EVALUATION OF NUTRITIONAL POTENTIAL OF SOME AQUATIC WEEDS OF NORTH EAST INDIA FOR FORMULATION OF FISH FEED FOR INDIAN MAJOR CARPS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

by

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Dedicated to my dearest parents

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Declaration

I hereby declare that due to the lack of proper facility at Tezpur University, following experiments / sample analyses were carried out at other institutes.

1. Taxonomical identifications of the plants were done at BSI, Itanagar, Arunachal Pradesh.
2. Mineral Analysis was done at Calcutta University, India.
3. Scanning Electron Microscopic (SEM) study was done by Dr. N. Saha at Regional Sophisticated Instrumentation Centre (RSIC), Shillong, India.
4. Thyroid hormone (T3, T4 and TSH) level in serum was determined by enzyme-linked fluorescent assay (ELFA) by using an ELISA Reader at Astha diagnostic laboratory, Tezpur.
5. Gas-Cromatography fatty acid profiling was done by Dr. B. G. Unni and Mr. M. G. Pathak at Regional Research Laboratory, Jorhat, India.

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List of Abbreviations / symbols

@	=	At the rate of
A:G	=	*Albumin : Globulin*
ALP	=	Alkaline Phosphatase
AND	=	Apparent nutrient digestibility
APD	=	Apparent protein digestibility
APS	=	Ammonium Persulphate
Ca	=	Calcium
cm	=	Centimeter
BWG	=	Body weight gain
°C	=	Degree Celcius
DCIP	=	2, 6-Dichlorophenol-indophenol
DWG	=	Daily weight gain
EDTA	=	Ethylene diaminetetra acetic acid
ELFA	=	Enzyme-linked fluorescent assay
ELISA	=	Enzyme Linked Immunosorbent Assay
et al.	=	And associates
ERE	=	Energy retention efficiency
FCR	=	Feed conversion ratio
GC	=	Gas chromatography
g	=	Gram
Hb	=	Haemoglobin
HDL	=	High density lipoprotein
hrs	=	Hours
kcal	=	Kilocalory
kD	=	*Kilo Dalton*
LDL	=	Low density lipoprotein
µl	=	Microlitre
µm	=	Micrometer
mA	=	Milli Ampere

MD	=	**Molecular dynamics**
ml	=	**Milli Litre**
mg	=	**milligram**
mm	=	***mimimeter***
N	=	**Normal**
nm	=	**Nanometer**
OD	=	**Optical Density**
PBS	=	**Phosphate buffered saline**
PER	=	**Protein efficiency ratio**
RBC	=	**Red Blood Cells**
rpm	=	**Revolution per minute**
SGOT	=	**Serum glutamic oxaloacetic transaminase**
SGPT	=	**Serum glutamic pyruvic transaminase**
SDS-PAGE	=	**Sodium Dodecyl Sulphate–Poly Acrylamide Gel Electrophoresis**
SGR	=	**Specific growth rate**
TCA	=	**Trichloroacetic acid**
TEMED	=	**N. N. N. N. Tetramethyl ethylenediamine**

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Chapter I
Introduction

CHAPTER I

INTRODUCTION

Aquaculture is a powerful instrument of livelihood for a large section of economically under-privileged population in India. More than 6 million fishers in the country depend on aquaculture and fisheries for their livelihood. Indian aquaculture is an important component of the global fisheries with India being the third largest producer of fish in the world and second in inland fish production. India's share in the world fish production has increased from 3.2 % in 1981 to 4.5 % at present (Planning Commission, Govt. of India, 2001).

1.1 Freshwater Aquaculture: A Global Scenario

The global production of fish and shellfish, in recent years, has shown a steady increase owing to continued growth in both aquaculture sectors as well as in capture fisheries (Ayyappan and Jena, 1999). With a total production of more than 32 mmt of finfish and shellfish, China continued to contribute the lion's share in both capture fisheries as well as aquaculture sector of the world. Infact, the Asian continent has remained in the forefront of global aquaculture production. Among the different groups of fishes being cultured around the world carps, barbels and other cyprinids form the most dominant groups with production level of 11.5 mmt obtained during 1996, sharing over 50.6% of the total aquaculture production and 88.6% of the freshwater production of finfishes and shellfishes (Ayyappan and Jena, 1999). Carps form the major component in aquaculture production, with species of silver carp, grass carp, common carp, bighead, crucian carp, rohu, catla and mrigal occupying the list of 10 major principal species of finfishes produced through aquaculture. These eight species of carp together contributed as much as 10.74 mmt, sharing over 71.22% of freshwater aquaculture produce and 40.7% produce (Ayyappan and Jena, 1999). Considering the average annual growth rate of about 14% in

last 5 years, in the global aquaculture production it is expected that the sector will play a major role in meeting the nutritional security of a large section of population and will also contribute greatly to the economy of major aquaculture producing countries of the globe (FAO, 1998).

1.1.1 AQUACULTURE PRODUCTION

According to FAO statistics, the contribution of aquaculture to global supplies of fish, crustaceans and mollusks continues to grow, increasing from 3.9 percent of total production by weight in 1970 to 29.9 percent in 2002. Aquaculture continues to grow more rapidly than all other animal food-producing sectors. Worldwide, the sector has grown at an average rate of 8.9 percent per year since 1970, compared with only 1.2 percent for capture fisheries and 2.8 percent for terrestrial farmed meat-production systems over the same period. Production from aquaculture has greatly outpaced population growth, with the world average per capita supply from aquaculture increasing from 0.7 kg in 1970 to 6.4 kg in 2002, representing an average annual growth rate of 7.2 percent, based largely on China-reported growth (De Silva et al., 2006).

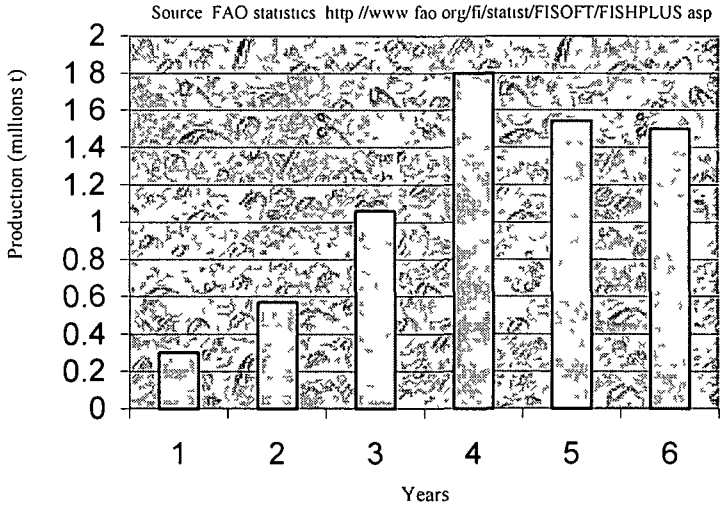
1.2 Scenario of Carp culture in the World

Carps are regarded very differently in different parts of the world. To many people in Western countries, carps are ornamental fish in ornamental ponds and aquaria. In one region, the Murray-Darling River system in southeastern Australia, feral common carp (*Cyprinus carpio*) are regarded as a pest. Yet carps and related species of the family Cyprinidae are a major source of animal protein for millions of people in many Asian countries. World cyprinid aquaculture production in 1998 was 14142298 mts, valued at US$ 14281091 (FAO, 2000). Cultured cyprinid production in 1998 accounted for 30.5% of world aquaculture production and 45.8% of global cultured fish and shellfish production. Furthermore, cultured cyprinid production in 1998 accounted for 78.1% of cultured fish and shellfish production from freshwater environments (FAO, 2000). As such, cyprinid culture is very important to the world aquaculture industry, outweighing all the other

species groups in its contribution to world aquaculture production (De Silva et al., 2006). It is evident that cyprinids constitute the major group of cultured freshwater fish and shellfish. Moreover, the contribution of cyprinids to world aquaculture production has remained steady throughout the last 15 years, contributing about 30%. Their contributions to total cultured fish and shellfish production (e.g. from marine and freshwater environments), and to total freshwater fish and shellfish production, have ranged from 42% and 48.6% respectively, in 1987 to 73.0% and 79.8%, respectively, in 1997. The slight decrease in cyprinid contribution in the early 1990s could be partially due to an upsurge in the culture of tilapia in freshwater and in mariculture (De Silva, 2003). In general the production of cyprinid species, in areas beyond their natural range of distribution, has increased in Asia, and the relative contribution of Chinese, Indian Major Carps, and common carp to inland aquaculture production has increased since 1996 (Fig.1.1).

Fig 1 1 Mean yearly cultured production of Indian Major cyprinids in nations where they occur naturally

Source FAO statistics http //www fao org/fi/statist/FISOFT/FISHPLUS asp



1=1981 year -1985 year, 2=1986 year-1990 year, 3= 1991year-1995 year, 4= 1996-00 year, 5= 2001 year, 6= 2002 year - 2006

1.2.1 Distribution

The family Cyprinidae is a typically freshwater group of fish with a very wide distribution; its members are collectively referred to as carps, barbels and minnows. Carps occur naturally in North America, Africa and Eurasia, but are absent from South America, Australasia and Madagascar (De Silva, et al., 2006).

1.2.2 Species

Distinguishing features of the group are the presence of pharyngeal teeth in oneto three rows, with not more than eight teeth in any one row, lips that are usually thin and an upper jaw that is usually bordered only by premaxillae. There are about 1600 species in the family Cyprinidae, making it the largest family of fish. The taxonomy of the family is correspondingly complex, with about 11 subfamilies and 275 genera. The greatest diversity of the group occurs in Asia (FAO, 2000).

Despite the large number of species in the family Cyprinidae, only a very small proportion is cultured commercially. Altogether, 29 species of carps are cultured globally (FAO, 2000). However, the predominant species cultured are the Chinese and Indian major carps and the common carp.

1.2.3 Culture Methods

The production of farmed aquatic animals is dependent upon the provision and supply of nutrient inputs, either directly in the form of food organisms and / or compound aqua feeds or indirectly in the form of fertilizers. Unlike terrestrial animal farming where production is restricted to a handful of warm blooded livestock species under relatively uniform rearing conditions, aquatic animal farming systems are currently based on the culture of a multitude of cold blooded species (in 2000 these included 131 finfish species, 42 mollusk species and 27 crustacean species) within an equally wide range of production units, farming systems and environments (Tacon and Forster, 2003).

As the Chinese begin to adopt modern aquaculture technologies, the situation is changing, but historically, prepared feeds have not been employed in Chinese polyculture ponds. Natural productivity is increased markedly by frequent, or even continuous, additions of manure and night soil. Organic fertilizers have been depended upon as sources of nutrition for the fishes stocked. Organic matter, in the form of agricultural wastes, has also been used, primarily for feeding grass carp, which will feed on various types of plants of aquatic or terrestrial origin. Fertilization promotes phytoplankton, zooplankton and benthos production in ponds. Common carp are bottom feeders that ingest benthic organisms such as worms, insects and mollusks. Mud carp are omnivorous and will consume detritus including decaying vegetation. Black carp feed on snails. Silver carp are able to filter phytoplankton from the water, though they also ingest zooplankton. Bighead carp selectively feed on zooplankton, while crucian carp consume plant fragments and zooplankton (Stickney, 2000).

Using the traditional polyculture approach, the Chinese have found ways of recycling livestock and human wastes and of utilizing all the food resources that are available. Production rates of about 8,000 kg/ha (7,140 lb/acre) are possible, using the Chinese approach to polyculture.

Polyculture is not limited to various carp species. To some extent in China, but more common in various other nations, is culture of one or two species of carp with fishes from other families. Outside of China, monoculture is much more common than polyculture. Regardless, the vast majority of carp produced, using either approach, are reared in earthen ponds. Production levels in ponds vary considerably from country to country, even within the same country, because of the levels of intensity that exist. With no pond fertilization or supplemental feeding, a yield of only a few hundred kg/ha (lb/acre) can be expected. Fertilization with manure increases natural productivity and consequently fish production levels. Supplemental feeding with grains has led to production levels of over 1,000 kg/ha (approximately 1,000 kg/ha) in countries such as Poland. Polyculture in Chinese ponds fertilized with manure and supplemented with agricultural wastes can

produce 1,500 kg/ha (about 1,500 lb/acre). Provision of high quality prepared feeds and the use of supplemental aeration to maintain dissolved oxygen levels can increase production dramatically (Stickney, 2000).

Carp production in raceways is not common, though a moderate percentage of the carp produced in Japan are reared in such systems. Cages are sometimes used to rear carp, since large natural or manmade water bodies (rivers, lakes and reservoirs) can be utilized effectively as aquaculture systems when cages are employed. For cage culture to be successful, some type of prepared feed should be provided, as natural productivity will usually not be adequate, except perhaps in the case of silver or bighead carp if plankton densities are sufficient (Stickney, 2000).

Similar to cage culture, in that it employs large water bodies for fish culture, is the blocking of bays and inlets with nets that confine stocked fish. In those situations, either natural food can be relied upon, if low stocking densities are employed and natural productivity is high, or culturists can provide supplemental feed to increase fish production (Stickney, 2000).

Carp have also been produced in rice-fish culture, another form of polyculture. Rice-fish culture has a long history in Asia and elsewhere in the world, though strong research programs on rice-fish farming systems dates only from the 1980s. In China alone, some 500,000 ha (1,250,000 acres) of rice paddies have been used in rice-fish culture. The approach involves stocking rice paddies with fish that will forage on insects and other organisms that become established in the rice fields. It is important, of course, that the fish do not negatively impact rice production, which is not a problem if common carp are employed. Because fish, such as carp, cannot be reared to market size at the same rate that a rice crop can be produced, it may be necessary to retain fish in the paddy while two or more rice corps are grown. That can be accomplished by digging a trench in the paddy (usually down the middle) that provides sufficient water to support the fish while the paddy is drained for harvesting. This approach works well in theory, but if it becomes necessary to apply pesticides to treat for rice pest invasions, the fish may also be killed,

even if the paddy is drained and the fish are isolated in the trench during spraying. Spray drift can be a major problem (Stickney, 2000).

Rice-fish polyculture is often practiced with combinations of grass carp, common carp and crucian carp, associated with high rice production. Rice-azolla-fish polyculture has also been employed. Yet another approach employed involves rotating crops between rice and fish.

Carps ponds are sometimes harvested with seines. Seines may also be employed to harvest fish from portions of large water bodies, such as bays, that are blocked off with nets to provide captive rearing areas. Dip nets are usually the method of choice for harvesting fish from cages. If a trench is provided in a rice-fish farming operation, the rice paddy can be drained, thereby forcing the majority of the fish into the trench, from which they can be removed with hand nets (Stickney, 2000).

1.2.4 Indian Major Carp Culture

A group of carp species known as the major Indian carps are produced predominantly on the Indian subcontinent. Included are the rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*) and catla (*Catla catla*). In addition to India, rohu and mrigal are being reared in Laos, Myanmar and Thailand; while catla is produced in India. Laos and Myanmar (De Silva, et al., 2006). With the exception of rohu production in Myanmar, where 74,000 tons were produced in 1995, production levels of these carp species are low outside India. There has been some research on the use of Indian carps in Egypt, but production there, if it is still underway, is not sufficient to make the FAO statistical reports (FAO, 1995).

All three species are native to India and spawn in the rivers of that country. Rohu and mrigal have a preference for vegetation and decaying plant matter as food, while catla feed primarily on zooplankton.

Historically, pond or bund spawning provides for environmental manipulations that induce natural spawning (Bardach et al., 1972). Grass is grown in large shallow ponds during the dry season, in which time, the brood fish are held in a pool within the pond. When the monsoon rains arrive, the levee between the holding pool and bulk of the pond is opened, and the fish enter the grassy area where they spawn. The eggs are collected with nets for incubation in small water bodies. Alternatively, hormone injections can be used to induce spawning. Grow out involves rearing in fertilized ponds, which may also receive supplemental feed.

The Indian major carps are cultured in natural or managed water bodies (mainly earthen ponds) with varying culture practices and degrees of intensification, ranging from extensive to more or less intensive systems. Production is expressed generally in terms of quantity per unit pond surface area. Natural production based on pond productivity alone is often very low, ranging from 200 to 300 kg / ha / yr. Liming and organic manuring can increase the production to 500 to 800 kg / ha/ yr. Use of formulated feed increases the productivity. Advances during the past decade in optimizing feed formulations has even led to a productivity of more than 10 to 12 tonnes / ha / yr (Tripathi et al., 2000). In India, production of these three species has increased tremendously over the past decade.

Earlier, in the absence of precise knowledge on the control of reproduction and breeding, farmers resorted to the collection of wild larvae and juveniles. Subsequently, management practices such as "bundh breeding" where a sudden gush of rainwater is forced into spawning ponds were and are even today used to induce natural spawning. Induced spawning techniques for the breeding of these species in captivity in a consistent manner and simple improvements in hatchery techniques for mass breeding (Jhingran and Pullin, 1988) has accelerated the development of the culture of Indian major carps, thus eliminating the dependence on the collection of wild seed from rivers.

Generally the larvae remain for 15 days in a "nursery pond" where they are stocked at 10 million larvae per hectare where they reach body weight of about 130 mg each. Fry produced are then transferred to "rearing ponds" where they are stocked at a rate of

3000,000 fry per hectare for about 45 days during which they grow to an average weight of 15 g. These fingerlings are then stocked into "production ponds" at a density ranging from 3,000 to 13,000 fingerlings per hectare and are grown for about a year before harvest. In the polyculture of these species, the relative proportions of the different species can vary from one farm to another. Generally, a ratio of catla:rohu:mrigal:calbasu of 3 : 5 :1 : 1 is considered good. This three pond system i.e. nursery, rearing and production-culture is still practiced throughout the Indian subcontinent. The natural productivity in all three ponds is enhanced by fertilization. Fish yield is further increased by supplementary feeding. It is a general contention that catla and mrigal grow faster than rohu. Catla and mrigal can attain from 1.5 to 2.0 kg, rohu, 1 kg and calbasu, 700 g after one year of culture. However field data indicate considerable variations in growth, depending upon management practices. Theoretical growth cueves based on field data indicate different growth curves (Mukhopadhyay and Kaushik, 2001).

Unlike total feed-based aquaculture practiced in intensive farming conditions, semi-intensive pond culture of the Indian major carps relies upon natural productivity to a large extent. Although culture techniques of the Indian major carps have been standardized, it is felt that there are opportunities for increasing the production through large scale adoption of better management practices and the optimization of the supply of various nutrients through supplementary feeding. To maximize the utilization of water resources, a transition from the traditional extensive culture system towards more intensive culture has occurred, over the last decade. This was achieved both by increasing the stock density of ponds and by increasing inputs of traditional feed mixtures, composed of locally available cereal brans and oil cakes. As the density exceeds the natural carrying capacity of ponds, shifts from natural food dependence to nutritionally complete exogenous feed becomes vital (Mukhopadhyay and Kaushik, 2001).

1.2.5 Global consumption of fish

During the past decades, per capita fish consumption has expanded globally along with economic growth and well-being. However, growth will not go on forever. There is a

limit to how much food – including fish each individual will consume, and long-term ceilings for consumption will be established. It is clear that the limit will be reached first by wealthy economies, and fastest in those where fish has been a staple food since ancient times – in Japan for example.

In 1997 the average consumption of fish per person in the world was 16 kg (FAO, 2000). The consumption varies greatly between different countries. There are several factors that determine the consumption of fish and shellfish, the main factors being the country's own supply of these products, the economy of the country and tradition.

It is also interesting to compare the consumption of fish to the consumption of meat in different countries. Statistics (FAO, 2000) show that the consumption of fish and shellfish for human food varies greatly in Europe and in other large countries such as Japan, USA and Russia. The highest consumption per capita in Europe is in Iceland with 90,7 kg per year. The lowest is in Eastern European countries such as Albania, Bosnia, Romania and Yugoslavia, where the consumption is under 3,5 kg per capita per year (FAO, 2000). When we look at the consumption of meat and fish on the different continents we can see that the consumption of meat varies more than the consumption of fish. The consumption of meat is six times higher in North and Central America than in Africa. The greatest difference in fish consumption is between Oceania and Africa, being three times higher in Oceania.

In the developed countries the image of fish is changing, it is moving away from being the basic food it once was and is becoming a culinary specialty. There are two main reasons for this: the vast majority of the population in these countries has the means to purchase adequate food and retailers are realizing that, to attract consumers, they have to sell a product that is more than just a basic foodstuff. Marketing campaigns launched for some fish products tend to affirm that the consumption of fish is an appropriate means of satisfying the consumer's need for variety and for nutritious, tasty, healthy and fashionable foods. The retailing of fish in these countries is no longer a question of satisfying a hungry consumer at a competitive price.

In the developing countries, fish is still very much an essential food. It contributes an important part of the animal protein in many people's diet. In the mid-1990s, fish provided more than 50 percent of the animal protein for the populations of 34 countries. Several Asian and some African countries fell into this category.

1.3 The Potential and Sustainability of Aquaculture in India

Similar to global trends, Indian aquaculture has shown significantly higher growth rate than that of capture fisheries during the last decade (Ayyappan and Jena, 2001). In India freshwater aquaculture alone contributes over 90% of the total aquaculture production. The sector has made notable strides in recent years with the component contributing to over $1/3^{rd}$ of total fish production in the country having growth rate of more than 6%. In the context of increasing food production on a sustainable basis, freshwater aquaculture has come out to be a major option not only for producing food of very high biological value but also recycling a host of organic waste material (Ayyappan and Jena, 2001, Jana and Jana, 2003).

The freshwater aquaculture resources of the country are huge in terms of 2.36 million ha (mha) of ponds and tanks and 1.07mha of beels, jheels and derelict water bodies in addition to long canals and a large number of small, medium and large reservoirs that could be put to different fish culture practices (Ayyappan and Jena, 2001, Gopakumar et al.,1999). Carps, catfishes, murrels, perch, featherbacks, large number of medium and minor carps form important components of culture practices in the country.

Over the last decade freshwater fish production has been developing from traditional extensive systems by increasing the fish stocking density to maximize the utilization of water resources. As density exceeds the natural carrying capacity, dependence shifts from a natural food to nutritionally favourable exogenous feed to achieve optimum growth and production (Mukhopahyay, 1998). A variety of ingredients of

plant and animal origin has been screened for incorporation in supplementary feed for carps and used either singly or in combination (Lakshmanan et al., 1967; Chakrabarty et al., 1973). Most of the diets formulated, however, are confined to only carp fry and fingerlings (Sen et al., 1980; Singh et.al., 1980; Mohanty and Swamy, 1986; Mohanty et al., 1990; Jafri et al., 1991; Mohanty and Das 1995; Paul et al., 1997; Jena et al., 1996; 1998, 1999). Several non-conventional feed ingredients have been evaluated for incorporation as feed supplements (Mukhopadhyay and Jena, 1999). In most cases, the grow-out supplementary feed comprises only oil cakes of mustard /groundnut and rice/wheat bran at 1:1 ratio by weight (Tripathi, 1990) However, as the cost of oil cakes and rice bran has been increasing rapidly, the farmers of the state like Andhra Pradesh are manipulatively using the combination of those two ingredients based on fish growth rate and pond productivity (Nandeesha, 1993). In polyculture systems, supplementary feeds are often given arbitrarily, though it is essential to provide optimum amount of feed to obtain maximum conversion of feed and growth of fish species. Thus, an understanding of the relationship between the rates of feeding and conversion of the food provided is of utmost importance in aquaculture, as it helps in avoiding wastage of a costly input, feed, both in the form of unutilized feed and non-assimilated fraction of the ingested. Such wastage not only adds to the cost of production but also would pollute the culture environment. On the contrary, when the quality of feed provided is too low, it adversely affects the fish growth too (Sen et al., 1980).

1.4 Scenario of Carp culture in India

Asian aquaculture has been contributing in great measure to the global fish basket (Ayyappan and Jena, 2001). The Indian subcontinent, with a rich biodiversity of fish species, has emerged as an important aquaculture country, particularly in freshwater environment. Carps form the mainstay of culture practices in the country, supported by a strong traditional knowledge base and scientific input in various aspects of biology, environment, nutrition and health management (Ayyappan and Jena, 2003). Carp culture is based on management practices developed over the years following intensive research utilizing different agro-ecological conditions. The production of carps through semi-

intensive and intensive polyculture systems results from stocking the appropriate size of fingerlings at desirable densities and species combinations, along with other factors, such as fertilization, supplementary feeding, aeration and water exchange (Ayyappan and Jena, 2003). Fish culture has a long history in India, and the species of Indian major carps, such as the catla, rohu and mrigal, in pond culture have been known to the farmers for many years (Chaudhuri et al., 1974). However, the production from these systems remained significantly low at 600 kg/ha/year (Jhingran, 1969) until the introduction of scientific composite carp culture technology during the 1970s. Further, the experiences gained over the years through experimentation have led to the gradual increase of fish production, particularly in ponds, with contributions of both Indian major carps and exotic carps. In 1996, national production reached 2 tons/ha/year (GOI, 1996), showing enormous potential.

Carps form the major component of aquaculture production in the country and contribute as much as 87% of the total aquaculture production of 1.768 million tons. Among the different groups of fish species cultured worldwide, carps and other cyprinids form the most dominant group, with production levels of 11.5 million tons during 1996, comprising over 50.6% of the total aquaculture production of finfish and shellfish (FAO, 1998). Besides the three Indian major carp species, other important medium and minor carp species being culture in certain parts of the country, though on a smaller scale, are Kalbasu, *Labeo calbasu*; bata, *Labeo bata*; fringed lipped carp, *Labeo fimbriatus*; reba, *Cirrhinus reba* and mola, *Amblypharyngodon mola*. Further, the country also possesses several other potential candidate carp species, endemic to different regions of the country, that could be cultured; the most important two species are pulchellus, *Puntius pulchelus*, and cauvery carp, *Cirrhinus cirrhosa* (Ayyappan and Jena, 2003).

The exotic species, such as silver carp, *Hypophthalmichthys molitrix*, and grass carp, *Ctenopharyngodon idella*, introduced during 1959 (Alikunhi and Sukumaran, 1964) to fill the two important niches of consuming phytoplankton and macro vegetation, respectively, along with the indigenous carp species, have also contributed significantly to

enhancing the yield rates from fish ponds. Further, the Bangkok-strain of common carp, *Cyprinus carpio* var. *communis*, introduced during 1957, has influenced aquaculture in India. The two components of Indian and exotic carps have established a compatible and wholesome aquaculture system, and the combinations are used in diverse habitats and practices such as ponds/tanks, paddy fields, sewage-fed waters, cages, pens and flow-through and other industrial aquaculture systems (Ayyappan and Jena, 1998).

Almost all freshwater aquaculture production in India is pond-based, and the different systems of culture practices include extensive and semi-intensive carp polyculture, sewage-fed fish culture, and integrated fish farming (Ayyappan and Jena, 2003).

1.5 Scenario of Carp culture in northeastern India

1.5.1 Geographical topography

Nature has endowed North East India with a distinct advantage of abundant water bodies and varied aquatic life. The North Eastern India, comprising Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura, is gifted with vast aquatic resources in the form of rivers, streams, lakes, reservoirs, floodplain wetlands, ponds and large areas under paddy-cum-fish farming system. The region lies between 21.50 ° to 29.50 ° North latitude and 85.50 ° to 97.50 ° East longitude and is bound on the north by China and Bhutan, on the west by Bangladesh and on the east by Myanmar. It covers an elevation from 15 to 5000m from sea level. The rivers Brahmaputra and Barak flowing through the different states along with myriads of rivulets and lentic water bodies, which harbour diversified fish fauna, form the principal drainage of North East India with their numerous tributaries. Owing to its diversity of topographic and climatic features, the aquatic resources of North Eastern India are rich in fish germplasm. The varied freshwater

resources of the region harbour 274 fish species out of the 806 freshwater fishes reported in India (Mahapatra et al., 2003).

1.5.2 Fishery Resources

All the eight states of this region are land-locked and thus they possess only freshwater resources. The main fishery resources of the region comprise: Rivers (18,968 km), Reservoirs (8,091 ha), Beels / Lakes (1,43,491 ha), Tanks and Ponds (42,782 ha). Besides, suitable paddy fields, low-lying areas and forests contribute towards additional source (94,577 ha) of in the region (Nath and Kalita, 2005). State- wise fishery resources are given in Table 1.

Arunachal Pradesh is the largest state in size and the thinnest in population out of all northeastern states.The state is blessed with a large number of natural water for fisheries development. Rivers in the state are mostly in the form of streams except Kameng and Subansiri in the west and Dihang, Dibang and Lohit in the east.

Assam is the second largest state in the region. The state is the homeland of two major river systems of the region viz., Brahmaputra and Barak. The state has maximum fishery resources in the region comprising rivers, beels/lakes, tanks/ponds, paddy fields, swamps, forests, fisheries etc.

The state of Manipur literally means "the land of jewels". It is a state having about 20% of its land area in its valley. Even with vast area of hilly terrain, the state is bestowed with vast fishery resources. The biggest freshwater lake of eastern India, Lohtak, (average size 19,150 ha) (27,800 ha full level and 10,500 ha dead storage), fall within this state.

Meghalaya, the abode of clouds, is a state with land area almost equally divided into three topographies of low, medium and high altitudes. The state is criss-crossed with a large number of rivers and streams of narrow width. There are about 98 big and small rivers/streams in the state, of which only nine are major ones.

The terrain of Mizoram is entirely hilly, with the exception of a few stretches of plain surface already under rice cultivation. Fishery resources of the state are also meager. There are no beels or marshy areas worth mentioning. The state is having one small lake (Palak).

In Nagaland fishery resources are meager for topographical reasons. The state is bestowed with a number of hill streams of narrow width. There are a few small water bodies as reservoirs with not much of fishery importance. Ponds and tanks are the main source for fish production in the State.

More than half of geographical area of Tripura is having hilly terrain with maximum elevation of 700 metres. Fishery resources of this state are limited due to constraint of topography and area. However, the state claims to be utilizing almost its entire available resources for fish production. There is only one reservoir (Gumti) in the state.

Sikkim state is entirely mountainous. Fishery resources are limited in the state and available only in the form of rivers/streams. However, resources in the form of ponds are being created in the state under the ongoing fisheries schemes (Nath and Kalita, 2005).

Table 1: Fishery Resources in North Eastern States

State	Rivers/Streams (kms.)	Beels/Lakes (ha)	Tanks/Ponds (ha)	Paddy fields (ha)	Other suitable water (ha)
Arunachal Pradesh	2000	2500 +110 (Cold water)	1000	2800	700
Assam	4820	100000	20000	20000	1517
Manipur	2000	40000	5000	40000	10000
Meghalaya	5600	394	1944	5000	3000
Mizoram	1748	32	1800	1560	-
Nagaland	1600	215	2000	10000	-
Sikkim	900	-	-	-	-
Tripura	1200	240	11038	-	-
Total	19868	143381 + 110	72782	79360	

Source Northeastern Council – Ten Year Perspective Plan

1.5.3 Diversity of fish fauna (Carps) of North East India

North East India is considered as one of the hot spots of freshwater fish biodiversity in the world (Kottelat and Whitten, 1996). It is a well recognised fact that there has been drastic reduction in abundance of the freshwater fishes in this region due to destruction of the habitat, overexploitation and other anthropogenic effects. Review of literature indicates that only limited information is available on fish germplasm resources of North East India with special reference to its potential as cultivable, sport and ornamental fishes. There has been a wide variation in the number of fishes reported from this region ranging from 172 (Ghosh and Lipton, 1982) to 267 (Sen, 2000).The North East Region shares its fish fauna predominantly with that of the Indo-Gangetic fauna and to a small extent with the Burmese and South China fish fauna (Yadav and Chandra, 1994). Scanning of literature shows that 172 fish species with reference to their economic importance were recorded by Ghosh and Lipton (1982) while Sen (1985) and Mahanta *et al.* (1998) recorded altogether 187 fish species from Assam and the neighbouring North Eastern states of India. Compilation of Yadav and Chandra (1994) listed a total of 129

species. Sinha (1996) in his comprehensive review gave a list of 230 fishes as available from North Eastern region. Recently, Nath and Dey (1997) recorded a total of 131 species from the drainages of Arunachal Pradesh. Sen (2000) has indicated that more number of species (267) has been reported from North East India. The various reports show a wide variation in the total number of species reported. In the present communication 186 potential food, sports and aquarium fish species belonging to 27 families under 84 genera, have been presented along with statewise distribution, abundance alongwith potential fisheries. While the list of 267 fish species given by Sen (2000) includes all indigenous and exotic species found in North East India, the list of 186 fish species given in the present paper is restricted to indigenous species for which their potential as cultivable, sport and ornamental value has been assessed. Many more species could be distributed in the drainages of North East (Nath and Kalita, 2005).

Indo-Burma has a remarkable freshwater fish fauna, with more than 1,260 documented species, or about 10 percent of the world's freshwater fishes. More than 560 of these species are endemic, as are 30 genera and one family, the Indostomidae, or armored sticklebacks, a family of strange fishes that may be remotely related to the marine sea moths.

1.5.4 How feed based carp culture is being practiced in North East India?

Fish is nevertheless, an indispensable item in the diet of the folks of northeastern India. But feed development, scientific fish farming on commercial lines is beyond reach for the poor farmers of this region. Regarding fish feed the age-old practice of dumping the ponds with rice bran / mustard oil cake without any necessary quantification is practiced till today. Though the fishery extension work by the fishery departments of both center and state governments are undergoing but yet this region will need decades to reach the pace with the rest of the country.

1.5.5 Researches already in North Eastern India in carp culture context

After the major breakthrough in Induced breeding of carps in 1957, several carp hatchery designs were developed in the past four decades in India for breeding of their eggs. Unfortunately, however most of these designs failed to attract farmers of this North Eastern Region of India. The modified Chinese circular type of hatchery was very much accepted here. However, due to its high-construction cost, only a handful of fish farmers could venture into the activities of major carp seed production. For marginal farmers, it is still a distant dream (Suresh, 2003).

1.5.6 Gap in the information

The present fish production of the region is about 7 % of the total inland fish production in the country. Present fish production level of the North Eastern States is just sufficient to provide about 6 kg per capita of fish to its present population against the standard nutritional requirement of 12 kg per capita (FAO, 1995). To provide the same, the region needs about 400,000 tones of fish. To partially offset the demand, substantial quantity of fish is imported daily into the region from other States of the country (Andhra Pradesh, Bihar, Uttar Pradesh etc.).

Indian Major Carp is all time favorite for the people of this region. But its price remains stagnant always. The major feed ingredients used by the people (that is, rice bran and mustard oil cake) without proper scientific infrastructure is cost effective because to procure 1 kg of fish 2 kg of feed is always needed (by assuming the FCR value 2:1). Therefore, there is a great demand for a scientifically developed feed for the carps for this region. It is generally advisable to make the use of local resources as fish feed as far as possible so that the farmers / fishery entrepreneurs do not have to bring the feed materials from other states and the huge transportation cost is avoided thus making available cost-effective feeds. In regard to natural resources again this region is enormously gifted, for example, the diversity of aquatic macrophytes available in this region though have potentials have hardly been attempted for such purposes.

Rice bran though is available in plenty has tremendous demand also as component of livestock and poultry feed and most of the time it is admixed with rice husk which reduces its nutritional value significantly.

Mustard oil cake has certain inherent antinutritional factors (trypsin inhibitor, saponnin, gossypol, calcium oxalate) because of which it is never recommended more than 20 % incorporation in aqua feed formulations unless these are inactivated before use. All these factors necessitate search for suitable alternative items that too from local sources to the extent possible.

Certain aquatic weeds which have been screened after due care and attention and also nutritional analyses have been found to be potential ingredient sources in carp feed formulations.

1.5.7 Aquatic weeds of Assam used as supplementary fish diet

A survey was carried out in Assam to unveil the hidden resources that could possibly be explored for the development of fish feeds. The state of Assam has been selected as major area for the present research due to the following reasons:

1. This region is exceptionally rich in both biodiversity and ethno-cultural heritage where maximum probability of finding botanical resources based on cultural knowledge have been predicted whereas the literature review have revealed that the area is least explored in the thrust area of aquatic weed research.
2. The ethnofisheries knowledge of the rural fishermen were found to be widely prevalent within their respective locality. It is due to the fact that their major occupation tends to be fishing and wet rice cultivation. However, it is ironical that only few of their vital techniques have been recorded so far in modern research literatures. The fish pond management systems and fish feeds used among them using wild aquatic weeds are still less well known to the scientific community and

concerted effort to know such a vital knowledge system in scientific line have not properly been made so far in commercial and industrial point of view.

3. Furthermore, in the area of aquatic weed based research, no such literatures are available. Therefore, ethnobiological survey was randomly done in both plain and hilly region of Assam to explore the weeds used among the rural fishermen as fish feed, by following Rao and Jain (1977) field method.

In all, 50 species were collected from different localities of Assam and all the species are used as fish feeds. The different fishing community of Assam and other parts of Northeast India has reported these aquatic weeds, but most of the species used are of less frequent. Only few species such as *Salvinia cuculata, Ipomoea reptans, Trapa natans* and *Lemna minor* were found to be used in frequent occasions as fish feed in Assam and many other parts of NE India whom the fishermen consider as less toxic, naturally available and sustainable.

1.5.8 Aquatic weeds of Assam and their possible use in fish feed

Currently there is no such authentic record of wetland and aquatic flora of Assam. However, the estimation of various workers put India's eastern and northeastern region for holding the majority of wetlands that supports nearly 80% of India's inland aquatic flora and fish genetic resources. Northeastern region in particular occupy a unique position by virtue of its 16 different agro climatic zones as found all 8 sister states, which is also found over India with varied topographic features, which supports proliferation of biological diversity in its diverse ecological habitats. The river valley of hilly states such as Arunachal Pradesh, and others host bulk of temperate aquatic macrophytes. Whereas the states of Tripura and Assam hold major parts of tropical and subtropical wetlands with high diversity index of aquatic macrophytes that serves a major dietary sources for the fish faunal diversity of the region. However, the perusal of literatures revealed that exhaustative floristic survey and taxonomical account of regional aquatic flora are still

insignificant. Kanjilal and his coworkers worked out Flora of Assam, which was a first account of regional flora of NE region published in 1934-40.

However, the text dealt only on higher economic plants of timber value and some genus of Poaceae and Cyperaceae but there is no mention of aquatic macrophytes and weeds that can be economically viable for fish feeds development. The ethnobotanical and floristic accounts of Balakrishna and Rao (1980-83), Sharma and Goel (2000) mentioned the predominant aquatic weeds such *Eichornia crassipes, Nymphea nauchali, Vallisneria, Nilumbo nucifera* and *Ipomoea fistulosa.* The British botanists initiated most of the floristic works pertaining to aquatic flora in India. Bentham and Hooker's Genera Plantarum (1862 – 1889) mentioned some aquatic flora of India which includes three species of Trapa - *Trapa natans* L. syn. *T. quadrispinosa* auct., non Roxb., published in J.D Hooker's Flora of British India 2: 590. 1879; Roxburgh first reported *Trapa natans* from Kashmir mentioned in his Flora Indica or Description of Indian Plants. Subramanyam (1962) under the aegis of CSIR first brought out the flora of aquatic angiosperms of India wherein taxonomic description of *Trapa natans* L. var. *bispinosa* Roxb. were mentioned.

The similar mentioned were made by the same author are *Ipomoea* species found in India such as *I. quomaclit, I. batatas, I. turpiatum, I. fistulosa and I. aquatica.* Out of 5 species, the *Ipomoea fistulosa* are emergent shrub growing in wetland while *Ipomoea aquatica* are obligate aquatic creeper that grow in water in floating conditions in ditches, ponds and stagnant water bodies and their degraded biomass remain available for fish food. The ethno botanical study of late 1850 and 1870s proved that the young leaves of *Ipomoea* spp. are used as vegetable as well as for medicinal purpose to improve digestion, enhance purgation and purify blood etc. by the tribes of Central and Northeast India. The plant was first described as *Ipomoea aquatica* Forskaal in Fl. Aeg. Arab. 44. 1775. *Lemna minor* L. were reported by Hooker in FBI (6: 556. 1893), Maheswari (1956), Subramanyam (1962).

Recently, the most illustrative aquatic plant manual of Fasset (2000) mentioned the current status of aquatic angiosperm species of both India and in global perspective. Ghose

(2005) have mentioned 150 aquatic and wetland plants from Kolkata (India) includes *Trapa natans* L. var. *bispinosa* Roxb.; *Lemna minor* L., and *Ipomoea aquatica* Forskaal. The taxonomy and reproduction of most of the aquatic macrophytes of pteridophytic groups found in floating condition were reported by Eames (1953, 1961). Among the Indian species reported by Eames (1953) are *Marsilea condensate, M agyptica, M brachypus, M. brachycarpa, M. rajasthanensis, M. quadrifolia. Azolla pinnata, A filiculoides* are distributed widely in Indian brakish water and ponds forming red water surface whereas *Azolla imbricata* is restricted to Eastern Himalayas and Arunachal Pradesh and States of Northeast India. The nitrogen fixing *Anabaena azollae,* a blue green alga is associated with circular cavity of the upper lobes of the leaves (Eames, 1961) play a significant ecological role.

