NUTRITIONAL REQUIREMENTS OF THE FRY OF GOLD-SPOT MULLET *LIZA PARSIA* (HAMILTON)

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AND TECHNOLOGY

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MAY 1989

CERTIFICATE

This is to certify that the thesis entitled **"NUTRITIONAL REQUIREMENTS OF THE FRY OF GOLD-SPOT MULLET LIZA PARSIA (HAMILTON)"** is the bonafide record of the work carried out by **KIRON VISWANATH** under my guidance and supervision and that no part thereof has been presented for any other Degree.

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DECLARATION

I hereby declare that this thesis entitled "NUTRITIONAL REQUIREMENTS OF THE FRY OF GOLD-SPOT MULLET LIZA PARSIA (HAMILTON)" has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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May, 1989.

(KIRON VISWANATH)

C O N T E N T S

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PREFACE

The food generating systems on the planet earth are the lone source of energy for sustenance of the human race. With the exploitation of terrestrial sources near maximum, man has turned his attention to the food resources of the aquatic environment. The potential of the world oceans to feed man has been portrayed in poetic terms since the dawn of time, with impressive promises including reports of huge krill sources in Antartic waters and offshore fishery stocks. With the expectation that sooner or later capture fisheries will level off, fluctuate or even decline, the obvious alternative source of production to fill world-wide increasing needs of fisheries products would be that of aquaculture. Indeed, the legendary promise of the oceans to feed all of human kind may yet be fulfilled, but if it is, it will most likely be via aquaculture.

Husbandry of aquatic organisms has been in practice for several centuries. On an ever-increasing scale, man has been supplementing the hunting yield from the seas, lakes and rivers by culturing the plants and animals of these waters. The techniques have been improved and have reached such perfections that today we have computer aided aquafarms in developed countries. The momentum aquaculture has gained throughout the world during the recent times is probably unparalleled in other branches of food production.

The last decade has witnessed the art of pisciculture being oriented in scientific lines in different parts of the world. The over-dependence on shellfishes for aquaculture is fast relegating fish culture to a lower slot, especially in maritime nations. Nevertheless, finfishes still contribute a major share (60.6%) to the world aquaculture production of 10.6 million metric tonnes (FAO., cited by Rhodes, 1988). An average growth rate of 7.5% per year would mean a world aquaculture production of about 22 million tons by the year 2000; which would be about 20% of the total world fisheries production (Rabanal, 1987).

In India freshwater fish culture has come a long way with the major and minor carps constituting the largest group. Brackishwater aquaculture is relatively a new area and the farmed fishes include the mullets, cichlids and milkfish. Mullets are euryhaline in nature and widely distributed in seas and estuaries of the tropics and sub-tropics. As a most suitable group for large scale fish farming in brackishwater impoundments (Gopalakrishnan and Ghosh, 1976), mullets have been increasingly used in systems of monoculture, mixed culture and polyculture (Bardach et al., 1972). Grey mullets form an important constituent in multiculture stocking practices in China (Lin, 1955), Israel (Pruginin et al., 1975) and in India (Job and Chacko, 1947; Pillay, 1949; Luther 1967).

Important among the 26 species of Indian grey mullets (Day, 1878) are Mugil cephalus (Linnaeus), Liza parsia (Hamilton) and Liza tade (Forskal). Liza parsia - the "gold-spot mullet" hold immense potential as a candidate species for coastal aquaculture.

Most tropical aquatic environments are naturally fertile and their natural fertility is renewed very rapidly. Natural food for many cultivable organisms can be grown to the maximum by proper management. However, enrichment of the environment can be done through rational fertilization. Still further increase in stocking rates, can yield increased crop if adequate feeding is done. Thus fish and shellfish nutrition is an important aspect of the multidisciplinary subject of aquaculture. The oldest and most classical studies in physiology have investigated the nutritional needs of the species of interest to aquaculture. The alimentary requirements for proteins, lipids, mineral salts and vitamins have been established for some temperate species. But, the nutritional requirements of only few tropical species have been studied. Before formulating a diet, a thorough knowledge of the nutrient requirement of the species is essential.

It is against this background that the present area of investigation has been identified. "Nutritional requirements of the fry of gold-spot mullet Liza parsia" is a comprehensive attempt to quantify the nutritional factors that are essential

for producing healthy fingerlings for stocking the farms. Aspects such as the protein and lipid requirements of the fry, the vitamin essentiality, nutritive evaluation of protein and lipid sources suitable for compounding diets were covered in this research project. The ultimate aim has been to evolve practical diets which could be applied in the nursery phase for juvenile production.

The thesis is divided into five parts. Part I forms the general introduction to the study. Part II discusses the common material and methods adopted for the work. Part III is divided into four sections, each dealing with specific aspects covered in the thesis; viz., (1) Dietary protein requirement, (2) Dietary lipid requirement, (3) Dietary vitamin requirement and (4) Nutritive value of protein/lipid sources and the evaluation of compounded diets. In part IV the consolidated summary of the thesis is presented. Part V consists of the references citied in the text followed by two research papers published in the relevant field.

This study has helped in elucidating many of the essential nutrients required for the fry of the mullet. The natural protein and the lipid sources identified and the compounded diets used in the field trial can be applied during the nursery phase of fish rearing.

A C K N O W L E D G E M E N T

I would like to record first my profound gratitude to Dr. R Paulraj, my guide, for accepting me whole heartedly as one of his Research Scholars.

Everything I have accomplished during my years of research, I owe to his grace, goodness and intelligent guidance.

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Dr. E G Silas, former Director and present Vice - Chancellor, Kerala Agricultural University, has always been to me a great source of inspiration by his challenging presence and forthright criticism.

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Mr. K Purushan, Associate Professor, Fisheries Station of Kerala Agricultural University and Dr. L Krishnan of C M F R I provided the animals for this study. I warmly record here my heartfelt thanks to them.

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LIST OF TABLES, FIGURES AND PLATES

T A B L E S

F I G U R E S

P L A T E S

PART I

GENERAL INTRODUCTION

Every animal requires energy for living - growth, maintenance and reproduction - which it must obtain from its food. Each animal starts life with a bit of food received from a parent, but it soon needs to fend itself. It must continue to feed with suitable food regularly or die. The regularity must suit the animal's ability to find and ingest food and to store energy. For most animals feeding is the dominant activity in their entire lives, because their need is constant and food is usually scarce.

The past fifty years have seen the understanding of the food requirements of man and his food animals advance to the stage where comparative nutrition is a recognised activity. With the increasing food demands of an expanding population, man has a growing need to understand the nutrition of those species upon which he feeds.

An understanding of the feeding activity of fish is useful to all who are concerned with any aspect of fisheries. If they want to improve the fish catch they should either develop better baits or learn about their feeding behaviour. Should they want to develop a rational method of exploiting a population they would need to know how food is a limiting factor and how it may be divided among competing animals. Should they culture animals, they would need to study intensively the nutritional requirements

of the animal inorder to obtain the best growth, at the least cost (Royce, 1984).

Fish farming on a global scale still largely depends upon natural food, with some supplementation with the by-products of other forms of agriculture or industry. At low culture densities, these diets are adequate as most of the nutrient requirements of fish are satisfied from natural food. However, at high densities fishes are dependent on artificial feeds, only benefiting little, if at all, from natural food. Thus at higher densities inadequate feeding leads to poor growth, nutritional disease and due to poor fish condition increased susceptibility to parasitic and bacterial infestations.

The production of nutritionally adequate diets for fish requires research quality control and biological evaluation. A well balanced diet, not only results in higher production but also provides the nutrients necessary to hasten recovery from diseases or aid the fish in overcoming the effects of environmental stress. In some cases a good quality diet may slow the progress of idiopathic diseases. Hence, nutritionally balanced and quality controlled diets for fish production are of critical importance.

It is difficult to imagine a time when no information existed about fish feeds. Schaperclaus (1933), Wood (1953), Lin (1959) and Huet (1960) reviewing the art of preparing feeds

showed that early formulae were based on attempts to duplicate the composition of natural foods. In 1927 McCay and Dilley attempted to grow trout fingerlings on various levels of purified protein, fat, carbohydrate and salts supplemented with known vitamins. Other early workers (Titcomb et al.,1928; Agersborg, 1934; Mc Laren et al., 1946; all cited by Halver, 1972) raised trout and salmon on mixtures of fish meal, vegetable oil meals, frozen liver and brewers yeast. John Halver pioneered the design and application of a test diet (Halver, 1957) and this was an impetus for the vital trend towards defined diets in fish nutrition. The first successful dry concentrate feed of known formulation was used by Phillips et al. (1964) for raising trout. Since then studies on nutritional requirements of fishes and shellfishes have been conducted with accelerated frequency. Such studies aid in feed formulation and manufacture. As a consequence of this basic and applied research on fish and shellfish nutrition, modern feeds are more nutritionally complete and more readily available than was true a few years ago.

In the past two decades there has been tremendous increase in research reports, with information on the nutrient requirements of fishes. The works of Halver (1972) as well as Cowey and Sargent (1972) dealt with macro and micronutrient requirements of fishes, along with known and proposed biochemical pathways in fishes, feed formulation strategy and feeding aspects of fish

culture. Since then much additional information has been gained on fish nutrition. Synopses have been compiled on chemical composition of feed ingredients commonly used in formulated fish feeds, recommendations have been made on test diet ingredient composition for determining the nutrient requirement of fishes and feed formulation strategies (NRC., 1973, 1981, 1983). Brief reviews of qualitative and quantitative nutrient requirements for specific groups of fishes are also available: for trout, salmon and catfish (NRC, 1973: Rumsey, 1978; Halver, 1982); carp (Jauncey, 1982); seabass, grouper and rabbit fish (Kanazawa, 1984) and shrimps and prawns (New, 1976). Cowey and Sargent (1979) reviewed the advances in protein, lipid, amino acid, fatty acid, vitamins and mineral requirement of fishes, since their early work (Cowey and Sargent, 1972). The feeding of the captive fishes in aquarium systems has been discussed by Cowey (1981). In an excellent review Millikin (1982) has delved into the interactions of various macro and micronutrients as related to artifical diet formulation for various life-stages of several species currently reared in large quantities in fish hatcheries. Other similar reviews include those of Rumsey (1977), Ketola (1978) and Stickney (1979). Cowey and Sargent (1977), Ketola (1982) and Watanabe (1982) have contributed outstanding, comprehensive reports on individual nutrients.

The widespread interest and importance of aquaculture nutrition is mirrored by the increasing number of symposia being

organised. The proceedings have been published from time to time and these are valuable documents in this subject. Being mentioned here are the works of Price et al. (1976), Halver and Tiews (1979), Castell et al. (1981) and Cowey et al. (1985). A more practical approach to this vital subject is adopted in the contributions of Lovell (1975), CMFRI (1982), Cho et al. (1985) and New (1987).

The demand and need for prepared aquatic animal feeds have continued to increase through the years. The wide body of published literature bears ample testimony to this. However, information on the requirements of tropical cultivable fishes, more so of the vital juvenile stages is scarce.

Although members of the Mugilidae are widely distributed, much attention has been given to their controlled mass production (Oren, 1981). Most of the available information, related to nutrition, on these commercially important fishes is limited to their natural feeding habits (Pillay, 1950, 1953; Sarojini, 1951, 1954; Bapat and Bal, 1952; Chidambaram and Kuriyan, 1952; Thomson, 1954; Suzuki, 1965; Odum, 1970; Ghosh et al., 1972; Pillay, 1972; Rangaswamy, 1973; Albertini-Berhaut, 1974; Mason and Marais, 1975; Zismann et al.,1975; Blaber, 1976; Chervinski, 1976; Moriarity, 1976; Blaber and Whitfield, 1977; Ching, 1977; DeSilva and Wijeyaratne, 1977; Perera and DeSilva, 1978; and Chan and Chua, 1979). Only a few studies exist on the

formulation of suitable artifical diets (Yashouv and Ben-Shachar, 1967; Vallet et al., 1970; Albertini-Berhaut and Vallet, 1971; Kuo et al., 1973; Ghosh et al., 1975; Nash and Kuo, 1975; Houde et al., 1976; Prasadam and Gopinathan, 1976; DeSilva and Perera, 1976; Bishara, 1978; Roy and Chakrabarti, 1979; Chakrabarti et al., 1984; Radhakrishna, 1984; Rangaswamy, 1984; Roy and Chakrabarti , 1984; Papaparaskeva-Papoutsoglou and Alexis, 1986; Kandasami et al., 1987).

This investigation is perhaps the first systematic attempt in determining the alimentary needs of a cultivable tropical brackishwater fish found in India.

PART II

GENERAL MATERIAL AND METHODS

All the experiments, except the field trial, were conducted in the wet laboratory of the Nutrition Section at the Central Marine Fisheries Research Institute, Cochin.

Experimental Design

Every experiment was carefully designed. The number of treatments varied with the experiment in question; there were three replicates for each treatment. Except for the experimental variables other biotic and abiotic parameters were quite homogeneous. The design permitted an unbiased outflow of data, aiding in successful statistical interpretations.

Experimental Facilities

Circular plastic aquaria (tubs), each having a diameter 54 cm and a height 30 cm, were used to rear the fry during the experiment. The tubs were arranged on wooden racks (Plate I)and the various treatments were randomly allotted.

Compressed oil-free air was supplied to each tub through air stone cubes (25 mm), connected to the main air delivery system by plastic tubing. The air supply was maintained uniformly throughout the experimental period, except while cleaning the aquaria or removing the left-over food/faecal matter.

Full strength sea water, collected from 20-30 m depth in the open sea, off Cochin, was transported to the laboratory in

PLATE 1. EXPERIMENTAL SET UP FOR THE LABORATORY BASED NUTRITION STUDIES.

jerry cans. This sea water was filtered clean using bolting silk, diluted with freshwater to experimental salinity level of 15ppt (Paulraj and Kiron, 1988) and kept in the sea water holding facility which consisted of a series of fibreglass tanks of 700 l. capacity, each equipped with a biological filter for further clarification. For reducing the bacterial load, the water stock was irradiated for 120 min every day using a 125 W U.V. lamp. The used seawater was recycled for two more runs following the procedures mentioned above.

Experimental Fishes

The fry of the mullet Liza parsia (Family: Mugilidae) were collected either from the Marine hatchery of Central Marine Fisheries Research Institute at Narakkal or from the Fisheries station of Kerala Agricultural University at Puduvaipu, both located in the Vypeen Island, off Cochin. The fishes were transported in plastic oxygenated seed transportation bags of 20 l. capacity, each holding over 100 fry in the ambient sea water, to the nutritional research facilities of the Institute at Cochin. Initially, in the laboratory, the fry were introduced into large fibreglass pools, gradually acclimatising them to the experimental salinity. During this transit phase, which lasted for two to three days, they were not fed. Subsequently, they were hand-graded to ensure minimum size/weight variation, and introduced at the rate of 25 fry per tub and fed a starter semi-moist diet to get them used to artificial diets. Feeding was

suspended a day prior to the start of an experiment. The entire acclimation period was fixed as two weeks.

On initiation of an experiment in each aquaria only 20 animals were maintained. The total lengths (in mm) and weight (in mg) were noted for each animal. The animals were weighed on a Mettler electronic balance. The entire procedure of recording these biological data was completed within seconds, giving least stress to the animal. The test diets were applied only from the next day, thus allowing the animals to recoup from the handling stress, if any. During the run of an experiment group-fish weights were recorded at regular intervals to minimise stress on the animals. When each experiment was terminated, individual lengths and weights were recorded.

At the commencement of every experiment, a sample of 20 to 30 fry were collected for proximate analysis to determine the initial body composition.

Experimental Diets

Semi-moist diets (moisture content 30-40%) were used for all the experimental studies as the initial feeding trials had indicated a preference for the same. Standard methods and formulation with suitable modifications were employed for the preparation of the diets. The ingredient composition of the

different diets applied will be described in the respective sections in part - III.

In general, the studies on nutrient requirements were conducted using purified ingredients (Table II). Casein and gelatin were protein sources; dextrin and cellulose constituted the carbohydrates and codliver oil and corn oil formed the lipid source in the artificial diets. The composition of the mineral mixture and vitamin mixture used were same for all experiments, except for the experiments on vitamin requirement. Casein was selected as the protein source as it was available in highly purified form and contains adequate amounts of almost all indispensable amino acids. Gelatin, the other protein source supplements arginine, which is quite low in casein (Halver, 1957; NRC, 1983), besides its function as a binder for the diets (McLaren et al., 1947). Though the utilization of dietary carbohydrates differ with its complexity, dextrin seems to be well utilized by a variety of fishes (NRC, 1983) and has therefore been used in the present study. Lipid sources included were corn oil (linoleic) and cod liver oil (linolenic), which provide both n-6 and n-3 essential fatty acids (Watanabe, 1982).

In the experiment to identify suitable natural ingredient sources of protein, powdered ingredients of both plant and animal origin were used in definite proportions for

TABLE I: QUANTITATIVE DIETARY PROTEIN REQUIREMENTS OF SEVERAL FISH SPECIES

SPECIES	DIETARY PROTEIN SOURCE	% PROTEIN REQUIREMENT	REFERENCE
Oncorhynchus tshwytscha	Casein/Gelatin	55	Delong et al., 1958
Oncorhynchus nerka	Casein/Gelatin	45	Halver et al., 1964
Cyprinus carpio *	Casein	38	Ogino & Saito, 1970
Pleuronectes platessa	Cod muscle	50	Cowey et al., 1972
Ictalurus punctatus	Casein	35	Lovell, 1972
Anguilla japonica *	Casein	44	Nose & Arai, 1973
Chrysophrys aurata *	Casein/Amino acids	38	Sabaut & Luquet,1973
Salmo qairdneri *	Casein	40-45	Zeitoun et al., 1973
Salmo qairdneri *	Fishmeal	40	Satia, 1974
Oncorhynchus kisutch	Casein	40	Zeitoun et al., 1974
Seriola quinqueradiata	Casein	55	Takeda, 1975
Ictalurus punctatus	Whole egg protein	32-36	Garling & Wilson,1976
Chrysophrys major	Casein	55	Yone, 1976
Ctenopharyngodon idella *	Casein	41-43	Dabrowski, 1977
Oreochromis aurea *	Soy/Fishmeal	36	Davis & Stickney,1978
Salmo qairdneri	Fishmeal composite	42	Austreng & Refstie, 1979
Chanos chanos *	Casein	40	Lim et al., 1979
Tilapia zilli *	Casein	35	Mazid et al.,1979
Fugu rubripes *	Casein	47(50)	Kanazawa et al.,1980
Micropterus dolomieui *	Fishmeal/Gelatin/ Amino acids	45	Anderson et al., 1981
Micropterus salmoides *	Fishmeal/Gelatin/ Amino acids	40-41	Anderson et al., 1981
Cyprinus carpio	Fishmeal	35	Jauncey, 1981
Oreochromis aurea *	Casein/Egg albumin	56	Winfree & Stickney, 1981
Oreochromis mossambicus *	Fishmeal	42	Jauncey, 1982
Oreochromis nilotica *	Fishmeal	35	Santiago et al.,1982
Channa micropeltes	Fishmeal	52	Wee & Tacon, 1982
Salvelinus alpinus	Fishmeal	36-43	Jobling & Wandsvik,1983
Morone saxatilis *	Fish/Soymeal	49	Millikin, 1983
Oreochromis nilotica *	Fishmeal	28-30	De Silva & Perera,1985
Oreochromis nilotica *	Casein/Gelatin	35	Teshima et al.,1985
Ictalurus punctatus *	Fishmeal	54-55	Winfree & Stickney,1985
Mugil capito	Casein/Fishmeal	24	Papaparskeva - Papoutsoglu & Alexis,1986

* Indicates fry or fingerling.

compounding the diets. Further details are included in the relevant chapters.

All the ingredients were pre-weighed for the respective lots of the experimental diets. Each time feed was prepared to supply a fortnights' ration. The ingredients were thoroughly ground, if necessary, either mechanically or manually and mixed in a waring blender. Fat-soluble vitamins were added on to the oil mixture.

In the first step, gelatin was allowed to dissolve in cold double-distilled water (30 ml for 100 g diet) taken in a container. Then it was boiled over a water bath; cellulose and dextrin were added on to the liquid gelatin and the contents were mixed. This was followed by adding casein and minerals and the diet was mixed thoroughly. Steam heating was done for over 10 minutes. After reducing the heat, oil mixture was added and the dough was thoroughly churned. After cooling (to room temperature), water soluble vitamin mixture was added and blended thoroughly. The pH of the diet was maintained near neutral. The overall moisture content of the diet ranged between 30 and 40% during different experiments. The approximate daily total feed allotment was cut into blocks, kept in air-tight plastic dishes and maintained in a frezeer. Everyday a block was taken, thawed, weighed for the respective treatments on a dry matter basis and the ration supplied as two meals.

Feeding Strategy and Collection of Left-Over Food and Faecal Matter

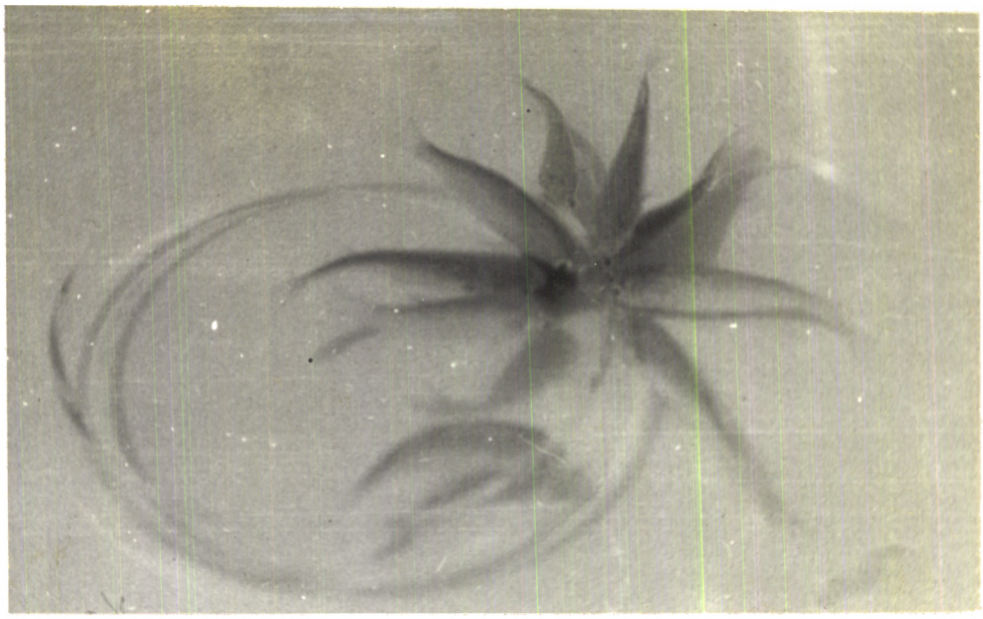
The fish were offered food at 7% of their wet body weight (Kiron and Paulraj, 1988) in two doses in petri dishes at 0800 hrs and 1500 hrs. The fishes fed actively by attacking the moist feed ball from all sides. The quantum of food offered was adjusted after weekly weight recordings.

The left-over food particles (rarely seen) were collected, an hour after the food was offered, from the food dish after carefully lifting it out of water. It was oven dried on aluminium foils and the weights determined.

Daily, before the first meal was provided faecal pellets were removed using a large volume bulbous filler. The pellets sucked up into the glass filler was delivered on to a bolting silk strainer, wherein the pellet was washed with a gentle stream of distilled water. After this, the pellets were transferred to aluminium foils and oven-dried at 60^{o} C for 36 hours. After weighing, the samples were stored in desiccator for further analyses.

Monitoring of (Water Quality) Experimental Conditions

Salinity was measured using an American Optical Refractometer. Dissolved oxygen was monitored using a Elico oxygen meter. At times the values of the above mentioned



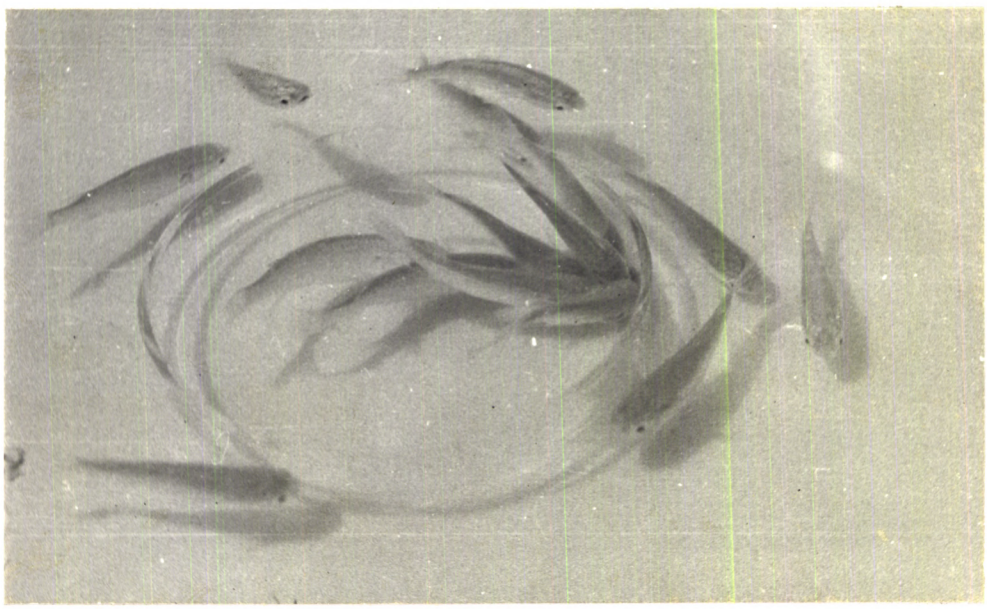


PLATE 2. FEEDING ACTIVITY OF THE FISHES IN THE EXPERIMENTAL AQUARIA.

parameters were cross checked with the respective titrimetric methods (Strickland and Parsons, 1972). Ammonia content in water was determined in samples drawn before and after water change in the experimental system. On collection, the water samples were fixed with 4% phenol solution and stored in refrigerator before it was taken up for analysis within 2 hrs. The ammonia concentration was determined using phenol-sodium hypochlorite method described by Solarzano (1969). pH of the water in the tanks were recorded every alternate day under room temperature using an Elico digital pH meter. Water temperature was recorded daily at 0730hrs and 1630 hrs. The experimental facility was located in well lighted rooms and hence the natural photo-period was effective.

Experimental Data Collection

Depending on the objective, the duration of the different experiments ranged from seven to twentyone weeks. Daily observations were made on the animals in each treatments - the general condition, swimming movements, acceptability of food, feeding behaviour, pattern of faecal output etc. On termination of an experiment, the animals were quick frozen to avoid post-mortem changes. Later, a sample was drawn for determining the moisture content. The rest of the animals, in each replicate, were freeze-dried and stored in desiccator for future biochemical analysis.

Survival : Deaths, if any, were immediately noted in the survival charts maintained for each experiment. The percentage of survival was calculated at the end of the experiment for each replicate and the mean was computed for each treatment.

$$\text{Percentage Survival} = \frac{N_o - N}{N_o} \times 100$$

where N_o is the initial number and N the number of dead fishes.

Growth rate : When each experiment was concluded, individual lengths and weights were taken as described earlier. For further calculations, mean lengths and mean weights were considered under each replicate. In all descriptions related to growth, gain in weight is considered a better mode of expression compared to length. The percentage weight increment was calculated as follows:

$$\text{Mean \% gain in weight} = \frac{W_t - W_o}{W_o} \times 100$$

where: W_o = mean initial weight

W_t = mean final weight.

Since growth pattern over a longer period was a monotonically decreasing function of time (Kruger, 1965), specific growth rate (SGR), was used for conveniently describing growth pattern. SGR was calculated as percent of daily growth rate.

$$SGR = \frac{W_t - W_o \times 100}{t/2\ (W_t + W_o)}$$

where t is the duration of the experiment in days and W_o and W_t same as above.

Condition factor (CF) was obtained from

$$CF = \frac{W_t}{L_t^3} \times 100$$

where W_t is the mean final weight and L_t is the mean final length.

Food Utilization Indices : The data accrued on food consumption during the experimental duration had been used for computing a host of related aspects. These include

$$\text{Feed Conversion Rate (FCR)} = \frac{\text{Feed intake}}{\text{Weight gain}}$$

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

The detailed studies on protein utilization in the experiments on protein requirement was made possible by calculating the net protein utilization (NPU), the productive protein value (PPV) and the apparent biological value (ABV).

$$NPU\ (\%) = \frac{\text{Final Body Nitrogen} - \text{Final Body Nitrogen at Zero Nitrogen intake}}{\text{Nitrogen intake}} \times 100$$

$$PPV\ (\%) = \frac{\text{Final Body Nitrogen} - \text{Initial Body Nitrogen}}{\text{Nitrogen intake}} \times 100$$

$ABV = \frac{PPV}{D_p}$, where D_p is the apparent digestibility of protein.

Regardless of the type of the nutrient, the most important aspect involved is the degree of release and uptake of the nutrient in the digestive tract. Digestibility of the feed nutrients was determined using the chromic oxide indicator method and expressed as :

$$\text{Digestibility} = 100 - 100 \times \frac{\%\ \text{indicator in feed}}{\%\ \text{indicator in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in feed}}$$

The nutrient retention efficiency (NRE) has been calculated for most of the experiments.

$$\text{NRE}(\%) = \frac{\text{Nutrient gain in body} \times 100}{\text{Nutrient intake} \times \text{Digestibility coefficient of nutrient.}}$$

Chemical Evaluation: Growth, food consumption and food conversion are the most important response parameters usually considered in nutrition experiments. Information about qualitative and quantitative metabolic alterations in the organism would be of greater value for better and deeper understanding of the influence of feeding regime. Chemical analyses of the fish carcass and the diets were performed to facilitate this.

Proximate analysis (CMFRI, 1982; AOAC,1984) was performed in duplicate samples on each diet and on a composite sample of fishes from each replicate. The moisture content was determined by oven drying at 100^{o}C overnight. The freeze dried fish and feed samples for chemical analysis were ground in a microgrinder.

Crude protein was determined by the micro-kjeldahl method using N x 6.25. Samples were extracted with petroleum ether for crude fat (Soxhlet method) determinations. Moisture-free ether extracted samples were digested with weak acid and then weak base, washed with acetone and finally the organic residue ignited to determine crude fibre. Crude fibre and fat were determined with the help of the semi automatic Fibretec and Soxtec systems (Tecator AB, Sweden). Ash was determined in samples after incineration in a muffle furnace at 550^{o}C. Nitrogen-free extract was calculated by difference from the above values. In the digestibility studies, the content of the chromic oxide indicator was measured spectrophotometrically (Furukawa and Tusukahara, 1966). Analytical measurements on pooled faecal samples were carried out wherever necessary using the same techniques described above. Gross energy was easily calculated from the nutrients. The productive energy value of feeds was determined using a Gallenkemp ballistic bomb calorimeter.

Pathological investigations: The animals under each treatment were carefully observed daily to detect any clinical abonormality. Proper record was maintained on the health of the experimental animals.

Histopathology served as a useful tool in the experiments designed to study the vitamin requirements. Section of gills, muscle tissue and liver were cut and scanned microscopically for pathological abberations. Fish tissues, preserved in 10% neutral

buffered formalin, were processed for light microscopy using standard histological techniques: normal as well as suspected cases were embedded in paraffin wax and sectioned at 6 µm followed by staining in Harris' haematoxylin and eosin (H & E).

Haematological observations were made on fishes fed diets deficient in vitamins. Blood samples were drawn from the cardiac region directly onto the clean glass slides, rapidly air-dried, methanol-fixed and stained by Giemsa method (Humason, 1972). The red blood cell morphology was assessed on the basis of the following criteria: abundance of immature cells, degenerative changes in cell populations, size variations within each of cell types and bizzare forms. The microphotographs were taken using an Olympus universal research microscope, VANOX - S Model PM 10 A.D.

Statistical analysis: The diet response data had been subjected to statistical analysis to arrive at valid conclusions. One way analysis of variance was performed to prove the treatment effects and the "t" test was employed to locate the significant difference between means. In the nutrient requirement experiments, second order polynomial relation has been established between weight gain/food conversion and nutrient levels. Using differential calculus in these cases, the optimum dose in relation to maximum weight gain has been identified.

PART III

1. DIETARY PROTEIN REQUIREMENT

1.1. INTRODUCTION

A successful artificial diet must meet the requirements for survival and growth of the fish being cultured. Consequently, aquatic diets must contain appropriate nutrient combinations which can be effectively and efficiently utilized. Protein, generally is the major constituent and the most expensive component in fish diets; and therefore it is of major concern to fish nutritionists.

Protein in the diet is basically utilized for three main purposes: (1) maintenance, the making good of tissue wear and tear, (ii) the repletion of depleted tissues, and (iii) growth or formation of new additional protein. The utilization of dietary protein is mainly influenced by its amino acid pattern, by the level of protein intake, by the level of other nutrients in the diet, by the caloric content of the diet and last but not the least by the physiological state of animal.

An animal's need for nitrogen and essential amino acids is met by dietary protein. Turn over and resynthesis of body components results in daily loss of endogenous protein. Coupled with this inefficiency, certain amino acids cannot be synthesised de novo or at a rate necessary for growth. Thus, there must be adequate protein in the diet to compensate for catabolic losses in addition to what is needed for growth. Moreover other dietary

components interact on a metabolic level and influence protein utilization. Apart from carbohydrates and lipids, amino acids too can be utilized for energy. In order to maximise weight gain while minimising protein intake, energy from these nutrient classes must be balanced (Lee and Putnam, 1973; Garling and Wilson, 1976). It should also be noted that as the animals' growth decrease, metabolic rate and protein requirement also decrease. To avoid waste, diets must also reflect current metabolic demands. Moreover, the protein requirement of a particular fish is influenced by many environmental and nutritional factors. A recent review examines some factors which influence protein utilization (Steffens, 1981).

The optimum dietary protein level required for maximum growth in farmed fishes is 50-300% higher than that of terrestrial animals (Cowey, 1975). These quantitative differences have been mainly attributed to the predominant carnivorous and omnivorous feeding habit of fishes and their apparent preferential use of protein over carbohydrate as dietary energy source. Unlike warm-blooded animals, fishes are aquatic ectotherms and hence do not need to spend a large proportion of energy in maintaining body temperature (Nijkamp et al., 1974). Moreover, as the fishes live in water the primary end product of nitrogen metabolism, ammonia, can be rapidly dissolved off by passive diffusion through permeable surfaces. As a result there

is no build up of toxic ammonia in the tissue, thereby doing away with the need of converting it into molecules such as urea or uric acid. Consequently, fishes derive more metabolic energy from catabolism of protein than do terrestrial animals, which must convert ammonia to non-toxic substances (Brett and Groves, 1979). The efficient mechanism possessed by fish for protein catabolism and excretion of nitrogen is seen by Smith et al. (1978) as one of the factors that contribute to the high energy efficiency of fish; besides other factors like cold-blooded existence, low energy cost of voluntary activity in water and low energy cost of reproduction.

Although there are several aspects of nutrition of fish that contrast with their terrestrial counterparts, that which has the greatest significance, at least from the view point of fish cultivation, is their demand for high levels of dietary protein. Apart from the biotic and abiotic factors (Austreng and Refstie, 1979; Cowey and Luquet, 1983) which affect the protein requirement of the fish, it should be remembered that protein is useful to the animal only when it can be digested and the degradation products - peptides and amino acids - absorbed. If protein in the diet is insufficient for the fish, it is withdrawn from the tissues to carry on the vital life functions, thereby resulting in rapid growth reduction. On the otherhand if excess protein is supplied, proportionately less will be used to make

new protein and the rest will be metabolized to produce energy. Hence, it is essential to determine optimum level of the nutrient to be fed to the fish. The amount of protein required in prepared diets is directly influenced by the amino acid composition of the diets. The minimum amount of dietary protein needed to supply adequate amino acids and produce maximum growth has been recommended for many fish species. Recommending an appropriate protein level, however, depends on culture practices and environmental conditions.

Based on feeding techniques pioneered and developed for terrestrial animals, the dietary protein requirements of fish were first investigated in chinook salmon (Oncorhynchus tshawytscha) by Delong et al. (1958). Fish were fed a balanced diet containing graded levels of a high quality protein (Casein - gelatin mixture supplemented with crystalline amino acids to stimulate the amino acid profile of whole hens' egg protein) over a ten week period, and the observed protein level giving optimum growth was taken as the requirement. Since these early studies, the approach used by the workers has changed very little, if at all, with the possible exception of the use by some researchers of maximum tissue protein retention or nitrogen balance in preference to weight gain as the criterion of requirement (Ogino, 1980). Protein requirements are usually expressed in terms of a fixed dietary percentage or as a ratio of protein to dietary energy. More than thirty fish species have

been examined in this manner and the results (Table I) show a uniformly high dietary protein requirement in the range 35-55%, or equivalent to 45-70% of the gross energy content of the diet in the form of protein. The use of different dietary protein sources, non-protein energy substitutes, feeding regimes, fish-age classes and methods for determination of dietary energy content and dietary requirement leaves little common ground for direct comparison to be made within or between species. However, some general conclusions can be drawn from the above studies and the present contribution could be discussed in such light.

1.2. MATERIAL AND METHODS

Carefully planned laboratory based experiments were performed to determine the requirement of dietary protein. The general material and methods described earlier (pages 7 to 18) were followed except for the minor variations described herein.

The fry of Liza parsia were hand-graded and alloted to different tubs, in groups of twenty. Their mean intial length measured 29.80mm (± 0.80) and the initial weight was 349.50 mg (± 8.15). Each tub was identified as one of the triplicate of the 13 treatment groups.

In all thirteen different dietary preparations, D1, D2, D3.....D13; (Table II) were offered to 39 groups. Casein and gelatin were the protein sources. Corn oil and cod liver oil in

TABLE II. INGREDIENT COMPOSITION OF THE DIETS USED IN THE EXPERIMENT ON PROTEIN REQUIREMENT

INGREDIENTS (g)	DIETS												
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13
Casein	0.0	3.8	7.6	11.4	15.2	19.0	22.8	26.6	30.4	34.2	38.0	41.8	45.6
Gelatin	0.0	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6	10.8	12.0	13.2	14.4
Dextrin	81.0	75.0	68.0	61.0	54.0	47.0	41.0	34.0	27.0	20.0	13.0	7.0	0.0
Cellulose	8.0	9.0	11.0	13.0	15.0	17.0	18.0	20.0	22.0	24.0	26.0	27.0	29.0
Corn oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Cod liver oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mineral mix *	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin mix *	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PROXIMATE COMPOSITION (%)													
Protein	0.4	4.9	9.7	14.5	20.3	25.2	29.1	34.1	39.2	44.3	49.8	54.5	59.0
Lipid	5.9	6.2	6.0	6.4	6.5	6.2	6.3	6.5	6.7	6.3	6.1	6.4	6.2
Fibre	9.2	10.3	11.7	13.9	15.7	17.6	18.8	20.5	22.5	24.3	26.1	27.3	29.0
Ash	3.8	4.5	4.6	4.6	4.7	4.7	4.7	4.9	4.8	5.2	5.1	5.2	5.4
Nitrogen free -extractives	80.8	74.1	68.0	60.8	52.9	46.1	41.2	34.2	27.1	19.5	12.7	6.9	0.2
ENERGY $kJ.g^{-1}$	16.3	16.3	16.3	16.3	16.4	16.4	16.3	16.3	16.4	16.3	16.3	16.3	16.3

* See Table II

equal proportions constituted the lipids. Dietary caloric adjustments were made by varying the dextrin content. Chromic oxide (1%) was included in the diet for digestibility studies. Table II gives the ingredient composition of the thirteen test diets that were prepared. Table III lists the vitamins and minerals utilised for diet preparation; the detailed procedure of which has been described in the general material and methods section (pages 9 to 11). The proximate analysis of the diets were performed and the gross energy content was calculated.

