EFFECTS OF DIETARY LIPIDS ON GROWTH, FEED EFFICIENCY AND CERTAIN BIOCHEMICAL CHANGES INCLUDING TISSUE FATTY ACID PROFILE IN THE FRY OF CARP *Catla catla* (Ham).

BY

Sangram Keshari Rout (B. F. Sc.)

A THESIS SUBMITTED TO
THE ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, BHUBANESWAR
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF FISHERY SCIENCE
IN
AQUACULTURE

POST GRADUATE DEPARTMENT OF AQUACULTURE
COLLEGE OF FISHERIES
Orissa University of Agriculture and Technology
BHUBANESWAR
1994

DEDICATED TO MY

BELOVED PARENTS

EFFECTS OF DIETARY LIPIDS ON GROWTH, FEED EFFICIENCY, AND CERTAIN BIOCHEMICAL CHANGES INCLUDING TISSUE FATTY-ACID PROFILE IN THE FRY OF CARP CATLA CATLA (Ham.)

THE THESIS SUBMITTED TO
THE ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY,
BHUBANESWAR

IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR
THE DEGREE OF

MASTER OF FISHERY SCIENCE
IN AQUACULTURE

BY

Sangram Keshari Rout, B.F.Sc

APPROVED BY.

CHAIRMAN,

Dr. P.K. Mukhopadhyay,
Head, Nutrition Division
Central Institute of Freshwater Aquaculture
Kausalyaganga, Bhubaneswar-751002.

M. Rudhi 27-12-94

Members: Sri M.Ranadhir
Director. C.I.F.A.

27.12.94
Dr R K Rath.

Dr. G.N.Patel
Reader
Postgraduate Department of Aquaculture
O.U.A.T. Bhubaneswar.

External Examiner. 27/12/94

Dr. P.K. Mukhopadhyay
Senior Scientist (Biochemistry)
Head, Nutrition Division
Central Institute of Fresh Water Aquaculture
Kausalyaganga, Bhubaneswar - 2.

CERTIFICATE

This is to certify that the thesis entitled "**Effects of Dietary Lipids on Growth, Feed Efficiency and Certain Biochemical Changes Including Tissue Fatty Acid Profile in the Fry of Carp *Catla Catla* (Ham.)**" submitted for the degree of **Master of Fisheries Science** in the subject of *Aquaculture* of the **Orissa University of Agriculture and Technology, Bhubaneswar**, is a faithful record of bonafide and original research work carried out by *Sri Sangram Keshari Rout* under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

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

31/8/94
(P.K. MUKHOPADHYAY)
Major Advisor
Head
Division of Nutrition
Central Institute of Freshwater Aquaculture
Kausalyaganga, Bhubaneswar-751002

ACKNOWLEDGEMENT

I deem it a privilege to express my profound sense of gratitude and indebtedness to Dr.P.K. Mukhopadhyay. Head of Nutrition division, Central Institute of Freshwater Aquaculture, Bhubaneswar, Orissa, for his scholastic guidance, constant supervision and ceaseless encouragement throughout the period of investigation and preparation of the dissertation.

I am grateful to Mr. M.Randhir, Director, C.I.F.A. and Dr. G.N.Patel Reader. P.G.Department of Aquaculture O.U.A.T, for their valuable suggestions, encouragement and timely facilities accorded to carry out the study and as members of my advisory committee.

I express my gratitude and indebtedness to Dr. P.C. Thomas, Director, College of Fisheries, O.U.A.T, for his supreme contribution to our colege and specially in making our base in the field of fisheries science.

My special obligation to Dr. S.D. Tripathi, former Director, C.I.F.A. Kausalyaganga and present Director Central Institute of Fisheries education, Versova, Bombay for his encouragement and help during the course at study.

I owe a great deal to Dr. S.N.Mohanty, Mr P.V. Rangacharyulu, Dr. B.N. Paul, Mr. S.K.Swain, Mr. S.Sarkar for their kind co-operation during my work.

I also acknowledge the help rendered by the staff of the Nutrition Division for their sincere help in maintenance during the research period.

I express my deep sense of thanks to Mr. M. Rout, Incharge Statistics and Computer Department, C.I.F.A and Senapati babu, Acharya babu, Satpathy babu of the same department for their assistance in my data analysis and preparing the graphs.

My special thanks to Bijaya babu typist, College of Home Science for his keen intrest in typing all my draft copies.

I extend my special thanks to M/s Acharya Associate, Jaydev Vihar, Bhubaneswar for helping me a lot in preparing my final manuscript.

I acknowledge with immense happiness the assistance rendered by all my friends, juniors for their co-operation throughout my study.

I feel it to be my pleasant duty to express my sincere thanks to Saswati Pattanaik, an immense source of inspiration for me, whose unstinted co-operation and encouragement helped me a lot in carrying out my work and submission of the thesis.

I bow my head before my grandmother, parents, Ma (Basanti Mohanty) for their supreme sacrifice and eternal benedictory in evolving this tiny personality. I always pray for their blessings to proceed on the way of life.

The award of Junior Research Fellowship by I.C.A.R is also greatly acknowledged.

Bhubaneswar.
31st August, 1994.

SANGRAM KESHARI ROUT

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION: GENERAL CONSIDERATIONS

Next to proteins, lipids form the major dietary components for fish. Fish require dietary lipid for use as an energy source, for cellular architecture and for maintenance of the integrity of the bio-membrane in terms of bio-membrane fluidity by the fatty acids of the phospholipids in particular. From the standpoint of practical aquaculture, dietary lipids play important role as energy and essential fatty acid sources. Thus nutritional quality of animal and plant lipid sources in enhancing growth of fish is of considerable importance. Moreover, dietary lipid level and fatty acid composition affect the chemical composition of farmed fish which is important with respect to organoleptic and nutritional quality of the product.

The function of dietary lipid in practical fish feed may thus be summarised in the following three points.

(i) Energy source (protein sparing effect)

(ii) Essential fatty acid source (species specificity)

(iii) Carrier of non-lipid nutrients (fat soluble vitamins. etc)

It is known that supplementation of lipid in the diet not only enhances growth of fish but feed efficiency is also improved by lipid supplementation. In any protein level, feed efficiency is generally proportional to the dietary lipid level; for example, 50 % fish meal diet with 10% lipid could produce the same feed efficiency as that of 60 % fish meal diet having 5% lipid. This is what is known as the protein sparing effect of lipid. It is now known that lipids rich in poly-unsaturated fatty acids (PUFA) are susceptible to auto- oxidation or rancidity. Animal fats like lard, tallow or hydrogenated oil are suitable in this respect as energy sources because of the greater resistance of these neutral lipid sources towards auto-oxidation. These mainly consist of saturated and mono-ethenoic fatty acid.

As dietary energy source various kinds of lipid can be used provided they are of high digestibility. Optimal dietary lipid concentration for inclusion in formulated feeds for fishes involve consideration of several factors including extrusion technique followed in feed processing. Fish also require certain fatty acids for their normal growth and well-being and inclusion of fatty acid of w-3 series or w-6 series or both are essential. The requirements differ from species to species and even between fresh water species also.

1.1 ESSENTIAL FATTY ACIDS

Fatty acids are the building blocks of lipids and only traces occur in cells and tissues. Fish lipid is generally rich in mono and polyunsaturated fatty acids, this is in contrast to other animal flesh, which is higher in saturated fatty acids. There are over 100 fatty acids that occur in nature. The general formulae for fatty acids is $CH_3(CH_2)_n$ COOH where 'n' is usually an even number. Most naturally occurring fatty acids are broadly of saturated and unsaturated type. The fatty acids having a straight unbranched carbon chain without a double bond is referred to as saturated fatty acid. Carbon chain having one double bond is termed as mono-unsaturated fatty acid and more than one double bond is known as polyunsaturated fatty acid (PUFA). PUFA having five or more numbers of double bonds are termed as highly unsaturated fatty acids (HUFA). The degree of unsaturation influences greatly the physical properties of the constituent lipids. The major functions of fatty acids are related to their role in maintaining flexibility and permeability of cell membranes and regulation of membrane bound enzyme activities. Bio-membranes consist of lipid bilayers with various type of proteins embeded in or associated with them. They contain about 25 % lipid with exact amount depending on the specific biomemebrane (plasma membrane, mitochondria, endoplasmic reticulum etc.) and the tissues from which it is derived. Maintenance of correct composition of lipid is critical for the functioning of the biomembrane.

Besides having a straight forward chemical formulae each fatty acid is given a specific numerical designation such as 14:0, 20:1; 18:3ω-3; 18:2 ω-6; 20:4 ω-6; 22:5 ω-6; 22:6 ω-3 etc. This nomenclature refers to length of carbon chain in the molecule, number of carbon-carbon double bonds present and the position of the first double bound. Following are some illustrations.

Linolenic acid 18:3 ω-3

CH_3 - CH_2 - CH=CH-CH_2CH=CH-CH_2CH=CH- $(CH_2)_7$ - COOH

Arachidonic acid 20: 4ω-6

CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH = CH-$(CH_2)_3$-COOH

In the designation 20:4 for example '20' means that there are 20 carbon atoms in the chain; 4 means that there are 4 carbon-carbon double bonds; ω-6 means that the first double bond numbering from the methyl end occurs after sixth carbon atom in the chain.

Fatty acyl groups provide the hydrophobic interior of all cell membranes forming an impermeable barrier to water and polar molecule and separating the cell contents from the extra-cellular medium. The degree of unsaturation of fatty acid is important in determining fluidity of the membrane and in providing the correct environment for membrane functions. Moreover degree of unsaturation of membrane fatty acids is also important in the process of adaptation to different environmental temperatures. In addition to these functions certain membrane phospholipid and their constituent fatty acids are metabolically very active having important role as precursors for prostaglandins. A large number of individual fatty acids have been isolated from fish tissues and identified using GLC and HPLC. The common fatty acids include fatty acids of linoleic family and linolenic family Nomenclature and bond positions of major long chain fatty acids are shown in Table.1 Polyunsaturated fatty acids (PUFA) of the tissues of terrestrial mammals including human being are generally dominated by the ω-6 series, whereas in both fresh and marine fish tissue we find ω-3 and ω-6 series. This is more particular in case of marine fish.

Several studies have shown that fish do not possess the Δ 12 and Δ 15 desaturase enzymes necessary to produce PUFAs 18:2 ω-6

Table-1 Nomenclature and bond positions of major long-chain fatty acids

Common name	Systematic name*	Abbreviation	Bond positions+
Palmitic acid	hexadecanoic acid	16:0	
Palmitoleic acid	9-hexadecanoic acid 6-hexadecanoic acid	16:1 (ω-7) 16:1 (ω-10)	Δ^{9} Δ^{6}
Stearic acid	octadecanoic acid	18:0	
Oleic acid	9-octadecanoic acid	18:1 (ω-9)	Δ^{9}
Linoleic acid	9,12-octadecadienoic acid	18:2 (ω-6)	$\Delta^{9,12}$
x-Linolenic acid	9,12,15-octadecadienoic acid	18:3 (ω-3)	$\Delta^{9,12,15}$
y-Linolenic acid	6,9,12-octadecadienoic acid	18:3 (ω-6)	$\Delta^{6,9,12}$
Arachidic acid	eicosanoic acid	20:0	
Dihomo-y-linolenic acid	8,11,14-eicosatrienoic acid	20:3 (ω-6)	$\Delta^{8,11,14}$
Mead acid	5,8,11-eicosatrienoic acid	20:3 (ω-9)	$\Delta^{5,8,11}$
Arachidonic acid	5,8,11,14-eicosatetraenoic acid	20:4 (ω-6)	$\Delta^{5,8,11,14}$
Timnodonic acid	5,8,11,14,17-eicosatetraenoic acid	20:5 (ω-3)	$\Delta^{5,8,11,14,17}$
Behenic acid	docosanoic acid	22:0	
Eurcic acid	13-docosenoic acid	22:1 (ω-9)	Δ^{13}
Adrenic acid	7,10,13,16-docosatetraenoic acid	22:4 (ω-6)	$\Delta^{7,10,13,16}$
Docosapentaenoic acid	4,7,10,13,16-docosatetraenoic acid	22:5 (ω-6)	$\Delta^{4,10,13,16}$
Clupanodonic acid	7,10,13,16,19-docosatetraenoic acid	22:5 (ω-3)	$\Delta^{7,10,13,16,19}$
Cervonic acid	4,7,10,13,16,19-docoshexaenoic acid	22:6 (ω-3)	$\Delta^{4,7,10,13,16,19}$
Lignoceric acid	tetracosanoic acid	24:0	
Nervonic acid	15-tetracosenoic acid	24:1 (ω-9)	Δ^{15}

* All double bonds are *cis* configuration.

\+ The bond position from the carboxyl end of the acyl group.

and 18:3 ω-3 respectively from endogenously synthesised 18:1 ω-9. Consequently both series are likely to be essential for normal growth and survival (Henderson and Tocher, 1987). The requirement for 18 carbon (C18) PUFA is associated with the ability of fish to convert them by desaturation and elongation to the longer chain 20 carbon (C 20) and 22 carbon (C 22) PUFA. Marine fish seem to lack one or more of the desaturase enzymes necessary to accomplish this process (Sargent *et al.,* 1989). Thus, strictly speaking only 20:5 ω-3, 22:6 ω-3 and 20:4 ω-6 can be termed essential fatty acids in these fish. With freshwater fish, 18 carbon PUFA seem to be true essential fatty acids, although only a relatively small number of fish have been studied to date.

1.2 BIOSYNTHESIS OF FATTY ACIDS

Although the enzymes have not been fully characterized, it is generally assumed that fatty acid biosynthesis in freshwater fish proceeds via pathways qualitatively similar to those which operate in mammals. Thus, it can be expected that the two carbon acetyl-CoA unit is carboxylated to malonyl-CoA, which is subsequently converted to fatty acids by the fatty acid synthetase complex via a series of condensation and reduction reactions involving the utilization of NADPH.

In omnivorous mammals, the acetyl-CoA used for fatty acid synthesis originates within the mitochondrion mainly from carbohydrate metabolism. The natural diet of carnivorous fish such as salmonids and the majority of fish species are rich in protein which consequently supplies a large part of the fishes' dietary energy along with lipid. Although salmonids and carp are able to utilize dietary carbohydrate as a source of energy, the rate of glucose oxidation in rainbow trout and carp has been shown to be considerably less than that of amino acid oxidation. In carnivorous fish, the enzymes involved in the conversion of amino acids to pyruvate and tricarboxylic acid cycle intermediates are active. Consequently, carbon derived from amino acids can be incorporated into citrate which can then leave the mitochondrion and become a substrate for ATP citrate lyase in the cytosol. The action of this enzyme generates cytosolic acetyl-CoA as a substrate for acetyl-CoA carboxylase.

The importance of ATP citrate lyase in fatty acid synthesis is obvious and its presence in tissues active in the production of fatty acids would seem obligatory. However, the enzyme does not appear to be ubiquitous in the lipogenic tissues of fish.

As in mammals, the NADPH required for fatty acid synthesis in fish is generated in the cytosol by glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (both enzymes of the hexose monophosphate shunt). NADP- malate dehydrogenase (malicenzyme) and NADP-isocitrate dehydrogenase.

Overall, although protein would seem to be the major natural source of carbon for fatty acid synthesis in fish. the relative contributions of carbohydrate and protein remain to be established.