Salvinia molesta, Salvinia cuculata and Salvinia rotundifolia are widely grow in water bodies and Wetland of eastern India (Kolkata, Orissa, and Northern India) and the states of Northeast India.

With above literature background, it revealed that there are not enough documentation work available related aquatic macrophytes or flora that serves as natural fish food that can be converted to fish diets through industrial process. Therefore the floristic and ethno botanical survey of aquatic plants possibly serves as diet for both cultivated and wild fish of Northeast India were conducted during the year 1999 – 2003. This region comprises of seven states viz Arunachal Pradesh, Assam, Nagaland, Meghalaya, Manipur, Tripura, Mizoram and Sikkim is world 12th biodiversity hotspots of the world (Myers et al., 2000). It has a total geographical area of 262,179 Sq. Km (8%) of India's landmass and harbors 50% (8500 species) of Indian angiospermic flora (Myers et al., 2000, Mao & Hyniewta, 2000). The region is not only a biological hotspot but also a biocultural hotspots where more than 150 ethnic tribes living in close association with nature by using age-old indigenous knowledge system that evolved through generation of trial as sources of their cultural and material wisdom (Nath, 2000). The Brahamaputra river of Assam and its tributaries, originated from Arunachal Pradesh, Nagaland, flood plain and riparian area are

the major wetland surveyed from Northeast India. These geopgraphical areas were visited in all season of the year and the phenology of observed aquatic flora were recorded.

The ethnobotanical information, parts used and types of aquatic macrophytes, ponds, fish faunal diversity of different wetlands of the villages were surveyed. The herbarium samples of plants were first consulted at the herbarium of BSI (Botanical Survey of India), Itanagar, and later consulted standard aquatic manual of Maheswari (1956), Subramanyam (1962) and Fasset (2000). The currently accepted author index and recent nomenclatural rules and changes were verified through IPNI and ePIC of Kew website which are in current use and approved in ICBN Saint Lious Code (Greuter et al, 2000).

1.5.9 Management of Aquatic Weeds

The management of aquatic weeds is a very important task to ensure the availability of water from the source to its end users. It also improves the conveyance efficiency. Water storage systems often get choked with the weeds and cause environmental pollution especially in low lying areas, adjoining irrigation and drainage channels. Management of aquatic weeds consists of two approaches viz. preventive and control of existing infestation (Aquatic weeds Report, 2002).

Aquatic weed can be brought under control to manageable limits by various methods. Broadly, these methods can be grouped under four groups:

1. Physical or Mechanical methods
2. Biological methods
3. Chemical methods, and
4. Cultural and physiological methods

Biological Control

Biological control is the use of host specific natural enemies to reduce the population density of a pest. Several insects and fungi have been identified as control agents for different aquatic weeds (Mathur, 2005).

1.5.10 Aquatic weed: Utilization for economic and social benefit

Utilization of the aquatic weeds has been given prime importance as a means to reduce the cost of removal of the weed. Utilization has become a part of weed management. It has been utilized as a feed, fertilizer and source of potash in different parts of the world. In Singapore its flowers are hawked while in Sri lanka flowers are offered in Buddhist temples. In Java the leaves, petioles and inflorescence are eaten, steamed, fried or cooked (Burkill, 1966; Slamet and Sukowati, 1975). In Philippines its leaves are used as fish traps and its ash is used for soap making (Azam, 1942). The plants are used as bedding material for the cultivation of mushrooms (Grist, 1959; Widyanto and Setiwan, 1977). Thick fibre petioles are used to make shoe soles. Water hyacinth can also be used in the proportion of artificial silk, oxalic acid, ethyl alcohol and in bakery (Nag, 1976).

1.5.11 Systematic classification of Indian Major Carps

(a) *Labeo rohita*

Phylum – Chordata
Subphylum – Vertebrata
Class – Actinopterygii (ray-finned fishes)
Order – Cypriniformes (Carps)
Family – Cyprinidae
Genus – Labeo
Species – *Labeo rohita*

(b) *Catla catla*

Phylum – Chordata
Subphylum – Vertebrata
Class – Actinopterygii (ray-finned fishes)
Order – Cypriniformes (Carps)
Family – Cyprinidae
Genus – Catla
Species – *Catla catla*

(c) *Cirrhinus mrigala*

Phylum – Chordata
Subphylum – Vertebrata
Class – Actinopterygii (ray-finned fishes)
Order – Cypriniformes (Carps)
Family – Cyprinidae
Genus – Cirrhinus
Species – *Cirrhinus mrigala*

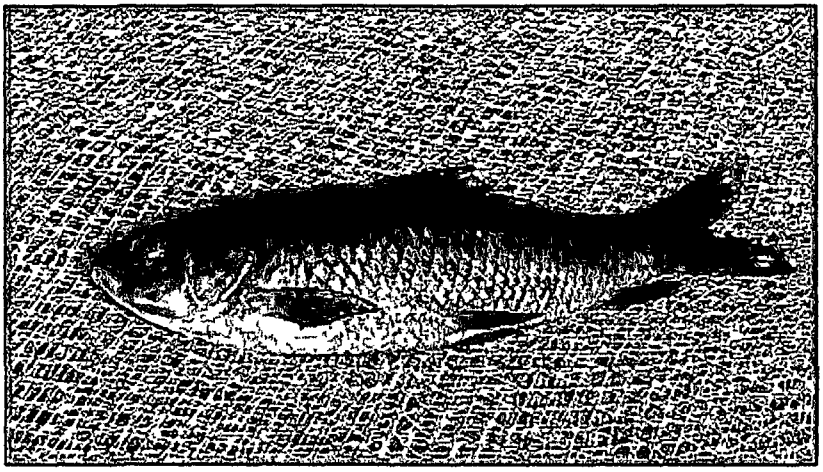


Plate (a): *Labeo rohita*

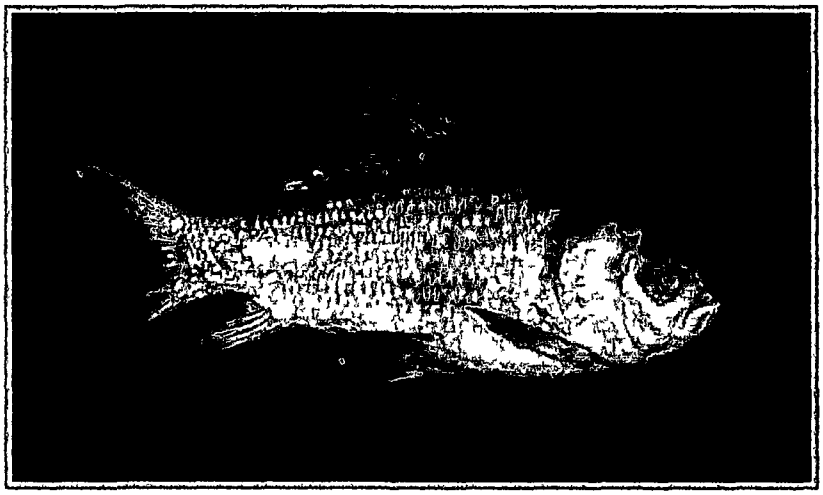


Plate (b): *Catla catla*

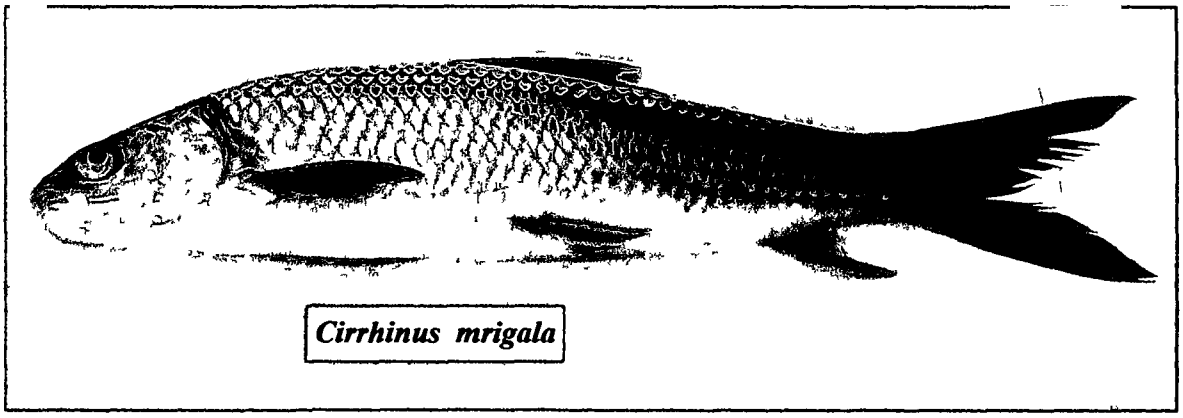


Plate (c) : *Cirrhinus mrigala*

1.5.12 Systematic classification of four aquatic weeds used in this study

(a) *Salvinia cuculata* Roxburgh

Kingdom – Plantae (plants)
Division – Pteridophyta
Sub-Division – Pteropsida
Class – Filicineae
Sub class – Leptosporangiatae
Order – Salviniales
Family – Salviniaceae
Genus – Salvinia
Species – *Salvinia cuculata*

(After Reimers, 1954, Smith, 1955)

(b) *Ipomoea reptans* Poir. *Syn. I. aquatica* Forsk.

Kingdom – Plantae (plants)
Subkingdom – Tracheobionta (vascular plants)
Superdivision – Spermatophyta (seed plants)
Division – Magnoliophyta (flowering plants)
Class – Magnoliopsida (Dicotyledons)
Sub class – Asteridae
Order – Solanales
Family – Convolvulaceae (Morning glory family)
Genus – Ipomoea L. (Morning glory family)
Species – *Ipomoea reptans* Poir.
(swamp morning - glory)

(After Cronquist, 1981)

(c) *Trapa natans* L.

Kingdom – Plantae (plants)
Subkingdom – Tracheobionta (vascular plants)
Superdivision – Spermatophyta (seed plants)
Division – Magnoliophyta (flowering plants)
Class – Magnoliopsida (Dicotyledons)
Sub class – Rosidae
Order – Myrtales
Family – Trapaceae (water chestnut family)
Genus – Trapa L. (water chestnut family)
Species – *Trapa natans* L.
(*After Cronquist, 1981*)

(d) *Lemna minor* L.

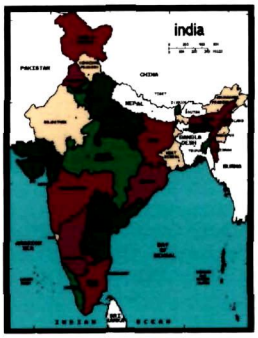
Kingdom – Plantae (plants)
Subkingdom – Tracheobionta (vascular plants)
Superdivision – Spermatophyta (seed plants)
Division – Magnoliophyta (flowering plants)
Class – Liliopsida (Monocotyledons)
Sub class – Arecidae (Arum)
Order – Arales (Arum)
Family – Lemnaceae (Lesser Duckweeds)
Genus – Lemna
Species – *Lemna minor*
(*After Cronquist, 1981*)

1.6 Aim and Objectives of the present study

The present work aims at achieving the following objectives.

1. A survey of various aquatic weeds / macrophytes commonly encountered in the ponds / beels of Assam and those are commonly used by the local people as natural fish feed.
2. Determination of nutritive value and presence of antinutrient factors in some of the selected aquatic weeds/ macrophytes for formulating cost effective fish feed with special reference to Indian major carps, viz. *Labeo rohita, Catla catla* and *Cirrhinus mrigala.*
3. Formulation of cost-effective, artificial diets from the selected aquatic weeds for Indian major carps and to evaluate the nutritive value of formulated diets.
4. Evaluation of aesthetic qualities in formulated diets by determining the growth performances of Indian major carps, along with nutrient turnovers from feed to fish tissue.

Fig. 1.2. Map of North Eastern States of India



Chapter II
Review of Literature

Chapter II

Review of literature

2.1 Aquatic weeds used as fish feed

Successful and sustainable aquaculture depend upon the provision of nutritionally adequate, environmental – friendly and economically viable artificial feeds. Feed is the major operational input in any culture system. But feed cost is considered the major recurring expenditure in any fish culture operation. Although the bulk of fish-meal is used in salmon, trout and marine fish farming in western countries, freshwater fish farming, largely that of carp, also consumes a substantial proportion. Given the current very rapid increase in the intensification of fresh-water farming in Asia, particularly in China, intense future competition for limited global supplies of fish-meal and fish oil are likely (Sargent and Tacon, 1999; Naylor et al., 2000). This predicted strong demand from Asia for available feed resources will have a considerable impact on the world commodity markets and feed prices in general (FAO, 1999). Fish-meal production is also rather localized in some in some regions of the world, as a result of which it is becoming more expensive and difficult to obtain in many countries practicing aquaculture. The need for alternative protein sources to replace fish-meal in aqua-feeds is therefore obvious and was strongly recommended by the Second International Symposium on Sustainable Aquaculture (1998) in Oslo, Norway. The very sustainability of the growing aquaculture industry depends on the progressive reduction of wild fish inputs into fish feed (Naylor et al., 2000).

Fish meal is a major source of dietary protein in fish and crustacean feeds but because of increasing cost, considerable variation in fish-meal quality and uncertainty of regular supply of reliable quality, it is essential that alternative sources of protein be identified for its partial or complete replacement (Gallagher, 1994), Tacon and Jackson (1985) and Kaushik (1990) reviewed the use of conventional and alternative protein sources in fish-feeds. In recent years serious efforts have been directed

towards the use of various non-conventional feed sources as ingredients of fish feed. Many efforts towards partial replacement of fishmeal have also proved encouraging (Wee, 1991).

2.1.1 Aquatic macrophytes

Water hyacinth (*Eichhornia crassipes*) is considered the least desired species among the aquatic macrophytes to be utilised by herbivorous fishes directly. But attempt have recently been made to use vegetative parts of water hyacinth as fish feed in semi digested form (Edwards, 1980; Edwards et al., 1985). Edwards et al. (1985) reported enhancement of growth and feed utilization of *Oreochromis niloticus* fed with diets containing 25-75 % composted water hyacinth with no significant change in physiological functions compared to the control diet of higher protein and energy levels. However, diets with 75% and 100% dried water hyacinth significantly inhibited growth compared to diets containing composted water hyacinth. A comparison of the proximate composition of the composted and dried water hyacinth meals indicated that while crude proteins were more or less similar, crude fiber and crude fat levels were approximately halved and the ash content approximately doubled by the composting process. The poor growth of fish fed on diets with higher levels of water hyacinth was attributed to lower feed consumption due to lesser palatability as well as higher crude fibre content in these diets. In another experiment, *Nile tilapia* recorded higher growth rate (110% of control) at 20% inclusion of water hyacinth meal in the diet than control animals fed on a chicken diet. However, inclusion of water hyacinth in feed formulations at 30% and above significantly inhibited growth (Klinavee et al., 1990).

Hasan et al. (1990) studied the use of water hyacinth for Indian major carp *Labeo rohita,* at 20 and 40% of total dietary protein and compared to control with fish meal as the sole source of protein. The specific growth rates obtained were 79 and 68% of control. The results also showed that apparent digestibility decreased with increase in level of water hyacinth meal. A 63 day trial with three species of Indian major carps fed on diets containing 25 – 35% water hyacinth also showed encouraging

results, both in the laboratory and under field conditions (Patnaik et al., 1989). However, trials conducted elsewhere (Hasan and Roy, 1994) to evaluate the suitability of leaf meal as a partial substitute for dietary fish-meal protein could not promote growth of Indian major carp fingerlings at 25 – 50% inclusion levels. Addition of molasses in the diet did not improve growth rate although feed conversion and protein utilization improved.

Fishes generally lack cellulose activity (Fish, 1960; Stickney and Shumway, 1974; Buddington, 1980) except species such as grass carp (Das and Tripathi, 1991). The intestinal microflora in fish cannot therefore degrade dietary fibre to any significant extent as they do not possess endogenous enzymes that catalyzes hydrolysis of cellulose content in the diet (Edwards et al., 1985) and therefore, Liang and Lovell (1971) did not consider it relevant to supplement dried water hyacinth meal in the diet of channel catfish, *Ictalurus punctatus.*

Murthy and Devaraj (1991) induced growth in *Ctenopharyngodon idella* and *Cyprinus caprio* fingerlings reared on diets made from leaf powder of *Pistia stratiodes.* Further, the total fish production obtained in experimental diets was about 23% higher than the conventional feed.

Use of leaf meal of two aquatic weeds *Ottelia alismoides* and *Nymphoides indicum,* as a protein source for Indian major carp fry at 30 – 50% inclusion level showed encouraging results compared to the conventional rice bran – groundnut oil cake mixture the specific growth rates obtained in diets with 30% and 50% inclusion levels of Ottelia meal were comparable to that of control in rohu, catla and mrigal. Inclusion of Nymphoides meal in the diet also resulted in growth increment similar to that of Ottelia in a 33-day rearing period (Patnaik et al., 1991).

Studies on utilization of *Ceratophylum demersum* as feed supplement revealed that its inclusion in feed formulations up to 20% along with other conventional ingredients not only increased survival rate, but significantly reduced FCR in *Nile tilapia* (Chiayvarcesajja et al., 1990; Klinavee et al., 1990). However, fresh

Ceratophyium might not promote fish growth, probably due to low crude protein and lipid contents (Chiayvarcesajja et al., 1990).

Salvinia cuculata commonly known as *water lettuce,* grows vigorously in some areas of tropical and subtropical zones during the rainy season. These free floating weeds are seldom consumed by fish as food in fresh condition. Ray and Das (1992) studied the nutritive value of composted *S. cuculata* fed to rohu fingerlings by incorporating it into the conventional diets at 20 – 80 % level. Interestingly, the diets devoid of *salvinia* resulted in lower growth rate than diets comprising 20 % composted *Salvinia,* though the former contained higher protein and energy levels. However, poor growth was recorded in rohu fry fed with diets containinglow content (10 %) of *Salvinia* powder (Mohanty and Swami, 1986).

2.1.2 Duckweeds

Duckweeds are highly productive with high protein content when cultivated in nutrient-rich waters (Hillman and Culley, 1978, Culley et al., 1981). Experiments revealing their inclusion in formulated fish feeds include *Lemna, Spirodela, Azolla etc.* Leptosorangiate fern, *Azolla,* has been successfully used as an organic nitrogenous fertilizer in agriculture. Besides this , *Azolla* is consumed by some macrophagus fish (Cassani, 1981; Autoine et al., 1987) and also enhances nitrogen fixation in semi-intensive piscicultural systems (Vincke and Micha, 1985). Its use in aquaculture in recent years as a biofertilizer too could replace the use of inorganic nitrogenous fertilizers in total (Ayyappan et al., 1993). The use of *Azolla* as a non-conventional feed supplement has been experimented with in many species. Pullin and Almazan (1983) though did not obtain encouraging results in feeding fresh Azolla to Nile tilapia; however, they noted its effective utilization by microphyte feeding species such as as *Tilapia zillii and T. rendalli.* Fresh *Azolla pinnata* as supplemental feed was found effective in enhancing growth of Nile tilapia fingerlings in cages in Laguna lake (Pantastico et al., 1986). A comparative appetency study showed fresh *A. pinnata* was less preferred to *Azolla filiculoides* by fingerlings of Nile tilapia (Antoine et al., 1986). At the same time, *A. pinnata* as a dietary protein source for Nile tilapia caused weight

loss in fingerlings when feed in the form of fresh biomass, powder or pellets. Thought fish responded favourably to low *Azolla* feeds of 10 % and 25 % levels, it was not found to be a complete fish diet due to low content of essential amino acids, tryptophan, and threonine (Almazan et al., 1986). Investigations by Micha et al., (1988) revealed that *Azolla* in the diet reduced the growth of *Oreochromis niloticus* and *Tilapia rendalli*. Similarly, two species, *O. niloticus* and *Cichlasoma melanurum* , showed growth retardation, high moisture and low lipid content when reared on 100 % *Azolla* or 50 % *Azolla* and 50 % pelleted feed than fish raised on a diet containing 100 % pelleted feed. However, the test animals did not show variations with regard to crude protein content (Antoine et al., 1987).

Incorporation of *A. pinnata* meal in the diet of *Nile tilapia* at five different levels from 8.5 to 42.5 % to replace fish-meal yielded positive growth response to increasing levels of dietary *Azolla* meal after 7 weeks of rearing, in spite of all feeds containing 35 % crude protein and 250 kcal digestible energy / 100g. this could be due to the presence of some W-6 fatty acids in *Azolla,* which constitute an essential dary requirement for Nile tilapia (Santiago et al., 1988). Similarly, inclusion of *Azolla* powder even at higher levels, from 30 – 60 %, in iso-nitrogenous and isocaloric diets fed to *Labeo rohita* fry showed identical results in improvement of growth performance and feed utilization efficiencies with increase in *Azolla* content in the diet (Mohanty and Dash, 1995). Experiments also showed dried *Azolla* powder up to a level of 25 % in the diet did not effect growth performance, feed utilization and carcass composition of *Etroplus suratensis* compared to fish fed with fish-meal-based diets. However, an increase in *Azolla* level above 25 % in the diet led to a depression in growth performance, food utilization and an altered muscle composition (Joseph et al., 1994). Use of *Azolla* was always found to be advantageous for inclusion in formulated diets as dry meal because of its concentrated nutrient content (Santiago et al., 1988).

Another species of Duckweed, *Spirodela,* was also found to be a potential plant material for inclusion in fish diets. Feeding Nile tilapia at 5 % resulted in optimum growth with an FCR of 4.0 (Edwards et al., 1984); contrarily, Hasan and

Edwards (1992) observed poor growth of the same species at feeding rates varying from 2.5 to 7.5 % of *Spirodela* with apparent FCR values lying between 3.1 to 5.9. Poor growth of fish under *Spirodela* based diets might be attributed to the high crude fibre content of the species.

Devaraj et al., (1981) studied the growth performance of *Cyprinus carpio* fed on a diet containing 40 % *Lemna minor* in an experiment trial conducted in cement cisterns and found the total weight gain in the test diet was 83 % of the control group fed on ground nut cake and rice bran (1 : 1.5). The species *Lemna perpusilla* fed ad libitum to Nile tilapia could provide a low FCR of 3.7 (Edwards et al., (1990). Hasan and Edwards (1992) advocated 3 – 5 % feeding rate of *Lemna* on a dry weight basis to achieve apparent feed conversion ratios ranging from 1.6 to 3.3 in tilapia. Although slow growth rate was recorded in the hybrid of *O niloticus* x *O aureus* fed *Lemna gibba,* the fish readily ingested and efficiently utilized the weed, resulting in a low FCR of 1.0 when cultured in a re-circulatory system under high stocking density (Gaigher et al., 1984).

According to Francis et al., (2001) the use of plant derived materials (eg aquatic weeds) such as legume seeds, different types of oilseed cakes, leaf meals, leaf protein concentrates and root tuber meals as fish feed ingredients is limited by the presence of a wide variety of anti-nutritional substances. Most of the fish feeds, do not lead to mortality, but could produce adverse effects and decrease productivity. Presently, there is a dearth of information in both India and abroad regarding the use of the four aquatic weeds viz. *Salvinia cuculata, Ipomoea reptans, Trapa natans and Lemna minor* as feeds for the Indian major carps – *L rohita, C catla* and *C. mrigala.* Only a few reports pertaining *Salvinia cuculata* (Ray and Das, 1992; Mohanty and Swami, 1986) and *Lemna minor* (Devaraj et al.,1981) are available till date. Unfortunately, the nutritive value of very few aquatic weeds have been assessed,(Dewanji, 1993, Banerjee and Matai, 1990) and literature on the anti-nutritional composition of the above mentioned four aquatic weeds are not available till date. Attempts have been made in the present study to investigate the nutritional potential and anti nutrient components of the four commonly available aquatic weeds

from northeast India, namely *Salvinia cuculata, Ipomoea reptans, Trapa natans and Lemna minor,* for ascertaining their suitability for use as fish feed.

It has been observed that a large share of available freshwater area of India is infested with aquatic weeds, which are posing great concerns for judicious utilization of pond sites. Studies have shown that some of the submerged weeds such as *Hydrilla, Najas,* and *Ceratophyllum,* and duckweed species such as *Spirodela, Lemna,* and *Wolffia,* can be effectively utilized as diet for fish species such as grass carp, *Tilapia zilli,* and *Puntius gonionotus.* Under a new system of carp culture known as weed-based culture, yields of over 4000 kg/ha/year have been achieved when fish are stocked at a density of 4000 fingerlings/ha with as much as 50 % grass carp and the other 50 % comprising five other carp species. No fertilizer was used except aquatic terrestrial vegetation applied for grass carp (Tripathi and Mishra 1986). Under similar experimental trials carried out with grass carp as the major species, Aravindakshan et al., (1999) recorded production of 2,407-2,517 kg/ha/year. Stocked at a density of 4,000 fingerlings/ha with grass carp at 40-50 %, and the remaining percentage comprising other carp species, the experiments did not utilize any other inputs except aquatic weeds like *Ceratophyllum* and *Najas,* provided at regular intervals as food for grass carp; fertilization was limited to single super phosphate at a rate of 100 kg/ha/year and lime (CaO) at 250 kg/ha/year at periodical intervals.

2.1.3 Terrestrial plants

Leaf meal from *Leucaena leucocephala* is a potential source of protein. It is a drought-resistance leguminous tree whose high-protein leaves have been widely used in animal feeds, particularly for ruminants in the tropics (Wee and Wang, 1987). However, the presence of mimosine has limited its use. *Leucaena* leaf meal contains 1.90 % mimosine and with increments in the amount of leaf meal, the mimosine content of the diet also increases (Santiago et al., 1988). Mimosine can be degraded to a relatively less toxic form through various methods of processing, thereby improving the nutritive value of *Leucaena* leaf meal (Wee and Wang, 1987). The growth responses of *T. mossambica* fed with dried leaf meal alone at 3 %, 6 % and 9 % body

weight, improved with increments in amount of leaf meal (Pantastico and Baldia, 1979). In another study Pantastico and Baldia (1980) found that increasing the *Leucaena* leaf meal concentration in supplementary diets (33.3 %, 66.7 % and 100 % leaf meal balanced by rice bran) improved the growth responses, with the diet containing 100 % *Leucaena* leading to the best weight gain although there were no statistically significant growth response differences among the three diets. However, poor growth performance was noted in *T. mossambicus* fed with processed leaf meal contributing 25 and 50 % of the total protein (Jackson et al., 1982). Studies also showed the possible inclusion of soaked leaf meal up to 25 % of the total protein with no adverse effects on the growth of *Nile tilapia*. Increase in levels of leaf meal incorporation led to increase in mimosine concentration and subsequent consumption led to poor growth performances. However, growth performance and feed utilization efficiencies of fish reared on diets containing the soaked *Leucaena* leaf meal were better than those fed commercial and sundried meal with little difference between the latter two at each level of inclusion (Wee and Wang, 1987).

Rohu fed on diets prepared with inclusion of 20 and 40 % total protein by *Leucaena* leaf meal in soaked and unsoaked form showed decrease in growth rate with increase in percentage of inclusion. The specific growth rate obtained was 86 and 75 % and 79 and 70 % of control with soaked and unsoaked *Leucaena* leaf meal respectively (Hasan et al., 1990). In another trial (Hasan et al., 1994) *Leucaena* leaf meal was supplemented at 25, 50 and 60 % protein level in the diet of Indian major carp (*Labeo rohita*) fingerlings both in soaked and unsoaked form. Use of *Leucaena* soaked for 48 hours promoted growth significantly with increased inclusion of dietary plant protein in and treatments. Though protein digestibility decreased with increasing plant protein in the diet, in terms of cost and feed and economic return, the diet containing 50 % inclusion of soaked *Leucaena* proved better than the control and other diets. Studies conducted by Saha and Ray (1998) showed that feeding one day with *Leucaena* leaf meal-based diet followed by three days of animal protein-based diet resulted in better performance of rohu in terms of live weight gain (%), SGR and FCR as compared to those fed continuously with animal protein-based diet.

A study to evaluate the impact of various levels of mimosine content in the diet showed that common carp (*Cyprinus carpio*) fed at 2.4 and 6 % mimosine in a basal diet exhibited very slow growth performance and protein efficiency ratio (Ter Meulen and El-Harith, 1983). Spawning activity was also suppressed at high intake levels of mimosine in the case of *Nile tilapia* (Wee and Wang, 1987). A similar study showed that growth in *Nile tilapia* was adversely affected at high levels of dietary *Leucaena* leaf meal (80 %) after the third week but the effect on reproduction was obvious only after the 15th week (Santiago et al., 1988). On the other hand, some researchers have found that inclusion of *Leucaena* leaf meal in the diet had no adverse effect on the reproductive behaviour of fish (Pantastico and Baldia, 1979, 1980; Ghatnekar et al., 1982).

Amino acid analysis of *Leucaena* leaf meal showed methionine levels ranging from 1.32 % (Glude, 1975) to 2.0 % (Jackson et al., 1982). Furthermore, a low protein digestibility value of only 26 % was reported in *Nile tilapia* fed with 95 % *Leucaena* leaf meal (Cruz and Magisa, 1983). Low digestibility (40 %) of *Leucaena* leaf meal was similarly reported in milkfish, *Chanos chanos* (Ferraris et al., 1986).

Cassava may be considered as two distinct crops' roots, rich in energy and foliage, rich in protein and pigments (Muller, 1977). The average protein content of dry cassava leaves is 25 % (Montaldo, 1977) and hence cassava is considered to be a low-cost protein source. However, the potential use of cassava leaves is constrained by the presence of cyanogenic glucosides which release toxic hydrocyanic acid on hydrolysis Ng and Wee, 1989). Evaluation of cassava leaf meal as a dietary protein source in many animal feeds showed that feed intake, growth and feed efficiency significantly decreased with increments in leaf meal content of the diet (Ross and Enriquez, 1969; Eggum, 1970; Lee and Hutagalung, 1972; Hutagalung et al., 1973). An assessment of nutritive value of cassava leaf meal in the pelleted feed for *Nile tilapia* at inclusion levels of 20-100 % of dietary protein either by soaked or sundried form, showed almost linear depression of growth performance and feed utilization efficiency with increased levels of cassava leaf meal in the diet (Ng and Wee,1989). It may not be possible to include even 20 % of the dietary protein from treated cassava

leaf meal without adversely affecting growth and protein utilization in various fish species. The prospects of using both the leaves and tubers as components of fish feed was also discussed by Varma and Ravi (1988) who advocate its use as an agglutinant in pelleting the feed because of its viscous and sticky starch.

2.2 Supplementary Feeding

The importance of feeding a supplementary diet for enhancing fish production has been reported by several workers (Ling 1967; Hickling 1971; Chaudhuri et al., 1975; Chakrabarty et al., 1979a; Nandeesha 1993). Using nitrogenous fertilizers, urea and ammonia sulphate, at 186 and 386 kg/ha without diet, Murty et al., (1978) obtained a net production of 2,275 kg/ha/year. With the addition of a supplementary diet, in addition to fertilizers at the above rates, a net production of 3,859 kg/ha/year was recorded. Khan et al., (1979) conducted detail experiments on the role of fertilizer and/or diet and registered production levels of 1,053-1,491 kg/ha/year in ponds without any treatment; 1,397-2,303 kg/ha/year in ponds which received fertilizers alone; 2,901-4,470 kg/ha/year in ponds which received diet alone; and 4,414-6,535 kg/ha/year in ponds with the addition of both fertilizers and diet. Further, Singh and Singh (1975) found that the weight of fish increased three to fourfold in 6 months when ponds were provided with both fertilizers and supplementary diet, as compared to the weights attained in ponds fertilized with cattle dung alone, Jena (1998) reported higher production of carps (stocked with catla, rohita, mrigala, and *H. molitrix)* ranging from 3,588 to 4,100 kg/ha/year with supplementary diet compared to 2,010 to 2,225 kg/ha/year in treatments without supplementary diet. The importance of a nutritious diet for carp culture has been stated, and several formulations have been used for carp fry and fingerlings (Sen et al., 1978; Singh et al., 1980; Mohanty et al , 1990; Jafri et al., 1991; Jena et al., 1996, 1998c, 1999). However, the conventional mixture of rice bran and peanut/mustard oil cake at equal proportions in dough form is still used extensively as the only form of supplementary diet in grow-out production of carps in India (Tripathi, 1990). Further, since the cost of oil cake and rice bran has been increasing rapidly, farmers in the state of Andhra Pradesh are using

combinations of these two ingredients based on fish growth rate and pond productivity. During the summer, when growth rates are high, fish are provided with a diet mixture consisting of 30-40 % oilcake and 60-70 % rice bran *ad libitum.* However, when the growth of fish is slower, particularly during the monsoon and winter seasons, fish are fed with rice bran alone or mixed with a small percentage of oil cake (Nandeesha, 1993). Other ingredients are also used based on their availability and cost, mostly in addition to rice bran and peanut cake or as a substitute for the later.

Jayram and Shetty (1981) demonstrated that a balanced diet, even without fish meal, could induce good growth in carps. Nandeesha et al., (1986) demonstrated successful replacement of fish meal with cheaper slaughter-house waste or silkworm fecal matter in diets while culturing carps in earthen ponds. However, in the case of commercial carp culture in India, the use of animal ingredients, especially in grow-out systems, is almost non-existent, except for the use of 15-20 % fish meal in the diet for broodstock production (Nandeesha, 1993). Studies on intensive carp culture carried out at the Central Institute of Freshwater Aquaculture, Bhubaneswar, over a period of five years, have utilized formulated diets composed of rice bran, peanut oil cake, soybean meal, fish meal, and vitamin-mineral mixture (with the following proximate composition : moisture - 9.06 %; crude protein – 30.27 %; crude lipid – 8.95 %; fiber – 9.55 %; gross energy – 3.49 kcal/g), and have reported production levels of over, 15 tons/ha/year (CIFA 1998).

2.3 Minerals

Phosphorus is a major mineral that must be supplied in the feed. However, much of the phosphorus in commercial fish diets may be released into the environment (Wiesmann et al., 1988) and this is influenced both by the availability of dietary phosphorus and the high levels of phosphorus encountered in feeds because of the high levels found in animal proteins such as fish meal. The effects of phosphorus wastes from intensive cage and pen culture have been reviewed by Beveridge (1996) and recent studies of intensive pond systems have also demonstrated the importance of dietary phosphorus in determining algal density and water quality. Consequently, it is

necessary to reduce phosphorus load in effluents and one way to achieve this is to reduce the levels in fish and shrimp diets.

Under practical farming conditions, mineral deficiency signs often arise from a dietary imbalance of calcium owing to the antagonistic effect of excess dietary calcium on the absorption of phosphorus (Nakamura, 1982). When there is an excess of calcium over phosphorus, the phosphorus is not absorbed by the intestine because it is combined with the calcium to form calcium phosphates that are biologically available (Andrews et al., 1973; Cowey and Sargent, 1979).

A phosphorus requirement has been determined for 10 species of fish including *Crysophrys major, Anguilla japonica, Salmo salar* L. 1758, *Cyprinus carpio* L. 1758, *Ictalurus punctatus* Rafinesque 1818, *Oreochromis niloticus* L. 1758, *Oreochromis aureus* Steindachner 1864, *Poecilia reticulata* Peters, 1859 and *Oncorhynchus mykiss* Walbaum 1792 (Sakamoto and Yone, 1973; Arai et al., 1974; Ketola 1975; Ogino & Takeda, 1976; Lovel, 1978; Watanabe et al., 1980; Wilson et al., 1982; NRC 1983; Viola et al., 1986; Robinson et al., 1987; Shim & Ho, 1989; Rodehutscord, 1996; Dougall et al., 1996). Reported phosphorus requirements vary from about 0.25-1.00 g kg^{-1} of the diet, although this rather wide range may be growth related.

A calcium requirement has been determined for 6 species of fish: *O. mykiss, A. japonica, I.. punctatus, C. carpio, O. aureus* and *C major* (Andrews et al., 1973; Sakamoto & Yone 1973; Arai et al., 1975; Ogino & Takeda 1976; Sakamoto & Yone 1976; Robinson et al., 1984, 1986, 1987). Reported calcium requirements vary from about 0.24-1.5 g kg^{-1} of the diet.

2.4 Growth performance and feed utilization by the different fishes fed on formulated diets

On the basis of the analysis of different sets of data, it was concluded that fish spend about 60% of their energy intake on metabolism and 40% on growth when fed *ad libitum* (Brett and Groves, 1979; Cui and Liu, 1990). Whilst this is a useful

generalization, the pattern of energy allocation by a species will depend on a wide variety of factors as described above.Closer analysis of the data presented by Cui and Liu (1990) shows that the proportion of energy devoted to growth in the six species investigated ranged from 21.3% to 63.4%. Similarly, Xie and Sun (1993) found that (*Silurus meridionalis,* a Chinese catfish, expended approximately 60% of the energy it consumed on growth. Since the behaviour of the species is likely to play an extremely important role in energy partitioning, it is unlikely that general hypotheses of fish energy budgets will prove to be a great use to the aquaculturist.

Fish feed to provide sufficient energy for their requirements (Nose and Halver, 1981), and hence dietary energy is important in regulating food intake. Feeding adequate amounts of energy is necessary for the economical production of fish, while feeding excess energy will result in obesity and deterioration of flesh quality.

2.4.1 Determining the quality of a diet

The quality of a feed is a function of how well that feed meets the nutrient requirements of an animal. Not only must the feed contain the correct proportions of nutrients, but the nutrients must be able to be digested and absorbed in a form that makes them available for providing energy and substrates for growth to the animal. This is termed bioavailability. The digestibility of the food is currently the primary determinant of bioavailability. The major problem with using digestibility is that it varies with species, source of nutrient, the temperature at which it is evaluated and often between two samples of exactly the same feedstuff that are treated in different ways (e.g. different heats of drying) (Pfeffer et al., 1991). These factors make it difficult to relate the data obtained by separate groups of workers, or even by one group of workers at one time with those obtained by the same group at different times. Nevertheless, digestibility remains the most widely used method of determining how much of a given food component is bioavailable.

Table 2.1 Digestibility of different components of feed by the African cat fish, *Clarius gariepinus*

Energy in diet (kj/g)	*Apparent protein digestibility (%)*	*Apparent fat digestibility (%)*	*Apparent energy digestibility (%)*
8.4	94.0	90.5	50.0
12.4	95.5	94.0	65.0
16.8	86.5	73.0	60.0

Source: Adapted from Machiels and Henken (1985)

There has been traditional reliance upon fish meal as a protein source in diets for farmed species. Fish meal is expensive relative to other protein sources and a number of investigations have addressed the effectiveness of replacing the fish meal with some other protein source, for instance some types of grain meal (e.g. cottonseed meal). It has generally been found that most alternative protein sources are able to replace fish meal to some extent. A number of factors affect the proportion of fish meal that can be replaced, and these depend upon the nature of protein source. In some grain meals, the essential amino acid composition is not adequate, while many others contain anti nutritional factors. Despite these limitations there can often be considerable cost advantage in replacing some of the fish meal with alternative protein sources.

2.4.2 Lipid as an energy source

Lipid is digested and metabolized with greater relative ease and so served as a much better source of energy for protein sparing than carbohydrate. Again, too much lipid can be included in the diet, which results in production of fatty fish.

The protein-sparing effect of lipid varies between species. The optimum dietary lipid requirement for some species is much less than 15-18% of the diet (Lie et al., 1988; De Silva et al., 1991). The effect is most clearly observed when the amount of dietary protein consumed is low, whether this is the result of a lower proportion of

protein in the diet (De Silva et al., 1991) or diminished rations (Beamish and Thomas, 1984).

Rainbow trout had reduced excretion of nitrogen (a measure of amino acid metabolism: section 3.6) when fed at 0.5% wet body weight per day a diet which contained higher amounts of lipid, but no effect of lipid level was observed when they were fed 0.1% wet body weight per day (Beamish and Thomas, 1984).

2.5 Relationship between thyroid hormone profile in fish serum and quality / quantity of food intake

The potential for disease-free, "off the shelf" foods that are lower in cost, nutritionally complete and well accepted by fish larvae, has driven research towards development of micro-particulate foods to supplement or replace live foods in fish hatcheries (Cahu & Zambonino-Infante 2001). The majority of research in this field has been performed with temperate species (Yúfera et al., 2000; Cahu & Zambonino-Infante, 2001; Koven et al., 2001) and there is a paucity of information on the specific nutritional compositions of formulated diets for tropical species such as barramundi (*Lates calcarifer*).

The relatively high growth rates of marine fish larvae would logically prescribe a high amino acid requirement (Ronnestad et al., 2003). However, few studies have quantitatively manipulated the protein component of diets for larval fish (Peres et al., 1996). Furthermore, although the inter action of dietary energy and protein has been very well documented for juvenile fish (e.g. Nankervis et al., 2000;Lee et al., 2002; Meyer & Fracalossi, 2004), no previous study has accounted for the influence of dietary energy on the protein requirement of larval fish. As amino acids and lipids are the major energy substrates in larval fish nutrition (Fyhn, 1989; Waranabe & Kiron, 1994), lipid-derived energy may influence quantitative protein requirements.