After the acclimatisation period or about two weeks, the fishes were reared on the experimental diets for 10 weeks. They were fed twice, a daily ration of 7% body weight. Left-over food was collected, as also the faecal matter which was later analysed for determining the nutrient digestibilities. The experimental conditions were monitored through the seventy days. The data collection procedure and analysis has been described earlier (pages. 13 to 18).

To study the effect of protein level on ammonia excretion, individual animals of known weight from respective treatments were maintained in metabolic chambers containing fresh sea water of salinity 15ppt. During the 48h acclimatisation period in the metabolic chamber the fish were fed the prescribed diet once a day. On the day of data collection, after the 1 h. feeding time, at 0800 hrs, the chamber was flushed with fresh sea

TABLE III: COMPOSITION OF THE MINERAL MIXTURE AND VITAMIN MIXTURE USED IN THE EXPERIMENTS

MINERAL MIXTURE	g/100 g.
Calcium biphosphate	13.580
Calcium lactate	32.700
Ferric citrate	2.970
Magnesium sulphate	13.200
Dibasic potassium phosphate	23.980
Sodium biphosphate	8.720
Sodium chloride	4.350
Aluminium chloride	0.015
Zinc sulphate	0.300
Cuprous chloride	0.010
Manganese sulphate	0.080
Potassium iodide	0.015
Cobaltous chloride	0.100
VITAMIN MIXTURE	**mg/g**
Choline chloride	500
Inositol	200
L-Ascorbic acid	100
Nicotinic acid	75
Calcium pantothenate	50
-Tocopherol acetate	40
Riboflavin	20
Thiamine hydrochloride	5
Pyridoxine hydrochloride	5
Menadione	4
Folic acid	1.5
Cyanocobalamin	1.1
Biotin	0.5
Cholicalciferol	0.2

water of known ammonia concentration. Only a 12th hour sample was collected to determine the excretion of ammonia (NH_3-N) as this could probably incorporate certain hourly variation of excretion which may appear during the said period. The same procedure was repeated to get four more values for each treatment group. A control chamber with no fish was also set up. The NH_3-N in the chamber water was determined by the phenol hypochlorite method (Solarzano, 1969). The mean value of the difference of intial and final ammonia contents of water samples was the NH_3-N excreted by each group.

1.3. RESULTS

The rearing conditions, for the fry of mullet Liza parsia, during the 10 weeks were, salinity = 15±1ppt, temperature = 27.4±2.1°C pH = 7.956±.114 and ammonia = 0.252± 0.113mg.$l,^{-1}$oxygen = 4.82 ±.33ppm.

The survival rate Table IV was significantly ($P<0.01$) influenced by dietary levels of protein. Higher levels (20%) of protein gave better survival rates (93-98%) with the best at 55% protein (Diet D11). The survival rates drastically slumped to 77% when protein was excluded from the diet (D1). The differences in the survival rates at higher protein levels were not statistically significant.

TABLE IV: RESULTS OF THE EXPERIMENT ON PROTEIN REQUIREMENT.

PARAMETERS	PROTEIN IN DIET (%)												
	D1 (0)	D2 (5)	D3 (10)	D4 (15)	D5 (20)	D6 (25)	D7 (30)	D8 (35)	D9 (40)	D10 (45)	D11 (50)	D12 (55)	D13 (60)
Survival (%)	77	83	87	87	90	93	92	95	93	95	93	98	95
Condition factor	1.101	1.203	1.167	1.249	1.283	1.306	1.240	1.245	1.202	1.274	1.228	1.256	1.278
Weight gained (g)	-	0.094	0.135	0.272	0.298	0.378	0.460	0.580	0.670	0.545	0.479	0.450	0.434
Net protein - utilisation (%)	-	41.58	35.32	27.58	29.65	27.02	23.65	28.03	27.30	22.25	19.69	17.57	16.53
Productive protein - value	-	22.73	21.89	22.85	23.99	23.10	20.65	25.05	25.18	19.89	17.81	15.88	14.86
Apparent digestibi - lity coefficient of protein	-	86.15	86.59	87.15	87.50	88.12	89.37	90.43	91.24	91.55	90.95	90.51	90.89
Apparent digestibi - lity coefficient of lipid	-	84.68	86.33	85.38	86.16	86.01	86.48	87.20	87.17	87.50	87.68	88.17	88.03
Apparent biological value	-	26.39	25.28	26.22	27.41	26.21	23.11	27.70	27.60	21.73	19.58	17.54	16.35

Condition factor was the best (1.31) in fishes fed 25% protein (D6). Except for those fed protein levels ranging from 0 to 15%, the values were almost similar and ranged between 1.24 and 1.30.

The protein inclusion had a profound influence on growth of the mullet fry. The best growth increment of 670mg was recorded when protein was included at 40% in the diet (D9). Growth was significantly different ($P<0.01$) from each other at all dietary protein levels. At 35% (D8) and 45% (D10) protein levels, growth recorded was 580mg and 545mg respectively. Only 94mg weight increment was recorded in fry fed diets (D1) without protein. The specific growth rate (Fig. 1) in the mullet fry fed 40% protein (D9) was 1.405 as compared to 0.344 when protein component was deleted from the diet. The growth pattern observed was parabolic with steady incremental gains upto 40% protein in diet followed by a gradual decline in the increment at higher levels of protein incorporation (Fig. 3; Table IV). By differential calculus it was found that the optimum % weight gain could be obtained when fry were fed a diet incorporating 43.54% protein.

The food conversion ratio was highest for fishes which were fed the protein free diet. The best conversion rate (1.63) was obtained for diet D9 (Fig.1). Although the rates for Diets D8 and D10 were significantly ($P<0.01$) different from that of

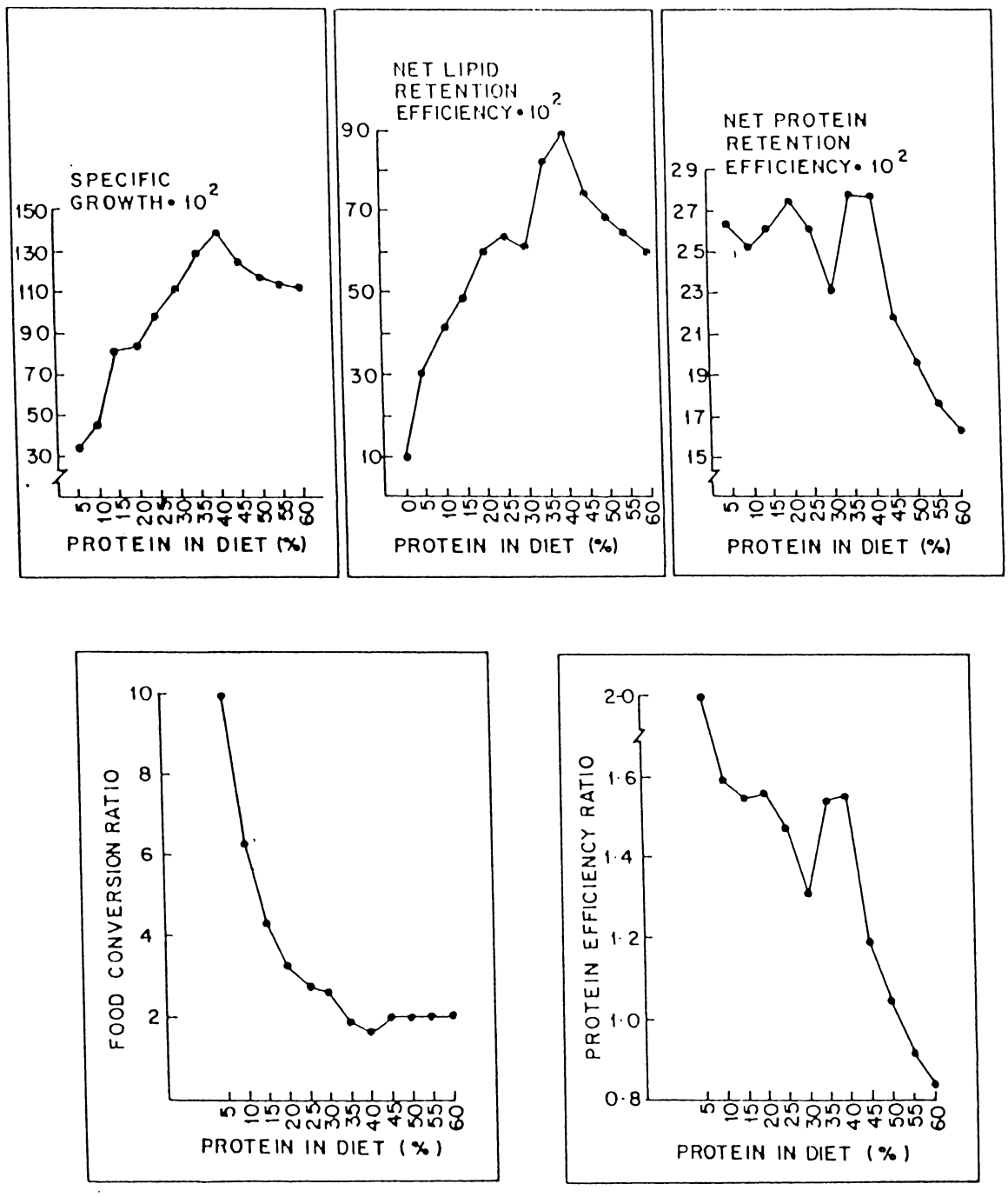


Fig.1. SPECIFIC GROWTH, NUTRIENT RETENTION EFFICIENCY, FOOD CONVERSION RATIO AND PROTEIN EFFICIENCY RATIO IN FISHES FED GRADED LEVELS OF PROTEIN.

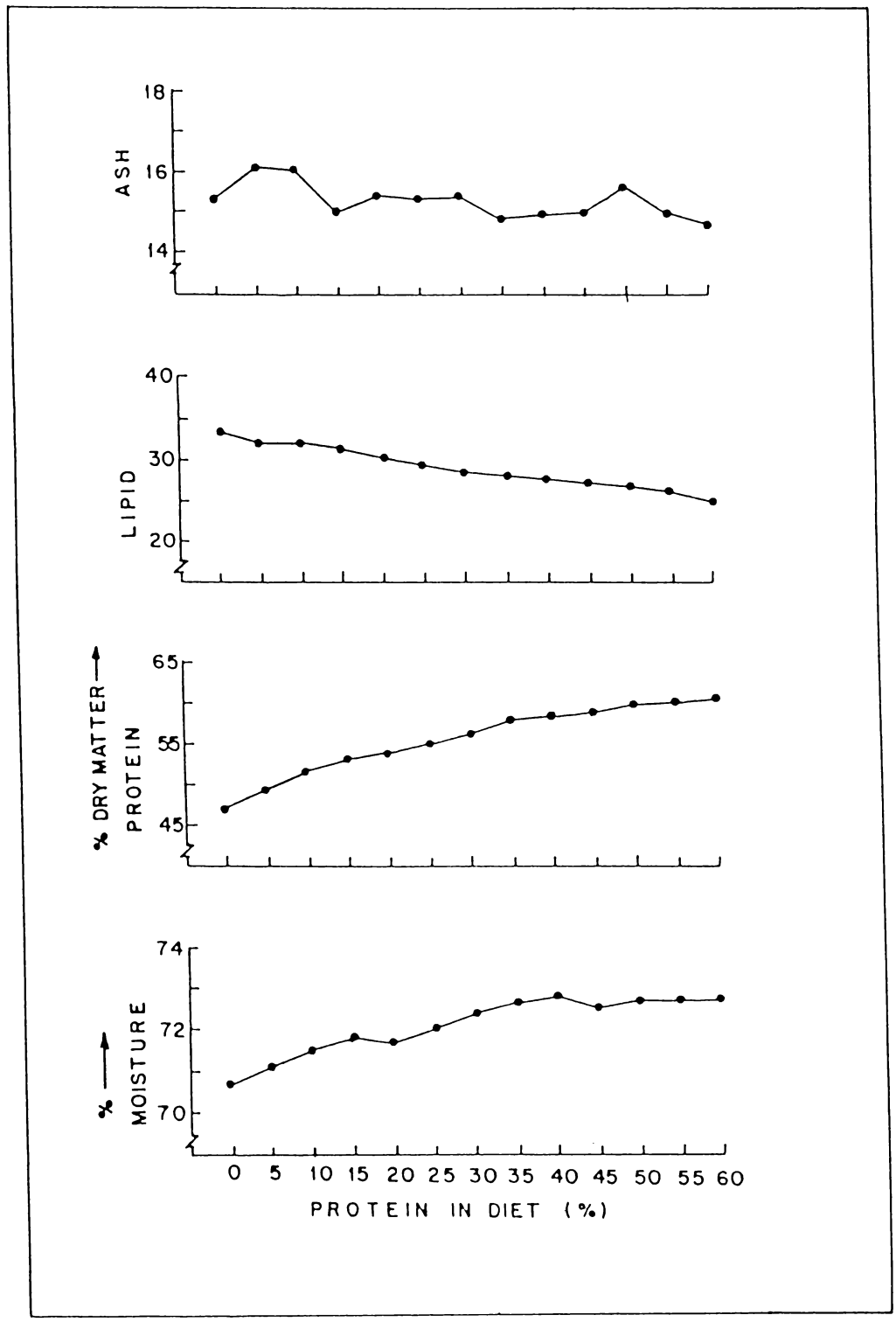
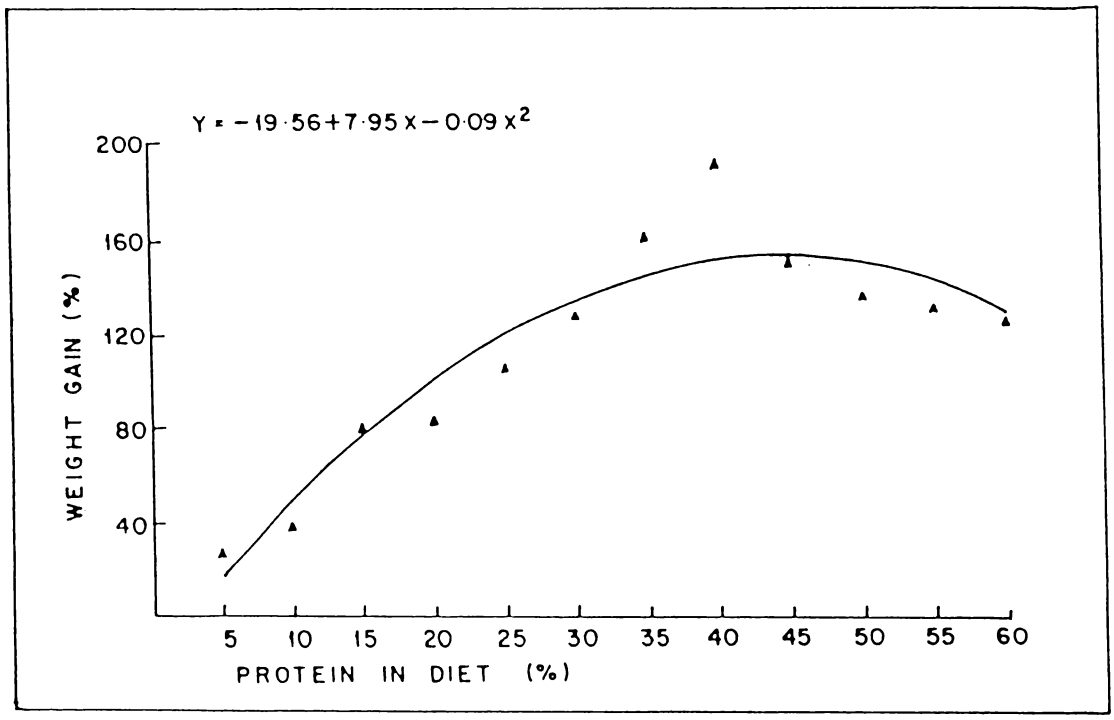


Fig.2. BODY COMPOSITION OF FISHES FED GRADED LEVELS OF PROTEIN.



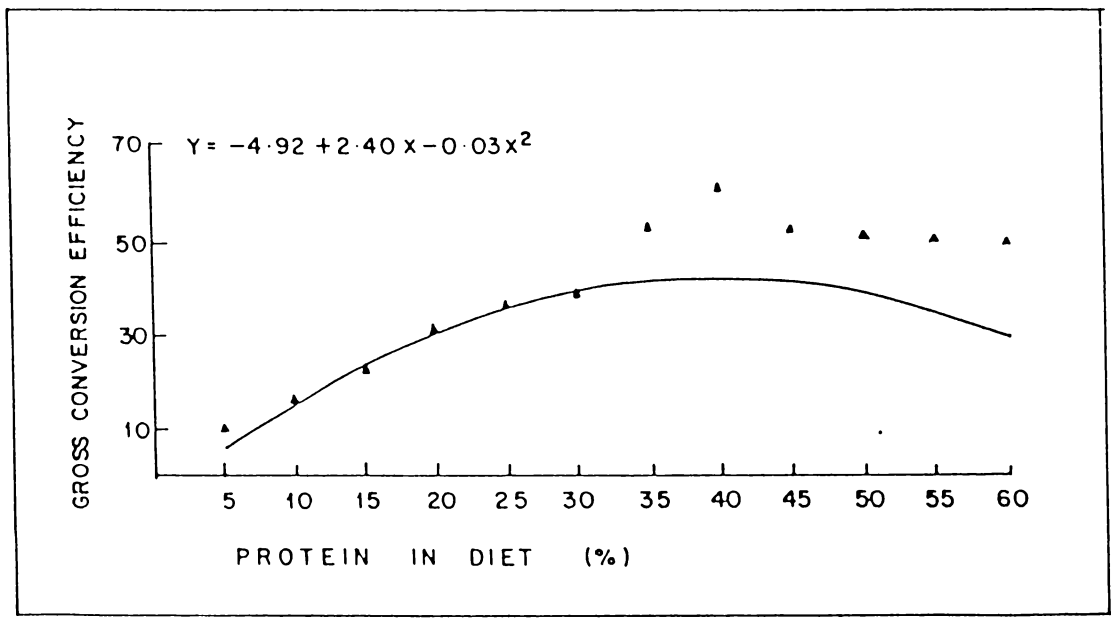


Fig.3. SECOND DEGREE POLYNOMIAL RELATION OF PERCENTAGE WEIGHT GAIN/GROSS CONVERSION EFFICIENCY IN FISHES AND DIETARY PROTEIN CONCENTRATION.

D9, the figures were closer to that of the best value. A second degree polynomial relationship was established between the gross conversion efficiency and the dietary protein levels (Fig.3).

The protein efficiency ratio (PER) decreased with increase in protein content in the diet (Fig.1). The range was between 0.84 for diet D13 to 2,01 for diet D2. Calculations on net protein utilization (NPU) indicate that an inverse relationship existed between the protein content in the diet and the NPU values (Table IV). Around 20% utilization was recorded at protein inclusion levels of over 45% in the diet. Productive protein values and apparent biological values of protein too exhibited a similar trend, (Table IV). For diet D8 (40% protein) and diet D9 (45% protein) the productive protein values were 25.18 and 19.89 respectively while the apparent biological values read as 27.60 and 21.73.

The carcass analysis revealed that the body moisture ranged between 70.72 (D1) and 72.74 (D9; Fig. 2). Dietary protein concentration seemed to significantly ($P<0.01$) influence the body protein levels. Only 46.99% protein was recorded in the dry tissues when no protein was incorporated in the diets (D1). The tissue protein content increased with dietary levels. At 60% dietary level, the protein content was 60.58%. Body lipid exhibited an inverse relationship; the maximum lipid content of 33.63% for diet D1 and lowest of 24.91 for diet D12. Body ash

was not significantly different between treatments. It ranged between 14.70% (D13) and 16.13% (D1). Calculations of nutrient retention efficiency of lipid and protein indicate significant influence of the treatments. Maximum lipid was retained at diets D8 and D9 (22.7 and 27.6%, not significantly different from each other at 1% level); maximum protein was retained (88.5%) when dietary protein level was 40% (Fig.1)

The protein digestibility (Table IV) was lowest at 5% level (D2 = 86.15%) and highest at 45% level (91.55%). Lipid was digested to the extent of 88.17% for Diet D12; the maximum of 84.68% for Diet D1.

The endogenous nitrogen excretion, i.e., nitrogen loss in fish fed the zero protein diet was calculated as 3.138$\pm$ 0.190 mg N. 100g fish $^{-1}d^{-1}$. The metabolic faecal nitrogen (N in faeces of fish fed zero protein) was found to be 21.23 mg N. 100g diet^{-1} . The NH_3 -N excreted by fishes under different dietary groups is given below:-

Diet	NH_3 -N excreted mg.g $^{-1}h^{-1}$	Diet	NH_3 -N excreted mg. g$^{-1}h^{-1}$
D1	0.006	D8	0.020
D2	0.011	D9	0.021
D3	0.013	D10	0.029
D4	0.014	D11	0.030
D5	0.016	D12	0.033
D6	0.017	D13	0.034
D7	0.018		

1.4. DISCUSSION

The importance of dietary protein was revealed by the fact that survival was lowest, when protein component was excluded from the diet. The utilisation of protein from the tissues of the protein-free diet fed fish would have rendered the fish weak and vulnerable to infection leading to higher mortality rate. This condition seems to be offset at higher protein levels, where a greater percentage survived. Though the condition factor was highest at diet D6, almost similar values were obtained for protein levels less than 30% indicating almost complementary performance.

The growth data reveals the best increment of 670 mg for 40% protein diet with a specific growth rate of 1.405. This is nearly 100mg more than the next best level. Thus, the observed data indicate that a level of 40% protein in the diet is optimum for growth when fed a daily ration of 7% body weight. Similar results were obtained by Lim *et al*. (1979) for fry of milkfish *Chanos chanos* fed a purified casein diet during a 30 day experiment. In the case of *Oreochromis nilotica* fingerlings the requirement was assessed as 35% (Teshima *et al*., 1985). Dupree and Sneed (1966) experimenting with channel catfish fingerlings fed casein, gluten or soyabean found lesser weight gains upto 40% protein. Halver (1976) generalised that the apparent protein requirements were essentially the same for salmon and trout fry

at about 50% with a reduction in requirement as the fish increased in size. However, it must be noted that the mathematically arrived at optimum level in the present experiment was marginally higher (43.5%). Almost the same value was obtained by Dabrowski (1977) for the fry of grass carp Ctenopharyngodon idella, when raised in a controlled environment.

Fishes given the protein-free diet showed abnormal and stunted growth and the increment recorded during the 70 days rearing period was merely 94mg. Protein is absolutely essential for the build up of new body tissue. If protein is excluded from the diet, no growth of the fish might be expected. In fact, a decrease in growth was noted in the initial phase of this experiment and similar observations have been made earlier (Dabrowski, 1977; Jauncey, 1982). A slight increase in weight, however, was reported by Sen et al. (1978) and Ogino et al. (1976) in carps. The slight weight gain may be due to the deposition of lipids in the body (Fig.2) which is mainly derived from carbohydrate metabolism. The protein-free diet had the highest carbohydrate level.The results indicate that raising the level of dietary protein upto 40% improves weight gain; but further increase induces a reduction in growth rate.The decrease can be attributed to the low non-protein energy in the diet. Lim et al. (1979) and Fah and Long (1984) have made similar observations in milkfish Chanos chanos and guppy Poecilia reticulata respectively. Prather and Lovell (1973) even

indicated that diets with high levels of protein and low amount of non-protein energy may be toxic to channel catfish Ictalurus punctatus.

On a critical analysis of the growth characteristics over the whole experimental period, the slight decrease in specific growth rate observed for dietary groups D10 and D13 (protein 45 - 60%) corresponds to that reported for grass carp (Dabrowski, 1977), eel (Nose and Arai, 1972) and tilapia (Jauncey, 1982) and confirms with the general pattern observed for high quality proteins (Harper,1965). The decrease in specific growth rate at protein levels above the optimum can be attributed to a reduction in available dietary energy for growth, perhaps due to the energy expended to deaminate and excrete excess of absorbed amino acids from these high protein diets.

The food conversion ratios decreased with increasing dietary protein levels up to 40%; beyond this the values were almost similar. The groups receiving diets D1 and D2 had a food conversion ratio more than three times the average of the remaining groups. The conversion values of fish on diet D1 were very high because irrespective of the amount of feed they consumed, the body weight gained was little. The FCRs obtained are on an average lower than those reported for Mugil capito in certain preliminary experiments by Vallet et al. (1970) and Papaparaskeva-Papoutsoglou and Alexis (1986).

Protein efficiency ratios and net protein utilisation values decreased with increasing dietary protein levels as observed for carp (Ogino and Saito, 1970) and tilapia (Mazid et al., 1979). The decrease was almost linear except at 35 and 40% protein levels. The apparent assimilation efficiency varied directly with dietary protein concentration as observed by Rychly (1980) and Beamish and Thomas (1984). The maximum values of percentage protein retained and PER observed in this experiment are lower than that recorded for rainbow trout fed diet with low or high energy content (Takeuchi et. al,, 1978) and almost similar to that in grey mullet (Papaparaskeva-Papoutsoglou and Alexis, 1986) and gilthead bream (Sabout and Luquet, 1973) and higher than those found by Cowey et al. (1970) for plaice. It seems therefore that L.parsia utilises protein for body growth less efficiently than do rainbow trout. It may also be that this mullet requires a better protein with more balanced amino acids profile than the protein sources (casein and gelatin) used in this study. Further more, the improvement in protein retention and efficiency with rise in carbohydrate levels indicate a protein sparing action. The net lipid retention efficiency indicates that more of dietary lipid is utilized for production of energy at low protein levels; thereby there has been a consistent increase in net lipid retention efficiency with a corresponding increase in protein in diet upto a level of 35%. Above this protein level, the lipid retention seems to decrease.

Thus it is apparent that protein limitation in diet results in increased utilization of lipid as a source of energy.

The apparent protein digestibility increased from about 86% in 5% protein diet to 91% in 45% protein diet. A similar increase in apparent protein digestibility has been observed in channel catfish (Page and Andrews, 1973), snakehead (Wee and Tacon, 1982) and rainbow trout (Nose, 1963); although in the case of rainbow trout when corrections were made for endogenous nitrogen losses, true protein digestibility remained constant. The low apparent protein digestibility observed in the fish fed the lower dietary protein levels 5 to 25% was probably due to the high carbohydrate content of the diets. The studies of Shimeno et al. (1978) has shown that high levels of purified carbohydrate (potato starch) had a deleterious effect on growth, feed efficiency and resulted in reduced protein and carbohydrate digestibility in yellow tail.

The trends discernible in fish carcass composition are an increase in body moisture and protein contents and a decrease in lipid content with increase in dietary protein. As regards the body moisture content, the present result was not in congruence with the observations in Ctenopharyngodon idella (Dabrowski, 1977) where no change was found. As in the present case, a decrease in carcass lipid content was observed in plaice

(Cowey et al, 1972), grouper (Tang et al, 1978), eel (Nose and Arai, 1972) and tilapia (Jauncey, 1982); with increasing dietary protein. It can be seen that fish which had high lipid content were actually those which had received diets containing high carbohydrates; since dietary lipid level was kept constant. It is thus clear that excess dietary carbohydrate was converted into body fat. The linear relationship between protein content of the diet and body protein as observed in the present case was also earlier reported by Ogino and Saito (1970) in young carp using casein as the protein source. Saita (1974) also showed a general increase in protein content in the carcass of rainbow trout in relation to the amount of dietary protein. Increase in protein content was also observed in plaice (Cowey et al., 1972), gilthead bream (Sabaut and Luquet, 1973) and eel (Nose and Arai, 1972). Body water and lipid levels appeared to be inversely related as has been noted for several other species (Kausch and Ballion-Cusmano, 1976; Dabrowska and Wojno, 1977; Grayton and Beamish, 1977; Murray et al., 1977; Atack et al., 1979; Jauncey, 1982). Body ash did not show any specific pattern in relation to the dietary regimes as has been noted with other fish species. (Phillips et al, 1966; Cowey et al., 1974; Elliot, 1976; Dabrowska and Wojno, 1977; Atack et al, 1979).

The apparent protein digestibility coefficient increased with increasing protein content in the diet. Since the metabolic faecal nitrogen is interrelated with dry feed intake

than protein content in the diet, it will represent a greater portion of nitrogen in faeces when fed low protein diets than for fish fed high protein diets (Austreng and Refstie, 1979). The value of metabolic faecal nitrogen obtained in this study (21.23 mg N.100g diet^{-1}) is almost equal to that recorded for carp fry by Syazuki (1960). The endogenous nitrogen loss recorded in tilapia by Jauncey (1982) was three times higher than the present values. This may be because the experimental tilapia were more than thrice the size of mullet used in the present experiment. Ogino *et al.* (1973) reported the endogenous nitrogen excretion of carp to be 7.2mg N.100g fish^{-1} . d^{-1} at 20^{o}C and 8.6 mg N.100g fish^{-1} . d^{-1} at 27^{o}C. On the contrary the same authors (1980) have recorded still higher values for carp and has attributed it to the higher metabolic rates in smaller fishes.

Practical artificial diets are formulated with highly digestible and nutritious components with well balanced energy content, the objective being to enhance utilization and to reduce faecal and metabolic losses (Hastings, 1969; Cho *et al.*, 1982). Some information is available on the quantitative relationship between the composition of natural diets and nitrogen excertion (Gerking, 1955; Iwata, 1970; Elliot, 1976; Guerin-Ancey, 1976). The major end product of protein catabolism in fish is ammonia. The formation of this requires no energy and it is easily eliminated by diffusion across the gills apparently in exchange with sodium, the latter facilitating a critical requirement for

salt balance (Maetz and GarciaRomeu, 1964; Brett and Groves, 1979). However reduction in fish growth may be caused by a high concentration of ammonia as pointed out by Soderberg et al. (1983). The proportion of total nitrogen excreted as ammonia appears to vary among species and feeding conditions from about 45-90% (From, 1963; Iwata, 1973). In this study it was observed, that the amount of NH_3 - N excreted increased with dietary protein concentration. Such a relationship has been established earlier by Gerking (1955), Beamish and Thomas (1984) and Degani et al., (1985). The quantum of ammonia excreted, 0.006-0.034 $mg.g^{-1}.\ h^{-1}$ falls within the range reported in literature (Brett and Groves, 1979). The linear relationship observed indicates that at higher protein levels, especially beyond the optimum (40%), a greater percentage of protein is catabolised for energy production. The lower ammonia excretion for diets D1 to D4 may be because the required energy is derived from the dietary carbohydrate to a certain extent thus partly sparing catabolism of assimilated nitrogen compounds.

The principal end products of nitrogen metabolism have been used to measure the efficiency of dietary protein utilization in several terrestrial and aquatic species (Eggum, 1970; Miles and Featherston, 1974; Garcia et al, 1981). The proportion of dietary nitrogen excreted or retained is dependent intially on the organisms' assimilation efficiency. Retention of assimilated

nitrogen is known to vary with diet composition (Rychly, 1980) and environmental conditions (Niimi and Beamish, 1974). The cumulative losses of N across the gills, in urine and in faeces provide for an estimate of nitrogen retained within the fish. In the case of L. parsia it was found that the net retention efficiency of protein ranged between 16% and 27% (Fig. 1). A similar range (15-24%) was reported for Nr efficiency in rainbow trout fed with approximately 36% protein (Smith and Thorpe, 1976). This was not different from the Nr efficiencies reported for other teleosts as well (Gerking, 1955; Savitz et al., 1977; Durbin and Durbin, 1981). The notable exceptions where higher values were reported included the researches of Kaushik (1980), Rychly (1980) and Beamish and Thomas (1984). Protein efficiency ratio was found to show an inverse relationship to that of NH3 -N excretion. This agrees with the result of Ming (1985) in rainbow trout. Garcia et al. (1981) while comparing the efficiency of protein utilization for different diets fed to rainbow trout found agreement between PER and PPV based on total ammonia excreted in 24h as observed in L. parsia, Savitz (1969) and Iwata (1970) point out that variations in ammonia may be influenced by nutritional and thermal history. Ammonia excretion is thus a valid index of dietary protein utilization.

Thus considering the various response parameters, a dietary level of around 40% is recommended while formulating diet for the nursery rearing of L. parsia.

2. DIETARY LIPID REQUIREMENT

2.1. INTRODUCTION

Fishes rely to a large extent, when compared to most mammals including man, on lipids (and also protein) rather than carbohydrates as a source of energy. Nevertheless, the overall lipid content of fish is relatively similar to most land animals. But, a variety of lipid classes and fatty acids are present in fish compared to those present in mammals. This diversity stems from the much larger range of species found in the aquatic environment and possibly because lipids fulfil certain essential functions which do not normally occur in land animals.

The two major categories of lipids identified in fishes are the polar and non-polar lipids. The polar lipids include phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine phosphatidylinositol, plasmalogens, sphingomyelins, cerebrosides and gangliosides. The non-polar or neutral lipids are sterol esters, triglycerides, alkyl-diacyl-glycerols and wax esters. All the lipid types contain fatty acids of different chain lengths and degree of saturation. Limited quantities of free fatty acids also occur in fish tissues.

Like in other animals, lipids fulfil two broad function in fishes also. They are involved in maintaining the structural integrity of a wide variety of biomembranes between and within cells. Lipids also have the major role as energy nutrient. The

provision of chemical energy in the form of ATP depends largely on the oxidation of fatty acid moieties. Though other moieties like glycerol are readily available they are relatively unimportant in the provision of energy from lipids. The beta-oxidation pathway in fish is especially important because the lipid content of natural fish diets usually exceed the carbohydrate content. As in terrestrial animals, the triglycerides provide the bulk of fatty acids for oxidation in fish although alkyl-diacyl-glycerols and wax esters when present will also be utilised (Cowey and Sargent, 1972). The other lipid classes are not known to serve, generally, as energy sources other than in conditions of prolonged starvation (Olley, 1961; Wilkins, 1967).

Unlike land animals, fishes store large quantities of lipid in their livers and muscles. The site of normal lipid storage is significant from the view point of nutritional status of fish as well as in understanding the overall energetics. While the triglycerides feature largely in energy provisions, it is the polarlipids together with cholesterol and its esters that feature largely in biomembranes.

Apart from these functions, dietary lipid acts as a vehicle for the absorption of fat soluble vitamins provided in the diet. Lipids are also important in the flavour and textural properties of feed consumed by fishes as well as similar

properties in fish themselves. Lipids are involved in many other aspects of metabolism: viz., precursor of steroid hormones and prostaglandins, and also in the activation of certain enzymes. Other specific and crucial roles in fishes include its involvement in embryo development (Leray et al., 1985) and in intestinal functions (DiConstanzo et al., 1983; Leray and Florentz, 1983).

Digestion, absorption and transport of lipid provide the animal with fatty acids which are used either as a source of energy, as structural cell wall elements or as precursors of cyclic or non cyclic derivatives which play an important hormone like role at the cellular level. All lipid classes in the diet will contribute fatty acids to the fish although on a normal diet, the bulk of them will be derived from triglycerides and phospholipids in about equal proportions. Dietary fat in animals influence the fatty acid composition of depot neutral lipid, the fatty acid composition of membrane phospholipids and the ratio of membrane phospholipid to membrane protein (Stubbs and Smith, 1984; Clandinin et al., 1983).

A number of reviews on fish nutrition have been published which contain information on the lipid requirements of fish (Castell, 1979; Cowey and Sargent, 1972, 1977, 1979; Hashimoto, 1975; Lee and Sinnhuber, 1972; Watanabe, 1982). Most studies indicate that carnivorous fishes like salmonids

efficiently utilize lipids in their diets, provided adequate amounts of choline, methionine and tocopherol are present in the ration. Fats have the distinct advantage of being almost completely digestible. Fish appear to be designed to metabolise fats efficiently as an energy source with a concomittant sparing effect on the protein requirement for maximum growth. The neutral lipid component of fish rations is, therefore, a useful entity in diet preparations and is particularly desirable in feeds of fry or fingerling which require high energy intake for rapid growth. This asset is not without constraint as some fishes like rainbow trout, salmon, plaice and seabream do not have sufficient ability to elongate short chain fatty acids and then unsaturate the carbon chain to convert simple animal or vegetable fats into polyunsaturated long chain fatty acids at the levels found in fish tissue lipids. The polyunsaturated fatty acids are essential for good fish growth and normal cell functions. The requirement of fish for polyunsaturated fatty acids of n-3 series creates problems with respect to feed storage. These types of fatty acids are very labile to oxidation. The products of oxidation may react with other nutrients such as protein and vitamins and reduce the available dietary levels or result in toxic oxidation products.

Numerous studies have been made of the effects of increasing the dietary energy intake by increasing the levels of lipid on food conversion, protein utilization and growth of

various species (Tiemeier et al., 1965; Stickney and Andrews, 1972; Lee and Putnam 1973; Sin, 1973 ; Adron et al., 1976; Higuera et al., 1977; Viola and Rappaport, 1979; Reinitz et al., 1978; Takeuchi et al., 1978 a,b,c; 1979).

Liza parsia being a warm water fish, studies on lipid requirement would prove to be useful as the ambient temperature in tropical waters improve lipid digestibility, absorption and utilization. Such efficacy of lipid as a nutrient has been demonstrated by Kayama and Tsuchiga (1959), Atherton and Aitken (1970) Shcherbina and Kazlauskene (1971) Stickney and Andrews (1972) and Andrews et al. (1978). The identification of an appropriate level of lipid that could be incorporated in practical diets for L.parsia is the ultimate motive of this experiment.

2.2. MATERIAL AND METHODS

Laboratory based experiments were performed to arrive at optimum lipid level for diet formulations. Experimental procedures were as described in part II (pages 7 to 18) except for the variations included here. Liza parsia fry were of mean intial length 28.80mm (± 0.80) and weight 335.86 mg (± 9.17). There were seven triplicate treatment groups; each replicate had 20 fry.

Seven experimental diets (Table V) were prepared (D1....D7) containing graded levels of lipid at 2,4,6,8,10,12% and a zero-lipid diet. Casein and gelatin were used at a constant proportion to provide 40% protein in the diet. Caloric adjustments were done using carbohydrate provided as dextrin. Corn oil and cod liver oil in equal ratio met the lipid requirement of each dietary formulation. Proximate analysis was done and the gross energy content was calculated (Table V).

After the initial acclimatisation period, the experimental diets were fed to the fish for a period of ten weeks; feeding a restricted ration of 7% body weight daily in two doses. Faecal matter was collected daily to calculate the nutrient digestibility. The experimental conditions were monitored regularly. The data collection procedures and analysis of results were as mentioned in part II (page 13 to 18).

2.3. RESULTS

The experimental conditions during the 10 week period were: salinity 15 $\pm$ 1 ppt; temperature 31.1 $\pm$ 3.0^{o}C; pH 7.638 $\pm$ 0.275; ammonia 0.285 $\pm$ 0.144 mg l^{-1} ; oxygen 4.86 $\pm$ 0.25 ppm.

Even though the survival rates (Table VI) were found to be influenced by the lipid levels in the diet, the difference among treatment were not significant especially at levels of 4%

TABLE V: INGREDIENT COMPOSITION OF THE DIETS USED IN THE EXPERIMENT ON LIPID REQUIREMENT

INGREDIENTS (g)	DIETS						
	D1	D2	D3	D4	D5	D6	D7
Casein	30.4	30.4	30.4	30.4	30.4	30.4	30.4
Gelatin	9.6	9.6	9.6	9.6	9.6	9.6	9.6
Dextrin	50.0	46.0	41.0	37.0	32.0	28.0	23.0
Cellulose	5.0	7.0	10.0	12.0	15.0	17.0	20.0
Corn oil	0.0	1.0	2.0	3.0	4.0	5.0	6.0
Cod liver oil	0.0	1.0	2.0	3.0	4.0	5.0	6.0
Mineral mix*	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin mix*	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PROXIMATE COMPOSITION %							
Protein	39.5	38.9	39.0	39.5	39.4	38.8	39.3
Lipid	0.0	2.0	3.8	5.7	7.9	9.9	11.5
Fibre	4.9	6.6	9.3	11.4	14.1	17.5	19.7
Ash	5.5	5.2	5.4	5.1	5.6	5.3	5.5
Nitrogen free extractives	49.6	45.4	40.7	36.2	31.8	27.4	23.0
ENERGY $kJ.g^{-1}$	17.8	17.7	17.6	17.7	17.8	17.7	17.6

* See Table III

TABLE VI: RESULTS OF THE EXPERIMENT ON LIPID REQUIREMENT.