1.3 ENZYMES INVOLVED IN LIPID METABOLISM

Until the mid-1970's, much of our understanding of lipid metabolism in fish was based on the rat model. However. it has now been established that the liver is the major site of fatty acid synthesis in fish and, in contrast to mammals. very little,if any, synthesis takes place in adipose tissue. Radiolabel studies have proved the elongation and desaturation pathways of dietary ω-9,ω-6 and ω-3 fatty acids in fish, which appear to differ from those in rats only in the significant production of dead-end elongation products. Enzyme studies have clearly demonstrated the presence and functionality of enzymes such as acetyl-1-CoA carboxylase, fatty acid synthetase, glycerol-3-phosphate acyltransferase, and CDP-choline-1 2-diglyceride choline-

PATHWAYS OF BIOSYNTHESIS OF POLYUNSATURATED FATTY ACID

Oleic acid sequence (ω-9) family

DE NOVO[a] 18:0, (Stearic acid) → Δ^9 Desaturation → 18:1,Δ^9 (Oleic acid) → Δ^6 Desaturation → 18:2,$\Delta^{6,9}$ → Elongation → 20:2,$\Delta^{8,11}$ → Δ^5 Desaturation → 20:3,$\Delta^{5,8,11}$[b]

18:1,Δ^9 (Oleic acid) → Elongation → 20:1, Δ^{11}

Linoleic acid sequence (ω-6) family

DIET 18:2, $\Delta^{9,12}$ (Linoleic aicd) → Δ^6 Desaturation → 18:3,$\Delta^{6,9,12}$ (γ-Linolenic acid) → Elongation → 20:3,$\Delta^{8,11,14}$ → Δ^5 Desaturation → 20:4,$\Delta^{5,8,11,14}$ (Arachidonic acid) → Elongation → 22:4,$\Delta^{7,10,13,16}$

18:2, $\Delta^{9,12}$ (Linoleic aicd) → Elongation → 20:2,$\Delta^{11,14}$

Linolenic acid sequence (ω-3) family

DIET 18:3, $\Delta^{9,12,15}$ (Linolenic acid) → Δ^6 Desaturation → 18:4, $\Delta^{6,9,12,15}$ → Elongation → 20:4, $\Delta^{8,11,14,17}$ → Δ^5 Desaturation → 20:5, $\Delta^{5,8,11,14,17}$ (Eicosapentaenoic acid) → Elongation → 22:5, $\Delta^{7,10,13,16,19}$ → Δ^4 Desaturation → 22:6, $\Delta^{4,7,10,13,16,19}$ (Docosahexaenoic acid)

18:3, $\Delta^{9,12,15}$ (Linolenic acid) → Elongation → 20:3, $\Delta^{11,14,17}$

---

[a]Stearic acid (18:0) is formed by de novo synthesis and linoleic and linolenic acid are obtained from dietary sources.
[b]20:3, $\Delta^{5,8,11}$ is a fatty acid that normally occurs in trace amounts. This fatty acid will increase during essential fatty acid deficiency.
Ref: Green, D.H.S. and D.P. Selivonchick (1987) Lipid metabolism in fish. *Prog. Lipid Res.* 26: 53-85.

phosphotransferase in various fish species. Enzyme regulation and triacylglycerol biosynthesis in the intestine is not well understood, although it has been suggested that a combination of nonspecific lipases and acyltransferases in epithelial cells are operative in the biosynthesis of triacylglycerols from ingested lipid precursors.

1.5 ESSENTIAL FATTY ACID REQUIREMENT OF FRESHWATER FISHES

During the last few years nutritional aspects of essential fatty acids (EFA) have been extensively studied. Among and various species salmonids were the first experimental species in several studies and many investigators have demonstrated that rainbow trout *Salmo gairdneri* require dietary fatty acids of the linolenic family (ω-3 series) for maximum growth, feed conversion and freedom from pathology (Lee *et al,* 1967, Higashi *et al,* 1964, 1966, Castell *et al,* 1972 a,b,c, Watanabe *et al* 1974 abc). *Nicolaids and Woodall* (1962) initially found impaired pigmentation in chinook salmon fed diets deficient in linolenic acid 18:3 $_{\omega\text{-}3}$. The ability of freshwater fishes to utilise linoleic acid 18:2 ω-6 and/or linolenic acid 18:3 ω-3 as EFA has been demonstrated by many nutritional studies (Takeuchi *et al,* 1979, 1980. Takeuchi and Watanabe, 1982, Yu *et al,* 1982). The requirement for these two PUFA is associated with the ability of the fish to invest them by desaturation and elongation to the long chain 20 carbon and 22 carbon PUFA which are essential constituents of biomembrane phospholipids (Henderson and Tocher, 1987. Castell *et al.*, (1972) found that the requirement of rainbow trout for 18:3ω-3 is 1% in the diets and no combination of 18:3$_{\omega\text{-}3}$ with 18:2 ω-6 resulted in as fast as growth rate or as efficient a feed conversion ratio as 1 % of 18:3 ω-3 alone in the diet. Inclusion of 18:2 ω-6 in the diet resulted in some improvement in growth and feed conversion compared with EFA deficient diet, However, ω-6 fatty acids, did not prevent some EFA deficiency symptoms such as 'shock syndrom'. Later experiments of Watanabe et al (1974 a b) place the linolenic requirement of the species at between 0.8 to 1.6%. Fish fed on diets containing less than 0.5% 18:3 (ω-3) exhibited retarded growth, erosion of the caudal fin and a shock syndrome.

The channel catfish, one of the most important warm water fish of North America is found not to utilise 18:3 ω-3 as efficiently as do salmonids. The carp *Cyprinus carpio* was found to be similar to cat fish in EFA requirement. The growth rate of channel catfish was effectively improved by supplementation of 1 % ω-3 HUFA (Satoh *et al*, 1989).

Studies with tilapia on the relative efficiencies of ω-6 and ω-3 PUFA as EFA have provided contradictory results. 18:2 ω-6 has been found to be more effective in the growth promotion of *Tilapia zilli and T. nilotica* than 18:3 ω-3, 20: 4 ω-6 or longer chain PUFA (Kanazawa *et al.*, 1980; Teshima *et al.*, 1982, Takeuchi *et al.*, 1983). Such findings implied that enzymes of elongation and desaturation in tilapia efficiently convert C-18 PUFA to longer chain PUFA and that there enzymes favour 18:2 ω-6 over 18:3 ω-3 as substrates.

Studies with other warm water fish indicated a requirement of both type of acids. A level of 0.5 % of each acid was found to be sufficient for Japanese eel *Anguilla japonica.* Cold water fish such as trout, solmon and marine fish depend more on the linolenic type of acids. It seems that the incorporation of these acids, which have a lower melting point than linoleates, into the membrane phospholipid facilitates permeability through the membranes in cold temperature and in the higher salt concentration of the marine medium.

Studies to determine essential fatty acids of grass carp *Ctenopharyngodon idella* indicated vertebral column curvature is one of the EFA deficiency symptoms and that the requirement of grass carp for dietary ω-6 + ω-3 fatty acids was found to be 1 % and 0.5 - 1 % respectively.

Adding saturated lipid (50 % methyl laurate) resulted in a positive growth response and supplements of 18:2 ω-6 or 18:3 ω-3 to the diet for 22 weeks resulted in little further improvement in growth and feed conversion.The relatively low growth rate obtained in the fat-free group could be attributable to the lower caloric content due to the absence of fat. When the feeding trials were done with very young carp weighing about 0.65 g which had been kept on a fat free diet for four months before initiation of feeding trials, it was clearly shown that these fish have an EFA requirement for both 18:2 ω-6 and 18:3 ω-3 (Watanabe *et al.*, 1975 ; Takeuchi and Watanabe, 1977). The best weight gain and feed conversion were obtained in fish receiving a diet with both 1% 18:2 ω-6 and 1 % 18:3 ω-3. The results obtained in both carp and catfish seem to indicate that the requirement of these warmwater fish for EFA are less than that of the cold water fish such as ranbow trout. Kanazawa *et al.*, (1980) examined EFA requirement of *Tilapia zilli*. In tilapia growth promoting effects of 18:2 ω-6 and 20:5 ω-3 were found to be superior to those to 18:3 ω-3 and 20:4ω-3 indicating that the fish requires ω-6 fatty acids rather than ω-3 fatty acids. The dietary requirement of tilapia for 18:2 ω-6 or 20:4 ω-6 was about 1 % in the diets.

A reason for the difference observed in the essential role of 18:3 ω-3 between marine and fresh water fishes can be presumed from changes in fatty acid composition noted during a long period of feeding with a 18:3 ω-3 supplemented diet. In freshwater fishes the concentration of 20:5 ω-3 and 22:6 ω-3 in body lipid increased as a result of feeding 18:3 ω-3 but it was not so in marine fishes like plaice and red sea bream. From lower abilities to convert 18:3 ω-3 to ω-3 HUFA when compared to freshwater fishes. The following table (Table-2) summarises the essential fatty acid requirement of certain freshwater fish species.

Table-2 Essential fatty acid requirement of certain freshwater species.

Fish species	Requirement	Author(s).
Cyprinus carpio	1% 18:2ω-6 & 1% 18:3 ω-3 or (ω-3) HUFA	Watanabe *et al.* (1975)
Ctenopharyngodon idella	1% 18:2ω-6	Takeuchi *et al.* (1991)
Clarias batrachus	18:2 ω-6 &	Bandyopadhyay *et al.* (1982)
Heteropneustes fossilis	18:3 ω-3	
Tilapia zilli	1% 18:2 ω-6 or 1% 20:4 ω-6	Kanazawa *et al.* (1980)
Salmo gairdneri	1%18:3 ω-3	Castell *et al.* (1972)
Ictalurus punctatus	1% ω-6 HUFA	Satoh, *et al.* (1989)

REVIEW OF LITERATURE

Fish have always being important as a part of human diet. In addition to fish caught by traditional methods in wild cultured fish significantly contribute to the total amount of fish consumed by humans. Following table gives the general situation of aquaculture production in the country.

Table- 3 General situation of fisheries and aquaculture in India* (1991-92).

Parameter (Unit)	
Total fish production (million tonne)	4.14
Total inland fish production.(million tonne)	1.70
Aquaculture production. (million tonne)	0.98
Area under freshwater aquaculture (million ha)	0.59
Contribution of carp in freshwater aquaculture(%)	67.70

* Dehadrai (1992).

The economic importance of aquaculture has meant that considerable research have been carried out on lipid metabolism of several freshwater fish species in relation to their growth and development under culture conditions. Unfortunately relatively little work seem to have been carried out on lipids and fatty acid nutrition in various Indian carp species. Following is a brief account of some of the information available on the subject in some freshwater fish species.

Csengeri *et al.*, (1978) investigated fatty acid composition of various fish feeds and that of the carp *Cyprinus carpio* fed with different feeds. They found that fatty acid composition of the feed was equivocally reflected in the tissue fatty acid composition. The carp lipids contain high amount of oleic acid because of the high carbohydrate content of diet and were lower in polyunsaturated fatty acids.

Csengeri *et al.*, (1978) carried out investigations with young carp fed on diet containing varied amounts of linoleic and linolenic acid. The diet resulted in several differences in liver fatty acid composition and synthesis. Those high in carbohydrate and low in linolenic acid increased the rate of synthesis of oleic acid and resulted in low contents of PUFA in liver. Simultaneously, the ratio of eicosatrienoic to docosahexaenoic acid was high, showing an early stage of essential fatty acid deficiency of the carp.

Yu and Sinnhuber (1979) found that in salmon (*Oncorhynchus kisutch*) the optimum level of dietary ω-3 fatty acids ranged from 1 to 2.5 % and that dietary ω-6 fatty acids higher than 1 % depress fish growth, and feed conversion. The concentration of 22:6ω-3 and total ω-3 fatty acids in phospholipid were nearly identical regardless of the dietary 18:2ω-6 concentration.

Dupree *et al* (1979) investigated the effects of different levels of menhaden oil and cod liver oil in the diet of channel catfish in regard to weight gain, tissue protein deposition and fatty acid composition of the whole fish. Corn oil was inferior to the fish oil at all level and suppressed growth at higher levels. The fatty acid composition of the lipids of the whole catfish fed diets supplemented with increasingly higher levels of either corn oil or menhaden oil become increasingly more similar to the composition of dietary lipid.

El. sayed *et al.,* (1984) studied seasonal variations of total lipids, free fatty acids, triglycerides, phospholipid and cholesterol content of freshwater fish *Tilapia nilotica*. Effects of different dietary lipid source on muscle and egg fatty acid composition of coho salmon reared in freshwater net pens was studied by Hardy (1989). Fatty acid profiles of the fish muscle and developing eggs reflected dietary fatty acid profile after two months of feeding. They found that salmonids differ from many marine species in the importance of dietary lipid source on reproductive performance, as long as dietary ω-3 level is at least 1 % of the diet.

Taskeredzic *et al.*, (1989) conducted a series of experiments in order to evaluate the effects of various oils of both animal and vegetable origin upon the growth performance of juvenile rainbow trout. Of all the oils examined dietary supplementation with 7% sun flower oil produced the best results over a 10 week period compared to other treatment groups (rape seed oil, soya oil, fish oil and control).

Kheyyali *et al.*, (1989) investigated the effects of dietary carbohydrate and lipid levels on hepatopancreatic enzymes, growth and body composition in common carp *C. carpio.* Their results indicated that the species can utilise a relatively large amount of both carbohydrate and lipid in the diet and suggested that metabolism in hepatopancreas was regulated to accelerate glycolysis, lipogenesis and glycogen formation and to depress gluconeogenesis and amino acid degradation when fed high carbohydrate diets. While to depress glycolysis, gluconeogenesis and lipogenesis as well as to facilitate fatty acid utilisation when fed high lipid diets.

Anderson and Arthington(1989) reported that silver perch reared on high lipid diets accumulated significantly more body fat than those reared on fat-free diet. Dietary fatty acids accumulated in both depot lipids and phospholipid of the fish. On transfer from a high lipid diet to a lipid-free diet there was a preferential incorporation of both arachidonic (20:4ω-6) and docosahexanoic (22:6ω-3.) acids into phospholipids during lipid turnover. On the basis of their studies they concluded that silver perch have a dietary requirement for fatty acids of both ω-3 and ω-6 series.

Belsare and Belsare (1990) studied the lipid content of three Indian major carp species i.e., *L.rohita, C.catla and C. mrigala* feeding them with high lipid content diets. Significant increase in the content of total phospholipid and free fatty acid could be noted in muscle, liver of these fishes exposed to high lipid diet for six weeks.

Viola and Arieli (1990) succeeded in enriching nutritional quality of carp in respect of ω-3 fatty acids without impairment of storage stability or organoleptic qualities by feeding the fish for four months with formulated feeds containing 5% of fish oil in diet.

Total lipids and fatty acids in the liver of some freshwater fishes like *Tor putitora, L. calbasu and C. mrigala,* were quantified by Qazi *et al.*, (1990). The proportions of unsaturated fatty acids were higher than the saturated fatty acids and the predominant fatty acids recorded were 18:0, 19:3ω-6, 20:3ω-3 etc.

Hanley (1991) studied the effects of feeding supplementary diet containing varying levels of lipid on growth, food conversion and body composition of tilapia *Oreochromis nilotica.* Increase in dietary lipid level effected a significant increase in the level of carcass and visceral lipid.

A comparison of gill lipids in terms of lipid classes and fatty acid composition were compared among tilapia, carp, rainbow trout, white fish, yamme by Sampekalo *et al.,* (1992). There was no difference in total lipid content of the gills among the fishes. The main component was triglycerides in all the species and their content of phospholipid. was 0.7 - 1.5 %:, being highest in phosphatidyl choline followed by phosphatidyl ethanolamine.

A quantitative comparison between diet and body fatty acid composition in wild *northern pike* was made by Schwalme (1992). Among the individual PUFAs the largest differences occurred in 20:5ω-3 and 22:6ω-3 which comprised on an average 9.6 and 14.7% weight respectively of diet lipids and 5.9 and 18.3 % weight respectively of pike lipids. The close similarity in fatty acid composition between pike and their diet suggested that pike may have limited ability to elongate and desaturate (18C) PUFAs and may require specific long chain PUFAs in their diets.

