UTILISATION OF COCOA POD HUSK AS A FEED INGREDIENT FOR *LABEO ROHITA* (HAMILTON) FINGERLINGS

By

R. NAVYA

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Dedicated to

My loving

Parents

DECLARATION

I hereby declare that this thesis entitled **"UTILISATION OF COCOA POD HUSK AS A FEED INGREDIENT FOR *LABEO ROHITA* (HAMILTON) FINGERLINGS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University of Society.

R. NAVYA

Panangad

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CERTIFICATE

Certified that this thesis entitled **"UTILISATION OF COCOA POD HUSK AS A FEED INGREDIENT FOR *LABEO ROHITA* (HAMILTON) FINGERLINGS"** is a record of research work done independently by Miss. R. NAVYA, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Dr. S. SHYAMA

Panangad
11.10. 2007

(Chairperson, Advisory Committee)
Associate Professor
Department of Aquaculture
College of Fisheries
Panangad, Kochi.

NAME AND DESIGNATION OF MEMBERS OF ADVISORY COMMITTEE/ EXAMINATION COMMITTEE

Chairperson:

Dr. S. Shyama
Associate Professor
Department of Aquaculture
College of Fisheries
Panangad

Members:

Dr. C. Mohanakumaran Nair
Professor and Head
Department of Aquaculture
College of Fisheries
Panangad

Dr.Lizy Behanan
Professor
Department of Processing Technology
College of Fisheries
Panangad

Smt. Malika, V
Associate Professor
Department of Management Studies
College of Fisheries
Panangad

External Examiner

Dr. L. Krishnan
Principal Scientist
Central Marine Fisheries Research Institute
Cochin - 682018

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(R. NAVYA)

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Introduction

1. Introduction

Aquaculture can be considered a global production method with over 180 countries reporting some level of aquaculture production. Aquaculture activity is particularly high in Asia, accounting for up to 91 percent of the production by volume and 82 percent by value (Asche and Khatun, 2006). The contribution of aquaculture to the global fish production has increased ten-fold from 0.64 million tonnes in 1950 to 59.4 million tonnes in 2004 (Tacon *et al*., 2006). Carp species form the mainstay of aquaculture production in the subcontinent and despite the international recognition of markets for other species such as tilapia, carps continue to contribute significantly to global aquaculture production. In India, carps account for nearly 2 million MT per annum, comprising mainly of Indian major carps, with Chinese carps and common carp also contributing to overall national production (FAO, 2004).

The growth and health of a fish depends upon the intake of food containing 40 or so essential nutrients (Tacon, 2002). The form in which these nutrients are supplied varies with the farming system and the feeding strategy employed. For example, in the extensive farming system, these nutrients are supplied mainly by natural food organisms produced within the pond ecosystem. In semi-intensive farming system they are usually supplied by a combination of natural food organisms and externally supplied supplementary feed inputs, while in intensive farming system these nutrients are usually supplied almost entirely by a nutritionally complete compound aquafeed, either alone or combined with a natural food item of high nutrient value such as whole fish, brine shrimp or clams. The majority of nutritionists and aquafeed manufacturers focus extensively on the formulation, manufacture, and application of nutritionally complete artificial diets or aquafeeds, irrespective of the proposed farming system and stocking density leading to over nitrification, wastage of nutrients and consequent pollution of the pond ecosystems.

The growth in aquaculture has led to an increase in the use of feeds applied to water for improved production. Likewise production of feeds for aquaculture has been widely recognised as one of the fastest expanding agricultural sectors in the world with annual growth rates in excess of 30% per year (Tacon, 2002). Feed cost can account for well over 70% of the operating cost in intensive fish production, with protein being the most expensive component (Cheng *et al*., 2004). This aspect, incidentally, is of still more

concern from the point of impact on the environment. One of the most daunting challenges facing aquaculture is the identification and utilisation of sustainable alternate protein and lipid sources – a challenge that must be met if the industry is to survive and flourish (Craig and McLean, 2006).

Fishmeal is widely used as the main source of dietary protein for most commercially farmed species (El-Sayed, 1998). Both finfish and crustacean aquaculture sectors are still highly dependent upon marine capture fisheries for the key dietary inputs like fishmeal, fish oil and low value trash fish. This literal 'fishmeal trap' has led to the view, that aquaculture is environmentally damaging since it necessitates increased fishing activity, to meet the incessant demand for fishmeal for inclusion in aquafeeds. Increased production of carnivorous and omnivorous aquaculture species have increased the proportion of fishmeal going into aquaculture feeds from 12% in 1984 to 37% in 1997 (Tacon, 2003). The use of mixed feeding schedule which is a recent concept is highly promising. Here a high protein diet (with optimum protein requirement) is alternated with a low protein diet (with 10% less than the optimal requirement); each of these diets being fed alternately as per specific feeding schedules. Experimental results showed that fish maintained on mixed schedule diets performed better or equally well as those steadily fed on a high protein diet (DeSilva, 2006). Moreover, reducing feed costs is considered to be crucial for ensuring long term sustainability of the aquaculture industry.

Indian major carps are currently fed supplemental diets, formulated on an empirical basis, using combinations of a few conventional feedstuffs such as rice bran and various kinds of oil cakes. However, there is increasing emphasis on the development of low-cost, balanced rations for these fish species, which will involve utilisation of less expensive, agro-based by-products (Erfanullah and Jafri, 1998).

The inclusion of alternate, perennially available plant protein sources, with low seasonal variability in nutritional quality, is therefore a dire necessity in aquaculture. Although successful total, dietary substitution of fishmeal has been demonstrated only in a few cases, a variety of ingredients ranging from oilseed meals, pulses, leaf meals, agricultural by-products etc. have been shown to be capable of replacing fishmeal, up to 40% inclusion level without compromising on the growth performance of cultured species (DeSilva, 2006). Soybean meal is the most commonly employed plant protein

source in aquaculture feeds (Refstie *et al*., 2001). In general, protein sources, of plant origin are believed to be less suitable as fishmeal replacements, on account of relatively inferior indispensable amino acid profiles, presence of antinutritional factors and/or low palatability (Dong *et al*., 2000). However, the adoption of varied approaches like improved processing techniques, ensilation, amino acid supplementation etc. have been of immense help in overcoming the inherent disadvantages of plant protein sources (Davies and Morris, 1997).

Of late, increased emphasis is being laid on the environmental impacts of aquaculture, principally from the pollution standpoint. The nutrients most commonly implicated in aquaculture based pollution are phosphorus and nitrogen. Several studies have indicated that the nutrient rich aquafeeds employed in culture systems contribute significantly to hypernutrification. Fishmeal, a major component of aquafeeds is implicated as one of the key sources of phosphorus in effluents. Consequently, the use of alternative protein ingredients that possess highly digestible protein and low phosphorus levels, as compared to fishmeal will be decisive in fish feed formulation strategies. Of late, this is evident as the adoption of multipronged strategies aimed at limiting feed based hypernutrification (Cheng *et al*., 2004).

In India, work on aquaculture nutrition has also centred on screening and assessment of the potential of locally available ingredients in carp diets. This approach focuses on the dual benefits of possible reduction in feed costs and formulation of low pollution feeds. The rohu, *Labeo rohita* is the most widely cultured of the Indian major carps (Murthy and Varghese, 1996). The economic success of controlled production of rohu largely depends on the cost of feeds, particularly the cost of protein materials incorporated in the diets. Relatively little research has been carried out on digestibility and utilisation of tropical feedstuffs using this species.

An agricultural by-product, cocoa pod husk is used for the study because of its availability throughout the year and in bulk. Cocoa, *Theobroma cacao* is an economic cash crop in tropical African, Asian, and Latin American countries. Cocoa is cultivated for its beans, which are contained in large red or yellow pods growing directly on the stems and branches of the tree. The beans are embedded in white mucilage (up to one third the total weight of the pod). Both the mucilage and beans are taken out of the pod

and fermented. The fermented dried beans are then processed in chocolate factories. Cocoa pod husks constitute about 75% of the fruit and contain lower theobromine levels (a toxic alkaloid, 3-7-dimethylxanthine) than other cocoa by-products and is, therefore more suited as a feed ingredient. Their use in fish diets and the results indicate a positive potential/ value (Fagbenro, 1988a, 1988b). Studies conducted by Fagbenro (1992) in diets for *Clarias isheriensis* have yielded encouraging results. Later on Falaye *et al*. (1999) studied the growth response of tilapia (*Oreochromis niloticus*) fed varying levels of cocoa husk diets and reported that 10% inclusion level is optimum for growth.

Rohu, besides being fast growing and tasty, also has a good market demand, in addition to thriving on feeds, formulated from a wide variety of alternate feed ingredients. Successful growth in trials involving cocoa pod husk have also been reported in livestock and poultry (Osei *et al*., 1991, Fleischer *et al*., 1991, Sobamiwa and Longe, 1994, Ridzwan *et al*., 1995).

The present study aims at assessing the potential of cocoa pod husk meal as a partial or total substituent in diets for the Indian major carp, *Labeo rohita*.

Review of literature

2. Review of literature

Carps are the dominant species in freshwater aquaculture. Carp farming for consumption dates back to more than 2000 years ago in China. Nowadays, carp culture is widely practiced in East Asia, Southeast Asia, South Asia, Israel, Central Asia and some Eastern European countries (Billard and Gall, 1995). In 1999 the world carp production from aquaculture was 14.9 Million Tonnes, accounting for 44.7% of the total aquaculture production (FAO, 2000). As per the report of FAO (2004), carps account for 40% of total aquaculture production of fish, crustaceans and molluscs. Of the top ten aquaculture finfish in single species production, eight are carps, the other two being tilapia and salmon. The popularisation of carp culture is due to various reasons:

- Carp feed low in the food chain.
- It is possible to integrate carp culture with crop farming and/or animal husbandry, as widely practiced in some Asian countries. This reduces the production cost, makes efficient use of land and water and reduces disposal/ discharge of waste materials into the environment.
- Carp thrive equally well on plant proteins and animal protein feeds.

For grow-out culture, compound feeds consisting mainly of plant ingredients and with 30-40% crude protein are widely used (Shetty and Nandeesha, 1988). Due to their fast growing nature and taste, Indian major carps enjoy a prime position in the Indian aquaculture scenario. These highly priced fishes, though originally inhabitants of the Ganga river network in North India and the rivers of Pakistan, Bangladesh, Nepal and Burma, have also been transplanted into other rivers in central as well as peninsular India.

However, carp farming is mostly pond based with several species being stocked in the same pond (polyculture). Fishes like tilapia, catfish and *Macrobrachium* species are also stocked together with carp in culture systems. Single-species culture, or monoculture, is rare in carp culture except in flow-through systems. Intensity of pond carp culture ranges from extensive to intensive.

When the natural food supply is found insufficient to sustain the standing crop supplementary feeds are provided.The availability of supplementary feed is one of the key inputs in semi-intensive and improved extensive culture systems for enhancing fish production (Azim and Wahab, 2003). The lack of an appropriate diet which can supplement the available natural food in nursery ponds has been identified as one of the major reasons for low survival (Chakraborty *et al*., 1973). Although supplementary feeding is considered essential, relatively few field trials have been conducted to evaluate the dietary influence on growth and survival rates of the various Indian carp species (Lakshmanan *et al*., 1967). Supplementary feed is a major input in the nursery rearing of fry, often in the form of finely powdered rice bran and groundnut oil cake in a 1:1 ratio. Various types of supplementary feeds have been formulated using a number of ingredients of both plant and animal origin for rearing Indian major carps (Chakraborty *et al*., 1973; Singh *et al*., 1977; Sen *et al*., 1978; Mohanty *et al*., 1990). The common ingredients in supplementary feeds are rice bran, wheat bran, groundnut oil cake, mustard oil cake and some other agricultural wastes (Azim and Wahab, 2003). In the commercial production of fish in Indian aquaculture farms, oil seed cake meals particularly groundnut oil cake is used as a predominant protein source for carp (Devi *et al*., 1999).

