) (U mg^{-1} Hb) and Lipid peroxidation (LPO) (nmol MDA G Hb $^{-1}$) in blood haemolysate improved with significant decrease values of SOD in D6 (0.16) and LPO in D2, D5 (0.44) and D5 (0.47) after 120 days of feeding.

5.1.6 Serum Transaminases

Liver profile in terms of alanine transaminase (ALT) and aspartate aminotransferase (AST) (Ul^{-1}) enzyme activities at the completion of experiment revealed significantly higher values in all the silage treatments.

5.1.7 Lipid profile

Lipid profile (mg dl^{-1}) in terms of triglycerides, cholesterol, high density lipids (HDL), low density lipids (LDL) and very low density lipids (VLDL) indicated an increase in lipid content in all the treatments with maximum value in D3 (56.00, 366.53, 110.00 and 11.32) i.e diet with 100% replacement of fishmeal with silage except HDL which decreased in all the treatments with maximum value in D1 (53.00).

5.2 Growth performance, health status and meat quality of Pangas catfish (*Pangasianodon hypophthalmus*) fingerling fed on fish silage and linseed oil supplemented formulated feeds for grow-out production – Experiment II

The experimental fish, Pangas (*P. hypophthalmus*) reared (fry to fingerling) during Experiment I and overwintered (November, 2018 to February, 2019) was used for conducting Experiment II. For experimental study, 40 uniformly sized Pangas fingerlings (average body weight 55g, average standard length 18cm) were stocked in each experimental tank ($80m^2$) in triplicate. 1-2 inch thick soil was spread at the bottom of the tanks before filling of water. Borewell water was used for initial filling and exchange of water during the experimental period. The fish was fed on similar diets as for experiment I for 120 days (May – August 2019). After 120 days of experimental period, all the 6 diets (D1-D6) were supplemented with linseed oil @ 5 % (95% formulated diet + 5 % linseed oil) as finishing diets and fish was fed for further 30 days during the month of September 2019. The fishes were fed @ 5-3% (I^{st} month- 5%, 2^{nd} and 3^{rd} month- 4%, 4^{th} and 5^{th} month- 3%) twice daily during the experimental period of 150 days during experiment II. Observations were recorded w.r.t physico-chemical parameters viz., temperature, pH, dissolved oxygen (DO), total alkalinity (TA), ammonical nitrogen (NH_3-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N) and orthophosphate ($O\text{-}PO_4^{3-}$) at fortnight interval; survival and fish growth at 30 day interval and hematological parameters, biochemical parameters, antioxidant status, serum transaminases, lipid profile and flesh quality after

completion of experiment (150 days). After feeding with linseed oil supplemented diets for 30 days, fish product (fish fingers) and fillet were prepared and meat quality of the fresh and refrigerated (4°C) product (0, 3, 6 day) and frozen (-20°C) fillet (0, 20, 30, 35, 40 days), sensory evaluation of fresh and refrigerated (4°) product (0, 3, 6 day) and texture analysis of refrigerated (4°C) product (0, 3, 6 day) and frozen (-20°C) fillet (0, 20, 30, 35, 40 days) was analysed.

5.2.1 Physico-chemical parameters of water

The water quality parameters viz. temperature, pH, DO, TA, NH_3-N, NO_2^--N, NO_3^--N and O-PO_4^{3-} varied from 30.26-34.86°C, 7.26-7.95, 10.00-12.80 mgl^{-1}, 126-188 $CaCO_3$ mgl^{-1}, 0.01-0.09 mgl^{-1}, 0.014-0.099 mgl^{-1} and 0.01-0.09 mgl^{-1}, respectively in all the treatments (D1-D6). All the water quality parameters remained in optimum range for fish culture throughout the experimental study in all the treatments.

5.2.2 Survival and growth parameters

Fish survival was 100% in control and all the treatments at the completion of experiment, which revealed that the diets having only fish meal, fish silage, fish meal and fish silage, plant protein and fish silage together or only plant protein were equally acceptable by pangas. At the end of the experimental period (150 days), final total body length (cm) and total body length gain was significantly higher ($p \leq 0.05$) in D3 (33.68) and D5 (15.68) and minimum in D1 (28.91 and 10.14). At the end of experimental period, significantly higher ($p \leq 0.05$) fish growth in terms of final body weight (FBW), NWG and SGR was observed in D5 (274.36g, 217.14 and 1.00), while minimum FBW and NWG in D1 (194.57g and 135.98) and SGR in D6 (0.74). The differences for condition factor were insignificant ($p \geq 0.05$) for all the treatments and control, with maximum value in D5 (0.99). Significantly lower ($p \leq 0.05$) value for FCR was observed in D4 and D5 (2.21 and 2.37) and maximum in D1 (3.63). Likewise, PER was also significantly higher in D5 (3.08) and minimum in D1 and D2 (1.99).

5.2.3 Haematological Parameters

Haematological parameters including Hb (g%) and Ht (%) enhanced significantly with fish silage supplementation in all the treatments, however,

significant improvement was observed in D5 (10.30 and 33.33) i.e. diet replacing plant protein sources with fish silage @ 50%.

5.2.4 Biochemical parameters

Among different treatments, significant improvement observed in terms of enhanced values for serum total protein (g dl^{-1}), globulin (g dl^{-1}) and albumin (g dl^{-1}) with no change in Alb/Glb ratio (g dl^{-1}) with maximum enhancement in D5 (6.96, 0.96 and 6.00).