Growth in fish has been shown to being regulated by hormones, including thyroid hormones (L-Thyroxine, T4 and Triiodothyronine, T3), growth hormone, insulin-like growth factors, sex steroids and cortisol (Eales, 1985; Gray & Kelley, 1991; MacKenzie et al., 1998). These hormones mediate the effects of extrinsic processes, such as nutrition, on growth. Although there is strong association between thyroid hormones and growth (Hey et al., 1996), survival (Ayson & Lam, 1993) development (Kim & Brown, 1997) and metamorphic success (Soffientino & Specker, 2001; Gavlik et al., 2002) in fish larvae, data are lacking on the link between larval nutrition and these hormonal processes.

Differences in growth performance between the two rainbow trout strains have been attributed to either the capacity for feed consumption (Valente et al., 1998a, 2001), or the capacity for growth (Valente et al., 1998b, 1999), the fast growing strain displayed the highest voluntary feed intake, although they showed similar nitrogen or energy retention. Furthermore, the sustained higher requirement of muscle fibers, associated with a higher capacity for synthesizing proteins, observed in the fast-growing strain could endow this strain with the potential to maintain rapid somatic growth and to accomplish further muscle growth (Valente et al., 1999), these differences observed during the juvenile to adult phase, could proceed through differences in hormonal status and specially in anabolic hormones such as growth hormone (GH) or thyroid hormones (T3 and T4). These hormones are known to participate in the regulation of growth and development (Donaldson et al., 1979; Weatherley and Gill, 1987). However, in the case of GH, it is quite difficult to establish a relationship between circulating levels and growth rate, partly because if the daily episodic character of its secretion (Le Bail et al., Gomez et al., 1996).

There is evidence that growth hormone and thyroid hormones enhance fish growth by stimulating greater voluntary food intake (appetite), by improving food conversion (Rasmussen et al., 2001), and by stimulating protein synthesis (Fauconneau et al., 1996). Other hormones whose secretion is indirectly or directly stimulated by both GH and thyroid hormones may also be involved (Donaldson et al.,

1979). Altered metabolic state due to nutrient and /or other endocrine action may itself influence blood hormone levels (Leatherland et al., 1977).

Chapter III
Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Indian major carp (*L. rohita, C. catla* and *C. mrigala*) fingerlings were procured from local fisherman. Control feed (mustard oil cake and rice bran) was purchased from a grocery shop at Tezpur. Silkworm pupae were collected from Assam Sericulture Farm, Jamuguri, District- Sonitpur, Assam. Vitamin mineral premix was purchased from Veterinary College, Khanapara, Guwahati, Assam.

3.2 Methods

3.2.1 A survey of various aquatic weeds / macrophytes of Assam used by local people as natural fish feed – A Field Study

Actual field survey was conducted among the rural fisherman of Assam to know the plants (weeds or macrophytes) they use as natural fish feed. The people were asked the following questionnaires and the data was entered in the data sheet:

(a) Local name of the plant they used as fish feed.
(b) Which part of the plant is used as fish feed?
(c) What are the local fishes being farmed?
(d) What is the method of fish feed preparation by utilizing these plants (fish feed processing procedure)?
(e) The quantity of these plants they use for feeding?

References have also being collected from few books written on vernacular languages and pamphlets on useful plants of North East India. The most used plants (fish feed) were collected, photographed and taxonomically identified at Botanical Survey of India, Itanagar, Arunachal Pradesh.

3.2.2 Biochemical / proximate analysis of four selected plants

The proximate analysis of four selected plants viz. *Salvinia cuculata, Ipomoea reptans, Trapa natans* and *Lemna minor* was done as described below.

3.2.2.1 Estimation of moisture content

Moisture content was determined by the method of Association of Official Analytical Chemists (A.O.A.C., 1990). 2.5 gm of plant sample was taken in a pre-weighed dry petridish and kept in a hot-air oven at 100 – 105° C and a cover was put over the petridish keeping a little open space. Heating was continued until constant weight is achieved. It was cooled in a dessicator and weight was taken.

Calculation:

% Moisture = {(Weight of sample before drying - weight of sample after drying) / Wt. of sample taken} x 100

3.2.2.2 Estimation of crude protein

Estimation of crude protein content was done as described below (section 3.2.9.2).

3.2.2.3 Estimation of crude fibre content

Estimation of crude fibre content was done as described below (section 3.2.9.5).

3.2.2.4 Estimation of crude lipid content

Estimation of crude lipid content was done as described below (section 3.2.9.3).

3.2.2.5 Estimation of ash content

Estimation of ash content was done as described below (section 3.2.9.6).

3.2.2.6 Estimation of total carbohydrate content

Estimation of total carbohydrate was estimated by summing up nitrogen free extract (NFE) with crude fibre.

3.2.2.7 Estimation of nitrogen free extract (NFE) content

Estimation of nitrogen free extract (NFE) content was done as described below (section 3.2.9.7).

3.2.2.8 Estimation of gross energy content

Estimation of gross energy content was done as described below (section 3.2.9.8).

3.2.2.9 Determination of vitamin contents of plants

Estimation of vitamin contents of plants viz. vitamin E, vitamin A and vitamin C was done first by extracting the vitamins followed by their quantification as described in sections 3.2.9.10.1, 3.2.9.10.2 and 3.2.9.10.3 respectively.

3.2.2.10 Mineral analysis

The ash of the plant samples was moistened with a small amount of glass-distilled water and 5 ml of 6 N hydrochloric acid (AR grade) was added to it. The mixture was evaporated to dryness on a boiling water bath. Another 5 ml of

hydrochloric acid was added and the solution was evaporated to dryness as before. 4 ml of hydrochloric acid and a few ml of double distilled water were then added and the solution warmed over a boiling water bath and filtered into a 100 ml volumetric flask using Whatman No. 40 filter paper. After cooling, the volume was made up to 100 ml and suitable aliquots were used for the estimation of calcium, sodium, potassium, zinc, copper, magnesium and phosphorus. The mineral elements of the plants viz. Na^{+}, K^{+} and Ca^{++} were estimated by flame photometry, whereas Zn^{++}, Cu^{+} and Mg^{++} contents were measured by atomic absorption spectrophotometry (Hitachi U 2000) using standard reference chemicals. The total phosphorus content was determined as described by Nahapetian and Bassiri (1975) and later modified by Umoren et. al (2005). To 0.5 ml of the digest, 4 ml of deionised water, 3 ml of 0.75 M H_2SO_4, 0.4 ml of 10% (w/v) (NH_4) 6 Mo_7O_2. $4H_2O$ and 0.4 ml of 2% (w / v) ascorbic acid were added and mixed. The solution was allowed to stand for 20min, and the absorbance reading was recorded at 660 nm. KH_2PO_4 was used as the standard.

3.2.2.11 Determination of anti-nutritional components

3.2.2.11.1 Trypsin inhibitory activity

The activity of trypsin inhibitors in the samples was determined by using benzoyl –DL-arginine-paranitroanilide (BAPNA) as a substrate. The trypsin inhibitory activity was expressed as the amount of trypsin inhibitor (TI) in gram present per 100 gram of sample (Kakade et al., 1974 and Chitra and Sadasivam, 1986). 0 to 1ml of extract in duplicate set of test tubes were pipetted out, one to serve as endogenous (E) and the other test (T). Volume was made up to 2 ml with buffer in the endogenous set, whereas volume was made up to 1 ml in the test set. 1 ml of trypsin solution (20 mg) was added to each test tube in the test set. 1 ml of buffer and 1 ml of trypsin solution for standard (S) was pipetted out into a separate test tube. All the tubes were incubated in water – bath at 37°C. After a few minutes, 2.5 ml of substrate (1 mg BAPNA) to each tube was added. The reaction was allowed to proceed for 10-60 min at 37°C. the reaction was stopped by adding 0.5 ml of 30% glacial acetic acid. The absorbance was read at 410 nm in a spectrophotometer.

3.2.2.11.2 Phytic acid content

The phytic acid content of the samples was determined spectrophotometrically using Hitachi U 2000 uv-vis spectrophotometer (Vaintraub and Lapteva, 1988). Ground samples (0.5gm each) were stirred using a magnetic stirrer in 10ml 3.5% HCL for 1 h. The contents were centrifused at 3000gm for 10 min to obtain supernatants. A suitable aliquot of the supernatant was diluted with 3.5% HCL to make up to the 3 ml mark. 1 ml of Wade reagent (0.03% solution of $FeCl_3$. 6 H_2O containing 0.3% sulphosalicylic acid) was added and it was centrifuged again. The absorbance was measured at 500 nm. Phytic acid was used as a standard.

3.2.2.11.3 Tannin content

Total phenols (tannin) from the plants was isolated as described by Makkar (1994) and then estimated by Folin-Denis reagent (Makkar and Goodchild, 1996). 1 or 2gm of the plant sample was extracted with 50% methanol in a water bath at 95^{O} C for 10 minutes. The pooled extract was made up to 10 ml. 0.5 ml extract was mixed with equal quantity of distilled water and treated with 5 ml sodium carbonate (Na_2CO_3) (0.1 N NaOH). After 10 minutes 0.5 ml of Folin-Ciocalteu reagent (diluted 1:2 with distilled water) was added. The absorbance was measured at 725 nm. Tannic acid was used as standard.

3.2.2.11.4 Calcium oxalate content

Calcium oxalate in the sample was isolated according to the method of Jones (1988) and was refined by Danielson (1993). One gram of sample (40 ml in volume) was taken and gently crushed with a pre-cleaned mortar and pestle. The crushed sample was placed in a clean 600 ml beaker. In fumehood, 30 % (v / v) hydrogen peroxide (H_2O_2) was added to cover the material. The beaker was swirled by hand until all material was covered with peroxide and then was left to stand for five minutes. Approximately 0.25 gm of potassium dichromate crystals were added over the material

and the beaker was swirled to help in the dispersion of the chemical throughout the sample. A delayed, violent bubbling resulted (to prevent the sample from bubbling over 95% ethyl alcohol was sprayed over the sample). When the reaction began to abate, 1-2 ml of hydrogen peroxide was added to renew oxidation. When all of the organic material was digested, the samples were kept intact until the reaction ceased and the and the temperature in the beaker decreased. The supernatant was poured carefully into 12 ml glass test tubes and concentrated by centrifugation. The remains were rinsed with distilled water and centrifuged three times to eliminate any hydrogen peroxide and potassium dichromate residues. After decanting the last water wash, 8 ml of 95 % (v / v) ethyl alcohol was added. The samples were transferred to 1 gm vials for storage. A plastic 12 ml graduated centrifuge tube marked at 1 ml intervals was used to quantify the sample residue. The calcium oxalate volume was estimated by concentrating the extracted calcium oxalate in the graduated centrifuge tubes and measuring the volume of residue.

3.2.3 Diet preparation

Composition of the diets used in the present study is presented in Table 3.1. The basal diet without addition of aquatic weeds is referred to as control (C) diet. Initially pre-estimated amount of basic ingredients viz. silkworm pupae, rice bran, mustard oil cake were cooked at 50 to 60° C for 3 h, cooled to room temperature, followed by thoroughly mixing with vitamin-mineral premix, grounded, sieved and then weighed. A dough was made by mixing the dry weighed samples with warm water. Pellets of 2 mm diameter were made by using a commercial pelletiser and the pellets were dried at 60 ° C, packed in air tight containers and stored in refrigerator. Five diets (F_1 to F_4 and C) were formulated by adding a predetermined amount of any of the four cooked aquatic weeds viz. *S. cuculata, I. reptans, T natans* and *L. minor* respectively to the basal diet so that the total protein content of the formulated diets should range between 26 and 28 g% (Table 3.1). Chromic oxide (1 % w / w) was added to each formulated diet as an external digestibility marker.

Table 3.1. Ingredient proportions of formulated diets for Indian major carp fingerlings.

Components/ composition	Formulated Diets				
	F1	F2	F3	F4	Control
	Ingredients (g %)				
Mustard oil cake	45	45	50	45	45
Silk worm pupae	10	10	10	10	8
Rice bran	23	33	15	33	45
Salvinia cuculata	20	-	-	-	-
Ipomoea reptans	-	10	-	-	-
Trapa natans	-	-	23	-	-
Lemna minor	-	-	-	10	-
Vitamin mineral premix*	2	2	2	2	2
Chromic oxide	1.0	1.0	1.0	1.0	1.0

*Vitamin premix (mg or IU/g premix): retinol palmitate, 500,000 IU; thiamin, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10; cholecalciferol, 50,000 IU; α -tocoferol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25

Table 3.2. Ingredient proportions of formulated diets (*I. reptans* based) for Indian major carp fingerlings.

Components/composition	Formulated Diets				
	IR-1	IR-2	IR-3	IR-4	Control
	Ingredients (g %)				
Mustard oil cake	45	45	45	45	45
Silk worm pupae	10	10	10	10	8
Rice bran	41	35	31	7	45
Vitamin mineral premix*	2	2	2	2	2
Ipomoea reptans	1	18	38	65	-
Chromic oxide	1.0	1.0	1.0	1.0	1.0

*Vitamin premix (mg or IU/g premix): retinol palmitate, 500,000 IU; thiamin, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10; cholecalciferol, 50,000 IU; α -tocoferol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25

3.2.4 Diet preparation for optimization of *Ipomoea reptans* supplemented dietary protein requirement for growth performance of Indian major carp fingerlings

Four diets (IR-1 to IR-4) were formulated so that the total protein content of the formulated diets range as 25, 30, 35 and 40 g% (w/w) (Table 3.2).

3.2.5 Proximate analysis of feed

Dry matter (DM), organic matter (OM), crude protein (CP), moisture, ash, ether extract (EE), crude fiber (CF) and nitrogen-free extract (NFE) were determined by the methods of Association of Official Analytical Chemists (A.O.A.C., 1990). Chromic oxide levels in the feeds and faecal samples were estimated spectrophometrically (Bolin et al. 1952). Proximate analyses of fish carcass were done at the beginning and termination of feeding experiment.

3.2.5.1 Estimation of organic matter

Dry matter content (DM) was determined after drying in an oven at 60°C to constant weight. Organic matter (OM) was calculated by subtracting the total ash value from the dry matter (DM).

3.2.5.2 Estimation of crude protein

Estimation of crude protein content was done as described below (section 3.2.9.2).

3.2.5.3 Estimation of crude lipid (CL) or ether extract (EE)

Estimation of crude lipid content was done as described below (section 3.2.9.3).

3.2.5.4 Estimation of ash content

Estimation of ash content was done as described below (section 3.2.9.6).

3.2.5.5 Estimation of crude fibre (CF)

Estimation of crude fibre content was done as described below (section 3.2.9.5).

3.2.5.6 Estimation of nitrogen free extract (NFE)

Estimation of nitrogen free extract (NFE) content was done as described below (section 3.2.9.7).

3.2.5.7 Estimation of total carbohydrate content

Estimation of total carbohydrate content was done as described above (section 3.2.9.4).

3.2.5.8 Estimation of gross energy content

Estimation of gross energy content was done as described below (section 3.2.9.8).

3.2.5.9 Estimation of protein / energy content *(P/E)*

Estimation of protein / energy ratio was done by dividing the protein content with energy content.

3.2.6 Fish feeding experiment

Fingerlings of *L. rohita* (average weight 47.3±2.0 g and length 14.7± 1.1 cm), *C. catla* (average weight 41.2 g and length 15.0 cm) and *C. mrigala* (average weight 43.2 g and length 15.6 cm respectively) advanced fingerlings were brought from a local pond and acclimatized for 48 h under laboratory condition (without feeding) in stored

tap water. The fingerlings were then hand-sorted and distributed in 15 glass aquaria (each having 50 l capacity) at a stocking density of 5 fishes per aquarium with three replications for each diet, with aeration from air compressors through air stones. Daily about 50% of water from each tank was replaced with clean, fresh tap water. Tanks were indoors and the light cycle was 12h / 12h light / dark.

All groups of fish were fed their respective diet at 3% body weight for 60 days. Left over food materials was collected 1 h post feeding. Fishes were weighed and length measured every fortnight (Siddhuraju and Becker, 2001, Samantaray and Mohanty, 1997) and daily rations adjusted accordingly. The daily ration was divided into two equal parts and fed at 0930 and 1700 h. After one hour of feed offered, the left over feed was collected by siphoning to determine weight of residue and to calculate actual feed consumption and feed conversion ratio by the fish per day on a dry matter basis after drying in an oven (100 ± 2 ° C for 12 h) of feed offered and feed residues. The faecal samples released by the fish were collected daily from each aquaria by pipetting. The oven dried faecal samples were analyzed for digestibility estimation. The experiment was conducted for 60 days and during this period, water temperature was recorded everyday and it ranged from 25^0 C to 28^0 C.

3.2.7 Fish feeding experiment for optimization of *Ipomoea reptans* supplemented dietary protein requirement for growth performance of Indian major carp fingerlings

L. rohita fingerlings (average weight 42.2 ± 2.0 g and length 15.0 ± 0.2 cm), *C.catla* fingerlings (average weight 47.4 ± 1.0 g and length 15.0 ± 0.2 cm) and *C.mrigala* fingerlings (average weight 45.0 ± 1.5 g and length 14.8 ± 0.3 cm) were brought from local ponds and acclimatized for 48 h under laboratory condition in stored tap water. Fish feeding experiment was same as in section 3.2.5.

3.2.8 Monitoring the growth performance of fish post feeding the diets (at the end of 15, 30, 45 and 60 days)

Growth performance and nutrient utilization by fish were monitored and analysed in terms of feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR), and energy retention (ER) according to Castell and Tiews (1980). At the end of 15, 30, 45 and 60 days feeding trial, fish were weighed and apparent nutrient digestibility (% AND), feed conversion ratio (FCR), specific growth rate (SGR, % per day), protein efficiency ratio (PER), body weight gain (BWG g), daily weight gain (DWG g / day), feed intake (g / 100g average body weight / day) of the fingerlings were calculated using standard methods (Steffens, 1989). Visual symptom of any disease was also screened along with the natural activity of the fingerlings.

Calculations.

Feed conversion ratio (FCR) = Total feed intake/ Total live weight gained

Protein efficiency ratio (PER) = Total live weight gained/ Total protein intake

Energy retention (ER) = (Total body energy/ Total dietary energy intake) x 100

Specific growth rate (SGR) = ($\log_e$ final weight-$\log_e$initial weight/ number of days) x 100

Body weight gain (BWG) = (100/initial weight) x final weight

Increase in length (IL) = (100/initial length) x final length

The apparent nutrient digestibility (%) was calculated according to Maynard et al. (1979) using the formula

Apparent nutrient digestibility (AND) = 100 – 100 x {(% Cr_2O_3 in feed / % Cr_2O_3 in faecal matter) x (% nutrient in faecal matter / % nutrient in feed)}

3.2.9 Carcass composition of fish

Carcass composition of fish at the onset and post feeding experiment was determined by analyzing organic matter, crude protein, crude lipid, crude fibre, ash, nitrogen free extract, total carbohydrate and gross energy.

3.2.9.1 Determination of organic matter

Estimation of organic matter content was done as described previously in section 3.2.5.1.

3.2.9.2 Determination of crude protein

The crude protein content was determined by the Kjeldahl procedure, AOAC Method 920; the factor N X 6.25 was used to convert nitrogen into crude protein. 0.1gm of the sample, a pinch of digestion mixture (copper sulphate and potassium sulphate in 1: 9 ratio) and 5ml of H_2SO_4 (conc.) was taken in a digestion tube, 2 sets of the fish tissue samples were made and put it in the digestion chamber. After digestion, distilled water was added and shaken. Volume was made up to 100 ml in the digestion flask. 5.0 ml of sample was transferred to Micro-kjeldahl distillation unit, and this 5.0 ml of (v / v) 40% NaOH was added. This was followed by addition of 5.0 ml of Toshiro (protein indicator, red colored, boric acid, bromocreesol green, methyl red) indicator and placed in the receptor site, the red color turns green. After that it was titrated against N/10 H_2SO_4. The green color again turns red and reading was taken. Amount of sample to be taken for titration is 100ml.

CHEMISTRY:

Ammonium Sulphate will form after digestion. In this ammoniun sulphate was added to (sodium hydroxide) NaOH to make the medium alkaline and ammonium hydroxide is formed. When heated ammonia (NH_3) will be free and then it will be trapped in the Boric acid indicator solution and ammonium borate will be formed.

Calculation:

Weight of Nitrogen (N) in 1000 ml of 1 N solution = 14 gm

Weight of N in 1 ml of 0.1 N solution = (14 x 0.1) / 1000 = 0.0014 gm

Volume of digested solution = 100ml

Volume of digested solution taken for distillation = 5 ml

Amount of 0.1 N H_2SO_4 required for titration = X ml

Therefore, % N = (X x 0.0014 x 100 x 100) / (5 ml x 0.1gm dry sample) = Y
Therefore, % protein = Y x 6.25

3.2.9.3 Determination of total lipid

3.2.9.3.1 Determination of crude lipid

Ether extract (EE) or crude lipid (CL) was determined by the method of Association of Official Analytical Chemists (A.O.A.C., 1990). 2 – 3 gm of dried powdered fish tissue samples was taken accurately either in an extraction thimble or in the silk bag. It was placed inside the soxhlet extraction unit. A dry and properly cleaned pre-weighed solvent / oil flask beneath the extraction unit was connected and required quantity (50 ml) of solvent (petroleum ether, b.p. 60 – 80 °) was poured and connected to the condenser. The heating rate was adjusted to give a condensation rate of 2-3 drops / per second and extraction was continued for 8 hours. By increasing the extraction rate the extraction time may be reduced. After completion of extraction the thimbles and ether are removed. The flask was dried at 105 ° C for 30 minutes in a hot air oven and cooled in the dessicator and weighed.

Calculation:
Weight of crude fat = Final weight of the oil flask - Initial weight of the oil flask.
Crude fat % = (Weight of fat / Weight of dry sample) x 100

3.2.9.3.2 Isolation of total lipid

Total lipid from blood / tissue or plant samples was isolated by the method of Folch et al. (1957). For analyzing total lipid content of liver and muscle tissues, fishes were sacrificed; liver and muscle tissues were dissected out from fish approximately 12 h after the last feeding and stored at –20° C till analysis were done.1 gm of tissue or tissue fraction was homogenized with 2:1 (v/v) methanol-chloroform mixture in a final volume of 20.0 ml. The homogenate was mixed properly and filtered through a Whatman filter paper No.1. The filtrate was collected in a fresh tube and the residue was re-suspended in 5.0 ml of methanol-chloroform mixture, vortexed and then re-

filtered and the filtrate was collected in a fresh tube. This process was repeated once more and the filtrate was evaporated to dryness. The dried residue was weighed and re-suspended in chloroform at a final concentration of 5.0 mg / ml.

3.2.9.3.3 Estimation of total lipid

The total lipid was estimated spectrophotometrically as described by Nath and Chatterji (1962). Briefly, 0.2 ml of lipid extract was transferred in 4.8 ml of concentrated H_2SO_4 and kept in a boiling water bath for 10 min. The tubes were allowed to cool at room temperature and then 0.2 ml of the mixture was withdrawn and transferred in a fresh tube containing 3.8 ml of orthophosphoric acid followed by addition of 1 ml of 0.6 % (w/v) vanillin. The reaction was allowed to continue for 10 min at room temperature and absorbency of the pink color was read at 530 nm using a double beam spectrophotometer (Hitachi).

3.2.9.3.4 Extraction of Free Fatty acids from blood serum / liver

Extraction of Free Fatty acids from biological samples was done as described by Plummer (1996). To 0.5 gm tissue dissolved in water 1ml of concentrated HCl (pH: 1.0) was added and allowed to stay for overnight. A pasty solid will separate and float on top. These are fatty acids. The mixture was extracted with ethyl ether, when the liberated fatty acids go into the ether layer. The layers were separated in a separatory funnel. The ether layer was collected and evaporated to dryness by blowing air or nitrogen on to the surface. The pasty solid was stored and used for further analyses.

3.2.9.3.5 Identification and quantification of fatty acids

Fatty acids are identified by the method cited by Sadasivam and Manickam (1997). 5ml of methanolic sodium hydroxide solution was taken in a round bottomed flask containing the sample and refluxed for half an hour. 5ml of water and 1ml of concentrated HCl was added and extracted with 10ml of petroleum ether. The process was repeated two times more. The sample was now put into a rotary vacuum evaporator or a current of nitrogen. 10 ml of methyl alcohol and few drops of concentrated H_2SO_4

was added to the free fatty acids. The mixture was again refluxed for three hours and at the end of this period it was diluted with 10 ml of water and extracted with 10 ml of petroleum ether. Extraction was once again repeated for two more times. And allowed to dry in a desiccator. The mixture was allowed to dry. The methyl esters are now ready for GC analysis (model C 1000 having flame ionization detector or FID). Standard methyl esters were separately injected to calculate the retention time for individual esters.

The identification of methyl esters of fatty acids was based on a comparison of the retention times with those of authentic reference compounds (Sigma, St. Louis, USA). Log_{10} values of the retention time of authentic methyl esters of the fatty acids were plotted against the number of carbon atoms. Unknown fatty acids were identified consulting the plots. The peak areas in the chromatogram were measured with a planimeter and the relative percentage of individual fatty acids was calculated. The amount of individual fatty acids was then determined from the starting material (lipid) used for methylation. The amount of fatty acids after calculation was expressed in % g wet tissues.

3.2.9.4 Estimation of carbohydrate content

Total carbohydrate was quantitated by phenol-sulphuric acid method as described by Dubois et al. (1956) using D-glucose as a standard. The optical density of the reaction mixture was measured at 490 nm against a reagent blank. The carbohydrate content of the unknown fish tissue samples was calculated from the standard curve obtained by plotting optical density Vs concentration of D-glucose (0.1 mg/ml).

3.2.9.5 Determination of crude fibre

Crude fibre (CF) was determined by the methods of Association of Official Analytical Chemists (A.O.A.C., 1990). 2 to 5 gm finely powdered dried fat free fish tissue sample was taken in a conical flask connected by a condenser. 100 to 150 ml of 1.25 % H_2SO_4 solution was added, boiled and refluxed for 30 min. The sample was filtered with boiled distilled water for several times to get it free from sulphate. The

residue was transferred into a conical flask and 100 to 150 ml of NaOH (1.25%) solution was added and boiled for 30 min. Again it was filtered, washed with boiled distilled water to make it free from alkali. The residue was washed at least 3 times with rectified spirit to wash down the remaining fat. Then the residue was again washed 3 times with acetone. Now the residue was transferred into silica crucible and dried in a hot air oven at 100°C and cooled in a desiccator and weighed. After that the residue was ashed in a muffle furnace at 500°C for 3 hours, cooled in a desiccator and weighed.

Calculation:

% Crude fibre = {(Dry weight of residue – weight of ash) / weight of sample} x 100

3.2.9.6 Determination of ash content

Ash was determined by the methods of Association of Official Analytical Chemists (A.O.A.C., 1990). 1 to 4 gm of dried fish tissue sample was dried and weighed and put into preweighed silica crucible. It was incinerated at 550-600°C in a muffle furnace for 3 hours. After incineration the sample was removed from muffle furnace with the help of a tong and cooled in a desiccator and weighed.

Calculation:

Weight of empty silica crucible = X gm

Weight of silica crucible + Ash = Y gm

Weight of dry sample = W gm

Therefore, weight of ash = {(Y-X) / W} x 100

3.2.9.7 Determination of nitrogen free extract (NFE)

The nitrogen free extract (NFE) content was estimated as the weight difference using moisture, crude protein, lipid and ash content data.

Calculation:

NFE = Moisture content – crude protein – crude lipid - crude ash content.

3.2.9.8 Determination of gross energy content

The gross energy was determined by using an oxygen bomb calorimeter (Parr, USA), using benzoic acid as a standard. 1 gm of the fish tissue sample was dried and pelleted and the cotton / thread plug was firmly embedded. The pellet was weighed and then transferred into the cup (pellet-thread weight = sample weight). The cup containing the sample was placed into the supporting ring. A piece of nickel-chromium wire was stretched between the electrodes of the bomb winding the cotton wick. After assembling the bomb, oxygen was introduced to a filling pressure of 25-30 lb / mm or atm. pressure. The bomb / capsule was placed in a bucket containing 2 liter of distilled water at a temperature not > 28°C. The bucket was placed into position inside the calorimeter and the rolling arm was carefully rotated for appropriate positioning of the bomb. The stirrer was switched on and the thermometer was placed through the hole making sure that it must immerse in the water. After taking note of the temperature the ignition button was pressed. The final temperature was noted down after the running of temperature. The temperature within 5 minutes remained constant. Lastly the switched was turned off and bomb was removed.

Calculation:

Gross energy (Kcal/gm) = {(Rise in temperature - initial temperature) x Bomb equivalent or water equivalent –23} / Weight of sample x 1000

3.2.9.9 Estimation of hepatic and muscle glycogen level

Isolation and estimation of glycogen content of liver and muscle tissues were done as described by Plummer (1996). For analyzing the glycogen, of liver and muscle tissues, fishes were sacrificed; liver and muscle tissues were dissected out from fish approximately 12 h after the last feeding and stored at –20° C till analysis were done. 2 gm of the tissue sample are homogenized in TCA (Tri chloro acetic acid) and centrifuged in cold, filtered and the sediment is rehomogenised with 5% TCA and again centrifuged in cold and filtered. The two supernatants are combined and 45% ethanol was added twice to it to make up the volume. After mixing them well it is left

in the refrigerator overnight for precipitation, which is later on weighed for glycogen isolation and quantification.

3.2.9.10 Biochemical analysis of fish serum

For analyzing the serum of Indian major carp fingerlings were anaesthetized with 60 mg/ l tricaine methane sulphate blood was collected by heart puncture from each group (5 fish/group) at the end of experiment, serum was separated by conventional procedure and thyroid hormonal status in serum were determined as stated below (section 3.2.6.10.7). Biochemical analysis of fish serum at the onset and post feeding experiment was determined by analyzing vitamin A, E and C, lipid profile, protein content, enzyme profile, hormones.

3.2.9.10.1 Isolation and estimation of vitamin A content

Determination of Vitamin A contents of blood serum and / or tissue of Indian major carp fingerlings includes first extraction of total lipid material of serum / tissue (Folch et al., 1957) followed by extraction and estimation of Vitamin E by the procedure of Baker and Frank (1968). 2ml of lipid residue was dissolved in 2ml of absolute ethanol. Again 2ml of Xylene was added to it and mixed well and centrifuged. Xylene layer was carefully drawn off with a pascheu pipette and transferred to a clean-stoppered tube. To 1ml of xylene extract 1ml of 0.12% Dipyridyn or 2,2' - Bipyridyl GR reagent (prepared in absolute ethanol) was added and mixed well. 2ml of the mixture was pipetted out to a cuvette and absorbance was read at 460nm.

Calculation:

Carotenoid (mg/litre) $= 0.29 \times A_{460\,nm}$

3.2.9.10.2 Isolation and estimation of vitamin E content

Isolation:

For analyzing the vitamin E contents of liver and muscle tissues, fishes were sacrificed; liver and muscle tissues were dissected out from fish approximately 12 h

after the last feeding and stored at –20° C till analysis were done. Total lipid was extracted from the tissues (Folch et al., 1957) (previously mentioned in section 3.2.9.3.2), followed by extraction and estimation of Vitamin E by the procedure of Baker and Frank (1968).

Determination of Vitamin E contents of blood serum of Indian major carp fingerlings includes first extraction of total lipid material from serum (Folch et al., 1957) (previously mentioned in section 3.2.6.3.2), followed by extraction and estimation of Vitamin E by the procedure of Baker and Frank (1968).

Estimation

The Baker and Frank (1968) method proceeds as follows: 2ml of lipid residue was dissolved in 2ml of absolute ethanol. Again 2ml of Xylene was added to it and mixed well and centrifuged. Xylene layer was carefully drawn off with a pascheu pipette and transferred to a clean-stoppered tube. To 1ml of xylene extract 1ml of 0.12% Dipyridyn or 2,2′ - Bipyridyl GR reagent (prepared in absolute ethanol) was added and mixed well. 2ml of the mixture was pipetted out to a cuvette and absorbance was read at 460nm. To the same mixture 0.5ml of 0.2% (wt / v) of $FeCl_3$ (dissolved in absolute ethanol) was added and absorbance was read at 520 nm after 1.5 minutes. For standard 10 mg EVION capsule (Vitamin E) was used.

Calculation

$A'(\alpha\text{ - tocopherol}) = A_{520\,nm} - (0.29 \times A_{460\,nm})$

α - tocopherol (mg/litre) = (A′ of unknown sample / A′ of standard) x 10

3.2.9.10.3 Isolation and estimation of vitamin C content

Vitamin C (ascorbic acid) content was assayed spectrophotometrically following the method of Dabrowski and Hinterleitner (1989). 2 gm of tissue sample was homogenized with 1 ml of chloroform and 5.0 ml cold extractant (4 % TCA mixed with Metaphosphate-EDTA solution in equal parts). Then it was centrifuged and the supernatant was used for analysis. For analysis 1 ml of aliquot from each centrifuge (0,

10, 20, 30, 40,.....100 µg/ml) sample and 1 ml of each standard solution was taken. For standard, 40 mg of ascorbyl-2-polyphosphate was dissolved in 100 ml cold extracting solution. 1 ml of coupling reagent (1 g of 2, 4, dinitrophenyl hydrazine or DNPH in 50 ml of 50 % H_2SO_4 diluted to 100ml by distilled water and 2 g of thiourea) was added to all the tubes and incubated at 80° C for 3 h. Then the tubes were chilled in ice water and to each tube 2.5 ml ice-cold 50 % H_2SO_4 was added and thoroughly mixed. The solution was allowed to come down at room temperature and the absorbance was measured at 524 nm against a reference blank.

3.2.9.10.4 Estimation of lipid profile of serum

Lipid profile of serum (total cholesterol, HDL - cholesterol, triglyceride) of rohu, catla and mrigal fingerlings were analyzed spectrophotometrically by using the commercial diagnostic kits (Monoenzyme India limited, Secunderabad, India and Crest Biosystems, Goa, India) and following the instructions of the manufacturers.

3.2.9.10.5 Quantitation of serum protein

Protein content of serum (albumin, globulin and total protein) of rohu, catla and mrigal fingerlings were analyzed spectrophotometrically by using the commercial diagnostic kits (Monoenzyme India limited, Secunderabad, India and Crest Biosystems, Goa, India) and following the instructions of the manufacturers.

3.2.9.10.6 Analysis of enzyme profile of serum

Enzyme profile of serum (GOT, GPT and alkaline phosphatase) of rohu, catla and mrigal fingerlings were analyzed spectrophotometrically by using the commercial diagnostic kits (Monoenzyme India limited, Secunderabad, India and Crest Biosystems, Goa, India) and following the instructions of the manufacturers.

3.2.9.10.7 Assay of serum hormone

Thyroid hormone (T3, T4 and TSH) level in serum was determined by enzyme linked fluorescent assay (ELFA) by using an ELISA Reader (Mini VIDAS, Germany). Serum was diluted to 1:2 ratio, centrifuged and put into the ELISA Reader for assessing the thyroid hormones.

3.2.9.10.8 SDS-PAGE analysis of fish serum

SDS – PAGE (Sodium doedecyl Polyacralamide Gel Electrophoresis) was carried out with or without reduction of proteins by β – mercaptoetanol as described by Laemeli (1970). Briefly 20 µl of serum of the fingerlings (obtained before and post feeding the control and F_2 diets) were loaded into 15% gel. Separating gel containing 5% glycerol. Electrophoresis was carried out at a constant current of 15 mA until the dye front (bromophenol blue) reached the bottom of the gel. Before staining, proteins were fixed by incubating the gel in 20% TCA for 30 min followed by washing the gel several times in distill water. Protein bands were visualized by staining the gel with 1% (w/v) Coomassie brilliant blue R250 in methanol: acetic acid: water (4: 1: 5 v/v/v). Destained gels were scanned in Gel Doc. 1000 (BioRad). Mobility of the purified protein was compared with the following molecular weight markers; phosphorylase b (97,400), bovine serum albumin (66,000), ovalbumin (43,000), carbonic anhydrase (29,000), soyabean trypsin inhibitor (20,100) and lysozyme (14,300). Molecular weight of the unknown proteins was calculated using Bio-Rad Multi-Analyst ™ / PC version 1.1 software (Bio–Rad).

3.2.9.10.9 Antigenic cross-reactivity between fish serum and plant extracts

3.2.9.10.9.1 Preparation of plant extract

Plant extract were prepared according to Mahanta and Mukherjee (2001). Fresh leaves / roots / bark were shade dried and made to coarse powder. Two gm of powder of leaves / roots / bark was taken in a beaker and soaked with 100ml of H_2O with

continuous stirring for 2 h at room temperature. The extract was filtered through muslin cloth and filtrate was concentrated at -20°C under vaccum.

3.2.9.10.9.2 Gel-immunodiffusion test

Antigenic cross reactivity if any, between the plant extracts (*Salvinia cuculata, Trapa natans, Lemna minor* and *Ipomoea reptans)* and serum of the Indian major carps fingerlings fed with the different experimental diets was analyzed by agarose gel immunodiffusion. 1% (w/v) agarose was prepared in 20 mM phosphate buffer containing 150 mM NaCl, pH 7.4 containing 0.02% (w/v) sodium azide. Wells of suitable diameter were prepared on the plate using a puncher, one in the center and four surrounding the central well. The central well was filled with 10 µl serum while the surrounding wells were filled with 10 µl plant samples each. Gel plates were incubated in humid chamber at 37°C for 1-2 days till the appearance of precipitin line.

3.2.10 Scanning Electron Microscope (SEM) study of fish tissues

Tissue samples used for the study were heart, liver, kidney and intestine of Indian major carp fingerlings before the onset and after the completion of experiment. Tissues were fixed in 2.5% (v/v) glutaraldehyde prepared in 0.1M sodium cacodylate buffer at pH 7.4 for 6 h at 4 ^{0}C. Following primary fixation in glutaraldehyde, the tissues were washed in buffer overnight, post fixed in 1% (w/v) osmium tetraoxide for 1h and dehydrated through increasing concentrations of acetone. Dehydrated specimens were immersed in tetramethylsilane (TMS; Alfa Products; boiling point 26.3^0 C, surface tension, 10.2 dyne / cm at 20^o C) in a specimen vial for 10 min. The specimens (fixed tissues) were then removed from TMS, transferred to a glass slide and allowed to dry either at room temperature (25-26^o C) or in an incubator at about 26^o C, followed by drying in a critical point dryer (Sandri Pvt, Tousimis). The dry samples were secured horizontally to brass stubs with double-coated adhesive tape connected via a patch of silver paint to ensure charge conduction. Care was taken to avoid trapped air bubbles. A conductive coating was applied to the sample using JFC 1100 (Jeol) ion

sputter coater. A relatively low vacuum (10^{-3} torr) was established in the sputtering chamber, and the 'target' material used was gold. The coated samples were observed with a JSM- 35CF (Jeol) SEM operated at 15KV.

3.2.11 Analysis of digestive enzyme activities of fish intestine

Gut of individual fish was dissected out and 100 mg of tissue was homogenized with 1.0 ml of ice-cold 20 mM potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 10,000 x g for 15 min at 4°C and the supernatant was used for the assay of amylase, protease and lipase enzymes as described below.

3.2.11.1 Assay of amylase activity

The assay of amylase activity was based on the method of Bernfeld (1955). The activity was expressed as mg maltose liberated from starch / mg protein / hour. The increase in reducing power of buffered starch solution was measured with 3, 5 dinitrosalicylic acid at 540 nm. The assay mixture consists of 1 ml of buffer solution (0.1 M, pH 7.0), 1 ml starch as substrate, 100 µl of enzyme extract (pre incubated), 500 µl of NaCl solution were mixed well and incubated at 37^0 C for 30 minutes. The reaction was stopped b adding 0.5 ml 2 NaOH, 500 µl of dinitrosalicyclate acid in 2 N sodium hydroxide and 60% sodium potassium tartars was added abd geated ub a boiling water bath for 10 minutes. Then allowed to cool and the intensity of the colour developed following the reaction were measured at 540 nm against a reagent blank.

3.2.11.2 Assay of protease activity

Casienolytic activity was evaluated calorimetrically by the method of Ouyang and Teng (1976) as modified by Mukherjee and Maity (1998). 1 % (w / v) of casein in 0.1 M potassium – phosphate buffer, pH 8.0 was incubated with 50 µl of homogenate for 90 min at 37 ° C followed by addition of 0.5 ml of 10 % (w / v) ice-cold TCA to stop the reaction. After centrifugation of the mixture, supernatant was transferred to a fresh tube and 2.0 ml of 2 % (w / v) Na_2CO_3 in 0.1 M NaOH was added and the

reaction was allowed to continue for 10 min at room temperature followed by addition of 0.5 ml of Folin-Ciocalteu's reagent (1: 2 dilution). After 30 min, absorbance was measured at 660 nm. Casienolytic activity of the gut homogenate was calculated from the standard tyrosine curve. One unit (U) of casienolytic activity is defined as nmole equivalent of tyrosine formed per minute.