A major problem facing aquaculture is the limited supply and increasing cost of fishmeal, which is a key animal protein in formulated fish feeds. Plants are an alternate and relatively inexpensive source of protein in supplemental fish diets (Dorsa *et al*.., 1982; De Silva and Anderson, 1995). However, in India, relatively few studies have been conducted to determine the nutrient digestibility of locally available feed ingredients in carps (Jayaram and Shetty, 1980; Nandeesha *et al*., 1989; Nandeesha *et al*., 1991). Results to date indicate that total dietary replacement of fishmeal is possible without decline in growth or feed efficiency in the case of carps, tilapia, milkfish, channel catfish, Pacific white shrimp etc. (El-Sayed, 1998; Davis and Arnold, 2000; Khan *et al*., 2003; Cremer *et al*., 2003 and Muzinic *et al*., 2004). However, in the case of carnivorous fish species, studies have shown that fishmeal in the diet can be reduced atleast by half without adverse effect on the growth, while complete replacement does not appear feasible (Francis *et al*., 2001).

Substantial effort has been directed over past few decades in evaluating a wide range of potential alternatives to fishmeal for use in aquaculture diets. These ingredients

can generally be categorised into two groups - those of plant origin and terrestrial animal origin. Plant derived sources include soybean meal, protein concentrate and oils (Kaushik *et al.,* 1995; Refstie *et al.,* 1998), canola meal, protein concentrate and oils (Higgs *et al.,* 1982; Mwachireya *et al.,* 1999; Glencross *et al.,* 2003) and lupin meal and protein concentrate (Burel *et al.,* 1998; Booth *et al.,* 2001; Farhangi and Carter 2001; Glencross *et al*., 2003).

The ingredients of terrestrial animal origin include rendered meat meals (Bureau *et al.,* 1999, 2000; Stone *et al.,* 2000; Sugiura *et al*., 2000), blood meals (Bureau *et al.,* 1999) and poultry meals (Bureau *et al.,* 1999; Nengas *et al*., 1999).

Most species require feeds formulated to suit their metabolic needs. However, the information relating to nutrient requirements of indigenous carps is limited (Alam *et al*., 1996). The following account reviews in brief the nutrient requirements of cultivable freshwater fishes and important plant and animal protein sources.

2.1. Nutrient requirements of cultivable freshwater fishes

2.1.1. Proteins

Protein is the most important component of the diet of fish because protein intake generally determines growth. Protein is required in the diet to provide indispensable amino acids and nitrogen for synthesis of non-dispensable amino acids. Fishes do not have a true protein requirement but require a well balanced mixture of indispensable amino acids in the diet for growth and maintenance. The gross protein requirements of fish are known to vary among species, stage of growth, water temperature, salinity etc. (*NRC, 1983). Due to a relatively high need of essential amino acids, Mertz (1969) found that fish require a far greater quantity of dietary protein for rapid growth than birds and mammals. The optimal protein level of a casein diet for common carp defined by Ogino and Saito (1970) was 38%.

*National Research Council

Optimum protein requirement of Indian major carps ranges between 30-45% (Renukaradhya & Varghese, 1986; Mohanty *et. al.*, 1990).

Studies conducted by Sen *et al*. (1978) and Singh *et al*. (1980) indicated the optimum protein requirement of rohu fry to be 45%. However Mohanty *et al*. (1990) observed that a dietary protein level of 45% enhanced the growth of catla fry, while the same level inhibited the growth of rohu.

The optimum protein requirement in *Tilapia zilli* and *Sarotherodon mossambicus* (Jauncey, 1982) was found to range from 30 to 40%. However, the protein requirement may decrease in fish with advancement of growth (Renukaradhya and Varghese, 1986).

It was further advocated by Lim *et al*. (1979) and Singh *et al*. (1980) that protein content in feed higher than optimum level (> 45%) might depress the growth of animals. De Silva *et al*. (1989) reported that the growth rate in juvenile tilapia increased as dietary protein content was raised upto 30-34% level. Cold water fishes like rainbow trout may require a higher level of protein atleast 38% in their diet (NRC 1983).

Higher levels of dietary protein lead to a decrease in growth rate showing that excess levels of dietary protein can be wasteful and have a negative effect on the growth profile. Economically optimum dietary protein level suggests that protein content of the feed can therefore be significantly reduced from the biological optimum with only a small trade-off in growth, leading to more economical production (Wilkinson, 2003).

2.1.1.1. Essential amino acids

For nutritional purposes, amino acids may be divided into two groups; the essential amino acids (EAA), and the non-essential amino acids (NEAA). EAA are those amino acids that cannot be synthesized within the animal body or are synthesized at a rate insufficient to meet the physiological needs of the growing animal, and must therefore be supplied in a ready made form in the diet (Tacon, 1992). NEAA are those amino acids that can be synthesized in the body from a suitable carbon source and amino groups from other amino acids or simple compounds such as diammonium citrate, and consequently do not have to be supplied in a ready made form in the diet (Tacon, 1992). The amino acids essential for fish include threonine, valine, leucine, isoleucine, methionine, tryptophan, lysine, histidine, arginine and phenylalanine (Royes and Chapman, 2003). In

general the information on the protein requirement of fish will be of limited value unless the optimum needs of the 10 essential amino acids are known. The range of amino acid requirement expressed as % of protein of Indian major carps are as follows : lysine: 5.7-7.0; methionine : 1.5-3.5; arginine 4.8-5.8; tryptophan 0.8-0.9; threonine 3.9-4.2; valine 3.6-4.5; leucine 3.7-6.9; isoleucine 2.3-3.8; histidine 2.1-3.0; phenylalanine 3.7-8.0 (Ravi and Devaraj, 1991a; 1991b; Mohanty and Kaushik,1991; Khan and Jafri, 1993).

Studies by Ahmed and Khan (2004) revealed that the diet of fingerling *Cirrhinus mrigala* should contain arginine at 18.4g/kg dry diet corresponding to 46g/kg dietary protein for optimum growth and efficient feed utilisation. Similarly tryptophan requirement of *Cirrhinus mrigala* was found to be 0.38g/100g dry diet corresponding to 0.95g/100g dietary protein for optimum growth and efficient feed utilisation (Ahmed and Khan, 2005). Optimum dietary leucine requirement of fingerlings of *Labeo rohita* is estimated to be in the range of 1.5 – 1.57g per 100g of the dry diet (Abidi and Khan, 2007).

A protein source can be judged by its amino acid profile. Amino acid profiles of the whole-body tissue of a given fish species closely resembles its dietary requirement profile (Ngamsnae *et al*., 1999). Most of the plant protein sources are deficient in lysine and methionine whereas animal protein sources are deficient in sulphur containing amino acids (New, 1987). Dietary imbalances may arise from the presence of disproportionate levels of specific amino acids; including leucine/isoleucine antagonisms, and to a lesser extent arginine/lysine and cystine/methionine antagonisms. For example, blood meal is a rich source of valine, leucine and histidine, but is a very poor source of methionine and isoleucine. However, in view of the antagonistic effect of excess leucine on isoleucine, animals fed high dietary levels of blood meal suffer from an isoleucine deficiency caused by an excess of dietary leucine (Tacon, 1993). The amino acid profile of soy protein is generally superior to other plant proteins; though compared to menhaden meal protein, it is deficient in lysine, methionine, threonine and valine. The increased level of cystine compensates for the deficiency of methionine to some extent (NRC, 1983).

The dietary inclusion of purified amino acids has been attempted to compensate for deficiencies resulting from the presence of meals with unbalanced amino acid profile in a diet (O'Keefe, 2000). Fish fed rations in which a significant proportion of the dietary protein is supplied in the form of 'free' or crystalline amino acids generally display

suboptimal growth and feed conversion efficiency compared with animals fed protein-bound amino acids or 'whole' proteins (Walton *et al*., 1984). Furthermore, in carp individual free amino acids appear to be absorbed at varying rates from the gastro-intestinal tract, and consequently peak plasma concentrations of individual amino acids do not occur simultaneously (Plakas and Katayama, 1981).

Studies have indicated that amino acid supplementation of diets increased performance of Nile tilapia (Odum and Ejike, 1991), rainbow trout (Bai and Gatlin, 1994), and channel catfish (Robinson and Li, 1994). Webster *et al*. (1995) reported that the inclusion of essential amino acids in diets for blue catfish resulted in performance comparable to that obtained with diets with high fishmeal content. Davies and Morris (1997) made similar observations in the case of rainbow trout by. In contrast, studies have indicated that fish utilise synthetic amino acids less efficiently than protein bound forms (Yamada *et al*., 1981; Schuhmacher *et al*., 1997). Tantikitti and March (1995) observed that shorter ration intervals reduced the metabolic loss of nitrogen to stabilize amino acid plasma concentration and increase protein deposition. Furthermore, granules must have high stability in water so that losses of crystalline amino acids through leaching could be reduced because these losses occur in a higher proportion than that of protein – bound amino acids (Zarate and Lovell, 1997).

2.1.2 Lipids

Dietary lipids are a source of biochemical energy and fatty acids. The level of fat in the diet is an impotant factor in the diet of indigenous carps (Jayaram and Shetty, 1980). In fact, the lipids are the most energy rich of all classes of nutrients: gross energy value of lipid being 9.5 kcal/g, against protein for 5.6 kcal/g and carbohydrate for 4.1 kcal/g (Henken *et al*., 1986). In this respect, dietary lipids may be used to spare the more valuable protein for growth. As per FAO (1983) the minimum lipid requirement of carp fry and fingerlings is 8%. Lipids have a protein sparing effect in carps (Das *et.al*., 1991). The effectiveness of the protein sparing effect of carbohydrates and lipid is related to the ratio of protein to energy in the diet (P/E ratio) (Wilkinson, 2003). Takeuchi *et al*. (1978) observed a protein to lipid ratio of 35: 18 to be optimal for juvenile rainbow trout, provided the dietary protein and fat were of superior quality. An increased lipid level up

to 15 – 20% leads to substantially higher protein utilisation in rainbow trout. In juvenile carp, lipids and carbohydrates were found to be equally effective in satisfying the requirements for energy (Takeuchi *et al.*, 1979).

When cod liver oil was incoporated at a level of 15% in the diets of rohu it was found to lead to significant reduction in the overall digestibility of the diet (Sethuramalingam and Haniffa, 2001a).

It is seen that free fatty acids derived from triglycerides (fats and oils) serve as the major aerobic fuel source for energy metabolism of fish muscle. Mukhopadhyay (1993) observed that the optimum dietary requirement for grow-out stages of carp ranges from 7-9%. The optimum lipid level that enhances growth is seen to increase with increasing dietary protein level (Wilkinson, 2003). According to Du *et al*. (2005) the dietary lipid level of 20-40 g/kg led to best growth performance and survival in grass carp juveniles. Ogino *et al,* (1976) observed an inverse relationship between the dietary protein content and fat in fish in the case of carps.

Assessment of the digestibility of ingredients is essential to formulate cost effective feeds because its accurate determination gives greater idea about the bioavailability of the components in fish feeds. Studies on protein digestibility of fishes are available but information relating to other nutrients especially lipids are scarce (Appleford and Anderson, 1997).

2.1.2.1. Essential fatty acids

Fats are the fatty acid esters of glycerol and are the primary means by which animals store energy. The predominant PUFA in the tissues of freshwater fish belong to the linolenic (n-3) series. The concentration of n-6 PUFA in the tissues of fish is generally low, although higher levels are reported in freshwater fish species. It is generally believed that the dietary requirement (preferential) of fish for n-3 series EFA, over n-6 series, is fundamentally due to the lower water temperature in the aquatic environment (as compared with mammals) (Tacon, 1987). In fact, the lower the water temperature, the greater the incorporation of n-3 series PUFA into the tissues (New, 1987).