5.2.5 Antioxidant parameters

Antioxidant parameters at the completion of the experiment revealed significantly higher SOD (U mg^{-1} Hb) activity in D4, D5 and D6 with maximum value in D5 (0.54) and significantly low LPO (nmol MDA G Hb^{-1}) activity in D3, D4, D5 and D6 with minimum value in D5 (0.78). The alterations in antioxidant status revealed stronger tolerance against oxidation and hence improved health status of fish with silage incorporated diets.

5.2.6 Serum Transaminases

Liver profile in terms of alanine transaminase (ALT) and aspartate aminotransferase (AST) (Ul^{-1}) enzyme activities was studied at the completion of experiment. The liver profile in terms of AST (IU l^{-1}) and ALT (IU l^{-1}) improved in all the treatments with significantly reduced values for ALT and AST in D5 (16.00 and 135.25).

5.2.7 Lipid profile

Lipid profile (mg dl^{-1}) in terms of total cholesterol and triglycerides, high density lipids (HDL) and very low density lipids (VLDL) showed significant increase with maximum value in D5 (442.63, 268.06, 71.75 and 88.53), whereas low density lipids (LDL) revealed significant decline with minimum value in D5 (43.52).

5.2.8 Flesh quality

Flesh quality (g $100g^{-1}$) in terms of total proteins, total lipids, total carbohydrates, moisture and ash content at the completion of the experiment revealed significant improvement in protein, lipid and ash content of fish flesh in D5 (13.85,

8.12 and 1.32), along with decreased carbohydrates (0.43) without affecting the moisture content.

5.2.9 Meat quality

Meat quality in terms of pH, peroxide value (meq kg fat^{-1}), free fatty acid (mg $100g^{-1}$), titratable acidity (gl^{-1}) and total volatile base-nitrogen (mg $100g^{-1}$) of fresh (0 day), refrigerated (4ºC) product (3, 6 day) and frozen (-20ºC) fillet (0, 20, 30, 37, 40 days) revealed significant decrease in meat quality in terms of biochemical parameters of both product and fillet in all the treatments.

5.2.10 Sensory evaluation

Sensory evaluation in terms of appearance, odour/smell, crispiness, juiciness, texture, flavour, taste of fish product (fingers) was studied to check the overall acceptability of the fresh (0 day) and refrigerated (4°C) product at day 3 and 6 on hedonic scale of 1-9. The significant decrease in all parameters of sensory evaluation revealed that product was not acceptable after 6 days of storage.

5.2.11 Texture quality

Texture quality in terms of firmness (N) and work of shear (N.sec) for refrigerated fish fingers at day 0, 3 and 6; and in terms of work of shear (N.sec) and cutting strength (N) for frozen fish fillets studied at day 0, 20, 30, 35 and 40 revealed significant decrease in texture of both fish fingers and fish fillet with increase in storage time.

Collective results of meat quality (biochemical parameters), sensory quality and texture evaluation, fish fingers prepared from pangas fish were acceptable before 6 days, stored at 4°C with maximum acceptability of fingers prepared from fish fed on diet D5. Likewise, pangas fillet were acceptable till day 30, stored at -20°C in all treatments with significantly higher ($p \leq 0.05$) quality in terms of biochemical parameters and texture parameter in D5.

5.2.12 Relative productivity and economics of the treatments

Relative productivity and economics was calculated by considering the total feed cost, fish production (from $80m^2$ tank), cost of production for 1 kg fish and total return from fish sale. Among all the treatments, the total feed cost and cost of production for 1 kg fish was minimum in D5 (22.79 % and 49.54 % lower

than control) as compared to control, with maximum return from fish sale (62.18 % higher than control). Further, same treatment i.e. D5 showed improved fish growth and health status with special reference to blood metabolic profile (haematological, biochemical, antioxidant and liver transaminase) and flesh quality along with higher overall acceptability of product (fish fingers) prepared from the fish reared on diet D5.

Conclusions

Overall results of the present study (experiment I and experiment II) revealed that

- Fish silage supplementation in diet of pangas fry revealed significant improvement in survival and growth in D3 (100% fish meal replacement with fish silage); haematological and biochemical parameters showed improvement in D4 (25% replacement of plant protein sources with fish silage), whereas other parameters including antioxidant status, liver enzymes and lipid profile did not revealed any particular trend. Hence, based on overall results with special reference to growth, diet D3 (100% replacement of fish meal with fish silage) can be recommended for rearing pangas fry to fingerling under indoor culture conditions. In view of variations, field trials need to be conducted for better understanding of effect of fish silage coupled with multiple factors under natural conditions.

- Fish silage supplementation in diet of pangas fingerlings revealed significant improvement in survival, fish growth and health status with special reference to blood metabolic profile (haematological, biochemical, antioxidant and liver transaminase) and flesh quality in D5 (50% replacement of plant protein ingredients with fish silage).

- Further, product (fish fingers) prepared from pangas fish were acceptable before 6 days, stored at 4°C with maximum acceptability of fingers prepared from fish fed on diet D5. Likewise, pangas fillet prepared from fish fed on diet D5 were acceptable till day 30, stored at -20°C in all treatments with significantly higher ($p \leq 0.05$) quality in terms of biochemical and texture parameter.

- In terms of relative productivity and economics of all the treatments, all the treatments, the total feed cost and cost of production for 1 kg fish was minimum in D5 (22.79 % and 49.54 % lower than control) as compared to control, with maximum return from fish sale (62.18 % higher than control).

Hence, based on the results of present study, Diet D3 (100% fish meal replacement with fish silage) and D5 (50 % replacement of plant protein sources with fish silage) can be recommended for rearing pangas fry to fingerling and fingerling to grow out, for improved growth, overall health stratus and meat quality of fish product and fillet.

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