PARAMETERS	LIPID IN DIET (%)						
	D1 (0)	D2 (2)	D3 (4)	D4 (6)	D5 (8)	D6 (10)	D7 (12)
Survival (%)	87	93	97	98	98	97	97
Condition factor	1.23	1.32	1.34	1.30	1.30	1.28	1.29
Weight gained (g)	0.230	0.401	0.427	0.519	0.536	0.579	0.596
Apparent digestibi - lity coefficient of protein	92.87	92.58	92.50	92.41	92.15	91.95	91.86
Apparent digestibi - lity coefficient of lipid	-	83.04	83.72	84.29	84.51	84.41	84.25

and above. Very good survival (96-98%) was recorded at these levels. Even omission of lipid from the diet did not drastically reduce survival.

Lipid level in the diet significantly ($p < 0.01$) influenced the condition factor. Higher values, 1.34 and 1.32, were observed at 4% and 2% lipid inclusion (Table VI).

Dietary lipid levels seemed to distinctly reflect on the total weight gain of the fry. A well defined gradation was observed in the accrued data. The weight gain was best at 596 mg for diet D7(12%). Diet D6(10%) recorded a weight gain of 579 mg. On exclusion of lipid in the diet the gain was reduced by nearly three times (230 mg.). The specific growth rates (Fig.4)were 1.357 for diet D7, 1.333 for diet D6 and 0.731 for the lipid-free diet(D1). The specific growth pattern exhibited was almost linear, peaking off at about 6% lipid level. The percent weight gain and dietary lipid levels exhibited a second order polynomial relationship (Fig.6).

The poorest food conversion ratio was recorded for the lipid-free diet (D1=4.60). The conversion values did not significantly improve when content in the diet was in excess of 6%, though the best conversion rate of 1.91 was obtained with 12% lipid diet. A second order polynomial relationship (Fig.6) existed between the lipid in the diet and gross conversion

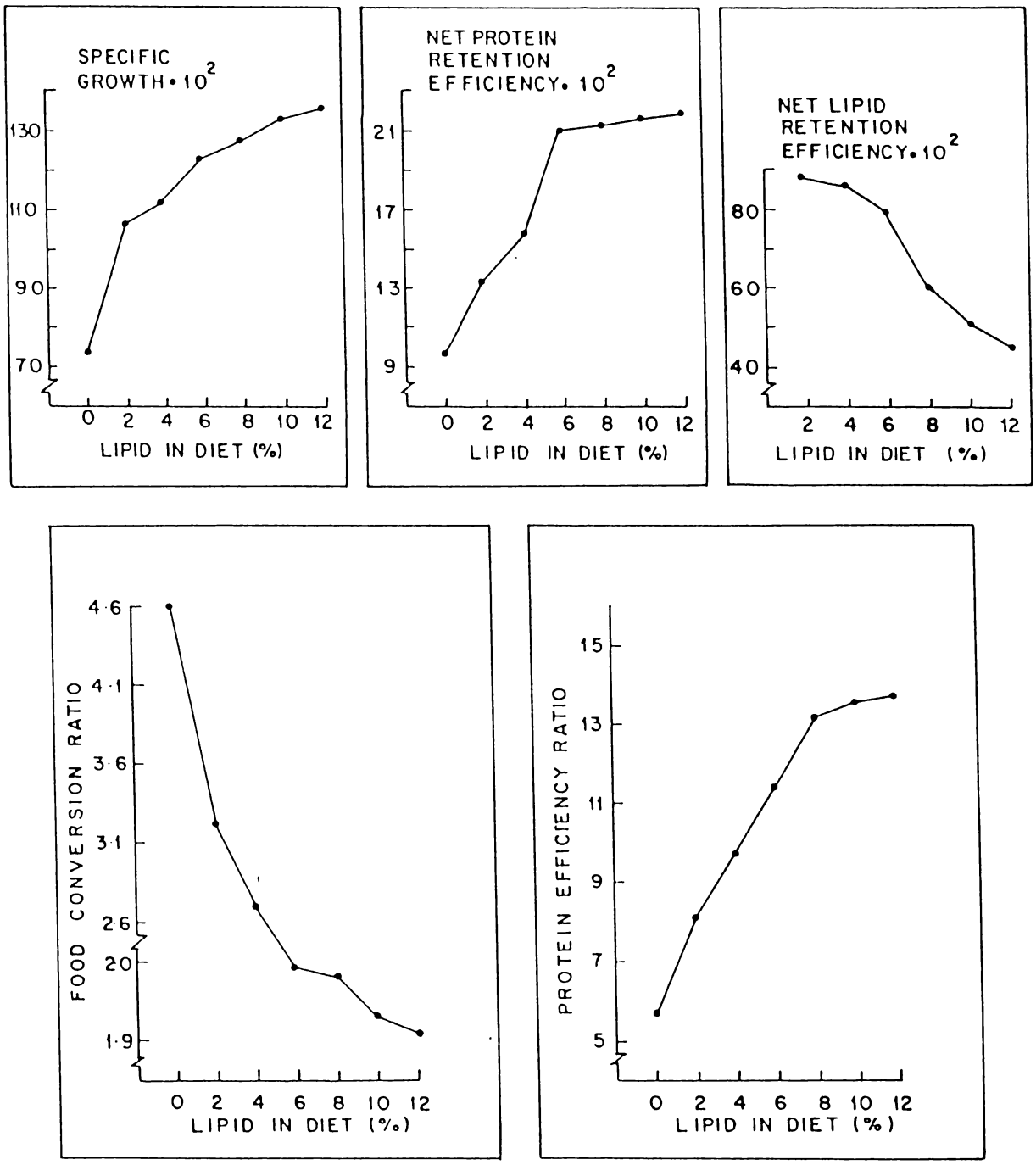


Fig.4. SPECIFIC GROWTH, NUTRIENT RETENTION EFFICIENCY, FOOD CONVERSION RATIO AND PROTEIN EFFICIENCY RATIO IN FISHES FED GRADED LEVELS OF LIPID.

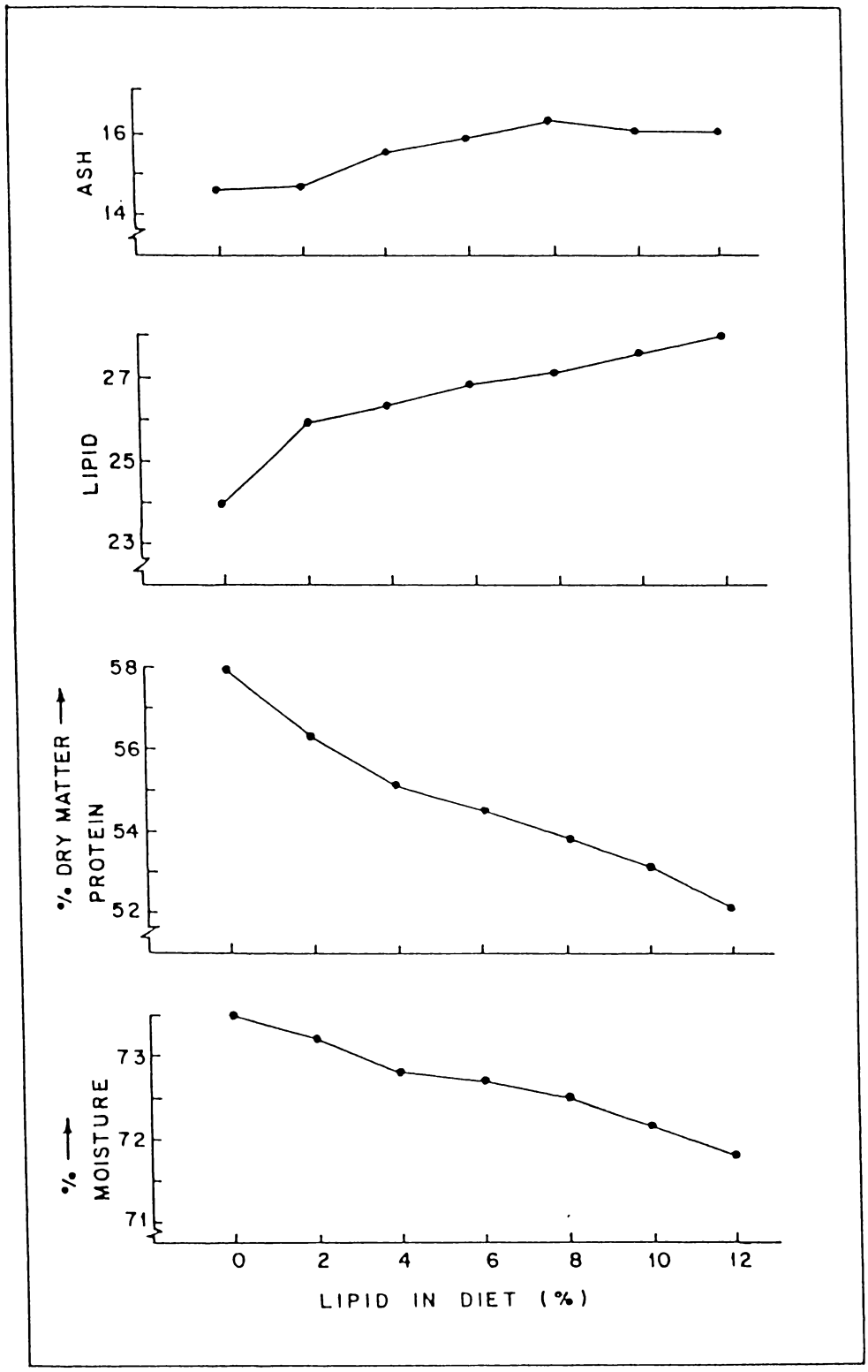
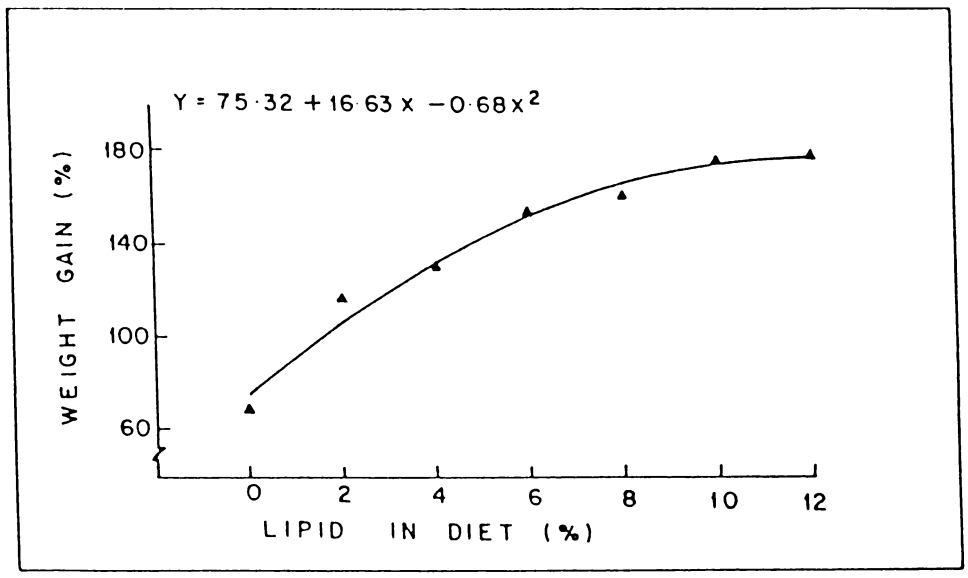


Fig.5. BODY COMPOSITION OF FISHES FED GRADED LEVELS OF LIPID.



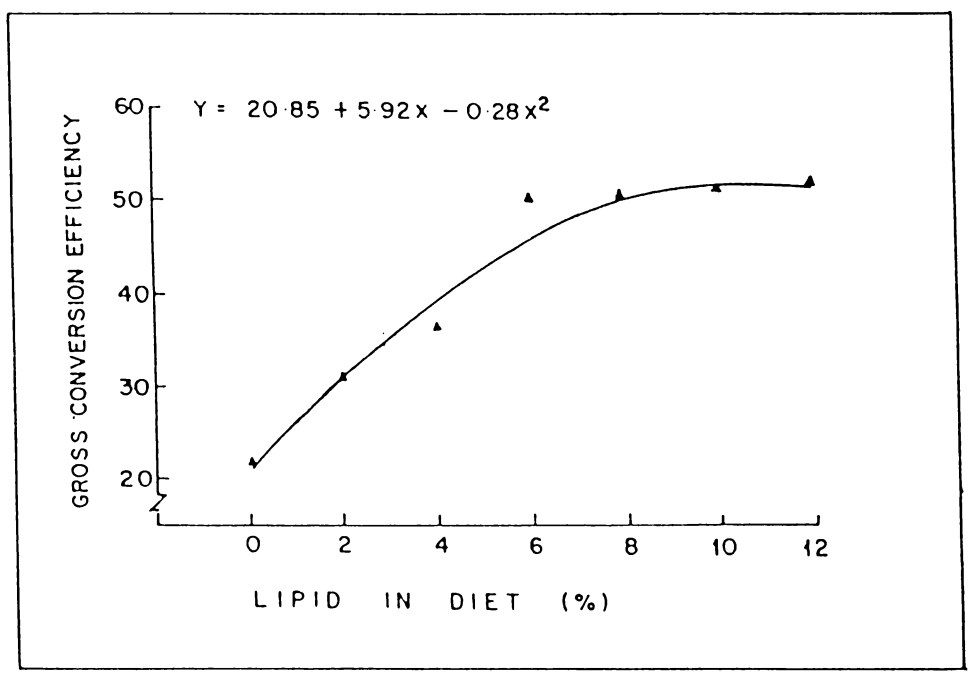


Fig.6. SECOND ORDER POLYNOMIAL RELATION OF PERCENTAGE WEIGHT GAIN/GROSS CONVERSION EFFICIENCY IN FISHES AND DIETARY LIPID CONCENTRATION.

efficiency. The calculated optimum level of lipid for the best conversion ratio was 10.74. The protein efficiency ratio was better at dietary lipid levels of 6% and above and the best ratio was at 12% lipid inclusion. For the zero lipid diet PER was 0.57 (Fig.4).

Proximate analysis of the fish revealed that all the components were significantly ($P < 0.01$) influenced by the level of lipid in the test diets. An inverse relation between body moisture and dietary lipid was evident from the analysis. For the lipid-free diet fed fish,moisture measured was 73.52%, whereas at 12% it was 71.80% (Fig.5). Protein content (Fig.5) in dry tissue also showed a similar trend with the maximum (57.93%) in the lipid-deficient diet. At 12% lipid in the diet, protein percentage recorded was only 52.06% . Dietary lipid influenced significantly the body lipid composition and a direct relationship was observed. The maximum body lipid of 27.96% was recorded for fishes fed the 12% lipid diet. No significant difference in ash content (Fig.5) was observed between the different treatments. Maximum ash content of 16.25% was recorded for 8% lipid diet.

The nutrient retention studies reveal that significant influence has been exerted by the dietary lipid level. Lipid was retained more when the percentage of dietary lipid was low. The lipid retention was lowest (45%) in the fishes fed 12% lipid

(Fig.4). On the contrary, protein was retained more in fishes when dietary lipid level was 6% and more and the retention values were not significantly different from each other. When the dietary lipid fraction was excluded, the protein retention efficiency of the fry was the lowest (9.5%)

A comparison of the data on apparent digestibility coefficient indicates that lipid diqestion was slightly improved at higher (6% and above) dietary lipid levels. The maximum value 84.51% was for the diet containing 8% lipid. A marginal decline in protein digestibility was recorded with the increase in dietary lipid level; the values ranging from 92.87% for diet D1 to 91.86% for diet D7.

2.4. DISCUSSION

The survival rates are a good indicator of the efficacy of feeds supplied to fry and fingerlings. The diets provided to Liza parsia fry in this experiment, except D1 where lipid component was excluded, proved to be very competitive as regards survival. The best survival of 98.33% was obtained when lipid was included at 6 to 8% in the diet. Even complete elimination of lipid did not drastically reduce survival rates. In salmon paars, Austreng (1979) and Bregstrom (1973) also recorded good survival in the same dietary lipid range.

The maximum weight gain of 596 mg (in 10 weeks) was recorded for the fry fed diets with the highest lipid level of 12%. This was 17 mg more than the weight gain recorded for diet with 10% lipid. The rate of increment had greatly slowed down above 6% lipid incorporation (Fig.6). However, the fat-free diet gave the lowest weight gain even as the diets were isocaloric, as observed in the case of carp and rainbow trout (Watanabe et al., 1975; Migita et al.,1973). The specific growth rate was highest for the maximum lipid diet (D7). Similar responses have been recorded in many other species. Channel catfish has been grown successfully on diets containing 10-12% lipid (Stickney and Andrews, 1971,1972; Page and Andrews 1973). In separate studies in the same fish Phillips et al.(1964) and Dupree et al. (1979) obtained maximum growth when fed 15% lipid. But Garling and Wilson (1977) had concluded that 15% lipid was excess and 1-12% levels proved better with comparable growth. On the contrary, Murray et al. (1977) found that the fry of this fish seemed to need only 5% lipid alongwith 25 to 35% protein. But this lower requirement for channel catfish can be equated to the lower metabolic need due to low rearing temperature.

Early workers like Higashi and Kitamikado (cited by Cowey and Sargent, 1972) et al. (1964) fed high levels of 25% and 34% lipid respectively to rainbow trout without any ill effect. Rainbow trout fingerlings have been raised on diets containing

upto 24% herring oil with excellent growth rates (Lee and Putnam, 1973). Yu et al. (1977) and Takeuchi (1978 a) also reported similar values (22% and 20% respectively) for the same species. Reintz et al. (1978) obtained increased weight gain at 16% and 21% lipid when protein level was 30% and 40% respectively. But earlier Ono et al. (1959) and later Watanabe et al.(1979) after testing lipid levels 5 to 25% recorded better growth at 15% lipid. Increase in fat content in diet from 8 to 16% resulted in improved growth in salmon (Bergstrom 1973; Austreng, 1979). Millikin (1982) has recommended 12-17% lipids for fingerlings of stripped bass. Adron et al., (1976) fed diet containing upto 9% to turbot Scophthalmus maximus and found that weight gain of the fish increased upto the maximum level of lipids used. In yellowtail Seriola quinqueradiata, Deshimaru et al.(1982) have observed 9% lipid as the optimum level. On the contrary, Takeuchi et al. (1979) interestingly found no effect on growth rate when diets with lipids ranging from 5 to 15% were fed to carp. A survey of the literature indicates that most fishes do have a optimum level of dietary lipid acceptance. In L.parsia fry too, this level seems to be around 6-8% of lipid, as revealed by the response parameters. However, lipid levels as high as 12% (the maximum used in the study) does not show any inhibiting effect.

Food conversion rate for the fry of L. parsia did not

vary markedly between lipid levels 6 and 12% though the best FCR was at the maximum level (12%) of lipid incorporation. This confirms to the results of Page and Andrews (1973) and Murray et al. (1977) in channel catfish. In, rainbow trout, Watanabe et al. (1979) did not find any significant pattern of variation in the conversion value though the highest lipid level (20%) recorded the best. Dupree et al. (1979) noted that the conversion efficiency in channel catfish with 5% oil was greater than that recorded in the lipid-free diet and that the efficiency decreased at levels above 15%. The protein efficiency ratio also improved with an increase in the amounts of lipid in the diet in L. parsia. Similar observations have been made by Watanabe et al., (1979) in Salmo gairdneri and Jauncey (1982) in Cyprinus carpio. Growth of the animal depends on proper utilization of ingested food and proteins. In this study on L. parsia food and protein utilization were significantly influenced by dietary lipid levels. The high food conversion rate and the low protein efficiency ratio recorded in the group fed lipid-free diet corroborates the previous statement. Inclusion of lipid in the diet significantly improved the two parameters upto about 8% level and above this the dietary lipid did not have any improved effect on food and protein utilization. The good conversion rates and protein efficiency ratios at the higher dietary lipid levels is attributed to the derivation of the maximum potential of energy from the increased

lipid content and maximum utilization of the ingested protein.

Dietary fat has the major impact on body composition. Cultured fish consistently have more body fat than wild fish and the amount and composition of body fat is directly related particularly to the amount of dietary energy supplied as fat (Buckley and Grooves, 1979). Inadequate or excess levels of dietary fat influences the amount of fat deposition, in addition to changes in fatty acid composition of body lipid. In the fry of L. parsia, a positive linear relationship was observed between dietary lipid and body fat. Tissue lipid levels alter significantly with lipid accretion at higher dietary incorporation. Increasing the lipid level to 12% resulted in whole fish lipid level ranging from 23.98% to 27.96%. The linear relationship between dietary lipid and body lipid as observed in L. parsia has been established for several other species; by Buhler and Halver (1961) in chinook salmon; Brett et al. (1969) in sock-eye salmon; Sin (1973) and Jauncey (1982) in mirror carp; Cowey et al. (1975) in plaice; Adron et al. (1976) in turbot; Ogino et al. (1976), Watanabe et al. (1979), Casteldine and Buckley (1980) in rainbow trout; and Garling and Wilson (1976) and Murray et al. (1977) in channel catfish. On the contrary, Seurman et al. (1979) did not find any influence of dietary lipid levels on the total lipid content in the muscle tissues of S. qairdneri. On making a similar observation in

salmonids, Wood *et al*.(1957) attributed it to variations in feeding level.

The protein content in the tissue showed a gradual decrease with increasing levels of lipid in the diet of *L. parsia* as observed in channel catfish by Dupree (1969) Stickney and Andrews (1971, 1972) and Page and Andrews (1973). It was also noted in the present study that increase in carcass lipid was concomittant with decrease in carcass moisture. Such a pattern has also been observed by Brett *et al*. (1969), Andrews and Stickney (1972), Papoutsoglou and Papoutsoglou (1978) Takeuchi (1978a) and Jauncey (1982) for several other species.

The digestibilities of both protein and lipid were fairly good and it seemed that the dietary lipid variation did not exert any influence. The protein digestibilities were marginally better at lower levels. The net protein retention efficiency increased as lipid in the diet increased; but the relationship was negative for lipid level in the diet and lipid retention efficiency.

Lipid at adequate levels spare protein for growth (Watanabe, 1982) in fishes. The main sparing effect of dietary lipid is to replace protein which could otherwise have been catabolised and used for energy production. This type of sparing action has been established for various species of fishes (Lee

and Putnam, 1973; Page and Andrews,1973; Takeuchi et al., 1978 a,b,c; Shimeno et al.,1980; Bromley and Smart 1981) The protein sparing action of lipid is more prominent in carnivorous fishes. Therefore, it is not surprising that we are unable to detect such a mechanism in L. parsia. At higher dietary lipid levels, we infact observe reduced body protein content. It could be that in this mullet, protein is a favoured energy nutrient compared to lipid. The protein retention almost doubles at around 8% lipid level compared to the lipid deficient diet, but still the quantum retained is low, further proving that a certain amount of protein is being catabolised. Comparing this data with the diqestibility values it is found that only about 6% lipid is utilised at dietary levels 8% and above. This again highlights the point that the optimal level of lipid acceptance is around 6% more so because the animal is predominantly not a carnivore.

Several studies have indicated that excessive dietary levels of lipid may result in lipid accumulation in cultured fish. The extra weight gain may end up being discarded with high levels of visceral fat during cleaning and gutting. Thus the design of practical diets is a compromise between a level that will permit good growth with little conversion to energy and an energy level concomittant with high rates of protein synthesis but not such as to lead to greater deposition of carcass lipid. Thus after reviewing all the responses in question, in this

study, it is recommended that dietary lipid inclusion can be at 6-8%. Such were also the recommendations of Phillips (1970) and the teams of Watanabe (1979) and Dupree (1979). Most commercial diets also contain less than 10% lipids due to technical reasons like difficulties encountered with the mechanics of pelletising processing and storing in high lipid diets.

3. DIETARY VITAMIN REQUIREMENT

3.1. INTRODUCTION

Vitamins are a chemically diverse group of vital complex organic substances usually of comparatively small molecular size. Even though they are required in the function of most forms of life, some organisms are unable to synthesize them. Vitamins are critical for the maintenance of normal metabolic and physiological functions. Deficiency diseases occur when they are totally absent from the diet. They are distributed in feedstuffs in small quantities and form a distinct entity from other major and minor food components (Cho et al., 1985).

The importance of vitamins as essential constituents in the diets of animals came to light in the early part of this century. During the past five decades active and rapid progress in vitamin research was made in almost all commercially important species. Little attention was paid on their mode of action in the early decades of the present century. Even so, the view was that they functioned as catalysts. Later on it has come to stay that many vitamins act as essential co-factors in enzyme systems functioning in various aspects of carbohydrate, fat and protein metabolism. (Cowey and Sargent, 1972)

The development of a vitamin deficiency syndrome occurs in several stages. First, the body is gradually depleted of the vitamin or coenzyme due to vitamin deficiency in the diet,

malabsorption or abnormal metabolism. Secondly, there is a fall in the activity of those enzymes dependent on the vitamin. Thirdly, as the vitamin activity is depressed a general decline in the well being of the animal is apparent with loss of appetite and hyperirritability. Finally the deficiency syndrome is perhaps manifest with diagnostic tissue pathology, permanent damage and death (Brin, 1967).

While contributions to vitamin nutrition of mammals and poultry are numerous (Mitchell, 1964) contributions from aquatic species are relatively less. Following the lead of nutritionists working with terrestrial animals, aquaculturists began in the 1940's to investigate specific vitamin requirements for trout. Schneberger (1941) demonstrated that injections of thiamine alleviated paralysis in rainbow trout (Salmo gairdneri) previously fed a minced fish diet. In the early 1950's Wolf (1951) adapted the use of purified test diets to fish, after which rapid progress was made in defining other vitamin requirements of trout. This test diet was refined and used extensively by Halver (1957 a,b). Halver's diet and its modifications have also been used for other species, and most successfully with catfish (Dupree, 1966). Information is also available for other salmonids, eel, carps, red sea bream, yellowtail, and herring.

The slow progress in vitamin nutrition research on aquatic organisms was partly due to the inherent problems posed by the aquatic medium. The major constraint is the leaching of vitamins from the diets when introduced into the water. The extent of this leaching is difficult to quantify, but it is probably reflected in the incremental differences of approximately two orders magnitude in the apparent requirement of fish over chick (Castell et al., 1981). The vitamin levels in the diets of fishes are only recommended levels as compared to poultry where the actual requirements are reflected due to minimal delivery problems. Another factor to be considered in vitamin research is the contribution from gut microbial flora in certain species which may mask the actual requirements. It has also been observed that since vitamins and their precursors are already present in the raw materials, blanket applications of vitamin premix in multi-ingredient diets may result in some excesses (New, 1976).

Exciting new data have appeared on the role of water - soluble vitamin intake and fish health with respect to disease vectors or other stressors which fish encounter in their culture environment. New knowledge has been accumulated on specific quantitative and qualitative vitamin requirements of fish with respect to different fish species, fish size and environment in which they are reared. Much of this information has been summarized in two recent publications of the National Academy of

Sciences (NRC 1981 ; NRC 1983). The FAO manual (1980) on fish feed technology contained more extensive descriptions of general physiology and biochemical functions of water - soluble vitamins and included techniques to minimise loss of these during fish feed manufacture. A more complete description of vitamin chemistry can be found in the treatise of Halver (1972).

Vitamin requirements are affected by size, age, and growth rate of fishes and environmental factors and nutrient relationships. Supplementary diets are formulated primarily to supply protein and energy with the presumption that fishes obtain some vitamins and other growth factors from food organisms present in the environment. The vitamin supplement added to a diet is termed as premix. A premix is formulated to supply the vitamins not present in the dietary ingredients or to compensate for vitamins not completely available and losses that occur during processing and storage. A vitamin allowance that meets only the minimum requirements (for ingredients and added premix) leaves little margin for safety. The level of each vitamin in a pelleted diet should be higher than the required level for several reasons. Certain vitamins may be destroyed during manufacture and storage. Inorder to ensure an adequate level of vitamins prone to oxidation in feeds, diets should be overfortified with protected forms of these vitamins, the use of oxidising fats should be eliminated, improper storage conditions should be avoided and feed should be used soon after pelleting.

Allowance should also be made for leaching of vitamins from the pellets.

So far four fat-soluble and eleven water-soluble vitamins are known to be required by fish. Many of the water - soluble vitamins function either directly or in a modified form as a coenzyme for one or more enzymes. None of the fat - soluble vitamins is known to function as a coenzyme. Among the fat-soluble vitamins, Vitamin A is involved in the metabolism of mucopolysaccharides and visual pigments and for general maintenance of epithelial tissues. Vitamin D functions in calcium homeostasis possibly by induction of co-binding proteins. Vitamin E is a lipid soluble antioxidant and may terminate peroxidative chain reactions among highly unsaturated fatty acids of biomembranes. Finally vitamin K is involved in the electron transport and oxidative phosphorylation and is also a cofactor in blood coagulation process. The rest of the vitamins are dealt at length here.

Thiamine functions metabolically as a coenzyme which has been characterized as thiamine pyrophosphate. This compound is an essential co-factor for the enzymic transfer of acyl groups from many substrates such as α-keto acids and ketophosphates. The reactions in which they partake include: 1.) the non-oxidative decarboxylation of keto acids to aldehydes, 2.) conversion of α-keto acids to acyl phosphates and formates and

3.) Oxidative decarboxylation of pyruvate. Essentiality of dietary thiamine has been verified for rainbow trout, brook trout and brown trout (McLaren et al., 1947; Phillips and Brockway, 1957), chinook salmon (Halver, 1957a), channel catfish (Dupree, 1966; Murai and Andrews, 1978), rainbow trout (Kitamura et al., 1967; Aoe et al., 1967, 1969), eel (Arai et al., 1972), red sea bream (Yone, 1975) and turbot (Cowey et al., 1975).

The riboflavin molecule is composed of a ribose moiety attached to an isoalloxazine nucleus. Two coenzyme forms of the vitamin occurs: flavin-mononucleotide and flavin-dinucleotide. These coenzymes function widely in the carbohydrate metabolism where their general role is that of hydrogen transfer from nicotinamide - adenine - dinucleotides to the cytochrome system. They are part of the large and complex system involved in the transfer of hydrogen from substrates to molecular oxygen. Because of its crucial role in metabolism and because it cannot be synthesised by animals, riboflavin is essential in diets of all animals including fish. Dietary riboflavin requirement has been reported for rainbow trout (McLaren et al., 1947; Kitamura et al.,1967 ; Poston et al., 1977; Takeuchi et al., 1980); brook trout, brown trout and lake trout (Phillips, 1970); Atlantic salmon (Phillips, 1959), channel catfish (Dupree, 1966; Murai and Andrews, 1978; Woodward, 1984), common carp (Aoe et al.,1967; Ogino, 1967), Japanese eel (Arai et al., 1972) and red sea bream (Yone, 1975).

Pyridoxine includes a family of closely related pyridine derivatives all of which occur naturally and represent different forms of vitamin B6. The active coenzymes are pyridoxal phosphate and pyridoxamine phosphate which are required for many enzymatic reactions in which amino acids are metabolized. Transamination reactions in which amino acid is converted into an α-keto acid and catabolized or an α-keto acid is converted to an amino acid are the most common types of reaction requiring pyridoxal phosphate. Other reactions requiring pyridoxal phosphate as a coenzyme include conversion of tryptophan to acetyl coenzyme A and pyruvate to cysteine. Important contributors on pyridoxine requirements in fishes were by McLaren et al. (1947), Phillips and Brockway (1957), Halver(1957a), Coates and Halver (1958), Phillips (1959), Ogino (1965), Dupree (1966), Kitamura et al. (1967), Sakaguchi et al. (1969), Arai et al. (1972), Yone (1975), Adron et al. (1978), Jruss (1978), Kissil et al. (1981), Halver (1982) and Herman (1985).

In animal tissues nicotinic acid is converted to nicotinic acid-mononucleotide and then to coenzyme forms nicotinamide-adenine-di nucleotide (Coenzyme I) and nicotinamide adenine-dinucleotide-phosphate (Coenzyme II). These coenzymes function as a part of a large number of oxidoreductases, collectively called pyridine linked dehydrogenases. More than two hundred dehydrogenases function in normal metabolism. They

act as hydrogen acceptors in various energy yielding and biosynthetic pathways. Niacin has been shown to be an essential dietary constituent for rainbow trout (McLaren et al., 1947), brook and brown trout (Phillips and Brockway, 1957), lake trout (Phillips, 1959), chinook salmon (Halver, 1957a), channel catfish (Dupree, 1966; Andrews & Murai, 1978), common carp (Aoe et al., 1967), Japanese eel (Arai et al., 1972), brook trout (Poston and Di Lorenzo, 1973) and red sea bream (Yone, 1975).

For the living organisms, pantothenic acid functions solely as a component of coenzyme A. While animals have an absolute requirement for pantothenate, they can synthesize coenzyme A from it by a series of enzymic reactions occuring generally in the liver. Coenzyme A forms high energy acyl groups which participate in reactions like fatty acid oxidation and other biological acetylations such as conversion of choline to acetyl choline and conversion of oxaloacetic acid to citric acid. Acetyl Coenzyme A is also required in reactions in which the carbon skeleton of amino acids enter into energy yielding metabolic pathways. Pantothenic acid essentiality in fishes has been demonstrated by McLaren et al.(1947), Phillips and Brockway (1957), Halver (1957), Coates and Halver (1958), Phillips (1959), Kitamura et al.(1967), Arai et al. (1972), Yone (1975), Murai and Andrews (1975) and Wilson et al.(1983).

Unlike the preceding water-soluble vitamins, choline

has no coenzyme function. It occurs in animal cells mainly as a constituent of phospholipids but also as acetyl choline and free choline. Choline has three known functions: as a precursor to the neurotransmitter acetyl choline, as a methyl donor in metabolic reactions and as a component in choline phosphoglycerides or phospholipids (structural role in membrane systems). Almost all the workers mentioned in the case of the preceding vitamin have pointed out the importance of choline too.

Inositol is a water-soluble growth factor for which no coenzyme function is known. Inositol, also known as myoinositol, is a sugar alcohol that is apparently not required in the diet of most animals. The only known function of inositol is as a component of the inositol phosphoglycerides that are found in many cells. Studies on inositol requirement of fishes in many include those of McLaren et al.(1947), Phillips and Brockway (1957), Halver (1957a), Coates and Halver (1958), Aoe and Masuda (1967), Arai et al. (1972), Yone (1975) and Burtle (1981).

Ascorbic acid is structurally one of the simplest vitamins. Most birds and mammals can synthesize it, but not fish. It has been proved that many fish require ascorbic acid for maximal growth. Ascorbic acid has non-specific activity in several areas of metabolism. It is a strong reducing agent loosing two hydrogen atoms to become dehydroascorbic acid. It is also a co-factor in the hydroxylation of proline to hydroxy

proline, a precursor to collagen. Thus ascorbic acid deficiency results in impaired collagen metabolism. Though some of the specific biochemical roles of ascorbic acid are known, its general physiological function is not, since it can be replaced in specific reactions by other reducing agents (Lehninger, 1975). A review of ascorbate metabolism in fish has been published by Tucker and Halver (1984). Extensive research has been conducted on the qualitative and quantitative ascorbic acid requirements of fish. These include those of McLaren et al. (1947), Kitamura et al. (1965), Hilton et al. (1978), Sato et al. (1978) and John et al. (1979) for rainbow trout; Poston (1967) for brook trout; Halver (1969), Yoshinaka et al. (1978) and Tucker and Halver (1984) for coho salmon; Sakaguchi et al. (1969) for yellow tail; Arai et al. (1972) for Japanese eel; Lovell (1973), Wilson and Poe (1973), Andrews and Murai (1975) and Lim and Lovell (1978) for channel catfish; Yone (1975) for red sea bream; Mahajan and Agrawal (1979) for snakehead and Mahajan and Agrawal (1980) for mrigal.

Test diets based on Halver's diet (1957a,b) have been used frequently to identify the essential vitamins. Values for dietary requirement of certain vitamins may depend on the method of assessment used - growth rate or tissue level - and where certain vitamins fulfil more than one metabolic role, the requirements for each may differ. Because many vitamins function

as coenzymes, one might logically regard the vitamin requirement as the dietary level of a vitamin which permits optimal activity of all those enzymes for which the vitamin serves (possibly in a modified form) as a coenzyme. Thus the correlation between vitamin intake and the activity of related enzymes in controlled experiments would be the ideal way to establish quantitative vitamin requirements (Jauncey, 1982). However, the type of experiments performed till date concentrate on growth and tissue level of the vitamin. The present experimental study too considers growth response as the criterion for arriving at vitamin requirement. In addition, pathomorphological techniques have been adopted wherever suitable.

The vitamin requirements of hardly any tropical brackishwater finfish has been studied. Hence an attempt is made here, though superficial to probe into the essentiality of certain water-soluble vitamins in the diet of the mullet Liza parsia. Besides, an experimental study has been carried out to determine optimal level of vitamin mixture in the diet.

3.2. MATERIAL AND METHODS

Experiments were conducted in the laboratory. The general techniques adopted in the conduct of the experiment has been same as described in part II (pages 7 to 18).

3.2.1 Qualitative requirements

Twentyfive fry of Liza parsia were alloted to each of the experimental aquaria. There were eight treatment groups and one control group, all in triplicate. The mean initial length of the fish was 25.65 mm (± 0.32) and the mean weight was 267.70 mg (± 9.60).

Test diets to examine the vitamin essentiality were prepared identically, except for the deletion of specific vitamin in each diet. Fish on test diet was compared with fish receiving a control diet having all the vitamins to discover signs of deficiency. Table VII indicates the composition of the experimental diets. Vitamin free casein and gelatin were the protein sources. Diets D1 to D8 were devoid of the respective vitamins, in order : choline, inositol, ascorbic acid, nicotinic acid, pantothenic acid, riboflavin , thiamine and pyridoxine. The control diet D9 had the full complement of vitamins. As and when the digestibility studies were conducted 1% chromic oxide was included in the diet; necessary adjustments being made with cellulose levels. The diets were maintained isocaloric to the extent possible. With the lapse of the acclimation period, the animals were put on experimental diets. They were fed a daily ration of 7% of their body weight, in two doses. The experiment was conducted over a period of 21 weeks. Relatively stress free, uniform conditions were ensured during the period. This was done

TABLE VII: INGREDIENT COMPOSITION OF THE DIETS USED IN THE EXPERIMENT QUALITATIVE VITAMIN REQUIREMENT

INGREDIENTS (g)	DIETS								
	D1	D2	D3	D4	D5	D6	D7	D8	D9
Cellulose	8.500	8.200	8.100	8.075	8.050	8.020	8.005	8.005	8.000
Vitamins									
Choline chloride	-	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Inositol	0.200	-	0.200	0.200	0.200	0.200	0.200	0.200	0.200
L-Ascorbic acid	0.100	0.100	-	0.100	0.100	0.100	0.100	0.100	0.100
Nicotinic acid	0.075	0.075	0.075	-	0.075	0.075	0.075	0.075	0.75
Calcium pantothenate	0.050	0.050	0.050	0.050	-	0.050	0.050	0.050	0.050
Riboflavin	0.020	0.020	0.020	0.020	0.020	-	0.020	0.020	0.20
Thiamine hydrochloride	0.005	0.005	0.005	0.005	0.005	0.005	-	0.005	0.005
Pyridoxine hydrochloride	0.005	0.005	0.005	0.005	0.005	0.005	0.005	-	0.005
Menadione	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Folic acid	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015
Cyanocobalamin	0.0011	0.0011	0.0011	0.0011	0.0011	0.0011	0.0011	0.0011	0.0011
Biotin	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
Cholecalciferol	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
-Tocopherol	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040	0.040

Other ingredients (g) Casein = 29.979, Gelatin = 12.295, Dextrin = 38.729
Corn oil = 3.000, Cod liver oil = 3.000, Mineral mix = 3.000
Mean Energy content = 18.36 kJ. g^{-1}

because tentative vitamin requirements of fish is related to the species, its environment and the physiological stress encountered. The response parameters considered were mortality, growth and food conversion, besides haematological and histological observations, wherever necessary.