It is a well known fact that polyunsaturated and longer chain fatty acids of the C22 or C24 n-3 series are required for maximum growth and diet utilisation in fish (Goncalves *et al*., 1989).

The dietary essential fatty acid requirement (EFA) of common carp has been elucidated to be 1%18:3n-3 (linolenic acid) +1%18:2n-6 (linoleic acid) or 0.5–1.0%HUFA n-3 (expressed as a percentage of dry diet) by Takeuchi and Watanabe, (1977). Apart form the differences in n-6 PUFA content of the tissues of freshwater and marine fish species, freshwater fish also generally have higher tissue concentrations of the shorter chain n-3 series (New, 1987). In general, marine fish, shrimp and mollusc oils are rich dietary sources of the n-3 series EFA. Plant oils are rich dietary sources of 18:2n-6, and contain little or no n-3 series EFA (with the exception of soybean oil, rapeseed oil and particularly linseed oil whose 18:3n-3 content may exceed 8, 7 and 56% respectively of the total fatty acids present). Plant oils whose 18:2n-6 constitutes 50% or more of the total fatty acids present include cottonseed oil, corn oil, sunflower seed oil and soybean oil (New, 1987).The carp species require both n-3 and n-6 fatty acids for growth as well as the favourable deposition of such fatty acids in the muscle (Mukhopadhyay and Rout, 1996).

2.1.3. Carbohydrates

Carbohydrates are the cheapest and most abundant source of energy for animals. The utilisation of carbohydrate by fishes, however, is governed by factors like source and complexity of carbohydrate and the presence of carbohydrate metabolizing enzymes (NRC, 1983). Although some information is available on the effects of dietary carbohydrate level in Indian major carps, the utilisation of different carbohydrates by these fish has not been investigated (Sen *et al*., 1978; Erfanullah and Jafri, 1993). Carbohydrates in feed materials range from easily digested sugars to complex cellulose molecules which cannot be digested by animals.

Studies have shown that adequate levels of non-protein energy sources, such as lipid and carbohydrate, in the diet can minimize the use of protein as a source of energy (Cho and Kaushik, 1990; Gomes *et al*., 1993). Carbohydrates supply energy besides sparing protein for growth in fish diets (Shimeno *et al*., 1981; Furuichi and Yone, 1982;

Lovell, 1989). However, the use of carbohydrate as a protein-sparing energy source has received less attention in omnivorous fish when compared with lipids (Page and Andrews, 1973).

Erfanullah and Jafri (1995) correlated the better utilisation of mono and disaccharides in cyprinids to the presence of specific enzymes and higher digestibility of such carbohydrates. Kawai and Ikeda (1971) based on their studies concluded that in common carp, carbohydrate digesting enzymes exist in varying levels. The occurrence of amyloclistic activity has been observed in the Indian major carps, including *L. rohita* by Dhage (1968). Endogenous cellulase activity has been reported in *L. rohita* indicating probably its preference for complex diets. The overall cellulase activity suggests that the fish could utilise all the ingested dietary energy sources and also indicates the possibility of including more carbohydrate in the diet of rohu. The presence of significant amylase, protease and lipase activity suggests the potential for digestion of non-conventional plant and animal food sources like cauliflower waste, chicken waste, prawn head waste, banana flower and groundnut leaf (Sethuramalingam and Haniffa, 2002).

The metabolisable energy (ME) values of carbohydrates for fish range from near zero for cellulose to about 3.8 kcal/g for easily digested sugars. Raw starch ranges from 1.2 to 2.0 kcal ME/g (Smith, 1989). Cooking of starch can increase the ME to about 3.2 kcal/g. Heat and moisture associated with the pelleting process improves the digestibility of starchy feed materials.

The value of carbohydrate in fish diets depends on the source and type of carbohydrate and the processing to which it has been subjected. Channel catfish and carp can utilise quite high levels of dietary carbohydrate; the natural diet of grass carp is very high in this component (New, 1987). Warmwater omnivorous or herbivorous fish speceis such as carp (*C. carpio*), channel catfish (*I. punctatus*), tilapia (*O. niloticus*), and eel (*A. japonica*) have been found to be more tolerant of high dietary carbohydrate levels; the dietary carbohydrate being effectively utilised as a dietary energy source or excess stored in the form of body lipid (Anderson *et. al*., 1984; Degani *et al*., 1986; Wilson, 1994). *L. rohita* being an omnivore, accepts plant protein and mixed diets with rich carbohydrate (Haniffa *et al*., 1987).

Mohapatra *et al*. (2003) observed that a diet containing 450g/kg gelatinized carbohydrate and 300g/kg crude protein was efficiently utilised by *Labeo rohita* fry.

2.1.3.1. Energy

Fish use much less energy for protein synthesis than do warm-blooded farm animals because they do not need to maintain a constant body temperature, need less energy to maintain position and move, and because the excretion of ammonia uses less energy in protein breakdown and excretion. However, excess or insufficient dietary energy levels result in reduced growth rates. Energy needs for maintenance and movement will be fulfilled before energy is used for growth. Thus if the energy/protein ratio is too low, protein will be used to satisfy energy requirements first; what is left will be available for growth (New, 1987).

A negative correlation has been reported between the rate of consumption and digestible energy content of the feed, indicating that fish feed primarily to satisfy their demand for energy. However, this also means that the quantity of essential nutrients tht are consumed will also be negatively correlated to the energy content of the feed. More frequent feeding over the course of a day can enhance the growth rate of some species (Wilkinson, 2003).

Studies on protein: energy in feed of carps indicated a feed with 38% protein and 400kcal/100g energy (having protein: energy of 95mg protein/kcal) should be enough for normal growth and development (Das *et. al*., 1991). A minimum 355 kcal/100g is, however, essential for efficient nutrient utilisation and growth in a 40% diet with 29.5% metabolisable energy as carbohydrate (Hassan and Jaffri, 1996). Ahmed (2007) found that for *Labeo rohita* fingerlings feeding in the range 6.5-7.0% body weight per day corresponding to 2.6-2.8g protein and 23.49-25.31 kcal energy per 100g of the diet per day is optimum for growth and efficient feed utilisation. For brood stock *Labeo rohita,* a dietary protein level of 250g/kg was found optimum with regard to its reproductive performance, egg quality and composition (Khan *et. al*., 2005).

2.1.4. Vitamins

Vitamins are complex organic compounds required in trace amounts for normal growth, reproduction, health and general metabolism. Water-soluble vitamins include eight well-recognized members of the vitamin B complex: thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, biotin, folic acid and vitamin B_{12}; the water-soluble essential nutritional factors: choline, inositol, ascorbic acid; and vitamins with less-defined activity for fish: p-aminobenzoic acid, lipoic acid and citrin. The most common vitamin deficiency in fish nutrition is that of vitamin B_1 (thiamine) (New, 1987). Choline, inositol and ascorbic acid are required in appreciable quantities in the diet and sometimes are not referred to as vitamins but as major dietary nutrients. Fat-soluble vitamins A, D, E, and K differ from the water-soluble vitamins in their accumulative action. In contrast to the fat soluble vitamins (A, D, E, K) the water soluble vitamins can readily be lost from the feed through leaching prior to ingestion by the fish or shrimp. In general, the smaller the feed particle size and the longer the feed remains uneaten in the water, the greater the loss of water soluble vitamins through leaching. Vitamin requirements have not been adequately studied in the Indian major carps except for vitamin-C. All the three species lack l-gulono γ-lactone oxidase, the terminal enzyme for the conversion of glucose to ascorbic acid (Mukhopadhyay *et.al*., 1996).This indicates that these species are unable to synthesis ascorbic acid de novo and exogenous supply alongwith feed is mandatory. However, when dietary supply was 1000mg/kg feed, growth, feed efficiency, tissue storage and vertebral collagen content were found to be optimum for *Labeo rohita* fry and fingerlings.

Misra *et al*. (2007) reported that elevated levels of vitamin-C could be recommended for optimum immunity, growth and survival of fingerlings of *Labeo rohita*.

2.1.5. Minerals

Mineral elements have a great diversity of uses within the animal body. The following mineral elements are recognized as essential for body functions in fish: calcium, phosphorus, sodium, molybdenum, chlorine, magnesium, iron, selenium, iodine, manganese, copper, cobalt and zinc. They provide strength and rigidity to bones in fish and the exoskeleton of crustacea. In body fluids they are involved mainly with the

maintenance of osmotic equilibrium with the aquatic environment and in the nervous and endocrine systems. They are components of enzymes, blood pigments and other organic compounds. They are essentially involved in the metabolic processes concerned with energy transport (New, 1987). According to FAO (1983) the mineral requirements of fish can be summarized as follows: Ca (0.5%), available P (0.7%), Mg (0.05%), Na (0.1-0.3%), K (0.1-0.3%), S (0.3-0.5%), Cl (0.1-0.5%), Fe (50-100mg/kg), Cu (1-4mg/kg), Mn (20-50mg/kg), Co (5-10 mg/kg), Zn (30-100 mg/kg), I (100-300mg/kg), Mo, Cr and F in trace levels.

Calcium is absorbed by fish from seawater but freshwater is low in calcium. However, since most feeding stuffs, particularly animal proteins, have high levels of calcium, calcium deficiency in fish through dietary insufficiency is most unlikely. On the other hand both seawater and freshwater contain very little phosphorus so this element is important from a dietary point of view. The level of phosphorus in feeds and feed components is therefore very important information. Some types of phosphorus are unavailable to fish and an assessment of the availability of phosphorus in the diet is essential. Generally animal sources of phosphorus are best absorbed by fish but some species, such as carp, do not absorb the element well from this source (New, 1987).

Inorganic sources of phosphorus vary in their availability but some in common use in feedstuffs are mostly high in availability. The phosphorus from plant sources is generally poorly available. The antinutritional factor phytic acid binds with phosphorus and makes it less available (Riche and Brown, 1996). Moreover, phytic acid forms insoluble complexes with other dietary elements such as zinc, and binds trypsin thus reducing protein digestibility (Cheryan, 1980, Singh and Krikorian, 1982; Liener, 1994). Reduced availability of protein and minerals cause poor feed economy and minerals and their subsequent loss to the environment (Vielma *et al*., 2004). Excessive excretion of phosphorus and nitrogen are the main water pollution concerns. Feed ingredients from alternative protein sources such as brewer's dried grains, brewer's dried yeast, spent hen meal, poultry by-product meal, feather meal, and spray-dried porcine plasma contain less phosphorus than fish meal. Therefore, substituting these alternative protein meals for fishmeal in trout diet formulations reduces the total phosphorus level of the diet, thereby lowering the levels of total phosphorus discharged into water (Cheng *et al.,* 2004).

Lower ash content is desirable in fish feeds because ash contributes minerals, especially phosphorus, which is a major environmental pollutant when over-formulated in fish feeds (Cheng *et al*., 2004).

A dietary supplementation of sodium chloride was found to enhance the fish growth in carps (Keshavnath *et al*., 2002). However, the levels of sodium chloride that induced the best growth differed with the species, it being 1% in rohu, 1.5% in mrigal and common carp.

2.2. Feed ingredients used in aqua feeds

Aquaculture feed ingredients tend to be mostly by-products of processing or milling industries, but also consist of natural products. Fishmeal is the single most expensive major ingredient in aquaculture feeds (Tacon, 1993). It is widely used as the main source of dietary protein for most commercially farmed fish species. In the mean time, the shortage in world production of fishmeal, coupled with increased demand and competition with terrestrial domestic animals, has further increased fishmeal prices (FAO, 1983). Recent research has shown that animal protein is not essential for normal growth of channel catfish under typical commercial culture conditions provided the amino acid requirements are met (Robinson and Li, 1994). Replacement of fishmeal with less expensive plant protein would lower feed cost (El-Saidy and Gaber, 2003). In general, protein sources of plant origin are believed to be less suitable as fishmeal replacements owing to indispensable amino acid profiles which may be markedly different to those required by many cultured teleosts (Akiyama, 1988). Additionally plant products contain a range of anti-nutritional factors which may lower the activity of digestive enzymes thereby reducing the digestibility of feed stuffs (Krogdahl, 1989). A combination of several plant protein sources is advisable, particularly if they have different limiting amino acids (e.g. soya deficient in lysine and cottonseed deficient in sulphur containing amino acids).