3.2.11.3 Assay of lipase activity

Lipase activity was assayed following the method of Winkler and Stuckman (1979). Ten milliliters of isopropanol containing 30 mg of *p* – nitrophenylpalmitate was mixed with 90 ml of 0.05 M Sörensen phosphate buffer, pH 8.0, containing 207 mg of sodium deoxycholate and 100 mg of gum arabic. A 2.4 – ml amount of this freshly prepared substrate solution was prewarmed at 37 ° C and then mixed with 0.1 ml of homogenate. After 15 min of incubation at 37 ° C, the OD_{410} was measured against an enzyme-free control. One enzyme unit is defined as 1 nmol of *p* – nitrophenol enzymatically released from the substrate ml^{-1} min^{-1} . Under the conditions described the extinction coefficient of *p* – nitrophenol is $\epsilon_{410} = 15{,}000\ cm^2\ mg^{-1}$.

3.2.12 Influence of diets in water quality

Water quality parameters were estimated as per standard method (APHA, 1989). The water temperature was recorded everyday and it ranged from 27°C to 30°C.

a) pH

10 ml of the sample was placed in a pH comparator tube and 0.2 ml of universal indicator was added, and shaked gently and the colour was matched against standard colour disc for that indicator in a pH comparator.

b) Dissolved oxygen

The water samples were collected in 100 ml glass stoppered bottle and immediately 1 ml of $MnSO_4$ followed by 1 ml of alkaline-iodide-azide reagents was

added. The solution was mixed thoroughly to develop a flocculent precipitate. 2 ml conc. H_2SO_4 was added to dissolve the precipitate and 50 ml of the dissolved solution was titrated with 0.025 N $Na_2S_2O_3$ using starch as indicator to the colourless end point.

Calculation:

Dissolved oxygen (ppm)

(ml of 0.025 N $Na_2S_2O_3$ required for titration x Nx 8x 1000) / ml of sample titrated

N= Normality of $Na_2S_2O_3$

c) Total alkalinity

(1) 20 ml of the sample was taken out in a conical flask and 2-3 drops of phenolphthalein indicator was added.

(2) If pink colour develops it was titrated with standardized 0.02 N H_2SO_4 till the pink colour disappears or pH is 8.3. The volume of H_2SO_4 consumed (A) was noted.

(3) 2-3 drops of methyl orange was added to the same flask and titration was continued till pH comes down to 4.5 or yellow colour changes to orange. The volume was noted.

Calculation:

Total alkalinity (as mg/l $CaCO_3$) ={(A + B) x N x 50, 000} / V

A= ml of standard H_2SO_4 used to titrate to pH 8.3.

B= ml. of standard H_2SO_4 used to titrate from pH 8.3 to pH 4.5.

N= Normality of acid used

V= Volume in ml of sample taken for test

d) Total ammonia

Standard ammonia solution $(NH_4)_2SO_4$ (0.367 g) was dissolved in 100 ml of distilled water and chloroform (1 ml) was added. 25 ml of this solution was diluted to

250 ml to get 10 ppm NH_4 solution. 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm and 1 ppm of solution was prepared by taking 1, 2, 3, 4, 5 ml of the solution and diluted to 50 ml. A reference blank was also prepared. The colour was developed by following the procedure given below. A standard curve was plotted after measuring the developed colour.

To the sample (50 ml), phenol solution (2 ml), sodium nitroprusside (2 ml) and oxidising reagent (5 ml) are added and allowed to stand for 1 h. The colour developed and was measured at 640 nm against a reference blank.

e) Nitrite

1. If sample contains suspended solids, it was filtered through a 0.45μm pore diameter membrane filter.
2. 50 ml clear sample was neutralized to pH 7 or to a portion diluted to 50 ml, 1 ml sulfanilamide solution was added and the reagent was allowed to react for 2-8 minutes.
3. 1 ml NED-dihydrochloirde solution was added and mixed immediately. Absorbance was measured after 10 minutes but before 2 hrs at 543 nm.
4. Blank was prepared in the same way.

f) Nitrates

To the sample (50 ml), buffer reagent (2 ml) and reducing agent (1 ml) were added and kept in a dark place for 20 hours and acetone (2 ml) and sulphanilimide (1 ml) were added followed by NNED (1 ml) and mixed thoroughly. The colour developed after 2 h was measured at 543 nm against a reference blank.

3.2.13 Statistical analysis

Data are presented as means ± SD. The effects of dietary treatments on the measured response variables were studied with the help of one-way analysis of

variance (ANOVA) by using the software Systat-10. A probability level of $p<0.05$ was considered statistically significant.

Chapter IV
Results

CHAPTER IV

Results

4.1 A survey of aquatic weeds of Assam used as supplementary fish diet

Present survey was carried out in Assam to unveil the hidden resources that could possibly be explored for the development of fish feeds. The state of Assam has been selected as major area for the present research due to the following reasons:

1. This region is exceptionally rich in both biodiversity and ethno cultural heritage where maximum probability of finding botanical resources based on cultural knowledge have been predicted whereas the literature review have revealed that the area is least explored in the thrust area of aquatic weed research.

2. The ethnofisheries knowledge the rural fishermen were found to be widely prevalent within their respective locality. It is due to the fact that their major occupation tends to be fishing and wet rice cultivation. However, it is ironical that only few of their vital techniques have been recorded so far in modern research literatures. The fish pond management systems and fish feeds used among them using wild aquatic weeds are still less well known to the scientific community and concerted effort to know such a vital knowledge system in scientific line have not properly been made so far in commercial and industrial point of view.

More particularly, in the area of aquatic weed based research, no such literatures are available. Therefore, ethnobiological survey was randomly done in both plain and hilly region of Assam to explore the weeds used among the rural fishermen as fish feed, by following Rao and Jain (1977) field method. Comparative study on plant used by both hill and plain region were assessed through semi – structure questionnaire method. The photographs were collected on the spot for the aquatic weeds used as fish feeds and frequency of using

single plants by different fishermen were noted and critically screened out after field survey.

In all, 50 species (Table 4.1) were collected from different localities of Assam and all the species are used as fish feeds; 19 species are used as vegetable and food sources; 7 species are used as fodder crop sources; 4 species are used as green manure; 1 species is used as herbicide; 26 medicinal species are used to cure different ailments.

These plants have been reported by the different fishing community of Assam and other parts of Northeast India, but most of the species used are of less frequent. Only few species such as *Salvinia cuculata, Ipomoea reptans, Trapa natans* and *Lemna minor* were found to be used in frequent occasions as fish feed in Assam and many other parts of NE India whom the fishermen consider as less toxic, naturally available and sustainable. Furthermore, few species such as *S. cuculata* and *L. minor* are encouraged to grow naturally in ponds to serve the nutritional needs of the fishes. But *I. reptans* and *T. natans* are harvested in wild and processed for fish feeds using the traditional fish feed processing techniques. The harvesting and processing technique involves collection, sundry, and subsequently pounded to powder and thrown to fishponds.

It is also revealed that majority of the plants used as fish feeds are reported from the tribes of Assam such as Bodo, Kachari, Deori, Mishing etc. and the other states of Northeastern states of India hardly use these plants as fish feed as they rely on natural water sources such as rivers, streams and natural ponds as fishery sources. The artificial pond management system has not been known prior to 1950 or 1960. With governments initiative they have recently been introduced with such activities.

Therefore, considering the above-mentioned socio-cultural and other backgrounds, the present work has concentrated on four aquatic weeds to know their biochemical and nutritional potential. It was envisaged that developing fish feeds from these naturally abundant aquatic weeds would lead to the industrial processing for commercial use in large sector.

Table 4.1. Enumeration of 50 aquatic plants of Assam in alphabetical order with botanical & vernacular name, locality and ecological status:

SN	Botanical Name	Vernacular Name	Family	Ecological Status	Part used	Source of data
1	*Acanthus illicifolius* L.	Hargoza	Acanthaceae	AqH/T/ST/C/E	Whole plant	Lakhimpur, Sonitpur, Nowgoan districts.
2	*Acorus calamus* L.	Buch	Araceae	AqH/T/ST/C/E	-do-	Lakhimpur, Sonitpur, Nowgoan districts.
3	*Alocasia macrorhiza* Schott.	Pani Kochu	Araceae	MH/T/ST/Tm/E	-do-	Lakhimpur, Sonitpur, Nowgoan districts.
4	*Alternanthera philoxeroides* (Mart.) Griseb.	Jalsakhi	Amaranthaceae	EH/T/ST/C	-do-	All the districts.
5	*Alternanthera sessilis* (L.) R. Brown ex ADC	Sanchi	Amaranthaceae	EH/T/ST/Tm/C	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Sibsagar, Kamrup, Nalbari, Goalpara, Morigoan, districts.
6	*Aponogeton appendiculatus* H.Bruggen	Ghechu	Aponogetonaceae	AqH/T/ST/C/Sub	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Sibsagar, Kamrup, Nalbari, Goalpara, Morigoan, districts.
7	*Aponogeton undulatus* Roxb.	Gechuhua	Aponogetonaceae	AqH/T/ST/C/F	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Karbianglong districts.
8	*Azolla pinnata* R. Brown	Kutipana	Azollaceae	AqH/T/ST/C/F	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Cachar districts.
9	*Bacopa monnieri* (L.) Pennel	Brahmi	Scrophulariaceae	AqH/T/ST/C/E	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Cachar districts.
10	*Blyxa aubertii* L.C.Richard	Jhanji	Hydrocharitaceae	AqH/T/ST/C/Sub	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Cachar districts.
11	*Burmannia caelestis* D.Don	Prajapati	Burmanniaceae	AqH/T/ST/C/E	-do-	Sonitpur, Jorhat, Nowgoan, Cachar districts.
12	*Ceratophyllum demersum* L.	Bottle brush	Ceratophyllaceae	AqH/T/ST/C/Sub	Leaves	All the districts.
13	*Caldesia parnassifolia* (Bassi ex L.)Parl.	Pani hak	Alismataceae	AqH/T/ST/C/F	-do-	All the districts.
14	*Commelina benghalensis* L.	Anchara	Commelinaceae	Aq/T/ST/C/E	Stem	All the districts.
15	*Eclipta alba* (L.) Hasska.	Kesuti	Asteraceae	AqH/T/ST/C/E	-do-	Sonitpur, Jorhat, Nowgoan, Cachar, Nalbari, Goalpara districts.
16	*Eichornia crassipes* (Mart.) Solm.	Kochupan	Pontederiaceae	AqH/T/ST/C/F	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Cachar districts.
17	*Euryale ferox* Salisb.	Katapadma	Nympaeaceae	AqH/T/ST/C/F	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Cachar districts.
18	*Fimbristylis bisumbellata* (Forsk.) Bubani	Joina	Cyperaceae	AqH/T/ST/C/E	-do-	Sonitpur, Jorhat, Nowgoan, Cachar, Nalbari districts.

SN	Botanical Name	Vernacular Name	Family	Ecological Status	Part used	Source of data
19	*Fuirena ciliaris* (L.) Roxb.	Bancola	Cyperaceae	AqH/T/ST/C/E	-do-	Sonitpur, Jorhat, Nowgoan, Cachar, Nalbari districts.
20	*Hydrilla verticellata* (L.) Royle	Ruisag	Hydrocharitaceae	AqH/T/ST/C/Sub	-do-	Sonitpur, Jorhat, Nowgoan, Cachar, Nalbari districts.
21	*Hydrophilla polysperma* (L.f.) Blume	Kulekara	Acanthaceae	AqH/T/ST/C/E	-do-	All the districts.
22	*Ipomoea reptans* Forssk.	Kalmi Hak	Convolvulaceae	Aq.CrH/T/ST/C/F	-do-	Sonitpur, Jorhat, Nowgoan, Cachar, Nalbari districts.
23	*Ipomoea fistulosa* Mart. ex Choisy	Dol Kalmi	Convolvulaceae	AqH/T/ST/C/E	Whole plant	Sonitpur, Jorhat, Nowgoan, Cachar, Nalbari districts.
24	*Lemna minor* L.	Kudipana,	Lemnaceae	AqH/T/ST/C/FF	-do-	Sonitpur, Jorhat, Nowgoan, Cachar, Nalbari districts.
25	*Limnophila indica* (L.) Buce	Korpur	Scrophulariaceae	AqH/T/ST/C/Sub	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Cachar districts.
26	*Lindernia antipoda* (L.) Alsto	Pimpernel	Scrophulariaceae	AqH/T/ST/C/E	-do-	All the districts.
27	*Ludwigia adscendens* L. ex H. Hara	Keshru bon	Onagraceae	AqH/T/ST/C/E	-do-	All the districts.
28	*Marsilea minuta* L.	Sushina	Marsileaceae	AqH/T/ST/C/FF	-do-	All the districts.
29	*Marsilea minuta* L.	Sashna bon	Marsileaceae	AqH/T/ST/C/F	-do-	All the districts.
30	*Monocharia hastaba* (L) Solms Laubach	Kechur	Pontederiaceae	AqH/T/ST/Tm/C/E	-do-	All the districts.
31	*Monochoria vaginalis* (N.L. Burman) Kunth	Nukha	Pontederiaceae	AqH/T/ST/Tm/E	-do-	Sonitpur, Jorhat, Nowgoan, Cachar, Nalbari districts.
32	*Myriophyllum tuberculatum* Roxb.	Janji	Halograceae	AqH/T/ST/Sub	-do-	Sonitpur, Jorhat, Nowgoan, Cachar, Nalbari districts.
33	*Nelumbo nucifera* Gaertn	Kamal	Nelumbonaceae	AqH/T/ST/C/F	-do-	All the districts.
34	*Neptunia oleracea* Loureiro	Pani Najaka	Fabaceae	AqSr/T/ST/C/F	-do-	All the districts.
35	*Nymphaea nouchali* W.L.Burman	Sundi	Nymphaeaceae	AqH/T/ST/C/F	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Cachar districts.
36	*Nymphaea pubescens* Willd.	Shaluk	Nymphaeaceae	AqH/T/ST/C/F	-do-	All the districts.
37	*Nymphiodes hydrophylla* (Lour.) Kuntz	Suanjuka	Nymphaeaceae	Aqh/T/ST/L/F	Leaves	All the districts.
38	*Oenanthe javanica* (Blume) ADC	Suda hak	Apiaceae	AqH/T/ST/C/E	-do-	All the districts.
39	*Ottelia alismoides* (L) Persoon	Panikala	Hydrocharitaceae	AqH/T/ST/C/E	-do-	All the districts.
40	*Pistia stratioides* L.	Matardalpana	Lemnaceae	AqH/T/ST/Tm/C/F	-do-	All the districts.

41	*Polygonum barbatum* L.	Jola bon	Polygonaceae	AqH/T/ST/C/E	-do-	All the districts.
SN	**Botanical Name**	**Vernacular Name**	**Family**	**Ecological Status**	**Part used**	**Source of data**
42	*Potamogeton crispus* L.	Panika	Potamogetonaceae	AqH/T/ST/C/Sub	-do-	All the districts.
43	*Potemogeton nodosus* Poiret	Pondweed	Potemogetonaceae	AqH/T/ST/C/Sub	Whole plant	All the districts.
44	*Rotala rotundifolia* (Ham. ex Roxb.) Kochne	Sunkar hak	Lythraceae	AqH/T/ST/C/E	-do-	All the districts.
45	*Rumex maritima* L.	Nuna Janj	Polygonaceae	AqH/T/ST/C/E	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Cachar districts.
46	*Salvinia cucullata* Roxb.	Water fern	Salviniaceae	AqH/T/ST/Tm/C/F	-do-	Lakhimpur, Sonitpur, Jorhat, Nowgoan, Cachar districts.
47	*Trapa natans* L. Var. *bispinosa* (Roxb.) Mak.	Panipal	Trapaceae	AqH/T/ST/Tm/C/E	-do-	All the districts.
48	*Utricularia aurea* Lour.	Pata jhanji	Lentibulariaceae	AqH/T/ST/C/E	-do-	All the districts.
49	*Utricularia stellaris* L.f.	Jhanji	Lentibulariaceae	AqH/T/ST/C/E	-do-	All the districts.
50	*Veronica anagalis-aquatica* L.	Speed well	Scrophulariaceae	AqH/T/ST/C/E	-do-	All the districts.

Legend used in Table: ES = ecological Status; T = tropical; ST = subtropical; Tm = temperate; Tr = tree; H = herb; Sr = shrubs; Eh = emergent herb; AqH = aquatic herbs, S = submerged; F = floating; FrL = free floating; r = rooted; C = common; R = rare

PHOTOGRAPHIC PLATES OF AQUATIC WEEDS OF ASSAM

Plate No. 01: ***Alternanthera philoxeroides*** **(Mart) Grisebach; 02:** ***Marsilea minuta*** **L.; 03:** ***Acorus calamus*** **L.; 04:** ***Hygrophila polysperma*** **(L.f.) Blume; 05:** ***Nymphaea pubescens*** **Willd.; 06:** ***Eclipta alba*** **(L.) Hasska.**

Plate No. 07 & 07A: ***Ipomoea fistulosa*** **Mart. ex Choisy; 08:** ***Ipomoea reptans*** **L. (*I. aquatica* Forskaal); 09:** ***Ludwigia adscendens*** **L. ex H. Hara; 10:** ***Nymphaea nouchali*** **N.L Burman; 11:** ***Trapa natans*** **L. var. *bispinosa* (Roxb.) Makino.**

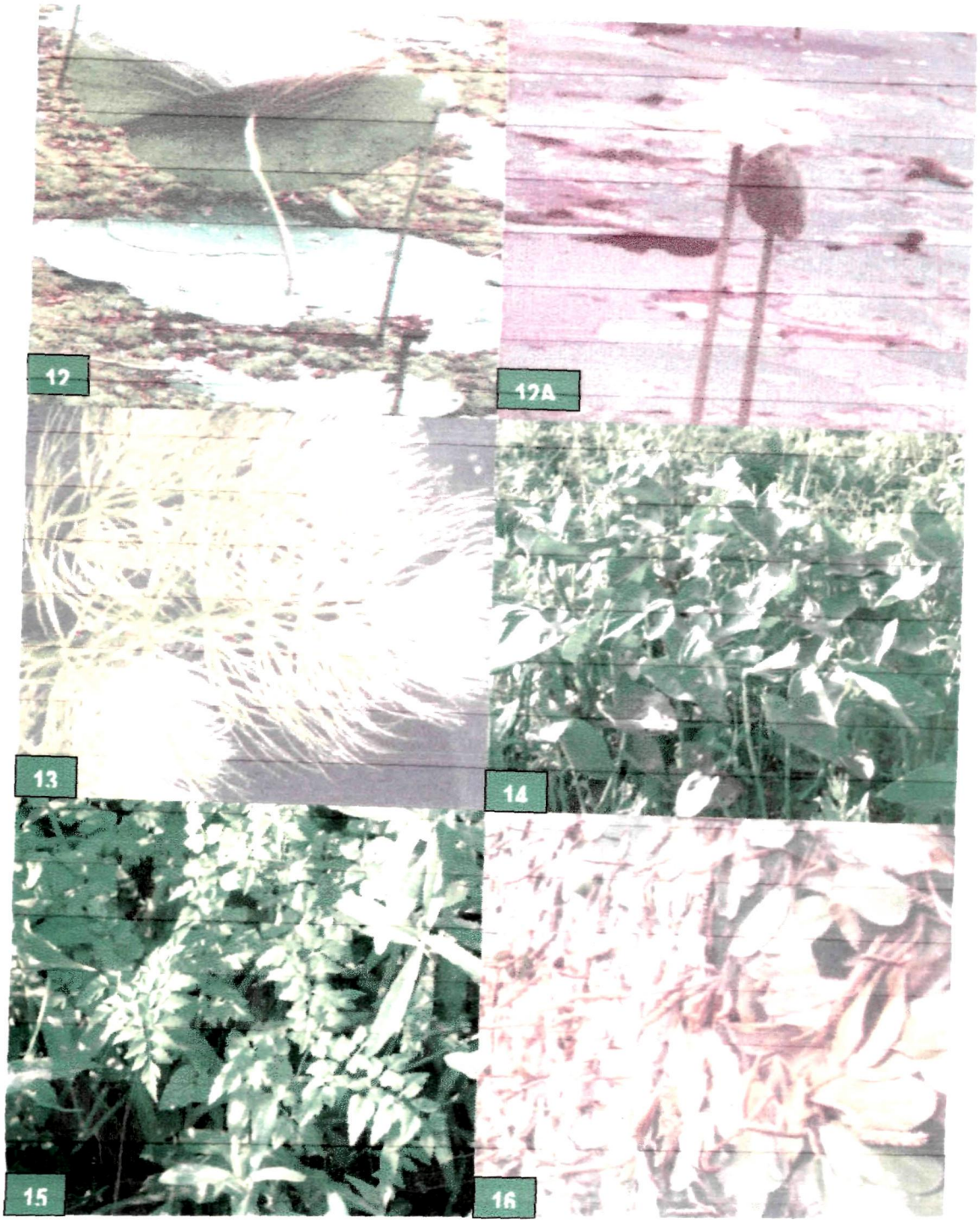


Plate No. 12 & 12A: *Nelumbo nucifera* Gaerten; 13: *Ceratophyllum demersum* L.; 14: *Monochoria vaginalis* (N.L. Burman)Kunth; 15: *Oenanthe javanica* (Blume) ADC; 16: *Potamogeton nodosus* Poiret

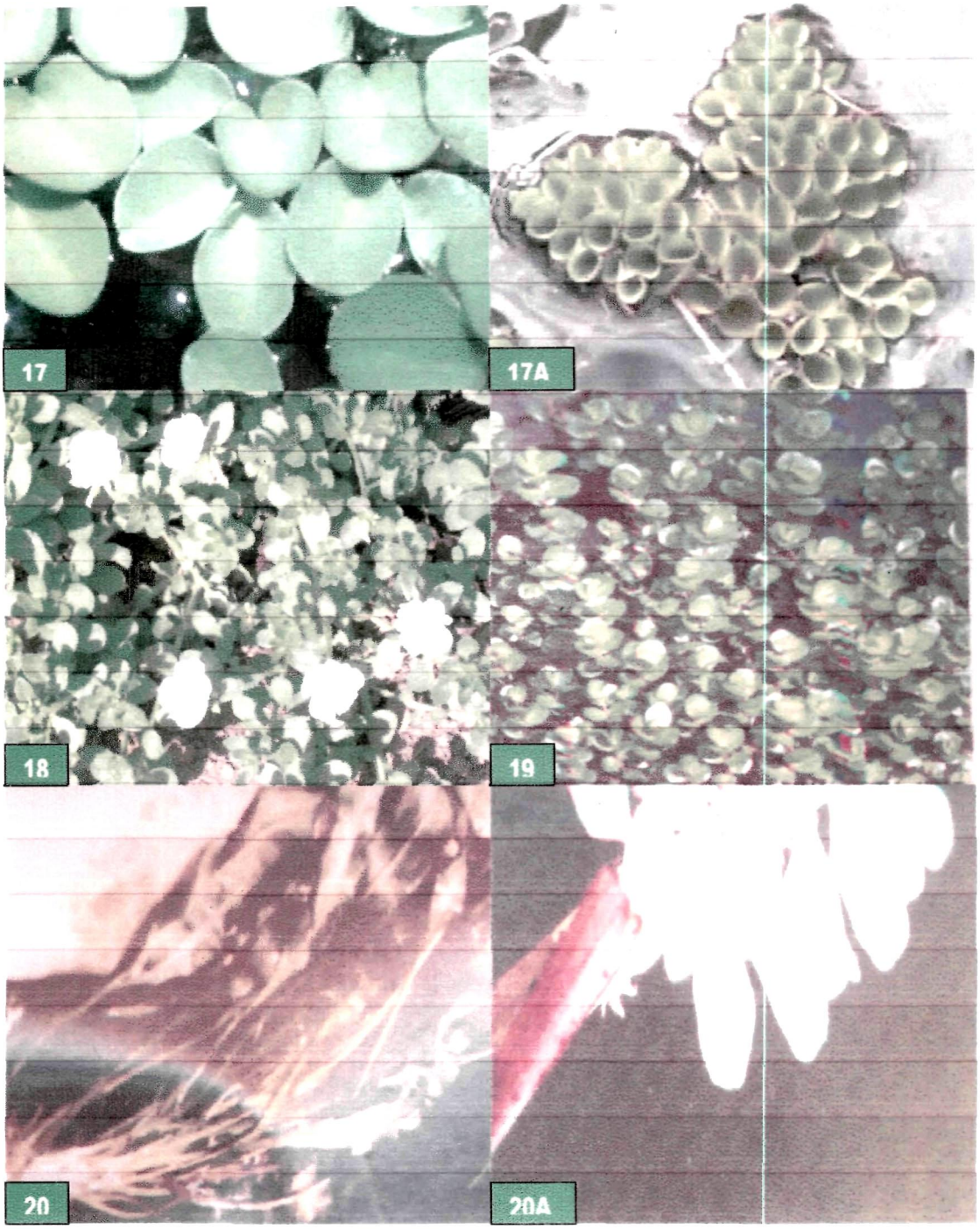


Plate No. 17 & 17A: ***Salvinia cuculata*** **Roxb.; 18:** ***Bacopa monieri*** **(L.)Pennel; 19:** ***Rotala rotundifolia*** **(Ham. ex Roxb.) Koehne; 20 & 20A:** ***Aponogeton appendiculatus*** **H. Bruggen.**

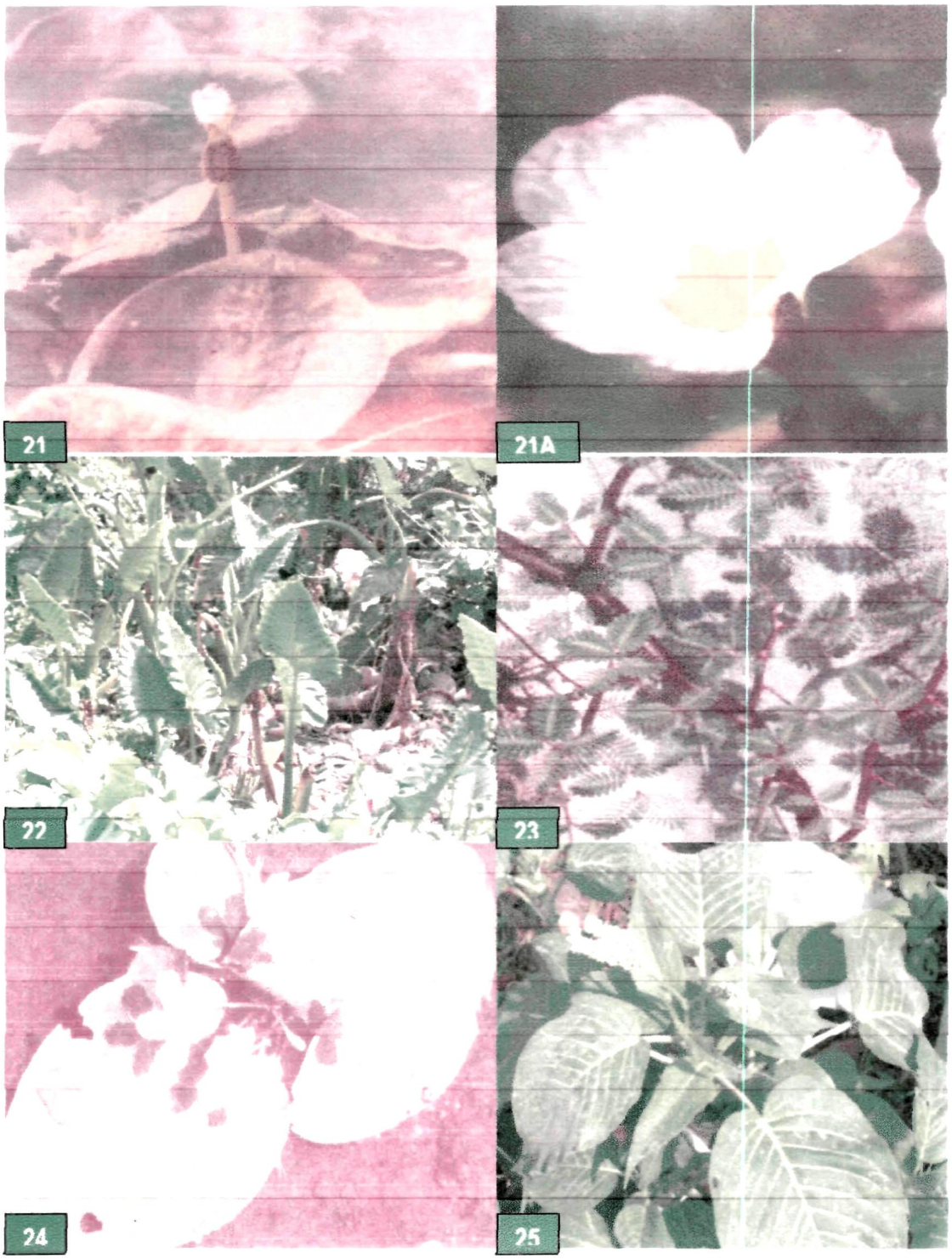


Plate No. 21 & 21A: ***Ottelia alismoides*** **(L.)Persoon; 22:** ***Alocasia macrorhiza*** **Schott.; 23:** ***Neptunia oleracea*** **Loureiro; 24:** ***Nymphoides hydrophylla*** **(Lour.) Kuntze; 25:** ***Polygonum barbatum*** **L.**

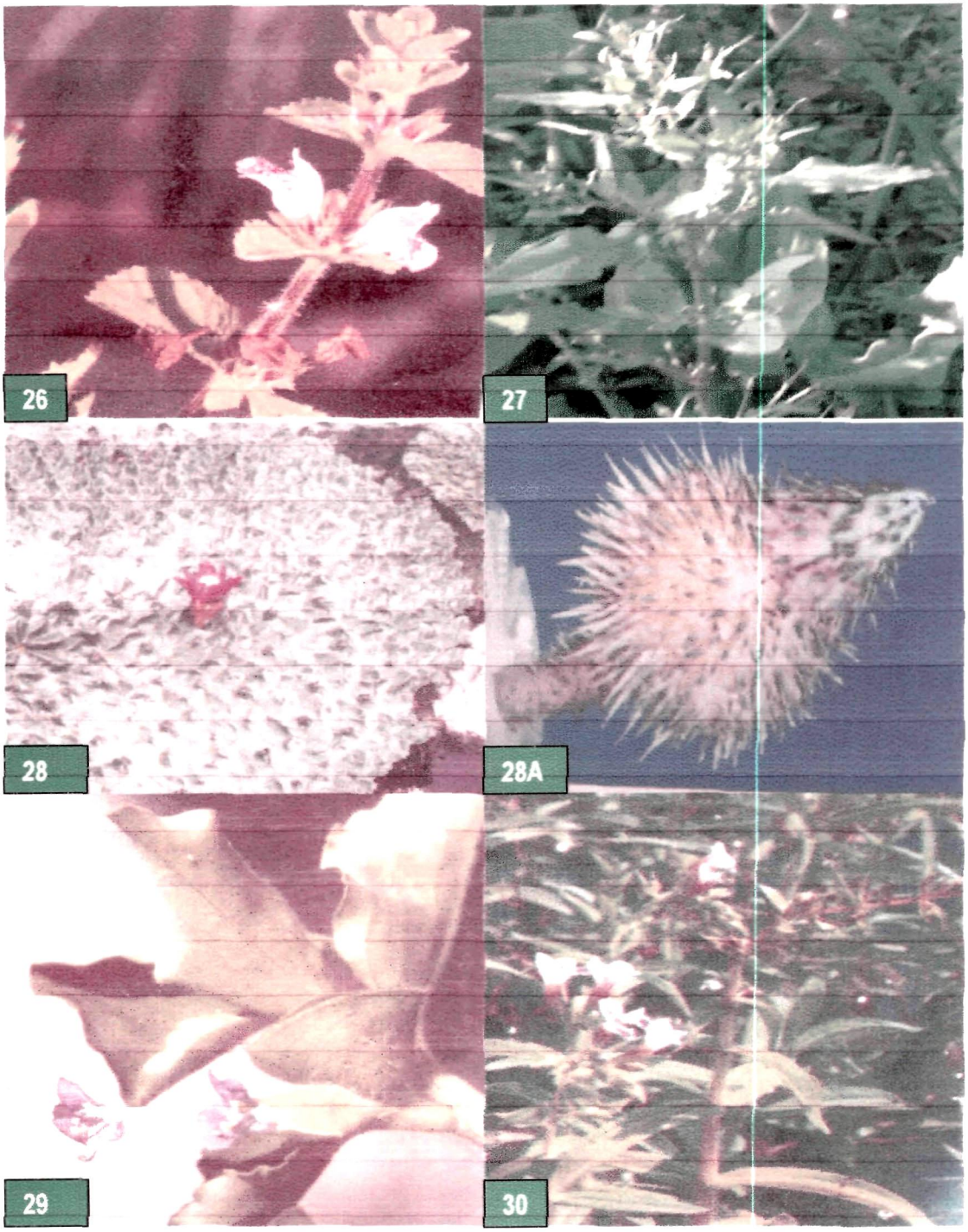


Plate No. 26: *Hygrophila defformis* (L.f.) Blume; 27: *Rumex maritima* L.; 28 & 28A: *Euryale ferox* Salisb & its fruiting body; 29: *Commelina benghalensis* L.; 30: *Limnophila indica* (L.) Buce.

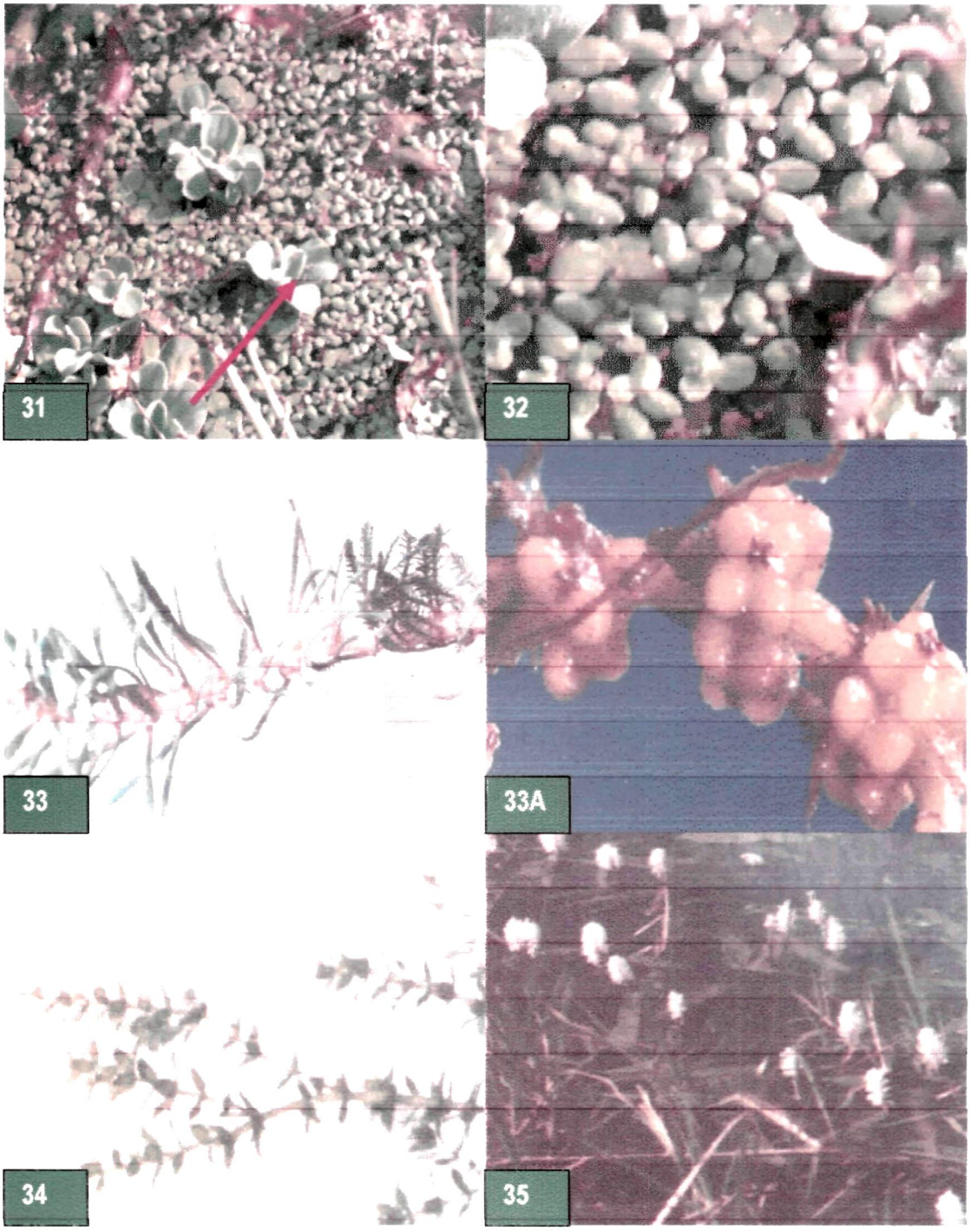


Plate No.: 31: *Pistia stratioites* L. with *Lemna minor* L.; 32: Close up view of *Lemna minor* L.; 33: *Myriophyllum tuberculatum* Roxb.; 33A: Close up view of *M. tuberculatum* fruits; 34: *Hydrilla verticellata* (L.) Royle; 35: *Aponogeton undulatus* Roxb. in flowering stage.

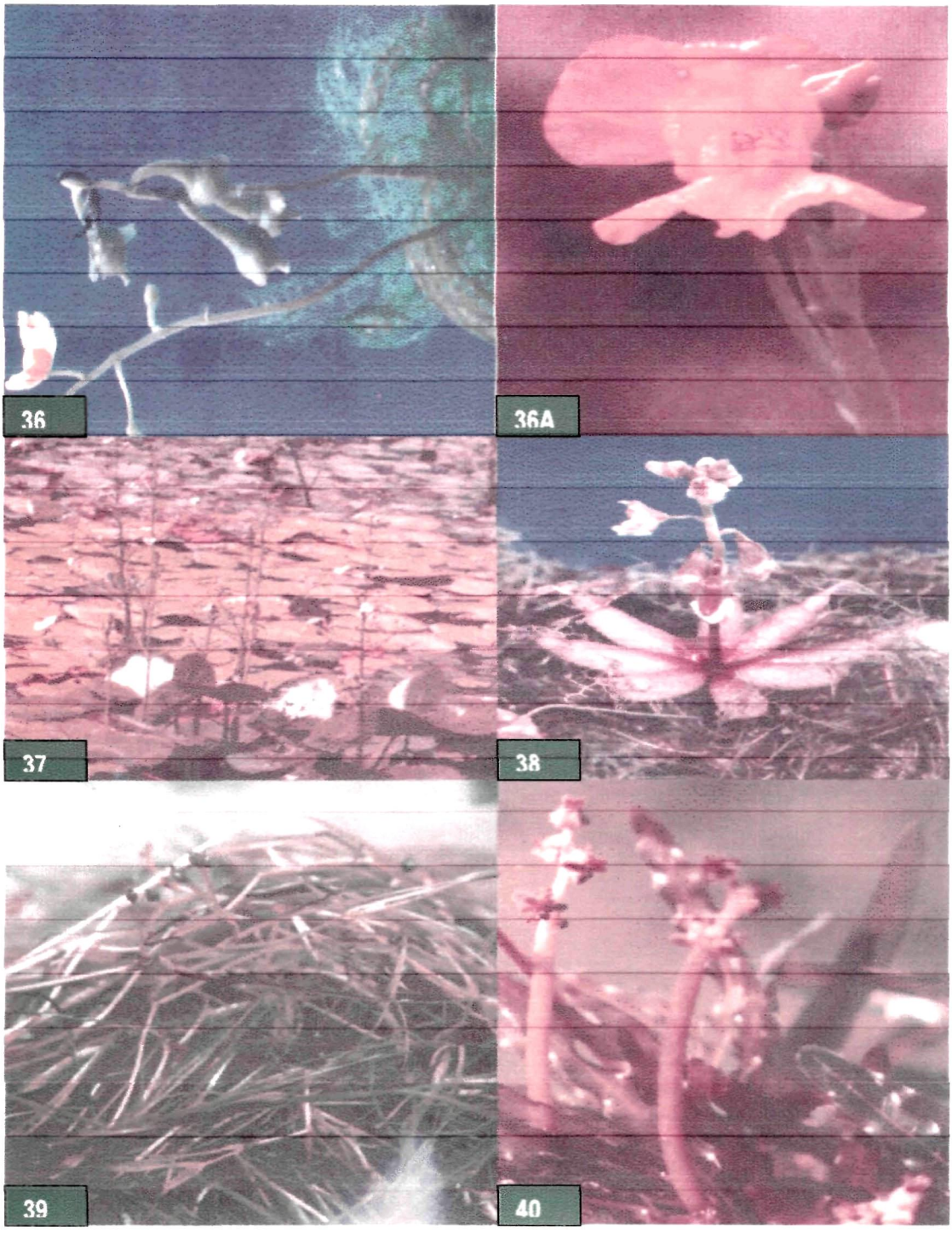


Plate No. 36 & 36A: *Utricularia aurea* Lour. & its yellow flower; 37: *Caldesia parnassifolia* (Bassi ex L.) Parl. var. *major* (Mitcheli) Buchenau; 38. *Utricularia stellaris* L.f.; 39: *Ruppia maritima* L.; 40: *Potamogeton crispus* L.

