GROWTH RESPONSE OF *LABEO ROHITA* (HAMILTON) FRY TO SOYABEAN MEAL BASED DIETS.

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Dedicated To

My Beloved

Grandparents &

Capt. Sanjeet Bhattacharya

DECLARATION

I hereby declare that this thesis entitled "GROWTH RESPONSE OF *LABEO ROHITA* (HAMILTON) FRY TO SOYABEAN MEAL BASED DIETS" is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, or other similar title, of any other University or Society.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1 INTRODUCTION

Aquaculture with its great and as yet semi-exploited potential is playing a key role in the total world food supply. It is estimated that more than 300 species of fish are cultivated the world over. Freshwater fish culture is a widely studied area of aquaculture. During the last 15 years, immense progress has been made in developing new techniques, enabling the use of increased areas of water for fish culture(Barnabe, 1990). Asia continues to lead in world aquaculture production. Most of the Asian countries are switching over to semi-intensive and intensive culture practices for finfish and shellfish. Undoubtedly, such changes emphasize the role of supplementary feeding and the urgent need for developing low-cost diets. The latter is still in its early stages of development, particularly with respect to finfish culture. The success of aquaculture depends upon the provision of nutritionally balanced, environment friendly and economically viable artificial diets. In spite of the fact that information on the nutrition of fish is of crucial importance in furthering fish culture, it has lagged behind that of other farmed animals. Among the fishes, the nutrient requirements of coldwater fishes like chinook salmon and rainbow trout and warm water fishes like channel catfish and common carp (NRC, 1983) have been studied the most. Scanty information is available on other species like tilapia and the Indian and Chinese carps that are widely cultured in Asia. Most of the reported nutrient requirements have been determined using feeding studies with growth as the primary response parameter. Feeding trials still remain the most useful method in obtaining results that have direct application in feeding practices.

Carps are the mainstay of Indian freshwater aquaculture. Rohu ,*Labeo rohita* is the most widely cultured among the Indian major carps. An efficient nursery rearing practice is necessary for making available the maximum number of quality seed for culture purpose. Formulation of feeds, which gives good survival, growth and food conversion ratio, either to be used as supplementary feeds in ponds or as complete diets in intensive systems has also become essential. The more intensive the system, the greater is the importance of supplementary feed and higher is the proportion of feed cost to the total production cost (Hepher, 1988). Feed cost may account for upto 40-60% of the total production cost in aquaculture systems (FAO, 1983). The most important and expensive component of fish diets is protein. In general, fish species require higher levels of dietary protein for optimum growth than poultry or cattle (Tacon and Cowey, 1987). To supply the adequate quantity of dietary protein, fishmeal of marine origin, which has amino acid profile that closely matches the requirement pattern of the fish, is widely used at levels ranging from 25 to 65% in fish feeds (Tacon and Jackson, 1985). Of late, fish meal is becoming increasingly scarce, moreover it is expensive. This has made the identification of cost effective replacement for fish meal protein in aquaculture feeds an urgent necessity. Research along these lines involves the evaluation of inexpensive, readily available and nutritious protein sources, which can supply all the nutritional needs of the cultured fish. One approach involves the increased utilization of ingredients of plant origin. Over the years, the use of oil seed meals in fish diets have picked up. Soyabean meal rates high as a prime alternate protein source in fish feeds. The superior nutrient profile of soyabean coupled with the spurt in its production in many parts of the world have prompted extensive studies on its utility in fish feeds. With

advanced processing techniques, its nutritive value has been enhanced to such an extent that it is now considered a conventional ingredient in aquaculture.

The use of soyabean meal in aquaculture feeds, probably, was initiated when it was initially realized that, it contains a high level of protein (averaging 40-45%) and secondly, the amino acid profile within the protein, with the exception of methionine, is very good and consistent with the requirements of most of the cultured fish species (Lovell, 1998). The anti-nutritional factors reportedly present in soyabean meal include trypsin-inhibitor and haemagglutinins, which fortunately can be eliminated through processing techniques involving heat treatment. Advances in processing techniques, such as micronization, extrusion, and expansion have produced better quality products, which are more digestible with increased bioavailability of nutrients and reduced levels of anti-nutritional factors (Tacon and Jackson, 1985).

The successful transformation of soyabean meal, from being a by-product of the soy oil extraction industry to a conventional fish feed ingredient is an example of the potential value of plant proteins as animal feeds and the initial importance of a systematic approach in evaluating and upgrading a product. An examination of the investigative steps into the development of soyabean meal as a major fish feed ingredient can serve as a model that can be similarly applied to other non-conventional plant origin feed stuffs.

Development of quality feed with soyabean meal as an alternate protein source for fish meal will reduce the reliance of the fish feed manufacturing industry on fish meal and ensure the farmers a relatively stable, less expensive and high

quality feed. With this in view, different levels of substitution of fishmeal by soyabean meal were tested in formulated diets for *L.rohita* fry.

The present study aims at evaluating soyabean meal as a partial /complete substitute for fish meal in diets for rohu based on the growth performance, food conversion efficiency, enzyme profile and carcass composition.

2. REVIEW OF LITERATURE

2.1. Nutritional Requirements of warm water fishes: -

2.1.1. Protein: -

Supplementary feeding is an important tool for augmenting fish production. In fish nutrition, protein is the most important nutrient, because of its decisive role in the growth profile and its direct bearing on the cost of the feed.

In fish, the capacity to synthesise amino acids de-novo from carbon skeleton is limited. Most of the protein, therefore must be supplied through the diet (Hepher, 1988). In fishes, the optimum dietary requirement of protein for maximal growth is found to be 50-300% higher than that for the terrestrial animals (Cowey, 1979).

In carps, the protein requirement for the maintenance have been estimated to be in the range of 1-2 g protein /kg body weight per day (Kaushik, 1995). Satoh (1991), reviewing the protein requirement of the common carp, observed maximum growth with 10-12 g / kg body weight / day which is not much different from that recorded for other teleosts.

Detailed studies on the protein requirements of cultivable warm water fishes have been done by Dabrowski (1977) and Sen *et al.* (1978).

The dietary protein requirement for best growth rate, food conversion ratio and specific growth rate in carps was studied by Francis and Ramanathan (1995). The optimum dietary protein requirement for better growth and food conversion efficiency depends on the environmental conditions, age, size and genetic factors.

The protein requirement of fishes decreases with increase in size and age in several warm water fish species (Renukaradhya and Varghese, 1986; Khan and Jafri, 1991).

Reviewing the protein requirement of fishes, Wilson and Halver (1980) emphasised that the dietary protein requirement of fish is influenced by the protein - energy balance, the amino acid composition, the digestibility of protein and the amount of non-protein sources in the diet. Studies done by Kirchgessner *et al.* (1986) and Pongmaneerat and Watanabe(1993) have shown that the digestibility of protein from normally used practical or purified diet ingredients is high in all fishes.

The optimum protein requirement of grass carp fry is 41-43% (Dabrowski, 1977), while that of rohu (Sen *et al.* 1978) and mrigal fry (Singh and Sinha, 1981) is 45%. Catla fry require a protein content of 40% in the diet (Khan and Jafri, 1991).

The studies conducted to assess the low protein high energy diets in carps showed that the optimum digestible energy of diets containing 32 - 37% crude protein enriched with carbohydrate or lipid are better than a commercial carp diet containing higher levels of crude protein (Watanabe *et al.*, 1987).

Mao *et al.* (1985) have reported the increase in protein efficiency ratio and net protein utilisation with decreasing protein levels and with increasing levels of carbohydrate, fat, minerals and fibre.

Utilisation of protein by fish is influenced by the calorific content of the diet, with growth occurring only if the ration contains sufficient energy in proper ratio (Garling and Wilson, 1976; Cowey and Sargent, 1977). In catla, the relationship between energy and protein is calculated as 8 k cal/g protein (Singh and Bhanot,

1988). When isocaloric diets were compared in *C. mrigala* better growth performance was recorded upto protein levels of 30-35% (Hassan and Jafri, 1996).

Growth and protein utilization also depend on the level of feeding. Rangacharyalu *et al.* (1991)observed that maximum growth of *L. rohita* was obtained at 5% level of feeding. The studies on the effects of feeding frequency on protein digestibility indicated the digestibility to be maximum when the fishes were fed twice daily.

In fishes, when the dietary protein and energy levels are in excess of the requirement, the surplus protein is used for energy provision leading to fat deposition, thereby adding to the caloric value of flesh. (Lee and Putnam, 1973). Hassan *et al.* (1991) have stated that the higher calorie content in the early stages may prove advantageous to fish released in the natural environment for stock replenishment.

In carps, protein utilization was found to be better with diets containing whole protein than with mixtures of amino acids (Murai *et al.*,1981, Kaushik and Dabrowski, 1983). Dabrowski (1983) observed significant absorption of protein macromolecules throughout the digestive tract of common carp. McLean and Donaldson (1990) reported that the absorption of macromolecules was several times higher in stomachless fishes like carp, than in fish having a functional stomach. Hertz *et al.* (1992) found that orally delivered peptides were absorbed by common carp, although direct evidence of potency of such biologically or immunologically active molecules is lacking.

It was evident from the work of Sen *et al.* (1978), Singh *et al.*(1980) and Khan and Jafri (1991) that surplus levels of protein significantly depress growth in carps. These observations can be linked to a possible reduction in dietary energy available for normal growth due to the diversion of extra energy towards the deamination and excretion of excessive amounts of amino acids. (Khan and Jafri, 1991).

2.1.1.1. Essential Amino Acids:

Fishes require a well-balanced mixture of essential and non-essential amino acids. The essential amino acid requirement of warm water fishes has been reviewed by Wilson (1985 & 1989).

Feeding studies conducted for different fish species have employed the supplementation of the specific essential amino acids like arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The biological value of a protein source is decided by its amino acid profile (Khan and Jafri ,1993)

The importance of A/E ratios (relative proportion of essential amino acids to that of the sum of essential amino acids) is now well established. Even marginal deficiencies or imbalances in the essential amino acid contents can have marked adverse effects. (Kaushik, 1995). Murai *et al.* (1984) found that as the A/E ratio increases from 80 to 95, nitrogen retention per unit nitrogen intake increased from 5 to nearly 40%.

Borlongan and Benitez (1990) stated that the more closely the essential amino acid profile of the dietary protein approximates the amino acid requirement of the fish, the higher is the nutritional value of that protein. In practical feed formulation, therefore, the knowledge of the quantitative amino acid requirement of the fish is of utmost importance.

Ravi and Devaraj (1991) studied the essential amino acid needs of *Catla catla*, and observed it to be very similar to the recommended values for common carp (Ogino, 1980). Aoe *et al.* (1970) and Khan and Jafri (1993) have reported poor growth in common carp and rohu, respectively, when fed with a diet free of essential amino acids.

Comparison of data on the whole body essential amino acid profile and the requirement profile of common carp and catla showed that these two patterns are correlated (Mambrini and Kaushik, 1993). Good correlation between dietary amino acid concentration and the dietary protein level has been observed in the carp (Zebian, 1977)

It was observed by Murai *et al.* (1984) that when carps were fed a diet with amino acid as the sole nitrogenous source, urinary excretion of amino acids increased, accounting for almost 36% of the total nitrogen in the urine.

It was evident from the work done by Yamada *et al.* (1981) that increasing the feeding frequency significantly improves the amino acid availability, leading to growth rates comparable to those obtained with a whole protein diet.

Studies have also indicated that the adequate treatment (protection by coating with casein or agar, pH adjustment etc.) of synthetic amino acids incorporated into diets improves its utilization in common carp and several other fishes (Murai *et al.*, 1982, 1989).

In the case of protein sources with marked amino acid imbalances, their utilization by fish can be enhanced significantly, if incorporated in diets after supplementation with adequate amounts of essential amino acids (Murai *et al.*, 1982,1984)

A relatively constant essential amino acid requirement profile of Indian major carps was reported by Mohanty and Kaushik (1991).

In the early stages, the relative composition of the free soluble amino acid pool showed little variation and the predominant amino acids detected were leucine, lysine, valine, isoleucine, alanine and serine (Ronnestad, 1992). Identical profiles might have resulted from the hydrolysis of the common yolk protein phosvitin - corresponding to the water uptake during swelling.

NRC (1983) and Tacon and Jackson (1985) observed that indispensable amino acids like arginine, lysine, methionine and tryptophan are limiting in feed stuffs of plant origin used in fish diets.

Murthy and Varghese (1996,1997) reported that for the growth and survival of juveniles of the Indian major carp, *Labeo rohita* the optimum requirement of threonine and lysine was respectively 1.71% and 2.27% of the diet, which

corresponds to 4.18% and 5.68% of the dietary protein, respectively. Similarly, the dietary tryptophan requirement was estimated to be 1.13 % of the dietary protein.

Khan and Jafri (1993), while estimating the requirements of arginine, methonine, tryptophan and lysine of *L rohita* fry, found 1.0 %, 1.2%, 0.2% and 2.0 % of the diet to be the optimum levels for the respective amino acids.

2.1.2. Lipids:

Lipids are a major source of metabolic energy in fish. Being highly digestible, it has greater sparing action than dietary carbohydrates or proteins (Ellis and Reigh, 1991). Since dietary lipid levels have a decisive influence on the feed utilization and flesh quality, appropriate amounts of lipid need to be incorporated in fish diets.

Besides providing energy, dietary lipids serve as a source of essential fatty acids. Watanabe (1982) reviewed the role of lipids in fish nutrition, pointing out the need for essential fatty acids. Several workers have studied the influence of dietary lipids on growth and fatty acid composition of teleosts. (Yingst and Stickney, 1979; Borlongan and Parazo, 1991).

According to Cowey and Sargent (1977), Cho *et al.* (1985) and Mukhopadhyay and Raut (1996) the lipids are almost completely digestible by fish and are preferred over carbohydrate as an energy source. In fishes, lipids are a source of energy besides playing a key role in biomembrane activity.

In practical diets for many cyprinids dietary fat levels of less than 12% are considered optimal. Grass carp, however, has a much lower dietary lipid requirement of 4% (Ding, 1991).

Murai *et al.* (1985) observed that at any given crude protein level in the diet, an increase in the dietary fat content led to reduced growth and decreased feed efficiency or protein retention in juvenile carp. However, other studies have demonstrated the protein sparing effects of lipids in common carp to be similar to those in several other teleosts. (Watanabe *et al.*, 1987)

According to Berg and Storebakken (1991) and De Silva *et.al.* (1991) growth ceased in fishes fed a diet having higher than optimal protein to fat ratio. Jafri and Farooq (1995) observed a reduction in the growth of *C. mrigala* when fed with higher than optimal levels of lipid.

Growth in common carp was unaffected by the increasing lipid content upto an inclusion level of 20 %, though the body lipid deposition, especially the visceral fat deposition increased with the increased dietary fat levels (Murai *et al.* 1985).

Studies by Takeuchi *et al.* (1979) relating to the quality of dietary fat suggest that hydrogenated fish oil or beef tallow could be added (6%) as an energy source in diets of common carp, provided high quality marine fish oil was also added.

2.1.2.1. Essential Fatty Acids:

Studies on the quantitative essential fatty acids requirement of carps have been inconclusive. It has been difficult to induce signs of fatty acid deficiencies in young carp even after long term feeding with fat free diets, although it is found that

the n-3 highly unsaturated fatty acids are required for normal growth and survival, especially during the larval development. Lipids are the main source of energy from the gastrula stage onwards in fish embryos. (Vetter *et al.*, 1983). The loss of lipid reserves of larval fish on food deprivation, underscores its primary role (Ehrlich, 1974)

Eicosapentaenoic acid (20:5 n-3) is one of the most essential fatty acids in fish (Kanazawa *et al.*, 1979). Kanazawa (1985) suggested that the rapidly growing larvae of fish need relatively large amount of extraneous eicosapentaenoic acid .

2.1.2.2. Phospholipids:

In the intestinal lumen, which is an aqueous environment, the phospholipids are known to act as emulsifiers. According to Kanazawa (1993) and Sargent *et al.* (1994) the need for phospholipids in certain fishes are significantly higher during the larval stages.

Radunz --Neto *et al.* (1994) observed that the addition of phospholipids was important to obtain initial survival and growth of first feeding carp larvae, as also observed by Szlaminska *et al.* (1993) in the gold fish, *Carassius auratus*. A diet with 2% phospholipids provided better larval performance than a diet with only 1 % phospholipid (Radunz -Neto *et al.*, 1995).

Phospholipids, therefore could be of significance in allowing the absorption of dietary lipids like cholesterol and triglycerides, as suggested by Kanazawa (1993) and Koven *et al.* (1993).

It is noteworthy, that fish larvae always have different types of phospholipid classes at their disposal during embryonic development and subsequent stages in their natural feeding habitat (Kanazawa ,1985). These phospholipids originate from the egg reserves (Fraser *et al.*, 1988) and later on from the live food organisms (Teshima *et al.*, 1987)

Kanazawa (1993) has observed that fish larvae have a limited ability to synthesise the phospholipids at a rate sufficient to fulfil the demand for building and renewal of cellular membranes. Radunz- Neto *et al.* (1994) and Geurden *et al.* (1995) observed that the adult fish did not exhibit any adverse effect on growth, even

when fed with a phospholipid deficient diet . Similarly, larval sensitivity to phospholipid deficiency is also restricted to the first 2-3 weeks of feeding.

2.1.3. Carbohydrates:

The dietary utilization of carbohydrate by carps has received much attention, on account of the herbivorous nature of carps.

Omnivorous and herbivorous fishes are known to utilize high carbohydrate diets (Shimeno *et al.*, 1981). Furkawa and Ogaswara (1952) observed that the addition of 5% of cellulose enhanced nitrogen retention and growth in common carp.

According to Bergot *et. al.*(1986) cellulose was not at all digested by rainbow trout and common carp , and no significant activity of cellulase could be detected in grass carp and gold fish too.

The utilisation of the carbohydrates by fishes was studied by Furuchi (1983) and it was presumed that carp (*Cyprinus carpio*) is a potential diabetic, which can be attributed to the absolute insufficiency of insulin. The differences in the ability to utilise carbohydrate among the different species is mainly caused by the differences in the activity of glycolytic and glycogenic enzymes, which is controlled by insulin secreted after feeding (Tacon and Jackson ,1985) .

Some information is available on the effects of dietary carbohydrate levels in the Indian major carps (Sen *et al.*, 1978; Erfanullah and Jafri, 1993; 1995). The common carp utilises complex polysaccharides more efficiently than simple sugars, unlike salmonids and eels (Furuchi and Yone, 1980; Shimeno *et al.*, 1981).

The utilization efficiency of carbohydrate in carps increases with increased feeding frequency. Murai *et al.* (1983) reported improvement in the utilization of glucose and maltose in common carp when the feeding frequency was increased to four times a day . Comparing the efficiency of various conventional and formulated feeds for rearing carp spawn, Kotwal *et al.*(1982) concluded that high protein and low carbohydrate diets lead to better growth.

Dhage (1968) observed a compensatory increase in the secretion of pancreatic amylase in common carp when fed wheat and also described marked amylase activity in Indian major carps. Kawai and Ikeda (1972) also pointed out increasing levels of the carbohydrate digesting enzymes amylase and maltase in carps when fed with carbohydrate rich diet. Smith (1989) studied the significance of high levels of carbohydrate metabolising enzymes in the intestine of the fishes particularly in herbivores like carps where the utilization of carbohydrate was optimum. Kheyyali *et al.* (1989) measured the activities of several enzymes of intermediary metabolism in the hepatopancreas of carp, where the dietary carbohydrate promoted an increase in the activity of glycolytic and lipogenic enzymes.

Das and Tripathi (1991) in their studies on fingerling and adult grass carp, observed the pattern of distribution of digestive enzymes to be related to the type of diet ingested by the fish. They concluded that the presence of cellulase activity suggests the possibility of providing cellulose as an ingredient in the diet.