Terrestrial animal by-product meals, including poultry by-product meal, hydrolysed feather meal, blood meal and meat and bone meal have been used to replace fishmeal in diets. They have high protein contents and favourable essential amino acid profiles (NRC, 1983). They are also available at low cost. However, these feeds may be

deficient in one or more of the essential amino acids, especially lysine, isoleucine and methionine (Tacon and Jackson, 1985). Therefore, if proper ratio of these by-products is maintained in the diet, the quality of this diet is likely to improve (Yang et al., 2004). The use of non-fishmeal protein sources may necessitate the use of amino acid supplements to restore the amino acid profile of the feed to a level which matches the requirement of the target species (Davies and Morris, 1997).

2.2.1. Plant protein sources:

Terrestrial plant protein sources include protein-rich oilseed and grain by-product meals, including soybean, rapeseed, corn gluten, wheat gluten, pea and lupin meals, palm oil, soybean oil, maize oil, rapeseed oil, canola oil, coconut oil, sunflower oil, linseed oil and olive oil (Hertrampf and Piedad-Pascual, 2000).

2.2.1.1. Soybean meal:

Soybean meal is considered to be the most nutritious of plant proteins, and this is used as a major protein source in many fish diets (Devi *et al*., 1999). Among the ingredients being investigated as alternatives to fishmeal, the products derived from soybeans (*Glycine max*) are some of the most promising (Lim *et al*., 1998; Hardy, 1999; Storebakken *et al*., 2000; Swick, 2002) because of the security of supply, price and protein/amino acid composition. Examining the work done with various fish species, it is apparent that there are a number of limitations to the use of soy protein products in feeds, despite its overall good nutritional qualities. Among these limitations, the most important are amino acid imbalance (especially the deficiency of methionine), poor palatability in some fish, presence of phytic acid, a naturally occurring compound that reduces the bioavailability of phosphorus and some other minerals and presence of trypsin inhibitor, which deactivates digestive enzyme trypsin, with subsequent reduction in protein digestibility (Francis *et al*., 2001).

According to Webster *et al*. (1995) fishmeal can be totally replaced by soybean meal in diets of blue catfish, *Ictalurus furcatus*. Olli *et al*. (1995) observed that for salmon in seawater and for rainbow trout in freshwater, soybean meal could replace fishmeal to the extent of 25% and 40% respectively. However, the effect of soybean meal (either in

raw or processed form) has not been fully investigated, particularly in species found in tropical warm water (Balogun and Ologhobo, 1989). Studies in rainbow trout (*Onchorhynchus mykiss*) showed that they could utilise soybean meal enriched with cystine and tryptophan almost as well as protein from fishmeal (Dabrowska and Wojno, 1977). Davies and Morris (1997) in feeding trials conducted with rainbow trout found that there was no significant restoration in growth, feed efficiency and apparent net protein utilisation when solvent extracted soybean meal was used to replace 66% of the fishmeal without amino acid supplementation. However, methionine and lysine supplementation to these diet improved percentage weight gain and specific growth rate.

Abdelizi *et al*. (1998) reported that 400g/kg soyflour diet and 400g/kg soybean meal diet was acceptable to rainbow trout diets when compared to commercial trout diets.

In red drum (*Sciaenops ocellatus*) diets in which 90% of the protein from soybean meal gained as much weight as fish fed a diet with 100% of protein from fishmeal, and also that soybean meal inclusion can be extended to 95% with supplementation of 2% glycine (McGoogan and Gatlin, 1997). However, in milk fish (*Chanos chanos*) diets containing more than 33% soybean meal replacement of fishmeal, there was a trend to lower growth and feed efficiency (Shiau *et al*., 1988). Phytic acid is an antinutritional factor present in many seeds including soybean which cannot be destroyed by heat treatment. Top spraying of soybean meal based diets with phytase improves protein and mineral digestibilities but not lysine utilisation in rainbow trout (Vielma *et al*., 2004). Khan *et al*. (2003) found that soybean meal was more effective than groundnut meal or canola meal as fishmeal was replaced in feeds for fingerling, *Labeo rohita* and could completely replace fishmeal when supplemented with methionine and minerals.

Defatted soybean meal when fed to mrigal showed that 400g/kg inclusion in a diet of 350 g/kg overall protein elicits good growth response and survival (Jose *et al*., 2006).

Studies by Goda *et al*. (2007) revealed that satisfactory growth and feed utilisation responses could be achieved through the replacement of fishmeal by soybean meal in the diet of African cat fish.

2.2.1.2. Cottonseed meal:

Cotton seed is perhaps the second most abundant source of plant protein in the world (Tacon *et al*., 2006). Cottonseed meals are low in lysine content and high in fibre. Cottonseed protein compared to that of soybean is very high in arginine (O'Keefe, 2000). When Wilson *et al*. (1981) studied the true availability of amino acids in channel catfish it was found to be lower in cottonseed meal than in soybean meal. However, the use of cottonseed meal in feeds is limited due to the presence of anti-nutritional compounds free gossypol and cycloproprenoid fatty acids (El- Saidy, 1999). Gossypol occurs naturally in the pigment glands of cottonseed, and is reported to decrease feed utilisation and growth in fish. (Fowler, 1980; Dorsa *et al*., 1982; Usmani *et al*., 1997). Studies conducted in *Labeo rohita* using solvent extracted cotton seed meal indicates that growth and conversion efficiencies are significantly affected at 6.5% level of cottonseed meal, containing 0.036% of gossypol. However, chinook salmon and coho salmon have been reported to tolerate diets containing up to 34 and 22% cottonseed meal, respectively (Fowler, 1980).

Numerous studies have been conducted to determine the level of cottonseed meal that can be incorporated in Nile tilapia diets without affecting their growth performance (El-Sayed, 1999; Mbahinzireki *et al*., 2001). Results have shown that prepressed solvent extracted cottonseed meal could replace up to 50% of fish meal in juvenile Nile tilapia diets without requiring lysine supplementation. Similar results were observed in rainbow trout juveniles (Luo *et al*., 2006).

When growth response of indigenous and exotic carps to different protein sources such as cottonseed meal, fishmeal and maize gluten meal was studied both Indian major carps and exotic carps showed a better growth with fishmeal diet (Alam *et al*., 1996). Similarly in Tilapia evaluation of some plant proteins viz. copra, groundnut, soya, sunflower, rapeseed, cottonseed and leucaena meals were tested individually at various inclusion levels in comparison with a control diet (30% protein) containing fishmeal (Jackson *et al*., 1982). All diets produced lower growth rates than the controls.

The presence of gossypol in cottonseed meal was identified as the major limiting factor for acceptance and utilisation of cottonseed meal based diets. Adding iron (Fe) to

the cotton seed meal based diets (1:1 iron to free gossypol ratio) for Nile tilapia reduced the negative effects of gossypol and improved growth performance and feed utilisation and can totally replace fishmeal in tilapia diets (El-Saidy and Gaber, 2004).

2.2.1.3. Rapeseed and canola meal:

Canola is the name given to selected varieties of rapeseed that are low in glucosinolate and erucic acid (Thiessen *et al*., 2004). High levels of glucosinolates have been considered to be the most important limiting factor to the use of rapeseed meals in fish diets (Higgs *et al*., 1982). Heat treatment of rapesesed inactivates the myrosinase activity which has an antithyrogenic effect whereas dehulling removes the fibre content and improves the digestible energy content (Yurkowski *et al*., 1978). The replacement of 10 and 20% of soybean by improved varieties of rapeseed meal in swine and poultry diets has proved to be without negative efforts (Gomes *et al*., 1993).

Canola meal, resulting from the solvent extraction of canola oil, is readily available worldwide. Relative to the nutrient requirements of carnivorous fish such as Atlantic salmon and rainbow trout, the amino acid profile of canola meal protein is similar to that of herring meal protein and superior to soybean meal protein (Higgs *et al*., 1995; Mwachireya *et al*., 1999). In some studies, the utilisation of 20 to 25% of rapeseed or canola meal in diets for salmonids did not result in any negative effects on growth and digestibility (Higgs *et al*., 1982; Gomes *et al*., 1993). Dabrowski and Kozlowska (1981) suggested the possibility of replacing 50% of fish meal by rapeseed meal in diets for carp, while Davies *et al*. (1990) proposed a limit of 15% of rapeseed meal in diets for tilapia. However, studies by Jackson *et al*. (1982) found that at 25% plant protein level in the diets containing rapeseed meal growth was comparable to that with fishmeal based diet. There are evidences to suggest that high levels of glucosinolates can produce reduced growth rates.

An industrial source of canola protein isolate produced by first extracting canola meal with aqueous salt solution and then recovering the protein fraction, had the best nutrient composition and lowest levels of antinutritional factors and an excellent substitute for premium quality fishmeal in diets for salmonids (Mwachireya *et al*., 1999).

Thiessen *et al*. (2004) found that dephytinized canola protein concentrate has potential to replace substantial levels of fishmeal in diets for carnivorous fish without compromising performance.

2.2.1.4 Sunflower oil meal:

Owing to its high sulphur amino acid content (Gohl, 1981) sunflower seed meal has been widely used in poultry and cattle feeding. In fish feeding it has been tested with species such as *Oreochromis mossambicus* (Jackson *et al*., 1982), *O. niloticus* (Sintayehu et al., 1996; *Onchorhynchus mykiss* (Tacon *et al*., 1984) and *Anguilla anguilla* (Garcia *et al*., 1998). These studies showed positive results at low inclusion levels (50%), but growth reduction at high inclusion levels, which may be due to high fibre content in sunflower meal. One of the reasons for its inclusion at high levels is due to the good amino acid profile and absence of known toxic factors. A study was carried out to examine the nutritional qualities of sunflower seed meal as an ingredient in *Tilapia rendalli* diets (Olvera *et al*., 2002). The experimental results show that it is possible to replace animal protein in tilapia fry diets with sunflower seed meal, with optimum growth response at a 20% substitution level. These results also show a reduction in growth and feeding efficiency as plant protein content increased beyond 20%. The growth reduction observed at high inclusion levels of sunflower meal could be related to dietary amino acid profile. This is possible considering that sunflower meal is deficient in phenylalanine.

2.2.1.5 Unconventional protein sources:

Single Cell Protein (SCP), algal protein, bacterial protein and Brewer's dried grains have been tried in fishes.

Crude protein levels in brewer's by-products ranged from 208 to 423g/kg which qualified them as protein ingredient by conventional definition (>20%). Brewer's dried yeast is produced from beer fermentation; during fermentation a surplus of yeast is produced (Cheng *et al*., 2004).

A study conducted by Matty and Smith (1978) observed that at 25% inclusion level of yeast in rainbow trout diets induced better weight gain and FCE than commercial trout feeds.

In practical diets, only 0.5 -15% brewer's dried yeast is recommended because of its high cost and bitter flavour (Tacon and Akiyama, 1997).

When the brewer's dried yeast (30%) was combined with casein-gelatin (70%) based diet in rainbow trout a positive growth response was obtained (Cheng *et al*., 2004).

Several trials have been conducted to test the possibility of replacing traditional protein sources (fish and soybean meals) in aquafeeds with an alternative, organically certified yeast protein. Recent trials in tilapia by Craig and McLean (2006) showed that yeast could effectively replace 100% of the fishmeal protein source in tilapia aquafeeds. It could also significantly reduce the muscle lipid levels in the edible component of fish, which provides a leaner and potentially healthier product for the market place. However, when the same was fed to a marine carnivorous fish *Cobia* 25% inclusion level of yeast was found to be acceptable.