A detailed analysis of the fatty acid composition of five fresh water fishes of China was done by *Liu* (1992). These included grass carp, common carp, silver carp, variegated carp and round headed bream.

A PERUSAL OF THE AVAILABLE LITERATURE THROUGH COMPUTER SEARCH USING ASFA CD-ROM AS WELL AS ALL AVAILABLE CURRENT JOURNALS (BOTH NATIONAL AND INTERNATIONAL) INCLUDING THE STUDIES ALREADY REFERRED TO ABOVE INDICATE THAT STUDIES RELATED TO LIPIDS PARTICULARLY ASPECTS OF FATTY ACID NUTRITION ARE MEAGRE OR RATHER NON-

EXISTENT IN CASE OF INDIAN MAJOR CARP SPECIES ALTHOUGH THESE FORM THE MOST IMPORTANT AND POPULAR CULTURED FOOD FISH IN FRESHWATER AQUACULTURE SECTOR IN THE COUNTRY. IN VIEW OF THIS LACUNA, THE PRESENT INVESTIGATION WAS UNDERTAKEN WITH THE SOLE OBJECTIVE TO STUDY THE EFFECTS OF DIFFERENT DIETARY LIPID SOURCES ON GROWTH, ASSOCIATED NUTRITIONAL INDICES, TISSUE FATTY ACID COMPOSITION AND SOME TISSUE BIOCHEMICAL CHANGES IN THE FRY OF THE CARP, *CATLA CATLA*. IT IS EXPECTED THAT THE INFORMATION IN CONCERT WITH THOSE ALREADY AVAILABLE DATA BASE RELATED TO STUDIES IN THE FIELD OF LIPID AND FATTY ACID NUTRITION SHOULD BE USEFUL TO DEVELOP SUITABLE FORMULATED FEED FOR THE SPECIES IN CARP POLYCULTURE FOR ACHIEVING OPTIMUM YIELD.

INTRODUCTION

The Indian major carp *Catla catla* is an important freshwater aquaculture species alongwith two others viz. rohu and mrigal in the composite fish culture system commonly pratctised. In fact, carp culture is now a rapidly growing industry country-wide alongwith simultaneous development of the segment of aquaculture feed industry. Information is rapidly accumulating on nutritional requirements, practial feed formulations, factors underlying more cost-effective conversion rates and so on. The results were consolidated in the form of a review article by Mukhopadhyay *et al.*, (1991). In spite of all these developments information relating to lipid metabolism, esential fatty acid requirements, role of phospholipids in growing carp species are grossly lacking. It is well known that dietary lipids, besides providing energy, serve as a source of essential fatty acids. Watanabe (1982) has reviewed the role of lipids in fish nutrition pointing out the need for essential fatty acids. The protein sparing effect of dietary lipid has been investigated in several freshwater fish species. (Ramachandra Nair and Gopakumar, 1981; Viola and Arieli, 1983; Das *et al.*, 1991). Lipids are almost completely digestible by fish and seem to be favoured over carbohydrate as an energy source. (Cowey and Sergeant 1977; Cho *et al.*, 1985). It is in this context that the quality of dietary lipid is of immense importance than perhaps its quantity. Our understanding with respect to the suitability of specific oils for incorporation into carp feed is still inadequate and thus studies which attempt to redress this imbalance will be meaningful for furtther advancement of carp culture in the country. The present study was undertaken to investigate the effects of different lipid sources upon the growth and tissue biochemical changes in the fry of the carp *Catla catla.*

MATERIALS AND METHODS

4.1 MAINTENANCE OF EXPERIMENTAL FISH

Healthy fry of Catla (av.wt. 0.12 ± 0.009g) were taken at random from the stock raised from fish spawned and reared at Central Institute of Freshwater Aquaculture (CIFA) farm ponds. The fry were acclimated to the laboratory conditions for one week maintained in fibre glass tanks. Supplementary aeration was provided through air stones using a compressor. The fry were fed with prepared diet thrice daily on a rigid schedule and tank water was replenished by siphoning out about two-third volume of water daily.

The fry were randomly distributed in fifteen plastic containers (fifteen numbers in each) each having 20 lit. water. Aeration was continuously provided as mentioned above. All the groups were fed with lipid free diet for 21 days given thrice a day slowly to satiation level prior to the start of the experiment. Each tank was cleaned daily taking utmost care to avoid injury to experimental fish and a hygienic condition maintained to the extent possible all through to avoid disease infestation. Only the disinfected and stored tap water was used in exchanging the water.

The acclimatised fry (av.wt. 0.28 ± 0.4 g) were then treated with five sets of experimental diets (each in triplicate) including one compounded diet commonly used to feed the fish at CIFA carp culture ponds.

The fry were reared for eight weeks (July- Sept, 1993) during which they were fed thrice daily from previously weighed pots containing the feeds. Sampling to record changes in body weight was done at weekly intervals. Feed pots were simultaneously weighed to know the amount of feed consumed in seven days period.

The water temperature was recorded daily at 11 a.m. The air temperature data was available through courtesy of Mr. M.D.Mantri from Institute's Meteorological laboratory. Dissolved oxygen was measured by using a portable automatic HANNA oxygen anlyzer (Model no-HI 8543; Orbit International Ltd, Hyderabad). The dissolved ammonia was determined spectrophotometrically (Strickland and Parsons, 1972). Briefly the method is as follows

Reagents Required

(a) Phenol alcohol (0.5%)-10g of phenol was dissolved in 100 m of 95% V/V ethyl alcohol.

(b) Na-nitroprusside solution-1g of Na-nitropruside was dissolved in 200 ml distilled water and was stored in an amber bottle.

(c) Alkaline citrate solution:- 100g of trisodium citrate and 5g of NaOH was dissolved in 500ml of distilled water.

(d) Oxidising agent:- 10 ml of Na- hypochlorite solution and 40 ml of alkaline citrate solution (1:4) were mixed and prepared fresh.

Procedure

To 50 ml of water sample taken in 100 ml beaker, 2 ml. of phenol alcohol solution and 2ml of Na-nitroprusside solution were added. Then 5ml of oxidising agent was added. The whole volume was kept undisturbed for one hour. Then the extinction was measured in a double beam Hitachi spectrophotometer, (model no.150-20) at 640 nm.

The water and air temperature are presented in table-4. The dissolved oxygen ranged between 5.0 to 6.4 ppm, the dissolved ammonia 4.04-10.68 mg atom N/l and PH 7,0 to 7.5.

Table 4. Fluctuation in air and water temperatures during experimental period (Taken at 11 AM at CIFA, Kausalyaganga, Bhubaneswar.

Dates	Days after stocking	Air temperature (°C)	Water temperature (°C)
29.7.93	0	32.5	33.6
5.8.93	7	32.3	33.4
12.8.93	14	28.8	30.5
19.8.93	21	29.4	30.2
26.8.93	28	29.4	30.0
2.9.93	35	32.1	33.5
9.9.93	42	29.5	32.0
16.9.93	49	30.6	31.3
23.9.93	56	31.5	32.2

4.2 EXPERIMENTAL DIETS

Three isoenergetic and isonitrogenous semipurified diets were formulated to know the efficay of various oil sources in the diets of the experimental fish species. The various oil sources listed include sunflower oil, cod liver oil and a mixture (1:1) of both. A lipid free basal diet was formulated by replacing oil with required quantity of dextrin to maintain the energy value of the feed. A formulated feed using finely powdered and sieved groundnut oil cake, powdered rice bran, roasted soybean meal, fish meal was prepared. The ingredient and proximate composition of the experimental diets have been given in Table-5 and shown fig.1. The composition of mineral and vitamin premix used are shown in Table-6.

Preparation of diets

Calculated quantities of dietary ingredients were weighed on a precision electronic balance (Scientech 5220, USA). A known quantity of water was then taken in a beaker and heated to a temperature of about 50°C. Gelatin was dissolved into part of it with mild stirring while heating the content on a heating mantle. In another portion of water starch was added and dissolved with stirring till clear solution was obtained. This was followed by the addition of dextrin. Both the mixture

Table - 5 **Ingredient composition of the experimental diets (Diet code D_1 to D_5)**

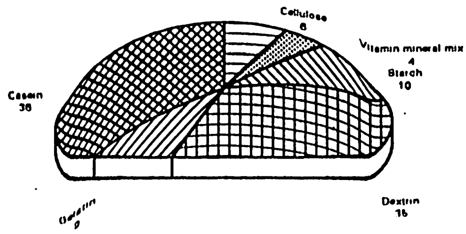
Ingredients (g/100g)	Diet Code				
	D_1	D_2	D_3	D_4	D_5
Casein (Fat free)	36	36	36	36	-
Gelatin	9	9	9	9	-
Dextrin	35	25	25	25	-
Starch	10	10	10	10	-
Vitamin mineral premix	4	4	4	4	2
Cellulose	6	11	11	11	-
Sunflower oil	0	5	0	2.5	3.5
Cod liver oil	0	0	5	2.5	1.5
Powdered groundnut oilcake	-	-	-	-	33
Powdered rice bran	-	-	-	-	35
Roasted powdered soybean	-	-	-	-	20
Powdered fish meal	-	-	-	-	5

were combined together and rest of the dietary ingredients were added to this which was followed by various mechanical stirring to form a thick paste like substance. This was then poured into a tray and then placed into a refrigerator to form gel. It was then cut into small cubes and kept in dark plastic container for storing in deep freeze until used.

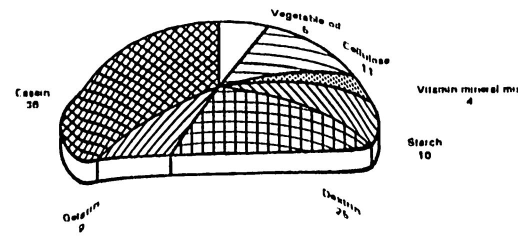
Table - 6 Composition of Vitamin-mineral premix (100g mix contains) used in carp diet preparation

Thiamine hydrochloride	5 mg
Riboflavin	20 mg
Pyridoxine hydrochloride	5 mg
Choline chloride	500 mg
Nicotinic acid	75 mg
Calcium pantothenate	50 mg
Inositol	200 mg
Biotin	5 mg
Folic acid	5 mg
Cyanocobalamine	5 mg
Vitamin D3	2000 I.U.
Menadione	5 mg
Ascorbic acid	150 mg
α-tocopheryl acetate	40 mg
Vitamin A	5000 I.U
Calcium lactate	2.5 g
Diacalcium phosphate	70.0 g
Dipotasium hydrogen phosphate	8.0 g
Potasium chloride	5.5 g
Sodium chloride	6.5 g
Disodium hydrogen phosphate	2.0 g
Magnesium sulphate	2.5 g
Ferric citrate	1.0 g
Zinc sulphate	50 mg
Potasium iodate	100 mg
Cobaltous chloride	50 mg
Cupric carbonate	10 mg
Selenium dioxide	5 mg
Manganese sulphate	100 mg
Aluminium Cholride	20 mg

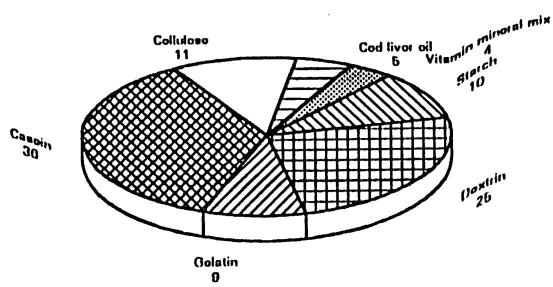
Fig. 1: Formulation of Experimental Diets



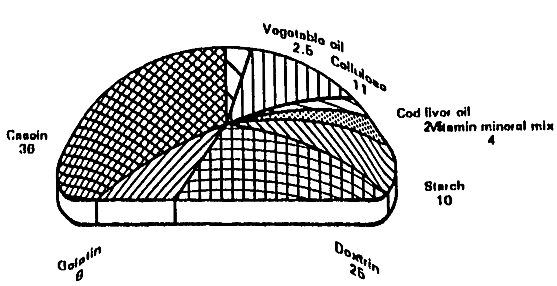
DIET-1



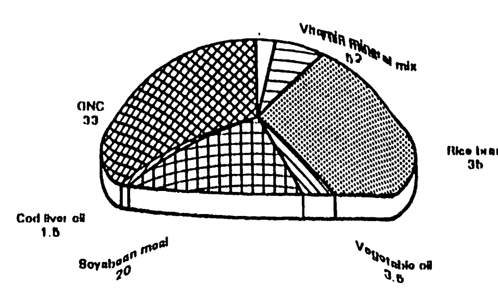
DIET-2



DIET-3



DIET-4



DIET-5

For preparing the practical formulated diet the powdered and sieved ingredients were mixed thoroughly and then required amount of boiled water added. The bulk was made into stiff dough, autoclaved, it was then pelleted using a pellet making machcine (California pellet mill, USA). Pellets were then dried in an oven on flat enamel trays at about 60° for 24 hours, The dry pellets were stored under room temperature in a dark plastic container for feeding purposes. (stored not more than three week at a time). The proximate composition of feed is given in Table-5.

4.3 CHEMICAL ANALYSIS

Upon termination of the experiment the fish from each container were weighed individually and a representative sample of fish from each container was sacrificed for

a) muscle fillet proximate composition.(Nx6.25; ether extract; total ash; crude fibre & NFE)

b) muscle RNA, DNA and protein content.

c) Intestinal lipase activity

d) Fatty acid composition of wholebody tissue lipid.

Five fish were selected randomly from each container at the end of the bioassay trial and pooled treatment-wise. The pooled samples were oven-dried and analysed for crude protein in a kjeltec system, crude lipid by petroleum ether extraction method in a soxtech system. Crude fibre using a fibretech system. Total ash was determined by incineration at 550°c for 12 hours in a muffle furnace. Nitrogen free extract (NFE) was calculated by difference.

NFE = 100 - (% crude protein + % Crude lipid + % crude fibre + % total ash).

From another set of pooled samples the muscle tissue was collected and homogenised in icecold 0. 89% Nacl solution. Three subsamples were then made out of the homogenate. The total muscle protein, muscle DNA and muscle RNA were estimated following Folin's method (Lowry *et al.*, 1951) for protein and diphenylamine method of Giles and Myres (1965) for DNA and orcinol method of Brown(1946) for RNA.

4.3.1 Estimation of Protein

To the homogenate of a weighed portion of muscle tissue in physiological saline solution was added equal volume of cold 10%

TCA; this was then centrifuged and supernatant discarded. To the precipitate 5 ml of ethanol: ether (1:1) was added and stirred with a thin glass rod. It was then centrifuged and to the precipitate 5 ml of ethanol: ether (1.1) was again added, placed on hot water in a beaker and stirred; then again the mixture was centrifuged. The precipitate left was homogenised with distilled water and 0.1 ml of Na-deoxycholate (0.15%) was added to make the solution clear.

Reagents

a) Alkaline sodium carbonate solution (2% NO_2 CO_3 in 0.1N NaOH.)

b) Copper sulphate-sodium potassium tartarate solution (0.5% $CuSO_4$ in 1% Na- K- tartarate). Prepared fresh by mixing stock solutions.

c) Alkaline solution. prepared on day af use by mixing 50ml of (a) and 1ml of (b)

d) Folin-ciocalteau reagent. (Diluted the commercial reagent (Sigma) with an equal volume of water on the day of use. This is a solution of sodium tungstate and sodium molybdate in phosphoric and hydrochloric acid).

e) Standard protein (bovine serum albumin solution 0.2mg/ml).