Erfanullah and Jafri (1995) obtained maximum growth (100%) in terms of percentage live weight gain with sucrose followed by fructose (85%), glucose (78%)

and dextrin (71%) diets in *L. rohita*. They also observed a relatively increased body fat deposition in rohu with sucrose-based feed, presumably due to lipogenesis from the dietary carbohydrate source.

Mao *et al.* (1985) observed the optimum carbohydrate requirement to be 0.95-1.28g for juvenile grass carp. In carp 30% was found to be the optimal level of dietary carbohydrate. Higher levels (40%) led to growth retardation (Furuchi and Yone, 1980). Wang and Song (1984) have described the pattern of reduction in the rate of digestion of proteins in carps when the carbohydrate level was increased from 30%- 43%.

Hassan and Jafri (1996) observed the optimum weight gain in *Cirrhinus mrigala* at a dietary carbohydrate level of 34% , when levels of 20% to 40% were studied.

2.1.4. Energy:

Energy is essential in that it contributes to the utilization of all nutrients in a diet. It is measured physically as calories of heat and physiologically as change in body weight (gain or loss), oxygen consumption and metabolic activity. Hastings (1979) observed that in fishes gross food conversion efficiency and energy efficiency are closely related.

Precise information on the energy requirement for maintenance and growth of cyprinids is scarce. A number of studies have dealt with the basal metabolic rates as affected by body weight and water temperature (Kaushik, 1995). Based on such studies of Yamamoto *et al.* (1978), Hepher (1988), Cui and Liu (1990), and

Chakrabarty *et al.* (1992) it was concluded that the basal metabolic rate and body weight closely relate with the temperature.

Weight specific basal (resting) metabolic rates of carps were found to decrease with a weight exponent of 0.20. Similar to other teleosts, both fasting metabolic rates and maintenance energy requirements of carp are affected by water temperature. Analysis of data from the literature cited above shows that the resting metabolic rates of carp at low water temperature (10-17^{0}C) are extremely low. But Schwarz *et. al.* (1983) found that the maintenance energy needs of common carp were reduced at low temperature: 19 and 45kj DE/kg $BW^{0.75}$/day at 10^{0}C and 20^{0}C, respectively.

Dietary energy also influences the carcass composition of fat (Zeitler *et al.*, 1984). A positive correlation was noted in *Cirrhinus mrigala* between the dietary energy level and carcass fat content and inverse relationship between the calorie density and carcass protein or ash content (Hassan and Jafri, 1996). A reduction in growth rate with dietary energy levels exceeding 367k.cal/g. was also noticed.

2.1.5. Vitamins:

In general, fishes require four fat soluble vitamins (A, D, E, and K) and eleven water soluble vitamins. Of this, thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, folic acid and vitamin B_{12} are required in small quantities and they function as co-enzymes.

Myo-inositol, choline and biotin are required in higher quantities. Vitamin requirement in fish varies with species, age, size, growth rate and physiological

conditions, requirement being particularly more at the time of wound healing and stress. Some fishes have the ability to synthesise the vitamin from glucose substrates or amino acid, while in some others intestinal micro flora can synthesise vitamins (Hepher, 1988; Halver, 1989). These sources reduce the dependence on dietary sources for vitamin in fishes.

Dabrowski *et al.* (1988) observed that the vitamin C requirement of common carp (*Cyprinus carpio*) fry ranges from 37.8-62.3 mg/g unlike the adults of the species which need lower concentrations 20.5-40.2 mg/g of ascorbate.

Common carp has the ability to oxidise gulonolactone to 2- keto gulanolactone (Yamamoto *et al.*, 1978) which subsequently forms ascorbic acid spontaneously. However, scurvy occurred in common carp when fed with the vitamin depleted diet (Kitamura *et al.*, 1965). There appears to be some disagreement regarding the exact requirement of ascorbic acid in common carp (Dabrowski *et al.* ,1988).

Takeuchi *et. al.* (1989) has described the requirement of grass carp fingerlings for alpha-tocopherol and deficiency syndromes in fishes fed with vitamin E depleted diet.

Wang *et al.* (1989) reported the requirement of choline in the diet of fingerlings of black carp.

2.1.6. Minerals:

Dietary requirement of minerals in fishes have been reviewed by Cowey and Sargent (1972) and Nose and Arai (1976). In general, minerals required by fish

include calcium, magnesium, phosphorus and a number of trace elements like iron, copper, manganese, selenium, zinc, chromium, cobalt, boron and molybdenum.

Fishes have the ability to absorb the inorganic elements from the surrounding water as well as from their diet. This ability to exchange inorganic ions across the gill membranes and the body surface makes it difficult to elucidate the nutritional functions of dietary minerals (Nose and Arai, 1976). Ions absorbed from the external medium have nutritional and osmoregulatory implications.

Even though boron and molybdenum were shown to improve growth in common carp (George, 1970), the species was found to be less sensitive to the depletion of dietary molybdenum than trout (Ogino and Kanizono, 1975).

Common carp showed good growth on a purified diet containing calcium at levels as low as 30 mg/100g if adequate quantities of phospholipids are provided in the diet (Ogino and Kanizono, 1975).

Phosphorus requirement (estimated at 0.6-0.7 % of dry diet for carp) is of significance, because of its potential importance in eutrophication, especially in static water culture systems. It has been estimated that the assimilation of phosphorous from fish meal has been found to be lower in carp than in rainbow trout (Satoh, 1991). According to Satoh (1991), such differences probably originate from the lack of gastric digestion in the stomachless carp.

Satoh *et al.* (1989) reported that an excessive dietary supply of (7 %) tri-calcium phosphate reduced the absorption of Zn, Mn and phosphorus by nearly 50%. Satoh *et al.* (1983) observed that the depletion of manganese (Mn) had a much

greater growth-depressing effect than the removal of Mg, Cu, Zn or Co. They also observed that Zn deficiency had a much less deleterious effect in common carp than in rainbow trout. Excess dietary Zn also decreases absorption of Zn in carp. (Brafield and Koodie, 1991).

2.2. Early biology of fish related to feeding: -

The initial nourishment for the developing fish egg is drawn from the egg yolk. The nutrient content of the eggs varies with species, but the dynamics of yolk absorption are similar among groups. Embryonic growth in fish depends on the composition of yolk and its digestion by the syncytium or analogous tissue, the absorption and transport of yolk nutrients to the developing tissue for somatic organisation and to meet the metabolic demands for survival. An increase in the protein component with growth is observed in all the three species of Indian major carps until 48 hr.which may be due to the morphogenetic processes undergone by the larvae (Heming and Buddington, 1988). Love (1980) suggested that the increase in the levels of free amino acids during the early development reflects the protein synthetic activity. Jurca *et al.*. (1975) also observed quantitative changes in the soluble proteins during the early development in these three species of carps.

The decline in lipid levels with development observed in all the three species suggests that lipids are utilised to meet the energy needs of the growing larvae (Watanabe, 1982).

In rohu, the glucose levels in the developing larvae increased up to 48 hours but showed a decline after 72 hours (Sharma *et al.*,1990)

Carbohydrates, lipids and proteins are consumed prior to hatching, while the latter two are catabolised also for hatching. Growth during the endogenous period is also influenced by abiotic factors such as temperature regimes, oxygen availability, pH and photoperiod.

As and when the yolk reserves are completely utilized, the feeding capabilities are developed and therefore larval survival ultimately depends on availability of quality food in sufficient quantities. The rapid development of the mouth, development of alimentary tract, enzyme systems etc. are followed by the exogenous nutrition.

The nutritional requirement of fish embryos and eleuthero - embryos has not yet been identified. Nevertheless, they can be expected to match the composition of the yolk that caters for the needs of the pre-feeding fish. As the physiological capabilities of larvae are limited, specific diets are required, be they live food organisms or formulated feeds.

2.2.1. Larval nutrition in cyprinids:

Potential specific growth rates and protein synthesis rates are much higher in larvae than in juveniles or adult carp (Fauconneau, 1984). Recently, multidisciplinary approaches (enzymatic activities, histocytology of the liver, DNA-RNA ratio, etc.) have been developed parallel to zootechnical performance (survival, growth, food conversion ratio, etc.) to assess the quality of larval feeds (Storch *et al.*, 1983).

It is accepted that the proteolytic activity has an influence on the larval growth (Ueberscher, 1988). The proteolytic activity depends on the nature of the food, the development stage and the species concerned. According to Dabrowki and Glogowski (1977) and Lauff and Hofer (1984), the use of natural food to start larval rearing could contribute up to 80% of the total proteolytic activity, due to protease in the natural food.

Bryant and Matty (1981) found that common carp larvae with an initial body weight ≥ 9.5mg have a better ability to utilise and survive on artificial diet alone than smaller larvae. But Dabrowski (1979) opined that the larvae of cyprinids could be transferred directly to a dry diet at a still smaller size (wet weight 5-6mg).

2.2.2. Changes in body composition:

Changes in crude chemical composition, an important aspect of fish quality, result from stimulation or alteration of the turnover and the retention of the chemical components, *viz.*, proteins, lipids, carbohydrates and mineral with normal and altered development of the specific tissues (Fauconneau *et al.*, 1995). Changes in body composition over a growth period are partly attributed to body size and growth rates and partly to dietary factors. In general, protein content of the fish (percentage wet matter) and amino acid profiles show little variation and are little affected by the size of the fish or by nutritional factors. Data obtained with carp (Schwarz and Kirchgessner (1988), Foken and Becker (1993) confirm this general observation.

Similarly, in well growing carp, the mineral composition of the whole body is also relatively stable and is little affected by dietary changes in the protein and

energy levels (Kirchgessner and Schwarz, 1986). On the other hand, fat content increases with increasing size and the growth rates are affected by dietary factors and are inversely related to water content (Zeitler *et al.*, 1984, Foken and Becker, 1993). An excess supply of non-protein energy appears to induce a greater deposition of fat even in the fingerlings of common carp (Murai *et al.*, 1985).

Improvement in growth and feed utilization can only be achieved through a quantitative knowledge of the digestibility, biological value and anti-nutritional componenet of the protein ingredients, besides the recognition of possible nutrient interactions (Kaushik, 1995).

Diet has often been shown to have profound influence on the body composition of fish. But the changes in the level of dietary nutrients do not affect the body protein and ash because their level in the body tissue is specified by the genetic code. (Brett *et al.*, 1969; Buckley and Groves, 1979; Huissman *et al.*,1979).

Among the various constituents, carcass fat has been reported to show the greatest fluctuations in carp (Zeitler *et al.*, 1984). A positive correlation was noted in *Cirrhinus mrigala* between the dietary energy level and the carcass fat content (Hassan and Jafri, 1996). They also observed an inverse relationship between dietary calorie density and carcass protein or ash content. When carcass protein in mrigal was calculated on a fat free basis no notable difference occurred among the fish groups receiving various experimental diets. The negative correlation between the dietary energy levels and the carcass protein or ash content may be linked to the diluting effect of carcass fat. This becomes clear from the inverse relationship found between carcass fat and protein or ash.

Buckley and Groves (1979) and Jafri and Anwar (1995) observed the highest lipid deposition (31%) in *C. mrigala* when fed with 7% dietary lipid. The gross energy retention increased with increasing dietary lipid levels, the maximum (78%) being in fish fed a diet containing 13% lipid (Jafri and Anwar, 1995).

The inverse relationship between body fat and moisture noted in mrigal fingerlings fed variable levels of dietary lipid was also reported in common carp (Takeuchi *et al.*, 1979 ;Viola *et al.* ,1988). The body constituents were found influenced by the dietary energy but no effect of dietary protein on body composition was evident in carps (Hassan *et al.*, 1990). They further observed the negative correlation with dietary energy, irrespective of the protein level in the diet. The whole body moisture followed a pattern of changes similar to that of body protein, in relation to body fat. An inverse relationship also existed between body fat and ash. The final moisture levels of fish were lower and the fat levels higher as compared with those of the initial moisture content of fish. Crude fat content showed a slightly increasing trend, while ash content exhibited a slightly decreasing trend (Yamamoto *et al.*, 1997).

The chemical composition of flesh mainly depends on the composition of the diets and digestibility of the nutrients (Dabrowski and Kozak, 1979; Jayaram and Shetty, 1980a). An inverse relationship between moisture and protein was observed in catla (Nandeesha *et al.*, 1989) and between moisture and fat in tilapia (Jauncey, 1982; Edwards *et al.*, 1985).

During the ontogeny of carp, there is an early increase in the protein content of the whole body carcass and muscle (Takeuchi *et al.*, 1979; Hossain and Jauncey,

1989). Only minor changes were observed in the protein component even if the carps were starved (Shimeno *et al.*, 1990) or fed a deficient or imbalanced diet (Viola *et al.*, 1992).

The amino acid composition of protein synthesised in the different life stages has been found to be very similar for different species (Zeitler *et al.*, 1984).

The fat content of whole body and flesh increases regularly with increase in the size of carp, and is associated with decrease in water content as a general law for living organisms (Fauconneau *et al.*, 1995).

The most effective compounds in the diet that stimulate nitrogen retention are lipids and to a lesser extent carbohydrates. Another factor affecting fat content is of genetic origin. (Takeuchi *et al.*, 1979; Viola *et al.*, 1981; Zeitler *et al.* (1984).

The fatty acid composition and especially the content of polyunsaturated fatty acids are essentially controlled by the fatty acid composition of the dietary lipids (Viola *et al.*, 1981,1988).

2.3. Protein sources of animal origin:

The development of aquaculture techniques for any organism depends mainly on the availability of appropriate protein rich feed. There are many reports on nutrition and feed conversion studies, involving the use of various animal protein sources in fish diets (Jeyachandran and Paulraj, 1976; Siddique *et al.*, 1988)

2.3.1. Fish meal:

Aquaculture of many fish species is dependent upon the use of fish meal as the major, if not sole, dietary protein source (Rumsey ,1994). It is a rich source of energy and minerals, and is highly digestible and palatable for most fishes (Lovell, 1989). Several experiments were carried out to determine the optimum protein requirement of fish especially when fed on fish meal as a sole protein source. Wee and Tacon (1982) reported the requirement of juvenile snake head *Channa striatus* to be 52%, while it is 47% for juvenile American eel *Anguilla anguilla* (Tibbets *et al.*, 1999).

The utility of tilapia meal for salmonid diets was investigated by Foltz *et al.*. (1982). They observed that upto 40% replacement of herring meal by tilapia meal is possible without any significant difference in growth and weight gain.

Wood *et al.* (1985) found low temperature dried fish meal to be a rich source of protein for the mirror carp *Cyprinus carpio*. Kamaruddin *et al.* (1989) conducted a digestibility study of red tilapia on six different feedstuffs, fish meal, shrimp meal, copra meal, corn meal, soyabean meal and rice bran. The results showed that, despite low digestibility of carbohydrates, fish meal was the second most digestible feedstuff (99.45%) after rice bran (99.85%). Similarly, fish meal was found to have the highest apparent digestion coefficient for energy (95.41%), while soyabean meal (77.38%) was found to have the lowest (Law1984, 1986).

2.3.2. Blood meal and meat meal:

Blood meal-ruminant mixture has been used as a partial or complete substitute for fish meal in commercial catfish rations. The results indicated no significant difference in the protein conversion efficiency and food conversion ratio (Reece *et al.*, 1975). Asgard (1984) found that slaughter blood upto 20% to 30% of dietary protein had no negative effect on growth, health or organoleptic characters of salmonids. Luzier *et al.* (1995) reported the use of spray dried blood powder to replace the fish meal in diets for juvenile rainbow trout. They recommended 22.7% blood powder and 17.0% fish meal as model feed formulation for rainbow trout diet.

In studies conducted to evaluate the efficiency of meat meal based diet and fish meal based diet Jena *et al.* (1998) did not observe any differences in the growth and survival of rohu fry showing the possibility of substitution of fish meal and meat meal for carp seed rearing.

Bull *et al.* (1988) found that slaughter house waste, vegetable waste, poultry farm waste and press cake waste fared equally effective in diets for common carp. These diets did not differ significantly with respect to percentage weight gain, protein efficiency ratio, specific growth rate and food conversion ratio.

2.3.3. Shrimp head waste:

Robinette and Dearing (1978) and Kiran (1984) elucidated the value of shrimp by products meal as an alternate protein source in the diets of channel catfish and mullet *(Liza parsia).* However, the results indicated poor growth performance and the reason attributed was that shrimp byproduct meal is neither digestible nor as

palatable as fish meal and it is deficient in some unidentified growth factors. Contrary to this, Pfeffer and Meske (1978) obtained good growth and survival in carps fed feeds based on casein and shrimp meal. Similarly Afolabi *et al.* (1980) reported better protein efficiency ratio when shrimp and fish waste were used. Kamaruddin *et al.* (1989) tested shrimp meal,fish meal, soyabean meal and copra meal and reported that up to 99% shrimp meal and 96% fish meal were well digested by red tilapia.

Prawn head meal did not fare well as a fish meal substituent for *C.chanos* fry as revealed by poor growth, food conversion ratio and biochemical composition (Carolin,1991). This may be because of the low content of protein in prawn head waste as pointed out by Anil (1981), Srikanth (1986) and Kiran (1984). However there are reports of better growth performance when shrimp head waste is incorporated at lower levels in fish feeds. Supplementing fish meal with shrimp head meal @ 8% of dietary protein promoted good growth and survival of milkfish fry (Lim *et al.*, 1979). Similar observations have been made by Fowler and Banks (1976), who recorded a growth promoting effect of shrimp waste at 5% level and growth inhibition at 20%. Poor growth of carp fed with diets having shrimp waste as the major source of protein (35%) was also reported by Anil (1981).

Fagbenro and Bell O-olusoji (1997) reported the incorporation of the fermented shrimp head silage as a protein supplement into pelleted semi-purified diets for catfish (*Clarius gariepinus*). They recommended the use of shrimp head silage upto 20% of the dietary protein, which resulted in higher growth than the fish meal protein diet. Carver *et al.* (1993) investigated the possibility of using raw and

ensiled shrimp head and squid viscera, co-extruded with 47% solvent extracted soyabean meal as a valuable, high quality and inexpensive feed for rainbow trout. The food conversion and protein efficiency ratios and fast growth rates on these diets compares favourably with those obtained when fed commercially available catfish diets.

2.3.4. Squid meal and silkworm pupae:

Squid meal has been used successfully to replace rice bran in shrimp feeds in Asia (Devendra, 1988). Squid, shrimp and mussel meat extracts are known to be a good source of feeding stimulants, especially for carnivores like eel, red sea bream and sea bream and bass (Paulraj, 1989). When squid was used as feed for salmonids and rainbow trout, increase in growth was observed (Asgard, 1984).

Silkworm pupa is extensively used in carp diets in China and Japan (Hora and Pillay, 1962) and is known to induce good growth in carps. Superior growth of catla on silkworm pupa diet appears to be due to its high fat content, which spares protein for growth. Silkworm pupa is known to contain some attractants and appetite stimulants (Ina, 1976; Tsushima and Ina, 1978) which are known to result in better acceptability of the pellets and hence good growth. In studies employing catla (*Catla catla*) rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*), Jayaram and Shetty (1980 b) recorded better growth in catla and common carp with a diet containing non-defatted silkworm pupa. In contrast to non-defatted silkworm pupa, defatted silkworm pupa based diet induced poorer growth of catla (Nandeesha *et al.*, 1989, 1990). Habib *et al.* (1994) observed that a 100% silkworm pupa diet was the least

expensive one for *Clarius batrachus* and resulting in the highest production at the lowest cost.

2.3.5. Krill meal:

Luckowicz (1978) investigated the possibility of replacing fish meal in carp diets with krill (*Euphausia superba*) meal. Wojino and Dabrowski (1984) reported the use of krill meal for feeding the year old rainbow trout *(Salmo gairdneri)*. Lou and Chen (1980) evaluated the use of krill meal as a sole protein source for young tilapia. It was concluded that the protein efficiency ratio, net protein ratio and net protein utilisation were comparable to that of fish meal-based diets.