4.2 Analysis of feed composition of four aquatic weeds with an aim to develop fish feed

The proximate compositions of four most commonly used aquatic weeds as fish feed by local people of Assam , viz. *S. cuculata, I. reptans, T natans* and *L. minor* on a fresh weight basis are presented in Table 4.2. Moisture, organic matter and lipid contents were nearly identical in all the plants ($p>0.05$). Among these four plants, *I reptans* possessed the highest amount of crude protein (32.2 %) followed by *L. minor* (28.0 %), whereas *S. cuculata* and *T. natans* were characterized to possess comparatively less amount of crude protein (11% to 11.4 %). The ash content in these plants

Table 4.2. Proximate composition of four aquatic weeds from Assam (N. E. India) in dry matter basis (%). Each value represents mean ± SD of three determinations. Values in the same row within each experiment followed by different superscripts are significantly different ($P<0.05$)

Component	*S.cuculata*	*I.reptans*	*T.natans*	*L.minor*
1.Moisture	9.8 ± 1.1^{a}	10.8 ± 1.5^{a}	7.6 ± 0.7^{a}	8.8 ± 1.2^{a}
2.Organic matter	69.0 ± 0.1^{a}	70.0 ± 0.2^{a}	87.0 ± 1.1^{a}	75.0 ± 0.7^{a}
3.Crude protein (Nx6.25)	11.0 ± 0.1^{a}	32.2 ± 1.0^{b}	11.4 ± 0.2^{a}	28.0 ± 1.7^{b}
4.Crude lipid (ether extract)	7.0 ± 0.1^{a}	6.0 ± 0.8^{a}	8.0 ± 0.6^{a}	5.0 ± 0.1^{a}
5.Ash	31.2 ± 1.3^{a}	30.0 ± 1.2^{a}	13.3 ± 0.7^{b}	25.0 ± 1.6^{a}
6.Total Carbohydrate (NFE+ Crude fibre)	50.8 ± 1.1^{a}	31.8 ± 1.5^{b}	67.3 ± 0.9^{c}	42.0 ± 1.9^{d}
7.Crude fibre	20.0 ± 1.3^{a}	10.0 ± 1.2^{b}	4.2 ± 0.6^{c}	10.0 ± 0.5^{b}

ranged from 13.3 % to 31.2 %; the highest amount being displayed by *S. cuculata* (31.2 %), followed by *I.reptans* and *L.minor* (30.0 % and 25.0 %) respectively, whereas the lowest ash content was possessed by *T. natans* (13.3 %). Total carbohydrate (including starch) content of *T. natans* (67.3 %) was significantly higher ($p<0.001$) as compared to the three other plants under study, whereas *S. cuculata* possessed highest amount of crude fiber (20 %, w/w) followed by *I. reptans* and *L. minor* (10 %, w/w), whereas lowest in *T. natans* (4.2 %, w/w).

As shown in Table 4.3, all four plants exhibited remarkable similarity in possessing identical gross energy content (337.9 kcal/100g to 358.1 kcal/100g). However, result showed that protein / energy (P/E) value was highest in *I. reptans* (95.3 ± 1.0 mg protein/kcal) followed by *L. minor* (78.4 ± 1.5 mg protein/kcal), *T. natans* (32.7 ± 1.2 mg protein/kcal) and least P/E value was shown by *S. cuculata* (30.7 ± 0.9 mg protein/kcal).

Vitamin contents of these aquatic weeds are shown in Table 4.3. Vitamin E (α-tocopherol) content of *T. natans* (61.3 mg/100g) was significantly higher as compared to other three plants ($p<0.05$), and the remaining three aquatic weeds contained identical amount of vitamin E, whereas *I. reptans* was characterized as possessing highest amount of ascorbic acid (vitamin C)(4.0 mg /100g) and carotenoid (0.25mg/100g) (Table 4.3). The lowest levels of ascorbic acid and carotenoid contents were found in *S. cuculata* and *L. minor* respectively. The mineral compositions of the four aquatic weeds are presented in Table 4.3. A significant variation in metal contents was noticed among these plants, which may be attributed to differences in their genus and species level. Potassium and magnesium were the most abundant of the elements considered, followed by sodium and copper. Among these plants, *I. reptans* was shown to possess the highest amounts of K (41.4mg %), Mg (31.0 mg %) and Zn (1.7 mg %), whereas *S. cuculata* and *L. minor* contained the highest amount of Na (6.3 mg %) and Cu (0.14 mg %) respectively. Interestingly, the Ca content was identical in all the four weeds; but phosphorous level was highest in *I. reptans*.

Table 4.3. Energy values, vitamin contents and mineral ion concentration in the leaves of four aquatic weeds. Values are mean of triplicate determination. Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Properties	*S.cuculata*	*I.reptans*	*T.natans*	*L.minor*
Energy values				
Gross energy (kcal/100g)	358.1 ± 2.1[a]	337.9 ± 1.2[a]	347.2 ± 1.4[a]	357.8 ± 1.6[a]
P/E (mg protein/kcal)	30.7 ± 0.9[a]	95.3 ± 1.0[b]	32.7 ± 1.2[a]	78.4 ± 1.5[b]
Vitamins content				
VitaminE (mg/100g)	28.8 ± 0.5[a]	28.5 ± 0.4[a]	61.3 ± 0.7[b]	26.6 ± 0.3[a]
Vitamin C (mg/100g)	3.0 ± 0.1[a]	4.0 ± 0.2[a]	3.6 ± 0.1[a]	3.8 ± 0.2[a]
Carotenoid (mg/100g)	0.2 ± 0.04[a]	0.3 ± 0.01[a]	0.2 ± 0.02[a]	0.1± 0.01[a]
Mineral ions concentration				
Zn (mg%)	0.7 ± 0.02[a]	1.7± 0.01[b]	1.4± 0.08[b]	1.0 ± 0.07[a]
Mg (mg%)	17.8 ± 0.1[a]	31.0± 0.02[b]	25.1 ± 0.06[c]	20.3 ± 0.03[d]
Cu (mg%)	0.1± 0.01[a]	0.1± 0.08[a]	0.1± 0.005[a]	0.1± 0.01[a]
Ca(ppm)	2 .0± 0.1[a]	2.0± 0.004[a]	2.0± 0.04[a]	2.0± 0.02[a]
Na (mg%)	6.3 ± 1.0[a]	5.0± 1.0[a]	5.0± 0.2[a]	3.0± 0.2[b]
K (mg%)	17.5 ± 0.5[a]	41.4± 0.2[b]	27.7± 1.0[c]	20.0± 0.4[d]
P (g/kg)	1.0 ± 0.1[a]	1.5± 0.5[a]	0.9± 0.1[a]	1.0± 0.4[a]

Antinutrient contents of the four aquatic weeds are summarized in Table 4.4. Highest trypsin inhibitory activity was detected in *T. natans* (1.53%) and in *L. minor*, whereas lowest activity was detected in *S. cuculata* (1.13%). The latter plant was characterized to possess the highest amount of tannins (0.93%) and phytate (0.005%) compared to the three other aquatic weeds. Calcium oxalate concentration was highest in *L. minor* (3.5%) followed by *T. natans* (0.9%). Interestingly, among these aquatic weeds under study, *I. reptans* possessed the least amount of tested antinutrients.

Table 4.4. Concentration of some antinutritional factors in the leaves of four aquatic weeds. Values are mean ± S.D. of triplicate determinations. Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

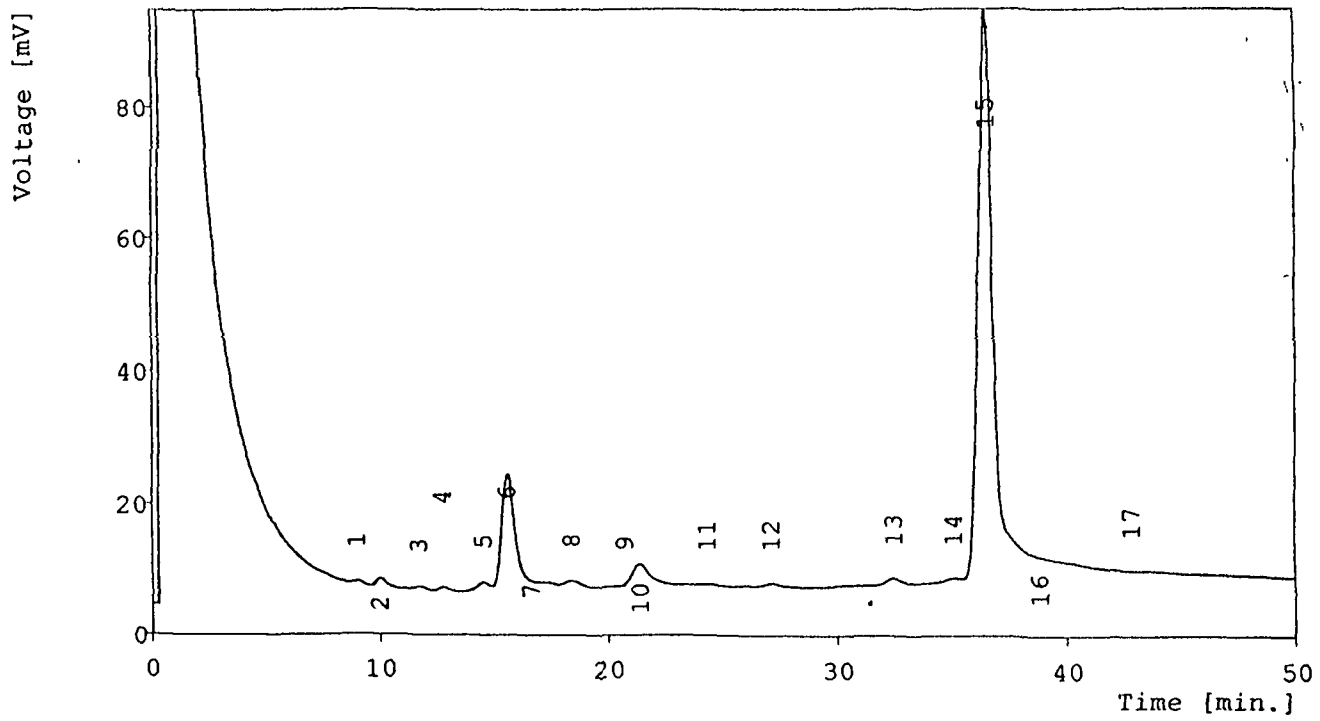
Component (g%)	Concentration in plants			
	S.cuculata	*I.reptans*	*T.natans*	*L.minor*
Trypsin Inhibitor	1.13 ± 0.1 [a]	1.34 ± 0.9 [a]	1.53 ± 0.2 [b]	1.47 ± 0.5 [a]
Calcium Oxalate	0.7 ± 0.01 [a]	0.6 ± 0.001 [a]	0.9 ± 0.2 [a]	3.5± 0.7 [b]
Tannin	0.9 ± 0.1 [a]	0.3 ± 0.01 [b]	0.5 ± 0.2 [b]	0.9± 0.3 [a]
Phytate	0.01 ± 0.001 [a]	0.004 ± 0.001 [b]	0.004 ± 0.002 [b]	0.004± 0.003 [b]

4.3 (a) Fatty acid composition of the four aquatic weeds of Assam

The fatty acid profile of the four aquatic plants show that both unsaturated and saturated fatty acids are present in the four plant samples. Gas chromatographic analysis showed the presence of 13-15 peaks, and they are identified as methyl esters of lauric, myristic, palmitic, palmitoleic, stearic, linoleic, linolenic, arachidonic, behenic, dicosadienoic and nervonic acids. Their concentrations were recorded in the range of 0.002 to 2.8g% dry powder of the whole plant. Furthermore, the concentration of unsaturated fatty acids was observed to be higher in *I reptans* when compared with the others. The level of C 24:1 and C 24:6 (Cis – 15 Tetracosenoic and Cis 4, 7, 10, 13, 16, 19- Docosahexanoic acids) was found to be high in the aquatic plant *I. reptans* than that of *S.cuculata, T. natans* and *L. minor.*

Sample ID : *P # 1*
Sample : fame
ISTD Amount : 0
Raw Data : tezuniv1
Primary : tezuniv1
Project : work4

Analyst : *mgp/GC-anc*
Inj. Volume : 1
From : Fri, 22th Dec, 2006 11:59:57
Calibration : (none)
Style : @report



G C fatty acid profile of *S. cuculata*

Sample ID : P # 2U
Sample : fame
ISTD Amount : 0
Raw Data : tezuniv
Primary : tezuniv
Project : work4

Analyst : mgp/GC-anc
Inj. Volume : 1
From : Fri, 22th Dec, 2006 10:44:21
Calibration : (none)
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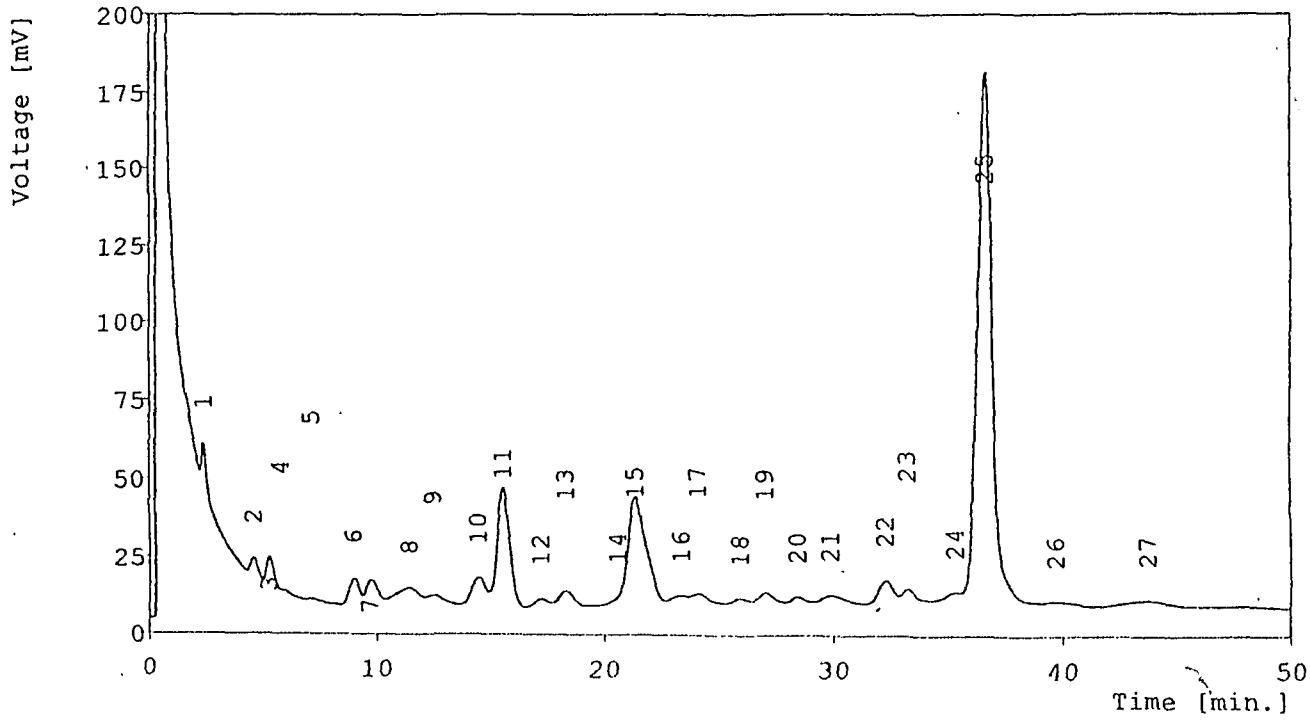

G C fatty acid profile of *I. reptans*

Fri. 22th Dec. 2006 15:16:12 TEZUNIV2

Sample ID : P # 3
Sample : fame
ISTD Amount : 0
Raw Data : tezuniv2
Primary : tezuniv2
Project : work4

Analyst : mgp/GC-anc
Inj. Volume : 1
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Calibration : (none)
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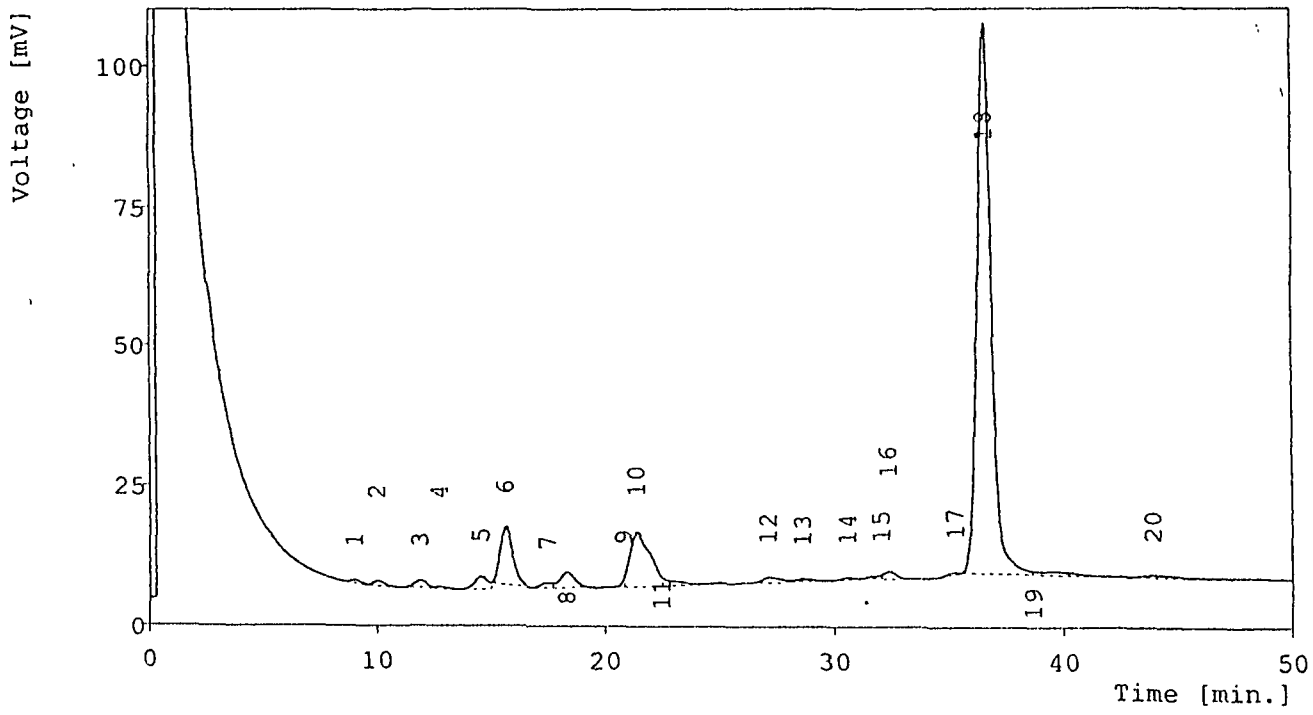
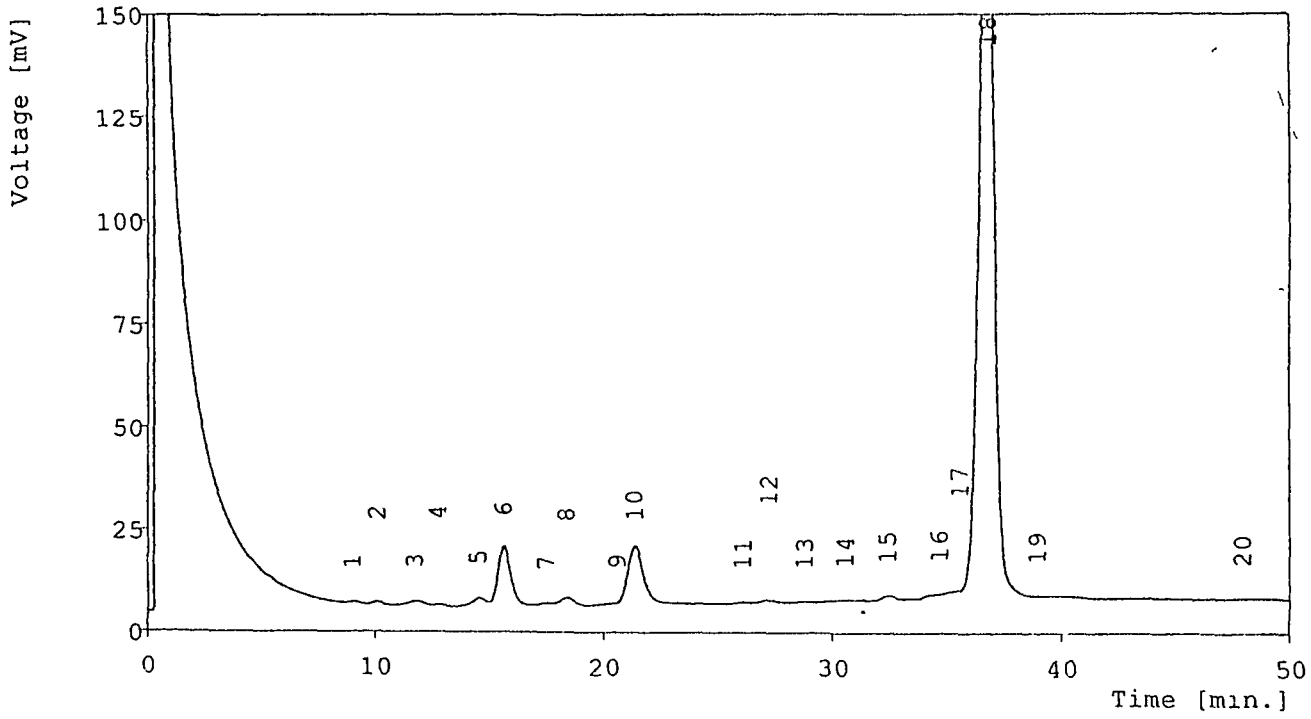


G C fatty acid profile of *T. natans*

Sample ID : P # 4
Sample : fame
ISTD Amount : 0
Raw Data : tezuniv3
Primary : tezuniv3
Project : work4

Analyst : mgp/GC-anc
Inj Volume : 1
From : Fri, 22th Dec, 2006 15:22 45
Calibration : (none)
Style : @report



G C fatty acid profile of *L minor*

Table 4.5. Fatty acid composition of the four aquatic weeds of Assam

Symbol	Common name	Systematic name	*g % of fatty acid*			
			S cuculata	***I. reptans***	***T. natans***	*L minor*
$C_{12\,0}$	Lauric acid	Duodecamoic acid	ND	0.06	ND	ND
$C_{14\,0}$	Myristic acid	Tetradecanoic aid	0.01	0.05	0.007	0.003
$C_{16\,0}$	Palmitic acid	Mexadecanoic acid	0.01	0.1	0.02	0.01
$C_{16\,1}$	Palmitoleic acid	9-Hexadecanoic acid	0.2	0.5	0.1	0.08
$C_{18\,0}$	Stearic acid	Octadecanoic acid	T	0.01	0.003	0.003
$C_{18\,2}$ $C_{18\,3}$	Linoleic acid α-Linolenic acid	9,12-Octadecanoic acid 9,12,15-Octadecatrienoic acid	0.04	0.6	0.2	0.1
$C_{20\,0}$	Arachidic acid	Eicosunoic acid	T	0.04	0.02	0.003
$C_{20\,4}$	Arachidonic acid	5,8,11,14-Eicosatetraenoic acid	0.005	0.02	0.007	0.002
$C_{22\,0}$	Behenic acid	Docasonoic acid	ND	0.1	0.003	T
$C_{22\,2}$ $C_{22\,3}$ $C_{22\,4}$	- - -	Cis-13,16-Dicosadienoic acid Cis-13,16,19-Dicosadienoic acid Cis-7,10,13,16-Dicosatrienoic acid	0.01	0.08	0.005	0.005
$C_{24\,1}$ $C_{24\,6}$	Nervonic acid -	Cis-15 Tetracosenoic acid Cis 4,7,10,13,16,19-Docosahexaenoic acid	1.1	2.8	1.2	1.3

ND = Not detected, T = Trace

4.3 (b) Growth performance and feed utilization by Indian major carp fingerlings (*L. rohita, C. catla and C. mrigala*) fed on aquatic weeds based formulated diets:

4.3.1 Proximate composition of feed

The proximate composition of feed is presented in Table 4.5. The feeds were formulated in such a manner so that the total protein content ranged between 26.0 g % and 28.0 g % on moisture free basis. Statistical analysis showed that except the crude lipid content and P/E value, proximate composition and energy values of these feeds did not differ significantly ($P>0.05$). Feed F_2 (containing *I. reptans*) was found to contain least amount of lipid and displayed highest P/E value as compared to other feeds ($P<0.001$).

4.3.2 Growth performance

It was observed that the carps fingerlings (*L rohita, C. catla* and *C mrigala*) readily accepted all the formulated feeds, as was evident from the voluntary feed intake level as well as survival rate in fish, because these values did not differ significantly among the different groups of carp fingerlings ($P>0.05$). Although these were isoprotein diets, however the best growth performance in terms of feed conversion ratio (FCR), gain in body weight (BWG), protein efficiency ratio (PER), protein retention efficiency (PRE) and specific growth rate (SGR) was demonstrated by *I reptans* based diet (F_2).

4.3.2.1 *L. rohita* fingerlings

The carcass composition of experimental *L rohita* fingerlings before and at the end of 60 days of experiment is shown in Table 4.6. Gross energy content and percent of total organic matter of the fingerlings before and at the end of 60 days feeding trial was found to be the same, irrespective of their diet ($P>0.05$). However, the proportion of crude lipid was significantly higher ($P<0.05$) in those groups of fish fed with the diets F_2, F_3 and F_4 as compared to diets F_1 and control. F_2 demonstrated 13.6 ± 1.0^b %, F_3

illustrated 10.0 ± 0.5^{b} % and F_4 showed 12.1 ± 1.3^{b} % followed by F_1 (6.8 ± 1.0^{a} %) and control (7.1 ± 1.4^{a} %).

Table 4.6 shows the growth responses and associated nutritional indices such as FCR, PER, BWG, DWG, APD etc. of rohu fingerlings fed the five experimental diets (F_1 to F_4 and C) for 60 days. It was observed that fish readily accepted all the formulated feeds, as it was evident from the voluntary feed intake level as well as survival rates in fish, because these values did not differ significantly among the different groups of rohu fingerlings. Although all feeds were formulated to contain nearly identical amount of protein, but the best growth performance in terms of feed conversion ratio (FCR) (1.8 ± 0.3^{a}), gain in body weight (BWG) (33.3 ± 1.4^{b}), protein efficiency ratio (PER) (1.0 ± 0.1^{b}) and specific growth rate (SGR) (1.0 ± 0.7^{b}) was demonstrated by *I. reptans* based diet (F_2). It was followed by the other diets. However, values for other nutritional indices such as ERE was insignificant in post feeding experiment and apparent protein digestibility (APD) was significant in F_2 and F_3 when compared with the other diets (Table 4.6).

Table 4.5. Ingredient proportions and proximate composition (g% DM) of formulated diets for Indian major carp fingerlings. The values for proximate composition represent mean ± S.D. of five determinations.

Components/ Composition	Formulated Diets				
	F1	F2	F3	F4	Control
	Ingredients (g%)				
Mustard oil cake	45	45	50	45	45
Silk worm pupae	10	10	10	10	8
Rice bran	23	33	15	33	45
Salvinia cuculata	20	-	-	-	-
Ipomoea reptans	-	10	-	-	-
Trapa natans	-	-	23	-	-
Lemna minor	-	-	-	10	-
Vitamin mineral premix*	2	2	2	2	2
Chromic oxide	1.0	1.0	1.0	1.0	1.0
	Proximate composition (g %)				
Organic matter	78.9±0.9[a]	81.1±0.8[a]	91.6±0.9[a]	91.1±0.7[a]	97.7±1.1[a]
Crude protein	26.5±1.7[a]	27.5±1.1[a]	27.7±1.4[a]	28.1±1.4[a]	28.2±1.6[a]
Crude lipid (ether extract)	7.8±1.4[a]	7.0±1.5[b]	8.1±2.5[a]	7.9±4.4[a]	8.0±2.6[a]
Ash	10.4±0.8[a]	7.2±1.6[a]	6.5±1.5[a]	8.2±1.3[a]	6.4±1.5[a]
Crude fibre	10.4±0.8[a]	7.4±1.1[a]	7.6±0.9[a]	7.7±1.2[a]	7.5±1.6[a]
Nitrogen free extract	43.2±2.5[a]	39.2±3.4[a]	48.2±4[a]	47.4±2.3[a]	54.0±2.1[a]
Total carbohydrate (NFE + crude fibre)	53.6±1.6[a]	46.6±1.7[a]	55.8±1.9[a]	55.1±1.7[a]	61.5±1.2[a]
	Energy values				
Gross energy (kcal/100g)	398.6±1.8[a]	360.6±1.6[a]	399.4±3.4[a]	399.1±2.8[a]	424.2±3.8[a]
P/E (mg protein/kcal)	66.5±1.8[a]	76.3±1.1[b]	69.4±2.3[a]	70.4±2[a]	66.5±3.1[a]
Chromic oxide (%)	0.92	0 95	0.97	1.0	0.95

*Vitamin premix (mg or IU/g premix): retinol palmitate, 500,000 IU; thiamin, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5; ascorbic acid, 10; cholecalciferol, 50,000 IU; α -tocoferol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; choline chloride, 100; biotin, 0.25

Table 4.6. Growth related performance and feed utilization by *L. rohita* fed different experimental diets (F1 to F4 and control) for 60 days. Values are mean ± S.D. of triplicate groups of fish, with 5 fish/group (n=5). Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Experimental diets Diet Code F1	F2	F3	F4	Control
A) Feed Intake (g/kg average body wt. /body)	0.9±0.1[a]	1.0±0.2[a]	0.9±0.2[a]	0.9±0.3[a]	0.9±0.4[a]
B) Growth performances					
1. DWG[1] (g day^{-1})	0.3±0.1[a]	0.6±0.2[b]	0.2±0.1[a]	0.2±0.09[a]	0.4±0.08[b]
2. BWG[2] (g)	16.4±1.1[a]	33.3±1.4[b]	13.4±1.3[a]	12.0±0.8[a]	21.6±1.9[b]
3. SGR[3] (% per day)	0.6±0.1[a]	1.0±0.7[b]	0.4±0.2[a]	\0.4±0.07[a]	0.7±0.1[a]
4. FCR[4]	3.3±1.0[b]	1.8±0.3[a]	4.2±1.1[b]	4.4±1.1[b]	2.3±1.0[b]
C) Whole body composition					
1. Organic matter	87.4±3.1[a]	87.1±1.5[a]	88.1±1.6[a]	88.0±1.6[a]	87.3±1.7[a]
2. Crude Protein (Nx6.25) (g/100g)	56.1±1.6[a]	64.2±1.7[b]	61.1±1.7[b]	60.2±1.7[a]	59.1±1.6[a]
3. Crude lipid (g/100g)	6.8 ±1.0[a]	13.6 ±1.0[b]	10.0±0.5[b]	12.1±1.3[b]	7.1±1.4[a]
4. Energy (kcal/g)	3.94± 0.6[a]	3.71± 0.5[a]	3.92± 1.0[a]	3.88± 0.4[a]	3.97± 0.7[a]
5. Total Carbohydrate	25.0 ± 8.1[a]	9.4± 2.4[c]	17.1 ± 9.7[b]	16.1± 8.1[b]	21.6± 12.1[b]
D) Nutrient Retention					
1. PER[5]	0.6±0.02[a]	1.0±0.1[b]	0.5±0.1[a]	0.4±0.1[a]	0.8±0.03[b]
2. ER[6]	10.5± 1.2[a]	35.5± 2.0[a]	25.5± 1.8[a]	23.8± 2.4[a]	21.2± 2.3[a]
E) Apparent nutrient digestibility (%)	77.4±4.1[a]	85.5±4.6[b]	87.7±4.9[b]	82.2±3.6[a]	82.3±3.8[a]

[1]DWG= Daily weight gain; [2]BWG=Body weight gain (%); [3]SGR =Specific growth rate; [4]FCR=Feed conversion ratio; [5]PER =Protein efficiency ratio; [6] ER=Energy retention efficiency

4.3.2.2 *C. catla* fingerlings

The carcass composition of experimental *C. catla* fingerlings shows that the whole body composition and energy content of *C. catla* fingerlings before and at the end of feeding trial did not differ significantly ($P>0.05$), irrespective of the supplied feed. However, the proportion of crude lipid was highest in those fish fed the diet F_2 ($P<0.05$), whereas fish on F_1 diet were characterized as possessing lowest percentage of tissue lipid. There was no change in gross energy content in all the groups of fishes ($P>0.05$).

Growth responses and associated nutritional indices such as FCR, PER, BWG, DWG etc. of catla fingerlings fed the five experimental diets (F_1 to F_4 and C) for 60 days was shown in the table. It was observed that fish readily accepted all the formulated diets as it was evident from the average food consumption in fish, because these values did not differ significantly among the different groups of fish. Although all the diets were formulated to contain nearly identical amount of protein, but the best growth performance in terms of feed conversion ratio (FCR), daily weight gain (DWG), gain in body weight (BWG) and apparent protein digestibility (APD) was demonstrated by *I. reptans* based diet (F_2). However, values for other nutritional indices such as PER, SGR and ERE were insignificant in different groups of *C. catla* fish post feeding experiment (Tables 4.7).

Table 4.7. Growth related performance and feed utilization by *C. catla* fed different experimental diets (F1 to F4 and control) for 60 days. Values are mean ± S.D. of triplicate groups of fish, with 5 fish/group (n=5). Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Experimental diets				
	Diet Code				
	F1	F2	F3	F4	Control
A) Feed Intake (g/kg average body wt. /body)	0.6±0.1[a]	1.1±0.2[a]	0.9±0.3[a]	1.0±0.3[a]	0.8±0.2[a]
B) Growth performances					
1. DWG[1] (g day^{-1})	0.1±0.01[a]	0.3±0.007[b]	0.2±0.02[a]	0.3±0.01[b]	0.3±0.03[b]
2. BWG[2] (g)	7.0±1.0[a]	18.2±1.1[b]	10.9±0.9[a]	15.6±0.8[a]	18.1±1.2[b]
3. SGR[3] (% per day)	0.3±0.3[a]	0.7±0.5[a]	0.3±0.2[a]	\0.5±0.1[a]	0.7±0.2[a]
4. FCR[4]	6.4±1.0[a]	2.5±0.6[b]	4.4±1.2[a]	3.3±1.0[b]	3.0±0.8[b]
C) Whole body composition					
1. Organic matter	90.9±4.0[a]	88.9±6.0[a]	89.1±7.2[a]	89.7±5.4[a]	89.5±11.4[a]
2. Crude Protein (Nx6.25) (g/100g)	51.2±4.2[a]	55.1±3.5[a]	52.7±6.5[a]	54.2±8.8[a]	53.1±5.8[a]
3. Crude lipid (g/100g)	3.5±0.9[a]	5.3±0.9[b]	4.5±0.5[a]	4.0±0.5[a]	5.1±0.8[b]
4. Energy (kcal/g)	4.1±1.2[a]	4.0±0.8[a]	4.1±0.9[a]	4.1±1.2[a]	4.1±1.3[a]
5. Total Carbohydrate	36.2±7.4[a]	28.5±5.0[a]	31.9±11.4[a]	31.5±7.3[a]	31.3±10.4[a]
D) Nutrient Retention					
1. PER[5]	0.3±0.2[a]	0.7±0.5[a]	0.4±0.3[a]	0.5±0.3[a]	0.3±0.1[a]
2. ER[6]	3.5± 1.3[a]	5.8± 0.6[a]	4.0± 0.7[a]	5.3± 0.7[a]	3.4± 0.6[a]
E) Apparent nutrient digestibility (%)	58.5±1.4[b]	54.2±3.3[a]	57.4±4.4[b]	52.3±4.6[a]	50.4±3.1[a]

[1]DWG=Daily weight gain; [2]BWG=Body weight gain (%); [3]SGR=Specific growth rate; [4]FCR=Feed conversion ratio; [5]PER=Protein efficiency ratio; [6] ER=Energy retention efficiency

4.3.2.3 *C. mrigala* fingerlings

Although the percent of total organic matter did not differ significantly (P>0.05) among the different groups of *C. mrigala* fish post-feed experiment, but the crude protein and crude lipid content was significantly higher in all the groups of fish at the end of experiment as compared to the initial fish (Tables 4.8). Total carbohydrate content was significantly high in the initial fish when compared with the other groups.

From the observed datas we have come to the conclusion that the mrigal fingerlings readily accepted all the formulated feeds, as was evident from the voluntary feed intake level, because these values did not differ significantly among the different groups of mrigal fingerlings (P>0.05). Although these were isonitrogenous diets, however the best growth performance in terms of feed conversion ratio (FCR), gain in body weight (BWG), protein efficiency ratio (PER), daily weight gain (DWG), apparent protein digestibility (APD) and specific growth rate (SGR) was demonstrated by *I. reptans* based diet (F_2) (Table 4.8).

Table 4.8. Growth related performance and feed utilization by *C. mrigala* fed different experimental diets (F1 to F4 and control) for 60 days. Values are mean ± S.D. of triplicate groups of fish, with 5 fish/group (n=5). Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Experimental diets				
	Diet Code				
	F1	F2	F3	F4	Control
A) Feed Intake (g/kg average body wt. /body)	0.5±0.1[a]	1.1±0.3[a]	0.6±0.2[a]	0.5±0.07[a]	0.6±0.09[a]
B) Growth performances					
1. DWG[1] (g day^{-1})	0.2±0.1[a]	0.4±0.1[a]	0.2±0.08[a]	0.3±0.2[a]	0.3±0.1[a]
2. BWG[2] (g)	10.9±1.1[a]	23.7±1.4[b]	11.4±1.2[a]	18.6±1.3[a]	20.2±1.0[b]
3. SGR[3] (% per day)	0.3±0.2[a]	0.8±0.2[a]	0.4±0.2[a]	\0.6±0.3[a]	0.6±0.3[a]
4. FCR[4]	5.0±0.4[a]	2.2±0.4[b]	4.8±0.6[a]	3.2±0.4[a]	2.7±0.5[b]
C) Whole body composition					
1. Organic matter	89.9±2.2[a]	88.2±0.5[a]	89.0±0.8[a]	89.4±1.1[a]	88.5±1.1[a]
2. Crude Protein (Nx6.25) (g/100g)	54.3±0.7[a]	60.8±0.6[b]	54.7±1.0[a]	57.1±0.7[a]	58.6±1.5[b]
3. Crude lipid (g/100g)	5.8±0.7[a]	7.4±0.7[b]	6.1±0.8[a]	6.0±0.8[a]	6.9±0.8[b]
4. Energy (kcal/g)	4.1±0.2[a]	4.0±0.2[a]	4.1±0.2[a]	4.1±0.4[a]	4.1±0.4[a]
5. Total Carbohydrate	29.8±0.8[a]	19±1.7[b]	25.8±2.0[a]	24.0±1.5[a]	22.5±2.3[b]
D) Nutrient *Retention*					
1. PER[5]	0.4±0.3[a]	0.9±0.4[a]	0.4±0.3[a]	0.7±0.4[a]	0.7±0.4[a]
2. ER[6]	0.2±0.2[a]	1.0±0.8[a]	2.4±1.2[a]	0.6±0.5[a]	2.1±1.2[a]
E) Apparent nutrient digestibility (%)	53.8±4.2[a]	56.4±4.5[b]	56.7±3.2[b]	53.7±3.1[a]	52.8±2.6[a]

[1]DWG= Daily weight gain; [2]BWG=Body weight gain (%); [3]SGR =Specific growth rate; [4]FCR=Feed conversion ratio; [5]PER =Protein efficiency ratio; [6] ER=Energy retention efficiency

4.3.3 Biochemical analysis of fish tissues

4.3.3.1 *L. rohita* fingerlings

Table 4.9 shows some biochemical composition of liver and muscle tissues of *L. rohita* fed with different plant – based formulated diets for 60 days. Hepatic and muscle glycogen, crude protein, crude lipid and vitamin E contents were highest on fish fed diet F_2 ($P<0.01$) when compared with those fed other diets.