Procedure

To 1 ml of the prepared sample, 5 ml of alkaline solution was added, mixed well and was allowed to stand for 10 minutes at room temperature. 0.5 ml of dilute Folin-Ciocalteau reagent was added after 10 minutes and mixed well immediately. After 30 minutes of addling Folin- Criocalteau reagent, the optical density was read at 750nm in a Hitachi double beam spectrophotometer., (modelno-150-20).

4.3.2 Estimation of DNA

To 5 ml of the tissue homogenate was added 5 ml cold 5% PCA. The tube contents were mixed with the help of a vortex mixer, placed in ice for 15 minutes and centrifuged at 30,000g (High speed regrigerated centrifuge) for 15 minutes at 4° C. The precipitate obtained was dispersed in 4 ml (10%) PCA. The tubes were covered with marbles and heated in a water bath at 70°C for 25 minutes. The contents were mixed gently thrice during heating. After cooling the tubes were centrifuged at 30,000g for 15 minutes at 4°C. The supernatant was collected in a graduated tube and precipitate washed with 4ml cold 10% PCA, mixed and centrifuged as before. The supernatant was added to the first collection and the total volume brought to 8ml with cold 10% PCA. The supernatant which contained the extracted DNA was quantified by the diphenylamine method of Giles and Myers (1965). Briefly the method consisted of mixing 1ml of the solution with 2ml water and 5ml of the diphenylamine reagent, heated on boiling water bath and reading the optical density at 595nm against standard DNA. (Calf thymus DNA, Sigma Chemical Co, USA). Diphenylamine reagent was prepared by dissolving 1g pure diphenylamine in 100ml of glacial acetic acid and 2.5ml of concentrated H_2SO_4 was added. This solution was prepared fresh.

4.3.3 Estimation of RNA

The freshly weighed muscle tissue was homogenised with normal saline solution containing 0.05 M phosphate buffer. Out of the homogenate 2-3ml was pipetted into a 15 ml capacity centrifuge tube and 5 ml of cold 5% PCA was added. Tube contents was mixed with a vortex mixer and was kept in ice for 15 to 20 minutes at 4°C. The supernatant was discarded and the precipitate was dispersed in 5 ml of cold 1% PCA. Tube contents were mixed with the help of a vortex mixer and was centrifuged again at high speed in the cold and the supernatant was discarded. The precipitate was dispersed and digested in 4 ml of 1 (N)KOH for 1-2 hrs at 30 to 40°c. During digestion the tubes were covered with marbles to limit water loss and contamination. After digestion the tubes were cooled to 4°C for 15 minutes. The contents were then acidified by addition of 5% PCA, mixed and centrifuged for 15 minutes at 30,000g. The supernatant was collected in 20 ml graduated tubes. The precipitate was washed twice with cold 5% PCA, mixed and centrifuged as described earlier. Two washings were added to the original supernatant and the total volume was brought to 20 ml with cold 5% PCA. This supernatant contained the extracted RNA which was quantified by the Orcinol reaction for pentose (Brown,1946). From this nucleic acid solution 2 ml was pipetted out and 3 ml of orcinol reagent was added. The total volume was heated on a boiling water bath for 20 minutes. Then it was cooled and the optical density was read at 665 nm by a Hitachi double beam spectrophotometer. (model no. 150-20)

Orcinol reagent was prepared by dissolving 100mg of ferric chloride ($FeCl_3$ $6H_2O$) in 100 ml of concentrated HCl to which 3.5 ml of 6% orcinol in alcohol was mixed.

4.3.4 Quantitative determination of lipase activity

Lipase was estimated by applying the method described by Seligman and Nachlas (1963) and α-napthyl laurate was allowed to act as substrate together with sodium taurocholate as the lipase activator.

The response of the reaction was evidenced by the liberation of α-napthal which was coupled with tetrazotized-o-dianisidine and the azodye formed was extracted with ethyl acetate and determined spectrophotometrically. Reaction was carried out in presence as well as in absence of taurocholate and the difference corresponded to lipase activity.

Regents

a) Buffer: (0.1.M) Tris (hydroxymethyl) amino methane/ HCl, PH 7.2.

b) Substrate: α-napthyl laurate 2mg/10ml in 1:9 acetone/water.

c) Sodium taurocholate (8x10^{-2}M) 890mg dissolved in 100ml of distilled water.

d) Tetrazotized-o-dianisidine (4mg /ml): 40 mg dissolved in cold distilled water just before use.

e) Glacial acetic acid

f) α - naphthol standard solution (0.1 mg/ml) 10mg was dissolved in 100 ml of warm distilled water.

Procedure

Enzymatic reaction: Each determination required 5 test tubes: two sample with taurocholate, two samples without tourocholate and a reagent blank pipetted successively into the tubes.

	Blank	with taurocholate	without taurocholate
Buffer solution	2ml	2ml	2ml
Sodium tourocholate	0.1ml	0.1ml	-
Distilled water	0.4ml	0.4ml	0.5ml
Enzyme preparation	-	0.1ml	0.1ml
Substrate solution	0.2ml	0.2ml	0.2ml

Then reaction mixture was incubated for one hour at 37°C. Then 1ml of tetrazotized-O-dianisidine was added in each tube and allowed to stand for 5 minutes. Then 2 ml of glacial acetic acid was added in each tube and the optical density was read at 540nm. Activity was expressed as mg of α-naphthol liberated per mg protein perhour.

4.3.5 Analysis for fatty acid composition in wholebody tissue of fish

Lipids were extracted with chloroform and methanol from the pooled dried sample from each treatment, using the method described by Bligh and Dyer (1959). The method is briefly as follows:

1 to 2g of weighed dry sample was homogenised with chlorform-methanol (2:1), centrifuged at 1000 rpm in a table top centrifuge and residue again homogenised in $CHCl_3$ CH_3 OH - water (2:1:1). After centrifugation the residue was again homogenised and

recentrifuged. The supernatauts were pooled. The chloroform layer containing total lipid was dried over anhydrous $Na_2 SO_4$.

The fatty acid methyl ester of extracted lipid was prepared by $H_2 SO_4$ catalysed methylation (Christie, 1982). Briefly the method was as follows:

To about 500 mg lipid sample added 10 ml toluene and 2 ml 1%. H_2SO_4 in dry methanol. This was incubated overnight at 50°C; cooled and then added 20 ml saturated aqueous $NaHCO_3$ and 50 ml hexane-diethylether (1:1) containing 0. 05% BHT. This was mixed and transferred to a separating funnel. Allowed the mixture to separate and collected the upper layer. Re-extracted the lower layer with 50 ml hexane-diethylether mixture. Concentrated the solution by evaporating the solvent from the pooled upper layer in a nitrogen stream. The methylester thus prepared was purified using thin layer chromatography on activated silicagel-G plates using the following solvent systems

a) Isopropyl alcohol: acetic acid (96:4) followed by
b) petroleum ether: ethylether: acetic acid (90:9:1).

The spots on the plates were detected with iodine vapour which was then encircled with fine dissecting needle.

Iodine was allowed to sublime before removal of the spots. The sample was eluted from the silica gel by pouring it in small glass vials containing n-hexane. The solvent containing the methyl-ester was separated through decantatation and was transferred into small vials, 5 μl amount of this methyl ester in n-hexane was injected into the gas chromatography (Pye Unicam, model GCD) under the following operating conditions.

a) Detector - FID

b) Glass column was packed with 10% diethylene glycol succinate.

c) Column temperature - 190°C

d) Injection port temperature - 210°C.

e) Detector temperature - 230°C.

f) Carrier gas (Nitrogen) flow rate : 50 -55 ml/min.

g) Recorder chart speed-640 mm/hr.

The peaks were identified by co-chromatography with known fatty acid methyl esters (obtained as gift from Dr. Roma Ghosh, Animal Physiology Research Unit. Centenary Building Bose Institute, Calcutta-54). The composition was calculated from the relation: height of the peak multiplied by width at half height and then corrected after multiplication by appropriate attenuation factors.

4.3.6 Growth and associated nutritional indices

The calculations done for different nutritional parameters are as follows:

a) **Feed conversion ratio (FCR).**

$$= \frac{\text{Total feed intake (dry weight)}}{\text{Total live weight gain.}}$$

b) **Specific growth rate (SGR)** as % per day

$$= \frac{\text{Log}_e \text{ final body weight (g)} - \text{Log}_e \text{ initial body wt.(g)}}{\text{Experimental period in days.}} \times 100$$

c) **Protein Efficiency Ratio (PER)**

$$= \frac{\text{Total live weight gain}}{\text{Total protein intake}}$$

d) **Percent protein deposited (PPD)**

$$= \frac{\text{Body protein gain x 100}}{\text{protein intake}} \times 100$$

e) **Energy retention Efficiency (ERE)**

$$= \frac{\text{Body energy gain}}{\text{Total energy intake}} \times 100$$

f) **Feed conversion efficiency (FCR)**

$$= \frac{\text{Wet weight gain}}{\text{Feed intake(dry)}} \times 100$$

g) **Average daily weight gain (ADG)**

$$= \frac{\text{Total weight gain}}{\text{Experimental period in days}} \times 100$$

h) **Gross feed conversion efficiency (GFCE)**

$$= \frac{\text{Dry body weight gain}}{\text{Dry feed intake}} \times 100$$

RESULTS

5.1 PHYSICO CHEMICAL CHARACTERISTICS OF WATER

During the experimental period, the air temperature fluctuated between 28.8°C to 32.5°C and water temperature from 30° to 33.6°C. The dissolved oxygen, dissolved ammonia and pH of water ranged from 5 to 6.4 ppm. 4.04 to 10.68 mg atom N/1 and 7.0 to 7.5 respectively.

5.2 DIETS

The ingredient composition and nutritional analysis of the diets (D_1 to D_6) are shown in table-5 and 7. The diets were respectively D^1 (lipid free diet using purified ingredients), D_2 (diet containing lipid as vegetable oil only), D_3 (diet containing lipid as cod liver oil only), D_4 (diet containing equal proportion of both vegetable oil and cod liver oil), D_6 (a formulated diet). Calculated values of essential amino acids and fatty acid profile are presented in table - 8 and 9.

5.3 RESPONSE OF FISH TOWARDS THE DIETS AND PERFORMANCE

In all sets of experimental trials, the fish accepted the diet fairly well and none of the diets elicited any apparent pathological effects

Table - 7 Proximate composition of experimental diets (% of dry matter)

Proximate composition	Diets				
	D_1	D_2	D_3	D_4	D_5
Dry matter	51.10	48.80	52.70	50.75	94.00
Organic matter	97.35	97.61	97.47	97.81	93.75
Crude protein	42.75	42.75	42.75	42.75	30.25
Crude lipid	0.00	4.81	4.89	4.90	8.57
Crude Fibre (CF)	6.00	10.90	11.20	10.89	9.75
NFE	48.60	39.15	38.63	39.27	35.18
Total carbohydrate (CF+NFE)	54.60	50.05	49.83	50.16	44.93
Crude Ash	2.65	2.39	2.53	2.36	16.25
Gross energy (kcal/100g)	463.81	490.22	490.07	491.52	434.45
Protein energy (kcal/100g)	239.40	239.40	239.40	239.40	169.40
Non-protein energy (kcal/100g)	224.41	250.82	250.67	252.12	265.05
Protein: energy ratio (mg protein/kcal)	91.17	87.20	87.23	86.97	69.63

Table 8 Essential amino acids (Calculated value) as percentage of protein in experimental diets

Amino acids	Casein	Gelatin
Arginine	1.37	0.69
Histidine	1.09	0.07
Isoleucine	1.88	0.11
Leucine	3.32	0.75
Lysine	3.01	0.32
Methionine	1.06	0.08
Phenylalanine	1.91	0.18
Threonine	1.57	0.15
Tryptophan	0.41	0.0
Valine	2.36	0.20

Ref: Halver, J.E. (1989). *Fish Nutrition* Second edition by Academic Press, Inc. New York.

Table 9 Essential fatty acids (Calculated value) in experimental diets

Diets	Fatty acids			
	18:2ω-6	18:3ω-3	20:5ω-3	22:6ω-3
D_1	0.0	0.0	0.0	0.0
D_2	3.5	0.5	0.0	0.0
D_3	0.25	0.05	0.80	0.70
D_4	1.88	0.05	0.4	0.35

Tacon, A.G.J. (1987) The nutrition and feeding of farmed fish and shrimp- A training mannual.1. The essential nutrients. FAO field Document, Project GCP/RLA/075/ITA; Field Document, No. 2/E, Brasilia, Brazil, 117 pp.

Tacon, A.G.J. (1987) The nutrition and feeding at farmed fish shrimp - A training manual 2. Nutrient sources and composition. FAO Field Document. Project GCP/RLA/075/ITA, Field Document No.5/e Brasilia, Brazil, 129 pp.

and the gross examination of fish did not reveal any from of abnormality. The growth response is indicated in fig. 2 . The growth performance and associated nutritional indices are summarised in table 10. The survival (percentage) has did not show any significant difference within the treatments Maximum survival was found in fishes treated with formulated diet and least survival was observed in the groups treated with lipid free diet.

Of the diets tested, the diets D_4 consisting of equal proportion of vegetable oil and fish oil was found to perform the best, the percentage increase in growth over initial weight in eight weeks was 380.35 ± 13.49. This was followed by diet D_2) (containing only vegetable oil) 319.5 ± 36.5; diet D_3 (containing only fish oil) 290.0 ± 31.9, diet D_1 (lipid free diet) 29.9. ± 30.8 and diet D_5 (a formulated diet) 164,4±44.9 (Table 10).

Specific growth rate, feed conversion ratio, protein efficiency ratio, percent protein deposited, energy retention efficiency, etc. followed the similar trend showing the least FCR in mixed lipid diet accompanied by best growth performances. figs.3, 4, 5, 6, 7.