2.3.6. Other animal protein sources:

Supplementation of various meals to fish meal diets, was studied by Akiyama *et al.* (1984), wherein fish meal was supplemented with silkworm pupae powder, beef liver and krill meal or earthworm powder in diets for chum salmon fry.

Use of terrestrial snails in the feed of *Oreochromis mossambicus* fingerlings have been reported by Shafiei and Costa (1989). In this study, fish fed with the flesh of the snail *Achatina fulica* showed higher growth rate than when fed chicken feed.

Abdulghani *et al.* (1997) reported the replacement of 10% soyabean meal with poultry meal in the diets for blue tilapia (*Oreochromis aureus*). Least growth was reported when soyabean meal was used as the sole protein source, but the feed mixture of soyabean meal and blood meal proved better compared to groundnut oil cake and soyabean meal when fed to mudfish *Clarius anguillaris* (Eyo, 1991). Ozdemir and Erkoyunca (1988) reported the use of hydrolysed leather meal in feeds

for the mirror carp *Cyprinus carpio*. Viola (1977) observed that poultry meal and feather meal could serve as a good substitute for fish meal in carp diets. However, Kerns and Roelofs (1977) found that the growth rate and food conversion efficiency were directly related to the level of poultry meal, which resulted in faster growth rate in fingerling of common carp as compared to that on a traditional feed mixtures of groundnut oil cake and rice bran. The importance of processed piggery waste as a feed material for common carp has been reported by Watson (1985).

Fagbenro *et al.* (1993)has reported the use of some amphibian meals (frog meal, toad meal, and tadpole meal) in the diets of mudfish. Poor protein digestibility was observed in the fishes fed with toad meal.

Hilton (1983) recorded reduced growth rate and feed utilization in rainbow trout (*Salmo gairdneri*) when high levels of worm (*Eudrilus eugeniae*) meal were included in the diet. However, there was no significant reduction in growth at an inclusion rate of 23%. Stafford and Tacon (1984) reported that worm (*Dendrodrilus subrubicundus*) meal at 71% led to significant decrease in growth and feed utilization in rainbow trout, but when included upto 36% gave growth almost comparable to the fish meal based control diet. Further trials on incorporation of earthworm (*Eisenia foetida*) meal in rainbow trout diets indicated no adverse effect at inclusion levels of 5 to 30%. Nandeesha *et. al.* (1989) also reported that incorporation of worm meal beyond 25% may not result in profitable growth in common carp (*Cyprinus carpio*). Velasquez *et al.* (1991) evaluated worm meal(*Eisenia foetida*) in the diets of rainbow trout (*Salmo gairdneri*). Significant decrease in lipid content was observed with increasing worm meal inclusion..

2.4. Protein sources of plant origin:

Protein sources originating from plants are the only products for which increased production in the future is likely. For the fast growth of the animals, protein that contains essential amino acids, in the same balance as those found in the body protein of the growing animals is desirable (Nose, 1979). However, since no single plant or animal source can provide all the essential amino acids at the adequate levels, a mixture of proteins is usually used in feed formulation. In recent years, considerable amount of research on partial or complete substitution of dietary fish meal by other plant protein sources has been conducted (Liquet, 1971). Due to the lower price and high market availability of vegetable protein sources with high protein content, the inclusion of these feedstuffs in freshwater fish feeds has increased substantially. Of late, it has been possible for the channel catfish industry to greatly reduce its dependence on fish meal. Most commercial catfish growout feeds now contain 0- 4% fish meal compared with >30 % in feeds produced prior to 1975. This reduction was achieved largely through the increased use of soyabean meal in catfish feeds and the substitution of fish meal by bone meal and blood meal.

Solvent extracted cotton seed can replace 20-35% of fish meal, rape seed 28-42% and sunflower 70% of fish meal in the diets of *Oreochromis mossambicus* (Jackson *et al.*, 1982), *Oreochromis niloticus* (Davis *et. al.*, 1989) and *C. carpio* (Shiau *et al.*, 1990), respectively.

Full fat soyabean meal can replace 58% and cottonseed meal 21% of fish meal in the diet of *Tilapia nilotica* without significant decrease in growth (Shiau *et al.*, 1990).

Roasted Indian mustard seed cake replaced upto 20% fish meal and groundnut oil cake up to 17% of fish meal in the diets of *C. carpio* (Jackson *et al.*, 1982). Autoclaved mustard oil cake improved growth performance and food utilization in the carp (Hossain and Jauncey, 1990). The baseline information in the potential use of legumes viz., pigeon pea (*Cajanus cajan*), mungo (*Phaseolus radiatus*), kidney bean (*P. vulgaris*) and soyabean (*Glycine max*) as protein sources for *C. chanos* is given by De-la pena *et al.* (1987). Martinez-palacois *et al.* (1988) and De Silva and Gunasekhara (1989) have tried jack bean (*Canvalia ensifarmis*) and green pea (*Phaseolus aureus*), respectively, in tilapia feeds.

Dried powder of *Nymphoides* and *Spirodella* mixed with rice bran has been found to be useful in rearing the fry of carp (Patnaik and Das, 1979). Water hyacinth (*Eichhornia crassipes*) has been successfully used in the feeds of *O. niloticus* (Edwards *et al.*, 1985). Rath and Dutta (1991)have reported the use of water hyacinth as a protein supplement in the feed of common carp and as an ingredient in the feed of *Clarius batrachus*. Faster growth rate and better food conversion efficiency was observed when *Azolla pinnata* was fed instead of fish meal based diet to Nile tilapia (SEAFDEC, 1984; Alamazan *et al.*, 1986). A mixture of plant proteins *Ceratophyllum demelsum, Eichhornia crassipes* and *Eleocharis ochrostachys* pellets have a potential as a partial replacement for fish meal in the diets for carps. (Teshima *et al.*, 1990).

Trials on the use of ipil ipil *(Leucaena leucocephala)* leaf meal for rearing tilapia to marketable size have given better results compared to the groundnut oil cake and rice bran based diets (Wee and Wang, 1987; Olevera Navao *et al.*, 1990). Hassan *et al.* (1990) also tried to evaluate *Leucaena* and water hyacinth leaf meal as dietary protein sources for the fry of Indian major carp, *Labeo rohita* (Hamilton).

Cassava (*Manihost esculenta*) leaf meal has been demonstrated to be a viable partial dietary protein source for Nile tilapia (Cruz and Fabian, 1980; Ng and Wee, 1989).

Hasan *et al.* (1991) evaluated the use of 20% to 40% of mustard, sesame and linseed oil cake as a partial substitute for dietary fish meal protein. Jackson *et al.* (1982) have shown that use of a combination of several plant protein sources is more advisable than a single form, as the protein sources have different limiting amino acids. Their experiments with Nile tilapia indicate that cotton seed, rape seed and sunflower seed promoted reasonable growth when provided at 50% of the total dietary protein, while copra, soyabean meal, groundnut oil cake might have yielded more favourable protein efficiency ratio in the diets, if they have been supplemented with limiting amino acids.

2.5. Replacement of animal protein with soyabean meal:

Various locally available plant protein sources have also been incorporated in fish diets instead of fish meal. Among these, soyabean meal appears to have been widely used as it is an economically viable oil seed protein available the world over. (Anon, 1978). Its amino acid profile is one of the best of all protein rich plant feed

stuffs, meeting the essential amino acid requirements of fish (NRC, 1983). The quality of a particular dietary protein source depends both on its digestibility and on the amino acid profile. Apart from the amino acid composition which is often unbalanced (NRC, 1981; Tacon and Cowey, 1985) endogenous anti-nutritional factors are the main factors limiting the use of high levels of soyabean meal in fish feeds (Satoh, *et al.* 1989). Many of these factors, such as protease inhibitors, can be inactivated by moist heat treatment, depending on the feed particle size, duration of the heat treatment and moisture conditions employed (Grant, 1989). Soyabean products are commonly used as a dietary protein source in the production of rainbow trout (Reinitz and Hitzel,1980) having also been included at low levels in diets for the production of large salmonids (Krogdahl, 1989) and catfish (Liquet, 1971). Preliminary results of its use in carp diets have been conflicting, as several workers have reported a reduction in both growth and feed conversion efficiency when higher levels of soyabean meal were used in place of fish meal (Hepher *et al.*, 1979) in diets of rainbow trout (Dabrowski *et al.*, 1989) and channel cat fish (Wilson and Poe, 1985).

However, the successful use of soyabean meal as a complete replacement for fish meal has been reported in *Oreochromis aureus* (Davis and Stickney, 1978) and as a replacement of 25-75% of the dietary protein in *Sarotherodon mossambicus* (Viola and Arieli, 1982).

Jafri and Farooq (1995) evaluated the protein digestibility of some low cost feedstuffs in fingerling Indian major carps. The highest digestibility (93-94%) was

noted for soyabean oil cake. Digestibility of protein from fish meal and slaughterhouse offal was relatively low ranging from74-76%.

Studies have been carried out to evaluate the effect of diets having soyabean meal in combination with squilla meal based diets enriched with sardine oil on the growth and organoleptic quality of common carp. Nandeesha *et al.* (1989) observed higher growth, yielding an increase of 25% body weight, in fishes fed 30% soyabean meal than those fed a fish meal based control diet. Effect of blood meal substituted soyabean diet on the growth rate of mudfish *Clarius anguillaris* fingerling was reported by Eyo and Akande (1990). It was found that 50% blood meal and 50% soyabean diet gave best mean weight gain, specific growth rate, food conversion ratio and protein efficiency ratio. Nandeesha *et al.* (1989) reported the use of defatted soyabean meal and non-defatted and defatted silkworm pupa in the diet of *Catla catla*. Blaziak (1989) studied the utilisation of wheat meal, meat meal and soyabean meal in carp diets and reported best food conversion ratio with 20% soyabean meal. Davis and Stickney (1978) observed the growth trend to be similar in tilapia (*Tilapia aurea*) when fed either soyabean protein diet or fish meal protein diet.

In India, studies on the utilisation of soyabean as a feed ingredient are scanty and pertain to the fingerling stage onwards (Sehgal *et al.*, 1976; Bhat *et al.*, 1986). Chakrabarty *et al.* (1973) demonstrated superiority of soyabean in combination with animal protein source over the traditional feed mixture of groundnut cake and rice bran in their experiments on Indian major carp spawn.

Eyo (1991) fed fingerlings of mudfish *C. anguillaris*, isonitrogenous feed mixtures containing blood meal, fish meal, groundnut oil cake and soyabean meal.

Least growth was recorded in the diet containing soyabean meal as the sole protein source.

Alternative dietary protein sources for use in the diets of farmed *Orechromis* spp. were reviewed by El-Sayed (1999), with emphasis on fishery by products, terrestrial animal by products, oilseed plants, aquatic plants, single cell proteins, grain legumes, plant protein concentrate and cereal by-products. The results obtained indicate that the growth rate and the rate of survival were significantly higher in the diet containing more than one protein source. Similar results have been obtained by Fernandez *et al.* (1999) by replacing fish meal with pea-seed meal, defatted soyabean meal and micronized wheat.

Several authors have also observed the poor palatability and the existence of anti nutritional components as affecting dietary utilization of soyabean meal by salmon (Fowler, 1980).

Feeding trails were undertaken using rainbow trout, to evaluate the efficiency of individual and multiple amino acid supplements in diets where soyabean meal was used as the principal protein source (Davis and Morris, 1997). Fish meal served as the reference protein. Solvent extracted soyabean meal could effectively replace approximately 66% of this protein source, but interestingly single or dual amino acid supplementation led to growth even inferior to that of the reference protein (Refstie *et al.*, 1997).

2.6. Unconventional protein sources:

Use of unconventional protein sources in fish feeds has been reviewed by Tacon and Jackson (1985) and Wee (1988).

Atack *et al.* (1979) reviewed a variety of novel proteins (herring meal, methanophilic bacterium, casein, petroleum yeast and soyabean protein) as the sole source of protein in carp diets. Further studies along this line have indicated that alkaline/petrochemical single cell proteins (SCP) *Candia lipolytica* can replace 25-50% (Tacon and Jackson, 1985) and the bacterial SCP *Methylophilus methylotrophus* 75% of fish meal in salmonids rations (Wee, 1988).

In general, dried algal SCP has only a lower feed value for fish than yeast SCP, bacterial SCP or fish meal (Matty and Smith, 1978; Atack *et al.*, 1979). However, the studies of Appler and Jauncey (1983) with *Tilapia* and Hepher *et al.*. (1979) with common carp have indicated that certain dried algal meals (*Cladophora glomerata, Scenedesmus obliqus, Chlorella spp., Oocystis spp.* and *Euglena spp.*) may be promising as a partial dietary replacement for fish meal, at relatively low inclusion levels in fish feeds (20% algae SCP). Recently *Spirulina maxima* was tried as a partial replacement for fish meal in the diets of *O. mossambicus* which resulted in the increased growth rate(Cho and Woo, 1990). *Spirulina* is also reported to improve the quality of Japanese flounder feed (Henson and Ronald, 1990).

Fish silage is a less expensive form of fish meal. Acid silages have been used in the feeds of salmonids. Asgard (1984) found that animal byproducts like acid preserved casein and dogfish wastes had no negative effect on growth, health or

organoleptic characters of salmonids. Silage produced from trash fish mixed with fish meal, soyabean meal and compounded pellets or silage from waste grown tilapia was also tested as a fish meal replacement in *Clarius* spp. (Wee *et al.* 1988)

Lapie and Bigneras – Benitez (1992) also reported similar growth in tilapia, when fed fish meal or silage prepared from ground fish offal. Manikandavelu *et al.*. (1992) reported considerably higher growth rate in common carp (*Cyprinus carpio*) fed with ensilage based diet than with a diet based on fish meal.

In North America efforts have been made to develop protein products such as fish hydrolysate, as an alternative protein source that might substitute fish meal. It is yet to be tested in warm water finfishes (Tacon and Jackson, 1985; Hardy, 1991).

In trials involving *Oreochromis mossambicus* fingerlings, Abalos *et al.* (1990) proved that live maggots can substitute upto 20% of the fish meal protein.

Leaf protein concentrate (LPC) and potato protein concentrate have also been evaluated as alternative dietary protein sources for grass carp (Tacon and Jackson, 1985).

2.7. Digestive enzymes:

Artificial nutrition of stomachless fish larvae is yet to be clearly understood. Kunitz (1947) mentioned that the inadequate proteolytic enzyme activity causes feeding difficulties. Aoe *et al.* (1970) suggested that hydrolysed protein has no better nutritive value for common carp than crude protein, while Nose *et al.* (1979) have shown that pH of the diet markedly affects absorption.

According to Walter (1984), carp can neither digest nor economically utilize artificial food if the feed is not made up of at least 50% natural food. Schaperclaus (1961) reported a list of various enzymes present in the carp digestive tract. According to him, protein is digested by trypsin and erepsin, but not pepsin, since the carp has no stomach; the lipids are hydrolysed by lipase, the carbohydrates by amylase and maltase, and lichenase attacks fibrous carbohydrates.

Jancarik (1964) showed that various substances are digested in the intestinal tract of the carp through the action of endogenous and exogenous enzymes; the latter activating the former. Starch is digested by enzymes present in the intestinal juices. Therefore, better digestion of starch was observed in carp, which had sufficient supply of natural food. Vegetable proteins (beans, peas, peanuts, soyabeans, etc) were better digested in the presence of crustaceans and worms, but the carp could readily digest animal protein in the feed. Jancarik (1964) concluded that natural food present in the diet was able to improve the efficiency of food digestion and utilization.

Palackova *et al.* (1988) reported the activity of digestive enzymes of carp fry, which differs with different water temperatures. Activity of trypsin varied without any definite trend when the water temperature was increased. Similarly irregular changes in alpha -amylase were observed when the temperature was decreased.

Uys and Hecht (1987) obtained the results from assays run on various digestive fluids occurring in the alimentary canal and associated organs of *Clarius gariepinus.* The pancreatic enzymes amylase, trypsin and chymotrypsin were found to be highly active depending upon the pH and temperature.

Some cereals are having anti-enzyme properties, which can inhibit fish amylase like the anti-enzyme albumin present in wheat. Natarajan *et al.* (1988) reported the alpha amylase activity in the intestinal content of the carp fed with autoclaved wheat.

Detailed reports also suggest that enzymatic activities are independent of season and age. The ratio of amylase to trypsin characterizes the constitution of the food. Marchand and Samain (1982) have described the specific levels of enzymes dependent on the ingested energetic rates.

Contents of protein and fat in the feed have shown to influence the activity of alkaline protease, trypsin and amylase in bester (*Acipenser ruthenus* × *Huso huso*). Results obtained indicate highest values of these two enzymes with protein 45% and fat 12% (Mezina *et al.*, 1982). According to Hofer *et al.* (1983) similar amylase and trypsin activities in gut were measured in cyprinids (*Alburnus alburnus alboreled*) and non-cyprinids.

Several comparative studies of the digestive enzymes in different fish species have been reported (Hofer *et al.*, 1983; Kuz mina, 1990). Kapoor *et al.* (1975) and Fange and Groves (1979) reported the proteolytic activity in the liver of carp and tench because of pancreatic infiltration. High proteolytic potential was found in non-carnivorous fish (Kuz'mina, 1990) and low proteolytic digestive potential was registered for eel and sea bream. Similar results were also noted in the Japanese eel (Kitamikado and Tachino, 1960) and European eel(Bulnheim, 1974).

Digestive enzyme pattern of two stomachless filter feeders, silver carp, *Hypothalmichthys molitrix* and bighead carp, *Aristichthys nobilis* have been

discussed by Bitterlich (1985). Activities of digestive enzymes, trypsin and amylase of microplanktophagous silver carp and macroplanktophagous bighead carp decreased sharply from the foregut to the hindgut indicating an efficient resorbtion mechanism. Kirilenko and Chigrinzkaya (1983) also reported the distribution of amylolytic, proteolytic and lipolytic enzymes along the alimentary canal of silver carp.

Ragyanszki (1980) carried out the investigations on the proteolytic digestive enzymes of carp fry. Results obtained showed that the chymotryptic activity was 2 to 9 times higher than the tryptic one. Similar results were found with the exception of lipolytic activity in the pike larvae *Esox lucius* (Szalminska *et al.*, 1993).

Kawai and Ikeda (1972) have discussed the studies on digestive enzymes. They observed very weak peptic activity in the eggs and development of maltase and amylase activities along with the growth after hatching. During the metamorphosis the proteolytic activity is recorded in the alkaline pH range, which increases with growth, but the main proteolytic activity appears to have an optimum in the acidic range in all the teleosts (Alliot *et al.* ,1981).

Growth and survival of the larvae were found to be better in common carp (*Cyprinus carpio*) when extracts of fish digestive enzymes from pancreas and intestine were added to the diet (Dabrowski., 1979).

Studies have been conducted to investigate the possible effects of protein-rich, carbohydrate-rich and lipid-rich feed on carps. Phadate and Srikar (1983) reported the highest protease activity in carps fed 35% protein. Hidalgo *et al.* (1999) compared the difference between the protease and amylase activities in fish with

different nutritional habits- rainbow trout (*O.mykiss*), gilthead sea bream (*Pargus aurata*), European eel (*Anguilla anguilla*), common carp (*C. carpio*), gold fish (*Carassius auratus*) and tench (*Tinca tinca*). Results obtained indicate the difference in digestive enzymes according to feeding habits. Observations on the digestive enzymes in the catfish *Pangasius pangasius* have also supported the relation to its food habits. Ghosh and Saigal (1984) reported the presence of amylase, invertase, lipase and protease in the anterior part of intestine. Escaffre and Kaushik (1995) evaluated the nutritional value of soy protein concentrate in feed for common carp. The growth performance and amylase specific activity are shown to correlate in common carp fry (*C. carpio*).