4.3.3.2 *C. catla* fingerlings

Table 4.10 shows some biochemical composition viz. glycogen, crude protein, crude lipid and vitamin E contents of liver and muscle tissues of *C catla* fish fed with different formulated diets for 60 days. Hepatic and muscle glycogen contents were lowest in fish fed the diet F_1 ($P<0.001$), whereas fish fed on diet F_2 displayed highest contents of crude protein and vitamin E in their hepatic as well as in muscle tissues ($P<0.01$). The crude lipid content of muscle and liver tissues was found to be the same ($P>0.05$) post feeding experiment.

4.3.3.3 *C. mrigala* fingerlings

Some biochemical composition viz. glycogen, crude protein, crude lipid and vitamin E contents of liver and muscle tissues of fish fed with different formulated diets for 60 days are shown in Table 4.11. Hepatic and muscle glycogen contents were lowest in fish fed the diet F_1 ($P<0.001$), whereas fish fed on diet F_2 displayed highest contents of crude protein and vitamin E in their hepatic as well as in muscle tissues ($P<0.01$). The total lipid content in the hepatic tissues of different groups of *C mrigala* fish differed significantly ($P<0.05$); the highest amount being displayed by fish fed on diets F_2, F_3 and control.

Table 4.9. Some biochemical composition of liver and muscle tissues of *L. rohita* fed with, different formulated diets for 60 days. Values are mean ± S.D. of triplicate group of fish, with 5 fish/group. Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Initial fish	Final fish (post 60 days feeding trial)				
		Diets				
		F1	F2	F3	F4	Control
	Hepatic tissue					
Glycogen (µg /g tissue)	25.5±1.7[a]	28.1±1.5[a]	38.3±1.9[b]	35.3±1.8[b]	31.2±1.7[a]	36.2±1.3[b]
Crude Protein (g %)	18.0±2.8[a]	20.2±1.6[a]	26.1±1.7[b]	21.3± 1.7[a]	23.1±1.7[a]	25.2±1.6[b]
Crude Lipid (g %)	1.4±0.6[a]	2.8±1.0[b]	4.0±1.0[c]	3.6±0.5[c]	2.2±1.3[b]	3.0±1.4[b]
Vitamin E (µg /g tissue)	10.5±1.6[a]	20.1±1.7[b]	28.5±1.8[c]	26.2±1.7[c]	14.1±1.7[a]	25.4±2[c]
	Muscle tissue					
Glycogen (µg /g tissue)	1.2±0.4[a]	2.0±0.7[a]	3.3±1.1[b]	2.3±0.8[a]	1.4±1.1[a]	2.7±1.0[b]
Crude Protein (g %)	11.0±2.8[a]	12.1±1.6[a]	15.2±1.7[b]	13.5± 1.7[a]	13.0±1.7[a]	14.3±1.6[a]
Crude Lipid (g %)	1.0±0.6[a]	1.2±1.0[a]	3.0±1.0[b]	2.2±0.5[b]	2.6±1.3[b]	1.7±1.4[b]
Vitamin E (µg /g tissue)	0.6±0.03[a]	0.9±0.04[a]	2.7±0.6[b]	1.9±0.4[b]	1.0±0.3[a]	1.2±0.5[a]

Table 4.10. Some biochemical composition of liver and muscle tissues of *C. catla* fed with, different formulated diets for 60 days. Values are mean ± S.D. of triplicate group of fish, with 5 fish/group. Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Initial fish	Final fish (post 60 days feeding trial)				
		Diets				
		F1	F2	F3	F4	Control
	Hepatic tissue					
Glycogen (µg /g tissue)	21.3±0.9[a]	25.2±0.7[a]	35.3±0.8[b]	33.2±0.7[b]	31.2±0.9[a]	34.2±1.0[b]
Crude Protein (g %)	16.1±0.7[a]	18.2±1.0[a]	24.1±0.6[b]	20.4±1.1[a]	21.1±0.5[a]	23.2±2.2[b]
Crude Lipid (g %)	1.2±0.7[a]	2.4±0.9[b]	3.5±0.7[b]	3.3±1.6[b]	2.1±1.6[b]	3.0±1.1[b]
Vitamin E (µg /g tissue)	8.7±0.5[a]	18.1±0.9[b]	25.5±0.9[b, c]	23.2±1.6[b, c]	12.1±1.0[b]	21.1±0.8[b]
	Muscle tissue					
Glycogen (µg /g tissue)	1.0±0.3[a]	1.8±0.3[a]	3.1±0.4[b]	2.1±0.4[a]	1.2±0.5[a]	2.5±0.4[b]
Crude Protein (g %)	9.2±0.3[a]	10.2±0.4[a]	13.2±0.5[b]	11.2±0.6[a]	11.0±0.4[a]	12.3±0.3[b]
Crude Lipid (g %)	0.8±0.5[a]	1.0±0.5[a]	2.1±0.5[a]	1.5±0.6[a]	1.7±0.3[a]	1.4±0.9[a]
Vitamin E (µg /g tissue)	0.4±0.3[a]	0.7±0.5[a]	2.4±0.5[b]	1.5±0.8[a]	0.8±0 7[a]	1.0±0.5[a]

Table 4.11. Some biochemical composition of liver and muscle tissues of *C. mrigala* fed with, different formulated diets for 60 days. Values are mean ± S.D. of triplicate group of fish, with 5 fish/group. Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Initial fish	Final fish (post 60 days feeding trial)				
		Diets				
		F1	F2	F3	F4	Control
	Hepatic tissue					
Glycogen (µg /g tissue)	27.1±1.4^{a}	31.2±3.2^{a}	41.1±2.9^{b}	38.3±5.1^{a}	35.4±3.5^{a}	40.1±3.0^{b}
Crude Protein (g %)	20.1±3.3^{a}	22.2±3.7^{a}	28.4±1.3^{b}	23.3±3.6^{a}	25.4±2.6^{a}	27.1±3.4^{b}
Crude Lipid (g %)	1.8±0.5^{a}	3.1±0.5^{b}	4.3±0.5^{b,c}	3.8±1.0^{b,c}	2.4±0.5^{a}	3.4±1.1^{b}
Vitamin E (µg /g tissue)	12.3±2.3^{a}	22.1±2.5^{b}	30.4±3.0^{c}	28.2±2.7^{c}	16.2±2.6^{a}	25.4±2.6^{b}
	Muscle tissue					
Glycogen (µg /g tissue)	1.4±0.8^{a}	2.4±1.6^{a}	3.7±1.2^{a}	2.7±1.0^{a}	1.7±1.2^{a}	2.9±0.9^{a}
Crude Protein (g %)	13.2±2.5^{a}	14.3±2.6^{a}	17.1±1.9^{a}	15.1±2.5^{a}	14.6±2.0^{a}	16.3±2.1^{a}
Crude Lipid (g %)	1.4±1.2^{a}	1.6±1.1^{a}	3.7±1.3^{a}	2.6±0.7^{a}	2.8±0.9^{a}	2.1±0.7^{a}
Vitamin E (µg /g tissue)	0.8±0.9^{a}	1.1±1.3^{a}	3.1±1.0^{b}	2.7±1.4^{b}	2.1±1.0^{b}	2.5±1.8^{b}

4.3.4 Biochemical analysis of fish serum

4.3.4.1 *L. rohita* fingerlings

Analysis of some biochemical profiles of *L. rohita* before the onset of experiment and at the end of feeding trial showed that SGOT (serum glutamate oxalate transaminase) level was enhanced in the serum of fish fed the diets F_3 and F_4 whereas SGPT (serum glutamate pyruvate transaminase) level was slightly decreased post feeding the diets F_4 and control compared to those fed the F_1, F_2 and F_3 feeds (Table 4.12). ALP (alkaline phosphatase) level in the serum showed significant increase in F_4. Total cholesterol and triglyceride levels were significantly higher in the serum of the fish fed F_3, whereas the fish fed the F_4 and the control displayed highest value of serum HDL - cholesterol. Total lipid content of serum was significantly lower in the fish fed F_2 and F_1 ($P<0.05$) when compared to other feeds and highest serum level of vitamin C was displayed by fish fed the diets F_1 and F_2, whereas the highest vitamin E level in the serum was shown in F_2, F_4 and control diets. The carotenoid level in serum was not much significant ($P>0.05$) except in F_3 diet. Total protein, albumin, globulin and uric acid contents of serum varied significantly ($P<0.001$) among the different groups of *L. rohita* fingerling fed varied formulated diets (Table 4.12).

4.3.4.2 *C. catla* fingerlings

SGOT level of serum were highest in the fish fed F_3 and F_4 diets and ALP level of serum was highest in F_4 followed by F_3 and control. Whereas SGPT level did not differ much significantly ($P>0.05$) except there was little increase in F_1 diet. Total cholesterol was highest in the serum of the fish fed F_3 diet followed by F_1; however serum of the fish fed the diets F_4 and control displayed highest value of HDL – cholesterol. Total lipid content in the serum was not significant ($P>0.05$) and triglyceride level in the serum was least in F_2. The fish did not show a significant difference in the total protein, albumin, globulin and uric acid content in their serum ($P>0.05$) before and after the experiment. Vitamin C in the serum did not show much

significance but was high in F_2 diet followed by control and F_1 diet and vitamin E level in the serum enhanced slightly in F_2, F_4 and control. Whereas carotenoid level in the serum did not differ significantly ($P>0.05$) among the different groups of fishes (Table 4.13).

4.3.4.3 *C. mrigala* fingerlings

SGOT level was more in the serum of fish fed F_3 followed by F_4 and F_1 diets respectively whereas ALP was highest in F_4, F_3 and control. SGPT level was not much significant in the different groups of fishes ($P>0.05$) but slight increase was observed in the fish fed the control, F_1, F_3 and F_2 diets. Total cholesterol level was pronounced in the serum of the fish F_3 followed by F_1 and serum of the fish fed the diets F_4, F_3 and F_1 displayed highest value of HDL – cholesterol. Total lipid content of serum was same in all the fishes ($P>0.05$), post – feeding experiment showed least amount of triglyceride in their serum post feeding the feed F_2 followed by F_1. Total protein was more in F_2 and F_3 fed fish serum, albumin and globulin was slightly more in the diets F_3, F_2 and F_1. Uric acid level in the serum enhanced in F_3, F_4 and control fishes and vitamin C level was pronounced in fish fed F_2 followed by control and F_1 diets. Vitamin E was high in the serum of the fishes fed diets F_2, F_4 and control ($P<0.001$) when compared among the different groups of *C. mrigala* fish fed different formulated diets whereas carotenoid level was same in all the groups of fishes ($P>0.05$)(Table4.14).

Table 4.12. Some biochemical profile of serum of *L. rohita* before and post feeding the different diets for 60 days. Values are mean ± S.D. of triplicate group of fish, with 5 fish/ group (n=5). Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Initial fish	Final fish (post 60 days feeding trial)				
		Diets				
		F1	F2	F3	F4	Control
SGOT (IU/L)	85.1±4.4^a	99.4±4.6^a	96.1±3.2^a	111.8±3.4^b	103.9±2.7^b	93.1±2.2^a
SGPT (IU/L)	54.1±2.8^a	63.5±2.1^b	60.6±4.3^b	61±4.6^b	56.7±3.5^a	58.9±2.9^a
ALP (IU/L)	454.1±3^a	472.7±2.9^a	519.1±3.1^b	681.1±3.9^b	795.1±3.7^c	674.9±4.3^b
Total cholesterol (mg/dl)	70.0±2.1^a	106.2±3.1^b	91.9±2.2^a	200.0±3.3^c	95.7±2.4^a	85.2±2.7^a
HDL cholesterol (mg/dl)	10.0±1.9^a	16.4±2.4^b	18.9±2.6^b	21.2±1.9^c	34.3±3.6^d	28.8±2.1^d
Total lipid (mg/dl)	0.1±0.01^a	0.2±0.02^a	0.2±0.01^a	0.4±0.02^b	0.4±0.03^b	0.4±0.04^b
Triglyceride (mg/dl)	15.4±2.8^a	25.2±2.9^b	20.1±3^a	48.1±2.1^c	33.7±2.9^b	29.6±2.3^b
Total protein (g/dl)	4.0±1.5^a	4.8±1.7^a	6.9±2.1^b	6.0±1.4^a	5.8±1.6^a	5.0±1.4^a
Albumin (g/dl)	0.3±0.2^a	1.1±0.7^b	1.4±0.5^b	1.7±0.3^c	0.8±0.3^b	0.6±0.1^a
Globulin (g/dl)	1.0±0.3^a	4.7±1.2^b	2.9±0.9^c	5.2±1.6^b	2.4±0.8^c	2.0±0.6^a
A/G ratio	0.29±0.1^a	0.2±0.2^a	0.5±0.04^a	0.3±0.03^a	0.3±0.1^a	0.3±0.2^a
Uric acid (mg/dl)	1.3±0.2^a	2.8±0.6^b	2.2±1.1^a	3.2±1.9^b	3.8±0.9^c	3.0±0.09^b
Vitamin C (mg/dl)	13.0±1.9^a	18.1±2.5^b	20±2.6^b	10.5±1.7^a	10.3±1.7^a	15.0±1.9^a
Vitamin E (mg/dl)	10.2±1.4^a	13.2±1.7^a	15.4±1.4^b	13.6±2^a	14.6±3^b	14.2±2.8^b
Carotenoid (µg /dl)	0.01±0.002^a	0.01±0.002^a	0.01±0.001^a	0.03±0.002^b	0.02±0.001^a	0.01±0.002^a

SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase
ALP: Alkaline phosphatase; HDL: High density lipid;
A/G: Albumin/globulin ratio

Table 4.13. Some biochemical profile of serum of *C. catla* before and post feeding the different diets for 60 days. Values are mean ± S.D. of triplicate group of fish, with 5 fish/ group (n=5). Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Initial fish	Final fish (post 60 days feeding trial)				
		Diets				
		F1	F2	F3	F4	Control
SGOT (IU/L)	72.1±2.0^a	85.2±1.5^a	82.1±1.8^a	99.8±1.8^b	88.9±1.6^b	80.3±1.8^a
SGPT (IU/L)	48.2±5.8^a	60.1±1.6^b	56.3±1.8^a	57.1±2.1^a	51.2±2.0^a	54.2±1.5^a
ALP (IU/L)	442.2±3.8^a	456.1±6.4^a	510.2±5.6^a	652.6±5.0^b	742.4±6.1^b	624.9±3.8^b
Total cholesterol (mg/dl)	60.0±2.4^a	101.1±1.7^b	81.9±2.9^a	169.1±2.4^b	85.7±1.7^a	77.2±1.9^b
HDL cholesterol (mg/dl)	9.5±1.5^a	14.2±2.4^a	18.1±1.6^b	20.1±2.6^b	33.1±1.9^c	27.4±1.7^c
Total lipid (mg/dl)	0.1±0.1^a	0.1±0.2^a	0.2±0.1^a	0.5±0.3^a	0.5±0.3^a	0.4± 0.2^a
Triglyceride (mg/dl)	14.2±1.7^a	21.1±1.9^b	18.1±1.7^a	36.2±1.7^b	29.7±2.7^b	27.6±2.4^b
Total protein (g/dl)	3.3±1.5^a	3.7±1.6^a	5.8±1.6^a	5.0±1.4^a	4.7±1.7^a	4.0±1.6^a
Albumin (g/dl)	0.2±0.1^a	0.8±0.2^a	1.1±1.0^a	1.4±1.0^a	0.5±0.4^a	0.6±0.4^a
Globulin (g/dl)	0.8±0.2^a	3.5±1.5^a	2.3±1.5^a	4.7±1.6^a	2.1±1.5^a	1.7±1.3^a
A/G ratio	0.3±0.2^a	0.2±0.1^a	0.5±0.4^a	0.3±0.3^a	0.2±0.2^a	0.4±0.3^a
Uric acid (mg/dl)	1.1±0.5^a	2.0±0.8^a	1.8±0.5^a	2.3±0.7^a	2.8±0.7^a	2.2±0.6^a
Vitamin C (mg/dl)	8.2±0.6^a	16.8±0.6^b	18.3±0.7^b	13.5±0.6^a	13.3±0.7^a	17.0±0.7^b
Vitamin E (mg/dl)	9.1±0.7^a	12.2±0.7^a	14.2±0.4^b	12.3±0.7^a	13.0±0.7^b	13.3±0.6^b
Carotenoid (µg /dl)	0.01±0.01^a	0.01±0.01^a	0.02±0.01^a	0.03±0.02^a	0.02±0.01^a	0.01±0.01^a

SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase
ALP: Alkaline phosphatase; HDL: High density lipid;
A/G: Albumin/globulin ratio

Table 4.14. Some biochemical profile of serum of *C. mrigala* before and post feeding the different diets for 60 days. Values are mean ± S.D. of triplicate group of fish, with 5 fish/ group (n=5). Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Initial fish	Final fish (post 60 days feeding trial)				
		Diets				
		F1	F2	F3	F4	Control
SGOT (IU/L)	81.2±2.7^a	97.2±1.4^b	85.1±3.0^a	112.4±2.6^b	102.4±2.2^b	90.3±2.4^a
SGPT (IU/L)	52.1±2.4^a	61.4±2.2^a	58.4±2.9^a	59.2±3.9^b	54.2±2.4^a	56.8±0.5^b
ALP (IU/L)	450.2±4.5^a	468.3±4.7^a	503.1±3.3^a	664.2±5.1^b	734.1±3.1^b	654.9±2.2^b
Total cholesterol (mg/dl)	75.1±3.4^a	112.1±4.6^b	85.9±3.3^a	189.1±4.5^b	90.7±3.2^a	82.1±4.3^a
HDL cholesterol (mg/dl)	11.1±2.9^a	17.2±0.6^b	9.2±1.6^a	22.1±3.3^b	36.2±3.8^b	30.3±4.4^b
Total lipid (mg/dl)	0.2±0.08^a	0.3±0.2^a	0.3±0.2^a	0.4±0.2^a	0.4±0.1^a	0.4±0.3^a
Triglyceride (mg/dl)	16.1±1.5^a	27.1±3.8^a	20.0±0.9^a	51.4±3.7^b	43.7±6.5^b	30.6±4.8^b
Total protein (g/dl)	4.8±0.6^a	5.3±0.7^a	7.3±0.7^b	6.5±0.8^b	5.9±0.5^a	5.4±0.7^a
Albumin (g/dl)	0.4±0.2^a	1.4±0.6^b	1.8±0.5^b	2.1±0.7^b	0.9±0.4^a	0.6±0.2^a
Globulin (g/dl)	1.5±0.5^a	4.9±0.4^b	3.2±0.4^b	5.5±0.6^b	2.8±0.6^a	2.3±0.4^a
A/G ratio	0.3±0.2^a	0.3±0.2^a	0.6±0.3^a	0.4±0.2^a	0.3±0.2^a	0.3±0.2^a
Uric acid (mg/dl)	1.5±0.3^a	2.6±0.5^a	2.0±0.6^a	3.4±0.6^b	4.1±0.5^b	2.9±1.2^b
Vitamin C (mg/dl)	10.3±1.7^a	16.1±1.0^b	22.0±2.9^b	11.5±1.3^a	11.3±1.4^a	20.1±1.9^b
Vitamin E (mg/dl)	11.3±2.3^a	14.7±2.3^a	17.4±1.9^b	14.5±3.3^a	15.1±2.7^b	15.9±1.9^b
Carotenoid (µg /dl)	0.02±0.01^a	0.02±0.01^a	0.03±0.02^a	0.04±0.02^a	0.03±0.2^a	0.02±0.01^a

SGOT. Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase
ALP: Alkaline phosphatase; HDL: High density lipid,
A/G: Albumin/globulin ratio

4.3.5 Level of hormones in fish serum

4.3.5.1 *L. rohita* fingerlings

Table 4.15 shows the thyroid hormones profile in *L. rohita* fingerlings before the onset and post feeding experiment. Thyroid stimulating hormone (TSH) level in the serum of *L. rohita* fingerlings decreased significantly post feedings the feeds F_1, F_3 and F_4 as compared to TSH concentration of initial fish as well as post feeding the feeds F_2 and control. The serum concentration of hormone T_4 enhanced significantly ($P<0.001$) in those groups of *L. rohita* fed the F_1 and F_2 for 60 days, whereas the serum concentration of another thyroid hormone T_3 was significantly higher ($P<0.001$) in fish post feeding the diet F_1

4.3.5.2 *C. catla* and *C. mrigala* fingerlings

In the present study, serum concentration of hormone T_4 was significantly higher ($P<0.05$) in those fish fed the diets F_1 and F_2, but the serum concentration of other two hormones (T_3 and TSH) did not differ significantly ($P>0.05$) among the groups of fish fed different formulated diets (Figs. 4.1, 4.2 and 4.3).

4.3.6 Gross examination of fish tissues by scanning electron microscopy (SEM)

A gross examination of fish tissues (heart, liver, kidney and intestine) obtained from different groups of fish under the SEM (Scanning Electron Microscope) did not reveal any sign of abnormality or pathogenesis in these tissues. SEM photograph of liver, heart and intestine of fishes fed control and F_2 diet are depicted in figs. 4.4 to 4.8.

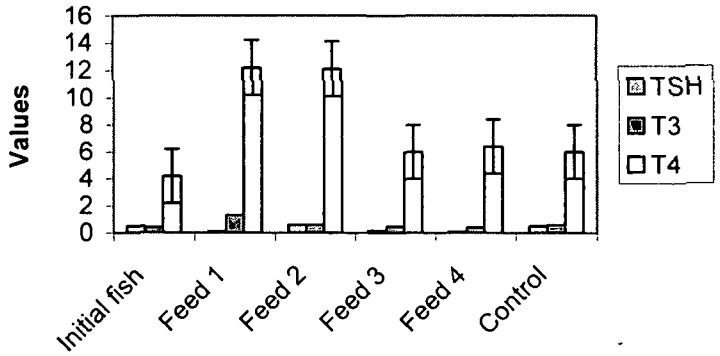


Fig 4.1 *L. rohita* fingerlings

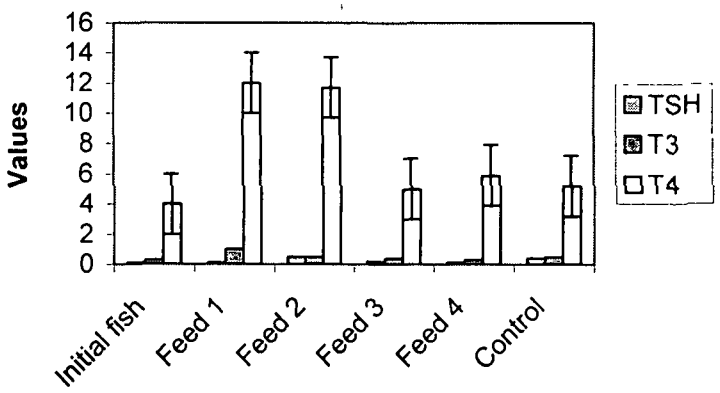


Fig 4.2 *C. catla* fingerlings

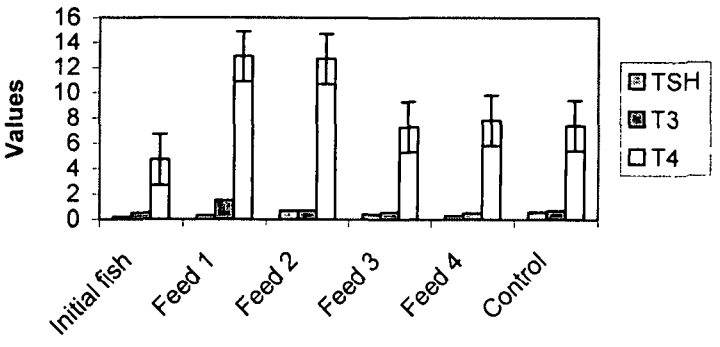


Fig 4.3 *C. mrigala* fingerlings

Figs 4.1–4.3: Hormones titer in the serum of Indian Major Carp fingerlings. Values are mean ± S.D. of triplicate groups of fish, with 5 fish/group (n=5). TSH: IU/ml, T3: nmol/l, T4: nmol/l.

Legend of the Figures

Fig 4.4a. SEM photograph of liver tissue of *L. rohita* fed control diet for 60 days.

Fig 4.4b. SEM photograph of liver tissue of *L. rohita* fed F_2 diet for 60 days.

Fig 4.5a. SEM photograph of intestine of *L. rohita* fed control diet for 60 days.

Fig 4.5b. SEM photograph of intestine of *L. rohita* fed F_2 diet for 60 days.

Fig 4.6a. SEM photograph of liver tissue of *C. catla* fish fed control diet for 60 days.

Fig 4.6b. SEM photograph of liver tissue of *C. catla* fish fed F_2 diet for 60 days.

Fig 4.7a. SEM photograph of heart tissue of *C. mrigala* fish fed control diet for 60 days.

Fig 4.7b. SEM photograph of heart tissue of *C. mrigala* fish fed F_2 diet for 60 days.

Fig 4.8a. SEM photograph of intestine of *C. catla* fish fed control diet for 60 days.

Fig 4.8b. SEM photograph of intestine of *C. catla* fish fed F_2 diet for 60 days.

Abbreviations: RBC: red blood cells; BV: blood vessels; SV: sinus venosus; LM: longitudinal muscle.

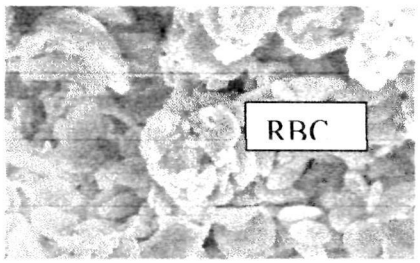


Fig 4.4a.

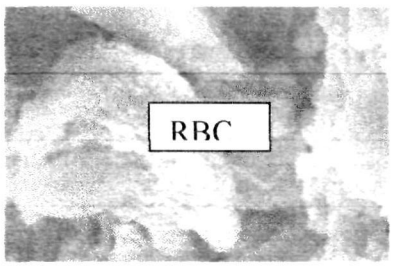


Fig 4.4b.

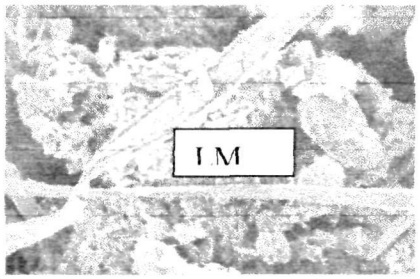


Fig 4.5a.

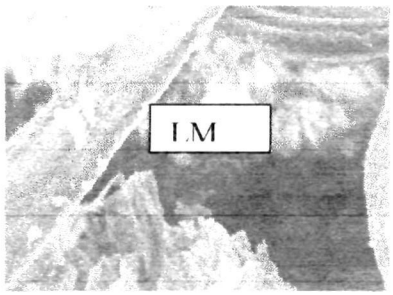


Fig 4.5b.

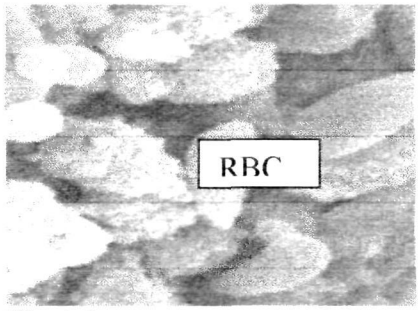


Fig 4.6a.

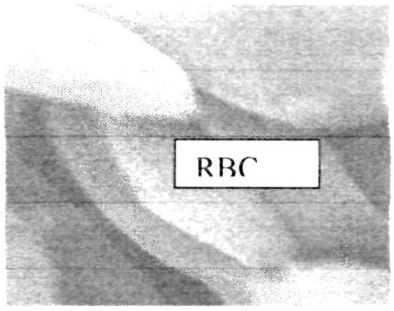


Fig 4.6b.

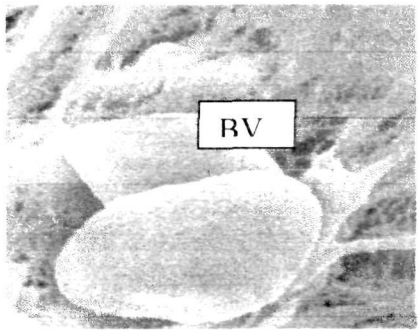


Fig 4.7a.

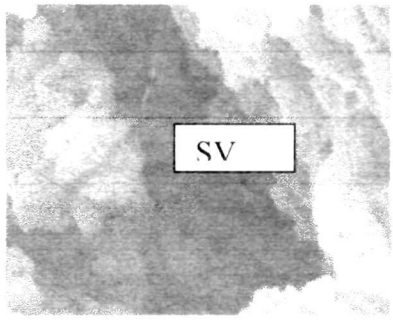


Fig 4.7b.

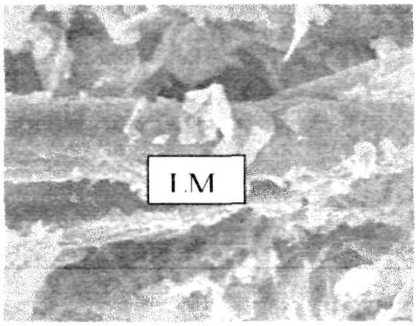


Fig 4.8a.

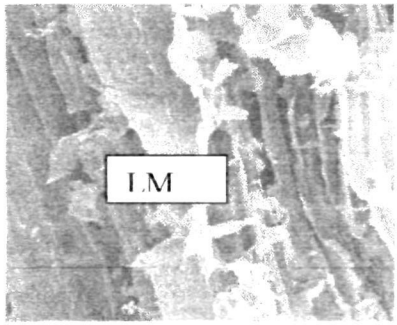


Fig 4.8b.

4.4 Optimization of *I. reptans* supplemented dietary protein requirement for maximum growth performance of Indian major carp fingerlings

4.4.1 Proximate composition of diets IR-1 to IR-4

The proximate composition of diets IR-1 to IR-4 is presented in Table 4.18. The diets were formulated in such a manner so that the total protein content of the formulated diets range as 25, 30, 35 and 40 g% (w/w). Statistical analysis showed that except the crude protein and P/E value content differed significantly (P>0.05) crude lipid was more in IR-3 and IR-4, energy values of these feeds did not differ significantly (P>0.05).

4.4.2 Proximate whole body composition of fish

The whole body protein and lipid contents were enhanced significantly, whereas the amount of nitrogen free extract as well as total carbohydrate contents decreased, from the initial values in all the experimental groups of fish post feeding the diets containing different levels of *I. reptans* protein (Tables 4.19 – 4.21). However, the maximum deposition of protein was achieved post feeding the diet IR-2 (~ 300 g protein / kg diet) to *L. rohita*, and this fish fed higher levels of *I. reptans* protein could not provide additional growth (Table 4.19).

In case of *C. catla* and *C. mrigala*, feeding the diet IR-1 (250 g protein / kg of diet) did not result any significance increase in the carcass protein content (p>0.05), compared to initial fish; however maximum deposition of protein was achieved post feeding the diets IR-3 (350 g protein / kg diet) and IR-4 (400 g protein / kg diet) over 60 days, with a corresponding decrease in total carbohydrate content of the body (Tables 4.20 and 4.21).

4.4.3 Growth performance and nutritional indices

Data on various nutritional parameters indicating growth performance, feed utilization and survival of three groups of fish post feeding the different levels of dietary protein are presented in Table 4.22. Survival of fish was not effected ($p>0.05$), however other nutritional indices were influenced by the protein levels of diets. Results of the present study showed that maximum increase in daily weight gain (DWG) and body weight gain (BWG), specific growth rate (SGR), as protein efficiency ratio (PER), and lower feed conversion ratio (FCR) were achieved post feeding the diet IR-2 to *L. rohita* and diets IR-3 and IR-4 to *C. catla* and *C. mrigala* respectively (Table 4.22).

However, increase in the dietary protein content (eg. > 300 g protein / kg diet for *L.rohita* and > 350 g protein / kg diet for *C. catla* and *C. mrigala*) did not result further improvement of the growth performance and feed utilization by these fish ($p> 0.05$).

4.4.4 Digestive enzymes profile

Results of enzyme activity are expressed as means ± SD of three replicates and data were analyzed by one way ANOVA. The level of significance was accepted at $P<0.05$. Intestinal protease, α-amylase and lipase enzyme activities of fish at the onset and post feeding trial are presented in table 4.23. Specific activity of all the tested enzymes was enhanced significantly post feeding the *I. reptans* based diets as well as control diet compared to initial fish; however control diet was less efficient than *I. reptans* diets in inducing the enzyme synthesis in fish intestine.

Amylase activity was measured in the rohu, catla and mrigal fingerlings after 60 days of post feeding the varying protein diets. A significant increase in intestinal amylase activity in the Indian major carp fingerlings was observed post feeding the diets IR-2 to IR-4. Thus, detectable amylase activity was observed in IR-2, IR-3 and IR-4 groups of fingerlings respectively. Specific enzyme activity increased along with increase in protein level.

Protease activity was also detected in the *L. rohita, C. catla* and *C. mrigala* fingerlings on 60^{th} day after post feeding the varying protein diets. It was observed that intestinal protease activity increased along with the increase of protein level in the diet. A significant increase was also observed in intestinal protease activity in the Indian major carp fingerlings post feeding the diets IR-2 to IR-4.

Lipase activity was broadly detectable in *C. catla* and *C. mrigala* fingerlings. Differences in protein content of diets could not enhance the intestinal lipase enzyme activity of *L. rohita*. In contrast to this, variation in protein content of diets influenced the synthesis of lipase enzyme in the intestine of *C. catla* and *C. mrigala* fingerlings and the optimum level of this enzyme was detected post feeding the diets IR-3 (350 g protein / kg diet) and IR-4 (400 g protein / kg diet) to *C. catla* and *C. mrigala*, respectively.

Table 4. 18. Proximate composition (g% DM) of formulated diets for Indian major carp fingerlings. The values for proximate composition represent mean ± S.D. of five determinations

Components/composition	*Formulated Diets*				
	IR-1	IR-2	IR-3	IR-4	Control
	Proximate composition (g %)				
Organic matter	93 7±2 0[a]	92 6±2 1[a]	92 2±2 2[a]	91 7 ± 2 0[a]	97 7±1 1[a]
Crude protein	25 9±1 0[a]	30 0±1 7[b]	35 1± 2 0[c]	40 0 ± 0 9[d]	28 2±1 6[a]
Crude lipid (ether extract)	6 7±0 7[a]	7 2±1 0[a]	8 4 ± 0 6[b]	9 0 ± 0 8[b]	8 0±2 6[a]
Ash	6 3 ± 1 3[a]	7 4 ± 1 2[b]	7 8 ± 0 5[b]	8 3 ± 1 5[c]	6 4±1 5[a]
Crude fibre	7 2 ± 1 1[a]	7 5 ± 1 0[a]	8 4 ± 0 7[b]	8 0 ± 1 3[b]	7 5±1 6[a]
Nitrogen free extract	53 9 ± 1 7[a]	47 9 ± 1 9[a]	40 3 ± 1 6[b]	34 7 ± 2 0[c]	54 0±2 1[a]
Total carbohydrate (NFE + crude fibre)	61 1 ± 2 1[a]	55 4 ± 2 0[b]	48 7 ± 3 1[b]	42 7 ± 2 4[c]	61 5±1 2[a]
	Energy values				
Gross energy (kcal/100g)	409 6±2 0[a]	408 8 ± 1 3[a]	411 9 ± 1 4[a]	410 4 ± 1 6[a]	424 2±3 8[a]
P/E (mg protein/kcal)	63 2 ± 2 1[a]	73 4 ± 1 2[b]	85 2 ± 1 5[c]	97 5 ± 2 0[d]	66 5±3 1[a]
Chromic oxide (%)	0 92	0 95	0 97	1 0	0 95

*Vitamin premix (mg or IU/g premix): retinol palmitate, 500,000 IU; thiamin, 5; riboflavin, 5; niacin, 25; folic acid, 1; pyridoxine, 5; cyanocobalamine, 5, ascorbic acid, 10, cholecalciferol, 50,000 IU, α -tocoferol, 2 5, menadione, 2, inositol, 25, pantothenic acid, 10, choline chloride, 100, biotin, 0 25

Table 4.19. Whole body composition (% fresh weight basis) and energy content (kcal/g) of *L. rohita* fingerlings fed with different diets for 60 days. Each value represents mean ± SD of five determinations. Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Composition before and post feeding the diets					
	Initial fish	Final fish (post 60 days feeding trial)				
		IR-1	IR-2	IR-3	IR-4	Control
Organic matter (%)	88.0 ± 2.2[a]	87.0 ± 1.5[a]	86.2 ± 1.5[a]	86.8 ± 2.3[a]	85.8 ± 3.4[a]	87.8 ± 1.7[a]
Crude Protein (%)	50.0 ± 2.8[a]	56.2 ± 1.7[b]	67.2 ± 2.1[c]	69.0 ± 2.7[c]	70.0 ± 2.8[c]	58.0 ± 1.6[b]
Crude lipid (%)	6.0 ±1.[a]	6.6 ± 1.4[a]	7.8 ± 1.0[b]	8.0 ± 1.0[b]	7.9 ± 1.6[b]	6.8 ± 1.3[a]
Ash (%)	12. 0±1.1[a]	13.0 ± 1.0[a]	13.8 ± 1.6[a]	13.2 ± 1.4[a]	14.2 ± 1.9[a]	12.2 ± 3.0[a]
Crude fibre (%)	3.1 ± 0.3[a]	2.6 ± 0.7[a]	2.5 ± 0.9[a]	2.6 ± 1.1[a]	3.0 ± 1.7[a]	2.9 ± 1.1[a]
Nitrogen free extract (%)	28.9 ± 4.1[a]	21.6 ± 2.2[b]	8.8 ± 3.1[c]	7.3 ± 1.5[c]	4.9 ± 3.3[d]	20.1 ± 5.2[b]
Total Carbohydrate (%)	32.0 ± 3.3[a]	24.2 ± 2.4[b]	11.4 ± 2.4[c]	9.9 ± 2.0[c]	7.9 ± 2.8[c]	23.0 ± 12.1[b]
Gross energy content (kcal/g)	393.0±4.2[a]	390.2±3.6[a]	393.9±5.1[a]	396.9±5.7[a]	395.9±3.7[a]	395.9±7.2[a]

Table 4.20. Whole body composition (% fresh weight basis) and energy content (kcal/g) of *C. catla* fingerlings fed with different diets for 60 days. Each value represents mean ± SD of five determinations. Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Composition before and post feeding the diets					
	Initial fish	Final fish (post 60 days feeding trial)				
		IR-1	IR-2	IR-3	IR-4	Control
Organic matter (%)	90.0 ± 3.0[a]	90.0 ± 7.9[a]	89.0 ± 6.0[a]	87.0 ± 6.0[a]	86.8 ± 5.0[a]	89.5±10.0[a]
Crude Protein (%)	50.0 ± 4.8[a]	52.0 ± 2.7[a]	56.0±3.3[a]	61.0 ± 3.1[b]	64.0 ± 3.3[b]	54.1 ± 5.8[a]
Crude lipid (%)	3.0 ± 0.5[a]	4.4 ± 0.3[b]	5.0 ± 0.7[b]	6.1 ± 0.9[c]	6.3 ± 1.1[c]	5.1± 0.8[b]
Ash (%)	10.0 ± 1.5[a]	10.0 ±1.1[a]	11.0 ±1.0[a]	13.0 ±1.2[b]	13.2 ± 2.1[b]	10.5 ±1.7[a]
Crude fibre (%)	3.2 ± 1.2[a]	2.9 ± 0.9[a]	2.8 ± 1.1[a]	3.8 ± 1.6[b]	4.1 ± 1.0[b]	3.3 ± 1.7[a]
Nitrogen free extract (%)	33.8 ± 7.6[a]	30.7 ± 5.5[a]	25.2 ± 8.5[b]	16.1 ± 5.3[c]	12.4 ± 4.8[d]	28.0 ± 9.4[b]
Total Carbohydrate (%)	37.0 ± 7.5[a]	33.6 ± 5.3[a]	28.0 ± 5.0[b]	19.9 ± 4.9[c]	16.5± 6.0[d]	31.3±10.4[a]
Gross energy content (kcal/g)	413.5±2.1[a]	408.4±6.1[a]	405.4 ± 5.3[a]	403.0 ± 3.8[a]	405.9±7.9[a]	406.7 ± 6.4[a]

Table 4.21. Whole body composition (% fresh weight basis) and energy content (kcal/g) of *C. mrigala* fingerlings fed with different diets for 60 days. Each value represents mean ± SD of five determinations. Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

Parameters	Composition before and post feeding the diets					
	Initial fish	Final fish (post 60 days feeding trial)				
		IR-1	IR-2	IR-3	IR-4	Control
Organic matter (%)	87.6 ± 3.6[a]	88.6 ± 3.8[a]	89.0± 3.5[a]	88.8 ± 2.2[a]	89.0 ± 3.4[a]	88.1 ±1.1[a]
Crude Protein (%)	51.1 ± 3.5[a]	53.0 ± 3.0[a]	57.0 ± 2.7[b]	64.0 ± 3.9[c]	67.0 ± 4.1[c]	58.6 ± 1.5[b]
Crude lipid (%)	5.4 ± 0.6[a]	6.1± 0.3[b]	7.1± 0.7[c]	6.8 ± 0.6[c]	7.0 ± 1.1[c]	7.0 ± 0.6[c]
Ash (%)	12.4 ± 0.8[a]	11.4 ± 1.0[a]	11.0 ± 1.4[a]	11.2 ± 1.8[a]	11.0 ± 1.4[a]	11.9 ±1.3[a]
Crude fibre (%)	2.4 ± 0.7[a]	2.9 ± 1.2[a]	2.7 ± 0.7[a]	3.3 ± 0.9[a]	3.3 ± 1.0[a]	4.2±1.5[b]
Nitrogen free extract (%)	28.7 ± 2.0[a]	25.6 ± 1.5[b]	22.2 ± 1.3[c]	14.7 ± 1.1[d]	11.7 ± 1.4[e]	18.3 ±1.4[c]
Total Carbohydrate (%)	31.1 ±1.5[a]	28.5 ± 2.1[b]	24.9 ± 1.8[c]	18.0 ± 1.2[d]	15.0 ± 1.2[e]	22.5±2.2[c]
Gross energy content (kcal/g)	391.4± 4.4[a]	398.0 ± 3.2[a]	397.5 ± 6.6[a]	407.9 ± 9.1[c]	410.8 ± 7.6[c]	403.4 ± 7.0[b]

Table 4.22 . Growth related performance and feed utilization by Indian major carp fingerlings fed different experimental diets for 60 days. Values are mean ± S.D. of triplicate groups of fish, with 5 fish/group (n=5). Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05).