Table-10 Effect of feeding different dietary lipids on growth, survival and certain nutritional indices in fry of *Catla catla*

Nutritional indices	Treatment diets				
	D_1	D_2	D_3	D_4	D_5
Average initial weight	0.29±0.08	0.26±0.007	0.31±0.06	26±0.01	0.28±0.01
Average final weight	0.99±0.29	1.08±0.12	1.20±0.12	1.23±0.04	0.74±0.15
Percent increase in growth	239.9±30.8	319.5±36.1	290±31.9	380.35±13.49	164.4±44.9
Average daily weight gain (%)	1.24±0.39	1.46±0.20	1.54±0.20	1.74±0.05	0.83±0.25
FCR	2.73±0.24	2.53±0.05	2.61±0.14	2.26±0.08	3.73±0.54
SGR	2.17±0.16	2.53±0.15	2.43±0.15	2.82±0.05	1.70±0.31
PER	0.86±0.08	0.93±0.02	0.89±0.05	1.04±0.04	0.89±0.13
PPD	13.00±0.95	13.00±0.17	14.53 ±0.65	14.87±0.55	16.20±7.79
ERE	7.43±0.62	8.49±0.1	8.97±0.43	9.58±0.36	6.44±0.73
FCE	36.73±0.59	39.53±0.78	38.30 ±2.11	44.30±0.01	26.97±3.88
GFCE	7.37±0.59	8.85±0.09	8.89±0.42	9.07±1.68	5.57±0.61
Survival(%)	82.22 ±3.85	88.67±4.05	84.00 ±3.46	93.33±6.67	97.78±3.85

FCR - Feed Coversion Ratio

SGR - Specific Growth Rate

PER - Protein Efficiency Ratio

PPD - Percent Protein Deposited

ERE - Energy Retention Efficiency

FCE - Feed Conversion Efficiency

GFCE - Gross Feed Coversion Efficiency

Fig. 2: Growth of Catla fry (g/fish) under various dietary treatments

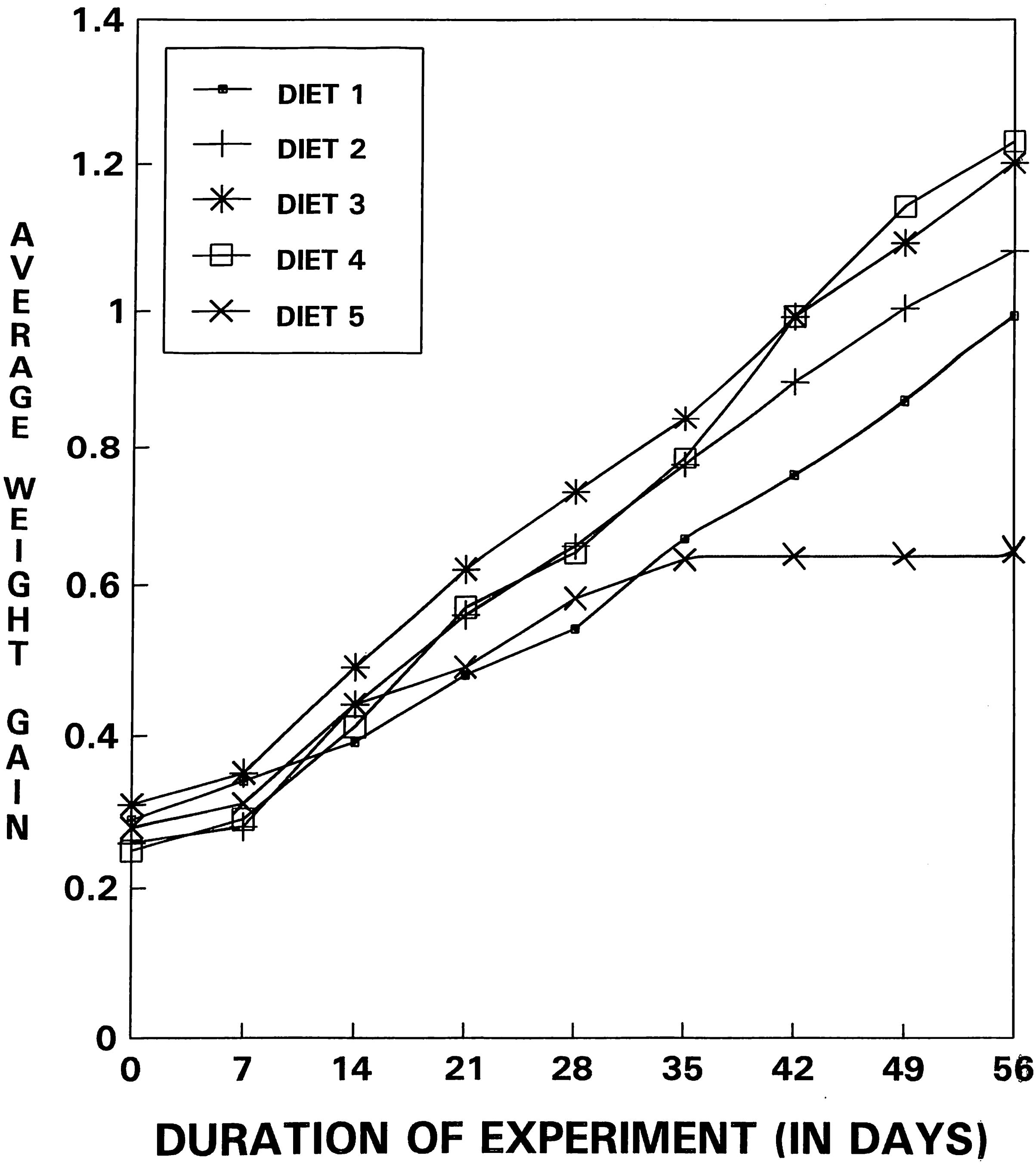


Fig. 3: Specific Growth Rate (SGR) of catla fry in relation to various diets

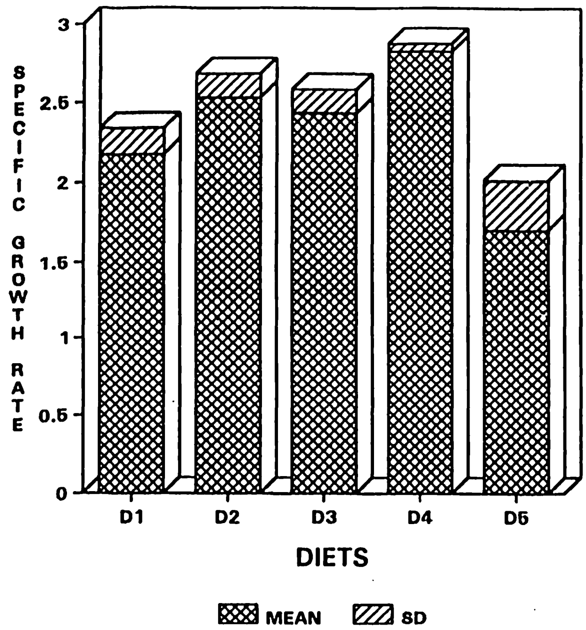


Fig. 4: Feed Conversion Ratio (FCR) of catla fry in relation to various diets

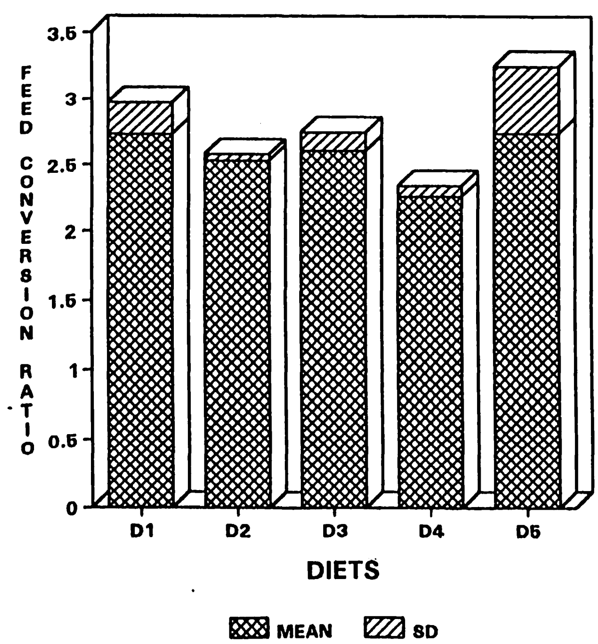


Fig. 5: Protein Efficiency Ratio (PER) (fresh weight basis) of catla fry in relation to various diets

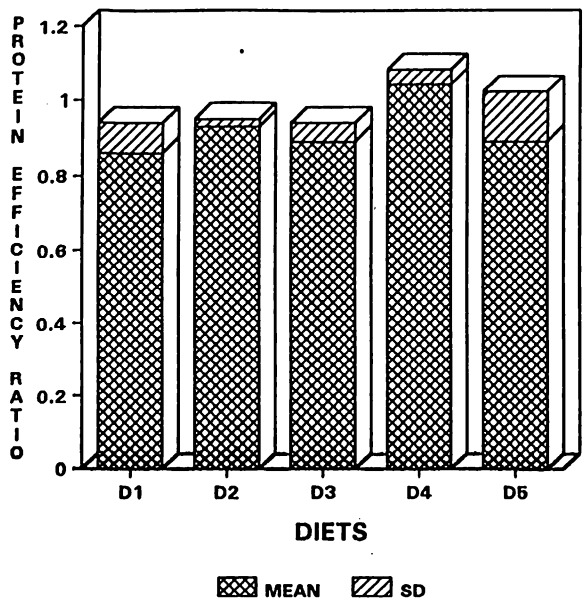


Fig. 6: % Protein Deposited (PPD) of catla fry in relation to various diets

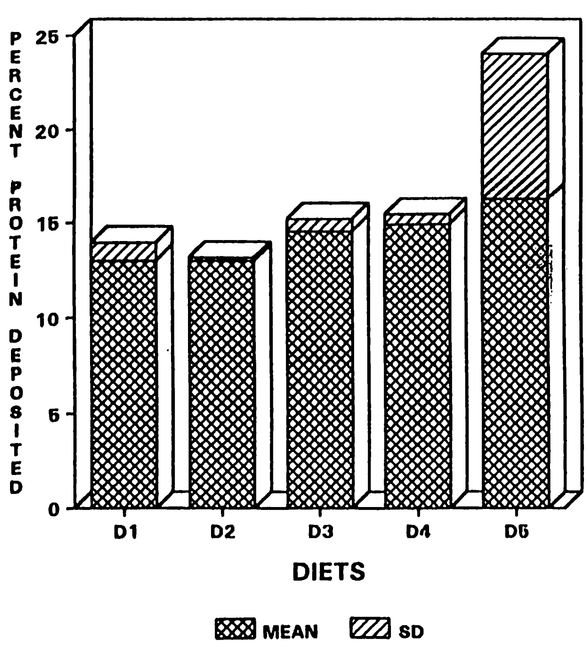
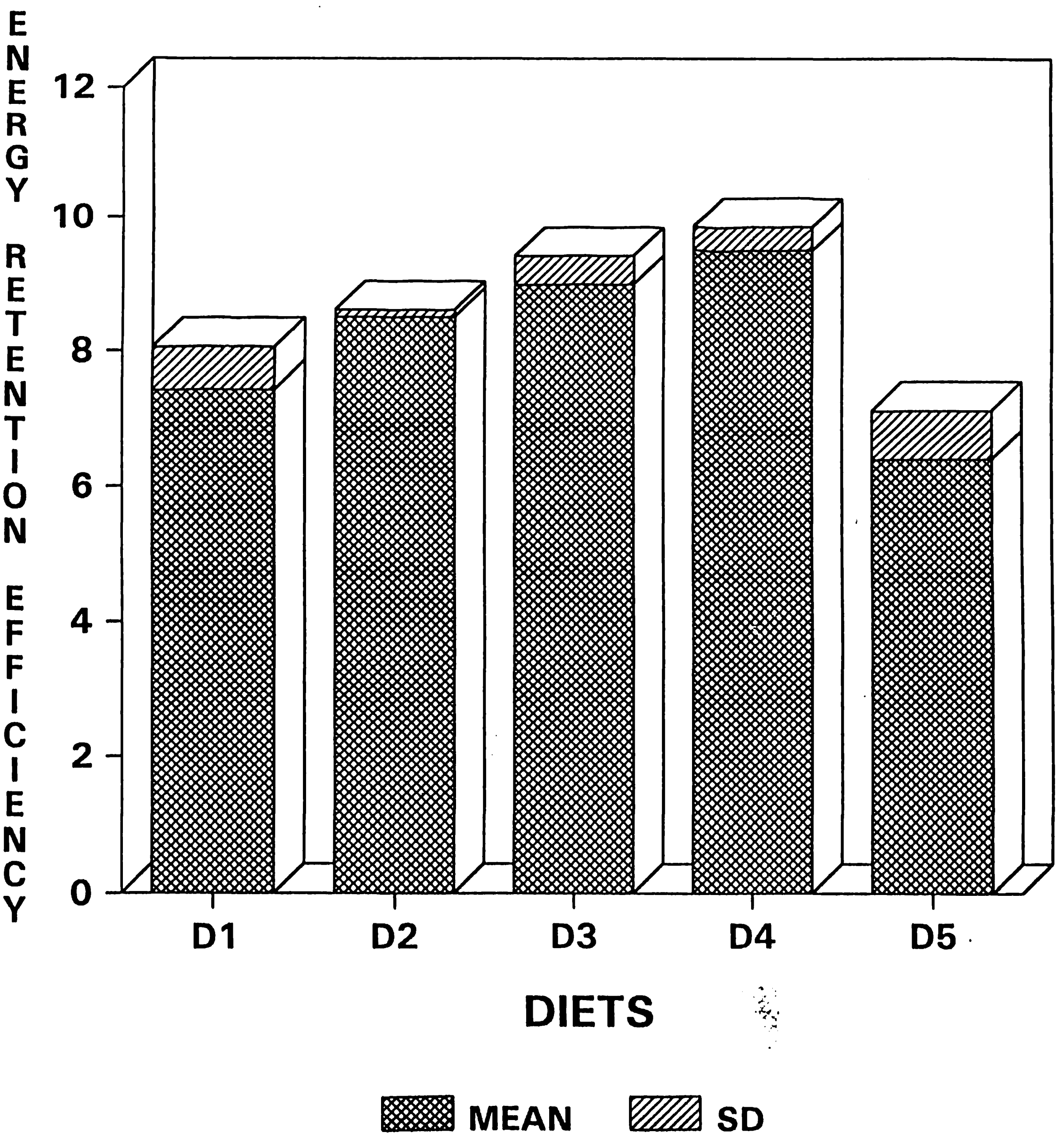


Fig.7: Energy Retention Efficiency (ERE) of catla fry in relation to various diets



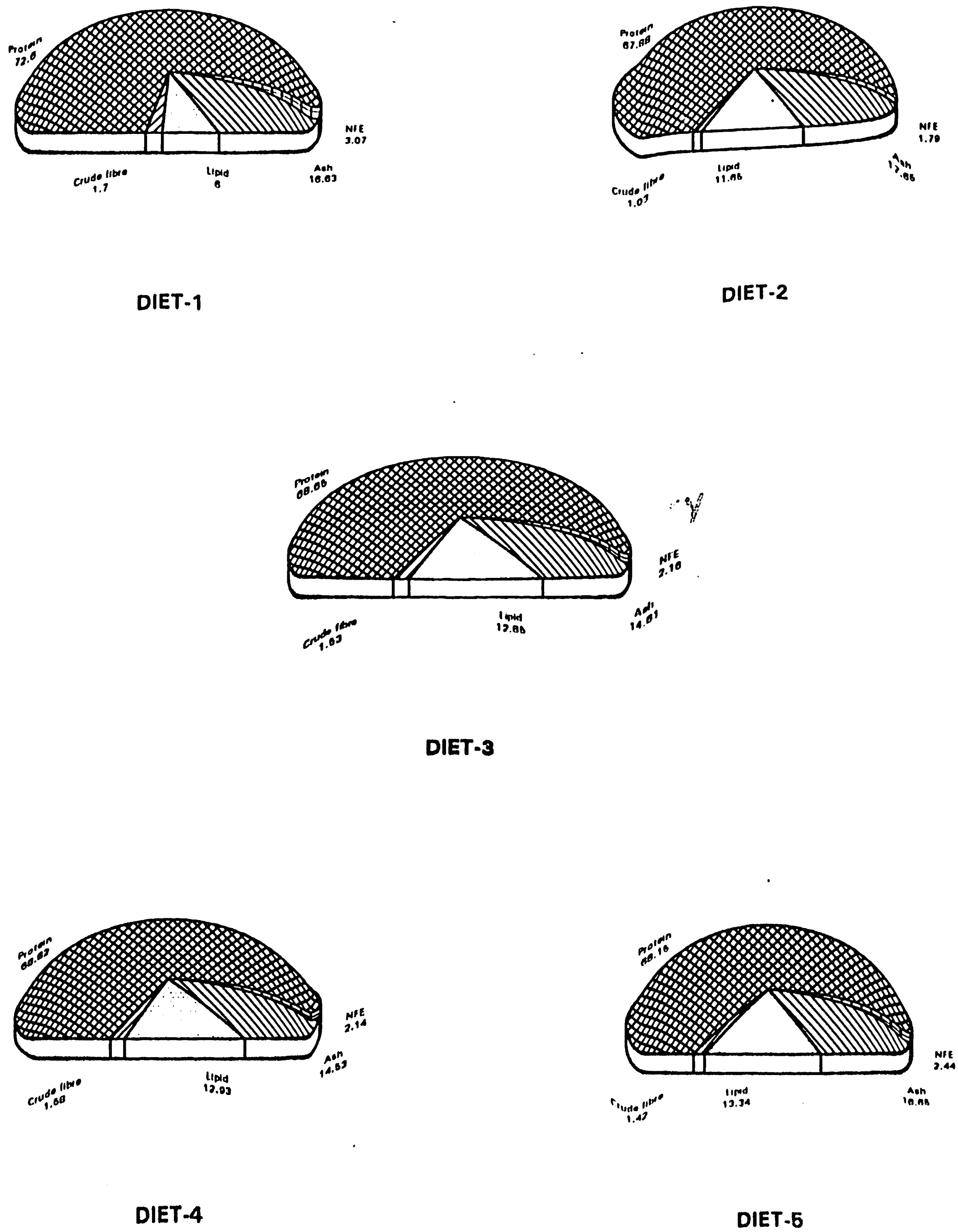
5.4 BODY COMPOSITION

The results of the body composition are presented in table-11 and shown in fig 8. The dry matter content was the least in lipid free diet (D_1) treated groups compared to other purified diets containing varied source of dietary lipids. The crude lipid content was also the lowest (6%) compared to all other test diets. The growth of the fish increased with increase in the dietary energy level recording maximum diet (D_4) treatment (521.96 k cal/100 gm) having P/E ratio of 131.85 mgprotein/ kcal energy.