Investigation carried out by Das and Tripathi (1991) indicate the activities of cellulase, amylase, protease and lipase in grass carp. The pattern of distribution and activity of the digestive enzymes were found to depend on the type of diet ingested by the fish.

Wu Tingting and Zhu Xhidoming (1994) reported that the lipase activity in various tissues differed with the species.

Changes in digestive enzyme activities are reported to be interrelated with the diet composition and highest protease activity was found in the fore intestine and lowest in the hind intestine in grass carp (Tian and Lin, 1993). Similarly, Fountoulaki *et al.* (1977) reviewed the digestive fluid volume and activity of digestive enzymes of gilt head bream (*Sparus aurata*). It was found from his studies that volume of digestive liquids increases initially, approaches maximum after feeding and reduces considerably after 24 hours.

Materials and methods

3. MATERIALS AND METHODS

The aim of the present study is to evaluate the growth response of the *L. rohita* fry to soyabean meal based diets.

3.1 Feed:

3.1.1. Raw materials:

The ingredients used for the preparation of the test diets were fishmeal, soyabean meal, groundnut oil cake, rice bran and tapioca flour.

All the ingredients except soyabean meal were procured locally. Defatted soyabean meal obtained from the Goswami Suppliers, Pune was heat processed at 105^0C for 90 minutes to eliminate the trypsin inhibitor and anti-nutritional factors such as heamagglutinin.

All the ingredients were dried, powdered and sieved to obtain uniform particle size (less than 400 micron).

3.1.2. Proximate analysis of the feed ingredients :

Proximate analysis of the ingredients was done prior to feed formulation (Table 1). The ingredients were analyzed for proximate composition to evaluate the nutrient status. Moisture, crude protein, crude fat, crude fibre and ash content were analysed following AOAC (1980) methods. The procedures adopted were as follows.

Moisture content was estimated by drying the sample at 105^{0}C until constant weight was reached. Microkjeldahl method (AOAC, 1980) was used to estimate the crude protein content. The nitrogen content was multiplied by a factor of 6.25 to get protein content. Fat content was estimated by extracting the sample in petroleum ether (60- 80^{0}C B.P.) in a Soxhlet apparatus for 6-8 hrs. The ash content was estimated by incinerating the sample at 550^{0}C ± 10^{0}C for 6 hours in a muffle furnace. The crude fibre was estimated by the method of Pearson (1976). Carbohydrate content was determined by the difference method of Hastings (1975). Nitrogen free extract = % .Dry matter – (% crude protein + % crude fat + % ash content + % crude fibre). Calorific content of the formulated diets was calculated using the energy factor 9 for fat, 4 for carbohydrate (Hastings,1975) and 5 for protein (Viola,1977) and expressed as Kcal/g.

Table 1

Proximate composition* of feed ingredients used in the formulation of feeds.

Parameter / Ingredient	Moisture %	Crude protein %	Crude fat%	Crude fiber%	Ash%	Nitrogen free extract%	Energy kcal/g.
Fish meal	7.03 (± 0.03)	78.98 (± 0.07)	4.12 (± 0.42)	2.23 (±0.31)	5.41 (±0.12)	2.23 (±0.14)	4.94 (±0.05)
Groundnut oil cake	8.26 (±0.09)	54.68 (±0.01)	16.51 (±0.12)	6.60 (±0.22)	4.80 (±0.19)	9.21 (±0.06)	5.02 (±0.02)
Rice bran	10.03 (±0.22)	9.18 (±0.47)	12.91 (±0.34)	18.28 (±0.07)	13.62 (±0.02)	36.06 (±0.65)	2.48 (±0.07)
Tapioca flour	7.63 (±0.24)	1.75 (±0.09)	0.54 (±0.65)	3.38 (±0.3)	0.44 (±0.03)	86.38 (±0.17)	3.68 (±0.05)
Soyabean meal	9.96 (±0.18)	52.5 (±0.76)	12.87 (±0.06)	6.20 (±0.16)	7.55 (±0.12)	10.97 (±0.01)	4.33 (±0.17)

Expressed on dry weight basis * Average of three values.

Figures in parentheses indicate SD

3.1.3. Formulation of test diets:

Test diets were formulated employing the method of Varghese *et al.*(1976)In the test diets soyabean meal progressively replaced the fish meal. The diets designated as 10%SM, 20% SM, and 30% SM contained 10%, 20% and 30% of soyabean meal, respectively. Fish meal based diet served as the control. The control diet was devoid of soyabean meal, while the 30% SM diet was fish meal free. The proportion of the ingredient used in the feed was adjusted to obtain final protein of 30%. The percentage proportion of ingredients in the test diets is given in Table 2.

3.1.4. Formulation and processing of test diets:

The procedure described by Jayaram and Shetty (1981) was adopted in the processing of the diet.

The experimental diets were formulated by replacing fishmeal protein with soyabean meal (SM) at inclusion levels of 10,20 and 30%. The fishmeal based control diet was devoid of soyabean meal. All the diets were made isonitrogenous with the overall protein content being kept at 30%.

For preparing the diets, all ingredients except vitamin mix were weighed in required proportion (Table 2) and mixed well. To this, the required quantity of water (1: 1.25 w/v) was added and the ingredients were mixed thoroughly to make a smooth dough. The prepared dough was cooked at 105^0C for 30 minutes. Cooked dough was cooled rapidly and to this, accurately weighed quantity of Supplevite - M (Table 2a) was added. Pelletizing was done in a hand-operated extruder with a diameter of 2 mm. in the die. The pelletised feeds were dried in a hot air oven at

60^0C for 12 hours to a moisture content of less than 10 %. The dried pellets were crumbled, cooled to room temperature and stored in airtight containers for further use.

3.1.5. Initial analysis of proximate composition:

The feeds were analysed for initial proximate composition by using the methodology as described earlier for proximate analysis of feed ingredients.

Table 2

Ingredient proportion of the formulated feeds.

Diets / Ingredients	Control	10%	20%	30%
Fish meal	18 %.	13 %	4 %.	-
Groundnut oil cake	23 %.	20 %	21 %.	20 %
Rice bran	43 %.	41 %	39 %.	34 %
Tapioca flour	15 %.	15 %	15 %.	15 %.
Soyabean meal	-	10 %.	20 %.	30 %
Supplevite –M*	1 %.	1 %	1 %.	1 %
Total	100	100	100	100

* Supplevite –M: Sarabhai Chemicals, Mumbai

Table 2a

Composition of *Supplevite – M (Vitamin mineral concentrate)

Each 250 mg provides	Quantity
Vitamin A	500,000 IU
Vitamin D_3	100,000 IU
B_2	0.2 g
E	75 units
K	0.1 g
Calcium pantothenate	0.25 g
Nicotinasmide	1 g
Vitamin B_{12}	0.6 g
Choline chloride	15 g
Calcium	75 g
Manganese	2.75 g
Iodine	0.1 g
Iron	0.75 g
Zinc	1.5 g
Copper	0.2g
Cobalt	0.045 g

*Supplevite –M: Sarabhai Chemicals, Mumbai

3.2. Experimental cisterns:

Present study was conducted in 16 uniform size circular cement cisterns devoid of soil base, the dimension of the cisterns being as follows,

Total capacity : 380 litres

Diameter : 90 cm

Height : 60 cm

Water depth : 50 cm height (315 litres)

The cisterns were flushed thoroughly to remove traces of soil particles and then allowed to dry for two days. They were then filled with water from a bore well to a depth of 50 ± 5 cm. This level was maintained through out the experimental period.

3.3. Initial enzyme analysis:

Initial enzyme analysis was done prior to initiation of the experiment.

The preparation of enzyme extract from the hepatopancreas of the sampled animals was done according to the method of Overnell (1973). The tissue was homogenized with 0.050M Tris buffer (pH - 8.0) containing 0.15 M $NaCl_2$, 0.02 M $CaCl_2$ and 0.1% Triton × 100 at 4^0C, using a high speed homogenizer. The resulting homogenate (1/10 W/V) was tissue centrifuged at 15,000 rpm for 30 minutes at 4^0C. The supernatant obtained was used in enzyme assay.

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3.4. Initial analysis of carcass composition:

Carcass composition of fishes fed the different diets were analyzed initially and also on termination of the experiment. Flesh was collected from the trunk region of several fishes of each treatment and the pooled samples were used for analysis of moisture, crude protein, crude fat and ash following AOAC (1980) methods.

3.5. Stocking:

Fry of rohu, *Labeo rohita* obtained from the college hatchery was used for the study. Stocking was done @ 10 fry /cistern, taking care to select uniform sized healthy fry for stocking.

3.6. Feeding:

Fishes were fed once daily in the morning @ 5% of body weight through out the experimental period of 126 days. Before commencement of the experiment, the fishes were acclimatised to the experimental diets for a week. Leftover feed of the previous day, if any, was removed prior to feeding. After every fish sampling the quantity of feed was readjusted according to the increased fish weight.

3.7. Quality of water:

Proper care was taken to maintain good water quality throughout the experimental period. During the course of the experiment half the quantity of water was changed once every fortnight to avoid deterioration in quality of water due to accumulated organic matter and metabolites.

3.8. Sampling frequency:

3.8.1. Fish sampling:

Fishes were sampled at fortnightly intervals to record the increments in length and weight. At the time of commencement of the experiment initial weight and length of the individual fishes introduced in each cistern were measured accurately. The length was measured to the nearest mm and weight to the nearest milligram.

During the experimental period 50% of the stocked fishes were caught and the length and weight were recorded. From this data, the average length and weight of fish from each cistern were found out. At the termination of the experiment all the cisterns were dewatered and the surviving fishes were collected and length and weight were recorded for further statistical analysis.

3.8.2. Water sampling:

Water samples were collected from all the experimental cisterns in morning hours on the days of sampling for recording pH, dissolved oxygen, total alkalinityand ammonia.

Temperature was determined by using mercury bulb thermometer with $0.1^{0}C$ accuracy. p^{H} was assessed by using the Universal pH indicator method, dissolved oxygen was determined by using the Standard Winkler method (Strickland and Parson 1972). Total alkalinity was determined following the acidimetric titration method (APHA, 1980) and ammonia was assessed by using the phenol hypochlorite spectrophotometric method (Stickland and Parson, 1972).

3.8.3. Plankton:

Plankton samples were taken fortnightly using a net of 20μ bolting silk. The plankton was assessed by filtering 50 litre of water. Plankton samples collected were assessed for biomass based on settlement volume. Since the cisterns were not fertilized and also due to frequent replenishment of water, the plankton biomass produced in the cisterns was meagre. No qualitative analysis of plankton was done.

3.9. Water stability of formulated feeds:

Water stability of the feeds was determined using the method of Jayaram and Shetty (1981). It was found out by determining the percentage dry matter recovered after exposing the pellets in water for 6 hours.

3.10. Storage studies of test diets:

All the formulated diets were evaluated for keeping quality. They were analysed for proximate composition after 4 months of storage employing the same procedure as described earlier.

3.11. Food conversion efficiency:

Food conversion efficiency was calculated by using the formula,

$$\text{FCE (\%)} = \frac{\text{Weight gain (g)}}{\text{Feed intake (g)}} \times 100$$

3.12. Protein efficiency ratio:

Protein efficiency ratio was calculated by using the formula,

$$\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Protein intake (g)}}$$

3.13. Specific growth rate (SGR) :

Specific growth rate was determined using the formula,

$$SGR\ (\%) = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100$$

where,

$T_2 - T_1$ = Period in days

W_2 = Weight at time T_2 and W_1 = Weight at time T_1

3.14 Determination of digestibility coefficient:

Digestibility coefficient was determined using the method described by Halver (1989). A set of 4 number of fishes each was introduced in fiber glass tank having water holding capacity of 80 litres. Fishes were acclimatized for a week to the test feeds. During the experimental period fishes in a tank were fed one of the experimental diets @ 5% of body weight once daily. Water quality was maintained by replacing 50% of water everyday. Fishes were sampled weekly and the individual length and weight were recorded. Faeces were collected for determining the digestibility coefficient for the first 15 days. Collected faeces was sieved through bolting silk, rinsed with distilled water to remove the unwanted particles adhering to it and then dried in an oven at 60^0C to constant weight. The dried faecal matter was homogenized before the analysis for the proximate composition. Experiment was

further continued for the period of 30 days in order to determine absolute conversion ratio (ACR). The fishes were sampled weekly and the quantity of feed was adjusted to the increased weight.

Digestibility coefficient:

It was calculated using the following formula :-

$$\text{Digestibility} = \frac{\text{Nutrient digested}}{\text{Nutrient ingested}} \times 100$$

Absolute conversion rate :

ACR was calculated using the following formula,

$$\text{ACR} = \frac{\text{Dry weight of feed given (g)}}{\text{Increase in wet wt. of fish (g)}}$$

3.15. Final enzyme analysis:

On the termination of the experiment final enzyme analysis was performed employing the methodology already discussed in the initial analysis of enzymes.

3.16. Final carcass composition:

Fishes from all the cisterns were analyzed for the proximate composition on the termination of the experiment employing the same procedure as described earlier.

3.17. Organoleptic evaluation:

The raw and cooked meat of rohu fed on different experimental diets was evaluated organoleptically for the attributes like texture, odour, flavour, etc. Cooked meat was prepared by cooking the raw flesh in 1.5 % salt solution. Grades were assigned to each of the attributes. The hedonic scale in the evaluation proforma were converted into numerical scale to get the mean panel scores for each attribute, and analyzed statistically by applying ANOVA

3.18 Statistical analysis:

The difference in the growth response of rohu fed on the experimental diets was statistically tested by the analysis of variance technique (Snedecor and Cochran, 1968). Organoleptic evaluation of the fish flesh from different treatments were also statistically analysed using the above method.

Results

4. RESULTS

The details of the observations made during the study are presented below.

4.1. Proximate composition of feed ingredients and formulated feeds:

4.1.1. Ingredients:

The percentage composition of the ingredients used in the feed formulation is presented in Table 1. The moisture content of the ingredients *viz.*, fish meal, groundnut oil cake, rice bran, tapioca flour and soyabean meal ranged from 7.03% to 10.03%, with the maximum being in rice bran and the minimum in fish meal.

The highest percentage crude protein content was recorded in fish meal (78.98), while it was the lowest in tapioca flour (1.75). The percentage crude protein of soyabean meal, groundnut oil cake and rice bran were 52.50, 54.68 and 9.18, respectively.

Crude fat content in the ingredients ranged from 0.54% for tapioca floor to 16.51% for groundnut oil cake .For the other ingredients the contents were 4.12%(fish meal), 12.87 % (soyabean meal) and 12.91% (rice bran).

Tapioca flour showed the highest percentage of carbohydrate of 86.38, where as the fish meal had the lowest content of 2.23.

The crude fiber content was the highest in the rice bran (18.28%),followed by groundnut oil cake(6.60%),soyabean meal (6.20%),tapioca flour (3.38%)and fish meal (2.23%).

The ash content of the ingredients varied from 0.44% to 13.62%, with the lowest being in tapioca flour and the highest in rice bran. Table 2 gives the percentage proportion of the ingredients in the formulated feeds.

4.1.2. Formulated feeds (Initial analysis):

Proximate composition of the formulated feeds is presented in Table 3. The moisture content in the four feeds ranged between 9.34%(10%SM) and 9.47%(20%SM).

The crude protein content ranged between 29.75% (30%SM) and 30.12% (control), while the fat content varied from 7.29 %(30%SM) to 8.06 % (20%SM). The percentage nitrogen free extract was the highest in 30%SM (40.15) and lowest in the control (38.61).

Crude fiber content was maximum in the control (5.32%) and minimum in 30%SM (4.37%).

The ash content ranged between 8.21% (10%SM) and 9.01% (30%SM). The calorific values of the four diets were almost similar, ranging from 4.00 in the control to 4.07 k cal/g in the 20%SM diet.

4.1.3. Initial water stability:

All the feeds tested had stability of over 80% at the end of 6hr.Stability was highest in diet 30% SM followed by 20% SM, 10% SM and control diets the values being 85.72 %, 84.02%, 83.29 % and 80.22%, respectively (Table 4).

4.1.4. Analysis after storage :

Results of proximate analysis of formulated feeds after a storage period of 4 months is given in table 5. Moisture and NFE (Nitrogen Free Extract) showed an increase after 4 months of storage. The maximum increase in the moisture content was in the 30% SM diet. The increment in NFE content of feeds after 4 months of storage was in the order of control, 10% SM, 20% SM and 30% SM.

The decrease in crude protein content was the maximum for 30%SM, while it was minimum for the control. The crude fat content of the feeds was found lowered in the order of 20%SM, 10%SM, control and 30%SM as in the same order of fat content in the fresh feeds. Treatment diet 30%SM showed maximum decrease in ash content among the feeds, while minimum decrease was for control .The decrease in crude fiber content was in the order of 30%SM, 10%SM, 20%SM and control.

Table 3

Proximate composition* of the formulated feeds (initial analysis)

Parameter / Diet	Moisture %	Crude protein %	Crude fat%	Ash%	Crude fiber%	NFE%	Energy K cal/g.
Control	9.37 (±0.09)	30.12 (±0.42)	7.42 (±0.26)	8.86 (±0.28)	5.32 (±0.03)	38.61 (±0.29)	4.00 (±0.062)
10% SM	9.34 (±0.14)	30.06 (±0.07)	7.78 (±0.16)	8.21 (±0.12)	4.49 (±0.18)	40.12 (±0.30)	4.06 (±0.025)
20% SM	9.47 (±0.09)	29.91 (±0.10)	8.06 (±0.03)	8.38 (±0.07)	4.72 (±0.3)	39.46 (±0.55)	4.07 (±0.03)
30% SM	9.43 (±0.09)	29.75 (±0.24)	7.29 (±0.3)	9.01 (±0.06)	4.37 (±0.09)	40.15 (±0.37)	4.01 (±0.15)

*Average of three values.

Figures in parentheses indicate S.D.

Table 4

Water stability *(%) of the formulated feeds (Initial analysis)

Feed / Duration	1 hr.	2 hr.	4 hr.	6 hr.
Control	86.24 (±0.01)	84.07 (±0.06)	82.53 (±0.02)	80.22 (±0.06)
10% SM	90.47 (±0.08)	89.29 (±0.09)	85.62 (±0.04)	83.29 (±0.01)
20% SM	90.82 (±0.08)	90.79 (±0.03)	85.21 (±0.04)	84.02 (±0.07)
30% SM	90.56 (±0.06)	90.14 (±0.09)	87.29 (±0.08)	85.72 (±0.02)

- Average of three values.
- Figures in parentheses indicate SD

Table 5

Proximate composition*of the formulated feeds. (Final analysis)

Parameter / Diet	Moisture %	Crude protein %	Crude fat%	Ash%	Crude fiber%	NFE%	Energy K cal/g.
Control	10.83 (±0.03)	29.27 (±0.11)	6.92 (±0.19)	8.27 (±0.1)	5.03 (±0.03)	39.68 (±0.21)	3.93 (±0.02)
10% SM	10.69 (±0.22)	29.18 (±0.07)	7.17 (±0.34)	7.53 (±0.12)	3.92 (±0.23)	41.51 (±0.17)	4.02 (±0.06)
20% SM	10.42 (±0.02)	28.83 (±0.11)	7.24 (±0.32)	7.58 (±0.02)	4.17 (±0.24)	41.76 (±0.16)	4.020 (±0.05)
30% SM	11.07 (±0.09)	28.62 (±0.07)	6.90 (±0.38)	7.78 (±0.21)	3.68 (±0.06)	41.93 (±0.17)	3.99 (±0.16)

- Average of three values.
- Figures in parentheses indicate SD

Table 6

Water stability* (%) of the formulated feeds (Final analysis)

Duration / Feed	1 hr.	2 hr.	4 hr.	6 hr.
Control	85.00 (±0.06)	84.50 (±0.02)	81.12 (±0.07)	79.31 (±0.01)
10% SM	89.35 (±0.07)	89.10 (±0.04)	84.17 (±0.06)	82.25 (±0.05)
20% SM	89.70 (±0.09)	89.62 (±0.07)	85.54 (±0.03)	83.48 (±0.07)
30% SM	89.70 (±0.02)	89.60 (±0.01)	86.02 (±0.02)	84.56 (±0.06)

- Average of three values.
- Figures in parentheses indicate SD

Table 7

Enzyme profile of rohu (initial and final)

Enzymatic activity	Initial enzymatic activity (µg/mg protein)	Average final enzymatic activity (µg/mg protein)			
		Control	10% SM	20% SM	30% SM
Protease	0.667 ± (0.28)	1.210 ± (0.23)	1.055 ± (0.14)	1.115 ± (0.17)	0.995 ± (0.09)
Amylase	1.357 ± (0.26)	2.24 ± (0.15)	2.26 ± (0.02)	2.32 ± (0.21)	2.37 ± (0.01)
Lipase	0.198 ± (0.07)	0.377 ± (0.023)	0.391 ± (0.15)	0.432 ± (0.05)	0.258 ± (0.02)

*Average of three values.