Brewer's dried grain, the material that is left over from malted barley after the starch has been converted to sugars and extracted or converted into ethanol is the main dried and milled by-product from the brewing industry. A study conducted by Cheng *et al*. (2004) in rainbow trout using a brewer's dried grain high protein diet showed higher ADC for dry matter and lysine which would lower amounts of organic matter loss via feaces compared to use of commercial brewer's dried grain.

2.2.1.6 Other plant protein sources:

When jack bean (*Canavalia ensiformis* Leguminosae) meal was used as a partial substitute for fishmeal in diets for tilapia the results were encouraging. However, caution must be exercised when using this protein source because of residues of non-thermolabile toxins in the meal (Martinez *et al*., 1988).

DeSilva and Gunasekhara (1989) found that green gram (*Phaseolus aureus* synonym *Vigna radiata*) can be included at 25% level in diets of tilapia without affecting the growth.

Nandeesha *et al*. (1991) suggested that it is desirable to use ingredients such as leaf meal at 15 -20% level rather than 30% level as recommended by Cho *et al*. (1985).

Ray and Das (1994) studied the performance of rohu with six aquatic macrophytes viz. *Lemna polyrrhiza, Eichhornia crassipes, Pistia stratiotes, Salvinia cuculata, Hydrilla verticillata* and *Nymphoides cristatum*. The results showed that carbohydrates in *S. cuculata* and *H. Verticillata* were poorly digested by rohu, while those in *E. crassipes, N. cristatum, P. stratiotes and L. polyrrhiza* were well digested.

Alam *et al*. (1996) compared the growth performance of indigenous and exotic carps using fishmeal, cottonseed and maize gluten as a protein source.

Deoiled salseed meal (*Shorea robusta*) can be incorporated up to a level of 30% in processed condition in *Labeo rohita* fingeling diets (Mukhopadhyay and Ray, 1997).

Studies conducted by Saha and Ray (1998a) revealed that chuni, a commercially available low cost cattle fodder can be used as a component in the supplementary diet for rohu fingerlings, partially substituting fishmeal up to 10%.

Omoregie (2001) studied the utilisation of mango seeds and palm kernel meal by juvenile *Labeo senegalensis*. Results from this investigation showed that their inclusion in fish diets at a 10% level seems to have no deleterious effects on the growth of the juveniles.

A duckweed-fed carp polyculture trial conducted in Bangladesh by Azim and Wahab (2003) reported positive growth rates for exotic Thai silver barb and common carp and indigenous catla whereas , indigenous rohu was not affected by duckweed.

2.2.1.7. Cocoa pod:

The possibilities of designing practical fish and shrimp diets with the use of plant feed stuffs have been reported by Lim and Dominy (1992). With the development of fish feed processing industry, it could be possible to put into profitable use some plant and animal by-products which are presently discarded as wastes (Falaye *et al*., 1999).

Cocoa pod husk, discarded as waste products from cocoa plantations, has been evaluated as an energy source in poultry and pig rations, but it is low in protein (Branckaert *et al*., 1973). Haines and Echeverria (1955) successfully replaced cornmeal with cocoa pod meal in cow rations. Cocoa bean meal has been effectively used to replace over 30% cereal grains with no toxic effects on performance of sheep (Adebowale, 1984).

Cocoa pod husk (CPH) diets in fish feeds indicate a positive potential/value (Fagbenro, 1992). In his studies on the use of cocoa pod husk as an energy source in low-cost aquaculture diets in *Clarias isheriensis* it was observed that Feed Conversion Ratio (FCR) values declined as dietary CPH level increased, but there was no significant difference among the treatments. A lack of significant deterioration in growth performance and feed efficiency indices showed that CPH could be incorporated into fish feeds and has potential as a dietary energy source.

Falaye *et al*. (1999) studied the growth performance of tilapia (*Oreochromis niloticus*) fingerlings fed varying levels of cocoa husk diets and found that beyond 10% inclusion level fish weight gains and specific growth rates are depressed over the control animals fed fishmeal based diets. The reduced growth was caused by high fibre content of cocoa husk.

2.2.2. Animal protein sources:

Animal protein meals from the rendering industry have been used in animal feeds since the middle of the 19th century (Cheng *et al*., 2004). Fishmeal is widely used as the main source of dietary protein for most commercially farmed species. Many authors have reported that between 30% and 75% of dietary fishmeal could be replaced by animal by-products. Next to fishery by-products, terrestrial vertebrate by-products usually constitute

the second major source of animal protein within aquafeeds for warmwater fish species (Tacon, 1993). Another advantage of using these animal by-products is their easy availability in the locality and low cost. With possible exception of liver meal and meat meal, the utilisation of most vertebrate by-product meals is usually limited by specific nutritional imbalances including imbalances of essential amino acids (EAA) and ash/mineral (Tacon, 1993).

2.2.2.1. Fishmeal:

Compared to other conventional animal and plant protein sources fishmeal is unique in that it is not only an excellent source of high quality animal protein with well balanced essential amino acid profile, but it is also a good source of digestible energy, essential minerals and trace elements, vitamins and rich in omega -3 fatty acids (Hertramp and Piedad- Pascual, 2000). Several experiments were carried out to determine the optimum requirement of fish especially when fed on fishmeal as a sole protein source. Wee and Tacon (1982) reported the requirement of juvenile snakehead *Channa striatus* to be 52%, while it is 47% for juvenile American eel *Anguilla anguilla* (Tibbets *et al*., 1999).

Jauncey (1982) observed that white fishmeal with 40% crude protein elicit maximum growth in juvenile tilapia (*Sarotherodon mossambicus*).

The utility of tilapia meal for salmonid diets was investigated by Foltz *et al*. (1982). They observed that up to 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 fishmeal to be a rich source of protein for mirror carp *Cyprinus carpio*. The supplementation of fishmeal with a single amino acid or a single organic acid gave poor growth performance in rainbow trout (Fauconneau, 1988).

Fish silage can be produced very easily compared to fishmeal and has got good storage characteristics (Jackson *et al.,* 1984). Its practical utility in fish diets has already

been worked out (Asgard and Austreng, 1981; Hardy *et al*., 1984; Jackson *et al*., 1984; Ittoop *et al.,* 2006).

Utilisation of 40% silage in a ration without previous neutrilization in eel diets resulted in loss of appetite and weight (Affandi, 1985). Studies by Goncalves *et al.* (1989) reported that diets incorporating fish silage after neutralization gave better growth performance in eel fingerlings. Wood *et al*. (1985) reported poor growth response in common carp with silage-based diets and attributed this to its low palatability and loss of nitrogen by leaching. When fish silage was used as an alternate protein source for spawn rearing of *Cirrhinus mrigala* growth response was comparable to control diet with groundnut and rice bran (Ittoop *et al*., 2006).

2.2.2.2 Replacement of fishmeal by other animal protein sources:

2.2.2.2.1 Poultry litter and blood meal:

There are reports on the use of poultry droppings as manure in aquaculture practices (Jhingran, 1991), informations on the utilisation of this waste product in formulation of fish feed are scanty (Kumar and Singh, 1984).

Kumar and Singh (1984) fed common carp fingerlings with pelletized poultry litter and obtained faster growth than with pelletized traditional feed, comprising a mixture of groundnut oil cake and rice bran. The deep poultry litter is rich both in vitamin B_{12} and B_2 (Riboflavin) in addition to phosphorus, calcium, etc. (Saha and Ray, 1998b) and therefore, it was used as a feed for common carp as such. At the same time it is deficient in tyrosine and lysine and of variable quality, *i.e*., in lipid and protein content (Tacon, 1993). When poultry litter was used as a substitute to fishmeal, better performance of rohu fingerling was observed with 20% incorporation (Saha and Ray, 1998b). However, Tacon (1993) reported maximum inclusion of 35% poulty litter in complete diets for omnivorous/herbivorous fish. The poor performance of fish at higher inclusion levels of 30% or above of poultry litter is probably due to its deficiency in tyrosine and lysine contents and also due to possible copper toxicity (Collins *et al*., 1993).

Blood from the slaughter of domestic animals makes up 7.5% of the total weight of a living animal, and about 50% of this may be drained off at slaughter. Inclusion of blood meal in diets of warmwater culturable fish has been reported by a number of workers (Asgard and Austreng, 1986; Hossain *et al*., 1992; Saha and Ray, 1998b). Tacon (1993) reported the observed dietary inclusion level of blood meal within practical complete diets for omnivorous/herbivorous fish species up to a maximum level of 20%. However, in studies with tilapia, *Oreochromis niloticus* fingerlings, Otubusin (1987) indicated levels of blood meal exceeding 50% of the dietary protein resulted reduction in growth performance and feed utilisation.

Hardy (1989) reported that blood meal is very deficient in isoleucine and methionine. When goat blood meal was used as a substitute to fishmeal, better performance of rohu fingerling was observed with 30% incorporation (Saha and Ray, 1998b).

2.2.2.2.2. Meat and bone meal (MBM):

MBM is a potential animal protein source because of their high protein contents and low price compared to fishmeal (Tacon and Jackson, 1985). Generally the substitution of MBM for fish meal is less than 300g/kg in fish feeds (Robaina *et al.,* 1997; Bureau *et al.,* 2000). Even higher replacements with positive results are reported in the case of Mozambique tilapia and rainbow trout (Davies *et al*., 1989; Watanabe and Pongmaneerat, 1991). MBM is deficient in lysine and methionine and considered to be of low nutritional value (Watanabe and Pongmaneerat, 1991).

Millamena (2002) successfully replaced 750, 800 and 900 g/kg fishmeal protein with MBM combined with other proteins sources in diets for Mozambique tilapia and rainbow trout. However, Yang *et al*. (2004) reported that MBM could replace up to 500g/kg of fish meal protein in diets for gibel carp (*Carassius auratus*) without negative effects on growth while 150g/kg replacement by MBM protein improved feed utilisation.

2.2.2.2.3 Krill meal:

Luckowicz (1979) investigated the possibility of replacing fishmeal in carp diets with krill (*Euphausia superba*) meal. Wojino and Dabrowski (1984) reported the use krill meal for feeding the year old rainbow trout (*Salmo gairdneri*). Olsen *et al*. (2006) tested the possibility of replacing fishmeal by krillmeal in diets of Atlantic salmon (*Salmo salar*). The results showed that moderate levels of krill meal (20-60% of krill protein) in diets increased the growth during the first half of the experiment compared with the fishmeal control while no growth difference was observed in the second half of the experiment.

2.2.2.2.4 Shrimp meal:

Shrimp meal as an alternate protein source in the diets of channel cat fish and mullet (*Liza parsia*) showed poor growth performance (Robinette and Dearing, 1978; Kiran, 1984). Contrary to this, Pfeffer and Meske (1978) obtained good growth and survival in carps fed feeds based on casein and shrimp meal. Liti *et al*. (2006) studied the effects of partial and complete replacement of freshwater shrimp meal (*Caridinea niloticus*) with a mixture of plant protein sources on growth performance of Nile tilapia in fertilized ponds. Although complete substitution of shrimp meal by plant protein did not affect the growth of tilapia, production cost was reduced by 36%. They also concluded that animal protein is not required in diets for production of tilapia in fertilized ponds.

Materials and methods

3. Materials and methods

The aim of the present study is to evaluate the potential of cocoa pod husk meal (CPM) as a partial substitute for fishmeal in the diets of *Labeo rohita* (Hamilton) fry by determining its growth performance and survival. In addition, digestibility co-efficient of diets was determined incorporating CPM at different inclusion levels (5, 10, 15, 20 and 25%) to ascertain the best level of incorporation of the ingredient. The duration of the experiment was 70 days.