Table-11 Proximate composition of muscle fillet (% dry weight) of catla fry fed with different experimental diets for eight weeks.

Proximate composition	Diets				
	D_1	D_2	D_3	D_4	D_5
Dry matter	18.06	19.61	18.67	18.98	17.84
Organic matter	83.37	85.19	82.35	85.47	83.15
Crude protein	72.60	68.65	67.88	68.82	66.15
Crude lipid	6.00	12.85	11.65	12.93	13.34
Crude fibre (CF)	1.70	1.53	1.03	1.58	1.42
NFE	3.07	2.16	1.79	2.14	2.44
Total carbohydrate (CF+NFE)	4.77	3.69	2.82	3.72	3.86
Crude Ash	16.63	14.81	17.65	14.53	16.65
Gross energy (kcal/100gm)	482.44	520.14	500.99	521.96	511.43
Protein energy kcal/100g	406.5	384.44	380.13	385.39	370.44
Non protein energy kcal /100g	75.88	135.70	120.86	136.57	140.99
Protein: energy ratio (mg protein/kcal)	150.48	131.98	135.49	131.85	129.34

Fig. 8: Carcass composition (% dry matter basis) of catla fry under various diet treatments



5.5 TISSUE BIOCHEMICAL COMPONENTS

The effect of diet treatment on certain tissue biochemical contents are presented in table-12 and shown in fig. 9,10 and 11. No significant change was observed in the protein content except in case of formulated diet treatment groups. The muscle RNA,DNA and protein content followed the similar trend as that of growth. Values of RNA:DNA, protein: RNA, protein: DNA ratios from the table shows that the RNA:DNA was slightly higher (4.5) in the diet (D_4) treated groups and protein: RNA ratio was the minimum of all treatment groups.

Table 12 Effect of various dietary treatments on the content of some biochemical indices in muscle tissue of *Catla catla*

Treatment diets.	Biochemical Parameters in muscle tissue					
	RNA (mg/g)	DNA (mg/g)	Protein (mg/g)	RNA:DNA	Protein : RNA	Protein :DNA
D_1	1.007 ±0.219	0.291 ±0.005	120.70 ±5.44	3.46	119.86	414.77
D_2	1.294 ±0.160	0.376 ±0.041	117.83 ±3.04	3.44	91.03	313.30
D_3	1.124 ±0.116	0.365 ±0.029	112.57 ±2.70	3.08	100.18	308.49
D_4	1.541 ±0.128	0.382 ±0.017	119.00 ±5.74	4.05	77.27	313.16
D_5	1.042 ±0.139	0.296 ±0.017	86.70 ±3.63	3.51	86.53	304.05

Fig.9: Muscle RNA:DNA of catla fry under various dietary treatments

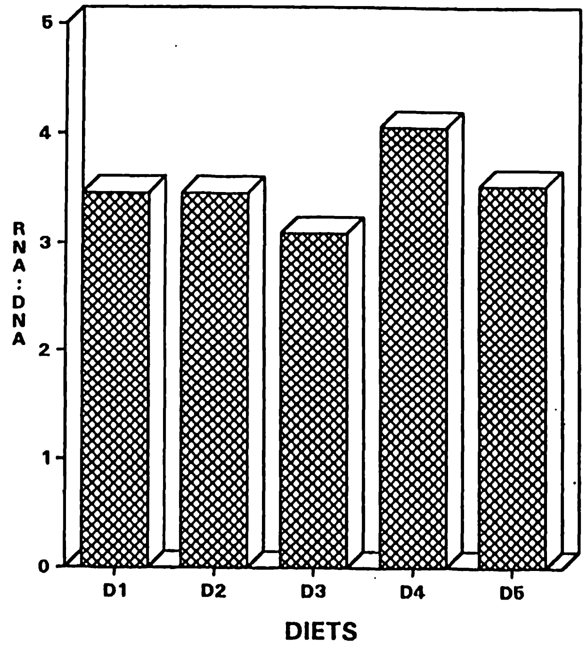


Fig. 10: Muscle protein : RNA of catla fry under various dietary treatments

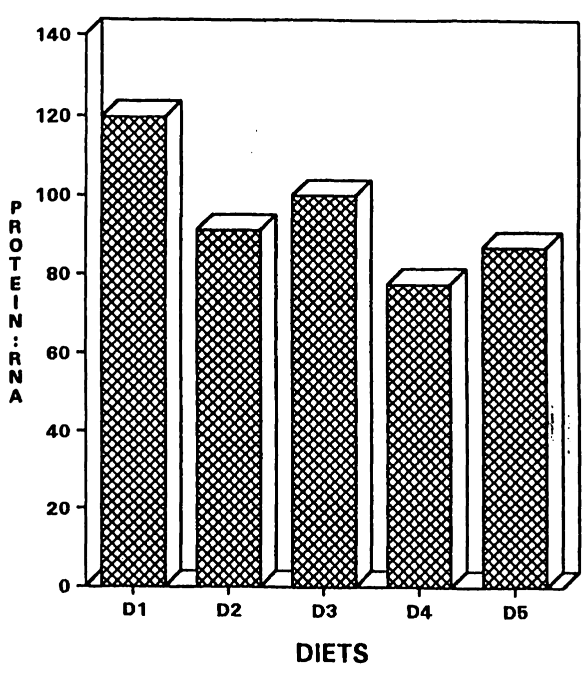


Fig. 11: Muscle protein : DNA of catla fry under various dietary treatments

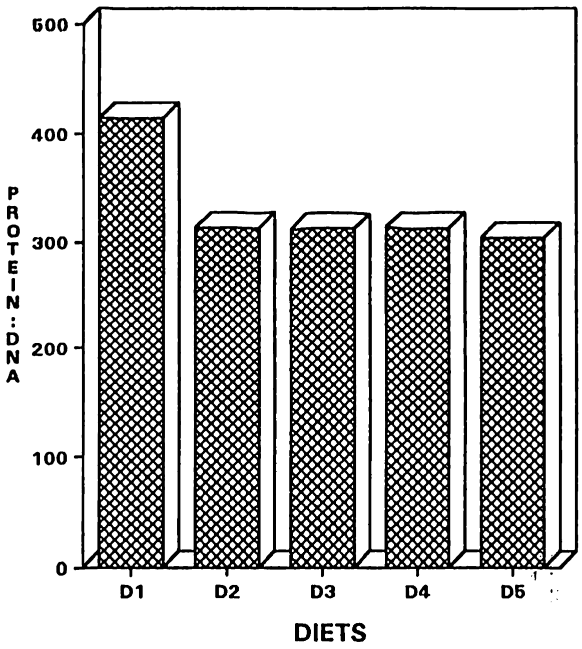
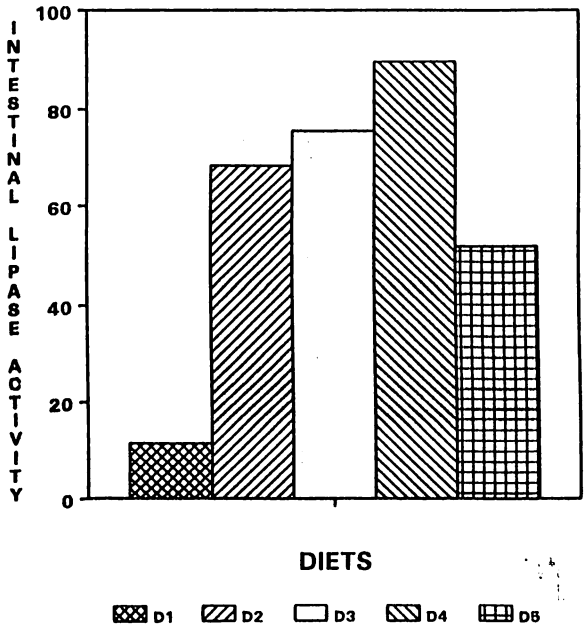


Fig. 12: Intestinal Lipase activity (µg α -napthol liberated/mg protein/hr) of catla fry under various dietary treatments



Intestinal lipase activity was significantly high in fishes treated with diet D_4 (mixed lipid diet), followed by diet D_3 (diet with fish oil only), diet-D_2 (diet with vegetable oil only) and diet-D_5 (the formulated diet) (Fig. 12). The values are 89.72, 75.53, 68.41, 51.59, 11.40 (μg α-napthal liberated/mg protein/ hr) respectively.

The fatty acid profile of the whole body tissue of fishes from different diet treatment are summarising in table-13 and 14. Of all the fatty acids analysed the following 18:2ω-6, 18:3ω-6, 20:5ω-3 22:6ω-3 were considered in particular. The fatty acid composition clearly reflected to that of the dietary lipids.

5.6 DATA ANALYSIS

Analysis of variance and Duncan's new multiple range test (SAS Institute, 1986 a, b) were used to determine difference in survival, weight gain, feed efficiency etc and proximate body composition of fish fed the experimental diets and also proximate composition of diets. The data have been presented as Mean ± S.D. The two way ANAOVA was used to identify significant differences of final weight again between treatments (Table-15).

Table 13 Fatty acid composition of whole-body tissue of Catla fry fed different sources of lipid in the diet.

Fatty acids	Treatment Diets									
	D_1		D_2		D_3		D_4		D_5	
	Peak Area	% of Fatty acid	Peak Area	% of Fatty acid	Peak Area	% of Fatty acid	Peak Area	% of Fatty acid	Peak Area	% of Fatty acid
14:0	4.58	7.97	4.56	6.52	4.58	5.496	4.58	5.40	4.62	8.81
15:0	0.27	0.47	0.27	0.38	0.24	0.29	0.48	0.57	0.48	0.91
16:0	4.00	6.96	3.18	4.55	4.54	5.54	4.52	5.33	5.31	10.13
16:1	2.22	3.86	1.04	1.49	2.15	2.58	3.64	4.29	1.17	2.23
18:0	-	-	0.24	0.34	1.40	1.68	2.16	2.55	8.76	16.71
18:1ω6	0.24	0.42	0.98	1.40	7.96	9.55	9.04	10.67	2.96	5.65
18:2ω6	1.12	1.95	4.14	5.92	0.95	1.14	1.96	2.31	1.76	3.36
18:3ω3	4.92	8.56	6.84	9.78	10.65	12.78	5.04	5.95	5.36	10.23
20:2ω6	0.88	1.53	3.50	5.00	6.10	7.32	5.68	6.70	3.60	6.87
20:3ω6	2.88	5.01	6.38	9.12	5.46	6.55	6.96	8.21	3.66	6.98
20:4ω6	1.76	3.06	6.46	9.24	7.20	8.64	5.40	6.37	4.20	8.01
20:5ω3	4.25	7.39	6.76	9.67	4.76	5.71	5.60	6.61	3.15	6.01
22:4ω6	3.92	6.82	6.10	8.72	4.75	5.70	6.80	8.02	0.96	1.83
22:5ω6	8.00	13.92	7.00	10.01	5.40	6.48	7.84	9.25	2.40	4.58
22:5ω3	7.20	12.53	6.30	9.01	7.60	9.12	4.44	5.24	0.78	1.49
22:6ω3	11.25	19.57	6.20	8.87	9.40	11.28	10.60	12.51	3.23	6.16

Table 14 Selected essential fatty acid composition of whole body tissue of Catla fry fed different source of lipid in the diet.

Fatty acids	Treatment Diets				
	D_1	D_2	D_3	D_4	D_5
18:2ω6	1.12	4.14	0.95	2.31	3.36
18:3ω3	4.92	6.84	10.65	5.95	10.23
20:5ω3	4.25	6.76	4.76	6.61	6.01
22:6ω3	11.25	6.20	9.40	12.51	6.61

Table -15 Analysis of variance for final average weight (g) of *Catla catla* fry from different dietary treatments.

Source of variation	Degree of Freedom	Sum of Squares	Mean Square	Observed F
Block	4	.382	.095	3.500
Treatments	2	.058	.029	1.060
Error	8	.218	.027	
Total	14	.66		

cv = 16.35%
Standard Error of treatment Mean = .074
Standard Error of Difference of two Means = .104
Critical Difference Value = .241

DISCUSSION

The results of the study confirmed that lipid is essential in the diet of this carp species (*Catla catla*) for growth and well being. It was also evident that the source of the lipids has significant effect on the growth performances. Following a period of eight weeks the highest weight gain and SGR could be achieved by the fish fed with the diet containing a mixture of vegetable oil and fish oil. Of the other diets, vegetable oil containing diet responded better than fish oil alone. Feeding on a lipid free diet resulted in low growth and a perusal of the data on FCR, SGR, PER, ERE revealed that these were not as poor as could be anticipated. It may thus appear that the fish is not very demanding in essential fatty acid requirement during this stage of growth. The highest growth actually occurred in fish given a diet containing a combination fish oil and vegetable oil i.e. a mixture of ω-3 and ω-6 fatty acids. Watanabe *et al.,* (1975a,b) reported that the carp *Cyprinus carpio* requires fatty acids of both linoleic and linolenic families for growth and proved that the essential fatty acid requirement (EFA) of this species was much lower than rainbow trout.

An examination of table 11 giving the proximate composition of the muscle fillet of the catla fry, fed different lipid sources (veg. oil, fish oil and mixture of veg. and fish oil) indicated no appreciable change in moisture, crude protein and lipid content. In lipid-free diet, however, tissue lipid level decreased significantly accompanied by increase in the protein and moisture content. The significant decrease in the lipid content in the fish under lipid free diet may indicate that, dietary lipid rather than carbohydrate forms an important energy source for the species.

The level of certain biochemical indices like RNA, DNA and protein in the liver tissue of ,catla fry under various dietary regime showed only incidental fluctuations, however slightly differential response to the higher side in regard to RNA: DNA (4.5) in tissues of fish which grew best under the diet treatment (D4) may indicate appropriate protein synthesis and energy storage for growth purpose. This might also be related to the protein sparing effect of lipid in this fish. Increased RNA: DNA corresponding to growth increment in fish species is well known (Lone and Matty, 1980; Kesavanath *et al.,* 1991).