Figures in parentheses indicate SD

Table 8

Proximate Composition * of the muscle of rohu (initial analysis)

Parameter	Moisture Content %	Crude protein%	Crude fat%	Ash%	Nitrogen free extract%	Energy k cal/g
Initial analysis	78.14 (±0.06)	15.05 (±0.01)	2.05 (±0.17)	3.41 (±0.35)	3.41 (±0.47)	1.07 (±0.11)

- Average of three values. Expressed in dry weight basis.
- Figures in parentheses indicate SD

4.1.5. Final stability of formulated feeds:

The water stability of formulated feeds analyzed after four months of storage is presented in Table 6. The dry matter retention ability of the diets was assessed over a period of 6 hours. The most stable feed at the end of 6 hours was 30%SM (84.56%), followed by 20%SM (83.48%), 10% SM (82.25%) and control (79.31%).

4.1.6. Initial enzyme activity:

Table 7 presents the protease, amylase and lipase activity in the test species on initiation of the experiment. Enzyme activity was found lower in digestive tract of rohu initially. Initially amylase activity was reported higher (1.357 µg/mg protein) followed by protease (0.667µg/mg protein) but lipase activity was found comparatively weak (0.198µg/mg protein).

4.1.7. Initial carcass composition:

Initial moisture content of fish was 78.14%, while the body protein content was 15.05%. The initial fat content was 2.05%. Initial NFE recorded was 3.41%, whereas energy content was 1.07 kcal/g (Table 8)

4.2. Monitoring of water quality parameters:

Range of temperature in the experimental tanks during the study period is given in Table 9. The water temperature ranged from 25.2^{0}C to 26.0^{0}C Temperature did not vary much between the experimental tanks. The range of air temperature was from 27.2 to 30.1^{0}C.

pH of the water fluctuated from 7.0 to 8.5 .The mean value of the pH and its range in each treatment during the experimental period is given in Table 10.

Dissolved oxygen content (ppm) of water recorded over the experimental period is shown in Table 11. The average values in different treatments varied from 4.2 to 6.6 mg/l. Dissolved oxygen trends were found to remain almost constant during the study period.

Total alkalinity ranged between 37.5 and 65.7 ppm. There were not much variations between the treatments (Table 12). Ammonia concentration was recorded in the range of 0.038 to 0.099 ppm which was within the tolerance limit of the experimental animals (Table 13).

Settled volume of plankton showed variation depending on temperature and ranged between 1.0 ml and 8.0 ml/ 50 L (Table 14).

4.3. Fish growth:

Average length and weight attained by rohu after 126 days of rearing is given in table 15 and 16.

The trend observed in average length and weight of rohu is given in fig.1 and 2,respectively. After 126 days average weight of fish in different treatments were 14.48 g (control), 14.50 g (10% SM), 15.04 g (20%SM) and 13.24 g (30%SM). Corresponding average length being 152.25 mm, 157.50 mm, 162.75 mm and 156.50 mm, respectively. Daily average increment in weight was 0.106g(control), 0.105g (10%SM), 0.110g (20%SM) 0.095 g (30% SM), respectively with corresponding increment of length being 1.049 mm, 1.074 mm,

1.102 mm and 1.067mm. Highest weight gain was achieved by 20% SM treatment followed by 10% SM., control and 30%SM. Initially growth of fish in different treatments was more or less similar, but it became distinctively different from the 42nd day onwards. The fish under 20%SM treatment showed faster growth leading to the highest average weight on termination. The trend in weight gain was more or less similar in fish fed the10%SM and control diets. Fish in 30% SM treatment showed poor growth throughout the experimental period (fig.2), showing the lowest average weight on termination, which was even lower than that of the control. Trend in gain in length followed a pattern slightly different from that of the gain in weight (fig. 1). Gain in length in different treatments did not differ much till day 70. Thereafter fish in 20%SM treatment showed faster growth, recording highest average length on termination. Gain in length was higher than the control in all the three treatments.

Table 9

Fluctuations in water and air temperature during the experimental period

Days of sampling	Air temperature $^{\theta}C$	Water temperature $^{\theta}C$
0	27.2	25.2 – 25.5
14	30.1	25.7 – 25.9
28	30.0	25.5 – 25.9
42	27.5	25.2 – 25.4
56	27.9	25.4 – 25.8
70	30.0	25.5 – 25.8
84	27.2	25.7 – 26.0
98	27.9	25.6 – 25.8
112	27.8	25.2 – 25.5
126	27.4	25.4 – 25.7

Table 10

Fluctuations in P^H in different treatments

Treatment	Days / Cistern	0	14	28	42	56	70	84	98	112	126
Control	1	7.5	7.8	7.8	8.5	8.2	8.4	7.2	8.5	7.4	7.3
	2	7.8	8.0	7.2	7.8	8.1	7.8	7.8	8.2	7.8	7.7
	3	7.0	8.0	8.0	8.2	8.5	7.5	7.8	8.5	8.0	7.2
	4	8.0	8.5	8.5	8.5	8.2	7.2	7.5	8.5	7.8	8.1
10%SM	1	8.0	8.0	8.5	8.2	8.2	7.6	7.8	8.0	7.8	7.9
	2	8.5	8.5	8.4	8.5	8.4	7.5	7.5	8.1	7.5	8.4
	3	7.5	7.1	7.8	8.4	8.0	8.2	7.2	8.5	8.0	7.8
	4	8.5	7.5	8.2	8.2	8.5	8.5	7.5	7.2	8.2	7.5
20%SM	1	7.2	7.5	8.5	7.8	8.3	8.1	7.2	7.8	8.2	8.5
	2	7.8	8.5	8.2	7.2	8.2	8.5	7.8	8.5	8.5	8.0
	3	7.5	8.2	8.5	7.5	8.4	8.4	7.5	8.0	8.0	7.8
	4	8.0	8.0	8.5	7.8	8.5	8.3	8.0	8.2	8.0	7.2
30%SM	1	8.0	8.0	8.1	7.2	7.8	8.4	8.0	8.5	8.2	8.5
	2	8.2	8.0	8.5	7.8	8.3	8.0	7.2	7.1	8.4	8.4
	3	8.5	8.2	8.2	7.5	8.5	8.2	7.5	7.8	8.5	7.3
	4	8.4	8.5	8.0	7.0	8.0	7.8	8.5	7.2	8.0	7.9

Table 11

Fluctuations in dissolved oxygen (ppm) in different treatments.

Treat-ment	Days / Cistern	0	14	28	42	56	70	84	98	112	126
Control	1	5.9	4.2	5.3	5.1	5.2	4.8	5.3	4.3	5.8	5.2
	2	6.3	5.0	5.4	5.4	6.1	5.2	5.6	4.7	5.2	5.6
	3	5.4	5.8	6.2	5.7	6.4	5.6	6.1	5.2	4.7	4.8
	4	6.5	6.2	6.1	5.4	6.1	5.9	5.2	5.7	6.1	4.3
	Mean ± SD	6.02 ±0.420	5.30 ±0.768	5.75 ±0.403	5.40 ±0.212	5.95 ±0.454	5.37 ±0.415	5.55 ±0.35	4.97 ±0.526	5.45 ±0.540	4.97 ±0.484
10%SM	1	5.2	5.2	5.6	6.5	5.6	6.2	4.8	5.6	6.1	4.9
	2	6.1	5.6	5.6	6.2	5.8	5.2	5.1	5.2	6.4	5.2
	3	5.6	5.4	5.2	5.8	5.2	5.4	5.8	5.8	5.2	6.1
	4	4.8	6.2	5.8	5.7	6.1	5.2	5.6	6.4	5.3	4.7
	Mean ± SD	5.42 ±0.481	5.60 ±0.374	5.55 ±0.217	6.05 ±0.320	5.67 ±0.326	5.51 ±0.434	5.32 ±0.396	5.75 ±0.0433	5.75 ±0.512	5.10 ±0.524
20%SM	1	5.3	6.2	5.1	5.1	5.1	6.0	5.6	6.3	5.2	5.7
	2	6.2	6.4	5.7	5.7	5.7	5.7	5.2	5.4	5.7	5.4
	3	6.4	6.1	5.4	5.5	5.4	5.1	4.7	4.7	5.4	5.8
	4	5.3	6.3	5.9	6.2	5.9	5.6	5.1	5.1	6.0	5.1
	Mean ± SD	5.8 ±0.502	6.25 ±0.113	5.52 ±0.303	5.62 ±0.390	5.52 ±0.307	5.6 ±0.324	5.15 ±0.320	5.37 ±0.588	5.5 ±0.309	5.23 ±0.494
30%SM	1	5.1	5.8	5.8	5.9	5.7	4.6	5.2	5.7	5.8	4.8
	2	5.9	6.4	6.2	6.2	5.1	4.8	5.4	4.3	5.9	5.2
	3	5.4	6.1	5.2	6.1	6.0	5.2	6.1	5.1	5.1	5.6
	4	6.1	6.6	6.4	5.7	5.3	6.1	5.8	5.9	5.4	5.9
	Mean ± SD	5.55 ±0.356	6.22 ±0.303	5.9 ±0.458	5.97 ±0.190	5.52 ±0.349	5.17 ±0.576	5.62 ±0.349	5.25 ±0.622	5.55 ±0.320	5.56 ±0.477

Table 12

Fluctuations in total alkalinity (mg/ l) in different treatments.

Treat-ment	Days / Cistern	0	14	28	42	56	70	84	98	112	126
Control	1	45.2	51.6	57.3	52.2	52.6	51.7	59.8	52.2	57.3	54.7
	2	41.4	48.7	53.8	54.6	59.2	57.6	48.3	61.6	54.6	56.3
	3	56.6	37.5	52.2	58.0	57.6	53.8	51.2	58.0	59.3	58.7
	4	47.8	42.6	47.2	61.6	63.5	54.1	58.0	53.8	63.6	61.2
	Mean ± SD	47.75 ± 5.59	50.1 ±5.36	52.62 ± 3.63	56.6 ± 3.54	58.22 ± 3.89	54.3 ± 2.11	54.32 ± 4.73	56.4 ± 3.67	58.72 ± 3.28	57.85 ±2.63
10%SM	1	42.4	52.7	53.4	59.8	65.7	52.2	43.8	52.8	46.3	47.8
	2	47.7	48.2	59.7	63.7	58.2	57.3	56.2	51.9	59.6	57.9
	3	42.5	39.6	62.5	48.3	53.7	51.6	54.8	49.3	63.3	51.3
	4	52.4	57.3	48.3	51.7	49.2	45.2	51.7	53.7	49.2	43.4
	Mean ± SD	46.25 ±4.14	49.95 ± 6.53	56.05 ± 5.41	55.87 ± 6.15	56.7 ± 6.09	51.57 ± 4.29	51.62 ± 4.80	51.92 ± 1.64	54.62 ± 7.04	54.52 ±5.3
20%SM	1	48.8	57.8	54.7	52.7	58.2	62.6	53.4	47.2	57.8	47.8
	2	47.2	61.6	63.7	48.7	52.6	42.4	59.2	53.4	61.6	55.7
	3	45.4	48.2	61.2	54.2	54.2	52.4	62.1	59.7	57.6	52.2
	4	42.2	39.2	64.8	56.7	54.8	38.3	57.3	62.5	63.1	62.4
	Mean ± SD	45.92 ± 2.45	51.72 ± 8.71	60.9 ± 3.74	53.07 ± 2.90	54.92 ± 2.0	48.92 ± 9.41	58.01 ± 3.15	55.70 ± 5.90	60.00 ± 2.38	54.52 ±5.33
30%SM	1	47.7	43.2	53.7	56.2	59.7	42.2	58.1	54.7	52.2	51.7
	2	53.6	49.2	48.2	47.2	61.7	45.4	57.7	48.2	51.7	54.2
	3	52.4	56.1	60.4	58.7	63.1	58.0	50.6	60.7	59.8	62.7
	4	58.2	63.2	49.2	63.2	60.6	59.2	45.7	53.2	48.3	50.8
	Mean ± SD	52.97 ± 3.73	52.92 ± 7.48	52.87 ± 4.81	56.32 ± 5.83	61.27 ± 1.26	51.22 ± 7.49	51.52 ± 4.41	54.22 ± 4.45	53.05 ± 4.19	54.85 ±4.70

Table 13

Fluctuations in NH_3-N (ppm) in different treatments.

Treat-ment	Days / Cistern	0	14	28	42	56	70	84	98	112	126
Control	1	0.076	0.072	0.083	0.068	0.063	0.060	0.081	0.049	0.084	0.075
	2	0.051	0.063	0.077	0.096	0.080	0.070	0.073	0.078	0.078	0.082
	3	0.066	0.051	0.064	0.098	0.077	0.057	0.061	0.062	0.071	0.043
	4	0.054	0.057	0.073	0.094	0.071	0.065	0.088	0.064	0.054	0.066
	Mean ± SD	0.061 ±0.009	0.060 ±0.0075	0.074 ±0.0069	0.089 ±0.012	0.072 ±0.0064	0.060 ±0.0057	0.075 ±0.010	0.063 ±0.010	0.0717 ±0.0112	0.066 ±0.0141
10%SM	1	0.052	0.052	0.084	0.076	0.046	0.074	0.052	0.087	0.072	0.052
	2	0.068	0.063	0.071	0.059	0.062	0.059	0.071	0.048	0.067	0.072
	3	0.061	0.072	0.062	0.063	0.079	0.067	0.064	0.062	0.075	0.087
	4	0.096	0.038	0.078	0.053	0.050	0.042	0.049	0.077	0.084	0.084
	Mean ± SD	0.069 ±0.016	0.056 ±0.012	0.073 ±0.008	0.062 ±0.008	0.059 ±0.012	0.065 ±0.011	0.059 ±0.008	0.068 ±0.001	0.074 ±0.061	0.073 ±0.013
20%SM	1	0.077	0.072	0.063	0.078	0.084	0.081	0.071	0.078	0.087	0.072
	2	0.098	0.097	0.077	0.064	0.079	0.049	0.084	0.062	0.072	0.078
	3	0.045	0.067	0.083	0.052	0.099	0.054	0.065	0.046	0.064	0.085
	4	0.094	0.078	0.071	0.070	0.085	0.063	0.052	0.059	0.038	0.052
	Mean ± SD	0.078 ±0.020	0.078 ±0.011	0.073 ±0.008	0.066 ±0.009	0.086 ±0.0074	0.061 ±0.0121	0.068 ±0.0115	0.061 ±0.011	0.074 ±0.0095	0.071 ±0.012
30%SM	1	0.072	0.052	0.038	0.063	0.054	0.089	0.034	0.054	0.047	0.075
	2	0.058	0.062	0.047	0.077	0.072	0.094	0.063	0.058	0.072	0.065
	3	0.042	0.071	0.067	0.076	0.069	0.062	0.079	0.042	0.069	0.068
	4	0.066	0.054	0.054	0.066	0.042	0.078	0.064	0.071	0.085	0.096
	Mean ± SD	0.059 ±0.011	0.059 ±0.0074	0.0515 ±0.010	0.070 ±0.006	0.060 ±0.011	0.080 ±0.012	0.06 ±0.016	0.056 ±0.010	0.068 ±0.013	0.076 ±0.012

Table 14

Fluctuations settled volume of plankton (ml/50 l) in different treatments

Treat-ment	Days / Cistern	0	14	28	42	56	70	84	98	112	126
Control	1	5.0	7.0	8.0	7.0	7.0	6.0	4.0	5.0	5.0	7.0
	2	7.0	8.0	4.0	7.0	2.0	5.0	2.0	7.0	7.0	5.0
	3	2.0	4.0	2.0	2.0	5.0	4.0	5.0	4.0	4.0	3.0
	4	5.0	5.0	4.0	5.0	4.0	7.0	7.0	5.0	5.0	2.0
	Mean ± SD	4.7 ±1.78	6.6 ±1.58	4.5 ±2.17	5.2 ±2.04	4.5 ±1 1.80	5.5 ±1.11	4.5 ±1.80	5.2 ±1.08	7.0 ±1.08	4.2 ±1.92
10%SM	1	5.0	2.0	2.0	5.0	2.0	4.0	5.0	5.0	5.0	5.0
	2	7.0	4.0	7.0	4.0	4.0	5.0	2.0	7.0	7.0	2.0
	3	8.0	2.0	8.0	7.0	7.0	7.0	7.0	7.0	5.0	5.0
	4	2.0	6.0	4.0	5.0	6.0	8.0	5.0	2.0	7.0	7.0
	Mean ± SD	5.5 ±2.29	3.5 ±1.65	5.2 ±2.38	5.2 ±1.08	4.7 ±1.92	6.0 ±1.58	4.7 ±1.78	5.2 ±2.04	5.6 ±9.4	4.7 ±1.78
20%SM	1	6.0	2.0	5.0	7.0	5.0	7.0	7.0	5.0	5.0	7.0
	2	3.0	4.0	6.0	4.0	4.0	5.0	5.0	4.0	4.0	2.0
	3	5.0	5.0	7.0	5.0	2.0	4.0	2.0	2.0	2.0	5.0
	4	7.0	7.0	8.0	7.0	7.0	2.0	4.0	6.0	7.0	6.0
	Mean ± SD	5.2 ±1.47	4.5 ±1.80	6.5 ±01.11	5.7 ±1.29	4.5 ±1.80	4.5 ±1.80	4.5 ±1.80	4.2 ±1.47	4.5 ±1.80	5.0 ±1.87
30%SM	1	3.0	4.0	4.0	4.0	5.0	4.0	5.0	2.0	7.0	6.0
	2	5.0	2.0	2.0	5.0	7.0	7.0	5.0	4.0	6.0	3.0
	3	2.0	4.0	3.0	6.0	4.0	8.0	6.0	5.0	4.0	5.0
	4	1.0	6.0	5.0	4.0	5.0	7.0	7.0	8.0	2.0	7.0
	Mean ± SD	2.7 ±1.47	4.0 ±1.41	3.5 ±1.11	4.7 ±1.82	5.2 ±1.08	6.5 ±1.59	5.7 ±1.29	4.7 ±2.16	4.7 ±1.92	5.2 ±1.42

(please continue ??)

4.4. Specific growth rate (%) :

The fluctuations in percentage SGR over the experimental period are presented in table 17. The average SGR in the different treatments were 2.18(control), 2.26(10%SM), 2.34 (20% SM) and 2.09 (30% SM). Highest SGR was observed in the first fortnight in all the treatments(fig. 3).

4.5. Survival and production of fish:

Survival percentage of rohu in different treatments is given in table 18. On an average the overall survival was about 90% and above.