3.1. Experimental set up

The experiment was conducted in the hatchery of Department of Aquaculture, College of Fisheries, Panangad. A static indoor rearing system comprising of eighteen circular flat bottom fibre glass tanks were used the study. The tanks have capacity of 83 litres, diameter 55 cm, height 35 cm, rim width 3 cm and thickness 4mm.The tanks were arranged in the hatchery which had provisions for subdued light penetration. The tanks were flushed thoroughly to remove any dirt traces and allowed to dry for one day. They were then filled with water from a bore well after sieving through a fine meshed nylon bolt cloth. The water was filled to a depth of 26±5 cm.

3.2. Experimental animals

Labeo rohita used in this feeding trial were obtained from a stock produced at the College of Fisheries, Panangad, Kerala Agricultural University. Fry of uniform size ranging in weight from 1.3 – 1.45g (mean 1.41±0.17g) were selected for the study. They were divided into eighteen groups of ten fish each. All experiments were carried out in triplicate, each group of fish being randomly assigned one of the six diets. The fish were acclimated to laboratory conditions for a period of one week prior to commencement of experiment during which the fish were fed with a reference diet (crude protein content of 30.29%). After acclimatization the fish were fed the experimental diets.

3.3. Preparation of experimental diets

3.3.1. Feed ingredients:

The ingredient proportion of the experimental diets is given in **Table.1**. The ingredients used for the preparation of the test diets were fishmeal, cocoapod husk meal, groundnut oil cake, wheat bran, tapioca flour and vitamin mixture. The ingredients except cocoa pod were procured locally. Cocoapod was collected from the Cocoa Research Unit of Kerala Agricultural University, Vellanikkara. Fresh cocopods were peeled and sundried and ground to a fine powder. Fishmeal was prepared from locally available species of silver belly according to procedure developed at CIFT, Cochin (Ramachandran *et al*., 2002). Vitamins were supplemented through Supplevit-M (Sarabhai chemicals, Mumbai). All the ingredients except vitamin mixture were sieved to get uniform particle size and packed in airtight bottles prior to feed formulation.

3.3.2. Proximate analysis of feed ingredients:

Proximate analysis of the ingredients was done prior to feed formulation to evaluate the nutrient status. Moisture, crude protein, crude fat, crude fibre and ash content were analyzed following AOAC (1990) methods.

The moisture level was estimated by heating the sample to 105ºC until a constant weight was reached. The crude protein content was estimated by Microkjeldahl's method (AOAC, 1990). The nitrogen content was multiplied by the factor 6.25 to arrive at crude protein content. Crude fat was extracted using petroleum ether (Boiling Point 40ºC to 60ºC) in a soxhlet extraction apparatus for 8 hours. The ash content was determined by burning the sample at 550ºC ±10ºC for 6 hours in a muffle furnace. The carbohydrate content, (Nitrogen Free Extract, NFE) was found out by difference method (Maynard *et al.,* 1979).

NFE = 100 – (% Crude protein on dry wt basis + % Crude fat on dry wt basis + % ash + % moisture + % fibre)

Table.1 Ingredient proportion of the test diets:

Treatment/ ingredient	Control	5% CPM	10% CPM	15% CPM	20% CPM	25% CPM
Cocoa pod husk meal	—	5	10	15	20	25
Fish meal	40	35	30	25	20	15
Wheat bran	21	20	20	17	14	9
Groundnut oil cake	13	15	21	28	35	43
Tapioca flour	25	24	19	14	10	7
Vitamins and mineral mixture	1	1	1	1	1	1
Total	100	100	100	100	100	100

The energy contents of the formulated diets were calculated using the average caloric conversion factors of 9.45, 4.10 and 5.65 Kcal/g for lipid, carbohydrate and protein, respectively and expressed as Kilocalories/ gram (Henken *et al*., 1986).

3.3.3. Formulation and processing of test diets:

All diets were isonitrogenous (29.93±0.36% crude protein content) and isocaloric (397.84±9.22KJ/100g). The diets were prepared following the methods of Olvera *et al*. (1990). A reference diet containing 30% protein was formulated using fishmeal. In each of the five experimental diets fishmeal was progressively replaced by cocoa pod meal.

For preparing the diets, requisite quantities of the ingredients with respect to each diet were mixed with a small quantity of water (1: 1.25 W/V) to form thick dough, using tapioca flour as the binder. The dough was steamed at 100°C for 10 minutes in a pressure cooker and cooled rapidly. The vitamin mixture (Supplevit-M) was added to all the diet mixtures and mixed thoroughly before pelletisation. Pelletising was done in a hand operated extruder to obtain pellets of about 2mm diameter. The pelletised feeds were dried in a hot air oven at 60ºC 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.

3.3.4. Proximate composition of formulated feeds:

All the six diets were analysed for moisture, ash, crude protein, crude fibre and total carbohydrate using standard methods (AOAC 1990). The calorific content of each diet was calculated using the conversion factors given by Henken *et al*. (1986).

3.4. Proximate analysis of carcass composition

Carcass composition of fish were analyzed initially and also on termination of the experiment according to standard procedures (AOAC 1990).

3.5. Experimental design and procedure

Fish in each tank fed one of the five experimental diets containing varying levels of cocoapod meal/ a diet lacking cocoapod meal, which served as control diet. Each diet was fed to fish in a set of three tanks.

The fish were weighed at the onset of the feeding trials, and were fed once daily (08.00 hours) at 5% of body weight per day. Feeding was preceded each day by cleaning the tanks of faecal materials and replacing two-third of the total water volume. Water quality in the experimental tanks was maintained by partial periodic replenishment. The study was conducted for a period of 70 days. After the acclimation period, all fish in each tank were weighed collectively at fortnightly intervals and individual length measurements were recorded. The quantity of feed given was readjusted following each sampling.

For digestibility assessment, faecal matter was collected for a period of 6 weeks. The method of voided faeces collection was adopted. The collected faecal samples per tank were dried at 105ºC for 10 hours and stored in airtight vials for subsequent analysis.

At the end of the experimental period, 6 – 8 individuals maintained on a particular diet were sacrificed and dried to a constant weight and the percentage moisture was determined. Dried samples of each group of fry were pooled together and finely ground and used for proximate analysis.

3.6. Water quality

Water quality parameters: temperature, pH, dissolved oxygen, total alkalinity and ammonia were monitored at weekly intervals following standard procedures recommended by APHA (1998).

Temperature was determined by using mercury bulb thermometer with 0.1ºC accuracy. pH was assessed by using the Universal pH indicator method, dissolved oxygen was determined by using the Standard Winkler method. Total Alkalinity was determined

following the acidimetric titration method (APHA, 1998) and ammonia was assessed by using the phenate method (Parson *et al*., 1984).

3.7. Water stability of formulated feeds

Water stability of the feeds was determined using the percentage dry matter recovered after exposing the pellets in water for 6 hours (Jayaram and Shetty, 1981).

3.8. Evaluation criteria

Parameters like growth (weight gain), specific growth rate (SGR) and Food Conversion Efficiency (FCE) were computed using appropriate formulae given by Lovell (1989). Protein Efficiency Ratio (PER) was computed based on the formulae given by Osborne *et al*. (1991) and Miller & Bender (1955) respectively. Nutrient digestibility was estimated by the methods of Furukawa & Tsukahara (1966).

3.8.1. Net weight gain

The increase in weight of fish during the experimental period when fed the different test diets. Net weight gain was calculated as

Net weight gain (g) = final weight (g) – initial weight (g)

3.8.2. Percentage increment in growth

The percentage growth of the animal was calculated using the following formula:

$$\text{Growth}(\%) = \frac{\text{Final measurement} - \text{Initial measurement}}{\text{Initial measurement}} \times \mathbf{100}$$

3.8.3. Specific Growth Rate (SGR)

Specific Growth Rate (SGR) was calculated as

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

Where W_1 - Weight at time T_1

W_2 - Weight at time T_2

The calculated value gives the average percentage increase in body weight per day over 70 days.

3.8.4. Survival rate

It is expressed in terms of percentage.

$$\text{Survival}(\%) = \frac{\text{No. of test animals introduced} - \text{No. of test animals harvested}}{\text{No. of test animals introduced}} \times 100$$

3.8.5. Food Conversion Ratio (FCR)

FCR is the ability with which an animal can convert the feed consumed into edible and other products. It is the most commonly used index to measure the efficiency of different diets used in the experiment.

$$\text{FCR} = \frac{\text{Feed intake on a dry matter basis}}{\text{Weight gain on wet matter basis}}$$

3.8.6. Protein Efficiency Ratio

PER is defined as the weight gain per unit intake of protein

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

3.8.7. Apparent digestibility co-efficient (ADC)

This is used to directly evaluate the quality of protein in the diet

$$\text{ADC} = \frac{\text{Protein digested}}{\text{Protein ingested}}$$

3.9. Statistical analysis

The experiment was carried out by using the Completely Randomized Design (CRD). All indices, such as growth, specific growth rate, food conversion efficiency, protein efficiency ratio, feed assimilation and apparent digestibility co-efficient were subjected to comparison using Analysis of Variance (Snedecor and Cochran, 1968) and treatment difference studied at 5% level of significance.

Results

4. Results

The growth performance of rohu fed different inclusion levels of cocoa pod husk diets was evaluated. The details of the observations made during the study are presented below. Six isonitrogenous diets are denoted as C (control), T_1 (5% CPM), T_2 (10% CPM), T_3 (15% CPM), T_4 (20% CPM) and T_5 (25% CPM) respectively.

4.1. Proximate composition of feed ingredients and formulated diets.

4.1.1. Feed ingredients.

The proximate composition of feed ingredients (fishmeal, cocoa pod husk meal, wheat bran, groundnut oil cake and tapioca flour are presented in the Table.2.

The moisture content of the feed ingredients ranged from 6 to 11.15%, the highest being for tapioca flour and lowest for groundnut oil cake. The lipid fraction of feed ingredients ranged from 0.38% (cocoa pod meal) to 6.87% (groundnut oil cake). Fish meal, wheat bran and tapioca flour had lipid contents of 5.86, 2.92 and 2.13% respectively.

The results of the crude protein estimation showed that fishmeal had the highest (58.31%) followed by groundnut oil cake (42.12%), wheat bran (14.44%), cocoa pod husk meal (7.35%) and lowest in tapioca flour (2.87%).

Crude fibre content of the ingredients was found to be in the range of 2.5 to 30.43%. Tapioca flour had the lowest and cocoa pod meal had the highest fibre content. The carbohydrate content of the tapioca flour was highest (80.2%) while, fishmeal had the lowest (3.83%).

The ash content of the ingredients was estimated to be in a range of 1.15 to 19.4% for tapioca flour and fishmeal respectively.

4.1.2. Formulated diets (initial analysis)

The results of proximate composition analysis of the formulated diets are presented in the Table. 3.

The crude protein content of the isonitrogenous diets were as follows; control diet (30.74%), 5% CPM diet (30.35%), 10%CPM (30.20%), 15% CPM (30.06%), 20% CPM (29.95%) and 25% CPM (30.01%).Moisture content of the diets were found to be less than 10% in all the diets, ranging from 3.1% (25% CPM) to 4.15% (control).

Crude lipid content of the diets ranged from 4.46 (20% CPM) to 4.84% (Control) and carbohydrate content from 30.9 (25% CPM) to 42.65% (5% CPM).

Crude fibre content of the diets increased as the cocoa pod meal inclusion increased. Crude fibre content of the diets was less than 16% and ash content was in a range of 12 (5% CPM) to 16% (25% CPM). Highest fibre content was noted in the 25% CPM diet and lowest in control diet.

4.1.3. Formulated feeds (Final analysis)

The results of the proximate analysis of formulated diets after a storage period of 4 months are presented in Table.4.

The moisture and NFE content increased during the storage period. 20% CPM and 25% CPM diet showed maximum increase in moisture content. NFE content increased negligibly in all the diets. However, crude protein content, fibre and ash content decreased after the storage period. Decrease in crude protein content was in the order of control diet followed by 15% CPM, 20% CPM, 5% CPM, 10 % CPM and 25% CPM diets.