3.2.2 Quantitative requirement

The experiment was conducted only to determine the effect of selected levels of the vitamin mix included in the diets.

Five diets (D1 to D5) containing the vitamin mixture at 0.5, 1, 1.5, 2 and 2.5% of the diet (Table VIII) were prepared. Cellulose level was adjusted to incorporate the specific amount of vitamin mix. The rest of the ingredients were the same as that used for the qualitative studies (Table VII). Each of the diet was fed to triplicate groups (25 numbers in each group) of Liza parsia fry of initial mean length 25.54 mm (± 0.26) and mean weight 263.67 mg (± 6.63). The experimental duration, conditions and methods were the same as described in the previous experiment. General data collection and analytical procedures have been described in Part II. The diet treatment resulting in low mortality, maximum growth and food conversion was selected as the vitamin level meeting the requirements.

Table VIII: INGREDIENT COMPOSITION OF THE DIETS USED IN THE EXPERIMENT ON QUANTITATIVE VITAMIN REQUIREMENT

INGREDIENTS (g)	DIETS				
	D1 (0.5)	D2 (1.0)	D3 (1.5)	D4 (2.0)	D5 (2.5)
Cellulose	8.500	8.000	7.500	7.000	6.500
Vitamins:					
Choline chloride	0.250	0.500	0.750	1.000	1.250
Inositol	0.100	0.200	0.300	0.400	0.500
L-Ascorbic acid	0.050	0.100	0.150	0.200	0.250
Nicotinic acid	0.038	0.075	0.112	0.150	0.190
Calcium pantothenate	0.025	0.050	0.075	0.100	0.125
Riboflavin	0.010	0.020	0.030	0.040	0.050
Thiamine hydrochloride	0.003	0.005	0.008	0.010	0.013
Pyridoxine hydrochloride	0.003	0.005	0.008	0.010	0.013
Menadione	0.002	0.004	0.006	0.008	0.010
Folic acid	0.0007	0.0015	0.0022	0.0030	0.0037
Cyanocobalamin	0.0006	0.0011	0.0017	0.0020	0.0028
Biotin	0.0002	0.0005	0.0007	0.0010	0.0012
Cholecalciferol	0.0001	0.0002	0.0003	0.0004	0.0005
-Tocopherol	0.020	0.040	0.060	0.080	0.100

Other ingredients (g) Casein = 29.979, Gelatin = 12.295, Dextrin = 38.729, Corn oil = 3.000, Mineral mix = 3.000 g.

Mean Energy content = 18.4 $kJ.g^{-1}$

* Values in parantheses indicate the percentage vitamin mix in diet.

3.3. RESULTS

The experimental conditions were as follows:

Salinity 15 ± 1ppt, temperature 31.2 ± 4.8° C , pH 7.787 ± 0.206, ammonia 0.363 ± 0.171 mgl^{-1}, and oxygen 5.31 ± 0.22ppm.

3.3.1 Qualitative requirements

Deletion of vitamins had a highly significant influence ($P < 0.01$) on the survival rates of the fry of L.parsia. The survival was very low when riboflavin (48%) and niacin (49.3%) were deleted. The rates were relatively poor in the case of pyridoxine (56%), choline (60%) and thiamine (70.7%) deleted diets. Survival was 88% in the control diet (D9) fed groups.

The influence of treatment on condition factor was prominent ($P < 0.01$) and the control value (1.30) was significantly ($P < 0.05$) superior to other experimental values. The factor was comparatively lowered when pyridoxine (1.16), riboflavin (1.18), choline (1.18) and ascorbic acid (1.19) were deleted from the diets (Table IX).

Growth was also significantly ($P < 0.01$) influenced by the different treatments in this experiment (Table IX). Over the 21 week period, the lowest weight gain of 599 mg was recorded for niacin deleted diets. For pyridoxine deleted diet it was 653 mg, pantothenic acid: 664 mg,thiamine: 744 mg, ascorbic acid: 808 mg,

TABLE IX. RESULTS OF THE EXPERIMENT ON QUALITATIVE VITAMIN REQUIREMENT

PARAMETERS	DIETS								
	D1	D2	D3	D4	D5	D6	D7	D8	D9
Survival (%)	60.00	81.00	77.00	49.00	76.00	48.00	71.00	56.00	88.00
Condition Factor	1.18	1.23	1.19	1.20	1.24	1.18	1.20	1.16	1.30
Weight gained (g)	0.850	0.966	0.808	0.599	0.664	0.845	0.744	0.653	1.132
Apparent digestibility coefficient of protein	91.32	92.14	92.39	89.71	91.09	91.46	90.36	90.05	92.58
Apparent digestibility coefficient of lipid	82.87	83.25	83.43	82.29	81.72	82.13	82.49	82.77	84.11

riboflavin: 845 mg, choline: 850 mg and inositol it was 966 mg. The weight gain in control diet fed groups (1132 mg) was above the highest value in treatment groups by 166 mg and the lowest value by 533 mg. The weight gains in the case of riboflavin and choline deleted diet were almost similar. The weight gains in the case of pantothenic acid and pyridoxine were also not significantly different.

The triweekly weight recordings were subjected to statistical analysis and it was observed that in the initial phase of the experiment the diets without pantothenic acid, riboflavin, thiamine and pyridoxine produced almost similar response as the control diet containing the vitamins (Fig. 7). By the end of the 9th week clear cut distinction could be made in the weight gain of different groups. Triweekly increments for choline, inositol and ascorbic acid deleted diets which were almost in par with the control group in the initial stage of the experiment were drastically reduced towards the final phase of the experiment. Regression analysis performed on the data could establish a positive relationship between weight gain and time in the different groups (Fig. 8). The growth pattern in diets without ascorbic acid (D3), riboflavin (D6), pantothenic acid (D5) and pyridoxine (D8) were almost identical. Specific growth rate also was the lowest for niacin deleted diet (0.712). The fishes fed all the vitamins (D9) had a specific growth rate of 0.923 (Fig.9).

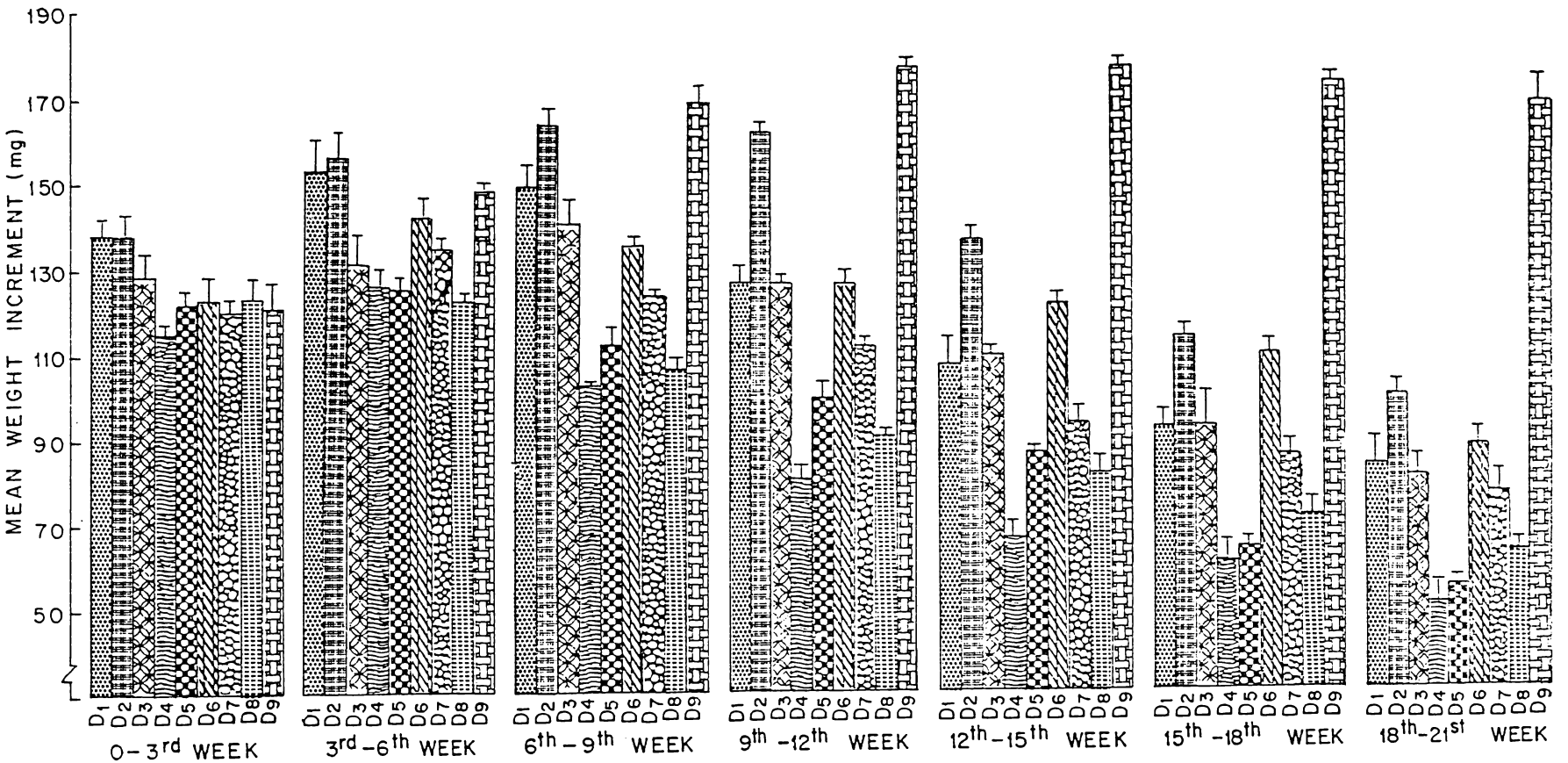


Fig.7. TRIWEEKLY WEIGHT INCREMENT IN FISHES FED A CONTROL DIET (D9) AND DIETS DEFICIENT IN DIFFERENT VITAMINS.

D1: CHOLINE, D2: INOSITOL, D3: ASCORBIC ACID, D4: NICOTINIC ACID, D5: PANTOTHENIC ACID, D6: RIBOFLAVIN, D7: THIAMINE, D8: PYRIDOXINE.

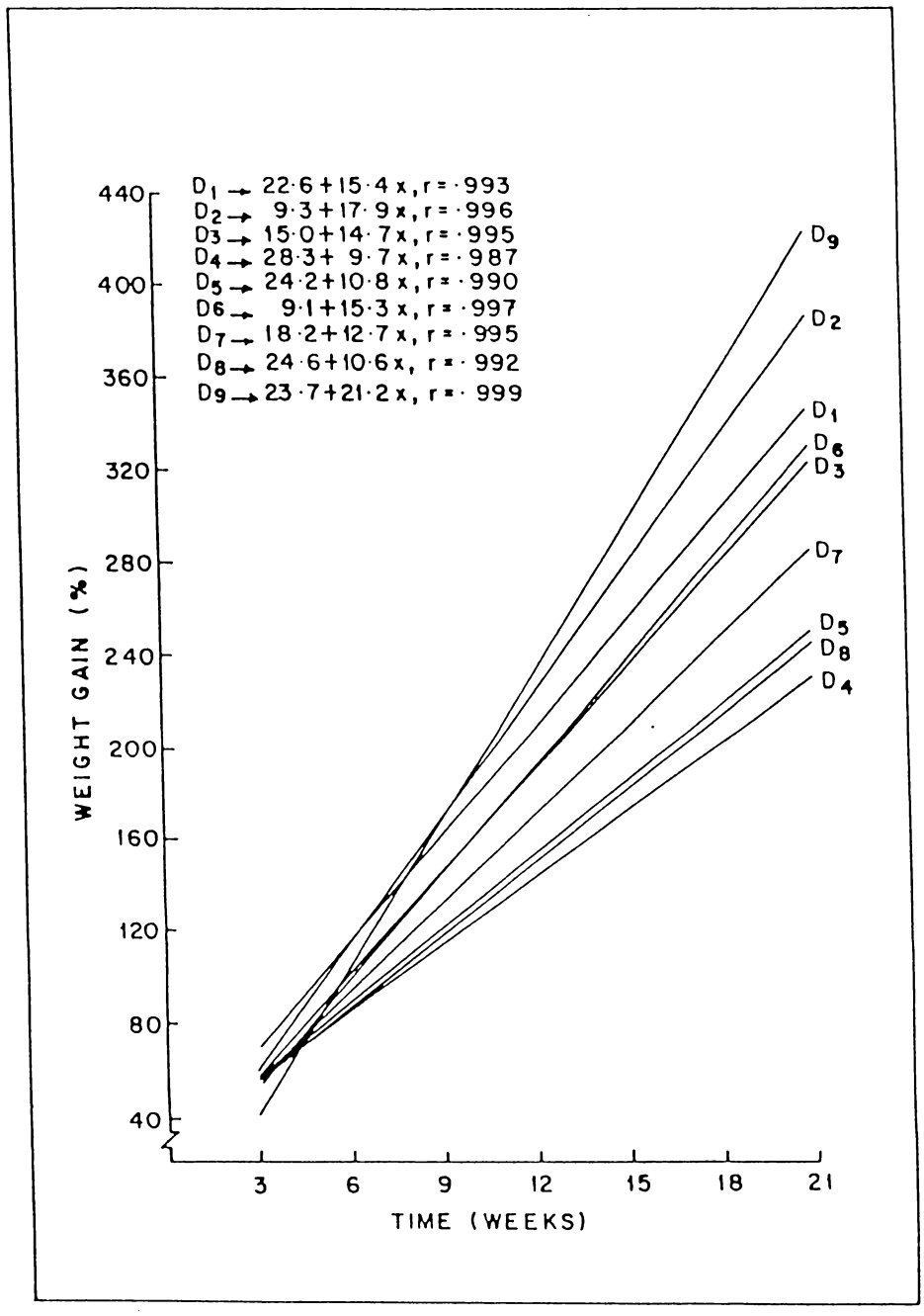


Fig.8. RELATION BETWEEN WEIGHT GAIN AND TIME IN FISHES FED DIETS DEFICIENT IN DIFFERENT VITAMINS.

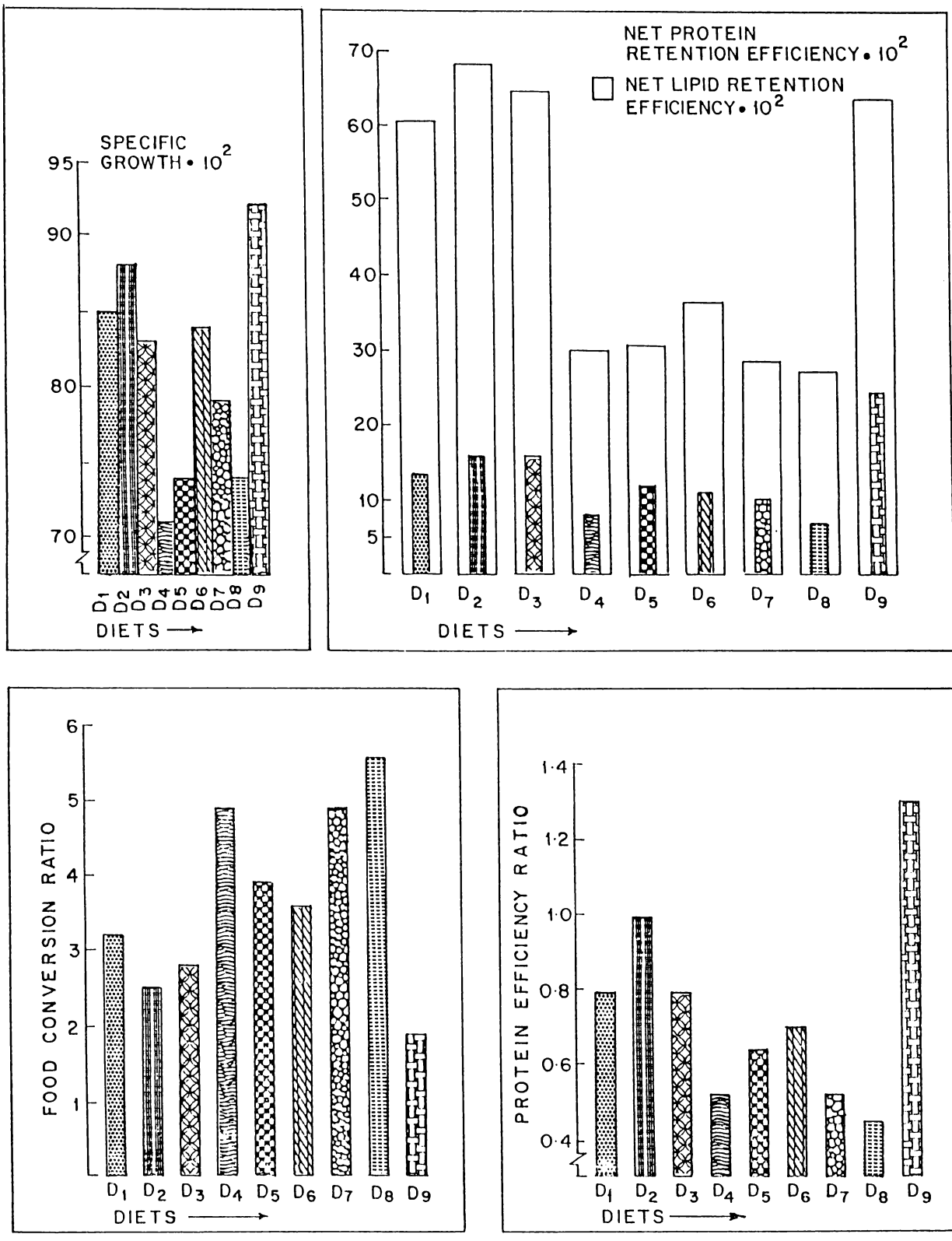


Fig.9. SPECIFIC GROWTH, NUTRIENT RETENTION EFFICIENCY, FOOD CONVERSION RATIO AND PROTEIN EFFICIENCY RATIO IN FISHES FED DIETS DEFICIENT IN DIFFERENT VITAMINS.

The food conversion ratios were also significantly ($P < 0.05$) influenced by the deletion of vitamins. The deletion of pyridoxine resulted in the poorest conversion (5.63). The conversion rate (4.85) when niacin was excluded from the diet was not significantly different from that of thiamine (4.78). The ratios obtained with pantothenic acid, riboflavin, choline, ascorbic acid and inositol deficient diet were 3.90, 3.58,3.19,2.82 and 2.52 respectively. For the control diet (D9), a comparatively better conversion of 1.92 was obtained.

The protein efficiency ratio was the lowest (0.45) when pyridoxine was deleted from the vitamin mix. The values obtained for other vitamin deficiency diet were niacin: 0.52 thiamine: 0.52, pantothenic acid: 0.64, riboflavin: 0.70, choline: 0.79, ascorbic acid: 0.89 and inositol: 0.99. The PER for the control diet D9 (1.30) was significantly different ($P < 0.05$) from those fed vitamin deficient diets (Fig. 9).

Deletion of different vitamins seemed to significantly influence the body composition of the fishes. Body moisture (70.37%) was lowest when ascorbic acid was removed from the diet. The body moisture content in control diet was only 72.39% whereas in the pantothenic acid deficient diet it was 73.29% (Fig 10). The body protein in control diet (61.51%) showed a minor edge over the content (61.08%) in thiamine deficient diet. The most significant difference (protein content = 51.23%) was that when

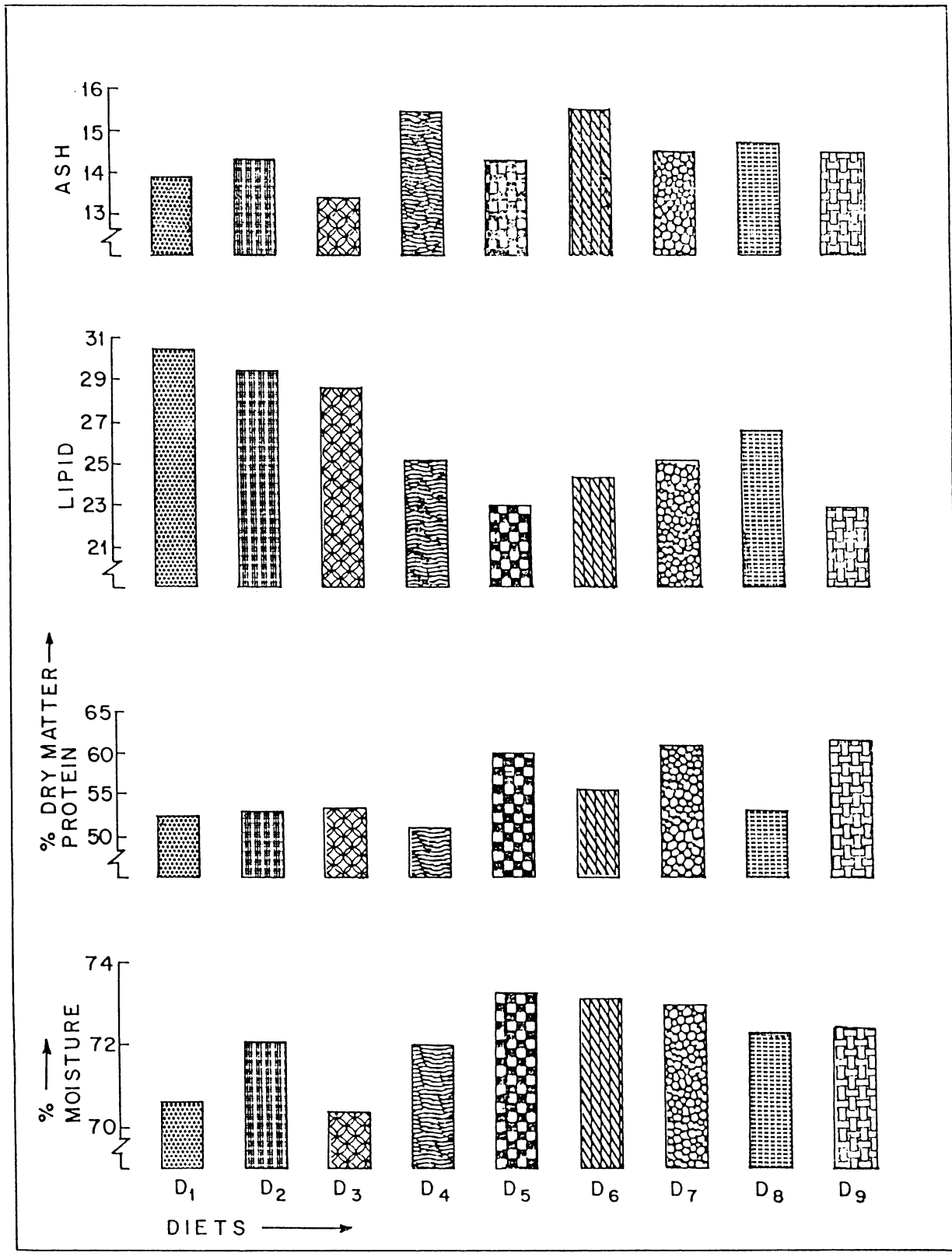


Fig.10. BODY COMPOSITION OF FISHES FED DIETS DEFICIENT IN DIFFERENT VITAMINS.

niacin was removed from the mix. The deletion of macro vitamins - choline, inositol and ascorbic acid as well as pyridoxine also resulted in reduced body protein (Fig 10). Removal of vitamins resulted in enhanced body lipid content as compared to the control. The lipid content of pantothenic acid free diet (23.03%) was not significantly different from that of the control diet (22.77%). When riboflavin was excluded from the vitamin mix the value was 24.41% . Exclusion of niacin and thiamine from the diet resulted in almost similar lipid values of 25.2 and 25.1% respectively. Ash content did not differ significantly from the control (14.53%) when pyridoxine (14.68%), thiamine (14.46%), pantothenic acid (14.34%) and inositol (14.25%) were excluded from the vitamin mix (Fig. 10).

Deletion of vitamins profoundly influenced ($P < 0.01$) the retention efficiency of nutrients (Fig.9). Protein retention was remarkably low at the deletion of pyridoxine (7.3%), niacin (8.2%) and thiamine (9.9%). The value for the control group (24.6%) was significantly ($P < 0.05$) higher than all other experimental groups. The retention efficiency of lipid too was lowest (27.1%) when pyridoxine was excluded from the diet. Equally poor were the values obtained for thiamine (28.6%) and niacin (29.9%) deficient diets. Comparatively poor recordings were made in the case of pantothenic acid (30.7%) and riboflavin (36.6%). The exclusion of macrovitamins (choline, ascorbic acid

and inositol) did not seem to affect the lipid retention efficiency and in the case of ascorbic acid and inositol it was slightly higher than the control value of 63.4%.

The apparent digestibility coefficient of protein (92.58%) was the highest for the control diet (D9) with all the vitamins. Deletion of ascorbic acid (D3 = 92.39%) and inositol (D2 = 92.14%) did not seem to alter the coefficient. The value recorded when niacin was deleted was the lowest (D4 = 89.71% ; Table IX). Lipid digestibility (84.11%) was also the best when all vitamins were present (D9). Lipid digestibility was comparatively low (81.72%) when pantothenic acid was deleted from the diet supplied to fishes. Lower digestibility coefficients were also recorded in the case of riboflavin (82.13%) and niacin (82.29%) deficient diets (Table IX).

The deficiency symptoms exhibited in L. parsia on deprivation of the specific water - soluble vitamins are indicated in Table X.

3.3.2 Quantitative requirements

Survival rates were not significantly influenced by the levels of vitamin mixture incorporated in the diet. The maximum survival of 90.7% was obtained when the mixture was included at 2.5% (Table XI).

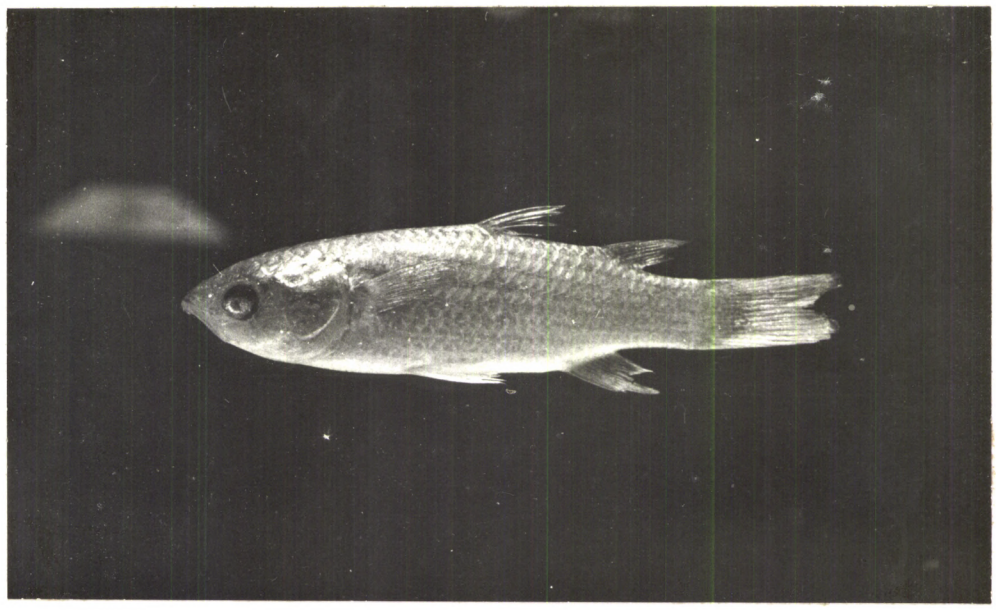
TABLE X: INDICATORS OF VITAMIN DEFICIENCY EXHIBITED BY THE FRY OF *L. PARSIA*

VITAMIN	SYMPTOM OBSERVED	APPROXIMATE PERIOD FOR ONSET (Weeks)	PERCENTAGE ANIMALS AFFECTED
Thiamine	Anorexia	3	All
	Reduced growth increment	9	All
	Mortality	15-18	29
	Uneasy rapid movements on shock resulting in disorientation	12	Almost All
	Subcutaneous haemorrhage leading to muscular dystrophy. (Plate 3,7).	-	40
Riboflavin	Anorexia and poor growth	12	All
	Mortality	12-15	All
	Finerosion	12	25
	Photophobia	10	Generally all
	Mono & bilateral corneal opacity (Plate 3)	18	20
Pyridoxine	Poor appetite and growth	6	All
	Mortality	12	44
	Hyper irritability and erratic swimming	15	All
Nicotinic acid	Poor growth	5	All
	Mortality	9	51
	Skin erosin and muscle damage (Plate 5,8)	-	50
Pantothenic acid	Anorexia	6	All
	Poor weight gain	9	All
	Mortality	15	24
	Warped operculum (Plate 4)	12	40
	Liver damage (Plate 10)	-	-
	Typical gill condition lamellae clubbed, filaments erode and mucous covered (Plate 11)	15	60
Choline	Anorexia and reduced growth	10	All
	Mortality	15	40
	Pale liver and haemorrhagic damage (Plate 9)	18	-
Inositol	Growth reduction	15	All
	Mortality	18	19
	Distended abdomen (Plate 4)	18	25
	Haemorrhagic fin box leading to muscle degeneration(Plate13)	18	10
Ascorbic acid	Growth reduction	15	All
	Mortality	15	20
	Scoliosis/lordosis (Plate 6)	15	50
	Anaemic condition (Plate 12)	-	25

TABLE XI. RESULTS OF THE EXPERIMENT ON QUANTITATIVE VITAMIN REQUIREMENT

PARAMETERS	VITAMIN MIX IN DIET (%)				
	D1 (0.5)	D2 (1.0)	D3 (1.5)	D4 (2.0)	D5 (2.5)
Survival (%)	85.00	88.00	84.00	88.00	91.00
Condition Factor	1.28	1.30	1.29	1.31	1.29
Weight gained (g)	1.070	1.132	1.148	1.153	1.143
Apparent digestibility coefficient of protein	92.16	92.58	92.43	92.47	92.25
Apparent digestibility coefficient of lipid	83.62	84.11	84.27	84.46	84.39

a



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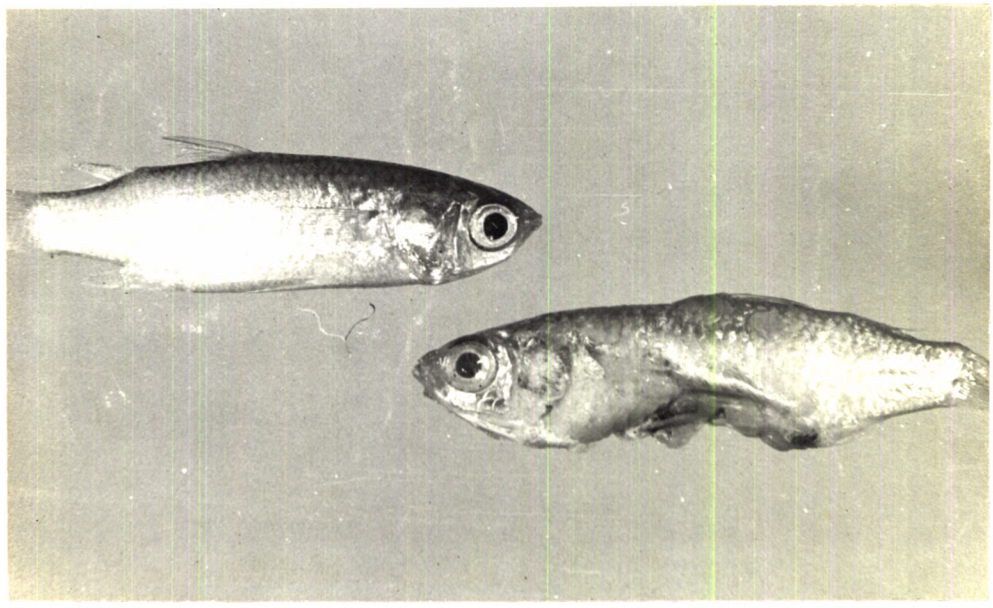
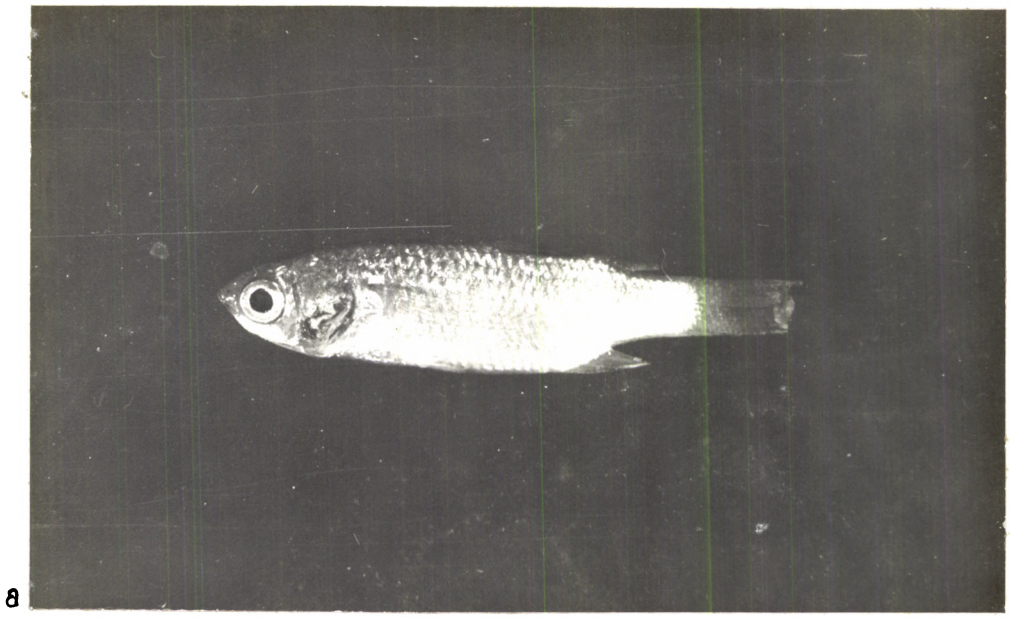


PLATE 3. VITAMIN DEFICIENCY SYMPTOMS

(a) Corneal opacity - Riboflavin

(b) Muscular dystrophy - Thiamine.



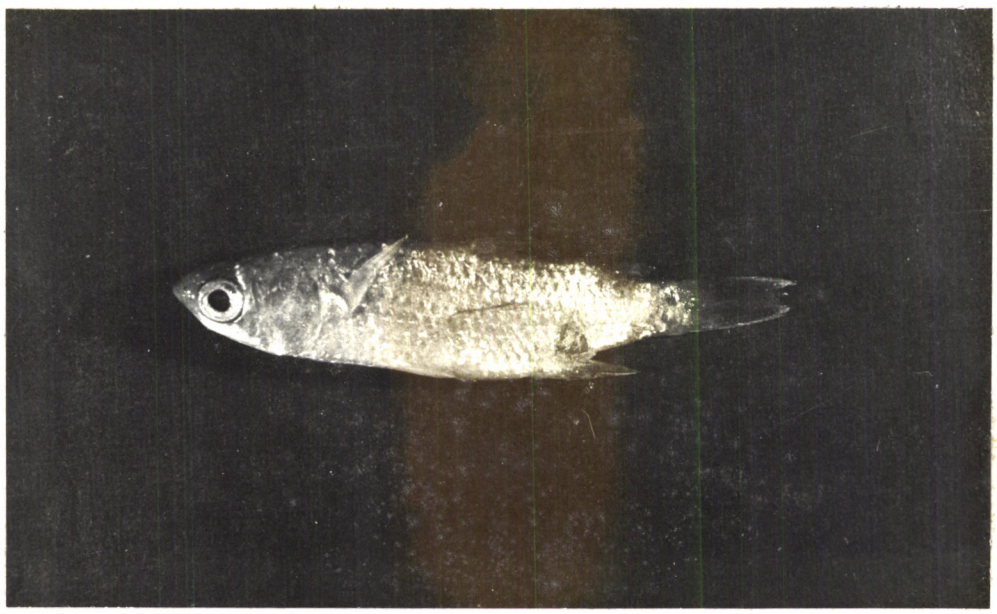
a

b

PLATE 4. VITAMIN DEFICIENCY SYMPTOMS

(a) Warped operculum - Pantothenic acid

(b) Distended stomach - Inositol.



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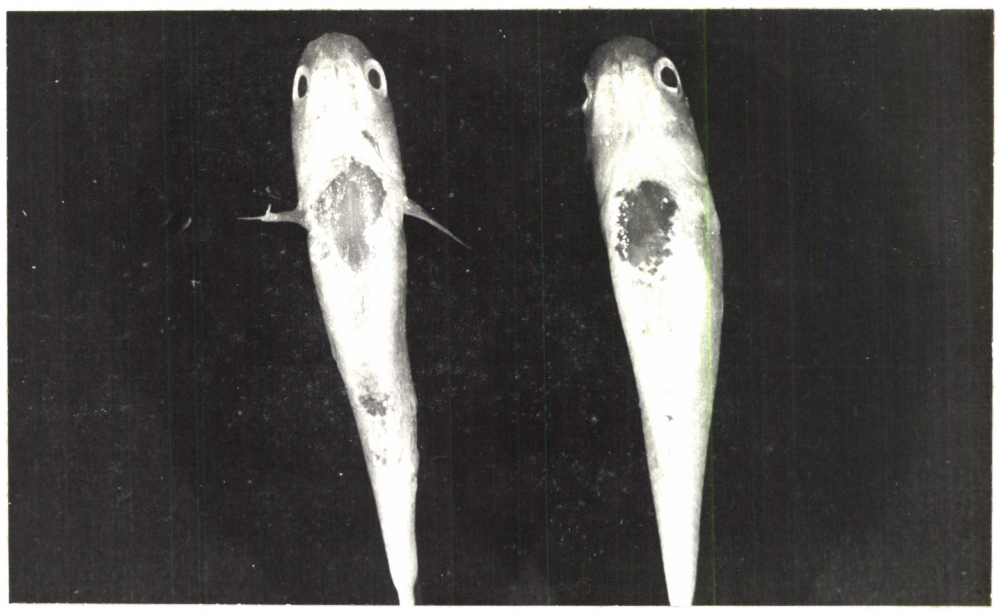
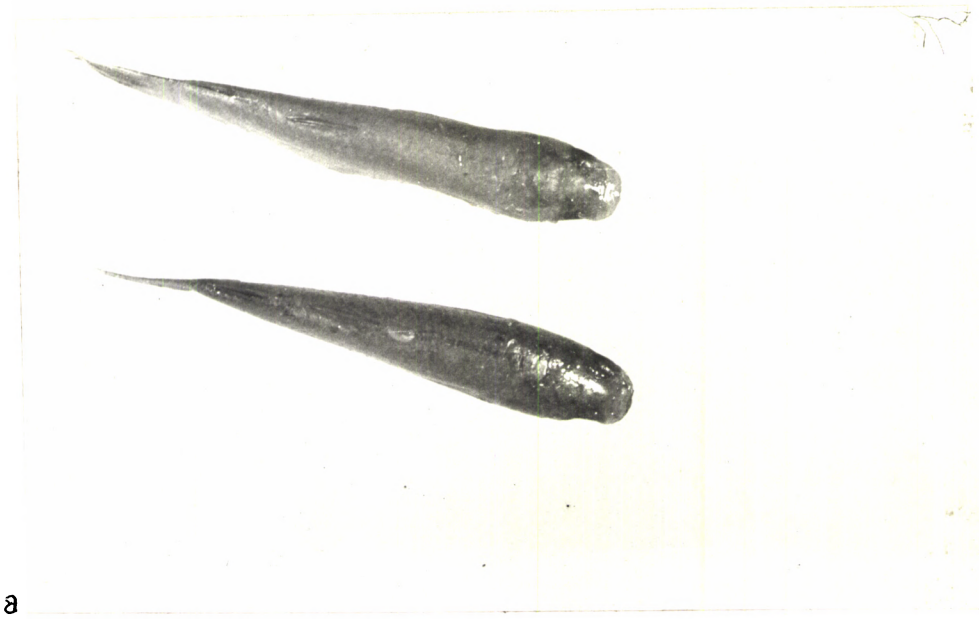


b

PLATE 5. VITAMIN DEFICIENCY SYMPTOMS

(a), (b) Skin erosion - Niacin.