Parameter	Experimental diets														
	Diet Code														
	IR-1	IR-2	IR-3	IR-4	Control	IR-1	IR-2	IR-3	IR-4	Control	IR-1	IR-2	IR-3	IR-4	Control
	L. rohita					*C. catla*					*C. mrigala*				
B) Growth performances															
1 DWG[1] (g day^{-1})	0 2±0 1^{a}	0 4±0 2^{b}	0 2±0 09^{a}	0 1±0 04^{a}	0 4±0 08^{b}	0 1±0 02^{a}	0 1±0 01^{a}	0 2±0 1^{b}	0 1±0 03^{a}	0 3±0 03^{b}	0 1±0 01^{a}	0 2±0 1^{a}	0 3±0 2^{b}	0 2±0 06^{a}	0 3±0 1^{b}
2 BWG[2] (g)	12 0±1 2^{a}	23 7±2 4^{b}	9 0±1 0^{a}	8 0±2 1^{a}	21 6±1 9^{b}	4 0±2 1^{a}	7 7±1 4^{a}	11 6±3 0^{b}	9 1±2 1^{a}	18 1±1 2^{b}	7 2±1 1^{a}	10 1±1 7^{a}	17 3±2 4^{b}	13 5±2 3^{a}	20 2±1 0^{b}
3 SGR[3] (% per day)	0 3±0 1^{a}	0 8±0 3^{b}	0 3±0 2^{a}	0 2±0 1^{a}	0 7±0 1^{b}	0 2±0 1^{a}	0 3±0 09^{a}	0 6±0 04^{b}	0 5±0 02^{a}	0 7±0 2^{a}	0 2±0 1^{a}	0 3±0 1^{a}	0 6±0 2^{b}	0 4±0 2^{a}	0 6±0 3^{b}
4 FCR[4]	4 6±1 2^{b}	2 2±0 8^{a}	6 1±1 0^{b}	6 9±2 1^{b}	2 3±0 6^{a}	4 2±1 2^{a}	2 5±1 0^{b}	1 3±0 4^{c}	2 0±0 6^{b}	3 0±0 8^{b}	3 0±0 3^{a}	2 4±1 0^{a}	1 2±0 7^{b}	2 1±0 6^{a}	2 7±0 5^{a}
D) Nutrient Retention															
1 PER[5]	0 5±0 2^{a}	0 8±0 3^{b}	0 3±0 1^{a}	0 2±0 1^{a}	0 8±0 03^{b}	0 1±0 02^{a}	0 3±0 1^{a}	0 4±0 07^{b}	0 3±0 03^{a}	0 3±0 1^{a}	0 2±0 1^{a}	0 4±0 2^{a}	0 7±0 05^{b}	0 5±0 1^{a}	0 7±0 4^{b}
3 ER[7]	12 1±1 2^{b}	17 1±1 0^{b}	14 6±0 9^{a}	12 1±1 3^{b}	21 2±2 3^{b}	2 4±0 8^{a}	4 9±1 0^{a}	7 3±1 3^{b}	6 3±1 2^{b}	3 4±0 6^{a}	19 5±1 2^{a}	19 5±2 0^{a}	22 0±4 1^{b}	20 0±3 7^{a}	19 5±3 1^{a}
E) Apparent digestibility (%)	69 1±4 6^{a}	86 7±4 1^{c}	82 9±3 1^{b}	85 0±5 2^{b}	82 3±3 8^{b}	45 9±3 1^{a}	50 1±3 3^{a}	60 1±4 1^{b}	55 0±4 7^{a}	50 4±3 1^{a}	48 6±7 1^{a}	51 3±5 2^{a}	56 8±4 3^{b}	54 3±3 4^{a}	52 8±2 6^{a}

[1]DWG= Daily weight gain; [2]BWG=Body weight gain; [3]SGR =Specific growth rate; [4]FCR=Feed conversion ratio; [5]PER =Protein efficiency ratio; [6] ER=Energy retention efficiency

Table 4.23. Enzyme activity of the fish intestine at the onset and post feeding experiment for 60 days. Each data represents mean ± S.D. of triplicate groups of fish, with 5 fish/group (n=5). Values in the same row within each experiment followed by different superscripts are significantly different (P<0.05)

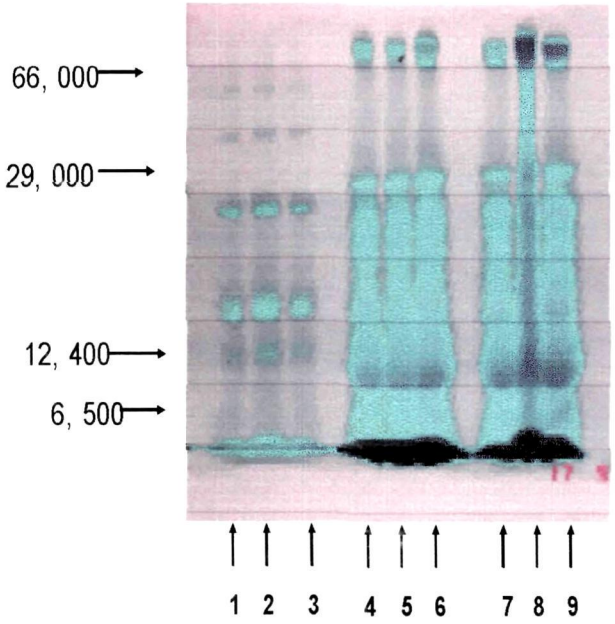
Intestinal Enzyme	Initial fish	Enzyme activity (Unit / mg protein) Final fish (post 60 days feeding trial) IR-1	IR-2	IR-3	IR-4	Control
	L. rohita					
Protease	114.0 ± 4.5 [a]	415.0 ± 11.9 [b]	472.0 ± 12.1 [c]	485.0 ± 13.0 [c]	481.0 ± 15.1 [c]	351.0 ± 11.0 [d]
Amylase	10 ± 0.7 [a]	16.0 ± 0.8 [b]	21.0 ± 1.2 [c]	22.1 ± 1.9 [c]	21.1 ± 2.1 [c]	15.1 ± 1.0 [b]
Lipase	108.0 ± 9.1 [a]	215.0 ± 15.3 [b]	217.0 ± 18.2 [b]	214.0 ± 16.0 [b]	219.0 ± 18.0 [b]	199.0 ± 13.1 [c]
	C. catla					
Protease	99.0 ± 8.2 [a]	372.0 ± 14.1 [b]	401.0 ± 16.2 [c]	461.0 ± 11.9 [d]	470.0 ± 19.0 [d]	352.0 ± 11.4 [c]
Amylase	12.1 ± 0.9 [a]	16.8 ± 3.2 [c]	17.2 ± 1.9 [c]	19.0 ± 3.2 [c]	18.8 ± 4.1 [c]	14.0 ± 1.8 [b]
Lipase	121 ± 12.1 [a]	244.0 ± 11.1 [b]	273.0 ± 13.1 [c]	291.2 ± 18.9 [d]	289.0 ± 16.5 [d]	237.2 ± 13.1 [b]
	C. mrigala					
Protease	102.0 ± 11.3 [a]	338.0 ± 16.2 [b]	389.0 ± 21.0 [b]	402.0 ± 11.1 [c]	410.0 ± 14.1 [c]	321.0 ± 12.1 [b]
Amylase	11.0 ± 1.3 [a]	16.2 ± 2.1 [b]	16.6 ± 2.1 [b]	19.8 ± 2.9 [c]	21.0 ± 3.1 [d]	16.0 ± 2.9 [b]
Lipase	114.0 ± 9.5 [a]	253.0 ± 14.2 [a]	277.0 ± 11.3 [b]	288.0 ± 13.4 [c]	291.0 ± 14.1 [c]	221.0 ± 13.1 [a]

4.4.5 SDS-PAGE of fish serum

SDS-PAGE of fish serum did not reveal any difference in protein profile of serum of rohu, catla and mrigal on post feeding the control as well as *I. reptans* based diets.

Fig 4.9

SDS-PAGE of crude serum of fish before and post feeding the control and *I. reptans* (F2) diet



Lane 1: *L.rohita* serum (initial diet)

Lane 2: *L.rohita* serum postfeed control diet

Lane 3: *L.rohita* serum postfeed F2 diet

Lane 4: *C.catla* serum (initial diet)

Lane 5: *C. catla serum post feed control diet*

Lane 6: *C.catla* serum postfeed F2 diet

Lane 7: *C.mrigala* serum (initial diet)

Lane 8: *C.mrigala* serum post feed control diet

Lane 9: C.mrigala serum postfeed F2 diet

4.4.6 Antigenic cross-reactivity between fish serum and plant extracts

Antigenic cross-reactivity test between fish serum and plant extracts showed that there was no reactivity between them.

4.4.7 Influence of diets in water quality

The water temperature was recorded everyday and it ranged from 27°C to 30°C. The diets did not have any negative effect in the water quality. Neither we could observe any significant change in the chemical properties of water, nor any foul odor was detected, all the investigated parameters were well within the normal range required for optimum culture of carps (Table 4.24).

Table 4.24. Water quality parameters after addition of diet. Values are taken after 60 days addition of diets

Parameters	Observed Range	Standard Range
1. Temperature (° C)	26.5 - 29.1	26.5-29.1
2. Dissolved Oxygen (ppm)	4 - 6.4	5 - 8
3. Total alkalinity (ppm)	133.6 - 138.4	80 - 250
4. Ammonia (ppm)	0.16 - 0.36	0.1 – 1.0
5. Nitrate (ppm)	0.30 – 0.32	0.1 – 1.0
6. Nitrite (ppm)	0.003 – 0.005	0.002 – 0.006
7. pH	6.6 – 8.4	6.5 - 9.0

Chapter V
Discussion

CHAPTER V

Discussion

5.1 A survey of aquatic weeds of Assam used as supplementary fish diet

Present survey was carried out in Assam to unveil the hidden resources that could possibly be explored for the development of fish feeds. The state of Assam has been selected as major area for the present research due to the following reasons:

1. This region is exceptionally rich in both biodiversity and ethno cultural heritage where maximum probability of finding botanical resources based on cultural knowledge have been predicted whereas the literature review have revealed that the area is least explored in the thrust area of aquatic weed research.

2. The ethnofisheries knowledge of the rural fishermen was found to be widely prevalent within their respective locality. It is due to the fact that their major occupation tends to be fishing and wet rice cultivation. However, it is ironical that only few of their vital techniques have been recorded so far in modern research literatures. The fish pond management systems and fish feeds used among them using wild aquatic weeds are still less well known to the scientific community and concerted effort to know such a vital knowledge system in scientific line have not properly been made so far in commercial and industrial point of view.

More particularly, in the area of aquatic weed based research, no such literatures are available. Therefore, ethnobiological survey was randomly done in both plain and hilly region of Assam to explore the weeds used among the rural fishermen as fish feed, by following Rao and Jain (1977) field method. Comparative study on plant used by both hill and plain region were assessed through semi – structure questionnaire method. The photographs were collected on the spot for the aquatic weeds used as fish feeds and

frequency of using single plants by different fishermen were noted and critically screened out after field survey.

Aquatic weeds are defined as those unwanted and undesirable vegetation which reproduce and grow in water and, if left unchecked, may choke the water body posing a serious menace to pisciculture, navigation, water supply conduits, channels etc. besides becoming hazardous to swimming, rowing and aquatic sports. Weed choking of a body of water severely restricts plankton production; limits the living space of fish; upsets the equilibrium of physico – chemical qualities of water; causes imbalance in dissolved oxygen budget; promotes accumulation of deposits leading to siltation; provides shelter to predatory and weed fishes, mollusks and aquatic insects and obstructs netting operations.

However, the magnitude of the problem and economic considerations have led to the thinking that the vast weed mass be turned to some productive use which will recoup some of the losses involved in controlling them. The extra advantage of utilization method is in producing valuable end products, such as fish feed, which plays a major role in dictating its ultimate nutritional and economic success. So far as aquatic weeds are concerned, no extensive report has been made in both India and abroad.

The reason for the selection of the four species of aquatic weeds viz., *S. cuculata, T. natans, L. minor* and *I. reptans* for the study, out of so many species of aquatic flora was, because they have been used frequently as fish feed in Assam and many other parts of North East India without analyzing their nutrient contents. Further, the local fishermen consider these plants to be less toxic, naturally available and sustainable. Moreover, few species such as *S. cuculata* and *L. minor* are encouraged to grow naturally in ponds to serve the nutritional needs of the fishes. Whereas *I. reptans* and *T. natans* may be harvested in wild and processed for fish feeds using the traditional fish feed processing techniques of this region.

5.2 Chemical analysis shows that four selected aquatic weeds are suitable for incorporation in fish diet

5.2.1 Proximate composition

The proximate composition analysis showed that all the tested aquatic plants / weeds are good sources of carbohydrate, protein and lipid. Carbohydrates are essential components of all living organisms, with roles as readily metabolized energy stores, as molecules, which facilitate transfer of energy throughout the organism, and as structural components. Oligosaccharides are often associated with proteins, forming glycoproteins, and with lipids, forming glycolipids which perform important biological functions. The most important carbohydrates in aquaculture nutrition are starch, chitin, sucrose and cellulose, and these are found in the greatest amounts in aquaculture feeds.

Starch is a complex of many α - linked glucose molecules and is a useful component of aquaculture diets since it is generally readily available to most aquatic animals. The accessibility of starch does vary between species, however, the maximum inclusion level in diet needs to be determined for each species of fish.

Although carbohydrates are important sources of energy in fish diet, the dietary carbohydrate requirement may vary depending upon the fish species, e.g. herbivorous fish can metabolize carbohydrates better than carnivorous species (Shiemeno, Hosakawa, and Takeda, 1979; Cowey and Sargent, 1979; Furuichi and Yone, 1981). It is quite reasonable to assume that because of the high protein and low carbohydrate contents, *I. reptans* and *L. minor* may be used as supplementary feed in commercial fish-feed, particularly for the formulation as carnivorous fish diet.

The main function of lipids in animals is either as high – energy storage molecules or as components of cell membranes. Lipid is digested and metabolized with greater

relative ease than carbohydrate and so serves as a much better source of energy for protein sparing (Anderson and DeSilva, 2003). Again, though too much lipid can be included in the diet, which results in production of 'fatty' fish and in growth retardation in crustaceans. The protein – sparing effect of lipid varies between species but appears to be optimal at about 15 – 18 % of the diet in fish (Lie et al., 1988; De Silva et al., 1991), particularly when coupled with low dietary protein levels (De Silva et al., 1991). The optimal dietary lipid level for crustaceans appears to be less than 10 % (D' Abramo, 1997).

Fasting fish often utilize lipid reserves as an energy source in preference to protein and carbohydrate. A study of Coho salmon indicated that this occurred initially as an increase in the rate of lipid breakdown, since the activities of lipid – synthesizing enzymes remained unchanged for a period of 2 days (Lin et al., 1977). However, significant decreases in lipid synthesis were noticeable after 23 days of food deprivation. There is also evidence that lipid mobilization in starved fish is selective, with shorter (C_{18} and C_{16}) and less saturated fatty acids being mobilized first (Sargent et al., 1989). However, again interspecific variation predominates.

Presence of optimum amount of protein in the diet is very much essential because proteins are the most abundant biomolecules in the cell and accounts for almost 50 % of dry weight and perform a variety of functions in the cell, like enzymatic catalysis of metabolic reactions, transport and storage and being a major component of fish muscle. Although there is considerable interaction between protein, carbohydrate and lipid as energy sources, there are some important differences between them. For the purposes of this discussion, it is assumed that the composition of protein provides a balanced mix of the EAAs (essential amino acid). This assumption makes it possible to ignore any growth – limiting effects associated with a restricted supply of EAAs (Anderson and DeSilva, 2003).

5.2.2 Energy values, vitamin contents and mineral ions concentrations

A major function of animal feeds is to provide energy to the animal. Energy is required for the chemical reactions that build new tissues, maintaining osmotic balance,

moving food through the digestive tract, respiration, reproduction, locomotion, etc. Animals obtain the energy they require from food or, in periods when they are deprived of food, from body stores.

Energy is not a nutrient itself, but is present in the chemical bonds that hold the molecules in the nutrients together. There are many different types of bonds, each containing a different amount of energy. Accordingly, the amounts of energy in the various nutrients that make up a feed are of great importance. In addition, the capacity of different species to utilize the energy contained in different nutrients varies considerably. For example, some species are able to use carbohydrates as a significant energy source, whereas the others utilize carbohydrates poorly and rely to a greater extent on protein for energy.

Table 5.1 Energy content of the major dietary nutrients (Anderson and DeSilva, 2003)

Nutrient	Gross energy (MJ / kg)	Digestible energy (MJ / kg)
Carbohydrate	17.15	8.37 – 12.55
Protein	23.01	15.90 – 17.78
Lipid	38.07	33.47

In the present study, P/E value was highest in *I. reptans* followed by *L. minor* and least in *S. cuculata.* Determination of protein to energy (P/E) ratio in fish diet is very important because the higher this ratio, the better is the diet. Generally, for achieving maximum growth, the P/E ratio in fish diet should range from 80 mg/ kcal to 100 mg/kcal (Akand, Hasan, and Habib, 1991; Arockiaraj, Muruganandam, Marimuthu, and Haniffa, 1999; Hasan, Moniruzzaman, and Farooque, 1990;). Therefore, on the basis of high gross energy as well as P/E values, it may be inferred that *I reptans* and *L minor* are suitable for incorporation in fish diet to reduce the cost of fish feed.

Vitamins are organic molecules that act as cofactors or substrates in some metabolic reactions. They are generally required in relatively small amounts in the diet and, if present in inadequate amounts, may result in nutrition – related disease, poor growth or increased susceptibility to infections (Anderson and DeSilva, 2003). The requirement for vitamins by different fish species varies greatly according to their usual feeding habit and their capacity to synthesize them. Vitamins are contained in the fresh products from which aquaculture diets are made. However, the processing method and the time can destroy these compounds, a problem not easily resolved. It is therefore very difficult to determine a generally recommended level of vitamin supplementation that will satisfy all species.

Vitamins are important constituents of diet including fish diet. For example; vitamin E, nature's most effective, lipid soluble antioxidant present in biological membranes, confers stability to the membranes (De Silva and Anderson, 1995). Vitamin E is considered a primary defense against lipid peroxidation, protecting polyunsaturated fatty acids in cell membranes from free radical attack through its free – radical quenching activity in biomembranes. Determination of vitamin E requirements is complicated by questions of whether requirements should focus on vitamin E intake that are adequate to prevent deficiency symptoms and allow normal physiological function or should consider higher vitamin E intake necessary to prevent peroxidation damage (Jacobson, 1987). It has been reported that approximate dietary vitamin E requirements for *C. mrigala* and *L. rohita* ranged between 99 mg/kg and 132.0 mg/kg of dry diet respectively for the normal growth and development of fish (Paul et al, 2004; Sau et al., 2004).

The Indian major carp fingerlings do require dietary supply of vitamin C for survival and normal growth (Sahoo, 2006). Their dietary vitamin C requirement is around 10 mg/100g diet (Mitra and Mukhopadhyay, 2003). Vitamin C requirement based on growth performance was 45 mg vitamin C eq. / kg for common carp larvae (Gouillou – Coustans et al.,1998). They found that required level of maximum tissue storage is higher than that needed for survival and maximum growth (350 mg ascorbic acid eq. / kg diet). With increasing dietary concentrations of vitamin C, it is expected that the tissue level

would reach maximum or saturation level. Beyond this level they would not increase along with a further increase in dietary concentration – the excess intake being rather excreted or metabolized (Cho and Cowey, 1993; Gouillou – Coustans and Kaushik, 2001).

Therefore, results of the present study indicate that sufficient amounts of vitamin E and C are present in these plants under study to meet the requirements of these vitamins for the proper growth and development of Indian major carps.

Carotenoid requirement for Indian major carps can be fulfilled by providing 4 mg to 6 mg carotenoid / kg of diet (ADCP, 1983). However, the carotenoid concentration in these weeds was much lower as compared to alfalfa meal and artificial astaxanthin (Harpaz et al., 1998), but it is worth to be mentioned here that this vitamin is mainly required for ornamental fish for pigmentation purpose (Meyers, 1994), and carps may not require a high content of this vitamin in their diet.

Minerals, or inorganic elements, are needed by animals to maintain many of their metabolic processes and to provide material for major structural elements (e.g. skeleton). Not all elements that are used in metabolism are required in fish diet. Aquatic animals, particularly marine animals, have an advantage in that their surrounding medium contains many of the elements needed for growth and survival.

The minerals required for normal metabolism can be divided into two groups:

1. Major minerals are required in large quantities and include calcium, phosphorus, magnesium, sodium, potassium, chlorine and sulphur.

2. Trace minerals are those required in trace amounts and include iron, iodine, manganese, copper, cobalt, zinc, selenium, molybdenum, fluorine, aluminum, nickel, vanadium, silicon, tin and chromium.

Table 5.2 Some important minerals and their functions (Anderson and DeSilva, 2003).

Mineral	Functions
Calcium	Component of skeleton, scales, teeth, exoskeleton, etc. Roles in physiological processes including metabolism, nerve and muscle function and osmoregulation.
Phosphorus	Component of bones and scales of fish and exoskeleton of crustaceans. Roles in metabolic reactions.
Sodium, potassium, chlorine	Appropriate levels of these ions needed for proper functioning of cells, in maintaining ion gradients between the inside and outside of cells and for maintaining nerve function.
Iron	Constituent of haemoglobin and cytochromes (proteins) important in energy metabolism.
Magnesium	Component of skeletal tissue. Important cofactor in a number of metabolic reactions. Important in maintaining muscle tone.
Manganese	Important cofactor in a number of metabolic reactions. Important in maintaining proper nerve cell function.
Copper, zinc	Important components of a number of metalloenzymes involved in a wide variety of metabolic processes. Approximately 20 different enzymes have been found to contain zinc.
Iodine	Important component of thyroid

	hormones; important in growth regulation.
Selenium	Constitutes an integral part of the enzyme glutathione peroxidase. Imparts protective effect against toxicityof heavy metals.
Cobalt	Component of vitamin B_{12}.
Chromium	Important in normal carbohydrate and lipid metabolism.
Sulphur	Required for the synthesis of the amino acid cysteine.

Mineral elements play an important role in regulating many vital physiological processes in the body, such as regulation of enzyme activity (cofactor or metalloenzyme), skeletal structures (e.g., calcium and phosphorus), neuromuscular irritability and for the clotting of blood (calcium). In fish and crustaceans, a dietary requirement has been established for only 11 minerals (Kaushik, 2001), which are calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), cobalt (Co), copper (Cu), iodine (I) and selenium (Se). Magnesium has a relationship with the protein concentrations in the blood serum of fish because 25% of the total serum magnesium is bound to albumin and 8% to globulin (Kroll and Elin, 1985). Non-availability of adequate quantities of minerals in diet affects fish growth and may cause irrecoverable deficiency diseases (De Silva and Anderson, 1995). Although diet is the main source of minerals for fish, some minerals can be absorbed from the environment (Lall and Bishop, 1977). But even then, despite the large amount of calcium in the water, the presence of a minimum amount of dietary calcium (~ 2.0 ppm) causes an increase in the final weight of the fish, indicating the absolute requirement of calcium in the fish diet (Chavez-Sanchez et al., 2000). Yueming Dersjant-Li et al., (2001) have shown that dietary Na/K ratios between 1.5 and 2.5 mol/mol produced the best growth for African catfish. The dietary Na/K ratios of the selected four weeds also ranged within this figure suggesting further their use in fish diet.

Plants appear to be able to accumulate many nutrient minerals from water and soil. Studies done by Guittikar et al. (1966) informed that the mineral requirements of the body are supplied by leguminous plants, especially magnesium and copper. Again, report of Riche and Brown (1999) revealed that the incorporation of plant protein feed stuffs into fish meal diets for rainbow trout increases phosphorus availability in the fish. Thus, it may be assumed that presence of adequate amounts of minerals in the four selected aquatic weeds may increase the growth of fish under study to a great extent, if incorporated in their diet.

5.2.3 Antinutritional factors present in these plants are within the tolerable limit of fish

Generally, when the composition of a feed is analyzed, more often the attention is directed to those components of feed which provide nutrition to the cultured species (De Silva and Anderson, 1995). However, in addition to nutrients, a feed may contain anti-nutrients, and the presence of significant amounts of such antinutritional factors in the weeds is of great concern since they might have a detrimental influence on the growth of the organisms. For instance, oxalate is a chelating agent, which binds calcium very effectively. Plants with high oxalate content may produce acute metabolic calcium deficiency syndrome (hypocalcemia) when fed as main feed to livestock (Checke and Shull, 1985). Trypsin inhibitor (TI) is a widespread antinutrient substance in many plant-derived nutritional ingredients that could be used in fish feed. It seems that below 5mg/g level of dietary TI, most cultured fish are able to compensate this antinutrient by increasing trypsin production (Francis, Makkar, and Becker, 2001). However, Makkar and Becker (1999) reported that carp are capable of tolerating high levels of TI (24.8 mg/g) in their diet.

Tannic acid is known to evoke growth-depressing effect in tilapia and rohu fish (Jackson, Capper, and Matty, 1982). Phytate chelates with certain metal ions, such as calcium, magnesium, zinc, copper and iron, to form insoluble complexes that are not readily broken down and may pass through the digestive tract unchanged, thus reducing

the bioavailibility of these minerals (Maga, 1982). In addition, phytates also form strong complexes with proteins that can lead to reduce digestibility of latter component (Richardson et al., 1985).

Present study documents that although antinutitional factors are present in these aquatic weeds under study; however their concentrations were within the tolerable limit of fish reinforcing the future use of these plants as for the formulation of cost effective, artificial fish – diet (Kalita et al., 2006).

5.3 Growth performance and feed utilization by Indian major carp fingerlings fed on aquatic weeds based formulated diets

Nutrition is the science of feeding the body to ensure its optimal development, health and maintenance. In all forms of animal husbandry, providing a supply of nutrients to match the requirements of the cultured animal is fundamental to achieving optimal growth and production efficiency and hence maximizing economic return (Anderson and De Silva, 2003).

Supplementary feed is considered as the most critical input for augmenting its production. The fish accepts a wide variety of agricultural byproducts in the form of pelleted or dough feed. Several studies have been carried out on the development of formulated feed for the species under controlled culture system (Mohanty et al., 1995, Mukhopadhyay and Ray, 1999, Mukhopadhyay and Ray, 2001, Khan et al., 2004, Biswas et al., 2006). In almost all these studies, ingredients, such as fishmeal, soybean meal, groundnut oil cake were liberally used. All these materials are becoming prohibitively costly for their continued use in aqua feeds in the foreseeable future. This has necessitated search for alternative cheap sources of protein for fish feed, which are available locally Assam. In this context, use of certain potential aquatic macrophytes offer excellent scope as these nutrient-laden leafy materials are naturally grown in the entire state without much agronomic care. It is being reported that these macrophytes containing substantial amount of protein and minerals are suitable for incorporation as a feedstuff for conversion into protein sources of high biological value (Edwards, 1987). Furthermore, in the light of

recent findings on bovine spongiform encephalopathy (BSE) and other transmittable diseases from animal meals, the use of rendered meat products, such as blood meal, bone meal and meat meal, in aquaculture has been prohibited in the European Union and the USA. Consequently, there has been an increase in attempts to explore the use of aquatic food product waste in aquaculture feeds (Gunasekera et al., 2002).

Another factor influencing selection of protein sources in aquatic animal feeds is the release of nutrients from uneaten food and faeces into the environment. These nutrients, particularly nitrogen and phosphorus, stimulate natural productivity and may lead to eutrophication. To prevent this, the nutritionist seeks to formulate feeds that are well utilized by the animal. Thus, efficient protein utilization (to limit nitrogen in the effluent) and phosphorus utilization are important. Efficient protein utilization is best achieved by a clear understanding of the amino acid requirements of the target species.

Protein is the major as well as the most expensive component in fish feed and although many workers discuss protein requirements, it is more appropriate to consider that aquatic animals have a requirement for a well – balanced mixture of essential and non – essential amino acids. The protein requirements range from 30 % to 56 % of the diet. These values can be considered to be estimated levels of protein required in diets, for most studies, sometimes incorrectly, have assumed that all amino acids are available for metabolism and protein synthesis. Khan et al (2005) observed that a dietary protein level of 25-30% is well suited for the optimum growth and reproductive performance of *L. rohita*. Therefore, in our initial study all the five feeds used for feeding trial were formulated in such a manner so that their protein content ranged between 26 % and 28 % (w/w) of diet. Crude lipid, ash and crude fibre contents of formulated feeds were not affected with variations in dietary protein and therefore, growth in fish is affected by dietary protein level; however, only few reports are available on this aspect. Gunasekera and Lam (1997) reported that *O. niloticus* fed low (10 g %) protein attained less weight than those fed high (20 and 35 g %) protein diets.

The result of the present study showed that the *L. rohita* fingerlings accepted the entire aquatic weed based diets quite well as was evident from the voluntary feed intake pattern by fish and percent survival data. However, the growth response of the fish fed on different feeds differed significantly showing superiority of F_2 over the other feeds. It is worthy to mention that since the gross energy content of the formulated diets did not vary significantly ($P > 0.05$), therefore it may be worthwhile to determine the aesthetic quality of a feed estimation of P/E (protein / energy) ratio of a feed is more important than determination of the gross energy content of that diet. It has been observed that lowest feed conversion ratio (FCR) and best growth of fish can be achieved if the P/E ratio of diet varies between 80 and 100 mg protein $kcal^{-1}$ diet (Akand et al., 1991; Samantaray and Mohanty, 1997). Therefore, it may be inferred that feed F_2 having the highest P/E ratio (76.3 ±1.1; mean ± S.D.) produced the best growth response in *L. rohita* fingerlings. Control diet possessing P/E value of 66.5±3.1 (mean ± SD) mg protein / kcal displayed the second best result in terms of fish growth and other nutritional indices. Interestingly, although the P/E values of feeds F_3 and F_4 are higher than that of the control diet, they produced less growth response in fish compared to latter diet. A possible explanation of this observed effect might be due to the presence of higher levels of antinutrients in these feeds compared to control diet (unpublished observation) attributing to the less growth of fish post feeding the F_3 and F_4 feeds.

Next to protein, energy sources that a balanced feed must contain to meet the energy needed for basal metabolism and growth of fish are carbohydrate and lipid (DeSilva and Anderson, 1995). It has been reported that lipid is favored over carbohydrate, and perhaps is better as a non-protein source of energy for carps (Mukhopadhyay and Rout, 1996). One of the reasons might be the fact that fish in general utilize dietary carbohydrates poorly (Furuichi and Yone, 1980), because carbohydrate fraction from plant sources is not very digestible (Siddharaju and Becker, 2001) whereas lipid component of a feed is almost completely digestible by fish. There is an apparent relationship between the natural diet of a species in the wild and its capacity to deal with dietary carbohydrate. Carnivorous fish such as yellowtail (*Seriola quinqueradiata*) respond less well to glucose tolerance tests than omnivores, such as common carp (*Cyprinus carpio*), which clear blood

glucose more rapidly (Furuichi and Yone, 1981) but slower than mammals. This information indicates that fish are unable to metabolise glucose quickly. When fish are presented with diets high in carbohydrate, the excess glucose appears to be used to synthesise glycogen (Palmer and Ryman, 1972). Glycogen has been shown to decline during prolonged starvation in the hepatopancreatic tissues of carp (Nagai and Ikeda, 1971) and in the liver of golden perch (*Macquaria ambigua*) (Collins and Anderson, 1995).

Murat et al. (1978) showed that the gluconeogenic pathway is more important in maintaining blood glucose levels in carp than breakdown of glycogen. From this information it is apparent that carbohydrate in aquaculture diets has to be carefully controlled, for the excess deposited, as glycogen is subsequently less readily available to the fish as an energy source than other stores.

When incorporating carbohydrate into diets for aquatic animals, it is preferable to utilize a carbohydrate that requires some degree of digestion, such as starch, rather than monosaccharides, such as glucose. This will at least allow a time lag between consumption of the carbohydrate and the appearance of glucose and other monosaccharides in the blood. The resultant slower increase in the plasma of the animal will result in a greater degree of catabolism of these substrates for energy. This preference for complex carbohydrates is one of the reasons that sucrose has not been widely used in aquatic animal diets.

Apart from satisfying the requirements of an aquatic animal for essential lipids (fatty acids and sterols), dietary lipid acts as a source of energy. In general, a 10 –20 % level of lipid in fish diets gives optimal growth rates without producing an excessively fatty carcass (Cowey and Sargent, 1979). Interspecific variation in the ability of different species to utilize lipid as a source of energy is prevalent. For example, when rainbow trout were fed diets with lipid levels from 5 % to 20 % and protein contents of 16 % to 48 %, the optimum ratio of protein to lipid was found to be 35 % protein to 18 % lipid (Takeuchi et al., 1978). However, carp fed diets with a fixed protein level of 32 %, with lipid varying

from 5 % to 15 % and with corresponding decreases in carbohydrate, did not show increased growth or food conversion rates (Takeuchi et al., 1979).

5.3.1 Requirement of essential fatty acids in fish diet

Essential fatty acids (n-3 or n-6) are the fatty acids, which are obtained generally from the diet of the fish because they are not biosynthesized by these animals. The n-3 and n-6 fatty acids play very important roles in the proper functioning of fishes, particularly in providing membrane fluidity and acting as precursors of some important hormones (Anderson and De Silva, 2003).

Growth and development of fishes are very much dependent on the nutritive value of their feeds (Hidalgo et al., 1987). The present study gives a report on the concentration of saturated, monounsaturated, polyunsaturated fatty acids and most particularly the essential fatty acids present in the four aquatic weeds. Such a study is of great importance because presence of essential fatty acids in fish feed determines the accuracy of a highly nutritive diet for fish.

The provisions of nutritionally high quality fish meal will keep the fingerlings healthy, uniform in size and will also enable them to produce healthy brood fish. Among these fatty acids, especially polyunsaturated group have been shown to be an essential requirement of many fishes (Watanabe et al., 1983). Our studies revealed the presence of higher concentration of total fatty acids in *I. reptans* as compared to the other three aquatic weeds. C 24:1 and C 24:6 are n-3 series polyunsaturated fatty acids (PUFA). They were observed in all the four aquatic weeds and *I. reptans* revealed presence of higher concentration of these PUFAs than the other three aquatic plants. Therefore, the results of the present study was in accordance with the observations by Lee et al., 1967; Castell et al., 1972; Sargent, 1976 where they have reported that fatty acids of the n-3 series especially linolenic acid are essential for the growth of fish.

In summary, it may be concluded that the presence of saturated and polyunsaturated fatty acids, are essential requirement for fish diet which are present in all the tested aquatic weeds particularly in *I. reptans*. Moreover incorporation of n-3 and n-6 fatty acids in the diet should not only ensure better growth performance and efficiency of feed utilization in the fishes but deposition of such fatty acids in the whole body tissue of the species as well. This will also have significance for the benefit of human health and nutrition as a whole.

5.3.2 Requirement of other nutrients in carps diet

It is reasonable to assume that apart of carbohydrates, lipids and proteins, presence of other nutritional factors such as vitamins and minerals, and either absence or presence of relatively low amounts (tolerable limit) of anti-nutritional factors in a feed are equally important in the context of a balanced fish diet. It has been reported that approximate dietary vitamin E requirements for Indian major carps range between 100 mg/kg and 132 mg/kg of dry diet (Paul et al., 2004, Sau et al., 2004), whereas dietary requirement of vitamin C is around 100 mg/kg diet (Mitra and Mukhopahyay, 2003). These vitamins act as natural antioxidants and are essential to protect the fatty acids and other oxidizable components in the feed. The results of our previous study have shown that *I. reptans* contains higher amounts of vitamins E and C, mineral ions and tolerable amounts of anti-nutrients (e.g. trypsin inhibitory activity, tannins, phytate and calcium oxalate) compared to other three plants, and this might be responsible for better growth performance of fish post feeding the *I. reptans* based diet.

In the present study, fish accepted all the experimental diets quite well as is evident from the average food consumption and percent survival data; however the growth response of *C. catla* and *C. mrigala* fed on the same diet is different. For example, although the percent of total organic matter is same ($P>0.05$) in all the groups of fish post feeding the different diets, however feeding the diets F_2 and control results an increase in the crude protein and carbohydrate contents of carcass of *C. mrigala* but not in *C. catla*. Therefore, our result also supports the earlier observation by Nandeesha et al. (1998) that a

species-specific difference seems to exist with respect to carcass nutrients contents. Our result also implies that like *Spirulina maxima* (Atack et al., 1979) and unlike *S platensis* (Nandeesha et al., 1998), *I reptans* has a tendency to increase the fat deposition in fish tissue, but it does not increase the lipid content of fish serum. Like meat, fat plays an integral part in the taste or quality of fish and it has been observed that fish having a high content in fat have a distinct flavor. Thus, the present study depicts that the feed F_2 containing least amounts of lipid and carbohydrate as compared to other feeds showed the best result in terms of growth performance of omnivorous fish like *C. catla* and *C. mrigala* and associated nutritional indices.

Present study shows that feed F_2 although contains the less amounts of lipid and carbohydrate and equal amounts of proteins as compared to other feeds but produced the best result in terms of growth performance and other nutritional indices of fishes like *L. rohita, C. catla and C. mrigala.* Lipid content may be less in feed F_2, but fat deposition in the fish tissues (i. e. both *n*-3 and *n*-6 PUFA) was more in the fingerlings fed with this diet thus increasing the taste or quality of the fish. Moreover feeding the diets F_2 and control results an increase in the crude protein and carbohydrate and vitamin E contents of the carcass. Thus F_2 is considered superior over the other diets

5.4 Biochemical and immunological analysis of serum did not reveal any pathological sign or disorder in fish post-feeding the diets

It is very necessary to know that whether the plant materials produce any slowly acting toxic metabolite (s) harmful for fish, in the process of digestion. Determination of serum level of some diagnostic enzymes post feeding the diets is one of the efficient way to determine the toxicity of plant material, if any. The use of enzymes in diagnosis dates back to the beginning of the present century when Wohlgemuth introduced his procedure for measuring urinary amylase activity (Wilkinson, 1976). Subsequently it was demonstrated that intracellular enzymes could be released into the circulation as the result of cell damage (Wilkinson, 1976).

Liver the biggest metabolic organ of the body performs very important physiological functions in the body starting from lipid, protein and carbohydrates metabolism to detoxification. Therefore, it is very essential to determine whether liver damage has occurred post feeding the diets. It is near universal current practice to report the results of both transaminase and alkaline phosphatase measurements, in response to a clinical request for 'liver function test'. Usually, these are the sole enzyme values routinely reported. The transaminases are the most important examples of a group of liver enzymes, which are present in high concentration in the cytoplasm of liver (Wroblewski and LaDue, 1956). With liver cell necrosis acute hepatocellular damage or abnormal membrane permeability the transaminases are released from the cell and their serum levels increases (Wroblewski and LaDue, 1955, Wilkinson, 1976). Aspartate transaminase (AST or SGOT) may be elevated in several non-liver diseases (e.g. heart or muscle necrosis), whereas non-hepatic alanine transaminase (ALT / SGPT) elevation is unusual (Wilkinson, 1976).

In liver, alkaline phosphatase activity is found at the sinusoidal surface of the liver cell (Wachstein and Meisel, 1958) and is the most familiar example of an enzyme especially elevated in cholestasis. However, bone and intestine may also contribute the level of this enzyme in serum (Hodson et al., 1962).