Table 2 Proximate composition* of feed ingredients used in the formulation of feed

Ingredients	Moisture (%)	Fat (%)	Protein (%)	Fibre (%)	Ash (%)	Carbohydrate (%)
Cocoa pod husk meal	8.4 (±0.20)	0.38 (±0.02)	7.35 (±0.22)	30.43 (±0.24)	11.09 (±0.91)	42.35 (±0.11)
Fish meal	9.6 (±0.10)	5.86 (±0.03)	58.31 (±0.06)	3.0 (±0.40)	19.4 (±1.10)	3.83 (±0.23)
Groundnut oil cake	6.0 (±0.30)	6.87 (±0.43)	42.12 (±0.13)	14.0 (±0.67)	2.31 (±0.19)	28.7 (±0.22)
Wheat bran	10.4 (±0.40)	2.92 (±0.32)	14.44 (±0.94)	12.9 (±0.70)	3.5 (±0.50)	55.84 (±0.46)
Tapioca flour	11.15 (±0.35)	2.13 (±0.17)	2.87 (±0.23)	2.5 (±0.50)	1.15 (±0.15)	80.2 (±0.21)

*Average of two values

Figures in parentheses denote standard deviation (SD)

Table 3. Proximate composition* of the formulated diets (Initial analysis)

Diets	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
Control	4.15 (±0.05)	4.84 (±0.00)	30.74 (±0.49)	14.0 (±0.01)	6.36 (±0.13)	38.91 (±0.23)
T1 - 5% CPM	3.55 (±0.05)	4.6 (±0.02)	30.35 (±0.32)	12.0 (±0.01)	7.85 (±0.03)	42.65 (±0.10)
T2 - 10% CPM	3.6 (±0.00)	4.67 (±0.02)	30.2 (±0.30)	13.5 (±0.03)	9.94 (±0.45)	39.09 (±0.12)
T3 - 15% CPM	3.5 (±0.10)	4.62 (±0.00)	30.06 (±0.26)	14.0 (±0.02)	11.77 (±0.12)	36.05 (±0.10)
T4 - 20% CPM	3.4 (±0.00)	4.46 (±0.15)	29.95 (±0.23)	13.5 (±0.00)	13.65 (±0.20)	35.04 (±0.31)
T5- 25% CPM	3.1 (±0.10)	4.57 (±0.01)	30.01 (±0.19)	16.0 (±0.15)	15.42 (±0.44)	30.9 (±0.52)

* Average of two values

Figures in parentheses indicate SD

Table 4 Proximate composition* of the formulated diets (Final analysis)

Diets	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
Control	5.20 (±0.05)	4.53 (±0.06)	29.55 (±0.03)	14.30 (±0.03)	6.24 (±0.02)	40.18 (±0.27)
5% CPM	4.25 (±0.13)	4.14 (±0.12)	29.85 (±0.80)	11.36 (±0.24)	7.49 (±0.45)	42.91 (±0.36)
10% CPM	4.15 (±0.23)	4.24 (±0.42)	29.78 (±0.45)	13.08 (±0.14)	9.43 (±0.11)	39.32 (±0.13)
15% CPM	4.30 (±0.07)	4.43 (±0.07)	29.20 (±0.30)	13.68 (±0.02)	11.45 (±0.35)	36.92 (±0.57)
20% CPM	4.78 (±0.01)	4.25 (±0.01)	29.36 (±0.40)	12.82 (±0.14)	13.18 (±0.26)	35.61 (±0.50)
25% CPM	4.48 (±0.00)	4.17 (±0.22)	29.69 (±0.20)	15.54 (±0.01)	14.64 (±0.30)	31.48 (±0.43)

* Average of two values

Figures in parentheses indicate SD

4.2. Water stability (%) of the formulated diets.

The results of the water stability experiment are given in the Table. 5. 10% CPM diet (81.33%) recorded highest water stability and control diet (77.33%) the least after 8 hours of immersion in water.

4.3. Proximate muscle composition of rohu fed experimental diets.

The biochemical composition of rohu muscle conducted at the beginning and termination of the experiment is presented in the Table. 6. Initial crude protein content of the muscle was 13.94%, fat (1.98%), NFE (1.7%), ash (5.71%) and moisture (76.67%).

At the end of the feeding trial, moisture content and ash content of fish from all treatments was lower than that of initial whole fish, whereas there was an increase in body protein content over that recorded for the initial whole fish (Table.6). Protein content was highest in 20% CPM (18.99%) and lowest in 25% CPM (18.2%). A similar trend was recorded for lipid content. Lipid content was highest in flesh of control treatment (3.84%) and lowest in 25% CPM (3.28%). Ash content also showed a decreasing trend and found to be lowest in 20% CPM diet (2.35%).

Table 5. Water stability* of the formulated diets (in %)

Feeds	2 hours	4 hours	6 hours	8 hours
Control	87.45 (±0.10)	84.2 (±0.50)	80.97 (±0.67)	77.33 (±0.25)
5% CPM	90.52 (±0.40)	87.50 (±0.01)	84.77 (±0.65)	80.62 (±0.50)
10% CPM	90.88 (±0.40)	88.20 (±0.30)	84.97 (±0.07)	81.33 (±0.61)
15% CPM	89.22 (±0.50)	86.75 (±0.10)	81.13 (±0.44)	79.58 (±0.30)
20% CPM	89.54 (±0.20)	86.81 (±0.40)	81.16 (±0.07)	78.81 (±0.40)
25% CPM	88.35 (±0.04)	85.03 (±0.12)	80.77 (±0.03)	78.62 (±0.80)

*Average of two values

Figures in parentheses indicate SD

Table 6. **Carcass composition* of rohu.**

Muscle composition (%)	Final value						
	Initial value	**control**	**5% CPM**	**10% CPM**	**15% CPM**	**20% CPM**	**25% CPM**
Moisture	76.67 (±0.03)	72.72 (±0.00)	72.49 (±0.10)	74.31 (±0.21)	73.7 (±0.00)	73.29 (±0.15)	74.25 (±0.11)
Crude protein	13.94 (±0.50)	18.27 (±0.35)	18.38 (±0.31)	18.3 (±0.60)	18.68 (±0.46)	18.99 (±0.28)	18.2 (±0.40)
Lipid	1.98 (±0.41)	3.84 (±0.12)	3.78 (±0.25)	3.65 (±0.11)	3.40 (±0.22)	3.37 (±0.31)	3.28 (±0.20)
Ash	5.71 (±0.13)	2.38 (±0.16)	2.61 (±0.10)	2.36 (±0.18)	2.48 (±0.17)	2.35 (±0.55)	2.46 (±0.31)
NFE	1.7 (±0.22)	1.79 (±0.40)	2.74 (±0.36)	1.38 (±0.02)	1.72 (±0.28)	2 (±0.30)	1.81 (±0.12)
Energy (Kcal/g)	104.4 (±0.30)	146.85 (±0.70)	150.8 (±0.48)	143.55 (±0.30)	144.91 (±0.60)	148.01 (±0.15)	141.25 (±0.31)

*Average of two values

Figures in parentheses indicate SD

4.4. Monitoring of water quality parameters.

4.4.1. Temperature.

Observed values of temperature in the experimental units over the culture period are presented in the Table. 7. Temperature values did not alter much in the units and was found to be in a range of 22.0- 26.5ºC.

4.4.2. pH.

pH values ranged from 6.5 to 7.5 in all the units throughout the culture period (Table. 8).

4.4.3. Dissolved oxygen.

Fluctuations in average values of dissolved oxygen values over the culture period ranged from 4.05 (Control) to 5.24 mg/l (25% CPM). The recorded values are presented in Table.9.

4.4.4. Total alkalinity.

Table.10 gives the fluctuations in total alkalinity over the experimental period. Total alkalinity values fluctuated between 70.05 (15% CPM) and 122. 85 mg/l $CaCo_3$ 25% CPM).

4.4.5. Ammonia-nitrogen value.

Fluctuations in ammonia-nitrogen values are presented in the Table.11. The values ranged between 0.034 and 0.074 ppm both being recorded in 15% CPM dietary treatments.

Table 7. Fluctuations in air and water temperature during the experimental period

Days of sampling	Air temperature	Water temperature
0	27.2	24.5-25.0
14	26.5	22.0-23.5
28	27.5	23.0-24.5
42	30.0	26.0-27.5
56	27.5	23.0-25.5
70	28.0	24.0-26.5

Table. 8 Fluctuations in pH in different treatments

Treatments	Days / Replications	0	14	28	42	56	70
Control	1	6.5	6.8	7.0	7.5	7.1	6.6
	2	6.5	7.0	7.0	7.3	6.8	6.7
	3	6.5	7.0	7.1	7.5	7.3	6.6
	Mean	**6.5**	**6.93**	**7.03**	**7.43**	**7.07**	**6.63**
	±SD	**0.0**	**0.09**	**0.05**	**0.09**	**0.20**	**0.05**
5% CPM	1	7.0	7.1	7.3	7.0	7.1	7.0
	2	6.5	6.6	6.8	7.1	7.0	6.8
	3	6.5	7	6.8	7.3	6.7	6.8
	Mean	**6.6**	**6.9**	**6.9**	**7.1**	**6.9**	**6.8**
	±SD	**0.24**	**0.22**	**0.24**	**0.12**	**0.17**	**0.09**
10% CPM	1	7.0	7.0	6.5	7.0	7.1	6.6
	2	6.8	6.8	7.0	7.0	7.0	6.8
	3	6.5	6.5	7.0	7.0	7.0	6.8
	Mean	**6.7**	**6.7**	**6.8**	**7.0**	**7.0**	**6.7**
	±SD	**0.20**	**0.20**	**0.24**	**0**	**0.05**	**0.09**
15% CPM	1	6.5	6.6	7.0	6.8	7.0	7.0
	2	7.0	7.0	6.8	7.0	6.9	6.5
	3	7.0	7.0	6.6	7.0	7.2	6.5
	Mean	**6.8**	**6.8**	**6.8**	**6.9**	**7.0**	**6.6**
	±SD	**0.24**	**0.19**	**0.16**	**0.09**	**0.12**	**0.24**
20% CPM	1	7.0	7.0	6.9	7.1	7.0	6.8
	2	6.8	6.8	7.3	7.1	7.0	7.0
	3	6.5	6.8	7.0	7.0	7.0	7.0
	Mean	**6.7**	**6.8**	**7.0**	**7.0**	**7.0**	**6.9**
	±SD	**0.20**	**0.09**	**0.17**	**0.05**	**0**	**0.09**
25% CPM	1	7.0	7.0	7.1	7.0	7.0	7.0
	2	7.0	7.0	6.8	6.9	7.1	6.7
	3	7.0	7.0	6.8	7.0	7.1	6.8
	Mean	**7.0**	**7.0**	**6.9**	**6.9**	**7.0**	**6.8**
	±SD	**0.00**	**0.00**	**0.14**	**0.04**	**0.04**	**0.12**