Fish production was maximum in 20% SM treatment followed by control, 10% SM and 30% SM.

4.6. Food conversion efficiency:

FCE was best with 20% SM (27.6%) indicating that it was the most efficient feed among the four (Table 19). Fish fed on 30% SM gave the poorest FCE of 24.5%

The control diet had given better FCE (25.7%) followed by 10% SM (24.5%).

4.7. Protein efficiency ratio:

PER was best with 20% SM (0.920). PER values of various feeds are given in Table 19. Control, 30% SM and 10%SM had 0.856, 0.822, 0.820, respectively.

4.8. Apparent nutrient digestibility:

Data regarding the digestibility coefficient of different feeds is presented in Table 20.

Protein digestibility was the highest for fish fed on the control diet (65.45 %) and lowest for 30%SM (59.60%). The protein digestibility of 20%SM and 10%SM was 64.58% and 60.81 %, respectively.

Lipid digestibility was maximum for fish fed on 20%SM (71.12%), while the values for 10%SM and control were 66.84 % and 70.65 %, respectively. Lipid from 30%SM was least digestible (66.15 %).

Digestibility of carbohydrate was maximum for 30%SM (88.77%) and was minimum for the control (56.32%).

Table 15

Growth in length(mm) of rohu in different treatments

Treatment	Days / Cistern	0	14	28	42	56	70	84	98	112	126	Net length gain	Increment/day
Control	1	22.00	38.00	59.00	69.00	98.00	116.00	123.00	134.00	141.00	156.00	134.00	1.063
	2	17.00	43.00	57.00	69.00	94.00	107.00	121.00	134.00	142.00	153.00	136.00	1.079
	3	21.00	38.00	56.00	77.00	88.00	112.00	120.00	132.00	144.00	149.00	128.00	1.015
	4	20.00	32.00	52.00	16.00	89.00	118.00	127.00	133.00	137.00	151.00	131.00	1.039
	Mean ± SD	20.00 ± 1.80	37.00 ± 3.82	56.00 ± 2.54	69.00 ± 5.61	92.20 ± 4.02	113.00 ± 4.20	122.00 ± 2.68	133.00 ± 0.82	141.00 ± 2.54	152.25 ± 2.58	132.00 ± 3.03	1.049 ± 0.01
10%SM	1	21.00	32.00	56.00	69.00	97.00	107.00	122.00	134.00	143.00	151.00	130.00	1.031
	2	21.00	36.00	49.00	78.00	84.00	118.00	127.00	132.00	144.00	153.00	132.00	1.047
	3	24.00	31.00	57.00	74.00	89.00	96.00	107.00	121.00	136.00	167.00	143.00	1.134
	4	22.00	37.00	54.00	71.00	92.00	101.00	112.00	125.00	137.00	159.00	137.00	1.087
	Mean ± SD	22.00 ± 1.20	34.00 ± 2.54	54.00 ± 3.08	73.00 ± 3.3	90.50 ± 4.71	105.50 ± 3.26	117.00 ± 3.35	128.50 ± 0.82	140.00 ± 3.53	157.50 ± 6.22	135.00 ± 5.02	1.074 ± 0.03
20%SM	1	29.00	37.00	59.00	76.00	96.00	127.00	131.00	138.00	152.00	168.00	139.00	1.103
	2	22.00	38.00	56.00	79.00	99.00	118.00	127.00	138.00	152.00	158.00	136.00	1.079
	3	20.00	34.00	57.00	78.00	104.00	119.00	126.00	133.00	140.00	166.00	146.00	1.158
	4	24.00	32.00	52.00	74.00	101.00	121.00	128.00	134.00	142.00	159.00	135.00	1.071
	Mean ± SD	23.00 ± 3.31	35.00 ± 2.38	56.00 ± 2.54	76.00 ± 1.91	100.00 ± 2.91	121.00 ± 3.49	128.00 ± 1.87	135.00 ± 2.27	146.00 ± 5.54	162.75 ± 4.32	139.00 ± 4.30	1.102 ± 0.03
30%SM	1	27.00	31.00	58.00	69.00	94.00	105.00	117.00	122.00	137.00	159.00	132.00	1.047
	2	22.00	28.00	53.00	72.00	98.00	108.00	115.00	121.00	129.00	158.00	136.00	1.079
	3	19.00	33.00	52.00	66.00	91.00	102.00	117.00	118.00	134.00	156.00	137.00	1.087
	4	20.00	34.00	46.00	75.00	86.00	104.00	113.00	121.00	136.00	153.00	133.00	1.055
	Mean ± SD	22.00 ± 3.01	31.50 ± 2.29	52.00 ± 4.26	70.00 ± 3.32	92.00 ± 4.38	105.00 ± 4.14	115.00 ± 1.65	120.00 ± 1.50	134.00 ± 3.08	156.50 ± 2.29	134.00 ± 2.06	1.067 ± 0.01

Fig.1

Average length(mm) attained by rohu in different treatments

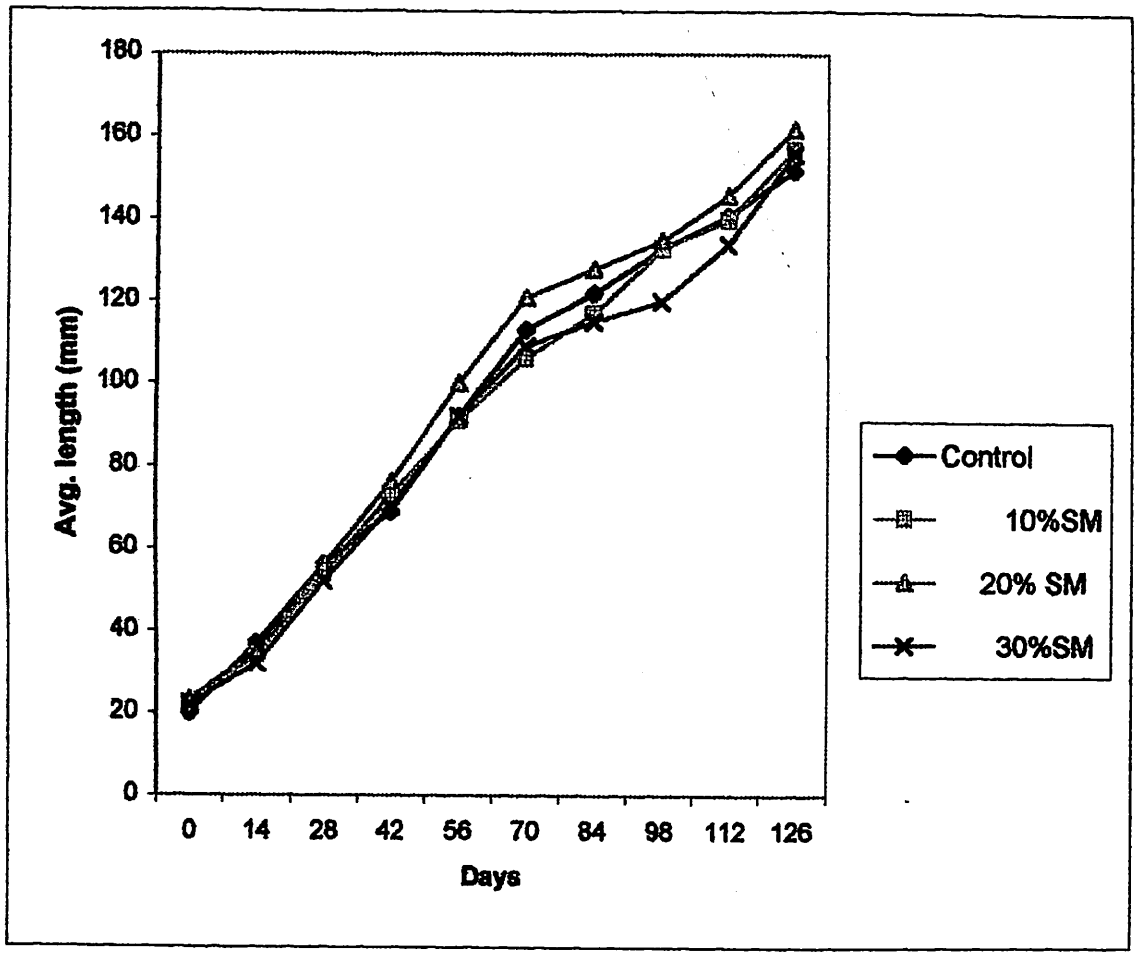


Table 16

Growth in weight (g) of rohu in different treatments

Treatment	Days / Cistern	0	14	28	42	56	70	84	98	112	126	Net wt. gain	Increment /day
Control	1	1.11	2.56	3.98	5.78	7.26	9.13	9.69	10.89	12.12	14.70	13.59	0.107
	2	0.99	2.71	3.81	5.54	7.12	8.69	10.24	11.03	12.26	14.42	13.43	0.106
	3	1.06	2.68	3.68	5.72	7.34	8.82	9.46	10.56	12.38	14.30	13.24	0.105
	4	1.06	2.43	3.75	5.61	6.82	9.21	9.98	10.72	11.89	14.51	13.45	0.106
	Mean ± SD	1.05 ± 0.04	2.59 ± 0.11	3.80 ± 0.11	5.66 ± 0.09	7.13 ± 0.19	8.96 ± 0.21	9.84 ± 0.29	10.82 ± 0.17	12.16 ± 0.18	14.48 ± 0.14	13.42 ± 0.16	0.106 ± 0.001
10%SM	1	1.08	2.13	3.77	5.42	7.26	9.03	9.87	10.84	12.23	13.9	12.82	0.101
	2	1.07	2.24	3.62	5.56	6.86	9.13	9.75	10.58	12.38	14.1	13.03	0.103
	3	1.12	2.48	3.67	5.46	6.95	8.72	9.42	10.89	11.78	15.1	13.98	0.110
	4	1.2	2.33	3.42	5.31	7.30	8.68	9.74	10.74	11.82	14.9	13.70	0.108
	Mean ± SD	1.11 ± 0.05	2.29 0.12	3.62 ± 0.12	5.43 ± 0.08	7.09 ± 0.19	8.89 ± 0.19	9.69 ± 0.16	10.76 ± 0.11	12.05 ± 0.25	14.50 ± 0.50	13.38 ± 0.16	0.105 ± 0.004
20%SM	1	1.18	2.63	3.92	5.87	7.92	9.51	10.30	11.78	13.26	15.31	14.13	0.112
	2	1.20	2.42	3.83	5.89	7.86	8.41	10.20	11.82	13.41	14.81	13.61	0.108
	3	1.01	2.68	3.96	5.68	7.67	8.72	9.47	10.59	12.68	15.14	14.13	0.112
	4	1.15	2.57	3.72	5.73	7.56	9.63	9.82	10.42	12.29	14.90	13.75	0.109
	Mean ± SD	1.13 ± 0.07	2.57 ± 0.09	3.85 ± 0.09	5.79 ± 0.08	7.75 ± 0.14	9.06 ± 0.51	9.94 ± 0.32	11.15 ± 0.65	12.91 ± 0.44	15.04 ± 0.18	13.90 ± 0.18	0.110 ± 0.005
30%SM	1	1.03	2.07	3.56	5.12	6.68	7.82	8.62	9.71	11.56	12.68	11.65	0.092
	2	1.12	2.12	3.28	4.98	6.31	7.38	7.98	9.61	10.29	13.71	12.59	0.099
	3	1.09	2.31	3.34	4.82	6.26	7.52	8.32	9.23	11.11	13.42	12.33	0.097
	4	1.10	2.24	3.42	4.01	6.52	7.66	8.50	9.54	11.24	13.17	12.07	0.095
	Mean ± SD	1.08 ± 0.03	2.18 ± 0.09	3.40 ± 0.01	4.73 ± 0.43	6.44 ± 0.16	7.59 ± 0.16	8.35 ± 0.24	9.52 ± 0.17	11.05 ± 0.46	13.24 ± 0.37	12.16 ± 0.37	0.095 ± 0.002

Table 16a

Analysis of variance of growth data.

Source	Sum of square	Degree of freedom	Mean sum of square	F
Diet	59045.55	3	19681.83	3.163**
Error	7466.50	12	6222.20	
Total	133712.05	15		

** Significant at 1%.

Fig. 2

Average weight (g) attained by rohu in different treatments.

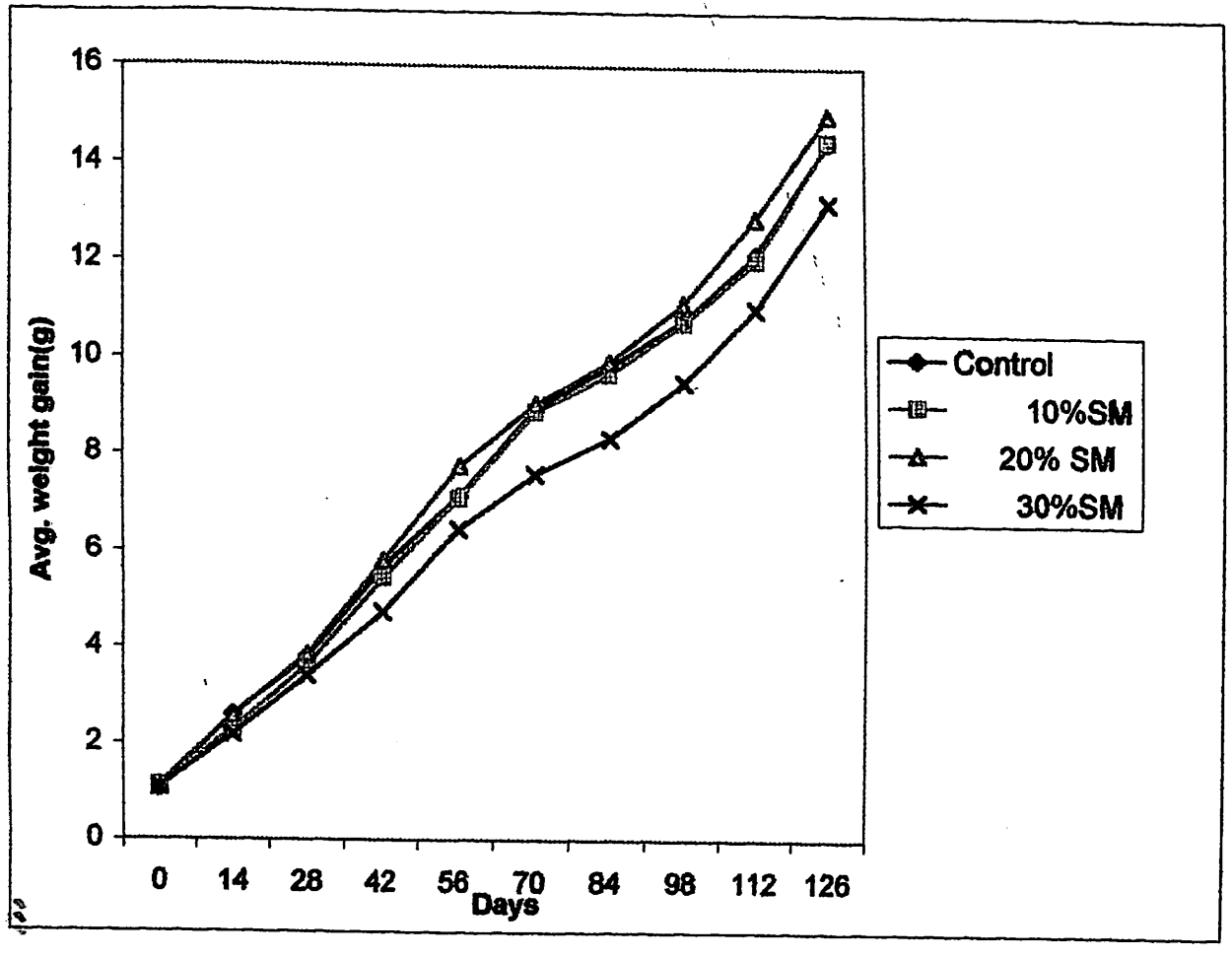


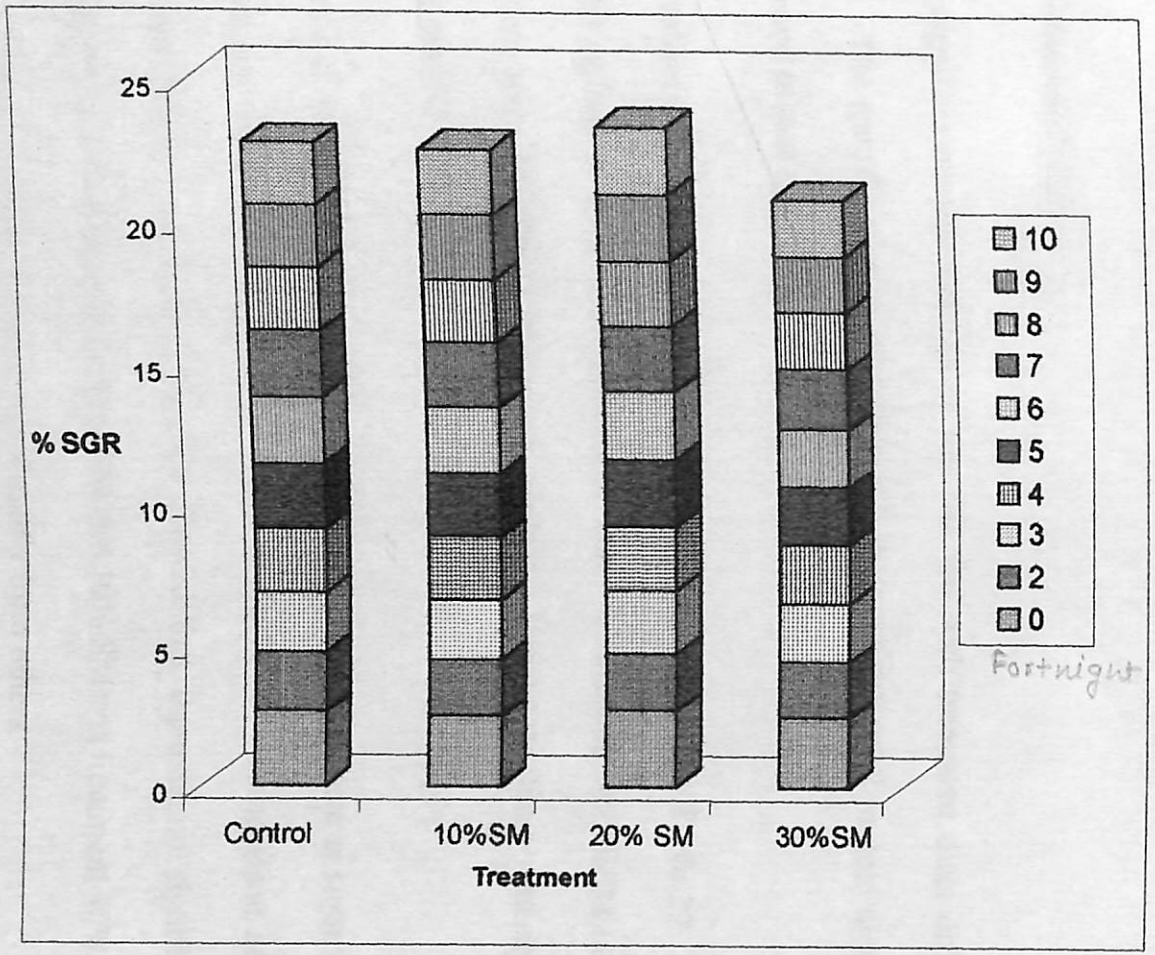
Table 17

Specific growth rate (SGR as %) of rohu in different treatments over the experimental period