In the present study, non of the tested enzymes were found to enhance significantly post feeding the aquatic weeds based diet, particularly the *I. reptans* based diet. Further, the serum levels of total cholesterol, LDL- cholesterol, triglycerides and uric acids were significantly lower in fish fed *I. reptans* based diet compared to other diets showing the superiority of former diet.

5.5 Scanning electron microscope (SEM) study of fish tissue did not show any pathological sign

Another way of determining the toxic effect of food components is the gross examinations of fish tissue before and post-feeding the diets under the electron microscope. For this reason, tissues were collected from fish and examined under SEM.

No lesion or abnormality was detected in tissues post feeding the aquatic weeds based diets reinforcing their consumption is safe for the fish.

5.6 Relationship between thyroid hormone profile in fish serum and quality / quantity of food intake

Many parameters including environmental, nutritional, generic and endogenous factors affect the fish growth (Sumprer, 1992). However, the role of endocrine status particularly for the anabolic hormone like thyroid hormones (T_3 and T_4) in fish development and growth remains poorly understood. From a pisciculture point of view, it is worthwhile to establish a relationship between fish growth and hormone level and the impact of fish diet in hormone profile of fish. There are only few evidences to show that thyroid hormones are known to participate in the growth and development of fish by stimulating greater voluntary food intake by improving food conversion (Le Bail and Bocut, 1997; Rasmussen et al., 2001), by stimulating protein synthesis (Fauconneau et al., 1996), and may be by influencing the secretion of other hormones (Donaldson et al., 1979). Although Valente et al. (2003) observed that plasma concentrations of T_3 and T_4 in rainbow tout (*Oncorhynchus mykiss*) were significantly higher ($P<0.05$) in fish fed to satiation compared to fish fed a restricted ration or starved.

The alterations in quantity / quality and pattern of food intake influence thyroid hormone kinetics in salmonid fish (Eales and Shostak, 1985) at levels ranging from neural control of TSH release to post receptor cellular action of thyroid hormones (Eales, 1988). However in the present study, a relationship between the pattern of food intake and thyroid hormone's status in the serum of Indian major carps could be established. For example, we could see the relationship between T_3 and T_4 hormones and also could prove that both of them are inversely proportional to TSH. T_4 concentration in the serum of Indian major carps post feeding the feeds F_1 and F_2 was higher in comparison to control or other diets ($P>0.05$). In a similar type of observation, Gomez et al. (1996) found no correlation between the quantity of food ingested and the average circulating GH levels in catheterized rainbow trout. Similarly, no relationship was found between appetite, time of a meal, or

level of food ration and T_3 during a daily period (Gomez et al., 1997). Therefore, further studies on both the thyroid hormones and their receptor systems, and the cellular response to these hormones are needed before this aspect of thyroid regulation of both growth in the Indian major carps can be fully understood.

5.7 Optimization of *I. reptans* supplemented dietary protein requirement for growth performance of Indian major carp fingerlings

5.7.1 Proximate whole body composition, growth performance and nutritional indices

Growth and development of fish are primarily dependent upon an adequate supply of nutrient (Halver et al., 2002), both in terms of quality and quantity, using well identified sources of such nutrients. It has been well established that there is a difference in the nutrient requirement of fish during all stages of development, such as – larvae, fry, fingerling, grower and brood stages (Mukhopadhyay et al., 2003; Mitra, 2003). Therefore, it is essential to estimate the nutrients requirement for a particular species of fish at a particular stage of development.

Proteins, making up about 65 – 75% of the total fish tissue on a dry – weight basis, are the principal constituents of fish tissue (Wilson, 2002). Inadequate protein in the diet causes a reduction of cessation of fish growth and a loss of weight, whereas supply of too much protein in diet may lead to the wastage of this nutrient (Wilson, 2002) and this enhances the operational cost in aquaculture. Although the *I. reptans* used in the present study was collected from Northeast India, however this aquatic weed is reported to occur in many parts of the world (Le Thi Men and Bui Hong Van, 1994; Ly Thi Luyen, 2003).

Present study showed that for the Indian major carp fingerlings, optimum level of protein requirement might vary, depending upon the carp species. For example, rohu fingerlings fed diets containing 300 g protein / kg diet demonstrated optimum growth performance, whereas for catla and mrigal, best growth performance was achieved post feeding the 350 g of crude *I. reptans* protein / kg of diet. It has been reported that same

factors like size and age of fish; fluctuation of water temperature etc. affects feed intake as well as the protein requirements of fish (Wilson, 2002). Since in the present study, these factors did not vary significantly, therefore their influence in regulating the protein intake by fish may be ruled out. This reinforces the species – specific difference in dietary protein requirement for optimum growth of Indian major carps. The optimum *I. reptans* protein requirement for *L. rohita* fingerlings observed in the present study is higher than *L. rohita* brood stock fed commercially available fish feed (Khan et al., 2005); however, the growth and associated nutritional indices of rohu fingerlings in the present study was higher compared to growth of *L. rohita* broodstock or fry fed commercial diet (Khan et al., 2005), *L. rohita* fingerlings fed yeast extract powder supplemented diet (Ghosh et al., 2005) and rohu fingerlings supplemented with diet containing sesame seed meal at 400 g / kg into a fish meal basal diet (Mukhopadhyay and Ray, 1995).

It has been observed that fish control the feed intake to meet their energy requirement (Kaushik and Medale, 1994), and fish fed the high energy content diet reached satiation before the low energy content diets, and this decrease in feed intake had a negative effect on fish growth (Silverstein et al., 1999; Watanabe et al., 2001; Borba et al., 2006). Although the feeds IR-2 and control are isoprotein diets ($p > 0.05$), but the better growth response of *L. rohita* when fed the former diet compared to latter diet may lead us to assume that higher energy content of the control diet resulted its less consumption by rohu, suggesting that protein or other nutrients were not consumed in adequate amounts (Silverstein et al., 1999; Watanabe et al., 2001) and further increase in protein content of diet may lead to wastage. Therefore, the present study advocate the inclusion of 300 g of *I. reptans* based protein / kg of diet for optimum growth response in *L. rohita* advanced fingerlings.

The above argument may also sound good to explain the observed growth performance of *C. catla* and *C. mrigala* fingerlings post feeding the *I. reptans* based diet. Although the three diets, viz., IR-2, IR-3 and IR-4 were isocaloric (Table 4.21), but the diet IR-3 containing optimum amount of protein necessary for the development and growth of catla and mrigal, produced the best result. It is to be noted that further increment of protein

concentration (>300 g / kg diet) by increasing the inclusion of *I. reptans* in the diet, also leads to enhancement of dietary antinutrient contents (Kalita et al., 2006), which did not favor further growth of fish.

Therefore, it may be inferred that for determining the aesthetic quality of a diet, estimation of P/E (protein / energy) ratio and digestible energy content of a feed are more important than determination of gross energy content.

5.7.2 Digestive enzymes profile

Enzymes function to break down nutrients in foods into compounds that can be absorbed across the brush border membrane of the enterocyte. These enzymes can be excreted into the lumen, bound to membranes, or contained in supranuclear vacuoles within the erythrocyte. Therefore, digestion can occur in fish using one or more digestive motifs : extracellular (or lumenal) digestion, membrane-linked digestion, and intracellular digestion (Halver and Hardy, 2005).

a) Amylases

Amylase enzyme acts on carbohydrate digestion. Amylase activity in rainbow trout increase in response to feeding, a rise in the temperature or salinity of the water, or when the fish are given a protein-rich diet. On the other hand, tilapia shows higher amylase activity when changed to a starch-rich diet. Amylase differs from species to species, and appears to be related to their feeding habits.

b) Proteases

The general digestive scheme for protein in monogastric animals, especially fish, occurs in the lumen of the digestive organs in a linear fashion, where protein is broken down to polypeptides by proteases, polypeptides are broken down into free amino acids by peptidases (both extracellular and membrane linked), and free amino acids are absorbed.

However, it has also been shown that protein assimilation in larval and adult fish can occur by pinocytosis and intracellular digestion in the posterior intestine (Watanabe, 1981, 1982; Gabaudan, 1984).

c) Lipases

The general digestive scheme for lipids is extracellular hydrolysis of lipids (Higgs and Dong, 2000) in the stomach, intestinal, and caecal lumen by a variety of lipases and colipases (Sargent et al., 1989; Smith, 1989). In fish, the short chain fatty acids (2-10 carbons) and glycerol are absorbed directly through the brush border of the enterocytes. Long-chain fatty acids (12 and more carbons) are cleaved by lipase and emulsified by bile salts to form negatively charged aggregates called micelles.

The gut enzyme profile is the indicator of nutrient digestibility and utilization (Das and Tripathi, 1991; Ghosh et al., 2001). Digestion and absorption of food particles and molecules generally take place along the brush border of the intestinal columnar epithelial cells where numerous digestive and absorptive enzymes are localized (Cahu and Jambnino-Infante, 1997; Tengjaroenkul et al., 2000). In the present experiment, the activity of the enzymes – protease, amylase and lipase were more in final fish (post feeding for 60 days) compared to initial fish, and this finding agrees well with the previous reports that with an increase in the age of fish, secretion of digestive enzymes enhances (Lauff and Hoefer, 1984; Kuz'mina, 2001; Chakrabarti et al., 2006). However, present study documents that apart from the age of a fish, type of the diet also plays a crucial role in enhancing the digestive enzyme activity of fish (Steffans, 1981; Mitra, 2003; Ghosh et al, 2005), may be by influencing the morphological structure of the digestive tract or through increasing the microbial flora in the gut of the fish (Hepher, 1988) that leads to enhance enzyme secretion (Ghosh et al., 2005). Since scanning electron microscope (SEM) study of fish intestine post feeding the *I. reptans* based diet did not reveal any gross morphological change (unpublished observation), therefore it seems that this plant is responsible for influencing the microbial flora of the gut of fish leading to higher digestive enzyme

synthesis. However, further research to elucidate the mechanism of action of *I. reptans* constituents in enhancing the digestive enzyme activity in fish is necessary.

5.7.3 Influence of diets in water quality

Water quality should be determined after the addition of diet in order to study any toxic or adverse effect of the water that may hamper the optimum health of the fish. The water temperature was recorded everyday and it ranged from 27°C to 30°C. The diets did not have any negative effect in the water quality. Over the 60 days indoor feeding trial with *I. reptans* based diets, neither we could observe any significant change in the chemical properties of water nor any foul odor was detected, and all the investigated parameters which are viz. pH, dissolved oxygen, total alkalinity, total ammonia, nitrite and nitrate were well within the normal range required for optimum culture of carps.

5.8 Enhancement of essential nutrients required for human health in Indian major carps post feeding the *I. reptans* based diet will lead to increase in consumption rate and more demand for fish

Last couple of decades, people have become more aware of fish as a healthier alternative to meat, mainly due to the problems with overweight and cardiovascular diseases that have become some of the severe problems in human healthcare in developed countries. The fish consumption per capita is expanding globally, and the pattern of fish consumption is changing gradually. In developed nations, the trend is that fish is increasingly becoming a culinary speciality rather than a basic food. In developing countries, fish is very much essential as food and a main source of protein. During the recent years, modern consumers demand fish / food for health, quality and safety. Therefore, apart from increasing the fish-production level; aquaculturist as well as fish farmers should give proper importance to enhancement of nutritive value of fish to cater or satisfy the demand of the modern consumers.

In the present study, feeding the fish with *I reptans* based diet not only leads to an increase in the growth of fish, but there is a subsequent enhancement of nutrient contents of fish as well these are considered as essential for human health. As such, human will not like to consume the aquatic weeds directly even if they are full of nutrients, however surely they will have a fascination for fish having high nutrient contents, those are derived from the highly nutritive aquatic weeds based diet. The following discussion relating the nutrient contents of fish post feeding the *I. reptans* based diet and essentiality of these nutrients in human health will make our understanding more clear.

1. The total protein content of serum as well as muscle of Indian major carps enhanced significantly post feeding the *I reptans* based diet compared to other diets. Proteins from fish have a high nutritional value because of containing more essential amino acids and hence considered as high biological value protein. In developing countries, fish is one of the important sources of dietary proteins, and human body can utilize proteins from fish better than proteins from beef, pork, chicken and milk. All the proteins from fish are adequate, important and digestible.
2. An analysis of lipid profile of the serum of fish post feeding the *I. reptans* based diet shows that these fish are more worthy for human consumption, even compared to fish fed the control (conventional) diet. Feeding the former diet leads to a significant decrease of total cholesterol, LDL-cholesterol as well as triglyceride levels of fish serum. Although cholesterol performs some important functions in the body, however a diet rich in cholesterol and triglycerides is not considered good for human health. A high plasma level of cholesterol and triglycerides in human often leads to the deposition of cholesteryl ester plaques on arterial walls, producing artherosclerosis and coronary occlusion diseases.

Further, fats from fish are healthier, because fish contains essential fatty acids that human body needs. Freshwater fish, e.g. Indian major carps can be just as good or even better as a source of essential fatty acids as marine fish and can be just as beneficial to human health (Steffens, 1997). In addition to

considerable amounts of *n*-3 polyunsaturated fatty acids, freshwater fish contain high levels of arachidonic acid than do marine species (Steffens, 1997). Omega-3 (*n*-3) fatty acids are critical for the development of the brain and retina and lower blood cholesterol level, thus give a protection against some chronic diseases. The essential fatty acids like linoleic and linoleneic acids which human is unable to synthesize, are present in adequate amount in fish and therefore consumption of the fish may lead to prevention of phrynoderma syndrome in human.

3. The high levels of vitamins like vitamin A, E and C in fish post feeding the *I reptans* diet should increase the demand of these fish for human consumption. It has been observed that daily dietary vitamin E intake of 10 to 30 mg in healthy adults will maintain serum vitamin E level in normal range (Mukherjee et al., 1998), and this vitamin can be obtained easily if people consume fish fed with *I. reptans* diet.

Since *I. reptans* is rich with some essential minerals like Ca, Fe, Zn it may be assumed that these minerals are eventually transmitted to fish fed with *I. reptans* based diet; and this group of fish can also contribute appreciable amounts of dietary calcium, heme iron and zinc, nutrients that tend to be low in people's diet.

Conclusions

The present study has demonstrated that among the four tested aquatic weeds *I. reptans* and *L. minor* could be important sources of proteins, vitamins and minerals suitable for incorporation in fish diet. Though antinutritional factors were found to be present in these weeds, their levels were within the tolerable limits and consumption of these plants would not result in any deleterious effect on the growth of fish, documenting further their utilization for the formulation of balanced fish diet. The fatty acid composition analysis of the four plants reinforces that all the four aquatic weeds are important sources of essential PUFAs, which are important requirement in fish diet for healthy growth.

The feeding experiments demonstrated that among the four tested aquatic weeds *I. reptans* is the most promising weed and can be used as a partial substitute of dietary fish meal protein for the Indian major carp fingerlings - *L. rohita, C. catla* and *C. mrigala*. It was observed that after partial incorporation of *I. reptans* in the diet and post feeding experiment, the cholesterol level in the blood was found to be low whereas the protein content was high in the fish. Further research to completely eliminate or to further reduce the anti-nutrients present in this plant for improving the quality of *I. reptans* based fish meal by employing various processing techniques, is required. Our study demonstrates that commercial exploitation of *I. reptans* for the formulation of efficient, low-cost fish feed for the Indian carps is highly promising. Further, after 60 days indoor feeding trial with *I. reptans* based diets, neither we could observe any significant change in the chemical properties of water nor any foul odor was detected, and all the investigated parameters were well within the normal range required for optimum culture of carps. Therefore, *I. reptans* based diet is an environment – friendly, low pollution feed suggesting its commercial exploitation as fish feed, is highly promising.

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Evaluation of the nutritional quality of four unexplored aquatic weeds from northeast India for the formulation of cost-effective fish feeds

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Abstract

A study was conducted to evaluate the nutritional potential of four commonly available, unexplored aquatic weeds namely, *Salvinia cuculata, Trapa natans, Lemna minor* and *Ipomoea reptans* from northeast India for ascertaining their suitability for utilization as supplementary fish feed with the aim to reduce the cost of commercial feeds. Results of proximate analysis showed that the crude protein content of the aquatic weeds ranged between 11.0% and 32.2% (w/w), whereas crude fibre and ash contents varied between 4.2% and 20% (w/w), and 13.3% and 31.2% (w/w), respectively. Protein to energy ratio (*P/E*) of these weeds ranged between 30.7 mg/kcal and 95.3 mg/kcal and the highest value was displayed by *I. reptans*. All these aquatic weeds contained high amounts of vitamins E and C and mineral elements required for the normal growth and development of fish.

Analysis of antinutritional factors showed that the concentration (g%) of trypsin inhibitor (TIA) ranged from 1.1% to 1.5%, calcium oxalate concentration ranged from 0.6% to 3.5%, tannin concentration ranged from 0.25% to 0.93% and phytate concentration ranged from 0.004% to 0.005% in these plant samples, and the amounts of these antinutritional components were within the tolerable limits of fish, particularly for carp. The present study demonstrates that commercial exploitation of these aquatic weeds, particularly *I. reptans* and *L. minor*, for the formulation of cost-effective and balanced artificial fish feeds appears to be highly promising.

Keywords: Antinutrient; Aquatic weeds; Gross energy; *Ipomoea reptans*; *Lemna minor*; Plant nutrients; *Salvinia cuculata*; *Trapa natans*

1. Introduction

The menace of aquatic weeds is reaching alarming problems in many parts of the world, but it is particularly severe in tropical countries, where abundant sunlight and favourable water temperature, increasing numbers of dams, barrage and irrigation channels foster aquatic plant growth. This problem is further aggravated because these unutilized weeds choke the water bodies, thus reducing the carrying capacity for aquaculture purposes. Such water bodies are often left unproductive with impeded light penetration and depletion of dissolved oxygen. Regrettably, there is hardly any simple or cost-effective way to control the infestation of these aquatic macrophytes in an environment-friendly manner. However, a perusal of the available literature shows that some of the aquatic weeds are highly nutritive and, therefore, one alternative solution to check the massive population of these weeds might be their utilization through incorporation as components of feedstuff for fish and prawn, in particular. In fact, significant effort has been directed toward evaluating the nutritive value of different non-conventional feed resources, including terrestrial and aquatic macrophytes, to formulate nutritionally balanced and cost-effective diets for fish and poultry (Edwards, Kamal, & Wee, 1985; Patra & Ray, 1988; Ray & Das, 1995; Wee & Wang, 1987).

The northeast (NE) part of India, which is considered as one of the hotspot regions of the world, has a rich heritage

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of biodiversity. This area contains an abundance of aquatic weeds that grow throughout the year. A few of them are consumed by local people but many remain unutilized and go to waste. Some of the aquatic weeds of the latter category, namely, *Salvinia cuculata, Trapa natans, Lemna minor* and *Ipomoea reptans,* are widely distributed in this part of the country without any agronomic care. Unfortunately, the nutritive value of these weeds, endemic to northeast India, has never been assessed. Fish-culture is one of the important occupations of this region, and therefore the use of some of these weeds as nutrient sources for fish feed formulation will not only at least partly replace the rather expensive, conventional commercial aqua feeds and assist the pisciculture of this region, but might restrict the alarming growth of these weeds that are affecting the ecosystem.

However, before advocating the utilization of these aquatic weeds for supplementation of fish feeds, there is an urgent need to explore their nutritional quality and anti-nutritional composition. The present study was undertaken to investigate the nutritional potential and anti-nutrient components of four commonly available aquatic weeds from northeast India, namely, *Salvinia cuculata, Trapa natans, Lemna minor* and *Ipomoea reptans*, for ascertaining their suitability for use as fish feed.

2. Materials and methods

2.1. Sample preparation

Samples of fresh, tender and green leaves, stem, roots, fruits and flowers of *Ipomoea reptans, Trapa natans, Lemna minor* and *Salvinia cuculata* were collected from ponds at several locations of the north eastern states of India and taxonomically identified. The samples were washed under running water and blotted dry. The moisture content of the leaf samples was determined at 60 °C (AOAC, 1990). The dried matter obtained was ground to a fine powder and stored at −5 °C in air-tight containers prior to further analysis.

2.2. Proximate analysis

Moisture, ash, ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) were determined by the methods of the Association of Official Analytical Chemists (AOAC, 1990). The crude protein content was determined by the Kjeldahl procedure, AOAC Method 920; the factor $N \times 6.25$ was used to convert nitrogen into crude protein. The crude lipid was extracted using a Soxhlet apparatus and quantity of lipid was determined gravimetrically. Crude fibre content was determined by the Fibretech system with repeated treatment of dilute H_2SO_4, followed by dilute NaOH and washing by water. The carbohydrate content was estimated as the weight difference using moisture, crude protein, lipid and ash content data.

Determination of α-tocopherol and carotene contents of plant materials first includes extraction of total lipid material from dried plant powder (Folch, Lees, & Sloane-Stanley, 1957), followed by extraction and estimation of α-tocopherol and carotene levels by the procedure of Baker and Frank (1968). For the extraction of ascorbic acid (vitamin C), 5.0 g of plant material were ground using a pestle and mortar in 50 ml of 4% (w/v) oxalic acid solution and filtered through a Whatman filter paper (No. 100). The ascorbic acid content was then determined volumetrically using 2,6-dichlorophenol indophenol dye (Sadasivam & Manickam, 1992). The gross energy was determined by using an adiabatic bomb calorimeter (IKA C-7000), using benzoic acid as a standard.

2.3 Mineral analysis

The mineral elements of the plants, namely, Na, K and Ca, were estimated by flame photometry, whereas Zn, Cu and Mg contents were measured by atomic absorption spectrophotometry (Carl Zeiss) using standard reference chemicals. The total phosphorus content was determined as described by Umoren, Essien, Ukorebi, and Essien (2005).

2.4 Determination of anti nutritional components

The activity of trypsin inhibitors in the samples was determined by using benzoyl-DL-arginine-paranitroanilide (BAPNA) as a substrate.The trypsin inhibitory activity was expressed as the amount of trypsin inhibitor (TI), in grammes, present per 100 g of sample (Kakade, Rachis, McGhee, & Puski, 1974). Total phenols (tannin) from the plants were isolated as described by Makkar (1994) and then estimated by the Folin–Denis reagent (Makkar & Goodchild, 1996). The tannin content of the samples was calculated as tannic acid equivalents from a standard graph. The phytic acid content of the samples was determined spectrophotometrically using a Hitachi U 2000 uv–vis spectrophotometer (Vaintraub & Lapteva, 1988). Phytic acid was used as a standard. For the estimation of calcium oxalate, the procedure of Jones (1988) was followed.

2.5. Statistical analysis

Data are presented as means ± SD. One-way analyses of variance (ANOVA) were carried out to compare the different values.

3. Results and discussion

3.1. Proximate composition

The proximate compositions of four aquatic weeds, namely, *S cuculata, I reptans, T. natans* and *L minor,* on a fresh weight basis, are presented in Table 1. Moisture, organic matter and lipid contents were nearly identical in

Table 1
Proximate composition of four aquatic weeds from northeast India on dry matter basis (%)

Component	*S. cuculata*	*I. reptans*	*T. natans*	*L. minor*
1. Moisture	9.8 ± 1.1	10.8 ± 1.5	7.6 ± 0.7	8.8 ± 1.2
2. Organic matter	69 ± 0.1	70.0 ± 0.2	87 ± 1.1	75.0 ± 0.7
3. Crude protein ($N \times 6.25$)	11.0 ± 0.1	32.2 ± 1.0	11.4 ± 0.2	28.0 ± 1.7
4. Crude lipid (ether extract)	7.0 ± 0.1	6.0 ± 0.8	8.0 ± 0.6	5.0 ± 0.1
5. Ash	31.2 ± 1.3	30.0 ± 1.2	13.3 ± 0.7	25.0 ± 1.6
6. Total carbohydrate (NFE + Crude fibre)	50.8 ± 1.1	31.8 ± 1.5	67.3 ± 0.9	42.0 ± 1.9
7. Crude fibre	20.0 ± 1.3	10.0 ± 1.2	4.2 ± 0.6	10.0 ± 0.5

Each value represents mean ± SD of three determinations.

all the plants. Among these four plants, *I. reptans* possessed the highest amount of crude protein (32.2%), followed by *L. minor* (28.0%); *S. cuculata*and *T. natans* had comparatively less crude protein (11–11.4%). The ash content in these plants ranged from 13.3% to 31.2%, the highest amount being displayed by *S. cuculata* and the lowest by *T. natans*. Total carbohydrate (including starch) content of *T. natans* (67.3%) was significantly higher ($p < 0.001$) than the three other plants under study, whereas *S. cuculata* possessed the highest amount of crude fibre (20%, w/w), followed by *I. reptans* and *L. minor* (10%, w/w).

Although carbohydrates are important sources of energy in fish diet, the dietary carbohydrate requirement may vary, depending upon the fish species; e.g. herbivorous fish can metabolize carbohydrates better than can carnivorous species (Cowey & Sargent, 1979; Furuichi & Yone, 1981; Shiemeno, Hosakawa, & Takeda, 1979). It is quite reasonable to assume that because of the high protein and low carbohydrate contents, *I. reptans* and *L. minor* may be used as supplementary feed in commercial fish feed, particularly for the formulation of carnivorous fish diet.

3.2. Energy values, vitamin contents and mineral ion concentrations

As shown in Table 2, all four plants exhibited remarkable similarity in possessing nearly the same gross energy value (338 kcal/100 g to 358 kcal/100 g). *P/E* value was highest in *I. reptans,* followed by *L. minor* and lowest in *S. cuculata.*

Determination of protein to energy (*P/E*) ratio in fish diet is very important because the higher this ratio, the better is the diet. Generally, for achieving maximum growth, the *P/E* ratio in fish diet should range from 80 mg/kcal to 100 mg/kcal (Akand, Hasan, & Habib, 1991; Arockiaraj, Muruganandam, Marimuthu, & Haniffa, 1999; Hasan, Moniruzzaman, & Farooque, 1990). Therefore, on the basis of high gross energy, as well as *P/E* values, it may be inferred that *I. reptans* and *L. minor* are suitable for incorporation in fish diet to reduce the cost of fish feed.

Vitamin contents of these aquatic weeds are shown in Table 2. Vitamin E (α-tocopherol) content of *T. natans* (61.3 mg/100 g) was significantly higher than those of the other three plants, whereas *I. reptans* was characterized as possessing the highest amounts of ascorbic acid (4.0 mg /100 g) and carotenoids (0.25 mg/100 g) (Table 2). The lowest levels of ascorbic acid and carotenoids were found in *S. cuculata* and *L. minor.*

Vitamins are important constituents of fish diet. Vitamin E, nature's most effective, lipid-soluble antioxidant, present in biological membranes, confers stability to the membranes (De Silva & Anderson, 1995). It has been reported that approximate dietary vitamin E requirements for *C. mrigala* and *L.rohita* ranged between 99 mg/kg and

Table 2
Energy values, vitamin contents and mineral ion concentrations in the leaves of four aquatic weeds

Properties	*S. cuculata*	*I. reptans*	*T. natans*	*L. minor*
Energy values				
Gross energy (kcal/100 g)	358 ± 2.1	338 ± 1.2	347 ± 1.4	358 ± 1.6
P/E (mg protein/kcal)	30.7 ± 0.9	95.3 ± 1.0	32.7 ± 1.2	78.4 ± 1.5
Vitamin content				
Vitamin E (mg/100 g)	28.8 ± 0.5	28.5 ± 0.4	61.3 ± 0.7	26.6 ± 0.3
Vitamin C (mg/100 g)	3.0 ± 0.1	4.0 ± 0.2	3.6 ± 0.1	3.8 ± 0.2
Carotenoid (mg/100 g)	0.2 ± 0.04	0.3 ± 0.01	0.2 ± 0.02	0.1 ± 0.01
Mineral ion concentration				
Zn (mg%)	0.7 ± 0.02	1.7 ± 0.01	1.4 ± 0.08	1.0 ± 0.07
Mg (mg%)	17.8 ± 0.1	31.0 ± 0.02	25.1 ± 0.06	20.3 ± 0.03
Cu (mg%)	0.1 ± 0.01	0.1 ± 0.08	0.1 ± 0.005	0.1 ± 0.01
Ca (ppm)	2.0 ± 0.1	2.0 ± 0.004	2.0 ± 0.04	2.0 ± 0.02
Na (mg%)	6.3 ± 1.0	5.0 ± 1.0	5.0 ± 0.2	3.0 ± 0.2
K (mg%)	17.5 ± 0.5	41.4 ± 0.2	27.7 ± 1.0	20.0 ± 0.4
P (g/kg)	1.0 ± 0.1	1.5 ± 0.5	0.9 ± 0.1	1.0 ± 0.4

Values are means of triplicate determinations.

132 mg/kg of dry diet, for the normal growth and development of fish (Paul, Sarkar, & Mohanty, 2004; Sau, Paul, Mohanta, & Mohanty, 2004), whereas dietary vitamin C requirement for Indian major carps is around 10 mg/100 g diet (Mitra & Mukhopadhyay, 2003). However, carotenoid requirement for Indian major carps can be fulfilled by providing 4 mg–6 mg of carotenoids/kg of diet (ADCP, 1983). Therefore, the present study indicates that sufficient amounts of vitamin E and C are present in these plants to meet the requirements of these vitamins for the proper growth and development of Indian major carps. However, the carotenoid concentration in these weeds was much lower than those of alfalfa meal and artificial astaxanthin (Harpaz, Rise, Arad, & Gur, 1998), but it should be noted that this vitamin is mainly required for ornamental fish for pigmentation purposes (Meyers, 1994), and carps may not require a high content of this vitamin in their diet.

The mineral compositions of the four aquatic weeds are presented in Table 2. A significant variation in metal contents was noticed among these plants, which may be attributed to differences in their genus and species level. As shown in Table 2, potassium and magnesium were the most abundant of the elements considered, followed by sodium and copper. Among these plants, *I. reptans* was shown to possess the highest amounts of K (41.4 mg%), Mg (31.0 mg%) and Zn (1.7 mg%), whereas *S. cuculata* and *L. minor* contained the highest amounts of Na (6.3 mg%) and Cu (0.14 mg%). Interestingly, the Ca content was identical in all the four weeds, but phosphorus level was highest in *I. reptans*. Mineral elements play an important role in regulating many vital physiological processes in the body, such as regulation of enzyme activity (cofactor or metalloenzyme), skeletal structures (e.g., calcium and phosphorus), neuromuscular irritability and clotting of blood (calcium). Magnesium has a relationship with the protein concentrations in the blood serum of fish because 25% of the total serum magnesium is bound to albumin and 8% to globulin (Kroll & Elin, 1985). Non-availability of adequate quantities of minerals in the diet affects fish growth and may cause irrecoverable deficiency diseases (De Silva & Anderson, 1995). Although diet is the main source of minerals for fish, some minerals can be absorbed from the environment (Lall & Bishop, 1977). But even then, despite the large amount of calcium in the water, the presence of a minimum amount of dietary calcium (~2.0 ppm) causes an increase in the final weight of the fish, indicating the absolute requirement for calcium in the fish diet (Chavez-Sanchez, Martinez-Palacios, Martinez-Perez, & Ross, 2000). Yueming Dersjant-Li, Wu, Verstegen, Schrama, and Verreth (2001) have shown that dietary Na/K ratios between 1.5 and 2.5 mol/mol produced the best growth for African catfish. The dietary Na/K ratios of the selected four weeds were also within this range, further suggesting their use in fish diet.

3.3. Anti-nutritional factors

Anti-nutrient contents of the four aquatic weeds are summarized in Table 3. Highest trypsin inhibitory activity was detected in *T. natans* (1.53%), followed by *L. minor*, whereas lowest activity was detected in *S. cuculata* (1.13%). The latter plant had higher amounts of tannins (0.93%) and phytate (0.005%) than had the three other aquatic weeds. Calcium oxalate concentration was highest in *L. minor* (3.5%), followed by *T. natans* (0.9%). Interestingly, among these aquatic weeds, *I. reptans* possessed the least amount of tested anti-nutrients.

Generally, when the composition of a feed is analyzed, attention is directed to those components of the feed which provide nutrition to the cultured species (De Silva & Anderson, 1995). However, in addition to nutrients, a feed may contain anti-nutrients, and the presence of significant amounts of such anti-nutritional factors in the weeds is of great concern since they may have a detrimental influence on the growth of the organisms. For instance, oxalate is a chelating agent, which binds calcium very effectively. Plants with high oxalate content may produce an acute metabolic calcium deficiency syndrome (hypocalcemia) when fed as the main feed to livestock (Checke & Shull, 1985). Trypsin inhibitor (TI) is a widespread anti-nutrient substance in many plant-derived nutritional ingredients that could be used in fish feed. It seems that below the 5 mg/g level of dietary TI, most cultured fish are able to compensate for this anti-nutrient by increasing trypsin production (Francis, Makkar, & Becker, 2001). However, Makkar and Becker (1999) reported that carp are capable of tolerating high levels of TI (24.8 mg/g) in their diet.

Tannic acid is known to cause a growth-depressing effect in tilapia and rohu fish (Jackson, Capper, & Matty, 1982). Phytate chelates with certain metal ions, such as calcium,

Table 3
Concentrations of some anti-nutritional factors in the leaves of four aquatic weeds

Component (g%)	Concentration in plants			
	S. cuculata	*I. reptans*	*T. natans*	*L. minor*
Trypsin inhibitor	1.13 ± 0.1	1.34 ± 0.9	1.53 ± 0.2	1.47 ± 0.5
Calcium oxalate	0.7 ± 0.01	0.6 ± 0.001	0.9 ± 0.2	3.5 ± 0.7
Tannin	0.9 ± 0.1	0.3 ± 0.01	0.5 ± 0.2	0.9 ± 0.3
Phytate	0.005 ± 0.001	0.004 ± 0.001	0.004 ± 0.002	0.004 ± 0.001

Values are means ± SD of triplicate determinations.

magnesium, zinc, copper and iron, to form insoluble complexes that are not readily broken down and may pass through the digestive tract unchanged, thus reducing the bioavailibility of these minerals (Maga, 1982) In addition, phytates also form strong complexes with proteins that can lead to reduced digestibility of the latter component (Richardson, Higgs, Beames, & Mcbride, 1985)

4. Conclusion

The present study has demonstrated that, among the tested aquatic weeds, *I reptans* and *L minor* could be important sources of proteins, vitamins and minerals, suitable for incorporation in fish diet Though anti-nutritional factors were found to be present in these weeds, their levels were within tolerable limits and consumption of these plants would not result in any deleterious effect on the growth of fish, further documenting their possible use for the formulation of balanced fish diet

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Addendum

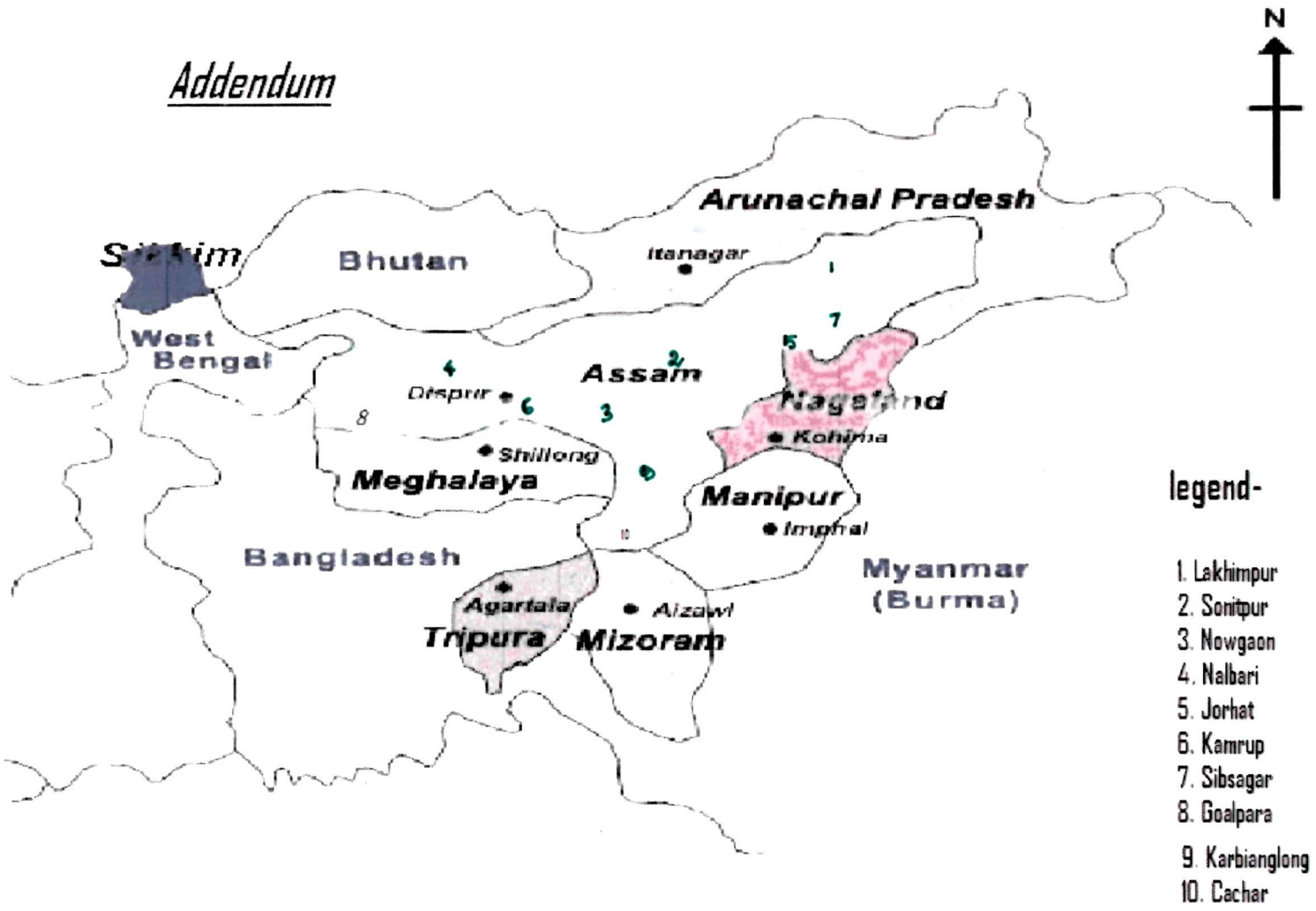

Fig. 1.2. Map of North Eastern States of India

Addendum

Table 1: Proximate composition of the individual components used for preparing fish basal diet

Component (g%)	Rice Bran	Silkworm Pupae	Mustard Oil Cake
Crude Protein	10.3	57.0	35.0
Crude Fibre	12.4	10.1	7.0
Moisture	6.9	7.8	6.6
Crude Lipid	6.1	10.1	7.5
Ash	11.5	9.8	9.2

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Addendum

Table 2: Comparison of fatty acid composition of the four aquatic weeds and four benthic algaes

Fatty acid	G % of fatty acid							
Saturated	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
12:0	-	0.06	-	-	1.3	-	-	-
14:0	0.01	0.05	0.007	0.003	1.5	4.7	2.9	-
16:0	0.01	0.1	0.02	0.01	3.2	4.0	4.1	6.4
18:0	T	0.01	0.003	0.003	1.4	7.2	6.3	10.8
20:0	T	0.04	0.02	0.003	6.5	3.7	8.7	12.5
Monounsaturated								
16:1	0.2	0.5	0.1	0.08	-	-	1.5	-
Polyunsaturated								
18:2	0.04	0.6	0.2	0.1	9.4	9.7	6.8	-
18:3					5.6	-	-	-

In the present study,

A stands for *Salvinia cuculata* (Kalita et al., 2006)

B stands for *Ipomoea reptans* (Kalita et al., 2006)

C stands for *Trapa natans* (Kalita et al., 2006)

D stands for *Lemna minor* (Kalita et al., 2006)

E stands for ***Ulva lactuca*** (Wahbeh, 1997)

F stands for ***Enteromorpha compressa*** (Wahbeh, 1997)

G stands for ***Padina pavonica*** (Wahbeh, 1997)

H stands for ***Laurencia obtuse*** (Wahbeh, 1997)

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Table 3: Fishery Resources in North Eastern States

State	Rivers/ Streams (kms.)	Beels/Lakes (ha)	Tanks/ Ponds (ha)	Paddy fields (ha)	Other suitable water (ha)	Total fishery resources in various states
Arunachal Pradesh	2000	2500 +110 (Cold water)	1000	2800	700	7110 ha and 2000 km
Assam	4820	100000	20000	20000	1517	141517 ha and 4820 km
Manipur	2000	40000	5000	40000	10000	95000 ha and 2000 km
Meghalaya	5600	394	1944	5000	3000	10338 ha and 5600 km
Mizoram	1748	32	1800	1560	-	3392 ha and 1748 km
Nagaland	1600	215	2000	10000	-	12215 ha and 1600 km
Sikkim	900	-	-	-	-	900 km
Tripura	1200	240	11038	-	-	11278 ha and 1200 km
Total	19868	143381 + 110	72782	79360		

Source: Northeastern Council (NEC) – Ten Year Perspective Plan,, 2004,18 pp

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