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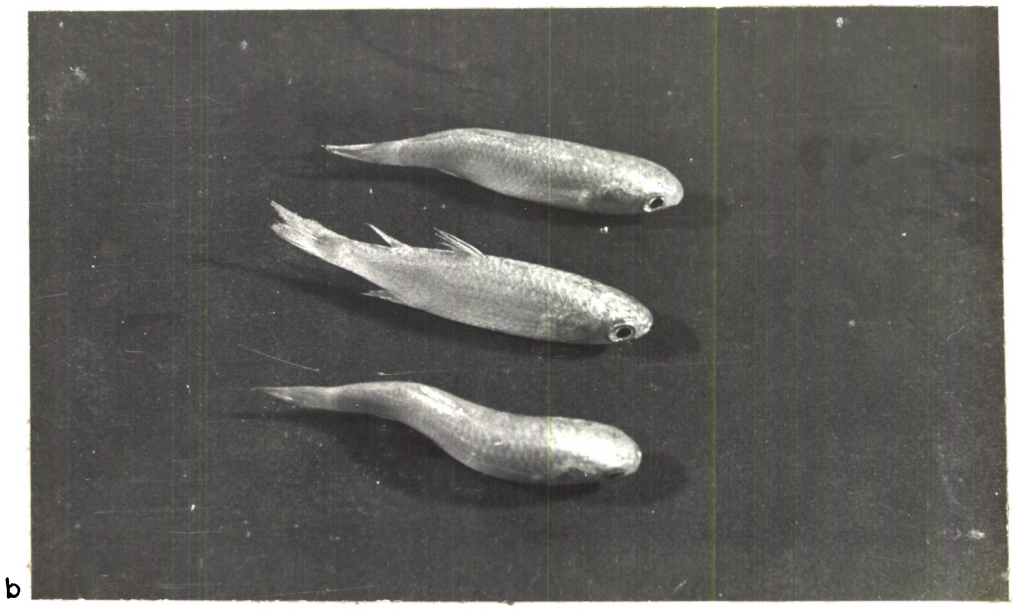


b

PLATE 6. VITAMIN DEFICIENCY SYMPTOMS

(a) Lordosis - Ascorbic acid

(b) Scoliosis - Ascorbic acid.

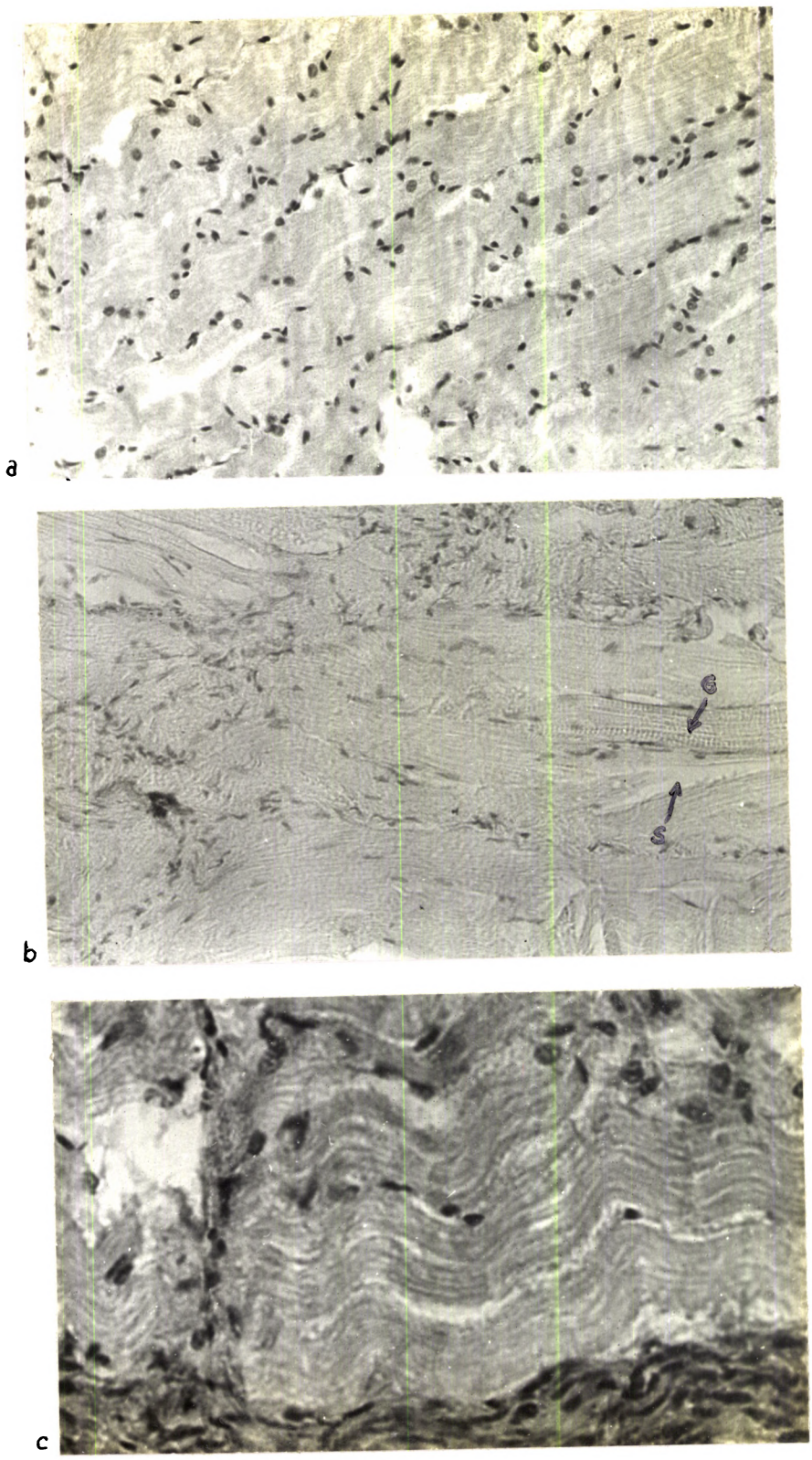


PLATE 7. HISTOPATHOLOGY OF VITAMIN DEFICIENCY; THIAMINE: a) Normal Muscle x 100; b) Muscle fibre has undergone longitudinal splitting (s) at several places; loss of striation and granular degeneration (G) x 100; c) Hyperfunctional changes in muscle indicated by necrosis (N) and fibrosis (F) x 200.

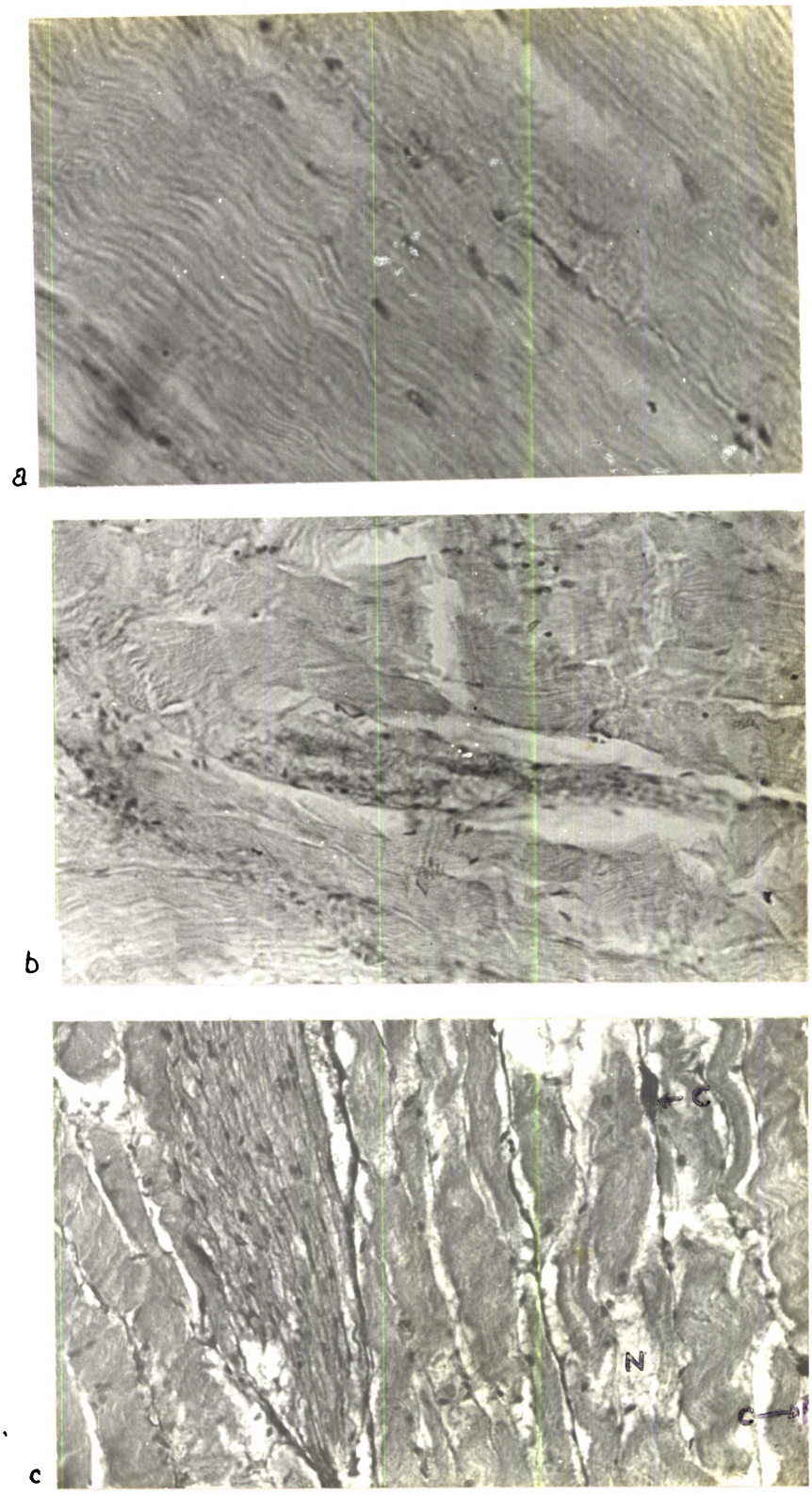
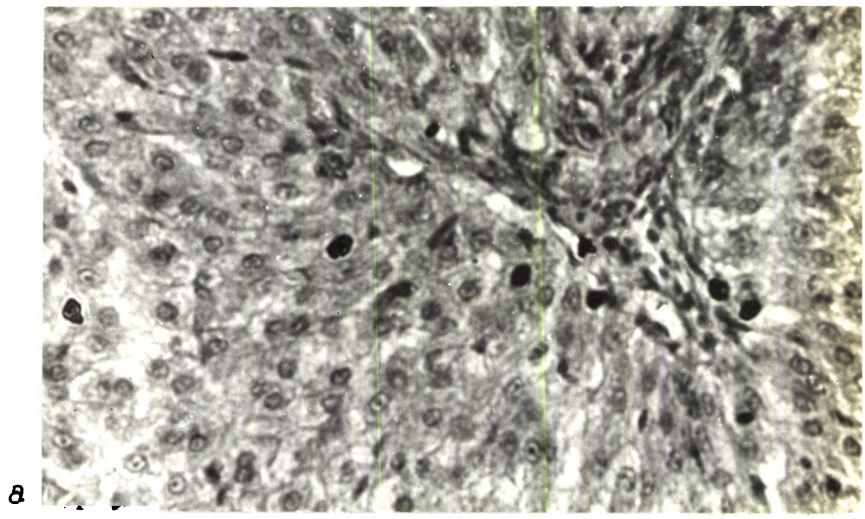


PLATE 8. HISTOPATHOLOGY OF VITAMIN DEFICIENCY; NIACIN: (a) Normal Muscle x 200; (b) Loss of striation, granular degeneration and liquefaction necrosis x 100; (c) Hyalinization leading to necrosis(N), note areas of calcium deposition (C) x 100.



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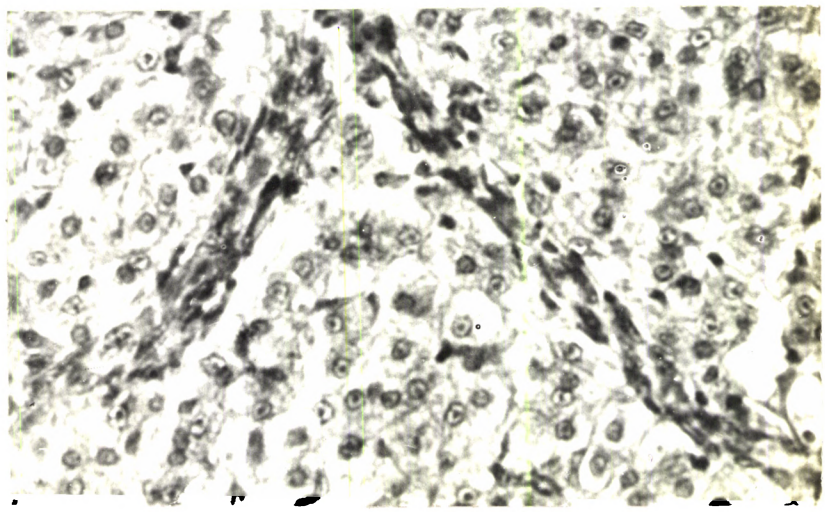
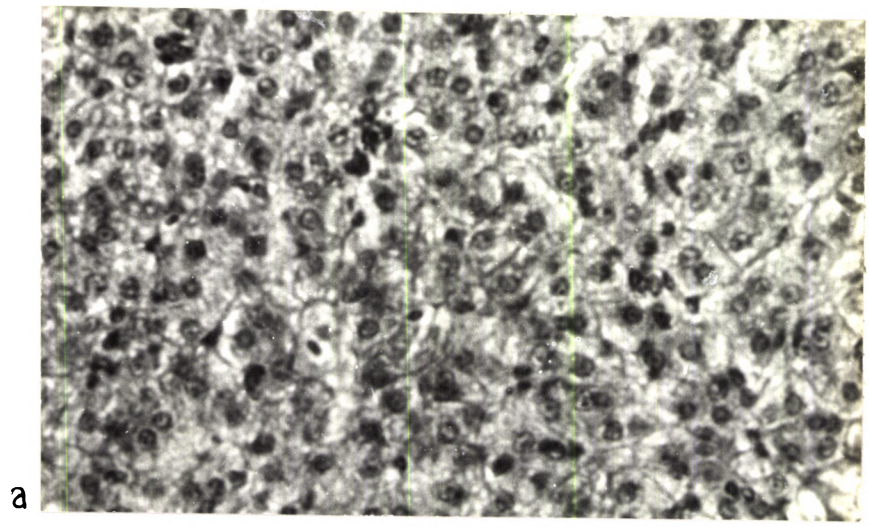


b

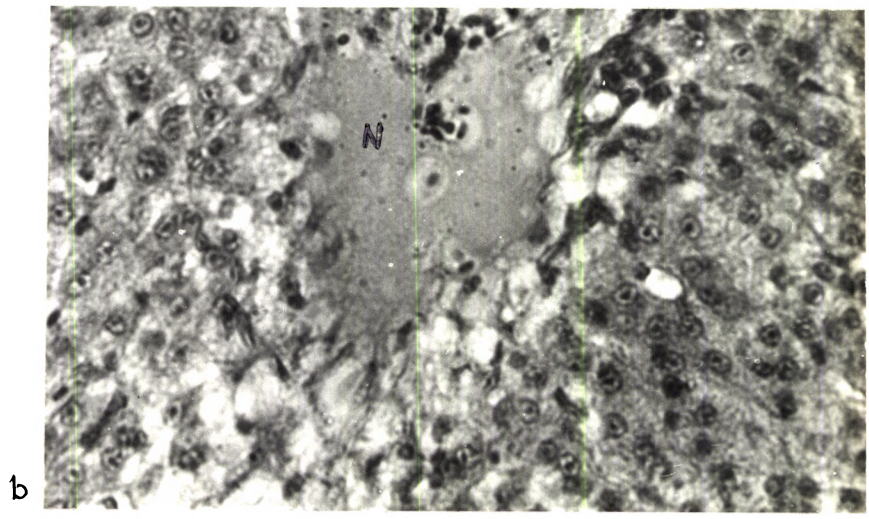
PLATE 9. HISTOPATHOLOGY OF VITAMIN DEFICIENCY; CHOLINE:

(a) Normal Liver x 200

(b) Central region with inflammatory cells x 200.



a



b

PLATE 10. HISTOPATHOLOGY OF VITAMIN DEFICIENCY; PANTOTHENIC ACID:
(a) Normal Liver x 200

(b) Area of necrosis (N) where proteinaceous exudate is found x 200.

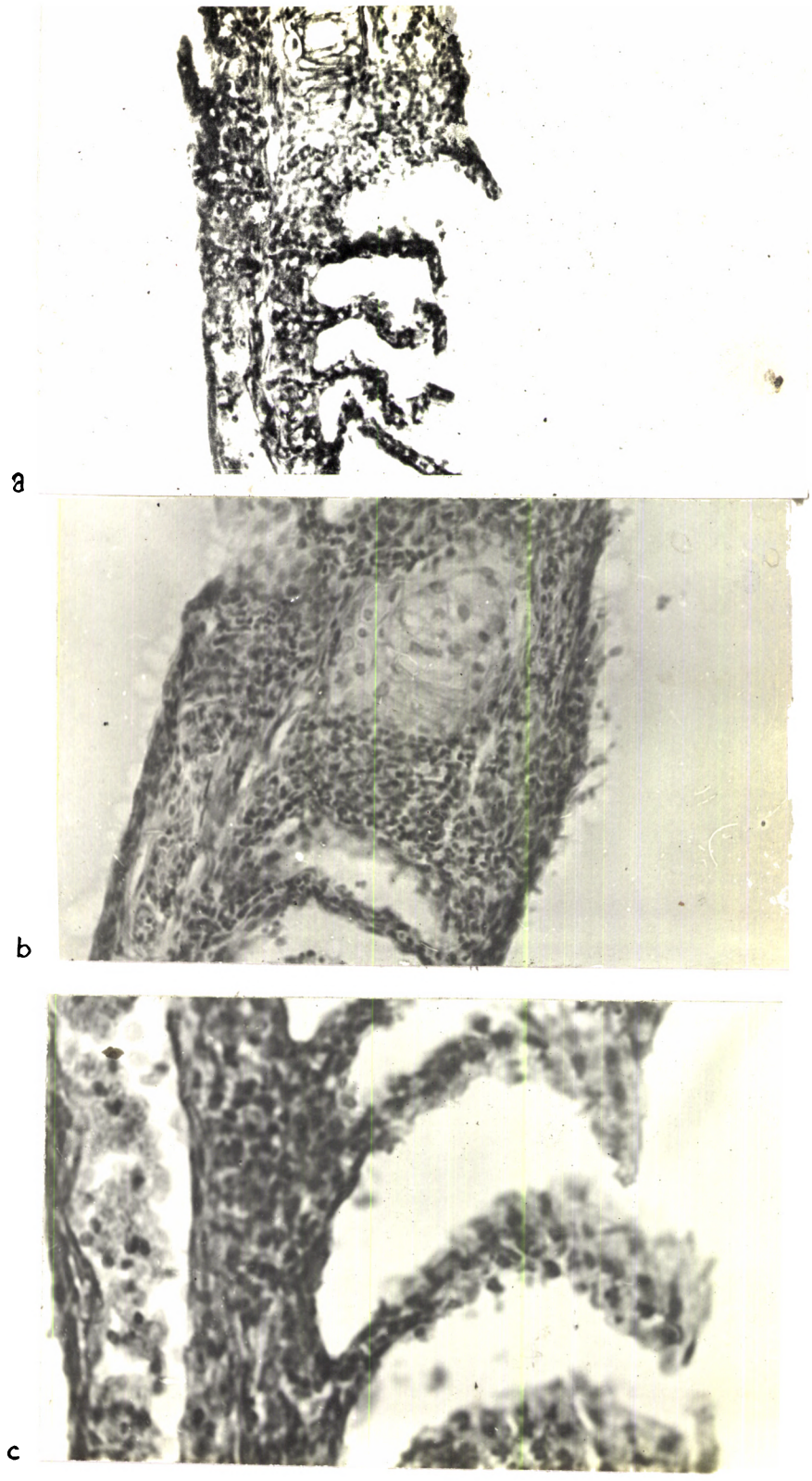
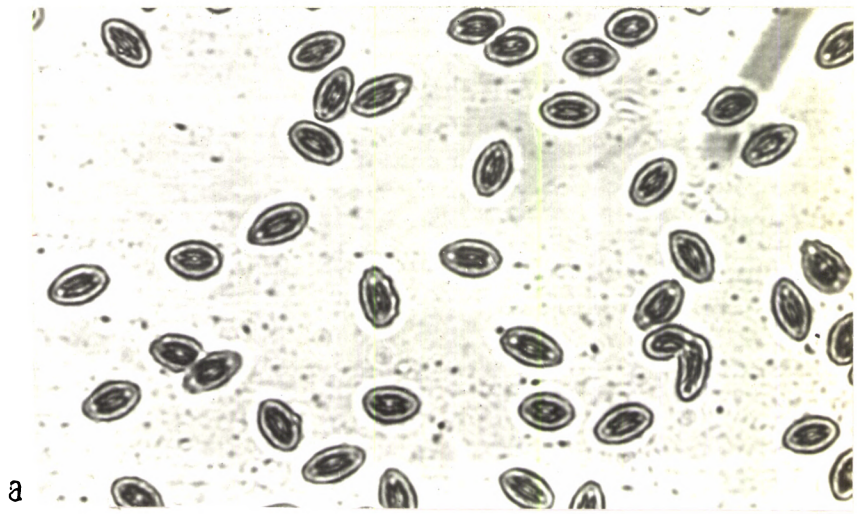


PLATE 11. HISTOPATHOLOGY OF VITAMIN DEFICIENCY; PANTOTHENIC ACID:
(a) Hyperplasia at the base of gill epithelium, lamella dialated at tip x 100, (b) Condition in (a) has worsened, inter lamellar space completely filled with cell x 100 (c) Epithelial hyperplasia x 200.



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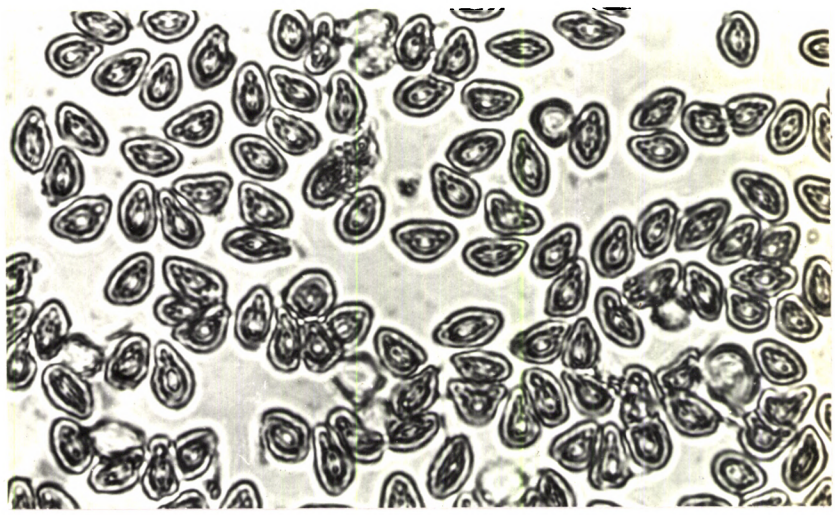

b

PLATE 12. HAEMATOLOGICAL OBSERVATION UNDER VITAMIN DEFICIENCY; ASCORBIC ACID:

(a) Normal blood cells x 200,

(b) Anisocytosis and poikilocytosis x 200.

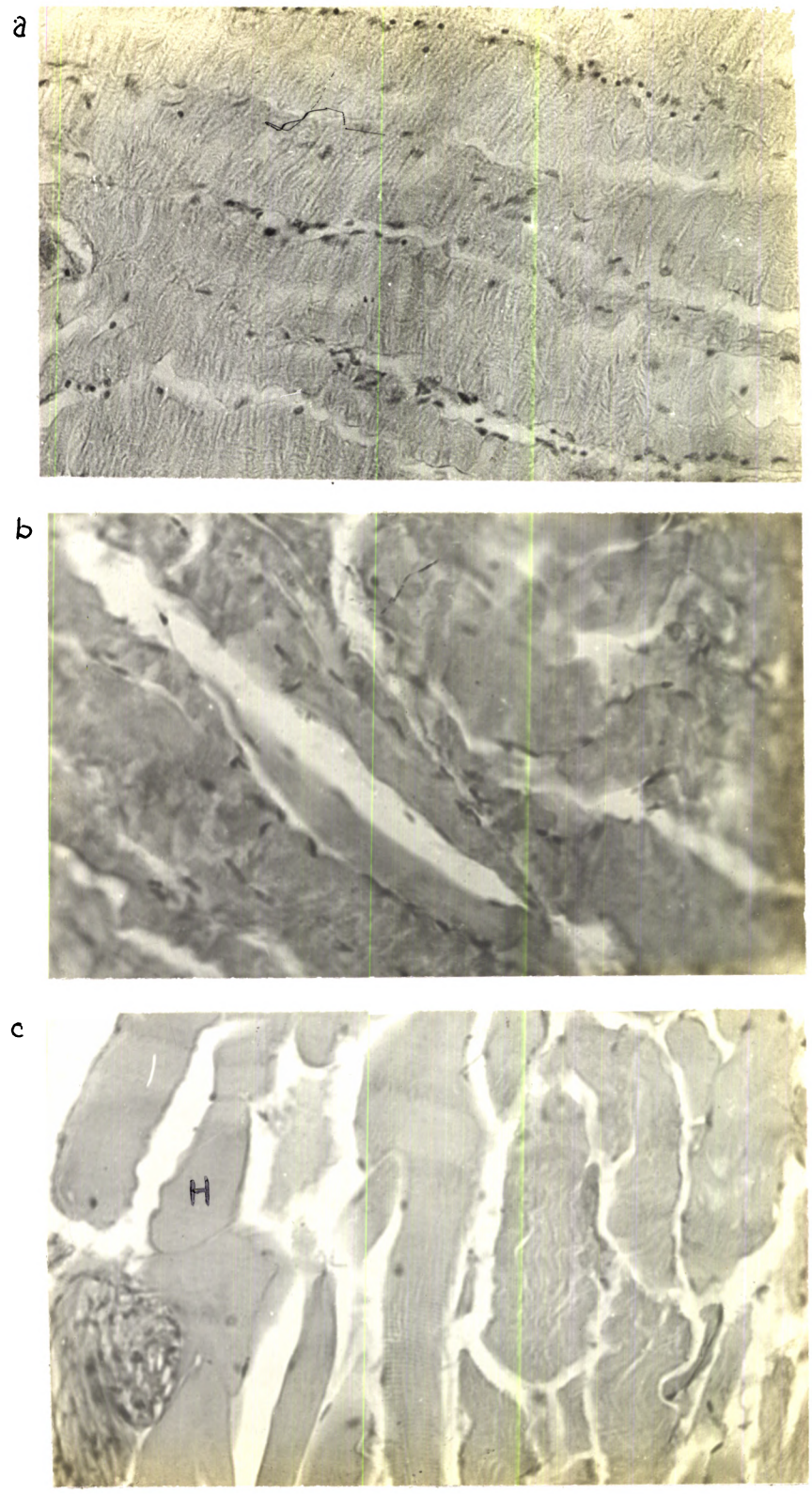

PLATE 13. HISTOPATHOLOGY OF VITAMIN DEFICIENCY; INOSITOL:

a) Normal muscle x 100; b&c) Muscle degeneration (H) indicates hyalinization x 100.

The condition factor too was not affected by the vitamin levels ($P = 0.01$). The value was 1.31 at 2% (D4) vitamin level. The factor at 0.5% vitamin level (D1 = 1.28) alone was significantly ($P < 0.05$) different from the others (Table XI).

The influence of dietary level of vitamin mixture was evident only after the experiment was half-way through. Statistical analysis of the 9th week growth data revealed no significant ($P < 0.01$) treatment effect. The maximum weight gain of 455 mg at this stage was recorded at 1.5% (D3) level of vitamin inclusion. But by the twentyfirst week, ie., at the termination of the experiment, growth was significantly ($P<0.01$) affected by the different levels of vitamin mix included in the diet. The maximum gain was recorded for diet D4 (1153 mg) with 2% of the vitamin mix. However, the weight gain for 1.5% level and above were not significantly ($P > 0.05$) different from each other. The lowest weight gain (1070 mg) was recorded for diet D1. In the case of specific growth rate (Fig.11) treatment influence was revealed at 5% significance. Though the highest figure of 0.935 was for diet D3, this was not significantly different from the values at D4 and D5. At 0.5% vitamin level the specific growth rate recorded was 0.914. Quadratic equation of the second order was employed to establish the relationship between percent weight gain and level of vitamin incorporation (Fig.13). Differentiating X and Y in the above equation, 1.974 say 2% was found to be the vitamin level giving maximum growth response.

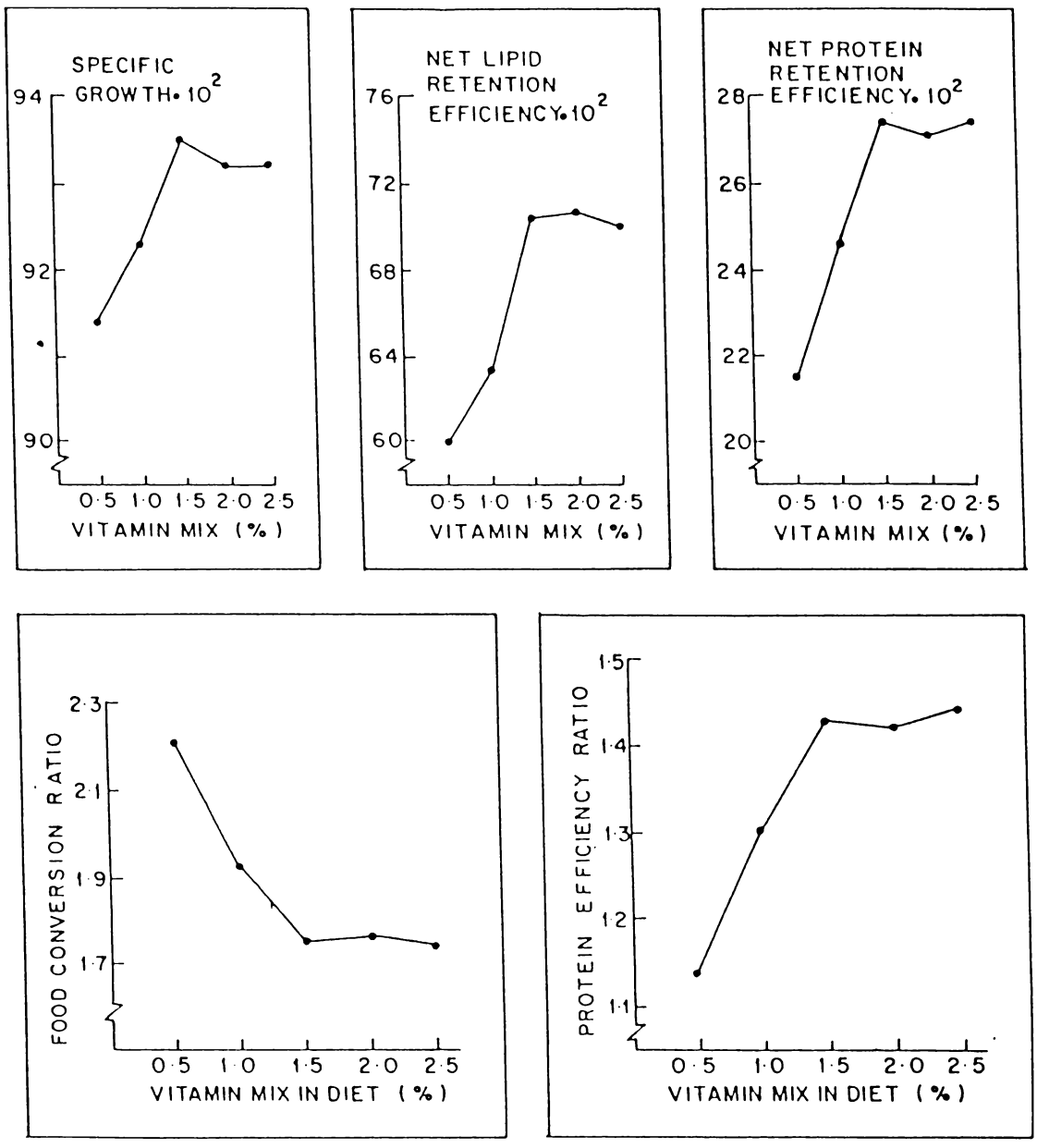

Fig.11. SPECIFIC GROWTH,NUTRIENT RETENTION EFFICIENCY, FOOD CONVERSION RATIO AND PROTEIN EFFICIENCY RATIO IN FISHES FED DIETS WITH GRADED LEVELS OF VITAMIN MIX.

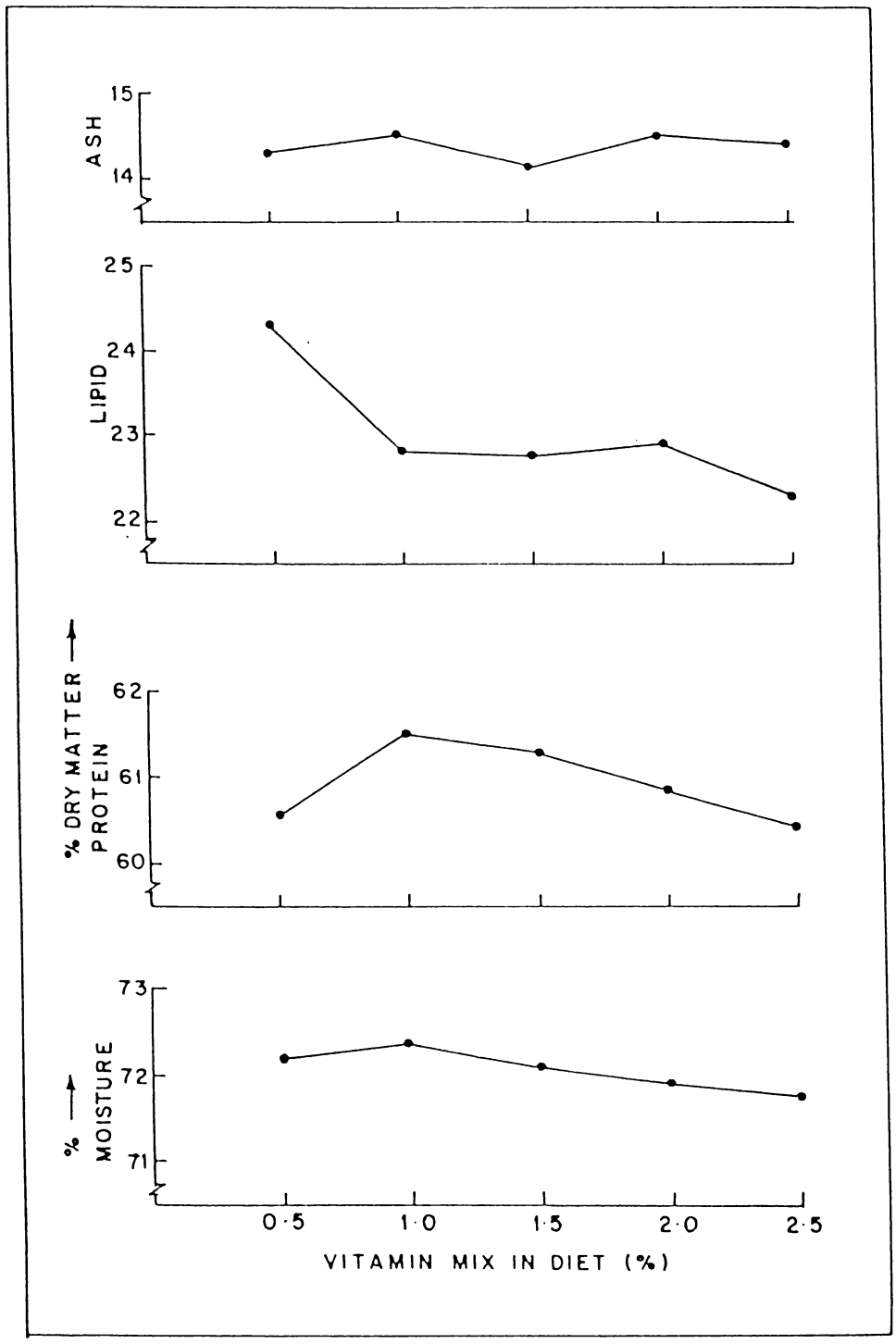

Fig.12. BODY COMPOSITION OF FISHES FED DIETS WITH GRADED LEVELS OF VITAMIN MIX.