Treat-ment	Days / Cistern	0	14	28	42	56	70	84	98	112	126	Average SGR
Control	1	2.73	2.05	2.16	2.27	2.32	2.32	2.27	2.17	2.27	2.20	
	2	2.68	2.11	2.23	2.31	2.27	2.42	2.32	2.28	2.22	2.27	
	3	2.57	2.06	2.07	2.17	2.33	2.39	2.34	2.32	2.06	2.16	
	4	2.64	2.10	2.15	2.34	2.39	2.37	2.30	2.31	2.26	2.12	
	Mean ± SD	2.65 ± 0.05	2.09 ± 0.04	2.15 ± 0.05	2.27 ± 0.06	2.32 ± 0.04	2.37 ± 0.03	2.30 ± 0.02	2.27 ± 0.05	2.20 ± 0.08	2.18 ± 0.05	2.18 ± 0.147
10%SM	1	2.42	2.02	2.17	2.27	2.34	2.38	2.32	2.27	2.28	2.29	
	2	2.56	2.04	2.21	2.17	2.27	2.32	2.26	2.21	2.23	2.32	
	3	2.48	2.03	2.03	2.32	2.13	2.27	2.24	2.26	2.30	2.36	
	4	2.59	1.93	2.11	2.36	2.29	2.33	2.31	2.29	2.32	2.38	
	Mean ± SD	2.51 ± 0.06	2.00 ± 0.04	2.13 ± 0.06	2.28 ± 0.07	2.25 ± 0.07	2.32 ± 0.03	2.28 ± 0.03	2.25 ± 0.02	2.28 ± 0.03	2.33 ± 0.03	2.26 ± 0.125
20%SM	1	2.81	2.03	2.37	2.31	2.37	2.31	2.26	2.24	2.34	2.42	
	2	2.74	1.99	2.33	2.46	2.41	2.38	2.34	2.28	2.36	2.38	
	3	2.57	2.14	2.28	2.11	2.37	2.42	2.38	2.32	2.32	2.34	
	4	2.78	2.03	2.17	2.38	2.28	2.46	2.39	2.36	2.39	2.37	
	Mean ± SD	2.72 ± 0.09	2.04 ± 0.05	2.28 ± 0.07	2.31 ± 0.12	2.25 ± 0.04	2.39 ± 0.05	2.34 ± 0.05	2.30 ± 0.04	2.35 ± 0.02	2.37 ± 0.02	2.34 ± 0.156
30%SM	1	2.57	2.04	2.17	2.19	2.13	2.11	2.12	1.98	2.03	2.11	
	2	2.46	1.98	2.06	2.17	1.92	2.0	2.17	2.02	1.82	2.09	
	3	2.49	1.99	2.12	2.08	2.01	2.03	2.20	2.11	1.99	1.98	
	4	2.57	1.96	1.97	2.11	2.1	2.06	2.02	2.06	2.05	1.80	
	Mean ± SD	2.50 ± 0.04	1.99 ± 0.02	2.10 ± 0.03	2.13 ± 0.04	2.04 ± 0.08	2.05 ± 0.04	2.12 ± 0.06	2.04 ± 0.04	1.97 ± 0.09	1.99 ± 0.12	2.09 ± 0.145

Fluctuations in specific growth rate (SGR) of rohu in different treatments

Fig. 3



4.9. Absolute conversion ratio:

Table 21 presents the average conversion ratio. The best ACR (3.521) was observed for the fish fed on 20%SM diet, followed by 10% SM (3.754), control (3.898) and 30%SM diet (4.090).

4.10. Enzymatic activity:

4.10.1 Protease activity:

The digestive enzyme activity of the fish fed with treatment diets differed significantly. The specific activity of protease in the total fish meal protein diet was higher compared to that of diet containing soyabean meal protein.

The values obtained for various treatments are presented in Table 22. The highest (1.210 µg/mg protein) was recorded for control, followed by20%SM (1.115 µg/mg protein) and 10%SM (1.050µg/mg protein). Lowest protease activity of (0.995 µg/mg protein) was recorded for 30% SM treatment.

Analysis of variance of protease activity data showed that there is significant difference between treatments ($P \leq 0.05$) as shown in Table 23. Comparison of the treatment means based on critical difference showed that there is no significant difference ($P \geq 0.05$) between treatments 20% SM and 10% SM and treatment 10% SM and 30% SM. However, control differed significantly from others.

4.10.2. Amylase activity:

The specific activity of amylase for various treatments is shown in Table 24. The highest was recorded for treatment 30% SM (2.37µg/mg protein) and lowest for

control (2.24 µg/mg protein). In the 20% SM and 10% SM treatments the values were 2.32 µg/mg protein and 2.26 µg/mg protein, respectively.

Analysis of variance of the data showed that there is significant difference between treatments ($P \leq 0.01$) as shown in Table 25. Comparison of the treatment means based on the critical difference showed that there is no significant difference ($P \geq 0.05$) in amylase activity between treatment 20% SM and 30% SM and also between treatment 10% SM and control.

4.10.3. Lipase Activity:

The highest specific lipase activity was observed in treatment 20% SM (0.432 µg/mg protein). The lipase activity of test diets 10% SM and control was 0.391µg/mg proteins and 0.377µg/mg protein, respectively (Table 26). The lowest lipase activity (0.258µg/mg protein) was recorded for the treatment 30% SM and the highest for the treatment 20% SM.

Analysis of variance of data showed that there is significant difference between treatments ($P \leq 0.01$) as shown in Table 27. Comparison of the treatment means based on critical difference showed that there is no significant difference ($P \geq 0.05$) between treatments 20% SM and 10% SM and between treatment 10% SM and control. Treatment 30% SM differed significantly from others.

Table 18

Survival (percentage)and net production of rohu in different treatments

Treatment	Cistern No.	% Survival	Net weight gain of fish (g)	Net production kg/ha /126 days
Control	1	100 (10)	13.59	2136.45
	2	90 (9)	13.43	1869.04
	3	90 (9)	13.24	1873.29
	4	90 (9)	13.45	1907.24
Mean		92.50	13.42	1946.50
10%SM	1	90 (9)	12.82	1842.16
	2	100 (10)	13.03	2048.42
	3	80 (8)	13.98	1670.17
	4	90 (9)	13.70	1768.58
Mean		90.00	13.38	1832.33
20%SM	1	90 (9)	14.13	1999.79
	2	90 (9)	13.61	1934.12
	3	100 (10)	14.13	2202.32
	4	80 (8)	13.75	1740.60
Mean		90.00	13.90	1969.20
30%SM	1	90 (9)	11.65	1768.58
	2	90 (9)	12.59	1754.58
	3	90 (9)	12.33	1740.29
	4	90 (9)	12.07	1697.84
Mean		90.00	12.36	1743.82

Figures in paratheses indicate the number of fish harvested.

Table 19

Food conversion efficiency (%) and protein efficiency ratio of rohu fry in different treatments

Treatment	Replication	FCE%	PER
Control	1 2 3 4	25.5 25.6 26.1 25.6	0.856 0.852 0.855 0.864
Mean ± SD		0.257 ± 0.0023	0.856 ± 0.005
10% SM	1 2 3 4	25.7 24.9 25.4 24.8	0.791 0.832 0.847 0.826
Mean ± SD		0.252 ± 0.0036	0.820 ± 0.020
20% SM	1 2 3 4	28.0 27.0 27.8 27.6	0.926 0.907 0.928 0.922
Mean ± SD		0.276 ± 0.0029	0.920 ± 0.008
30% SM	1 2 3 4	24.8 24.8 24.6 24.1	0.832 0.830 0.820 0.806
Mean ± SD		0.245 ±0.0028	0.822 ± 0.010

Table 20

Digestion coefficient of the experimental diets fed to rohu in different treatments

Treatment	Feed	Dry matter	Protein		Fat		Fibre		Ash		NFE	Digestibility coefficient			
			%	g	%	g	%	g	%	g	g	Protein	Fat	Fibre	NFE
Control	Consumed	3.677		1.0310		0.2720		0.1695		0.4470	1.681				
	Excreted	1.034	30.00	0.120	7.42	0.0341	4.61	0.0485	12.16	0.0426	0.1623	65.45	70.65	71.82	56.32
	Assimilated		1.6		0.62		0.47		4.12						
	(g)	2.643		1.0866		0.2070		0.1210		0.4041	0.8187				
10% SM	Consumed	3.277		0.9831		0.2549		0.1500		0.6010	1.288				
	Excreted	1.112	30.00	0.2131	7.78	0.110	4.58	0.0440	18.37	0.1351	0.6591	60.81	66.84	65.84	77.22
	Assimilated		19.17		5.47		3.96		12.18						
	(g)	2.165		0.7699		0.1941		0.1060		0.4662	0.6289				
20% SM	Consumed	3.832		1.1496		0.3088		0.1502		0.6886	1.5348				
	Excreted	1.120	30.00	0.1699	8.06	0.108	3.92	0.0348	17.97	0.1520	0.7306	64.58	71.12	70.52	83.04
	Assimilated		15.17		2.92		3.11		13.58						
	(g)	2.712		0.9797		0.2761		0.1154		0.5366	0.8042				
			14.83												
30% SM	Consumed	3.85		1.185		0.2879		0.1674		0.7038	1.6059				
	Excreted	1.22	30.00	0.1434	7.29	0.0344	4.24	0.0439	17.82	0.1500	0.9682	59.60	6.15	68.06	88.77
	Assimilated		23.23		6.12		3.60		12.30						
	(g)	2.63		0.9016		0.2134		0.1234		0.5538	0.9376				
									5.52						

Table 21

Absolute conversion factor of diets fed to rohu

Treatment	No. of days	Feed given (g) / week	Wt. of fish (g)	Growth increment/week (g)	Conversion factor /week	Conversion factor for 42 days	Average growth /day (g)
Control	0	-	4.74	-	-	3.898	-
	7	1.911	5.47	0.73	2.617		0.104
	14	2.156	6.17	0.70	3.080		0.100
	21	2.324	6.65	0.48	4.840		0.068
	28	2.527	7.23	0.58	4.356		0.082
	35	2.765	7.93	0.70	3.951		0.100
	42	3.045	8.60	0.67	4.544		0.097
10%SM	0	-	4.87	-	-	3.754	-
	7	1.876	5.36	0.49	3.828		0.070
	14	2.044	5.84	0.48	4.258		0.068
	21	2.247	6.43	0.59	3.808		0.084
	28	2.513	7.19	0.76	3.306		0.108
	35	2.807	8.02	0.83	3.381		0.118
	42	3.080	8.80	0.78	3.948		0.111
20%SM	0	-	4.68	-	-	3.521	-
	7	1.876	5.37	0.69	2.718		0.098
	14	2.177	6.23	0.86	2.531		0.122
	21	2.520	7.20	0.97	2.597		0.138
	28	2.807	8.03	0.83	3.381		0.118
	35	3.017	8.62	0.59	5.113		0.084
	42	3.255	9.30	0.68	4.786		0.097
30%SM	0	-	4.78	-	-	4.090	-
	7	1.834	5.25	0.47	3.902		0.067
	14	2.037	5.82	0.57	3.573		0.081
	21	2.219	6.34	0.52	4.267		0.074
	28	2.422	6.92	0.58	4.175		0.080
	35	2.625	7.50	0.58	4.525		0.080
	42	2.870	8.20	0.70	4.100		0.100

4.11. Final biochemical composition of the muscle of the fish:

Table 28 gives the results of analysis of carcass composition on termination of the experiment.

Moisture content was found to decrease in fish in all treatments, with maximum being in the control as compared to the initial carcass composition (Table 8).

Maximum increase in protein content was found when fed with the control (20.96%). This was followed by 20%SM (20.16%) and 10%SM (19.84%). The above data also reflects the same order of performance in terms of protein digestibility coefficient of various diets. Protein synthesis was low (18.02%) in fish fed on 30%SM.

Final fat content has shown different patterns of deposition. Maximum was in fish fed on 20%SM (4.97%). Fish fed on the control showed better fat deposition (4.82%) compared to those fed with 10%SM diet (4.17%)and 30% SM (3.12%). The NFE (Nitrogen Free Extract) content in general showed slight increase in the fish fed on feeds containing soyabean meal

Energy content showed an increase in its value after fish were fed with various feeds, the maximum being in those fed on the control (1.76 %) followed by 20%SM diet (1.74%). Minimum energy content was recorded for 30% SM diet (1.45%).

Table 22

Protease activity (µg/mg protein) in digestive tract of rohu in different treatments

Treat-ment	Replication	Final protease activity (µg/mg protein)	
		Total protease activity	Specific protease activity
Control	1 2 3 4	9.25 8.91 9.41 9.58	1.19 1.16 1.28 1.21
Mean ± SD		0.667 ± 0.138	1.21 ± 0.04
10% SM	1 2 3 4	8.58 8.25 9.08 9.25	1.03 0.99 1.09 1.11
Mean ± SD		0.705 ± 0.138	1.055 ± 0.047
20% SM	1 2 3 4	9.91 9.66 10.66 10.08	1.11 1.07 1.13 1.15
Mean ± SD		0.635± 0.0141	1.115 ± 0.02
30% SM	1 2 3 4	8.16 8.5 8.33 8.19	0.98 1.02 1.00 0.98
Mean ± SD		0.572 ± 0.104	0.995 ± 016

Table 23

Analysis of variance of specific protease activity

Source	Sum of square	Degree of freedom	Mean sum of square	F
Diet	0.1009	3	0.03363	14.78 **
Error	0.0273	12	0.002275	
Total	0.1282	15		

** Significant at 1%.

Comparison based on critical difference :

Treatment	Control	10% SM	20% SM	30% SM
Mean	1.21	1.05	1.11	0.99

Critical Difference = 0.0884

Control 20% SM 10% SM 30% SM

Table 24

Amylase activity(µg/mg protein) in digestive tract of rohu in different treatments

Treat-ment	Replication	Final amylase activity (µg/mg protein)	
		Total amylase activity	Specific amylase activity
Control	1 2 3 4	18.83 19.08 18.58 18.33	2.26 2.29 2.23 2.2
Mean ± SD		19.70 ± 0.279	2.24 ± 0.033
10% SM	1 2 3 4	19.00 19.41 18.66 18.33	2.28 2.33 2.24 2.2
Mean ± SD		18.85 ± 0.40	2.26 ± 0.048
20% SM	1 2 3 4	18.33 19.5 19.25 19.95	2.26 2.34 2.31 2.37
Mean ± SD		19.33 ± 0.33	2.32 ± 0.040
30% SM	1 2 3 4	19.66 19.33 20.08 19.91	2.36 2.32 2.41 2.39
Mean ± SD		19.74 ± 0.28	2.37 ± 0.040

Table 25

Analysis of variance of specific amylase activity

Source	Sum of square	Degree of freedom	Mean sum of square	F
Diet	0.03892	3	0.01297	6.198 **
Error	0.02497	12	0.00208	
Total	0.0639	15		

** Significant at 1%.

Comparison based on critical difference:

Treatment	Control	10% SM	20% SM	30% SM
Mean	2.24	2.26	2.32	2.37

Critical Difference = 0.0825

30% SM 20% SM 10% SM Control

Table 26

Lipase activity (µg/mg protein) in digestive tract of rohu in different treatments

Treatment	Replication	Final lipase activity (µg/mg protein)	
		Total lipase activity	Specific lipase activity
Control	1 2 3 4	3.35 2.68 3.57 2.89	0.421 0.322 0.421 0.342
Mean ± SD		3.14 ± 0.365	0.377 ± 0.04
10% SM	1 2 3 4	2.89 3.55 3.52 2.89	0.421 0.372 0.426 0.347
Mean ± SD		3.24 ± 0.263	0.391 ± 0.03
20% SM	1 2 3 4	3.25 4.13 3.71 3.30	0.391 0.496 0.446 0.396
Mean ± SD		3.59 ± 0.0355	0.432 ± 0.04
30% SM	1 2 3 4	2.47 1.83 2.26 2.02	0.297 0.22 0.272 0.243
Mean ± SD		2.145 ± 0.241	0.258 ± 0.029

Table 27

Analysis of variance of specific lipase activity

Source	Sum of square	Degree of freedom	Mean sum of square	F
Diet	0.06721	3	0.0224	11.72 **
Error	0.02295	12	0.00191	
Total	0.09016	15		

** Significant at 1%.

Comparison based on critical difference:

Treatment	Control	10% SM	20% SM	30% SM
Mean	0.377	0.391	0.432	0.258

Critical Difference = 0.0791

20% SM **10% SM** **Control** **30% SM**

4.12. Organoleptic evaluation:

Analysis of variance of the data showed that there is significant difference between treatments ($P \leq 0.01$) as shown in Table 29. Pair wise comparison of the treatment means based on critical difference showed that there is no significant difference ($P \geq 0.05$) between treatment 10% SM and 30% SM.

4.13 Statistical analysis:

The mean final weight of rohu in different treatments was subjected to one way analysis . Analysis of variance of data showed that the 20% SM and control are significantly different at 1% level of significance.

Table 28

Proximate composition* of muscle of rohu

in different treatments (final analysis)

Parameter / Treatment	Moisture Content %	Crude protein %	Crude fat%	Ash%	Nitrogen free extract %	Energy k cal/g.
Control	68.77 (±0.02)	20.96 (±0.13)	4.82 (±0.06)	1.87 (±0.19)	3.27 (±0.32)	1.76 (±0.03)
10% SM	71.9 (±0.05)	19.84 (±0.02)	4.17 (±0.08)	1.57 (±0.08)	3.42 (±0.09)	1.65 (±0.06)
20% SM	69.44 (±0.01)	20.16 (±0.32)	4.97 (±0.07)	1.69 (±0.01)	3.74 (±0.02)	1.74 (±0.51)
30% SM	73.09 (±0.01)	18.02 (±0.07)	3.12 (±0.02)	1.78 (±0.14)	3.92 (±0.17)	1.45 (±0.06)

- Expressed on dry weight basis. *Average of three values.
- Figures in parentheses indicate SD

Table 29

Analysis of variance of organoleptic evaluation

Source	Sum of square	Degree of freedom	Mean sum of square	F
Diet	1.15600	3	0.3853	12.81**
Parameter	0.08817	9	0.09796	3.25
Error	0.08117	27	0.03006	
Total	1.32534	39		

****Significant at 1% level .**

Comparison based on critical difference :

Treatment	Control	10% SM	20% SM	30% SM
Mean	33.75	29.95	32.81	29.94

Critical Difference = 0.1839

Control 20% SM 10% SM 30% SM

Discussion

5. DISCUSSION

5.1 Proximate composition of the formulated feeds:

Proximate composition of the ingredients used in feed formulation was carried out with a view to balancing the nutrient level to the extent possible. Fish meal with over 50% of protein is generally considered to be of good quality (NRC, 1977). Groundnut oil cake had optimum level of protein, fat and NFE. Higher level of fibre in rice bran indicates the presence of higher level of husk As expected, tapioca flour which was used to serve as binder had higher carbohydrate content. Soyabean meal had a protein content of 52.50% . Good quality soyabean meal is reported to contain 50 - 55% protein and the protein level of soyabean meal used in this study falls within this range.

5.1.1 Shelf life of formulated feed :

There was no drastic change in proximate composition of formulated feeds following four months of storage indicating that the quality of the diets developed was good. Shelf life of processed feed is dependent on the type of processing, storage temperature and moisture content (Hilton and Slinger,1976) . Increased moisture content could be due to the presence of hygroscopic ingredients mainly starch, which brings about softening of the pellets after storage (Hastings, 1971). The extent of moisture uptake also depends on the physical structure of the ingredient particles, porosity of feed and the relative humidity of the storage premises.

A good quality feed should maintain nutritional adequacy for few weeks of storage. Decrease in protein content was also observed in all the diets, which could be attributed to the increase in moisture content as observed by Jayaram and Shetty (1981). This is probably due to the break down of water soluble proteins owing to the moisture content and the deterioration of amino acids (Cho,1980). Crude fat content showed a decrease in all the diets.