The activity of the intestinal lipase was the least in fish maintained on lipid-free diet. This is what could be anticipated. A comparison of the lipase activity in fish from three treatment groups is e.g. vegetable oil treatment (D-2), fish oil treatment (D-3) and combination of vegetable and fish oil treatment, (D-4) indicated not much variation between vegetable oil and fish oil treatment groups. The D-4 group had, however, increased lipase activity in the intestine, Kawai and Ikeda (1972) and Das and Tripathy (1991) showed that the activity of the digestive enzymes in carp depend on the amount of substrate material in the diet. In this regard *Catta catla* which ingested a mixed lipid diet in this experiment had higher lipase activity than the groups fed either vegetable oil or fish oil only. Such superior response might indicate the effectiveness of the supplement containing a mixture of ω-3 and ω-6 fatty acids for this species in contrast to ω_{-3} and ω_{-6} fatty acids separately.

The Chromatographic record of methyl esters of the lipid showing fatty acid profile of the whole body tissue of fishes from different diet treatments showed that, the groups of fish fed lipid-free diet had decreased level of both w-3 and w-6 fatty acids indicating their utilisation of endogenous reserve resulting in EFA deficiencies; Increased level of 14:0, 15:0, 16:0, 16:1 suggest that the fish is capable of de-novo synthesis of these fatty acids as in many other species (Watanabe, 1975,, Satoh *et al.*, 1984, Olsen *et al.*, 1990, Takeuchi *et al.*, 1991,) In regard to tissue fatty acid composition of fish from vegetable oil diet treatment (D-2) fish oil diet treatment (D-3) and mixture of vegetable oil and fish oil diet treatment (D4) respectively, the composition of the fatty acid profile of wholebody tissue was influenced by the fatty acid profile of the experimental diets. For example 18: 2 ω-6 was the highest in D-3 treatment. 22:5 ω-3 and 22:6 ω-3 were also highest in D-3. D-4 treatment groups however had a fairly balanced composition of 18:2 ω-6, 18:3ω-3, 22:5ω-3 and 22:6ω-3 thus showing that this carp species require fatty acids of both the 18:2ω-6 and 18:3ω-3 families for growth.

CONCLUSION

In this experiment fry of *Catla catla* maintained on a lipid-free diet for eight weeks did not develop any morphological fatty acid deficiency symptom, although the growth was the lowest accompanied by similar nutritional responses. The result thus might indicate comparatively low EFA requirement of carp than many other fish species because this fish can grow without abnormality for a fairly long period with a diet sans fatty acid. From the data on growth, biochemical compositions, including the activity of intestinal lipase and tissue fatty acid profile, it could be found that the quality of the lipid source in the diet is more important than its quantity, particularly in view of organoleptic properties of the flesh and in affecting consumer preference. The tissue composition also reflects the dietary fatty acid composition.

The results of growth, feed efficiency, tissue fatty acid composition support a conclusion that instead of having ω-6 fatty acids (for example from sunflower oil) or ω-3 fatty acids (for example from cod liver oil) in the diet, the mixture of ω-6 and ω-3 provides a better dietary oil supplement for achieving optimum performance of this species under controlled culture system.

It remains to be seen on the basis of further studies that the positive role played by individual ω-3 and ω-6 fatty acids on growth of this fish species particularly when it is found in this experiment, that the fish requires both ω-6 and ω-3 fatty acids Such information on exact quantitative requirement of ω-3 and ω-6 fatty acids by this species and two other related species, rohu and mrigal may ensure maximal growth and deposition of such important fatty acids in fish lipids for the health benefit of the consumers.

In view of the fact that this species can survive and grow for a fairly long period with little EFA in the diet and because of the increasing cost of fish diet ingredients, particularly the lipid sources, the approach of Garcia *et al.*, (1981) and De-silva *et al.*, (1991) with the periods of alternate feeding of lipid-rich diet interrupted by periods of feeding lipid-free diet would enable mobilisation of EFAs in the fish thereby realising lower EFA requirement of fish without raising culture operation cost.

BIBLIOGRAPHY

Anderson, A.J., and Arthington, A.H. (1989). Effect of dietary lipid on the fatty acid composition of silver perch. (*Leiopotherapon bidyanus)*. Lipids. *Comp. Biochem. Physiol. 93B(3)*: 715-720.

Bandyopadhyay, G. Dutta, J. and Ghosh, S. (1982). Evidence for inability of the catfish, *Heteropneustrs fossilis* and *Clarias batrachus* to biosynthesis linoeic and linolenic acid. *Indian J. Biochem. Biophys*. **19**: 253 -256

Belsare, S.D. and Belsare, D.K. (1990) Lipid content of major carps under different feed trials. *Proc. Natl. Acad Sci (India)*. **60 (Part III)**: Sec.B: 233-227.

Bligh, E.G. and Dyer W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**: 911-917.

Brown, A.H. (1946). Determination of pentose in the presence of large quantities of glucose. *Arch. Biochem.*11: 269-278.

Castell,J.D., Sinnhuber,R.O., Wales,J.H. and Lee D.J. (1972a). Essential fatty acids in the diet of rainbow trout *(Salmo gairdneri)*. Growth, feed conversion and some gross deficiency symptoms. *J. Nutr.* **102**: 77-86.

Castell, J.D., Lee, D.J. and Sinnhuber, R.O. (1972c). Essential fatty acids in the diet of rainbow trout *(Salmo gairdeneri)* lipid metabolism and fatty acid composition. *J. Nutr.* **102**: 93-100.

Castell,J.D.,Sinnhuber R.O., Lee,D.J. and Wales, J.H. (1972b). Essential fatty acids in the diet of rainbow trout (*Salmo gairndneri*): Physiological symptoms of EFA deficiency. *J. Nutr.* **102**: 87-92.

Castell, L.D., Lee, D.J. and Sinnhuber, R.O. (1972) Essential fatty acids in the diet of rainbow trout *(Salmo gairdneri*) lipid metabolism and fatty acid composition. *J. Nutr.* **102**: 93-100.

Cho, C.Y., Cowey, C.B., and Watanabe, T. 1985. Finfish nutrition in Asia: Methodological approaches to research and development. *International Development Research Centre, Publication. Ottawa.* Canada.

Christie, W.W. (1982). Lipid analysis. Pergaman. Press, Oxford, UK.

Cowey, C.B. and Sargent, J.R. (1977). Lipid nutrition in fish. *Comp. Biochem. Physiol.* **57B**: 269-273.

Csengeri, I., Farkas, T., Majoros, F., Olah, J., Szalay, M. (1978). Effect of feeds on the fatty acid composition of carp (*Cyprinus carpio* - L). *Aquacultura. Hungarica*. **1**: 24-34.

Das, K.M., S.N. Mohanty, S. Sarkar (1991). Optimum dietary protein to energy ratio for Labeo rohita fingerlings, pp. 69-73. in S.S. De Silva (ed). Fish Nutrition research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop. *Asian Fish Soc. Spec. Publ.* 5, 205 p. Asian Fisheries society Manila, Phillippines.

Das, K.M. and Tripathi, S.D. (1991). Studies on the degestive enzymes of grass carp. *Ctenopharyngodon idella* (Val) *Aquaculture* **92**: 21-32.

Dehadriai, P.V.(1992) Aquaculture development in India. Government policies. Lecture presented at the Summer Institute on Aquaculture and Environment. Central Institute of Fisheries Education, Bombay (Ms.).

Desilva S.S. and F.B. Davy. (1992). Strategies for finish nutrition research for semi-intensive aquaculture in Asia. *Asian Fisheries Science* **5**: 129-144.

Greene, D.H.S. and Daniel P. Selivonchick. (1987) Lipid Metabolism in fish *Prog. Lipid Res.* **26**. pp 53-85.

Dupree, H.K., Gauglitz.JR, E.J., Hall. A.S and Houle, C.R. (1979). Effects at dietary lipids on the growth and acceptability (flavour) of channel catfish (*Ictalurus punctatus*) *Proc. world symp on finfish nutrition and fish feed tech.* Hamburg. 20-23 June.

El, Sayed, M.M., Ezzat, A.A., Kandeel, K.M. and Shaban, F.A. 1984. Biochemical studies on the lipid content of *Tilapia nilotica* and *Sparus aunatus. Comp. Biochem. Physiol.* **79B**: 589-594.

Garcia, M., Zamona S. and Lopez M.A. (1981). The influence of partial replacement of protein by fat in the diet on protein utilization by rainbow trout (*Salmo gairdnerii*). *Comp, Bio-chem. Physiol.* **68 B**: 457-460.

Giles, K.W., and A. Myers. (1965). An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature* **206**: 93.

Halver, J.E.(1989). *Fish nutrition*. Second Edition by Academic Press, Inc. San Diego, California.

Hanley,F., (1991) Effects of feeding supplementary diets containing varying levels at lipid on growth, food conversion, and body composition at Nile tilapia, *Oreochromis niloticus (L). Aquaculture* **93**: 323-334.

Hardy, R.W. (1989). Diet Preparation. In:*Fish nutrition*, second edition, edited by Halver, J.E. (1989). Academic Press Inc. San Diego, Califarnia.

Henderson, R.J. and Tocher, D.R. (1987). The lipid composition and biochemistry of freshwater fish *Prog.Lipid Res*. **26**: 281-347.

Higashi, H., Kaneko, T., Ushiyama. M. and Sugi hashi, T. (1964) Effects of dietary lipids on fish under cultiuatron II. Effect of ethyl linoleate, linolenate and ethyl esters of polyunsaturated fatty acids on deficiency of essential fatty acids in rainbow trout. *Bull. Jap. Soc Sci fish*. **30**: 778-783.

Higashi, H., Maneko, T., Ishii, S. and Sugihasi, T. (1966). Effect of ethyl linoleate, ethyl linolenate and ethyl esters of highly unsaturanted fatty acids on essential fatty acids deficiency in rainbow trout *J. Vitaminol.* **12**: 74-79.

Kanazawa, A., Teshima, S.T., Sakamoto, M. and Awal, M.A. (1980). Requirement of *Tilapia zilli* for essential fatty acids. *Bull. Jap. Soc. Sci. Fish.* **46**: 1353-1356.

Kawai, S. and Ikeda, S., (1972). Studies on digestive enzymes of fishes. II. Effect of dietary change on the activities of digestive enzymes in carp intestine. *Bull. Jap. Soc. Sci. Fish.*, **38**: 265-270.

Keshavanath, P., S.Shyama, M.C. Nandeesha and T.J. Varghese (1991). Influence of virginiamycin on growth and body composition of rohu (*Labeo rohita*) and common carp (*Cyprinus carprio*). p. 193-200. In S.S.

De Silva (ed). Fish nutrition research in Asia. *Proceedings of the Fourth Asian Fish Nutrition Workshop Asian Fish Soc. Speec. Publ. 5, 205 p. Asian Fisheries Society. Manila. Philippines.*

Kheyyali, D.; S. Shimeno and M. Tadeda. Effect of dietary carbohydrate and lipid levels on hepatopancreatic enzymes and body composition in carp. *Proc. Third Int. Symp. on feeding and nutrition in Fish*. Toba Aug. 28-Sept. 1. Japan, 1989. pp.451-460.

Lee, D.J., J.N. Roehm, T.C. Yu, and R.O. Sinnhuber (1967) Effects of W 3 fatty acids on the growth rate of rainbow trout *(Salmo gairdneri)* *J. Nutr.* **92**: 93.

Liu. Y (1992) Analysis at fatty acid composition of nine fresh water fishes in China. *Fish Aquat Sci No* **5569** pp 9.

Lone, K.p. and A.J. Matty, (1980). The effect of feeding testoterone on the growth and body composition of common carp (*Cyprinus carpio L.*). *Gen. Comp. Endocrinol.* **40**: 409-424.

Lowry, O.H., Rosebrough, N.J. Farr, A.L. and Randall. R.J., 1951. Protein measurement with Folin phenol reagent *J. Biol. Chem.* **193**: 265-275.

Mukhopadhyay, P.K., K.M., Das and S.N. Mohanty (1991). Freshwater fin fish and shellfish nutrition and diet development studies in India (Proc. *National Workshop* on *Animal Biotechnology* (Eds. Padmanaban, V.D. and Devraj, M). pp 6-14, published by Tamil Nadu Veterinary & Animal Sciences University, Madras.

Nicoloides, N. and Woodan, A.H. (1962). Impaired pigmentation in chinook salmon fed diets deficient in essential fatty acids. *J. Nutr,* **78**: 481-537.

Olsen, R.E., Henderson, R.J. and McAndrew, B.J. (1990). The conversion at linoleic and linolenic acid to longer chain polyunsatarated fatty acids by Tilapia *(Oreochromis)* nilotica in vivo. *Fish Physiol. Biochem.* **8**: 261-270.

Qazi, R., Iqbal. Q.J., Zoheer, K. (1990). Total Lipids and fatty acids in the livers of some freshwater fishes. Pak. *J. Zool.* Vol.**22(1)**: pp. 15-22.

Ramachandra Nair, K.G. and Gopakumar, K. (1981). Effect of dietary fat on deposition of fat and fatty acid composition of *Tilapia mossambicus*. *J. Food Sci. Technol.* **18**: 108-111.

Sampekalo, J., Takeuchi, T., Watanabe, T. (1992). Comparison of gill lipids between freshwater fish. *J. Tokyo. Univ. Fish.* Tokyo, suisandai kempo. 79(1) :71-76.

Sargent, J. R., Henderson, R.J. and Tocher, D.R. (1989). The Lipids. In:*Fish Nutrition*. (Edited by J.E.Halver). Academic press, NewYork. pp 153-218.

Satoh, S., Takeuchi, T. and Watanabe, T. (1984). Effects of stravation and environmental temperature on proximate and fatty acid and compositions of *Tilapia nilotica. Bull. Jap. Soc. Sci. Fish* **50**: 79-84.

Satoh, S., Poe, W.E. and Wilson. R.P. (1989). Effect of dietary ω-3 fatty acids on weight gain and liver polar lipid fatty acid composition of fingerling channel catfish *J. Nutr.* **119**: 23-28.

Schwalme, K. (1992). A quantitative comparison between diet and body fatty acid composition in wild northern pike (*Esox Laeins* L.) . *Fish physiol Biochem*: **10(2)**: 91-98.

Seligman, A.M. and M.M. Nachlas (1963). Lipase: In "Methods of Enzymatic Analysis" (Bergmeyer, H.U. ed). pp. 776-778: Academic Press. London.

Stirling, H.P. (1985). Chemical and Biological methods of water analysis for Aquaculturists *Inst. of Aquacult. Univ. of Stirlirg*, FK9 4LA Scotland. pp 89-91.

Strickland, J.D.H. and Parsons, T.R. (1972). *Apractical hand book of Sea-water analysis*. Bulletin No.167, 2nd edition, Ottawa, Fisheries Research Board of Canada, pp.310.

Tacon, A.G.J. (1987). The nutrition and feeding of farmed fish and shrimp- A training mannual.1. The essential nutrients. FAO Field Document, Project GCP/RLA/075/ITA; Field Document No. 2/E, Brasilia, Brazil, 117pp.

Tacon. A.J.J. (1987a) The nutrition and feeding at farmed fish and shrimp-A training mannual.2. Nutrient sources and composition. FAO Field Document. Project GCP/RLA/075/ITA, Field Document No. 5/E Brasilia, Brazil, 129pp.

Takechi, T., Satoh, S. and Watanabe, T. (1983). Requirement of *Tilapia nilotica* for essential fatty acids. *Bull. Jap. Soc. Sci. Fish*. **49**: 1127-1134.

Takeuchi,T., Watanabe,K. Yong, W.K. and watanabe, T. 1991. Essential fatty acid requirement of grass carp *(Ctenopharyngodon idella) Nippon Suisan Gakkaishi* :(Formerly *Bull Jap.Soc.Sci.Fish*).**57(3)** 467 -473.

Takeuchi, T. and Watanabe, T. (1977). Dietary levels of methyl laurate and essential fatty acid requirements of rainbow trout. *Bull. Jap. Soc. Sci. Fish.* **43**: 893-898.