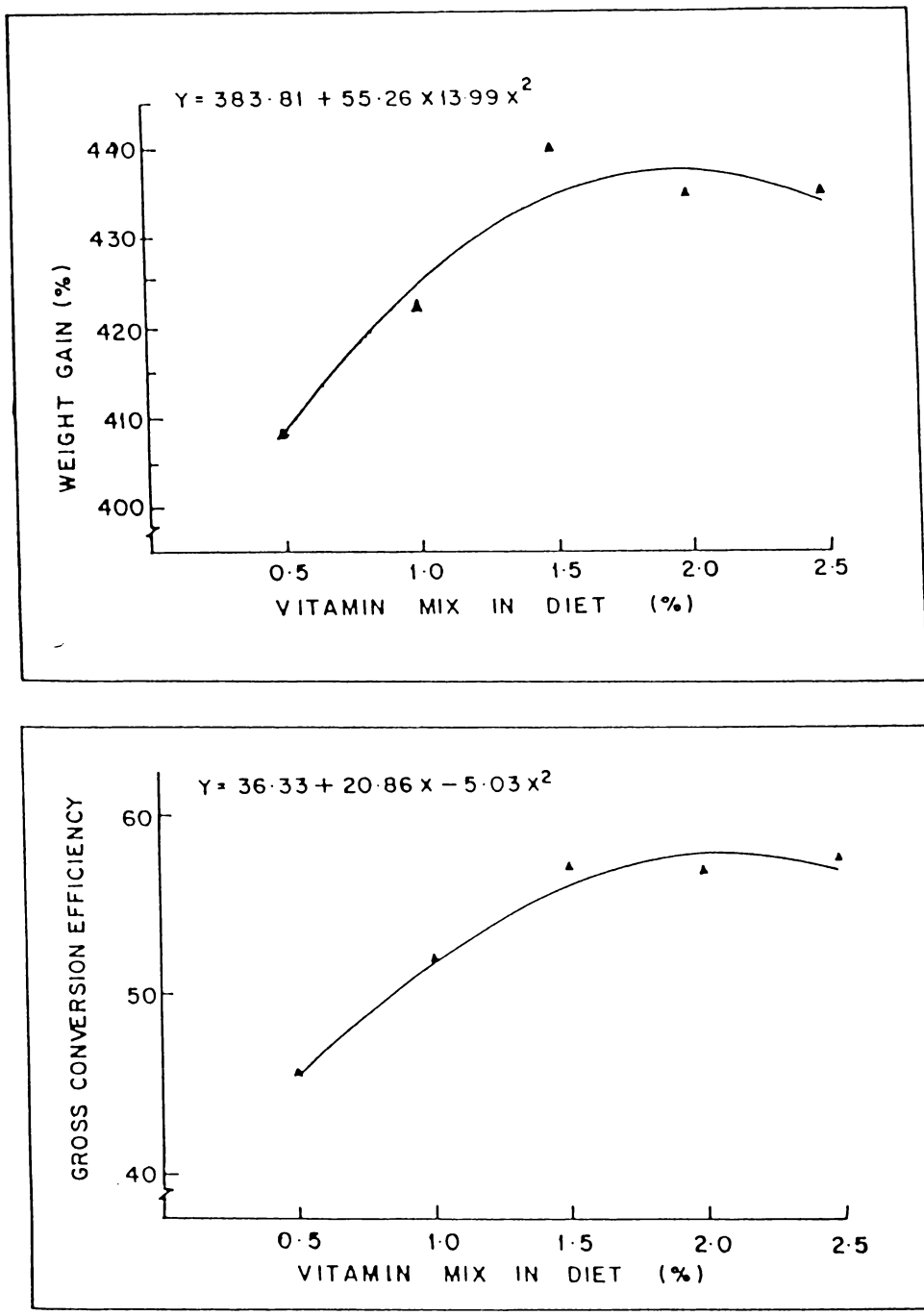

Fig.13. SECOND ORDER POLYNOMIAL RELATION OF PERCENTAGE WEIGHT GAIN/GROSS CONVERSION EFFICIENCY IN FISHES AND DIETARY CONCENTRATION OF VITAMIN MIX.

The best food conversion ratios were obtained when fishes were fed diets having 2.5% vitamin mix. Though the experimental levels of vitamins had influenced ($P < 0.01$) the conversion rates, the differences were not significant ($P > 0.05$) among levels 1.5, 2 and 2.5% (Fig.11). On fitting a second degree polynomial relationship between the vitamin levels and gross conversion efficiency, an inclusion level of 2.07 was found ideal (Fig.13).

The protein efficiency ratio was significantly low at 0.5% (1.14) and 1% (1.30) vitamin levels (Fig.11). It was not different ($P > 0.05$) among the next three higher levels and the maximum of 1.44 was obtained at 2.5% vitamin level.

Carcass analysis revealed that none of the proximate principles were significantly ($P = 0.01$) influenced by the level of vitamins incorporated in the diet (Fig.12). The maximum moisture content(%) was 72.39 (at 1%) and minimum 71.77 (at 2.5%). Highest body protein recorded at 1% vitamin level (61.51%) was not significantly different from that at 1.5% (61.29%). At 2.5% vitamin level protein percentage was 60.45%. Body lipid was maximum at 0.5% (24.28%). The next in order was 2% (22.86%). This was almost similar to the lipid content at all other remaining values. Maximum body ash was obtained when fishes were fed at 1% level (14.53%). The values for the other treatments were almost the same.

The nutrient retention efficiency was affected by the quantum of vitamin mix in the diet. Maximum protein was retained (27.5%) when diet D5 was offered, but this was nearly the same as that for D3 and D4 (Fig.11). Lipid retention efficiency was 70.7% at 2.0% vitamin level; and it was not significantly different from the values at 1.5 and 2.5% vitamin levels. At 0.5% (D1) the lowest retention efficiency (60.9%) was recorded.

The apparent digestibility coefficients for protein as well as for lipids did not differ significantly between treatments (Table XI).

3.4. DISCUSSION

3.4.1 Qualitative Requirements

3.4.1.1 Thiamine

Thiamine has been found to be an essential vitamin additive in the diets of *Liza parsia*. Though the mortality rate was not considerable, anorexia resulting in poor weight gain was noted. It was almost mid way through the experiment that the effect of deletion became more prominent, manifesting in reduced growth. The food conversion rates and protein effciency ratios were also very poor. The lipid retention efficiency seems to be affected probably because of the reduction of thiamine

pyrophosphate which could have impaired the dicarboxylation reactions in the metabolism. In this study, the carbohydrate levels in the feed were kept constant. Therefore, no relationships could be established between carbohydrate level and thiamine requirement as correlated by Aoe et al. (1969). Lipids in the diets were maintained at an optimum level to ensure that it does not impair the study. This was so because dietary fats affect thiamine requirement as carboxylase participates in the oxidation of fat through α-keto glutarate. Halver (1980) had made it clear that fish on a high fat diet and low thiamine intake might take longer time to develop deficiencies, thus leading to erroneous interpretations.

The deficiency symptoms observed in L. parsia are in common with what has been pointed out in the case of many other fishes. Anorexia and reduction in growth had been identified, as early as 1947, in trouts by McLaren et al. (1947). This was later confirmed in chinook salmon (Halver, 1957) channel catfish (Dupree, 1966) and Japanese eel (Arai et al., 1972). Loss of equilibrium was also reported in the above cases as observed in the present study. Violent movements were exhibited by the fish on being disturbed. A trunk winding symptom occured in eel Anguilla japonica (Hash imoto et al., 1970). In carp (Aoe et al., 1969) and in red sea bream Alvamis sp. (Yone, 1975), skin congestion and subcutaneous haemorrhage have been reported. In L. parsia this condition, noted in 20% of the animals, had even

further worsened resulting in muscular dystrophy (Plate 3). Histological studies (Plate 7) had confirmed the above observation. The muscle fibre under such conditons had extensive damage indicated by the granular degeneration and necrotic changes.

3.4.1.2 Riboflavin

The present experiment has indicated that riboflavin is an essential vitamin for L. parsia. The high mortality rates and poor growth with riboflavin deficient diet bear testimony to this fact. By about the 12th week the effect of deletion of the vitamin became evident as the fishes were lethargic indicating apparent muscular weakness. High mortalities have been recorded in salmonids too, fed unsupplemented diets. (Halver, 1957; Kitamura et al.,1967; and Steffans, 1970). However, Ogino (1967) and Aoe et al. (1967); Arai et al.(1972); and Dupree (1966) recorded only low mortalities in carp; eels and catfish respectively. The fishes under riboflavin deficiency were comparatively shorter than their counterparts, the control fishes. This could be a condition indicative of short body dwarfism, a symptom described in larger specimens of channel catfish (Murai and Andrews, 1978 b). The abnormal growth in channel catfish has been related to hypothyroidism. Several workers have demonstrated biochemical similarities between hypothyroidism and

riboflavin deficiency. Wolf and Rivulin (1970) suggested that thyroid hormone regulates the activities of flavoprotien enzymes by enhancing the synthesis of apoenzymes and coenzymes FMN, FAD to which enzymes are stably bound. It has also been suggested that a retarded synthesis of thyroid hormone by riboflavin deficiency is responsible for the lowered basal metabolic rate. The lethargic condition described in the mullet can be attributed to this. The food conversion rates and protein efficiency ratios were also poor as reported in channel catfish by Dupree (1966) and Murai and Andrews (1978 b). Aoe et al. (1967) have described similar conditions in common carp in a short time of 3 weeks. Another symptom in L. parsia was fin erosion, as was observed in rainbow trout by Poston et al. (1977) and Woodward (1984) and common carp by Takeuchi et al. (1980). It is of interest to note that fin erosion was a consistent feature of the deficiency syndrome. The mild degree of fin erosion had resulted in roughened, scalloped borders of the caudal and dorsal fins. Two very characteristic symptoms of riboflavin deficiency detected in L. parsia were photophobia and mono/bi-lateral corneal opacity (Plate 3). The full blown symptoms were exhibited in 20% of the animals, thus proving the involvement of this vitamin in retinal pigment during light adaptation. These conditions were also observed in salmon (Halver, 1957a), eels (Arai et al.,1972), rainbow trout (Poston et al.,1977; Hughes et al.,1981; Takeuchi et al.,1980). However, Aoe et al. (1967) and Woodward (1984) did

not observe such ocular abnormalities in carps and rainbow trout fry and fingerlings. Hughes and Rumsey (cited by Cowey et al.,1985) reviewed the many causes for lenticular cataracts induced by several nutrient deficiencies including riboflavin as one major cause for the disease. Woodward (1984) has concluded that the development of ocular opacities in riboflavin deficient trout appears to be dependent on an interaction between the vitamin deficiency and one or more additional conditons.

3.4.1.3 Pyridoxine

The low survival rates among fishes fed on diets deficient in pyridoxine is clearly indicative of the importance of the vitamin in the diet. Anorexia was exhibited within six weeks. Other non-specific conditions of reduced growth and poor feed conversion also occur. Similar reports on chinook salmon (Halver, 1957a) and yellow tail (Sakaguchi et al., 1969) are already available. Hyperirritability and erratic swimming were also detected in pyridoxine deficient diet fed L. parsia. Such nervous disorders have also been reported in common carp (Ogino, 1965), channel catfish (Andrews and Murai, 1979) and Japanese eel (Arai et al.,1972). In a very recent work on Atlantic salmon, (Salmo salar), Herman (1985) noted in addition to behavioural change, degenerative changes in kidneys, ovaries and liver. Similar experimentally induced deficiencies have been described

in rainbow trout (Smith *et al.*, 1974) and gilthead bream, *Sparusaurata* (Kissil *et al.*, 1981).

A large array of enzymes concerned in intermediary metabolism of amino acids require pyridoxal phosphate as coenzyme. Amongst the variety of reactions it is involved in, transaminations are the most important because when linked to synthesis and transformation of glutamate by specific enzymes, they form reaction sequences which are crucial to the assimilation and excretion of nitrogen (Cowey and Sargent, 1972). The low protein efficiency ratio in the fry of *L. parsia* fed on a diet lacking pyridoxine can be linked to the failure of the metabolic route discussed above. Vitamin B6 has also been proved to be influencing fat metabolism in other groups of animals (Sherman, 1950; Mueller 1964). Beare *et al.*, (1953), Sure and Easterling (1949) and Desikachar and McHenry (1954) have reported a striking decrease in total body fat on pyridoxine deficiency. A parallel could be drawn from the above discussion and the findings in *L. parsia*. The lipid retention has been found to be poor in the present experiment which may be due to the impairment of the lipid metabolism.

3.4.1.4 *Nicotinic Acid*

This vitamin is required by all living cells. Niacin in the form of niacinamide constitutes the enzymes involved in the

release of energy from all the three energy yielding nutrients. Hence, it becomes essential for the maintenance of life processes. The low survival rates in L. parsia deprived of niacin in the diets prove this point. Growth was poorest amongst all the treatments. High mortality and reduced growth as a consequence of nicotinic acid deficiency has been described in young carp. (Aoe et al.,1967), Japanese eel (Arai et al.,1972) and channel catfish (Andrews and Murai, 1978). Another prominent symptom detected in this mullet was skin erosion and the associated muscle damage (Plate 5,8); which was revealed through histological studies. Aoe et al.,(1967) and Andrews and Murai (1978) have described such conditions in other fishes. The histopathology of the muscle in the present case indicated loss of striation leading to granular degeneration and necrosis. However, Andrews and Murai (1978) did not observe any histological abnormalities. They have substantiated this by referring to reports in other animals (Gries and Scot, 1972) where death occured as a result of starvation, bacterial infections or biochemical abnormalities before histological abnormalities became evident. Previous reports on niacin deficiency signs in fishes have not been consistent. In rainbow trout McLaren et al.(1947) observed swollen gills, but Kitamura et al.(1967) failed to demonstrate deficiency signs other than slightly retarded growth. Chinook salmon have been reported to have signs such as jerk and difficult motion, weakness, lesions of stomach and colon, muscle

spasms and increased sensitivity to sunburn (Halver 1957a, Delong et al.,1958); but, silver salmon did not exhibit any deficiency symptoms. In channel catfish, tetany, high mortality after stress, lethargy and reduced co-ordination have been reported as niacin deficiency symptoms (Dupree, 1966). Since tetany, lethargy and reduced co-ordination and other neurological disorders were not observed in the present study, and yet growth depression, mortality and cutaneous damage were severe; the si gns were more similar to that in carp (Aoe et al.,1967), eel (Arai et al.,1972) and channel catfish (Murai and Andrews, 1978a). Since tryptophan is an in vivo precursor of niacin and niacin deficiency cannot be induced in most animals without reducing dietary tryptophan (Dalgliesh, 1956), it was surprising that deficiencies were quickly exhibited in L. parsia. Poston and Dilorenzo (1973) and Andrews and Murai (1978) have reported that brook trout and channel catfish cannot efficiently convert tryptophan to niacin. As the present diet was limiting in niacin, the results indicate tryptophan conversion inefficiency in L. parsia, as was in the case of brook trout and channel catfish.

3.4.1.5 Pantothenic Acid

Pantothenic acid plays a stellar role in general metabolic pathways and aid in the release of energy from all the three energy producing nutrients by way of the tricarboxylic acid cycle. Symptoms associated with pantothenic acid deficiency are

mostly non-specific and vary from species to species. Deficiency studies in higher vertebrates have shown retardation of growth, impairment of reproduction, imbalance of salt and water metabolism and reduction of coenzyme-A content in tissues leading to poor utilization of pyruvate (Chow, 1964). Exclusion of this vitamin from the premix has been identified to be the cause of growth retardation by the 9th week. Anorexia conditions exhibited led to poor conversion and protein efficiency ratios. In carp too, extremely poor weight gain was the consequence of pantothenic acid deletion (Ogino, 1967). The reduced amount of body fat recorded in these group of fishes may be due to the reduction in the synthesis and mobilization of fat. It is an established fact that pantothenic acid, as a component of coenzyme-A, is required for the synthesis of fat. A dietary insufficiency of pantothenic acid impairs the normal metabolism within mitochondrial rich cells undergoing rapid mitosis and high energy expenditure (Halver, 1982). Structures such as gill and kidney tubules which are involved in osmoregulation or active hydromineral homeostasis and pancreatic acinar cells almost constantly synthesize enzymes essential for digestion of fats, carbohydrates and proteins. A continual high level of energy is required for these activities. Pantothenic acid deficient trout (Poston and Page, 1982) showed conglutinated metamitachondrial lesions. These subcellular lesions which may be caused by anoxia within cellular energy transfer mechanisms (Hartroft, 1964;

Rouiller, 1964) intially appear as vacuoles or hyaline bodies and eventually lead to necrosis. Grossly the signs of pantothenic acid deficiency are manifested as a condition called dietary gill disease (Wolf, 1945; Halver, 1953), including clubbed exudate covered gill lamellae, swollen operculum, fused gill filament and abnormal swimming near the surface of the water. In salmon, trout and catfish such conditions were described (Halver, 1979; Dupree, 1966; Murai and Andrews, 1979; Wilson *et al.*,1983). Gill lesion observed in *L. parsia* were characterised by an epithelial hyperplasia and interlamellar tissue proliferation which was most marked at the distal end of the filaments (Plate 11). This was also observed in catfish fingerlings by Murai and Andrews (1979) and Wilson *et al.* (1983). In *L. parsia* another condition associated with the nutritional pathology was the pale liver. Sections revealed that necrotic changes were taking place resulting in foamy degeneration (Plate 10). Similar foci of necrosis was observed in cultured herring (*Clupea harengus*) by Blaxter *et al.* (1974).

3.4.1.6 Choline

Choline is an essential vitamin especially due to its structural role in biomembranes, as constituent or a phospholipid in a neurotransmitter and as a lipotropic and anti-haemorrhagic agent. From the present study it is evident that it is

unavoidable in the diet of gold-spot mullet too. Though the growth increment recorded was better than some of the previous reports the effect of the vitamin deletion on growth became evident by the 9th week. Survival was only 60%. Conversion efficiency was also poor. Similar manifestations of dietary deficiency has been reported in chinook salmon (Halver, 1957a), Coho salmon (Coates and Halver, 1958). As in the present study, pale moderately enlarged livers has been observed in trout (McLaren et al., 1947), and channel catfish (Dupree, 1966). The livers in L. parsia changed in colour from light tan to dark brown and exhibited a motted appearance with focal areas of discolouration. Inflammatory cells, with vacuolization in some cases, characterstic of damage, were identified histologically (Plate 9). Such pathomorphological observations has been made in the hepatic tissue of other animals under choline insult (Keith and Tryphonas, 1978). Other manifestations of choline deficiency include grey white intestine in eels (Arai et al.,1972) and haemorrhagic areas on kidney and intestine in redsea bream (Yone, 1975). The body composition studies in the present treatment could not reveal anything substantial.

3.4.1.7 Inositol

The essentiality of this vitamin is often debated. In the present study too, if growth and mortality can be considered

as good indicators of vitamin status, then the above view can be corroborated. A slight reduction in growth was the only sign exhibited in Japanese eel fed an inositol deficient diet for 15 weeks (Arai *et al.*,1972). Burtle (1981) has indicated that inositol is not normally required in the diet of channel catfish. He has demonstrated intestinal synthesis and *denovo* synthesis of inositol in the liver of channel catfish. However in rainbow trout (McLaren *et al.*,1947) and chinook salmon (Halver, 1957*a*) the non specific symptom of poor growth has been reported. Certain nutritional pathological indicators specific to inositol deprivation were noted in *L. parsia* - distended abdomen (Plate 4) and haemorrhagic fin base leading to muscle degeneration. Histological observations corroborate this inference (Plate 13). Yone (1975) related the distended abdomen to the ineffeciency in digestion and poor feed utilization; but this point is hard to prove based on the present study. Aoe and Masuda (1967) had indicated skin lesions in carp which in severe cases resulted in scales, fins and epidermis being sloughed off; and the muscle and bone being exposed. However, the proportion of carps exhibiting this deficiency sign was low as observed in *L. parsia*.

3.4.1.8 *Ascorbic acid*

Ascorbic acid performs numerous physiological functions in both plants and animals (Tolbert, 1979). The dietary needs for

salmonids, ictalurids and cyprinids for vitamin C has been reported over the last 2 decades. The deficiency syndromes explained are very many. In L. parsia too, some of these could be detected when vitamin C was excluded from the diet. The mortality rates (23%) were not very high in the present experiment. By about the 10th week a clear reduction in weight gain was recoded. Such reduction was reported in channel catfish by Lovell (1973), Andrews and Murai (1975), and Lim and Lovell (1978) and in yellow tail by Sakaguchi et al.(1969). Food conversion rate and protein efficiency ratio and in turn the retention efficiencies in the mullet fed vitamin C deficient diet were considerably better than many other treatments, probably indicating a less profound impact of vitamin C insult. The body proximate composition analysis did not reveal anything important that is worth mentioning. The major deficiency symptoms that has been described in different fishes include scoliosis / lordosis, an alteration of pigmentation, haemorrhagic area along the spinal column and ultimately a broken back. These symptoms collectively characterize the broken back syndrome. In L. parsia the scorbutic condition exhibited were scoliosis and lordosis (Plate 6). Scoliosis was indicated by a lateral curvature of the spinal column at approximately the mid length of the fish. There was usually external swelling near the damaged area. Most mullets showed scoliosis with or without lordosis which was characterised by a hump just posterior to the dorsal fin. These conditions have been described in coho salmon

(Halver et al., 1969); where extreme dislocation of the vertebrae and atrophy of spinal cord had occured in the area of acute deformity. Similar pronounced effects were observed in channel catfish (Lovell, 1973). Wound repair was also affected in ascorbic acid deficient yellow tail (Sakaguchi et al.,1969), the rate of repair being proportional to the ascorbic acid content of the food. Other signs of ascorbic acid deficiency in channel catfish were internal and external haemorrhage, fin erosion, dark skin color and reduced formation of bone, collagen and gill damage (Wilson and Poe,1973; Lim and Lovell, 1978).

Only very few attempts have been made to determine the impact of vitamin C on blood in fishes (Hilton et al., 1978; Andrews and Murai, 1975; Lim and Lovell, 1978; Agrawal and Mahajan, 1980). In the present study, the anaemic conditions induced by vitamin C deprivation is characterized in the blood picture (Plate 12) by the varying red blood cell size and shape (anisocytosis and piokilocytosis), cytoplasmic vacuolization, and disintegrated erythrocytes. A haematological assessment of the blood morphology of L. parsia fed diet deficient in ascorbic acid has reaffirmed the role of this vitamin in normal physiology of the animal. However, there are two reports, one in channel catfish (Dupree, 1966) and another in rainbow trout (Primbs and Sinnhuber, 1971) on the non-essentiality of vitamin C.

Fishes cannot generally synthesize vitamin C (Halver 1972, 1980). This inability in these group of animals may be due to genetic failure of enzyme synthesis or lack of expression (Levin, 1976). Wilson (1973) and Yamamoto et al. (1978) have postulated that the dietary essentiality of ascorbic acid is due to the absence of the enzyme L-gulonolactone oxidase which is required for the synthesis of ascorbic acid. A higher dietary level than that required to prevent deficiency signs may be required to provide maximum resistance to bacterial infections (Durve and Lovell, 1982) and under stress (Mayer et al., 1978; Agrawal et al., 1978).

3.4.2. Quantitative requirements

The common place knowledge that severe vitamin deficiency predisposes or exacerbates infections has been verified over the years by different workers. The present set of experiments on qualitative requirements of vitamins in L. parsia too, proved this point. The severity of the deficiency was decided based on the pathomorphology. To prevent the onset of symptoms it is necessary that the vitamins are provided in adequate quantities. Several experiments have been conducted in different species to determine the level of individual vitamins that are to be included in the premix. In this study on L. parsia, the minimum amount of vitamin premix, to be included in the diets to prevent the gross symptoms was determined.

As probably the minimum requirement was met by the dietary incorporation of all the vitamins, the mortalities recorded did not reveal anything striking. The growth data too did not exhibit any significant difference until towards the end of the experiment when 2% vitamin seemed to elicit better response. Considering gross conversion efficiency and weight gain, the level of incorporation of the vitamin mix at around 2% seemed to be the best. The protein efficiency ratio also indicate the levels 1.5 to 2% as appropriate. The nutrient retention efficiencies also was better in the range 1.5 to 2.5%. However, no relevant inference could be made from the results on body proximate composition.

Though no qualitative change could be pointed out in this experiment, the consequence of offering graded quantities of vitamin were reflected in certain factors governing the efficiency of food utilization and resultant growth. The experimental effects were roughly parallel to the quantum of mix and it would be inappropriate to identify an optimum level based on the performance differences between the groups. On the safer side it is advised that a vitamin mix above 1% should be incorporated in the diets of L. parsia fry. However, this experiment remains to be illustrative but not exhaustive. Further studies on quantitative requirements of individual vitamins are warranted to support the findings.

4. EVALUATION OF DIETARY PROTEIN AND LIPID SOURCES AND COMPOUNDED FEEDS

4.1. INTRODUCTION

The dramatic proliferation of interest in aquafarming has resulted in increased attention towards understanding cultural conditions that may determine the economic viability of a particular operation. Such efforts have been developing with emphasis on specific nutritional requirements of the candidate species and design of dietary preparations to optimize growth and achieve maximum survival under mass rearing conditions. Determination of nutrient requirements has been aimed at replacing live-food with fabricated artificial diets. Unlike conventional or natural food, artificial diets are not subject to seasonal variations in supply or nutrient composition and can be manufactured under strict quality control. Since the cost of feeds and feeding represent the single largest item in the operating budget any gains in the efficiency through improved nutrition and feeding technology resulting in greater growth and survival can make a significant contribution to the profitability.

As fish feeds become better formulated to comply with research findings of balanced nutrient requirements, and are texturized to improve acceptability and availability, they are likely to show improved utilization. The preparation of complete

diets, in accordance with known nutritional requirements, is clearly essential to the success of intensive aquatic animal husbandry. While the emphasis will be on complete rations, it is common in many culturing systems to have several different types and sources of feed constituting the total diet (Webber and Huguennin, 1979).

Feed formulations incorporate naturally available, nutritive, local and cheap ingredients to keep the diet cost-efficient. In this regard, a variety of natural feed sources are currently being utilised the world over.

Unlike most domesticated farm animals, the majority of the fish species currently farmed in intensive culture systems are either carnivorous or omnivorous and consequently require high protein diets. Hence, protein meals of animal origin particularly those of marine origin, are of great value in aquaculture feeds. Animal protein are generally rich in essential amino acids especially those (lysine and methionine) which are often limiting in plant proteins. At present high quality fish meal supply the major portion of protein in commercial rations formulated for fish culture operations. The fish meal content in the diets usually range between 25 and 65% by weight, with higher levels being used in starter and fingerling rations (Tacon and Jackson, 1985). In view of the high cost of good quality fish meal of relatively constant

chemical composition, the feed costs amount to 40 - 60% of the operating cost in intensive aquaculture (FAO, 1983). Besides, quality fish meal is in short supply in several countries, including India, while the demand is steadily increasing due to the accelerated development in animal husbandry and aquaculture. Therefore there is a definite need to identify alternate, ideally less expensive sources of good quality protein.

Unfortunately attempts by feed compounders and nutritionists alike to replace the fish meal component of practical fish feeds with alternate protein sources have met with only variable success and have generally led to reduced feed efficiency and growth. Protein sources which have been considered in this category include meat, bone meal, blood meal, poultry by-products, hydrolysed feather meal, soyabean meal, dried brewer's yeast, and corn gluten meal. These secondary protein sources are commonly incorporated at low levels in practical fish feeds (5 - 15%). Partial or total replacement of fish meals in commercial fish feed is by either conventional feed ingredients with enhanced nutritive value or by the use of new generation unconventional feed ingredients. The latter includes single cell proteins - algae, fungi (including yeasts)and bacteria which are produced by fermentation; plant protein concentrates like those of potato and leaf; whole food organisms - rotifers, copepods; and animal and food processing wastes -

rendered hide fleshings, activated sewage sludge dried coffee pulp, brewery and distillery wastes (Tacon and Jackson, 1985; FAO, 1983).

In the present study three plant protein sources - groundnut oil cake, soyabean meal and dried algae (Spirulina) and two animal protein sources - fish meal and prawn-head meal and three combinations of both plant and animal sources were tested to evaluate their efficacy in the diets of the mullet.

Lipids are generally recognized as important and highly digestible nutrients in fish diets and are widely used for improving feed efficiency. Several types of oils have been used in fish feeds. Since fish are incapable of de novo synthesis of n-6 and n-3 fatty acids, dietary sources of these are essential for normal growth and survival. Fishes generally require n-3 fatty acids rather than n-6 in contrast to terrestrial animals (Kanazawa, 1985). Marine fishes appear to have a greater requirement for highly unsaturated fatty acids than fresh water species (New, 1987). Dietary lipids are efficiently utilized, provided the essential fatty acids requirements are met (Watanabe, 1977; Viola and Rappaport, 1979; Yu and Sinnhuber, 1981; Gatlin and Stickney, 1982). In all these studies dietary lipid spared dietary protein or improved net protein utilization. Although the associated increased

deposition of body fat may not always be desirable, the increasing cost of fish meals and other important sources of protein for fish feeds make higher dietary lipids appear increasingly attractive. Fish oils and vegetable oils are the commonly used lipids, either singularly or in combination in fish diets. Although many vegetable lipids contain high levels of n-6 poly unsaturated fatty acids, the best sources (and the most expensive) of the n-3 highly unsaturated fatty acids are marine lipids. Animal fats have low total levels of poly unsaturated fatty acids (New, 1987). Many different natural animal tallows (solid above $40^{o}C$), lards (melting point b etween 20 - $40^{o}C$) and oils (liquid below $20^{o}C$) are available. The fish oils currently employed in dietary combinations include cod-liver oil, pollack liver oil, herring oil, menhaden oil and capelin oil. A variety of plant oils are being used in fish diets. These include soyabean oil, cotton seed oil, sunflower oil, sesame oil, groundnut oil, safflower oil and corn oil. Since fish oils and most plant oils are rich in poly unsaturated fatty acids, antioxidants are to be added to them during processing to delay the onset of rancidity.

In the present experiments, four plant lipids and four animal lipids were tested on the mullet Liza parsia. These included gingely oil, soyabean oil, groundnut oil, sunflower oil, sardine oil, shark liver oil, cod liver oil and beef tallow. Different combinations of these oils were also tested.

A prerequisite for long term culture of any fish is a practical diet that is nutritionally sound and reproducable, easy to handle and store, and capable of promoting growth and survival for a number of generations.The objective of feed formulations' is to supply the required nutrient density for optimal animal production (Conklin et al., 1977). In dietary formulations feedstuffs with generally similar properties may be substituted for one another and exchanges made within mixtures in accordance with market prices, local availability and nutrient composition. Particular regard should also be paid to essential nutrient content and balance of final diet and to an extent cultivist's preference (Cho et al., 1985). Thus different proportion of ingredients are combined to achieve the deserved nutrient balance. The chemical definition of the dietary composition through quantitative requirement studies and the subsequent identification of natural feed sources have aided in compounding practical diets. The efficacy of such formulations has to be tested in culture conditions. It is impossible, from field studies to state how efficient the feeds were, directly in promoting growth, and to what extend indirectly by raising primary productivity of the pond. However, the performance of the diets under pond trials alone can mark a particular diet as a successful formulation.

Intensification of mullet culture has made it essential to develop suitable feeds to be used either as a supplementary

diet in ponds or as a complete diet in tanks. In the final experiment included in this study five dietary preparations based on data accrued in the preceding experiment were fed to the fry of the mullet L. parsia. The main objective was to develop an appropriate starter diet formulation using different natural ingredient sources for the young ones.

4.2. MATERIAL AND METHODS

The experiments to evaluate the protein and lipid sources were conducted in the laboratory. The evaluation of compounded diets was undertaken in actual pond conditions. Variations to the general materials and methods (pages 7 to 8) adopted are discussed below under seperate heads.

4.2.1. Evaluation of Protein Sources

Twenty numbers of uniform sized fry of L. parsia were held in each experimental aquaria. There were eight test diets, each one tested in triplicate. The mean intial length of the fishes was 24.21 mm (±0.63) and the mean weight was 236.42 mg (±10.25).

Eight diets (D1 - D8) were compounded; three were based on plant proteins - groundnut cake, soyabean meal and an algal meal (Spirulina sp.); two were based on animal proteins - fish

meal and prawn waste; and three other formulations with both plant and animal sources. (Table XII). The energy content was adjusted by varying the level of potato starch and cellulose. The other additives were common to all diets. The diets were made iso nitrogenous and iso caloric to the extent possible.

The fishes were put on the test diets during the two week acclimatisation period itself. The daily food quota of 7% body weight was offered in two meals. Left-over food, if any and faecal matter were collected for digestibility determinations. The physico chemical conditions of the water were monitored regularly during the seven week study.

4.2.2. Evaluation of lipid sources.

Twenty similar sized fry were allocated to each of the thirty three experimental aquaria. There were eleven treatments, all in triplicate. The mean intial length and weight were 25.87mm ($\pm$0.53) and 267.39mg ($\pm$10.45) respectively.

Eleven test diets (D1 - D 11) were formulated (Table XIII). Four plant lipids - gingely oil (D1) soyabean oil (D2), groundnut. oil (D3) sunflower oil (D4) and four animal lipids - sardine oil (D5), beef tallow (D6), shark liver oil (D7) and cod-liver oil (D8) were tested in single lipid source diets. The mixed oil diets had the following combination in equal

TABLE XII. INGREDIENT COMPOSITION OF THE DIETS USED TO EVALUATE THE NUTRITIVE VALUE OF PROTEIN SOURCES

INGREDIENTS *	D1	D2	D3	D4	D5	D6	D7	D8
SINGLE NATURAL SOURCES :								
Groundnut cake	57.26							
Soyabean meal		53.64						
Algal meal			42.88					
Fish meal				45.38				
Prawn waste meal					64.67			
MIXED NATURAL SOURCES :**								
75% Plant + 25% Animal						55.34		
25% Plant + 75% Animal							55.13	
50% Plant + 50% Animal								55.25
Gelatin	12.30	12.30	12.30	12.30	12.30	12.30	12.30	12.30
Potato Starch	19.32	11.67	24.44	31.73	14.02	19.72	23.56	23.44
Cellulose powder	2.12	13.39	11.38	1.59	--	3.64	--	--
PROXIMATE COMPOSITION %								
Protein	39.46	39.57	40.04	40.10	40.83	39.96	40.25	40.05
Lipid	5.98	6.00	5.82	5.95	6.14	5.92	5.86	6.00
Fibre	14.07	16.59	16.93	6.42	11.17	13.66	8.73	9.44
Ash	10.55	8.23	8.14	17.44	24.59	9.98	17.46	14.10
Nitrogen Free Extractives	29.39	29.57	28.97	29.60	17.27	30.13	27.04	29.94
ENERGY $kJ.g^{-1}$	16.40	16.46	16.40	16.57	14.70	16.62	16.13	16.64

* Ingredients common to all diets (in g.): Corn Oil = 3.00; Cod liver Oil = 3.00; Mineral mix = 1.50; Vitamin mix = 1.50 and Chromic oxide 1.00.

** The required proportions of groundnut cake, soyabean meal, fish meal and prawn waste.

TABLE XIII. INGREDIENT COMPOSITION OF THE DIETS USED TO EVALUATE THE NUTRITIVE VALUE OF LIPID SOURCES

INGREDIENTS *	DIETS										
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11
Ground nut cake	28.63	28.63	28.63	28.63	28.63	28.63	28.63	28.63	28.63	28.63	28.63
Fish meal	22.69	22.69	22.69	22.69	22.69	22.69	22.69	22.69	22.69	22.69	22.69
Gelatin	12.30	12.30	12.30	12.30	12.30	12.30	12.30	12.30	12.30	12.30	12.30
Potato starch	27.38	27.38	27.38	27.38	27.38	27.38	27.38	27.38	27.38	27.38	27.38
SINGLE OIL SOURCES :											
Gingely oil	6.00										
Soyabean oil		6.00									
Grountnut oil			6.00								
Sunflower oil				6.00							
Sardine oil					6.00						
Beef Tallow						6.00					
Shark liver oil							6.00				
Cod liver oil								6.00			
MIXED OIL SOURCES											
Sardine oil :									6.00		
Groundnut oil :											
Soyabean oil :											
Shark liver oil :										6.00	
Sardine oil :											
Groundnut oil :											
Gingely oil :											6.00
Soyabean oil :											
Groundnut oil :											
PROXIMATE COMPOSITION %											
Protein	40.23	40.67	40.14	40.17	40.19	40.05	40.43	40.51	40.20	40.26	40.15
Lipid	6.04	6.02	6.20	6.09	6.10	6.17	5.98	5.95	6.05	6.00	6.00
Fibre	8.46	8.21	8.33	8.42	8.53	8.39	8.45	8.36	8.24	8.53	8.49
Ash	12.40	13.33	12.91	13.17	13.22	13.04	13.21	13.30	13.35	13.20	13.19
Nitrogen Free Extractives	31.67	31.19	32.15	32.06	31.94	32.233	31.72	31.81	31.67	31.79	31.52
ENERGY $kJ.g^{-1}$	16.99	17.00	17.11	17.06	17.05	17.09	17.02	16.96	16.99	17.00	16.93

* All diets included 1.50g Mineral mix and 0.5g Vitamin mix.

proportions: Diet D9 = sardine oil + groundnut oil + soyabean oil; D10 = shark liver oil + sardine oil + groundnut oil; and D11 = gingely oil + soyabean oil + groundnut oil. Fishmeal and groundnut cake were the protein sources common to all diets and the carbohydrate component was constituted by potato starch. Chromic oxide at 1% was incorporated for digestibility studies. Proximate analysis revealed the diets to be of fairly uniform compositions and energy.

After the initial acclimatisation as described in the protein source experiment, the animals were maintained on the experimental rations for seven weeks. They were fed twice, a daily ration of 7% body weight. Faecal matter was collected for finding out the digestibility. Uniform environmental conditions were ensured for all treatments.

4.2.3. Field trial of compounded feeds.

This experiment was conducted over a period of seven weeks to test the efficacy of five formulated diets under farm conditions. Uniform sized (initial length 25.20 mm, weight 248 mg) fry of L. parsia (100 nos in each hapa) were carefully allotted to the velon net hapas ($1m^3$) fixed in the brackishwater pond of the Central Marine Fisheries Research Institute at Narakkal, Cochin. (Plate 14). The pond enjoyed good water

PLATE 14. NET CAGES HOLDING THE FRY UNDER DIFFERENT TREATMENTS IN THE FIELD TRIAL OF COMPOUNDED DIETS.

exchange as it was directly connected to the main canal of the brackishwater system.

Five diets were compounded based on the data accrued from the experiments conducted in the laboratory. (Table XIV). The ingredients used in compounding the diets were groudnut cake, gingely cake, coconut cake, rice bran, mangrove leaves (Avicennia officinalis), fish meal and prawn waste. Tapioca powder and wheat powder also acted as binders. Mineral and vitamin mixtures were added in all the diets, except D5. The feed materials were included at maximum possible levels compatible with providing around 35% protein (except for D4, where it was 28% Diet D1 included all the feed stuffs listed above. In D2 coconut cake was removed and an increment was made in the level of mangrove leaves. In diet D3 the animal protein components (fish meal, prawn waste) were replaced by an increased amount of groundnut cake. Therefore, D3 was a plant based diet. Diet D4 was similar to D1 except that it had reduced protein percentage. Diet D5 was similar to D1 in ingredient composition except that it did not have vitamin and mineral mixtures.

The diets were tested in duplicate. Initially feeding was at 7% body weight, but only about 4% was ingested by the fish. Therefore the latter amount was offered to the fish during the subsequent feedings. Food was offered in a single ration at 0800 hrs. The percent weight gain, the relative daily gain and

TABLE XIV INGREDIENT COMPOSITION OF THE COMPOUNDED DIETS

INGREDIENTS	DIET 1	DIET 2	DIET 3	DIET 4	DIET 5
Groundnut cake	20.00	20.00	50.00	15.00	20.00
Gingely cake	10.00	10.00	10.00	7.50	10.00
Coconut cake	10.00	--	10.00	10.00	10.00
Rice bran	10.00	10.00	10.00	10.00	10.00
Wheat flour	5.00	15.00	5.00	5.00	5.00
Tapioca powder	8.00	8.00	8.00	18.00	8.00
Maida powder	5.00	5.00	5.00	10.00	5.00
Fish meal	20.00	20.00	--	15.00	20.00
Prawn waste meal	10.00	10.00	--	7.50	10.00
Cellulose powder	--	--	--	--	2.00
Mineral mix	1.50	1.50	1.50	1.50	--
Vitamin mix	0.50	0.50	0.50	0.50	--
PROXIMATE COMPOSITION (%)					
Protein	34.70	33.27	31.70	28.07	33.70
Lipid	7.81	6.72	7.83	6.92	7.81
Fibre	12.03	14.91	13.74	13.47	13.52
Ash	14.52	14.73	11.12	13.83	14.21
Nitrogen free extractives	30.13	29.58	35.37	37.80	30.26
ENERGY $kJ.g^{-1}$	16.13	15.29	16.35	15.58	15.92

apparent food conversion were calculated.The diets were tested in duplicate. They were offered to the fishes at 4% body weight in a single ration at 0800 hrs, on a feeding tray to avoid wastage.A control group of unfed fishes were also maintained. Weekly observations on growth were made during the experimental period. The conditions of the experiment should have eliminated any differential effect from environmental variables, extraneous food, feeding protocol etc., so that the observed differences in growth is a function of the diet alone.