According to Fowler and Banks (1976) there is no alteration in the nutritional adequacy in diets stored at room temperature for several weeks but storage for longer periods could have deleterious effects on the growth of fish.

The feeds employed in the present investigation maintained adequate nutritional quality over the period of storage of 126 days.

5.1.2 Water stability of formulated diets:

There was not much variation in the water stability of the diets after four months. In general water stability was found to be good after six hours with retention over 80%. Since carps are relatively slow feeders, it is recommended that the feeds developed should be stable for atleast two hours (Venugopal,1980;Anil,1981)

Higher stability was observed in soyabean meal even at lower levels of inclusion probably owing to higher levels of carbohydrates in soyabean meal . Better gelatinization of starch has been reported to increase the stability of diets (Boonyaratpalin and Lovell,1977).

The results of the water stability test of the diets indicate that all the diets were stable for five hours, stability started declining only thereafter. Since carps require diet stable for only two hours, test diets developed possess this primary character.

5.2 Water quality:

In fish culture, water quality can be defined as the suitability of water for survival and growth of fish. Water quality management forms an integral part of aquaculture systems. Knowledge of the complex interactions continuously taking place between the ecosystem and the stocked animals enables enhancement of survival and production, through appropriate manipulation of the aquatic environment. Artificial feeding has been found to profoundly influence the water quality. Moreover, food utilization is also found to be affected by water quality.

5.2.1 Temperature:

Rohu an inhabitant of tropical water can tolerate a temperature range of 24 to 32 ^{0}C (Alikunhi,1957). The temperature fluctuation observed over the experimental period ranged from 25.2 to 26 ^{0}C and is well within the tolerance range of carps in general and therefore could be considered conducive for the growth of rohu.

5.2.2 p^{H}:

Water p^{H} ranged from 7.0 to 8.5. Water suitable to culture is one, which has near neutral to slightly alkaline $p^{H,}$ ranging between 7 to 8 (Huet, 1975). Depasse (1956)

found that p^H range of 6.5 to 9.0 is suitable for fish culture. If waters are highly acidic with p^H values <6.5 or highly alkaline with values above 9.5 for longer periods, fish production and growth can be adversely affected (Mount, 1973). p^H values recorded in the present study is in the range that is generally accepted for fish culture.

5.2.3 Dissolved oxygen:

Dissolved oxygen is one of the most crucial factors in maintaining aquatic life. The effect of the dissolved oxygen on fish is determined by variety of factors including temperature which influences its solubility in water and metabolic rate of fish. Dissolved oxygen levels also influence the digestibility of feed. Kerns and Roelofs (1977) observed that artificial feeding is an important factor which determines the oxygen balance in culture ponds. In the present study, dissolved oxygen values varied only from 4.2 to 6.6 mg/ l which is in the normal range for cyprinids.

5.2.4 Total alkalinity:

Natural waters that contains 40 mg/l or more of total alkalinity are considered more productive than waters of low alkalinity (Mairs,1966). Waters of low alkalinity are poorly buffered against fluctuations in P^H and consequently rapid reduction in P^H occurs when carbon dioxide levels goes down . Total alkalinity values recorded in the present study ranged from 37.5 to 65.70 ppm and hence would not have been a limiting factor for fish growth.

5.2.5 Ammonia:

In the present study the ammonia concentration ranged between 0.038 and 0.099 mg/l.

Growth of fish is found impaired at unionized ammonia concentration of 0.12 mg/l or higher (Brown *et.al,* 1997). The concentrations of ammonia recorded in the present study were within this limits.

5.2.6 Plankton:

Plankton biomass in the experimental cisterns was rather meagre. The lack of soil base in cisterns and periodic replenishment of water can be regarded as reason for the low plankton production encountered in the present study.

5.3 Fish growth:

Among the various plant protein sources soyabean appears to be the most widely investigated substitute for animal protein in fish diets. It has been used in varying degree of success in the diet of trout (Tacon *et.al.* ,1983), catfish (Wilson and Poe,1985b),tilapia (Davis and Stickney,1978) and common carp (Murai *et.al.*,1986).Soyabean meal is becoming increasingly available in the country and its usage in fish diet formulation could help in reducing heavy dependence on fish meal. The growth trial conducted over a period of 126 days showed that 20% SM diet having 10% fish meal resulted in significantly superior growth of rohu as compared to that of the other diets. The overall average growth /day, both in terms of weight and length was

highest in the fish raised on 20%SM being 0.105g and 1.05 mm, respectively. So also the SGR (2.34) was the highest for the same treatment . Best growth recorded with 20% SM diet appears to be due to the balanced amino acid profile of the diet, which had 20% of soyabean meal in combination with 10% of fish meal. The consistently higher growth of fish recorded with 20% SM diet throughout the experimental period in the present study makes it clear that this combination is the best for rohu. The comparatively good growth of rohu obtained with soyabean meal - fish meal combination diet (20%SM) indicates the possibility of replacing fish meal only partially with soyabean meal. However, the combination 20% fish meal and 10% soyabean meal lead to growth even lower than that of the control, but higher than the growth on 30% SM diet.

Complete replacement of fish meal by soyabean meal in fish diet has not yeilded fruitful results unless enriched with amino acids but partial replacement has met with considerable success (Viola and Arieli ,1982; Balogun and Ologhobo, 1989). In the present study complete substitution of fish meal by soyabean meal (30% inclusion level) led to the lowest weight gain being even lower than that of the control. Though soyabean meal has one of the best amino acid profile of plant protein sources, methionine and lysine are limiting amino acids for most animals (Dani,1994). Bhat *et. al.* (1986) have recorded inferior growth of catla fed soyabean meal based diets. However, it is pertinent to state here that some progressive farmers in Andhra Pradesh use soyabean meal in carp diets with encouraging results. It is to be noted that the

farmers also used heavy fertilizers, the resultant plankton probably compensating for the deficient amino acid of the artificial diet.

Viola *et.al.*(1982) obtained good growth in common carp when fish meal was completely substituted with soyabean meal but enriched with lysine ,methionine and 10% oil. Murai *et. al* .(1986) also obtained 90% of weight gain in common carp employing soya flour diets enriched with essential amino acids compared with fish meal diet. In a subsequent study Murai *et. al.* (1989) found that supplementation of soya flour diet with 0.5% methionine alone is sufficient to enhance growth of common carp to the level obtained with fish meal.

The superior growth of rohu in the present study attaining final weight of 15.04 g in 20%SM as against 14.48 g in control diet appears to be due to the balanced amino acid profile brought about by combining 20% soyabean meal and 10% fish meal.

Soyabean meal is known to contain anti-nutritional factors other than trypsin inhibitors which are known to suppress growth in fishes (Wilson *et.al.*,1981;Wilson and Poe,1985a). It has also been reported that high activity of protease inhibitors in crude or inadequately heated soyabean meal adversely affects growth of fish (Dabrowski *et.al.*,1989). Smith (1977) suggested heating of soyabean meal at 175 to 195 ^{0}C for complete destruction of anti-nutritional factors, but this temperature is too high at which most of the nutrients could be destroyed. Spinelli *et.al.*(1979) suggested heating of soyabean meal at 110 ^{0}C for 15 minutes. This temperature was adopted in the present study and it appears to be quite sufficient to inactivate anti-nutritional factors, since no

adverse effects were noticed in any of the treatments. The lowest growth on 30 % SM diet could be due to the imbalance in amino acid profile resulting from complete elimination of fish meal from the diet.

In the present study, fish fed diets having combination of soyabean meal with fish meal showed better growth than those fed a soyabean meal based diet devoid of fish meal. The proportion of soyabean meal and fish meal in the diet also appears to influence the growth trend as evidenced by the relatively lower growth on 10% SM diet. Complete substitution of fish meal by soyabean meal appears to be unsuitable in diets for rohu as evidenced by the lowest growth attained in 30% SM diet. Thus, the growth results clearly indicate that use of soyabean meal in combination with fish meal is superior to use of soyabean meal alone or fish meal alone in formulated feeds for rohu suggesting the possible synergistic effect of fish meal and soyabean meal on growth.

5.4 Specific growth rate:

Specific growth rate can be considered as an index of growth in the evaluation of diets. The highest SGR (2.34) was attained in diet 20 %SM. The higher SGR recorded in first fortnight in all treatments could be due to the spurt in growth of fish as a result of thinning out from the stock tank at the beginning of the experiment. Higher SGR obtained in 20 % SM has indicated better utilization and efficient conversion of this diet by rohu.

5.5 Survival and gross production:

Overall survival was fairly high, minimum being 90 %. No correlation could be drawn between treatment and survival indicating that treatment has no influence on survival of fish . The highest production was obtained under treatment 20 %SM. It was seen that production was linked to growth rather than survival.

5.6 Food conversion efficiency:

Best food conversion efficiency was obtained with 20% SM, while the lowest was observed in 30% SM. Percentage food conversion efficiency did not differ much between 10%SM and control . The better food conversion efficiency of the diet containing combination of 20% soyabean meal and 10 % fish meal could probably be due to the favourable amino acid profile of the combination. The lower conversion efficiency noticed in 30% SM diet is reflected in comparatively poorer growth attained in that diet.

5.7. Protein efficiency ratio:

Protein efficiency ratio is used to evaluate the quality of dietary protein, those with high PER can be considered as better quality and those with low values as poor quality. In the current study the higher PER value was recorded for the treatment 20%SM followed by the control .

Chamundeshwari *et al.* (1999) reported PER values 2.38, 2.47 and 2.73 for the soyabean meal based diet with 20, 40 and 60% soyabean meal, respectively. In the current study PER values obtained was maximum with 20% soyabean meal in the diet but it was comparatively lower than above PER values.

5.8 Statistical analysis:

The mean final weight of rohu in different treatments was subjected to two way analysis. Significant difference was observed at 1% level of significance.

5.9. Apparent digestibility coefficient:

Digestibility determination along with chemical analysis allows a more thorough estimation of nutritive value of a particular protein source (Plakas and Katayama, 1981). The digestibility co-efficient is found to vary with the size of fish, microflora of intestine and digestive enzymes (Dabrowski, 1977). Digestibility may also be limited due to incomplete digestive action or incomplete absorption (Maynard and Loosli, 1978).

In the present study, fish fed with total soyabean meal (30%SM) diet showed only 59.60% protein digestibility .These lower values of protein digestibility of soyabean meal could be due to any trypsin inhibitor remining even after heat processing as observed by Smith (1977) and Dabrowski and Kozak (1979).

Apparent digestibility of the fat in the test feeds ranged from 66.15 to 71.12% . The lowest value (66.15%) was obtained for treatment 30%SM. It could be attributed to

the high amount of crude fiber in plant sources, which may lower the digestibility of nutrients because of rapid passage of food through the gut (Berder, 1967).

Higher value of lipid digestibility was observed in the treatment 20%SM (71.12%), which has also shown the maximum lipase activity in the digestive tract. Kirchgessner *et al.*, (1986) reported that mrigal yearling and grass carp fingerling have high digestibility coefficient (87.1% to 98.1%) of crude lipid from single ingredients as well as formulated feeds of plant origin, which is higher than the mean crude lipid digestibility (84%) observed in common carp.

The present results showed that carbohydrate digestibility was highest in total soyabean meal based diet (88.77%), followed by 20%SM (83.04%), 10%SM (77.22%) and control (56.32%). The lowest digestibility in the control diet could have been due to the higher level of inclusion of rice bran in it.

5.10. Absolute conversion ratio:

Inclusion of soyabean meal at a level of 20% showed better conversion in the present study . Poor ACR was observed in 30% SM diet . It could be because of the limited palatability of this diet, which comprised only soyabean meal protein. The better ACR obtained in 20%SM indicates the better utilization and conversion efficiency of this diet.

5.11. Digestive enzymes:

Results of the present physiological investigations reveal that in fry of *Labeo rohita* protease, amylase and lipase are present in different magnitude and in different regions of digestive tract. Changes in activities in gastric protease, and pancreatic and amylase were determined before the feeding studies (initial digestive enzyme activity) and on termination of the experiment (final digestive enzyme activities). Fish showed significant changes in enzyme activities and responded with increased enzyme levels in different treatments.

5.11.1. Protease activity:

The present investigation indicates that the level of protease activity was higher in fish fed with predominantly animal protein diets, i.e. control (1.21 µg/mg protein), followed by the 20%SM (1.115 µg /mg protein), where a combination of soyabean and fish meal protein sources were used, but the inclusion level of protein from soyabean meal was maximum (20%) in total dietary protein level. Although dietary protein level is known to influence the protease activity in fish (Steffens, 1994), it is evident from the present study that protease activity in fish is very much related to the dietary protein source. However, variations in the enzyme activity may be related to the structure of protein and duration of retention of feed in the digestive tract, which in turn depends on the fibre content and physical consistency of the diet. (Venkatesh *et al.*, 1986). A higher protease activity in the digestive tract of the fish fed the control feed (1.21 µg /mg

protein) may be due to a high crude fiber content (5.32%) compared to that of the other diets.

In the present study fish fed the control feed showed the highest proteolytic and lowest amylase activity, which is in agreement with the study of Ghosh (1976) correlating the enzyme profile to the diet. It indicates that fish fed on fish meal based diet was having more pronounced proteolytic activity because of high animal protein source in the diet. Similarly 30%SM diet showed highest amylase activity.

5.11.2. Amylase activity:

Results of present study showed that highest amylase activity has been recorded in 30%SM treatment (2.37 μg /mg protein), followed by 20%SM diet. It indicates that diet comprising of plant protein sources has higher amylase activity. Lowest amylase activity is recorded for control (2.24 μg /mg protein). Tian and Lin (1993) reported lower amylase activity in grass carp fed on soyabean meal. The present study observation is contrary to that, where the maximum amylase activity was observed in the 30%SM diet.

5.11.3. Lipase activity :

Normally, fats which are ingested by fish are adequately utilized together with their natural food (Kapoor *et al.*, 1975). In the present study the highest lipase activity was recorded for treatment 20% SM (0.432 μg /mg protein), followed by 10%SM

(0.391 μg /mg protein) and control (0.377 μg /mg protein). The lowest lipase activity was recorded for treatment 30%SM (0.258 μg /mg protein).

5.12. Biochemical composition of fish muscle:

The study of biochemical composition of fish is of paramount importance as the carcass composition allows the determination of optimum feeding rations. The growth of fish is influenced to a great extent by chemical contents of the diet, age, and sexual maturity (Reimers and Meske, 1977; Jayaram and Shetty, 1980a; Reinitz and Hitzel, 1980; Zeitler *et al.*, 1984).

In the present study, decrease in the moisture content and increase in protein, fat and fibre were observed in fish fed on different diets. Usually, in trials over long period of time a decline in water content and an increase in fat content with minor changes in protein and mineral content might be expected as weight increases with age in fish (Huisman *et al.*, 1979). The moisture content of fish fed on various feeds ranged from 68.77% (control) to 73.09%(30% SM). It seems to have an inverse relationship with fat content of fish, as reported by Love (1970).

Protein synthesis was in the order of control > 20%SM> 10%SM > 30%SM. A positive correlation of protein synthesis to the amount of protein in the feed has been reported in common carp by Jayaram and Shetty (1980b). However, no such relationship was observed in the present study. The protein deposition values in the present study are in agreement with protein digestibility coefficient.

The fat content of the carcass basically determines the quality of fish. Because of its high energy value, it is also important in the evaluation of nutrient utilization of feed. At the same time, among the nutrients, fat always shows greatest fluctuations. Fat content observed was maximum in fish fed on diet 20%SM (4.97) followed by control (4.82), while it was the lowest for the fish fed on 30%SM diet (3.12). The positive correlation between dietary lipid level and deposition of fat in fish flesh has been observed by Buckley and Groves (1979)and Reinitz and Hitzel (1980). The same pattern has also been observed in the present study, the order of fat deposition being 20%SM>Control>10%SM>30%SM. However, Borthakur (1983) and Anil (1981) found no such relationship between body fat deposition and fat content in feed. The above type of correlation could not be observed in the present study and fat content in the body was in agreement with the fat content of feed and lipase activity observed in the fish.

Variations in the calorific value of fish fed on different diets were between 1.45 kcal/ g (30%SM) to 1.76 kcal/g (control). The energy content of the carcass varied mainly with protein and fat content as reported by Buckley and Groves (1979).

5.13. Organoleptic evaluation:

Overall quality of flesh of the fish grown on fish meal based diet was superior. Similar results were obtained by Nandeesha *et. al.* (1989). They have reported that the overall quality of raw flesh of the non defatted silkworm pupae diet was significantly inferior to soyabean meal and fish meal based diets. Based on the mean panel score it could be observed that diet 20%SM is having comparatively better flesh quality but

10%SM and 30%SM diet possess poor quality of flesh. Among individual attributes, the texture of cooked flesh and colour of raw flesh were better in fish grown on control and 20%SM diet. Statistical analysis of the organoleptic evaluation has shown that there is no significant difference between the treatments.

As evident from the growth response , soyabean meal can be used as a partial substitute for fish meal in the diet for rohu, the level of inclusion being 20% SM . Complete substitution of fish meal by soyabean meal does not appear feasible.

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SUMMARY

The present study was undertaken to elucidate the effect of soyabean meal based diets on the growth, food conversion, body composition and enzyme profile of rohu, *Labeo rohita (Hamilton)* fry.

1. The feeds were formulated according to the method of Varghese *et al.* (1976) keeping the overall protein at 30%. A fish meal based diet served as the control. The test feeds contained soyabean meal incorporated at 10, 20 and 30% levels of inclusion. The control diet was devoid of soyabean meal, while the 30%SM diet was devoid of fishmeal. Fry of rohu were fed with experimental feeds at 5% level for 126 days.

2. The prepared feeds were analysed for the proximate composition and water stability. Protein was 30% on an average, whie fat content ranged from 7.26 to 8.06%.

3. The feeds were found to be of good nutritional quality at the end of four months of storage

4. Variations in the physiochemical parameters of water in the experimental cisterns were found favourable for fish growth. Plankton production range over the experimental period was meagre.

5. Fish fed the 20% SM treatment recorded the highest growth of rohu followed by 10% SM, control and 30% SM

6. The average SGR was the highest in 20% SM treatment . The highest SGR recorded in the first fortnight in all the treatments would be attributed to the spurt in growth rate due to thinning out.

7. One way analysis of growth data showed the existence of significant difference between different treatments at 1% level of significance

8. The overall survival was quite high. Fish growth was found correlated to production in different treatments

9. Fish fed the 20% SM diet recorded highest FCE and PER values.

10. Digestion coefficient studies revealed that the 20%SM diet led to higher feed assimilation and digestibility of nutrients.

11. Best conversion was obtained in fish fed the 20% SM diet.

12. Protease, lipase and amylase activity was found to be the highest in control, 20% SM and 30% SM treatments, respectively.

13. Evaluation of organoleptic characteristics of raw as well as cooked flesh of rohu, indicated no significant influence of soyabean meal on flesh quality.

ABSTRACT

Soyabean meal was evaluated as a partial/total substitute for fish meal in diet for *Labeo rohita* by incorporating at inclusion levels of 10%, 20% and 30%, respectively. Fish meal based diet served as the control. Diet containing 30% of soyabean meal (30%SM) was devoid of fish meal. The overall protein content of the diets was 30%. The study was conducted for a period of 126 days in circular cement cisterns of 350 litre capacity ; feeding being done at the rate 5% of the body weight. Best growth of rohu was attained on 20% SM diet, followed by 10% SM, control and 30% SM. The 30% SM diet was the most stable and the control diet showed the least stability. Fish fed 30% SM diet recorded the lowest average weight on termination of the experiment which was also reflected in the FCE and nutrient digestibility. The inclusion level of soyabean meal was found to influence the carcass composition and enzyme profile. Survival was not affected by soyabean meal incorporation; production was found related to growth rather than survival. Organoleptic quality of fish was not affected adversely by soyabean meal incorporation in the diet.