Takeuchi, T., Arai, S., Watanabe, T. and Shimma, Y. (1980). Requirement of eel, *Anguilla japonica* for essential fatty acids. *Bull. Jap Soc. Sci. Fish.* **46**: 345-353.

Takeuchi, T., Watanabe, T. and Nose. T. (1979). Requirement for essential fatty acids of chum salmon *(Oncorhynchus keta)* in freshwater environment. *Bull Jap. Soc. Sci. Fish.* **45**: 1319-1323.

Takeuchi, T. and Watanabe, T. (1982). Effects of various polyunsatur ated fatty acids on growth and fatty acid compositions of rainbow trout *(Salmo gairdnerii)*, Coho salmon *(Onchorhynchus kisutch)* and Chum salmon *(O. keta)*. *Bull. Jap. Soc. Sci. Fish.* **48**: 201-204.

Tesima, S.I.; Kanazawa, A. and Sakimoto, M. 1982. Essential fatty acids of *Tilapia nilotica*. *Mem. Fac Fish., Kagashima University* **31** 201-204.

Teskeredzic, Z., E. Teskeredzic and M. Tomec (1989). Theinfluence of four different kinds of Oil upon the growth of rainbow Trout.*(Salmo gairdnerii)*. Proc. *Third Int. Symp. On Feeding and Nutrition in Fish*. Toba Aug.28- Sept-1. Japan. 1989 pp. 245-250.

Viola, S. and Arieli. Y (1983). Nutrition studies with tilapia hybrids 2. The effects of oil supplements to practical diets for intensive aquaculture. *Bamidgeh*. **35**: 44-52.

Viola, S. and Arieli, Y. (1990) Effects of ω-3 fatty acid supplementation on storage stability and taste of carp and tilapia. *Israeli J. of Aquacult. Bamidgeh*. **42(2)**: 56-57.

Watanabe, T. Utsue, P., Kobayashi, I. and Ogino, C. (1975 a). Effect of dietary methyl linoleate and linolenate on growth of Carp-I. *Bull Jap. Soc. Sci. Fish*. **41(2)**: 257-262.

Watanabe, T., Takeuchi, T., and Ogino, C. (1975b). Effect of dietary methyl linoleate and linolenate on growth of Carp- II. *Bull. Jap. Soc. Sci. Fish.* **41(2)**: 263-269.

Watanabe, T., Takeuchi, T. and Ogino, C. (1975) Effect of dietary methyl linoleate and linolenate on growth at carp. II *Bull. Jap. Soc. Sci. Fish* **41**: 263 - 269.

Watanabe, T., Ogino.C., Koshiishi, P. and Matsunaga,T.(1974) Requirement of rainbow trout for essential fatty acids. *Bull Jap. Soc Sci. fish.* **40**: 493 - 499.

Watanabe, T., Takashima, F. and Ogino, C. (1974) Effect of dietary methyl linolenate on growth of rainbow trout. *Bull. Jap. Soc.Sci. Fish.* **40**: 181 -188.

Watanabe, T., Kobayashi, I., Utrie, O. and Ogino, C. (1974b) Effect at dietary linolenate on fatty acid composition of lipids in rainbow trout, *Bull. Jap, Soc, Sci. fish*. **40**: 387 - 392.

Watanabe. T. (1982). Lipid nutrition in fish, *Comp. Biochem. Physiol.* **73B**: 3-15.

Yu, T.C., Sinnhuber, R.O. and Hendreks, J.D. (1982). Reproduction and survival of rainbow trout *(Salmo gairdneri)* fed linolenic acid as the only source of essential fatty acids. *Lipids* **14**: 572-579.

Yu, T.C and sinnhuber, R.O. (1979) Effects of dietary $\omega3$ and and $\omega6$ fatty acids on growth and feed conversion efficiency of coho salmon *Oncorhynchus kisutch. Aquaculture* **16**: 31-38.

APPENDIX

Plate-1 A view of the CIFA farm - source of experimental fish species.

Plate-2 Feeding the carp in culture pond

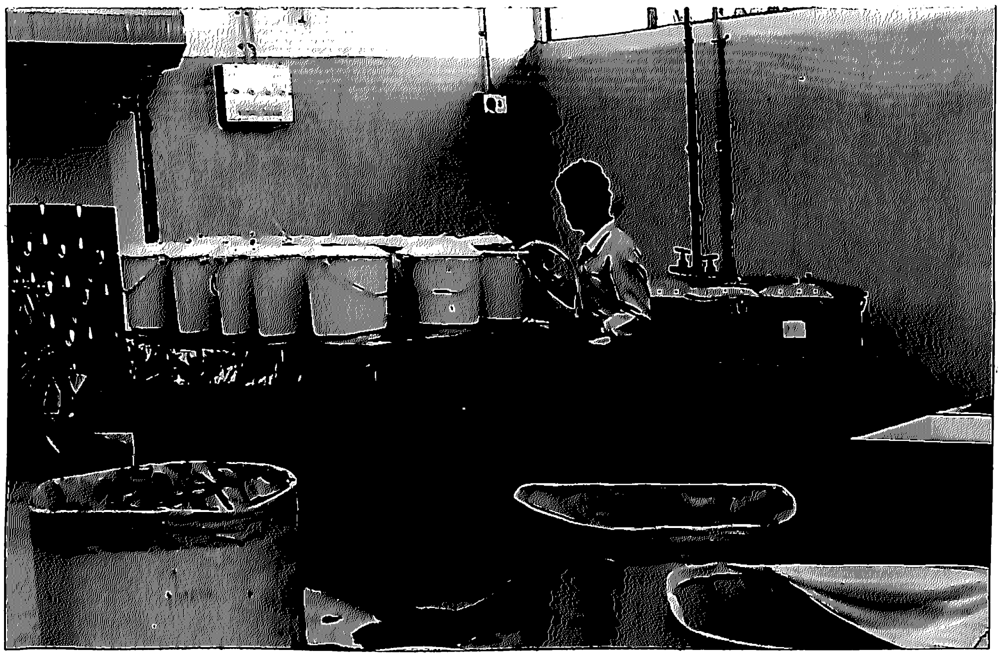


Plate-3 Experimental maintenance in wet-laboratory of CIFA.

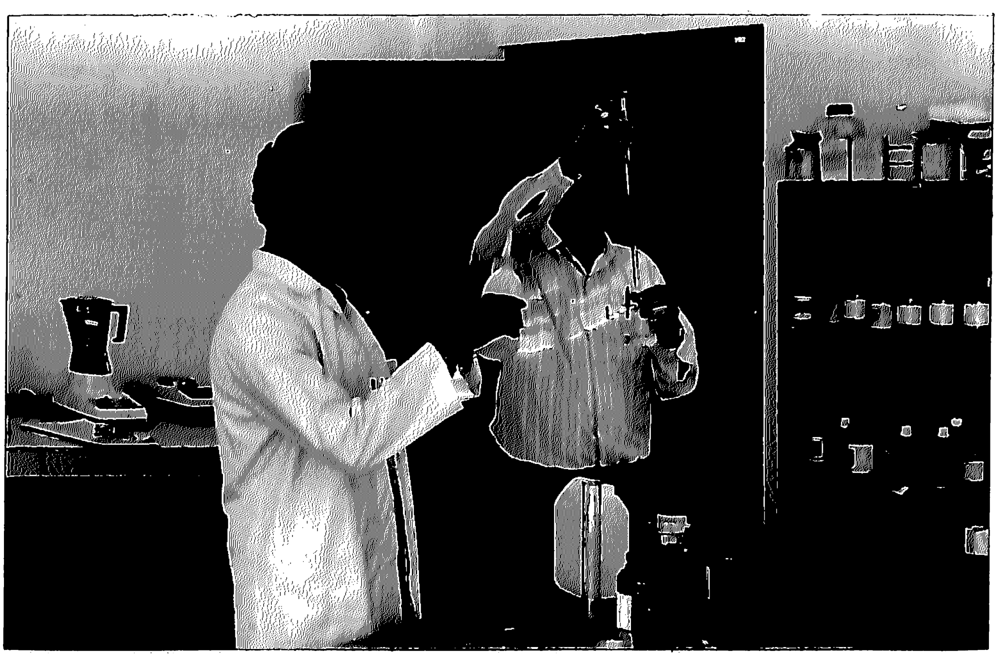


Plate-4 Biochemical analysis of the experimental fish samples in the nutrition laboratory.

Plate-5 The spectrophotometer for use in analysis of biochemical parameters.

Plate-6 Soxtech system used for lipid analysis.

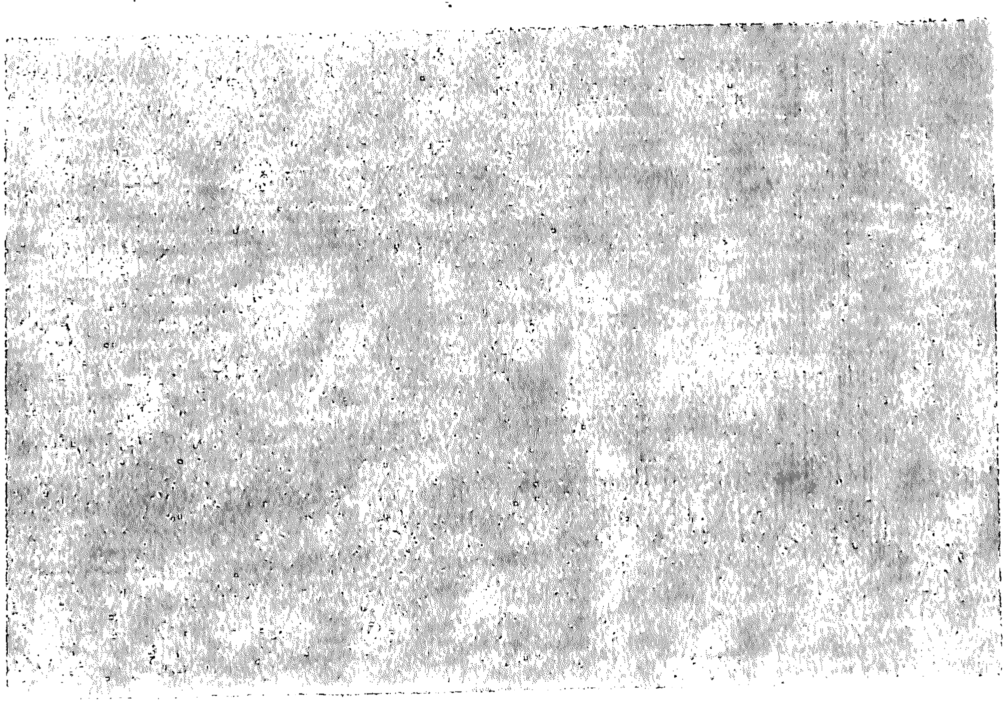


Plate 8 Chrom[illegible]

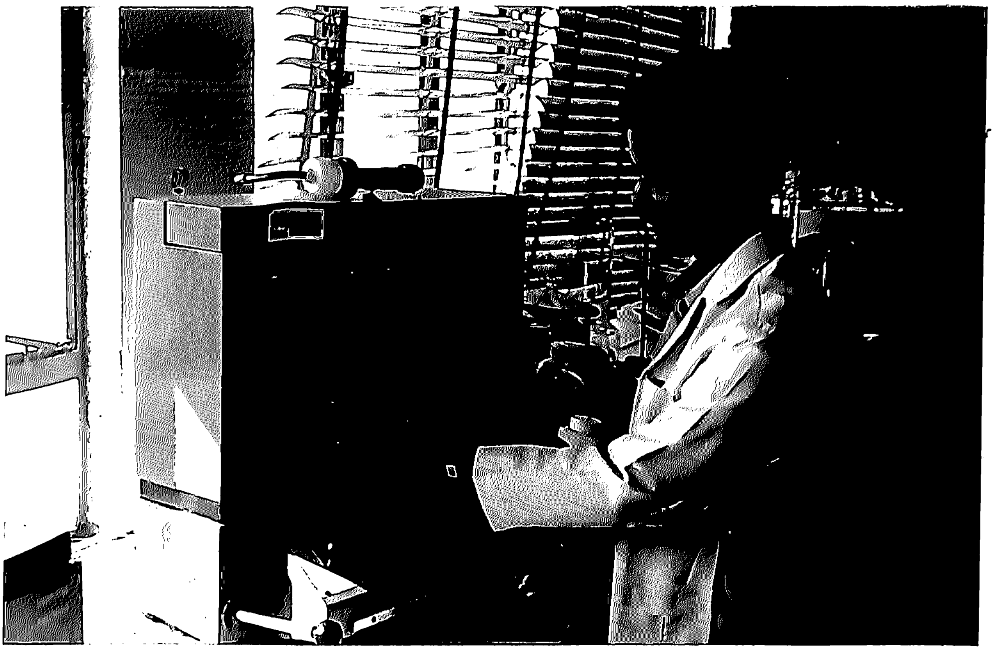


Plate-7 Fibreteeh system used to analyse fibre content.

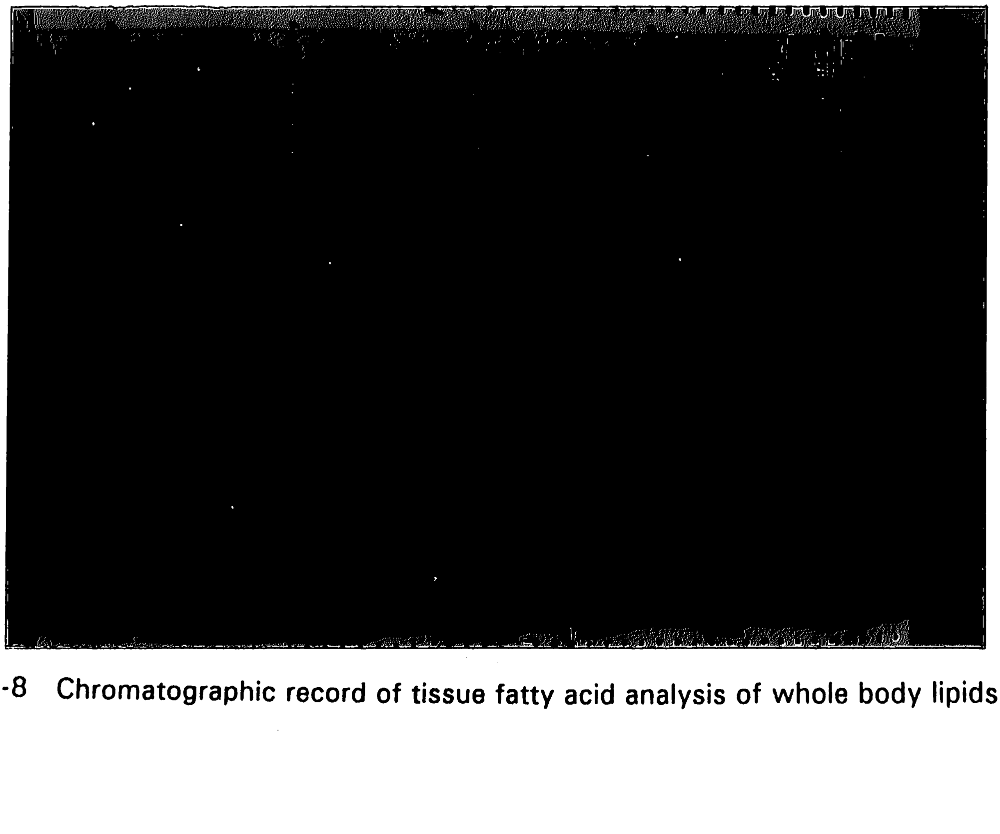


Plate-8 Chromatographic record of tissue fatty acid analysis of whole body lipids from carp.