4.3. RESULTS

4.3.1. Evaluation of protein sources

The experimental conditions recorded were:
salinity 15 $\pm$ 1ppt, temperature 27.4 $\pm$ 2.1^{o}C
pH 7.730 $\pm$ 1.80, ammonia 0.414 $\pm$ 0.109 mg l^{-1}
and oxygen 5.27 $\pm$0.15ppm.

The survival percentage varied when the diets containing different sources of protein were fed to fishes. Best survival (95%) was obtained with diet D4 containing fish meal. Equally good survival (92%) was obtained with diet D8 containing equal proportion of plant and animal protein sources. Lower survival values were recorded for soyabean meal based diet, D2 (75%) and prawn waste based diet D5 (70%; Table XV).

TABLE XV. RESULTS OF THE EXPERIMENT ON EVALUATION OF PROTEIN SOURCES

PARAMETERS	DIETS							
	D1	D2	D3	D4	D5	D6	D7	D8
Survival (%)	88.00	75.00	80.00	95.00	70.00	90.00	87.00	92.00
Condition Factor	1.28	1.29	1.29	1.23	1.28	1.23	1.25	1.24
Weight gained (g)	0.551	0.525	0.486	0.563	0.466	0.582	0.556	0.597
Apparent digestibility coefficient of protein	87.24	81.30	80.43	88.16	76.34	87.75	88.06	89.28
Apparent digestibility coefficient of lipid	91.15	90.86	91.99	91.85	92.18	91.83	92.66	92.49

The condition factor obtained for the different treatments ranged from 1.23 for fish meal diet (D4) to 1.30 for soyabean meal diet (D3). The values obtained for groundnut oil cake diet D1 (1.29), algae diet D3 (1.29) and prawn waste diet D5 (1.28) were not significantly different from each other (Table XV).

The total weight gain in the fry was significantly ($P < 0.05$) influenced by the protein sources in the diets. The maximum weight gain (597 mg) was obtained when both animal and plant protein sources were included in the diet (D8) at equal proportions (Fig. 14). The increment was 582 mg when the mixture of sources contained more of plant material (D6). The weight gain in the case of fish meal diet D4 (563 mg) was not significantly different from that of combination diet D7 containing more of animal sources in the mixture. Increment was least (466 mg) when the diet contained prawn waste as the protein source (Table XIV). Specific growth was maximum (2.29) for D8. The value for groundnut cake based diet fed group (D1) was 2.24 and fish meal fed group (D4) was 2.22. The values obtained for soyabean meal diet D2 (2.18) was not significantly different from that obtained for groups D6 and D7 (2.19), both containing plant and animal protein sources (Fig. 15).

Food conversion ratios were better (1.47) when a mixture of both plant and animal protein sources were included in the

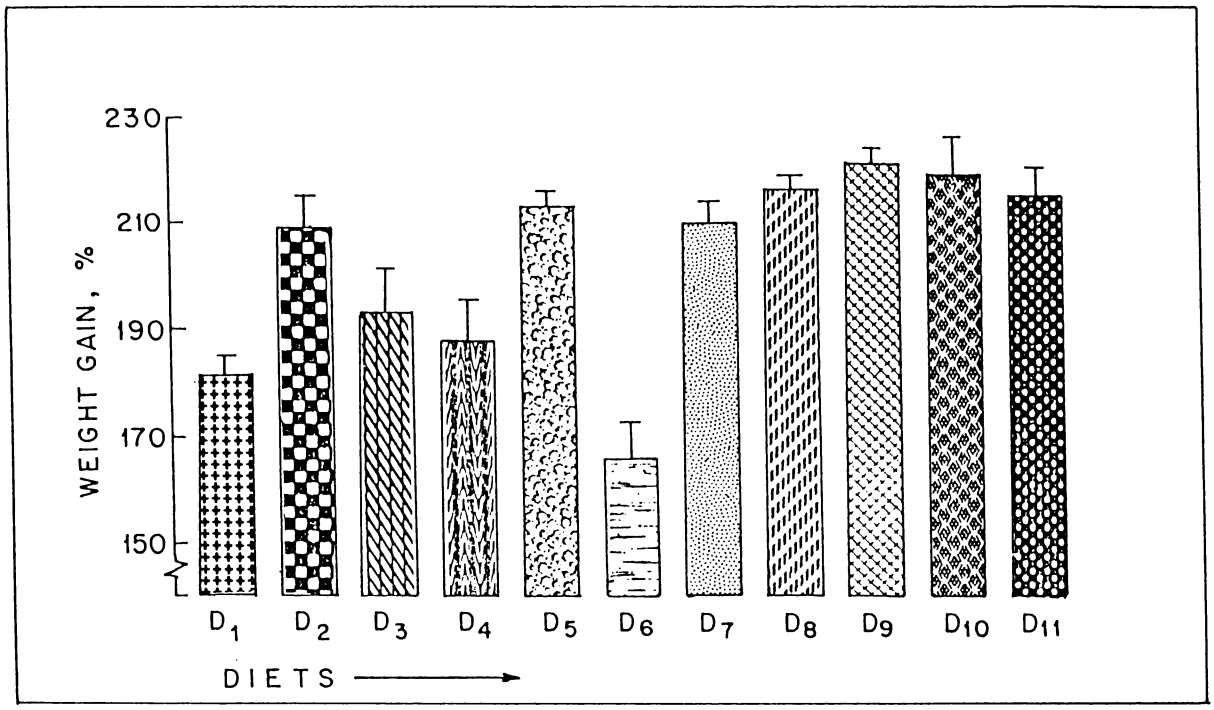

Fig.14. WEIGHT GAIN IN FISHES FED DIETS INCORPORATING DIFFERENT NATURAL SOURCES OF PROTEIN.

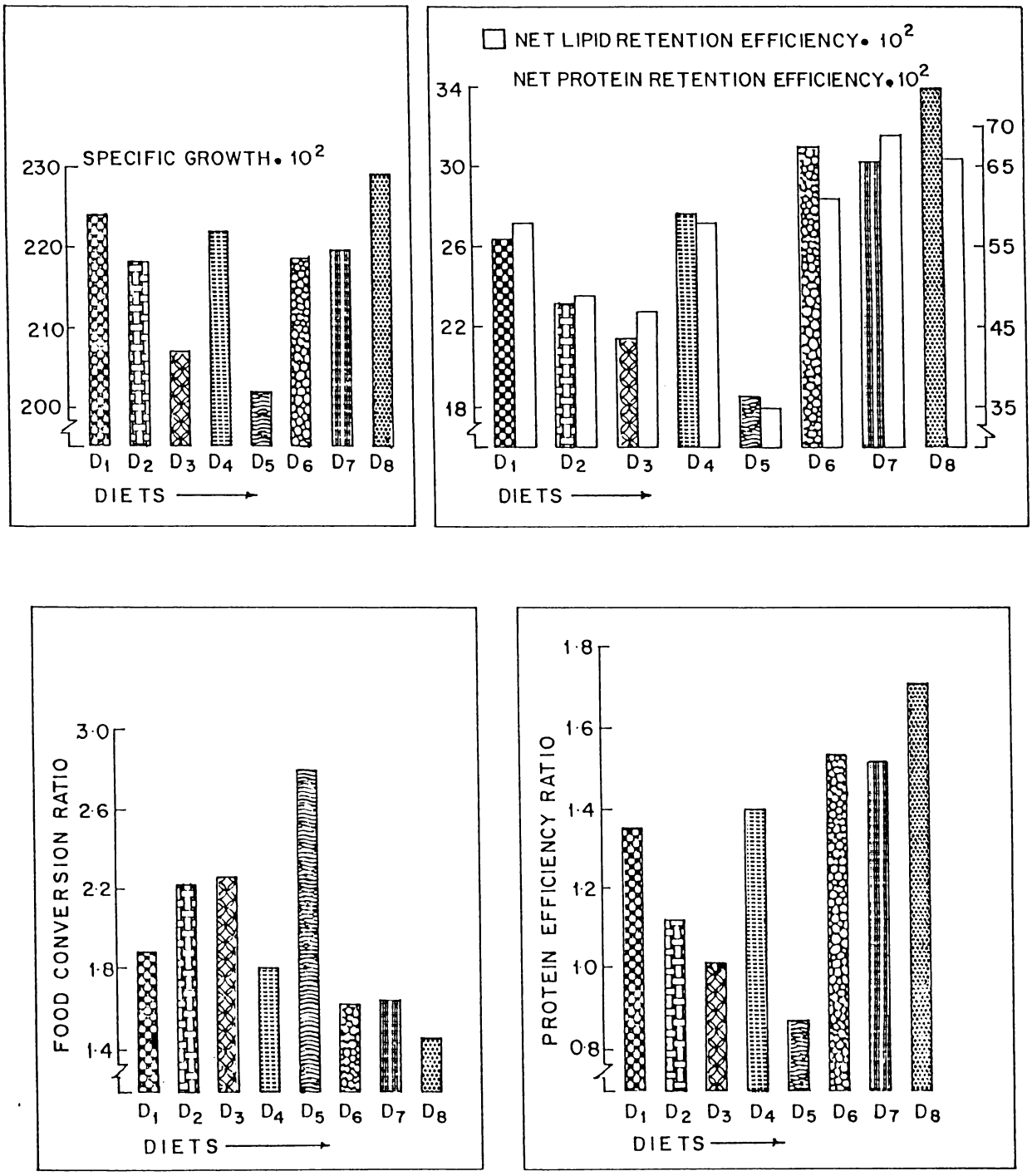

Fig.15. SPECIFIC GROWTH, NUTRIENT RETENTION EFFICIENCY, FOOD CONVERSION RATIO AND PROTEIN EFFICIENCY RATIO IN FISHES FED DIETS INCORPORATING DIFFERENT NATURAL SOURCES OF PROTEIN.

diets, with the best value for a diet containing 1:1 ratio of both the sources. A change in proportion slightly lowered the conversion rates. Diet with algal protein source could provide a conversion of only 2.46. Conversion ratio was the poorest (2.81) for the prawn waste diet (Fig. 15).

Protein efficiency ratio too was best for diet D8 (1.70) containing equal amount of plant and animal protein sources. The other combined sources diets D6 and D7 also provided good ratios of 1.54 and 1.52 respectively. PER was relatively poor for the algal source diet (1.01) but the lowest (0.87) was for prawn waste meal fed group (Fig. 15)

Proximate analysis revealed that the body composition is significantly ($P < 0.01$) influenced by the dietary protein sources. The moisture content varied between 73.33% for diet D8 and 72.12 for diet D5 (Fig.16). The application of dietary protein sources of both plant and animal origin,in different proportions (Diets D8, D7 and D6) resulted in higher percentages of protein (62% - 63.98%) in the body. The diet containing fish meal as the protein source also resulted in good protein deposition (62.72%). Maximum body lipid (22.38%) was noted for the fish fed diet D7 in which more of animal protein source was incorporated. But this was not significantly different from the values recorded for algal diet fed groups (D3 = 22.20%) and soyabean diet fed groups (D2 = 22.10%). Lipid was the least in

the groups fed the diet with prawn meal. There was no significant difference in the value (20.99%) obtained for plant sources dominant diet (D6).Maximum body ash was found in fishes fed on prawn meal diet D5 (15.6%). The algae diet fed group recorded a value of 15.3%. The ash content in fish groups fed diets containing soyabean meal (D2), fish meal (D4) and the combination of protein sources D6, D7 and D8 were not significantly different from each other (Fig 16).

Nutrient retention efficiency was significantly ($P < 0.01$) influenced by the source of protein used in the diets. Mixed protein source diets proved to be the best retainers of protein, with the diet D8 showing a retention efficiency of 33.9%. Amongst the individual sources,Diet D4 with fish meal achieved an efficiency of 27.6% as compared to 26.4% of diet D1 containing groundnut cake. Retention efficiency was the lowest for the diet with prawn meal, recording 18.5% for protein and 35.2% for lipid. Lipid retention efficiency was maximum (68.7%) in the animals fed the diet containing more of animal protein. The efficiency was equally good in the case of diets D8 and D6 (65.7% and 61.2% respectively). Amongst the single protein source diets, fish meal based diets recorded the best lipid retention efficiency of 58.1% (Fig 15). The apparent protein digestibility coefficient was the highest for the diet (89.28%) containing a mixture of both plant and animal protein sources in

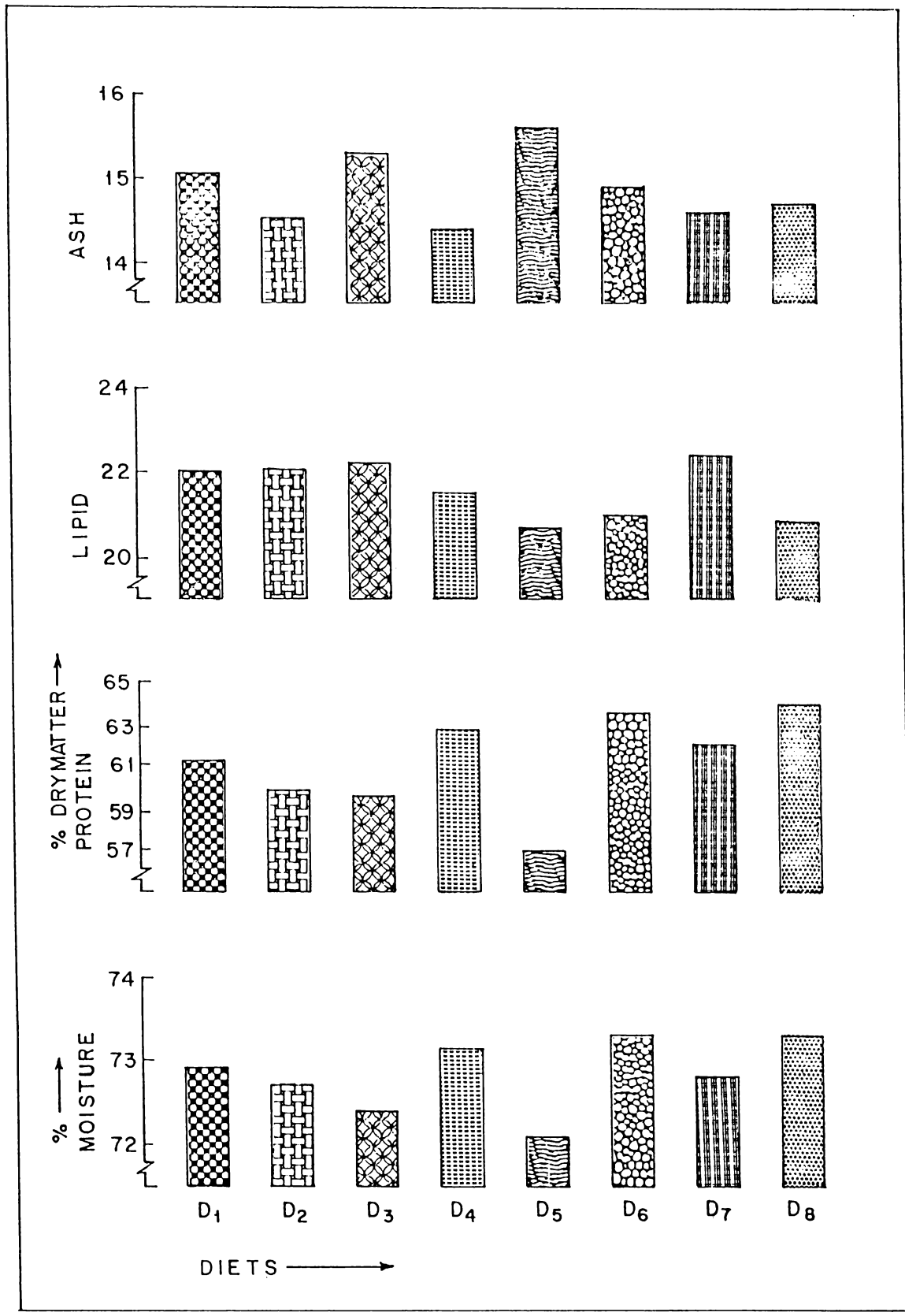

Fig.16. BODY COMPOSITION OF FISHES FED DIETS INCORPORATING DIFFERENT NATURAL SOURCES OF PROTEIN.

the ratio 1:1. Diet with fish meal and diets D6 and D7 with mixture of plant and animal protein sources also had better coefficient than the other diets (Table XVI). The lowest value was recorded for prawn waste meal (76.34%). Lipid digestion seemed to be better in the combined diets, especially when the animal component was more (D7=92.66%). Soyabean meal had comparatively poor digestibility (90.86%).

4.3.2. Evaluation of lipid sources

The conditions that prevailed during the 7 week feeding experiment were salinity 15±1 ppt, temperature 28.1 ±1.7°C; pH 7.815 ± 0.152, ammonia 0.325 ± 0.117 mg l^{-1} and oxygen 5.05± 0.36ppm.

The different lipid sources in the diet supplied to the fry of the mullet significantly ($P < 0.05$) influenced the survival. Survival was best (98%) in cod liver diet (D8) and diet D9containing a mixture of sardine oil, soyabean and groundnut oil. Good survival rates (95%) were also obtained for diets having groundnut oil (D3), sardine oil (D5) and the mixture containing more of animal oils (D10). Survival was poor (76%) when beef tallow was the lipid source in the diet.

Condition factor was better in fishes fed diets containing either gingely oil (D1=1.28), shark liver oil

TABLE XVI RESULTS OF THE EXPERIMENT ON EVALUATION OF LIPID SOURCES

PARAMETERS	DIETS										
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11
Survival (%)	83.00	90.00	95.00	85.00	95.00	77.00	93.00	98.00	98.00	95.00	90.00
Condition Factor	1.28	1.23	1.24	1.27	1.22	1.26	1.27	1.20	1.22	1.19	1.21
Weight gained (g)	0.495	0.542	0.523	0.504	0.571	0.434	0.532	0.575	0.623	0.594	0.568
Apparent digestibility coefficient of protein	87.91	89.04	88.20	88.39	89.21	87.20	88.74	89.45	89.24	88.94	88.36
Apparent digestibility coefficient of lipid	82.83	86.49	83.33	83.52	95.32	76.31	95.01	96.16	95.49	94.98	89.17

(D7=1.27) or sunflower oil (D4=1.27). The oil mixture containing more of plant oils when supplied to the fry resulted in a factor of only 1.19.

The dietary lipid sources significantly ($P < 0.01$) influenced the growth of fishes. The best response (623 mg) was obtained for the diet D9 containing a mixture of oils with the plant oils predominating. The weight gain was 594 mg when another diet (D10) with oil mixture containing more of animal oils were fed. Among the single oil sources tested, cod liver oil (575 mg) and sardine oil (571 mg) provided almost similar gains. Soyabean oil proved to be the best in promoting growth (weight gain, 542 mg) amongst plant lipids. The lowest weight increment of 434 mg was for the diet with beef tallow as lipid source (Table XVI). The percentage weight gains are graphically represented in Fig.17. The maximum specific growth was recorded for diet D9 with more of plant oils in a mixture; but it was not significantly different from the specific growth obtained for diets D8, D10, D11 (Fig.18)

The best conversion ratio (1.70) was obtained with the diet containing the oil mixture (D9). Nearly equal value (1.75) was obtained when fed diet D10 with more of animal oils. The ratios for cod liver oil (D8 = 1.8) plant oil mixture (D11 = 1.81) and sardine oil (D5 = 1.83) were not significantly different from each other. Conversion was very poor for the diet

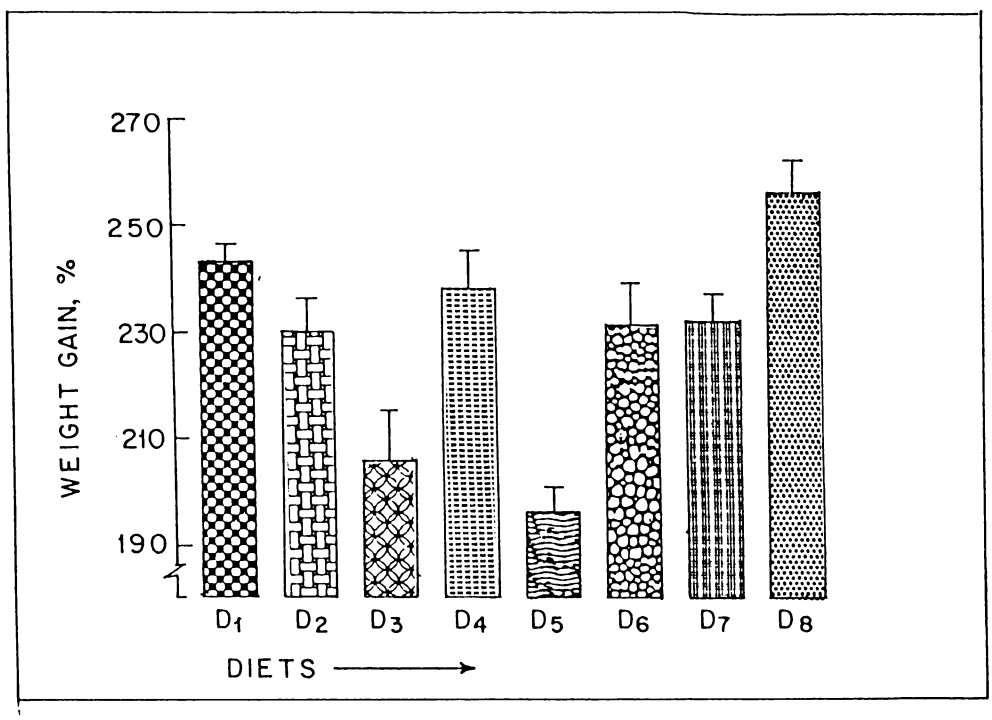

Fig.17. WEIGHT GAIN IN FISHES FED DIETS CORPORATING DIFFERENT NATURAL SOURCES OF LIPID.

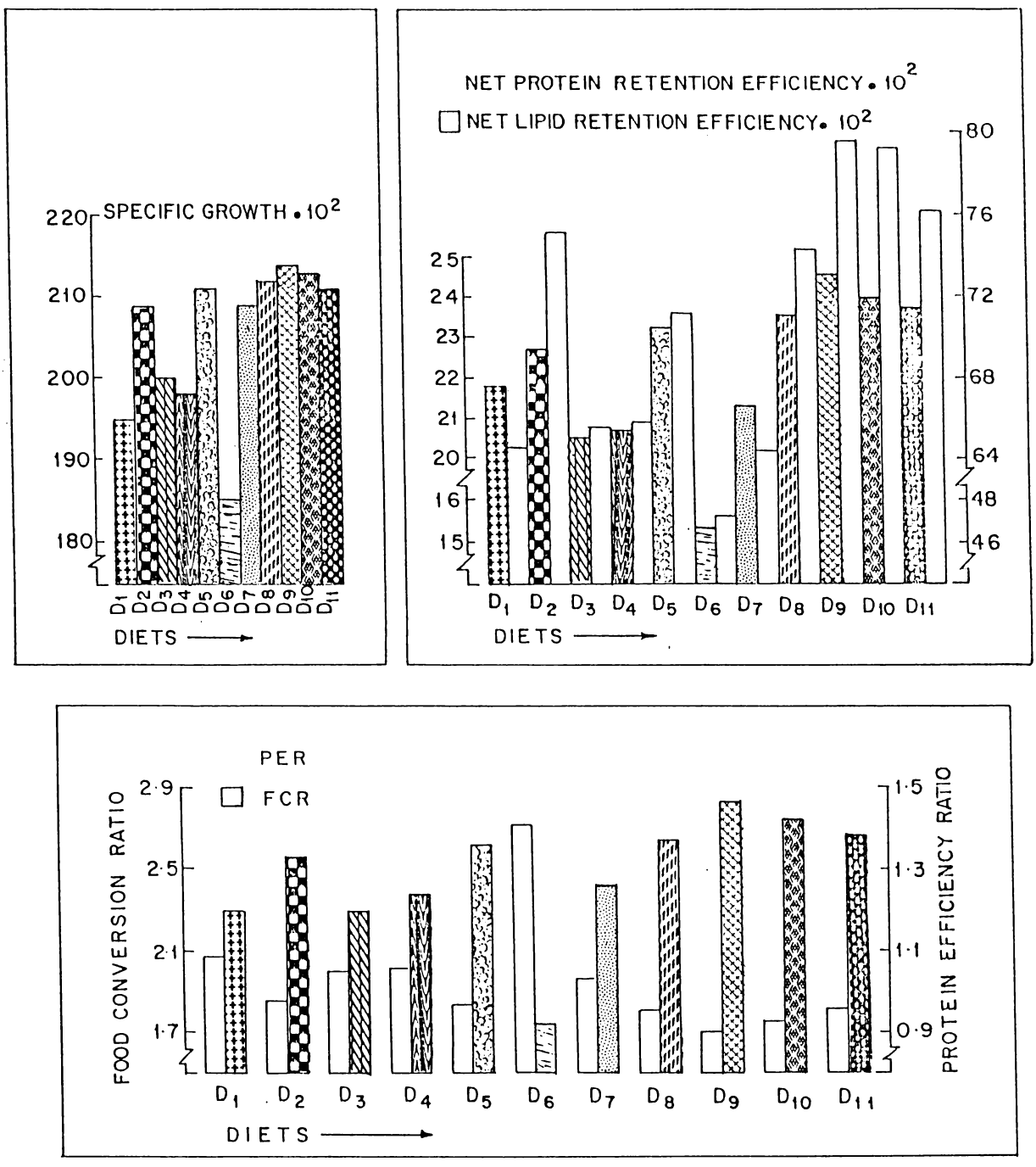

Fig.18. SPECIFIC GROWTH, NUTRIENT RETENTION EFFICIENCY, FOOD CONVERSION RATIO AND PROTEIN EFFICIENCY RATIO IN FISHES FED DIETS INCORPORATING DIFFERENT NATURAL SOURCES OF LIPID.

with beef tallow (D6). Protein efficiency ratio was the best (1.46) when diet D9 was fed to the fishes. Equally good ratio of 1.42 was obtained with diet D10. The ratios obtained for the diets containing cod liver oil (D8) and plant oil mixture (D11) and sardine oil (D5) were similar. Beef tallow based diet proved to be the least efficient (0.92; Fig. 18).

The different dietary lipids offered to the fry had highly significant ($P < 0.01$) effect on their body composition (Fig. 19). The moisture content ranged between 72.17(D9) and 73.26% (D6).

Maximum body protein (61.70%) was recorded in fish fed the diet containing a mixture of plant oils alone (D11). This was however not significantly different from the response of soyabean oil diet (61.57%). The protein percentage recorded for diets with ground nut oil (D3 = 61.26%), sardine oil (D5 = 61.24%), codliver oil (D8 = 61.20%) and shark liver oil (D7 = 61.08) were not significantly different from each other . The body lipid percentage was highest for diet D9 (26.45%) with a mixture of oils. But this was not significantly different from values obtained for Diets D10 and D8 = 26.33 and Diet D5 = 26.20 (Fig.19). Ash content was highest in the fish group fed beef tallow (14.76%) in the diet. The percentages of 14.46 and 14.37 obtained for groups fed on diet containing gingely oil and sunflower oil (D4) were not significantly different. The

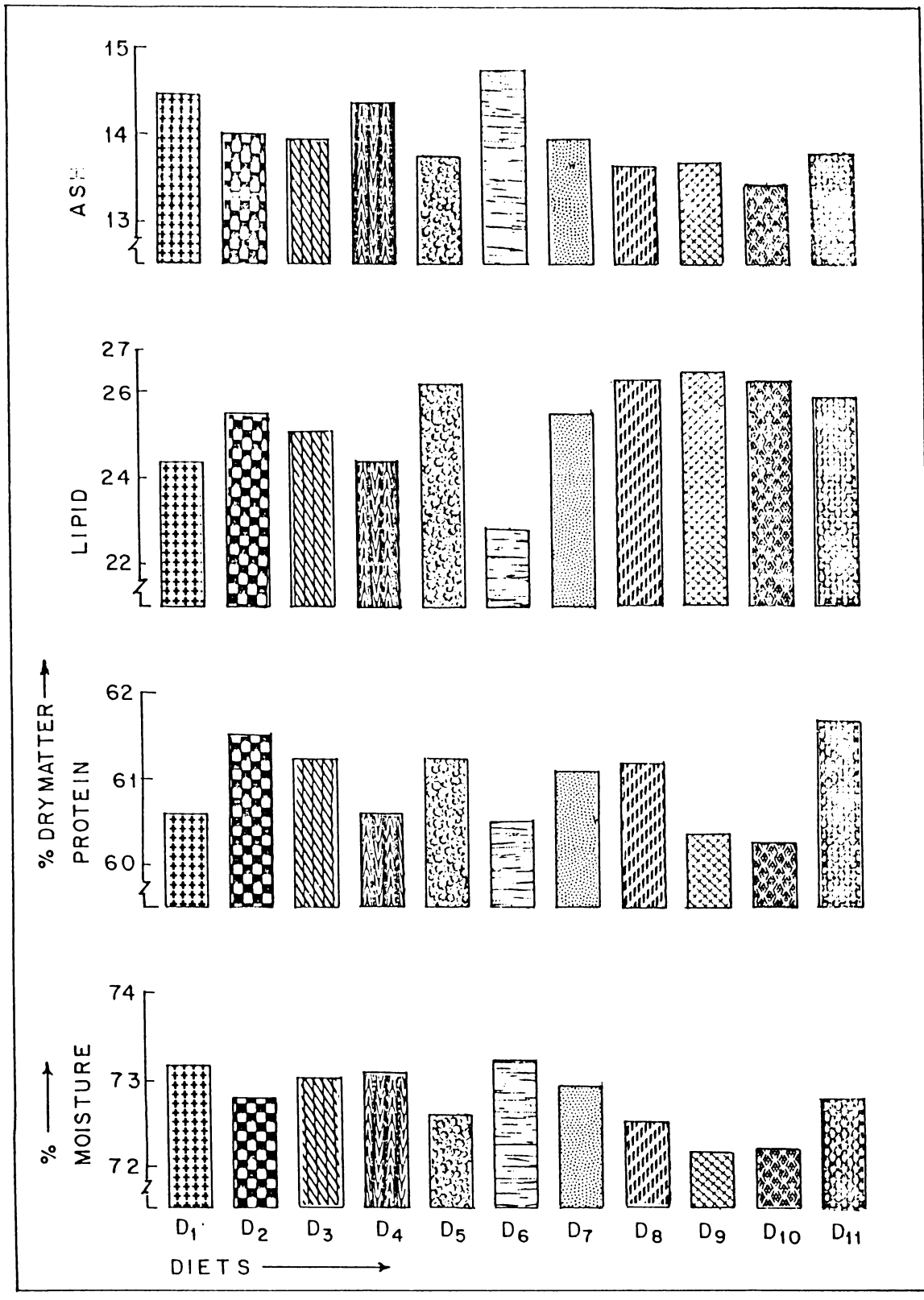

Fig.19. BODY COMPOSITION OF FISHES FED DIETS INCORPORATING DIFFERENT NATURAL SOURCES OF LIPID.

first group supplied a diet with an oil mixture containing more of animal oil (D10) had the lowest ash (13.42%) level.

The nutrient retention also varied significantly ($P < 0.01$) with the different oils included in the diets. The maximum retention efficiency for protein was obtained with diet D9(24.5%). The values for diets D10 (23.2%) D11 (23.6%) and D5 (23.2%) were not significantly different. Diet with beef tallow recorded a value of only 15.3% (Fig.18). A wide variation in lipid retention efficiency was noted when different sources of lipid were employed in the diets. Oil mixtures containing both plant and animal lipids were retained better D9 = 79.6%, D10 = 79.3%.Diets with plant oil mixture (D11 = 76.2%),codliver oil (D8 = 74.3%) and soyabean oil (D2 = 75.2%) recorded a performance which did not vary significantly. Lipid retention efficiency was least (47.1%) when beef tallow was the lipid source.

Good protein digestibility was recorded for diets D8 with cod liver oil (89.45%), D9 with more of plant lipids in the mixture (89.24%), D5 with sardine oil (89.21%) and D2 with soyabean oil (89.04%; Table XVI). Highest digestibility coefficient for lipid (96.16%) was obtained when fed the diet with cod liver oil. The oil mixture in diet D9 gave an apparent digestibility coefficient of 95.49%. Sardine oil was also well digested (coefficient = 95.32%). The digestibility coefficient was very poor (76.31%) in the case of beef tallow.

4.3.3. Field trial of compounded feeds

The abiotic conditions that prevailed in the pond during the seven week experimental period were: salinity 14 ± 2.5ppt, temperature 30.5± 2.1°C and pH 7.85 ± 0.20.

Five compounded diets were offered to five duplicate groups of fishes. Percentage survival was above 90 in all cases, 97% being recorded for diet D1. The maximum gain of 1289 mg was obtained for diet D1. This was followed by diet D5 (1247 mg) and diet D3 (1208 mg; Table XVII). The weight gained by the fish in treatments were over twice that recorded in the control (unfed) groups. The percentage wet weight gain was 520 for D1 as against 230 for unfed control (Fig.20). Diet D4 produced a weight gain of only 430%. The condition factor of fishes fed on different diets were 1.61 for D1, 1.49 for D3, 1.47 for D5, 1.31 for D2 and 1.28 for D5. In the unfed group it was 1.03. The relative daily weight gain was better for D1 (10.6%) and D5 (10.3%) than that for D4 (8.79%). The apparent conversion ratio was best (1.22) for diet D1 followed by D5 with a value of 1.23. Diet D4 recorded a comparatively poor conversion of 1.300. The gross conversion efficiency for diet D4 was 77% as against 82% recorded for D1.

Analysis of stomach content of the unfed fishes revealed remains of crustaceans (copepods/amphipods), nematodes, diatoms, plant fragments etc.

TABLE XVII RESULTS OF THE FIELD TRIAL OF COMPOUNDED DIETS

DIET	SURVIVAL	WEIGHT GAINED (g)
Diet 1	97	1.289
Diet 2	93	1.093
Diet 3	96	1.208
Diet 4	94	1.068
Diet 5	95	1.247
Control (unfed)	90	0.571

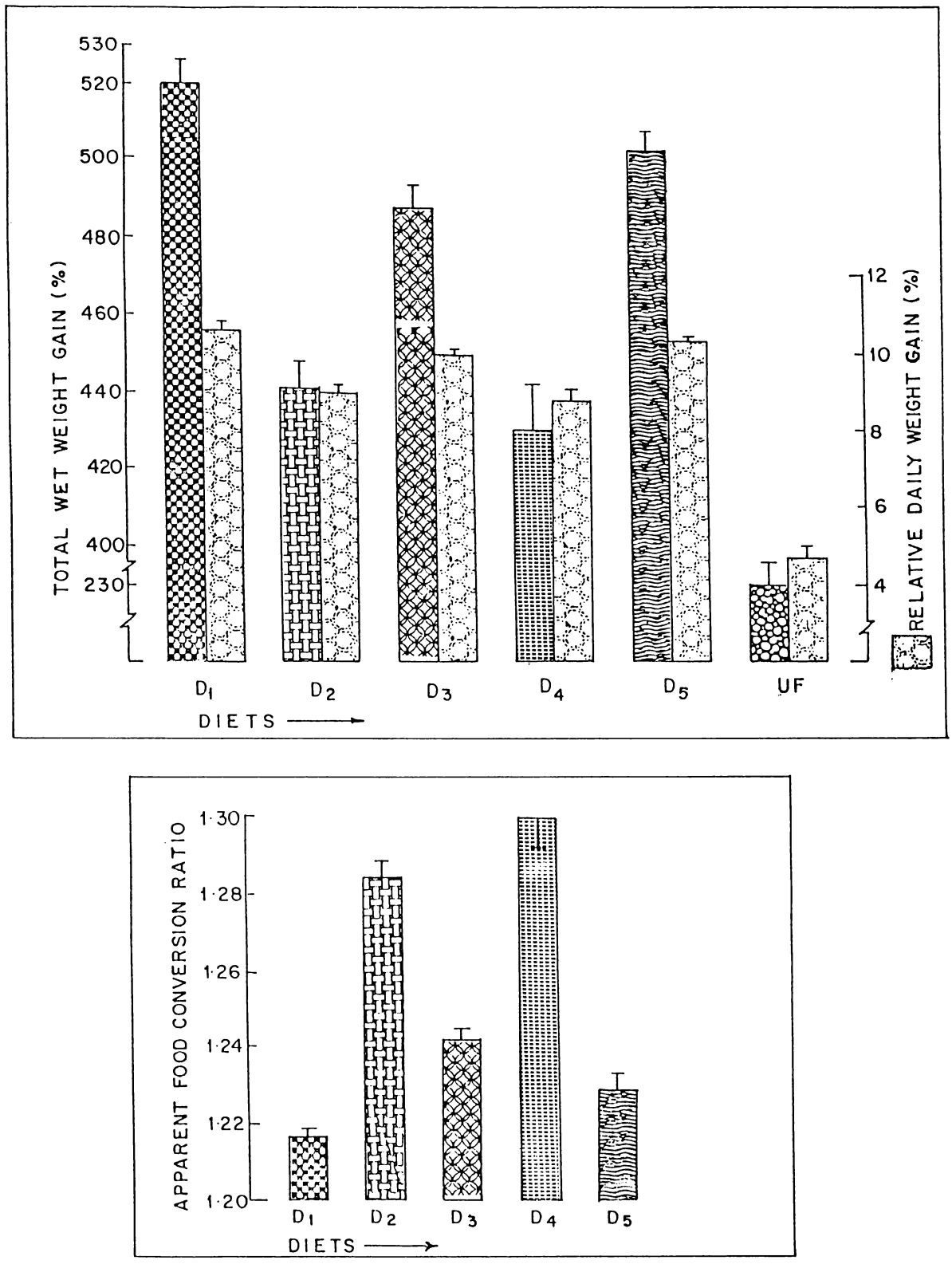

Fig.20. WEIGHT GAIN AND APPARENT FOOD CONVERSION RATIO IN FISHES FED COMPOUNDED DIETS UNDER FIELD CONDITIONS.

4.4. DISCUSSION

The growth potential of a fish is influenced to a great extent by the food quality (Pandiyan, 1967). The fry of Liza parsia exhibited feed preferences when a variety of diets, formulated from a host of natural sources, were offered. The findings of these experiments are discussed based on the relative nutritive value of the feed stuffs.

4.4.1. Nutritive Value of Protein Sources

The plant sources studied were ground nut cake (D1) soyabean meal (D2) and algal meal - Spirulina (D3). Among these groundnut oil cake seems to be a better protein source as it turned out the maximum survival and growth of fish besides good conversion of ingested food and protein. However the condition factor did not reveal any significant difference between the diets (D1, D2 and D3). The poor conversion value for the fish group fed on diet with algal meal reflected on the weight gain and the protein efficiency ratio. Body protein content in fishes fed plant proteins (D1, D2 and D3) was less compared to those on animal protein diets. The percentage lipid was almost the same in all the groups fed plant proteins and it was slightly higher than that recorded for other groups. The mixed protein sources diets (D6, D7 and D8), the fish meal diet (D4) and the

groundnut diet (D1) retained more of the nutrient as they were better digested.

Groundnut cake seems to be a plant protein source fairly accepted by Liza parsia. All the perfomance parameters discussed earlier point out this fact. Das (1976) has also identified groundnut cake as a highly preferred feed stuff for grey mullet Mugil cephalus. In tilapia Sarotherodon mossambicus (Jackson et al. 1982) groundnut cake was found to be an accepted protein source but at low inclusion levels. However, Wu and Jan (1977) reported poor growth in Tilapia aurea when fed on an all groundnut protein diet. The deficiency of methionine and lysine in the ingredient is a limiting factor for maximum utilization.

Soyabean meal is used extensively, next only to fish meal, in commercial feeds, because of its high nutritive value and palatability (Robinson and Daniel, 1987). In the present experiment on Liza parsia a fairly good weight increment was recorded with soyabean meal in the diet inspite of a slightly greater mortality. The protein digestibility was lower to that of groundnut cake, probably indicative of a partial trypsin inhibition. Soyabean meal was used successfully in feeding salmonids as a substitute for fish meal when the lacking amino acids were supplemented (Dabrowska and Wojno, 1977; Smith, 1977). However there are many reports on inferior growth rates when fed soyabean meal in the diets. Davis and Stickney (1978) reported a

32% decrease in growth rate and Wu and Jan (1977) recorded a 27% decrease when soyabean was the protein source instead of fish meal in the diets of Tilapia aurea. Poor growth was also noted in plaice and carp (Cowey et al., 1971; Dabrowski and Kozak, 1979; Viola et al., 1983). The trypsin inhibitor in soyabean is responsible for its poor utilization because it interferes with the protein digestion (Smith, 1971; Dabrowski and Kozak, 1979). But Nose (1971), Atack and Matty (1979) and Atack et al. (1979) found unimpaired protein digestibilities and obtained biological value of soyabean meal as high as those of fish meal. The principal conclusions on soyabean meals arrived at by Viola et al. (1982) are that it is deficient in available energy and lysine in addition to methionine. These deficiencies can be remedied by proper supplements of oil and amino acids.

The use of unicellular algal meal as feed for warmwater fish has been reported by several workers (Terao, 1960; Ahmad, 1966 ; Stanley and Jones, 1976 ; Meske and Pruss, 1977). The species usually employed for the meal are Chlorella, Scenedesmus, Spirulina, Euglena, Oocystis and Micratinum. In general the dried cell material of most algae contain 50 to 65% protein (Tamiya, 1975) but the cost of production hampers large scale utilization. Digestibility of algae is lower than that of fish meal but comparable to other vegetable protein sources (Hepher et al., 1979). Although the algal diet was well accepted,

the poor growth in L. parsia could be attributed to amino acid limitation as pointed out by Atack and Matty (1979) in trouts. The inferior performance of the algal diet in L. parsia can also be due to the lower digestibility recorded. A similar observation has been made earlier by Hepher and co-workers (Hepher et al.,1979) when identifying alternate protein sources.

As in the present study, low protein effeciency ratio has been reported in mirror carp fingerlings for the same species of algae (Atack et al., 1979). The proximate composition of gold-spot mullet fed the algal incorporated diet showed a higher percentage of fat and lower protein levels. In black seabream and yellowtail (Nakagawa et al., 1984,1985) also algal diets induced lipid accumulation. It seems, more energy from the diet is channelized for synthesis of lipid. This may be one of the reasons for excess fat being deposited in edible tissues. Algal meal though a good source in terms of protein content does not seem to be an ideal one for use in diet as a major component.

The dominance of fish meal in feeds of cultivated fishes has challenged nutritionists for many years. Successful replacements by other protein feed stuffs have been reported, mostly of animal origin: milk or whey powder (Meske et al., 1977), feather meal and meat meal (Tiews et al., 1976), krill meal (Pfeffer and Meske, 1978), single cell protein (Atack et al., 1979) and algal meal (Sandbank and Hepher, 1978). But most

of these items are scarce or as expensive as fish meal. On the other hand most attempts to replace fish meal by plant proteins like soyabean meal have led to reduced growth and low food conversion rates in carp and other fishes (Nose, 1971; Koops et al., 1976; Atack and Matty, 1979; Atack et al., 1979). Growth, food conversion, protein efficiency ratio and protein retention data suggests fish meal to be the best source of protein for L. parsia fry. The utilization of fish meal by the fry indicates that the essential amino acid content in the ingredient is quite well balanced to the requirements of the mullet.

The quality of shrimp meal determines how best the ingredient could be incorporated in the diets of L. parsia. The mullet fry does not seem to relish this protein source as revealed by the poor growth and conversion. The proximate composition of the animal also gave an indication of the poor quality of the meal. The high ash content can be attributed to this. Care should be taken in its inclusion as an ingredient for it may affect pellet stability too (New, 1987).

Diets with mixed protein sources offered to the mullet in the present study proved superior to the diets based on individual protein sources. Equal proportions of animal and plant proteins seemed to be the best formulation for the young ones of Liza parsia. The groups of fishes fed on the above diet

ranked better as regards growth, conversion efficiency, protein efficiency and nutrient retention. The digestibility coefficient also was better than that recorded for the counterparts offered mono dietary sources.Mathavan et al. (1976) also observed in Oreochormis mossambica that supplementation of animal matter to vegetable food increased the digestibility fraction, thereby improving growth performance. Pandiyan and Vivekanandan (1985) have also established that when fed exclusively on plants or detritus, the absorption efficiency of herbivores/detritivores was lower than that recorded for carnivores. The higher absorption efficiency exhibited by a herbivore when fed exclusively on an animal diet and the high protein requirement suggest that animal matter is essential for herbivorous and detritivorous fishes and that these fishes neither will nor can consume and absorb a sufficient quantity of plant/detrital material to meet their metabolic energy demands (Menzel, 1959; Kitchell and Windell, 1970).

The main protein source suitable for fish feed must have high protein content because of the higher protein requirements of fish especially in the younger stages. Thus animal and plant protein sources have been used in various combinations. For fast growing animals, the protein which contains essential amino acid in such a balance as those found in protein of growing animal is evaluated as high in quality (Nose,1970). The essential amino

acid composition of whole body has been revealed to be remarkably similar between freshwater/seawater fish and between egg, yolk sac, fry and fingerlings (Halver, 1957a). Fish meal showed similar patterns in essential amino acid contents as the fish of low or high fat content, from freshwater/seawater origin (March 1967). Thus fish meal can be the animal protein of best quality for most fishes. Other proteins show specificity in that their amino acid composition is different from that of whole fish. Therefore, these materials should be used in combination with fish meal to ensure well balanced amino acid composition. In conclusion, it could be stated that a combination diet incorporating plant and animal sources is ideal for rearing the fry of Liza parsia.

4.4.2. Nutritive value of lipid sources

Though the use of lipid is widely recommended in fish feeds, most commercial formulations conveniently avoid oil supplements because the individual feed stuffs constituting the diet contain a good percentage of lipids. Moreover it has also been noted that the natural foods of mullets (Quasim, 1972; Kurian, 1975) contain appreciable percentage of lipids. As fats with high melting point are poorly absorbed, fish oil and vegetable oils are commonly used singularly or in combination in the fish diets. Several species of oils have been used for

feeding the fry in this experiment. The relevance of the findings in formulation of starter diets have been discussed irrespective of the fact that only few attempts have been made earlier in comparing the nutritive value of natural lipid sources.

The vegetable oil sources tested were gingely oil, soyabean oil, groundnut oil and sunflower oil. Among these, soyabean oil evoked the best growth response with better FCR and PER, though morta lity was slightly more than that observed in fish groups fed diet with groundnut oil. Higher body protein was also recorded in these fishes. The efficient utilization of soyabean oil as an energy source would have spared protein for growth. That the other oils were not as efficient in sparing protein was indicated by the fact that their body protein levels were comparatively lower even as the diets were almost isocaloric. Sin (1973) had earlier found a protein sparing effect by soyabean oil in small carps. The lipid retention efficiency in the case of soyabean fed fishes was high and this preferential response can be attributed to the higher content of n-3 series fatty acids (about 6.8% of the lipid) in this oil (New, 1987). Groundnut, ginge ly and sunflower oils had very little amount of the n-3 fatty acids (Shephard et al., 1978). Among the animal oils tested cod liver oil and sardine oil induced the best growth, FCR and PER. Body protein was also high

in these groups. Similar observations have been made in channel catfish groups fed fish oils (Dupree et al., (1979). Protein and lipid retention efficiency was also superior in fish fry fed codliver oil and sardine oil. Hardy et al. (1979) found heavier pacific salmon when fed codliver oil compared to beef tallow. The improved performances of codliver and sardine oil can be primarily related to the high amount of n-3 fatty acids in them (Chandge, 1987), especially because energy would not have been a limiting factor. The relative drops in response to shark liver oil in diet is probably due to the large quantity of squalene it contains, inspite of high essential fatty acid levels. Its growth inhibiting effect in fish has been demonstrated by Kayama (1964). Beef tallow is found to be a poor dietary source for Liza parsia due to the low levels of poly unsaturated fatty acids in it. (Stickney and Mc Geachin, 1985). Besides poor digestibility due to high melting point, as observed in this mullet, has been reported earlier (New, 1987). Stickney and Mc Geachin (1985) has recorded that tilapia grew slowly when fed a diet containing 10% beef tallow. This observation has been related to the low levels of poly unsaturated fatty acids in the diet.

The diet containing a mixture of sardine oil, groundnut oil and soyabean oil was the best among the diets tested in this experiment. The combination of shark liver oil, sardine oil and

groundnut oil was superior to the diet incorporating a mixture of plant oils alone - gingely oil, soya bean oil and groundnut oil. Growth and its related parameters, the nutrient retention efficiencies etc., point out the superior performance of the diets incorporating oil mixtures. The increased digestibility of lipids when fed in combination resulted in better food conversion and protein efficiency ratio. Even though the apparent digestibility of plant lipids is poor (Takeuchi et al., 1979) the values could be improved with the addition of marine lipids which contain n-3 highly unsaturated fatty acids. Recent investigations (Read 1981, Chandge, 1987) have established that a mixture of plant and marine lipids are more effective than only animal or plant lipids for promoting growth in prawns.

Farmed fish seem to accumulate more lipids than wild ones (Love, 1970; Barnabe, 1980; Oshima et al., 1982) and this is associated with a fall in food conversion (Bromley and Smart, 1981). Moreover, it is increasingly evident that alterations in dietary lipid can lead to changes in a variety of cellular and sub cellular enzymes and functions which may be related to differences in fatty acid composition produced within tissue structures by dietary means (Holman, 1964). Hence a judicious selection of natural lipid sources is warranted. In L. parsia it is concluded that the lipid additive should include both plant and marine oils for better performance.

4.4.3. Nutritive evaluation of compounded feeds in field conditions

In the pond trial with L. parsia five compounded feeds were tested. In addition to the sources identified in the previous experiment, coconut cake, mangrove leaves (Avicennia officinalis), tapioca (Cassava) powder and wheat flour were employed in the formulation. They were identified as the locally available cheap ingredients to keep the cost of the diet low. An unfed control group of animals were also maintained.

The survival rates observed in all groups were above 90%. The wholesome diet D1 incorporating all the mentioned ingredients, maintaining a protein level of about 35% resulted in the best weight increment. A similar diet (D5) but for the exclusion of mineral and vitamin mixture produced almost equivalent weight gains. This brings to light the non-essentiality of these two costly components of the diet when supplementary feeding is adopted. Probably the minimal requirements are met from the natural food available to the fry in culture ponds as well as from the mixed ingredients included in the diet. Thus if the food productivity of the pond and the composition of the ingredients in the feeds are known addition of vitamins and minerals in diets can be avoided. The diet D3 wherein the animal protein sources were completely replaced by plant ingredients, not much of a change was recorded compared to

diets D1 and D5. This observation seems questionable especially because conclusions to the contrary were drawn in the experiment to identify the protein sources. It can be explained that, again, the fry may be meeting its requirement of animal proteins by feeding on the zooplankton in the water body. This was confirmed by microscopic examination of gut contents when remains of animal origin were detected. Performance was poor when coconut cake was replaced by more of mangrove leaves in the diet (D2). Probably the digestibility is affected in the fry when fibrous component in the diet is increased. Proximate analysis of the feed had indicated a greater percentage of fibre. Too much fibrous material in the diet has been reported to lower feed efficiency (Cruz, 1975). This is more so because the experimental animals were in the fry stage. One mechanism believed to account for this effect is, dilution of nutrients in the ration by the indigestible fraction. Another probability is that fibrous materials, which are structural carbohydrates in plant feed stuffs, prevent digestive enzymes from acting upon the digestible fraction of the feed (Ellis and Pfander, 1958).

The performance of Diet D4 was the poorest. This can be attributed mainly to the lowering of the protein content and higher inclusion level of carbohydrate in this diet. Probably the titre of amylase in the young animal is insufficient to fully digest the carbohydrate. Thus among the diets, D5 seems to be an

adoptable formulation for commercial application cost-wise and efficiency-wise. However, it is interesting to note that unfed animals could gain around one third the weight obtained in the fed groups. Hence, if some school of thought holds that artificial feeding is not necessary; it is prerogative to demonstrate to them that additional feeding would double or treble the gain during the same span of time. This is amply demonstrated in the present experiment.

If one can characterize the total biota of the culture environment, well enough to model the ecosystem, then natural food organisms may be identified, characterized and certain ones encouraged to grow so that artificial feeds can then be designed truly to supplement the ration provided by natural forage. Programming the seasonal availability of natural feeds and integrating the formulated supplement into a total least cost feeding regime made to meet the predetermined nutrient requirements would result in truly technically and economically sophisticated feeds and feeding regimes. (Webber and Huguenin, 1979). The ultimate challenge in fish husbandry still remains to evaluate, modify and formulate commercial and potentially available feed stuffs into aquaculture diets that provide for optimum growth, food conversion and physical well-being of the animal throughout its life cycle.

PART IV

S U M M A R Y

The aim of the study was to quantify the requirement of some of the nutrients, identify some suitable protein and lipid sources and to determine the suitability of some compounded feeds, all for the successful rearing of the fry of Liza parsia in the nursery phase.

A series of statistically designed feeding experiments in the laboratory preceeded a pond trial of a few compounded diets. The duration of the experiments ranged from 7 to 21 weeks and the abiotic factors were monitored regularly. Food was offered at 7% of the body weight to the fry maintained in the laboratory. The response of the fry to the diets were guaged from survival, growth, food utilization and biochemical indices.

To determine the protein requirement, isocaloric purified diets incorporating graded levels of protein ranging from 0 to 60%, at intervals of five, were fed to the fry for a period of 10 weeks. Casein and gelatin were the main protein sources and the lipid component of the diet came from cod liver oil and corn oil. Dextrin was the carbohydrate source. Mineral and vitamin mixtures were included to balance the dietary composition. The animals clearly elicited response to varying quantity of protein in the diet. Based on growth, food conversion, protein utilization, digestibility and nutrient retention, the optimum level of dietary protein for the fry seems to be around 40%. It was also found that excretion of ammonia by the fry was directly related to the level of protein in the diet.

A ten week experiment was conducted to determine the optimum

level of lipid required in the diet of Liza parsia. Isocaloric, isoproteic diets were fed to the fry. The protein in the diet was fixed at 40% and the lipid levels ranged from 0 to 12 at intervals of 2%. Corn oil and cod liver oil were used in equal proportions. A well defined gradation was observed in the weight gains as the lipid level in the diet increased. Conversion efficiency also showed a similar trend. The retention efficiencies and digestibilities were better in the range 4-8% lipid levels. Considering the cummulative effects of all the aspects studied, a dietary lipid level of 6-8% is suggested for mullet fry.

Two experiments were conducted simultaneously for twenty one weeks; to determine the qualitative requirement of the major water soluble vitamins and to determine the optimum level of incorporation of the vitamin mixture in the diet. The deletion technique was adopted to determine the essentiality of choline, inositol, ascorbic acid, nicotinic acid, pantothenic acid, riboflavin, thiamine and pyridoxine. Survival was poor when riboflavin and niacin were deleted. Niacin deletion also resulted in poor growth. The food conversion was low when pyridoxine and niacin were excluded. Protein efficiency ratio was maximum affected when pyridoxine was not included in the diet. Body composition and nutrient retention efficiency data also prove the essentiality of the vitamins. A host of clinical symptoms associated with vitamin deficiency was recorded. They included anorexia , erratic movements, photophobia, fin degeneration, body lesions, haemorrhagic damage etc. The chara-

cteristic deficiency symptoms observed were corneal opacity for riboflavin, gill damage for pantothenic acid and scoliosis/ lordosis in the case of ascorbic acid. Histopathological and haematological observations were also made.

The optimum level of vitamin mixture to be included in the fish diet was also determined by incorporating the mixture at 0.5, 1, 1.5, 2 and 2.5% in the diet. Data on growth response, conversion efficiency and nutrient retention indicate vitamin levels of 1.5 to 2% is adequate in the diet. It is recommended that the vitamin level in the purified diet should not be below 1%.

A seven week experiment was conducted with diets containing selected protein sources. In all, three plant protein sources (groundnut cake, soyabean meal and algal meal) two animal protein sources (fish meal and prawn waste) and three combination of sources were tested. The protein content of the diets were fixed at 40%. Groundnut cake was identified as better among the plant sources and fish meal among the animal sources. The combination diets offered to the mullet proved to be superior to the diets based on individual feed stuffs. It is therefore suggested that equal proportion of plant and animal proteins (especially fish meal) be included when compounding diets.

The nutritive evaluation of a variety of lipid sources was completed in a seven week experiment. The plant oils tested were gingely, soya, groundnut and sunflower. The animal oils were from sardine, shark liver, cod liver and beef tallow. The mixed oil diets were of the following combination: sardine oil, ground-

nut oil, soyabean oil; shark liver oil, sardine oil, groundnut oil; and gingely oil, soyabean oil, groundnut oil. Cod liver oil and sardine oil were found to be better among the individual sources followed plant oil from soyabean. The diet containing the mixture of sardine oil, groun nut oil and soyabean oil was the best among the diet tested. It is recommended that a mixture of plant and marine oil should be used as lipid sources while compounding diets for mullet fry.

The pond trial was conducted over a period of seven weeks to test the efficacy of five formulated diets. The mullet fry were reared in velon hapas fixed in brackishwater ponds. Diets were tested in duplicate and the fishes were offered a daily ration of 4% body weight. The ingredients used in the diet preparation were groundnut cake, gingely cake, coconut cake, rice bran, mangrove leaves, fish meal and prawn waste. Tapioca powder and wheat flour were mainly intented as binders. The protein content of the diets was kept at 35% except for a lower protein diet were it was 28%. The diet incorporating all the mentioned ingredients, maintaining protein level at 35% provided the best weight gain. Exclusion of minerals and vitamins from diets applied in field condition did not seem to drastically affect growth. When plant ingredients alone was provided to the fry it was equally accepted under pond conditions. Lowering of protein in the diet induced a drop in weight gain of the fry in the pond. When no supplementary feed was provided, hardly 1/3 rd growth was recorded as compared to the wholesome diet with the protein level of 35%. This outlines the importance of artifical feeding in the nursery phase of Liza parsia.

PART V

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Influence of Salinity on the Growth and Feed Utilization in *Liza parsia* Fry

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Abstract

Liza parsia is one of the euryhaline species of finfish cultivated in the low saline coastal waters of India. Since salinity influences growth and food intake in euryhaline species, an experimental study was carried out to determine the effect of salinity levels 5‰ to 35‰ on growth and food conversion in *Liza parsia* fry. Salinity levels ranging from 5 to 25‰ did not significantly influence the survival (88.3% to 93%) but 30 to 35‰ salinity levels provided relatively low survival rates of 79.7 and 78% respectivley. Under the restricted ration (8% of body weight) food intake was not significantly influenced by salinity. Growth and food conversion rates were significantly influenced by salinity, levels 15 to 25‰ providing the best growth. This study shows that though *L. parsia* fry can tolerate salinities from 5‰ to 35‰, levels above 25‰ seem to be unfavourable for normal growth and feed utilization.

Introduction

In an excellent review, Kinne (1971) discussed the diverse effects of salinity on euryhaline species. Salinity is known to influence the distribution and abundance, survival and growth, as well as maturation and spawning of a variety of euryhaline species (Pearse and Gunther, 1957; Gunther, 1961). Responses of euryhaline fish species to environmental salinity changes have been well documented from the wild, with major emphasis on salinity induced variations in distribution. However, there is paucity of experimental evidence concerning the effects of salinity changes on euryhaline finfish. Knowledge of the response of cultivated finfish species to salinity changes would be of immense value in coastal aquaculture, especially in selection of sites as well as in maintaining desirable salinity levels, to achieve maximum survival, growth and efficient utilization of ingested food.

Ever since the pioneering experimental study on *Cyprinodon macularius* (Kinne, 1960) several attempts have been made to evaluate the influence of salinity on growth, food intake, food conversion, nutritional requirements or biochemical changes in the organs and tissues of euryhaline finfish (De Silva and Perera, 1976, 1985; Mukhopadhyay and Karmakar, 1981; Teshima *et al.*, 1984; and Jurss *et al.*, 1984, 1986). The ability to tolerate waters of a particular salinity varies, amongst other things, with the stage of development of the fish (Holliday, 1971). Hence, experimental studies are essential to ascertain the salinity preferences of different growth stages of fish. The objective of the present study was to understand the effect of salinity on survival, growth and feed utilization in the fry of the cultivated mullet, *Liza parsia*.

Material and Methods

The mullet fry for the experiments were collected from the estuarine creeks of Vypeen Island, Cochin, using a velon-screen net and transported under oxygen packing to the experimental facility. They were acclimatised for 48 hours in ambient salinity (13‰).

Sea water (35‰) for the experiment was collected from off-shore regions and the desired saline media was prepared by dilution. Salinity concentrations were measured with an American Optical Refractometer. The fry were segregated size-wise and transferred to circular plastic tubs (50 cm dia) and acclimatised to test salinities for one week. Fifteen fishes (3.3±0.3 cm total length and mean weight 0.434 g) were maintained in each of the three replicates per treatment. Individual fish weights were recorded after acclimatization. Water was aerated and temperature and salinity monitored daily. Water change was made once in two days. The fishes were maintained for 45 days and fed with a semi-moist purified diet, at 8% of the body weight, once a day at 0900 hrs. and the left-over food collected next morning. Group fish weights were recorded every 15 days and the ration supplied was adjusted accordingly. Analysis of variance was carried out on the data to examine the effect of salinity on growth and conversion efficiency. Students 't' test was used to find out the significance in results between the different treatments.

Results and Discussion

Data on food intake obtained from different treatment groups are given in Table 1. Food intake was not significantly affected by salinity levels when the restricted ration was offered. Food consumption depended on weight of fish.

Growth of the Fish (Fig. 1) was significantly influenced by the salinity level. The maximum weight gain (0.543±0.018 g) was at salinity 15‰, followed by almost similar gains at

20‰ (0.512 ± 0.043 g) and 25‰ (0.536 ± 0.028 g). Lower and higher salinity levels produced inferior weight gains. Analysis of variance indicated that salinity content of the medium has highly significant ($P < 0.001$) effect on weight gains. Significant difference was noted at 5, 10, 30 and 35‰ when compared to the weight gains in the range 15-25‰ ($P < 0.01$). The food conversion efficiency also was significantly affected by salinity. Salinities which resulted in good conversion ratios in the descending order are 25, 15, 20, 10 and 5. Gross conversion efficiency (Fig. 2) was comparable among salinity levels of 15, 20 and 25‰, while in other treatments they were significantly lower. Survival rates (Table 1) ranged from 78 to 93%. While there was no significant differences in survival rates between salinities 5 and 25‰, significantly low rates were observed at salinities 30‰ (79.7%) and 35‰ (78%).

Seasonal salinity variations are pronounced in estuaries, backwaters and lagoons of India, and therefore *Liza parisa* which is cultivated in coastal ponds is exposed to wide changes in salinity. The results indicate the salinity of water has significant influence on survival, growth and food-conversion efficiency of *L. parsia* fry. Salinities ranging from 5 to 35‰ are usually encountered in their nursery grounds. However, the fry had high survival rates at S‰ 5 to 25‰ indicating their preference for lower salinity levels. The results of the acclimatization tests indicate that the fry are not probably capable of regulating the internal osmotic and ionic concentration in fresh water.

Growth rate recorded from various salinity treatments indicate that for maximum growth the fry require relatively narrow ranges (15 to 25‰) of salinity. The gross conversion efficiency data also indicate that the above salinity range is ideal for better utilisation of ingested food. Thus salinity ranges of 15-25‰ is bio- energetically advantageous to the fry. The significantly lower gross conversion efficiency at higher and lower salinity levels indicate that the fish fry expend a greater proportion of the ingested food energy for maintainence and routine metabolism than for growth.

Usually salinity tolerances tend to decrease as test temperatures and concentration of dissolved gases decrease (Kinne, 1971). In the present study all treatments were maintained under almost similar laboratory conditions and hence not discussed further. However, variations in the ionic composition of various saline media as a result of mixing of fresh-water might influence tolerance levels. Besides, differences in salinity may also modify the specific gravity of water which may result in differences in swimming effort and activity levels (Holliday, 1971). These factors may necessitate diverting a certain amount of energy for physiological adjustment by fish which otherwise would have been used for tissue building.

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Table 1. Growth, conversion efficiency, and survival of *Liza parsia* at different salinities

Salinity (‰)	Initial weight (g)	Final weight (g)	% growth per day (g)	Total dry food consumed	Food Conversion ratio	% Survival
5	0·421 ±0·030	0·793 ±0·032	1·96 ±0·14	1·764 ±0·107	4·74 ±0·26	88·3
10	0·433 ±0·049	0·874 ±0·058	2·26 ±0·22	1·882 ±0·194	4·32 ±0·32	90·7
15	0·436 ±0·020	0·979 ±0·015	2·77 ±0·22	1·975 ±0·072	3·64 ±0·20	93·0
20	0·429 ±0·033	0·941 ±0·055	1·65 ±0·29	1·899 ±0·205	3·70 ±0·25	90·3
25	0·428 ±0·010	0·964 ±0·024	2·78 ±0·13	1·925 ±0·153	3·59 ±0·10	89·7
30	0·432 ±0·024	0·793 ±0·070	1·86 ±0·26	1·923 ±0·136	5·49 ±0·96	79·7
35	0·456 ±0·035	0·820 ±0·051	1·77 ±0·13	1·877 ±0·177	5·15 ±0·42	78·0

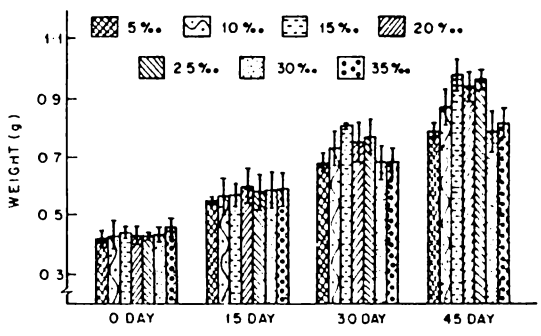

Fig. 1. Growth of *L. parsia* at different salinities

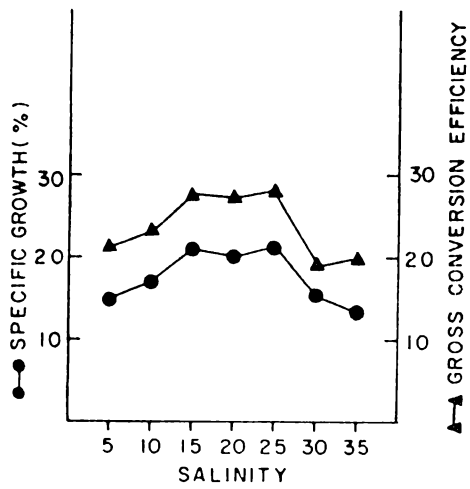

Fig. 2. Specific growth (weekly weight increment) and gross conversion efficiency of *L. parsia* exposed to different salinities

Food Ration for Rearing the Fry of the Mullet *Liza parsia*

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Abstract

The objective of this study was to determine the optimum feeding level for rearing the fry of a euryhaline mullet, *Liza parsia*. Six feeding levels, (4, 8, 12, 16, 20 and 24% of the body weight) were selected for experimental trial. Three groups, each with twelve fish fry (0.650 ± 0.025 g.) were fed on semi-moist purified diet at each of the above feeding levels, once a day for 5 weeks. Data on growth, food intake and food conversion were obtained. Food intake did not increase significantly beyond 8% feeding level. The growth increased linearly upto a feeding level of about 8%. Further increase in feeding level did not proportionately increase growth. The food conversion efficiency was better at the lower feeding levels, the best being at 4%.

Introduction

The influence of rate of feeding on growth rate, conversion efficiency, body composition and metabolism has been extensively studied by Gerking (1955, 1971) on *Lepomis macrochirus;* Pandian (1967) on *Megalops cyprinoides* and *Ophiocephalus striatus;* Brett *et al.* (1969) on *Oncorhynchus nerka;* Andrews and Stickney (1972) on *Ictalurus punctatus;* Pandian and Raghuraman (1972) on *Tilapia mossambica;* Reddy and Katre (1979) on *Heteropneustes fossilis;* Teshima *et al.* (1984) on *Chanos chanos* and Singh and Srivastava (1985) on *Heteropneustes fossilis.* Karmakar and Ghosh (1984) attempted to determine the optimum rates of feeding in *Liza parsia* using rice polish as the lone feed source. This feed is nutritionally inadequate especially because of its low protein and high fibre contents. That study thus remained empirical as the authors failed to consider important aspects like nutrient composition of the diet, food intake and conversion rate. The present work was therefore carried out using a standard reference diet of known composition to determine ration levels for nursery rearing, as well as for laboratory based studies with the fry of *L. parsia.*

Material and Methods

Fry of the mullet *L. parsia,* (size 3.6±0.2 cm and mean weight 0.625 g) were obtained from the fish farm of Kerala Agricultural University, Vypeen Island, Cochin. They were acclimatised to the laboratory conditions and artificial diets for ten days. Twelve animals were introduced into each of the eighteen circular plastic tubs holding about 40l of sea water. The fish were fed on a modified, Halvers H 440 purified test diet (Table 1) in semi-moist (moisture content about 35%) form. The selected feeding levels were 4, 8, 12, 16, 20 and 24%) (designated as F_4 F_8 F_{12} F_{16} F_{20} and F_{24}) of the live body weight of the fishes. Each of the ration was fed to three groups of fry and thus a total of 36 fish fry were maintained in each ration. The amount of feed consumed by the fish was determined after collecting the food left over, if any. Individual weights were recorded in the initial and final stages of the experiment. However, group weights were measured every week. Salinity was maintained at 15±1 ppt; temperature ranged between 30.3 and 32.2°C and pH varied between 8.03-8.32 during the experimental period.

Results and Discussion

Growth (Fig. 2) observed in the fish depended on the amount of food offered, the maximum (64.67%) being for the fish fed 24% of their body weight and the least (28.57%) for fish fed 4%. The weight increment in the 4% fed group was significantly less ($P<0.001$) than those of groups F_8 to F_{24}. The percent gain in weight at the higher feeding levels (F_8-F_{24}) was not significantly ($P>0.01$) different from each other Table 2). The correlation pattern of growth over the experimental duration in the different group is indicated in Fig. 1. The data on the weekly growth increment revealed that gains were greater during the initial weeks of the experiment. The overall growth exhibited by the fish in the present study gave a non-linear exponential relation when plotted against daily ration provided (Fig. 2).

The food consumption at the F_8 level was significantly ($P<0.001$) higher than at F_4. At higher feed levels the increase in consumption was not statistically significant ($P>0.001$). Maximum amount of left-over food was collected from the F_{24} groups. Fig. 2 also shows that feed offered above 8% body weight is wasted. The gross conversion efficiency showed an inverse relation with ration level (Table 2, Fig. 3). The Food conversion ratio ranged between 4.73 (F_4) and 5.97 (F_{16}). The protein

efficiency ratio was higher at the lower feed levels and the maximum was 0.48 at F_4 (Fig. 3). No significant difference in mortality rate was found between fish groups fed at different levels.

Consumption is the quantity of food eaten (as % body weight) by an animal in unit time of 24 hours (Fischer, 1979). The growth of fish is dependent on the quality and quantity of food offered. Under the present conditions, the growth of fish did not proportionately increase when fed in excess of 8% of the body weight. From Fig. 2 it is evident that the fry of *L. parsia* can consume a maximum of about 80 mg of feed/g live fish day^{-1}. This is comparable to the figure for an euryhaline fish *Tilapia mossambica:* 65 mg g day^{-1} (Pandian and Raghuraman, 1972) and that of an airbreathing fish *Ophiocephalus striatus:* 70 mg g day^{-1} (Pandian, 1967). The values obtained for other fishes were higher: *Gasterosteus aculeatus,* 120 mg/g day^{-1} (Beukema, 1968); *Mystus vittatus,* 157.6 mg/g day^{-1} (Arunachalam, 1978) and *Heteropneustes fossils,* 127.26 mg/g day^{-1} (Reddy and Katre, 1979). Karmakar and Ghosh (1984) had concluded that 12% body weight ration was ideal for the *L. parsia* fry having a mean weight of 142 mg using rice polish as the feed. The increased intake can be attributed to the small size of the fish, or to the nutritional imbalance of the rice polish. The percent food consumption (Fig. 2) in the fry of *L. parsia* did not increase much beyond the feed level of 8%, indicating that the maximum acceptable ration for the fry is about 8% of its body weight. Food satiety is probably reached around this level, thereby mechanisms inhibitory to feeding reflexes are induced resulting in no further increase in food consumption. This accounts for the increasing amounts of left-over food (Fig. 2) at higher feeding levels.

Fig. 1 indicates strong correlation between weight gain and time found in all treatments. The 'b' values also showed an increase with ration levels. From the growth-ration curve (Fig. 2) we can deduce a linear increase up to a level of 8%, beyond which the growth slowed down to a non-linear pattern. The drop in growth increment beyond the feeding level of F_8 was such there was no significant difference amongst the values. Brett *et al.* (1969), Edward *et al.* (1972), Allen and Wootton (1982) and Singh and Srivastava (1985) have described such a relationship. In the present study the grwoth rate at the different feeding levels are 7.13 mg/g day^{-1} (at 4%), 12.72 mg/g day^{-1} (at 8%), 13.13 mg/g day^{-1} (at 12%), 13.26 mg g day^{-1} (at 16%), 13.49 mg/g day^{-1} (at 20%) and 13.96 mg g day^{-1} (at 24%). In the catfish, *Heteropneustes fossilis,* Reddy and Katre (1979) reported a growth of 8.6 mg g day^{-1} at feed level of 4%. Since a limit has been noted in the maximum food ingested, the effective utilization for growth seems to be poorer at the higher levels of feeding.

Conversion efficiency decreased with increase in the level of food offered. The reduction in conversion efficiency may be attributed to the decrease in the efficiency of assimilation and digestion at higher rations (Werner and Blaxter, 1980). Increased SDA also may have contributed in lowering the conversion values.

The survival rates in the experiment were not affected by the feeding rates eventhough 8% mortality was observed at F_4. This is in contrast to the observations made by Karmaker and Ghosh (1984) wherein there was great variations in survival (31 to 88%), which the authors attribute to the high stocking density (3/1) of the fish. The superior survival rates obtained in this study clearly indicates that the feed supplied was nutritionally adequate.

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Table 1. Composition of experimental diet.

Ingredient	(g)
Casein	38
Dextrin	25
Gelatin	10
Starch	5
Cornoil	6
Codliver oil	4
Vitamin mix*	1
Mineral mix**	4
Cellulose	7

* Vitamins (g): Choline chloride 0.500; Inositol 0.200; L-Ascorbic acid 0.100; Nicotinic acid 0.075; Calcium pantothenate 0.050; Riboflavin 0.020; Thiamine hydrochloride 0.005; Pyridoxine hydrochloride 0.005; Menadione 0.004; Folic acid 0.0015; Cyanocobalamin 0.0011; Biotin 0.0005; L-Tocopherol acetate 0.040.

** Minerals (for 100g): Calcium biphosphate 13.58; Calcium lactate 32.70; Ferric citrate 2.97; Magnesium sulphate 13.20; Potassium phosphate dibasic 23.98; Sodium biphosphate 8.72; Sodium chloride 4.35; Zinc sulphate 0.300; Manganesesulphate 0.080; Cobalt chloride 0.100; Aluminium chloride 0.015; Potassium iodide 0.015; Cuprous chloride 0.010.

Table 2. Growth, food consumption and gross conversion efficiency of *Liza parsia* fry.

Daily ration % bodyweight F	Initial mean weight (g) W_o	Final mean weight (g) W_t	Total weight increment (%)	Daily rate of growth (%) G_d	Total food consumed (mg) FC	Gross conversion efficiency E
4	0.676 ± .014	0.869 ± 022	28.57[a] ± 1.07	0.713 ± 023	912.33[a] ± 27.80	23.13 ± .47
8	0.671 ± .029	1.056 ± 036	57.30[b] ± 1.35	1.272 ± 024	1844.33[b] ± 30.07	20.83 ± .12
12	0.630 ± .020	1.006 ± 040	59.67[b] ± 1.31	1.313 ± 022	2008.67[b] ± 79.46	18.70 ± .50
16	0.662 ± .008	1.062 ± 014	60.43[b] ± 1.03	1.326 ± 018	2389.33[b] ± 37.50	16.77 ± .15
20	0.641 ± .040	1.037 ± 067	61.80[b] ± 1.08	1.349 ± 018	2357.00[b] ± 91.43	16.80 ± .56
24	0.634 ± .011	1.044 ± 015	64.67[b] ± 1.26	1.396 ± 021	2417.66[b] ± 22.19	16.97 ± .32

t = duration of experiment = 35 days

$$G_d = \frac{W_t - W_o}{t\,2(W_o + W_t)} .100$$

$$E = \frac{W_t - W_o}{FC} .100$$

* Means not sharing a common superscript letter are significantly different ($P < 0.001$)

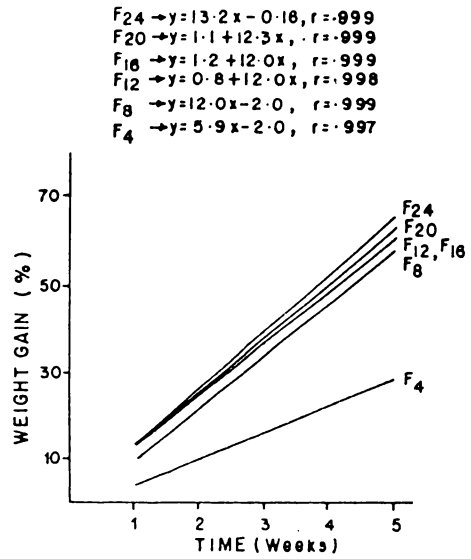

Fig. 1. Pattern of weight gain over time in the different groups.

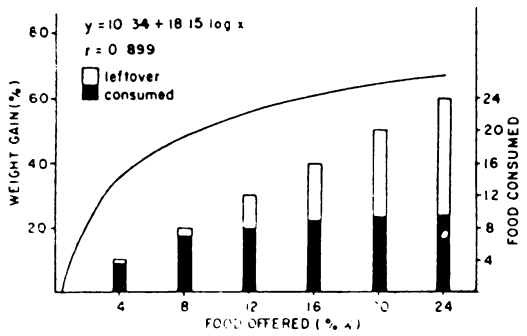

Fig. 2. Weight gain and food consumption in relation to feeding levels.

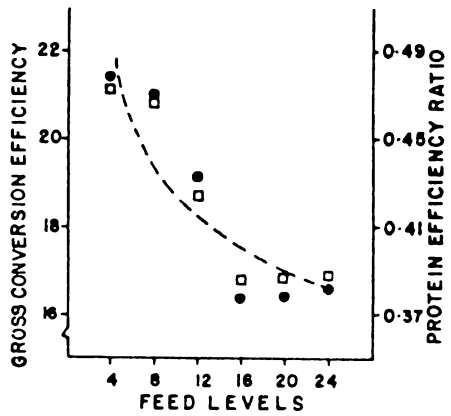

Fig. 3. Gross conversion efficiency (□) and Protein efficiency ratio (●) in relation to feeding levels.