Table 9. Fluctuations in dissolved oxygen (mg/l) in different treatments

Treatments	Days	0	14	28	42	56	70
	Replications						
Control	1	4.18	4.60	3.93	4.01	4.15	4.03
	2	3.87	5.28	4.29	4.31	3.78	4.12
	3	4.11	4.92	4.65	3.97	4.03	4.89
	Mean	**4.05**	**4.93**	**4.29**	**4.09**	**3.98**	**4.35**
	±SD	**0.13**	**0.28**	**0.29**	**0.15**	**0.15**	**0.39**
5% CPM	1	5.21	4.48	5.08	4.68	4.91	5.31
	2	4.38	5.12	5.11	4.76	4.97	5.25
	3	3.84	5.18	5.08	4.85	4.21	5.15
	Mean	**4.48**	**4.93**	**5.09**	**4.76**	**4.69**	**5.24**
	±SD	**0.56**	**0.32**	**0.01**	**0.07**	**0.35**	**0.06**
10% CPM	1	4.98	4.97	4.56	4.43	5.15	4.79
	2	4.75	5.27	4.53	4.9	5.08	4.98
	3	5.32	4.89	4.48	5.16	4.99	4.99
	Mean	**5.02**	**5.04**	**4.52**	**4.83**	**5.07**	**4.92**
	±SD	**0.23**	**0.16**	**0.03**	**0.30**	**0.06**	**0.09**
15% CPM	1	5.11	4.54	4.41	4.87	4.85	5.22
	2	4.31	5.18	5.06	5.07	5.02	5.43
	3	4.99	4.27	5.11	5.13	5.11	4.97
	Mean	**4.80**	**4.66**	**4.86**	**5.02**	**4.99**	**5.21**
	±SD	**0.35**	**0.38**	**0.32**	**0.11**	**0.11**	**0.19**
20% CPM	1	4.72	4.38	4.87	5.03	5.08	5.13
	2	4.97	5.14	4.66	5.11	4.24	4.25
	3	5.06	4.92	5.13	4.96	4.96	5.18
	Mean	**4.92**	**4.81**	**4.89**	**5.03**	**4.76**	**4.85**
	±SD	**0.14**	**0.32**	**0.19**	**0.06**	**0.37**	**0.43**
25% CPM	1	5.22	4.87	4.93	4.99	4.87	4.97
	2	5.11	4.48	5.08	5.21	4.04	4.99
	3	4.24	4.87	5.03	4.99	4.99	5.08
	Mean	**4.86**	**4.74**	**5.01**	**5.06**	**4.63**	**5.01**
	±SD	**0.44**	**0.18**	**0.06**	**0.10**	**0.42**	**0.05**

Table.10 Fluctuations in total alkalinity (mg/l $CaCO_3$) in different treatments

Treatments	Days / Replications	0	14	28	42	56	70
Control	1	78.01	68.93	75.44	110.11	119.27	121.22
	2	75.25	67.56	74.211	96.38	116.38	120.87
	3	74.21	73.7	73.08	108.58	112.56	123.33
	Average	**75.82**	**70.06**	**74.24**	**105.02**	**116.07**	**121.81**
	±SD	**1.96**	**3.22**	**1.18**	**7.52**	**3.36**	**1.33**
5% CPM	1	73.08	72.9	72.21	112.24	118.99	120.98
	2	72.21	72.67	73.95	100.04	103.28	114.31
	3	73.95	73.2	74.82	102.48	115.22	118.19
	Average	**73.08**	**72.92**	**73.66**	**104.92**	**112.49**	**117.82**
	±SD	**0.87**	**0.27**	**1.33**	**6.45**	**8.20**	**3.35**
10% CPM	1	74.28	73.8	74.28	109.8	109.87	116.24
	2	73.82	72.4	74.07	111.12	120.43	126.91
	3	75.23	72.8	73.21	103.56	118.4	112.53
	Average	**74.44**	**73.0**	**73.85**	**108.16**	**116.23**	**118.56**
	±SD	**0.72**	**0.72**	**0.57**	**4.04**	**5.60**	**7.46**
15% CPM	1	73.7	73.18	70.47	98.76	110.5	115.87
	2	73.68	67.24	73.27	112.3	105.2	118.95
	3	74.48	69.73	74.11	105.69	111.8	123.69
	Average	**73.95**	**70.05**	**72.62**	**105.58**	**109.17**	**119.50**
	±SD	**0.46**	**2.98**	**1.90**	**6.77**	**3.49**	**3.93**
20% CPM	1	72.67	72.12	74.23	98.36	113.67	125.41
	2	75.12	73.89	74.21	99.11	111.99	118.66
	3	73.18	74.13	72.86	102.3	118.76	114.89
	Average	**73.65**	**73.38**	**73.76**	**99.92**	**114.81**	**119.65**
	±SD	**1.29**	**1.09**	**0.79**	**2.09**	**3.53**	**5.33**
25% CPM	1	74.5	73.6	75.54	100.99	120.55	119.99
	2	73.38	73.2	74.87	112.3	113.65	125.42
	3	75.43	74.3	73.88	106.8	112.44	123.13
	Average	**74.44**	**73.7**	**74.76**	**106.69**	**115.55**	**122.85**
	±SD	**1.02**	**0.56**	**0.83**	**5.66**	**4.37**	**2.73**

Table.11. Fluctuations of ammonia-nitrogen (ppm) in different treatments

Treatments	Days / Replications	0	14	28	42	56	70
Control	1	0.068	0.059	0.051	0.059	0.067	0.057
	2	0.066	0.055	0.053	0.063	0.067	0.064
	3	0.043	0.053	0.061	0.064	0.068	0.068
	Average	**0.059**	**0.056**	**0.055**	**0.062**	**0.067**	**0.063**
	±SD	**0.011**	**0.002**	**0.004**	**0.002**	**0.000**	**0.004**
5% CPM	1	0.026	0.063	0.061	0.064	0.061	0.076
	2	0.021	0.065	0.062	0.044	0.075	0.078
	3	0.055	0.069	0.069	0.069	0.065	0.068
	Average	**0.034**	**0.066**	**0.064**	**0.059**	**0.067**	**0.074**
	±SD	**0.015**	**0.002**	**0.004**	**0.011**	**0.006**	**0.004**
10% CPM	1	0.057	0.069	0.065	0.064	0.067	0.071
	2	0.039	0.062	0.051	0.058	0.069	0.065
	3	0.056	0.059	0.06	0.063	0.068	0.063
	Average	**0.051**	**0.063**	**0.059**	**0.062**	**0.068**	**0.066**
	±SD	**0.008**	**0.004**	**0.006**	**0.003**	**0.001**	**0.003**
15% CPM	1	0.051	0.072	0.062	0.064	0.066	0.062
	2	0.057	0.071	0.062	0.063	0.069	0.069
	3	0.044	0.071	0.071	0.071	0.068	0.065
	Average	**0.051**	**0.071**	**0.065**	**0.066**	**0.068**	**0.065**
	±SD	**0.005**	**0.000**	**0.004**	**0.004**	**0.001**	**0.003**
20% CPM	1	0.056	0.064	0.062	0.084	0.066	0.063
	2	0.049	0.075	0.068	0.069	0.068	0.069
	3	0.049	0.068	0.063	0.064	0.069	0.065
	Average	**0.051**	**0.069**	**0.064**	**0.072**	**0.0677**	**0.066**
	±SD	**0.003**	**0.004**	**0.003**	**0.009**	**0.001**	**0.002**
25% CPM	1	0.067	0.066	0.063	0.069	0.061	0.062
	2	0.076	0.071	0.078	0.076	0.069	0.068
	3	0.0615	0.069	0.071	0.064	0.071	0.061
	Average	**0.068**	**0.069**	**0.071**	**0.069**	**0.067**	**0.064**
	±SD	**0.006**	**0.002**	**0.006**	**0.005**	**0.004**	**0.003**

4.5. Fish growth.

The average weight attained by rohu in different dietary treatments over the experimental period is presented in Table. 12. Average final weight of rohu in different treatments were 27.63 mg (Control), 29.67 mg (5% CPM), 29.43 mg (10% CPM), 29.93 mg (15% CPM), 31.5 mg (20% CPM) and 27.8 mg (25% CPM). The fish in 20% CPM recorded highest final weight. The trend observed in average weight of rohu is given in Fig. 1. Initially the growth pattern showed fluctuations which stabilized after 42 days of culture when the growth rate was faster. The growth pattern was more or less similar for 20% CPM and 15% CPM dietary treatments. The fish under 20% CPM showed faster growth and higher average weight on termination of experiment. Daily average increment in weight was 0.21, 0.22, 0.22, 0.23, 0.24, 0.20 mg in control, 5% CPM, 10% CPM, 15% CPM, 20% CPM and 25% CPM respectively (Table.13). The average weight gain of rohu in different dietary treatments is given in Table. 13. Highest weight gain was achieved by 20% CPM diet fed rohu and 25% CPM diet fed rohu exhibited poor growth performance showing the lowest average gain in weight on termination, which was even lower than that of control. Trend in average gain in weight is presented in Fig. 2. Highest percentage weight gain was noted in 20% CPM dietary treatment and lowest in the 25% CPM dietary treatment (Table. 13). Percentage weight gain also exhibited a similar pattern as in the case of average weight gain (Fig. 3).

The average length (mm) attained by rohu in different dietary treatments is presented in Table. 16. Average final length of rohu in different dietary treatments was 62.17mm (control), 66.17mm (5% CPM), 66.23mm (10% CPM), 66.07mm (15% CPM), 63.2mm (20% CPM) and 62.93mm (25% CPM). The trend observed in average length of rohu is given in Fig. 4.The growth pattern was almost similar in all the dietary treatments. The trend in gain in length followed a different pattern from that of gain in weight (Fig.5). Gain in length in different treatments were 17.33, 14.27, 17.9, 12.93, 16.67, 16.2 mm in control, 5% CPM, 10% CPM, 15% CPM, 20% CPM and 25% CPM respectively (Table.17). Fish under 10% CPM showed higher average length gain than control fishes. However, percentage gain in length was higher in the case of control fishes (Table. 17). The trend in percentage length gain is shown in Fig. 6.

Table. 12 Growth in weight (mg) of rohu in different treatments

Treatments	Days / Replications	0	14	28	42	56	70
Control	1	14.3	15	15.4	20.9	24.8	31.9
	2	12.3	15.4	15.6	16.4	19.97	25.8
	3	12.4	14.0	17.2	18.0	20.1	25.2
	Average	**13.0**	**14.8**	**16.07**	**18.43**	**21.62**	**27.63**
	±SD	**0.92**	**0.59**	**0.80**	**1.86**	**2.25**	**3.03**
5% CPM	1	16.9	16.1	20.1	20.5	23	29.3
	2	12.5	13.9	15.1	18.3	20.2	29.8
	3	14.6	16.4	17.2	19.5	23.2	29.9
	Average	**14.67**	**15.47**	**17.47**	**19.43**	**22.13**	**29.67**
	±SD	**1.79**	**1.11**	**2.05**	**0.89**	**1.37**	**0.26**
10% CPM	1	12.8	12.6	16.6	15.9	21	29.5
	2	13.9	14.7	17.2	20.1	21.7	30.5
	3	15.0	13.9	13.8	19.3	21	28.3
	Average	**13.9**	**13.73**	**15.87**	**18.43**	**21.23**	**29.43**
	±SD	**0.89**	**0.86**	**1.48**	**1.82**	**0.33**	**0.89**
15% CPM	1	14.0	14.6	17.5	20.7	24.5	31.1
	2	14.1	15.0	18.5	22.7	23.3	27.9
	3	14.1	16.3	15.9	17.9	24.4	30.8
	Average	**14.07**	**15.3**	**17.3**	**20.43**	**24.07**	**29.93**
	±SD	**0.05**	**0.72**	**1.07**	**1.97**	**0.54**	**1.44**
20% CPM	1	14.3	13.7	17.9	20.7	23.1	29.4
	2	14.4	16.0	19.3	18.6	25.3	33.0
	3	14.7	15.1	18.9	21.9	25.1	32.1
	Average	**14.47**	**14.93**	**18.7**	**20.4**	**24.5**	**31.5**
	±SD	**0.17**	**0.95**	**0.59**	**1.37**	**0.99**	**1.53**
25% CPM	1	14.4	13.3	17.5	19.9	22.8	29.0
	2	12.7	14.4	18.9	19.8	21.5	29.0
	3	14.7	13.5	18.5	18.2	20.9	25.4
	Average	**13.9**	**13.73**	**18.3**	**19.3**	**21.73**	**27.8**
	±SD	**0.88**	**0.48**	**0.59**	**0.78**	**0.79**	**1.69**

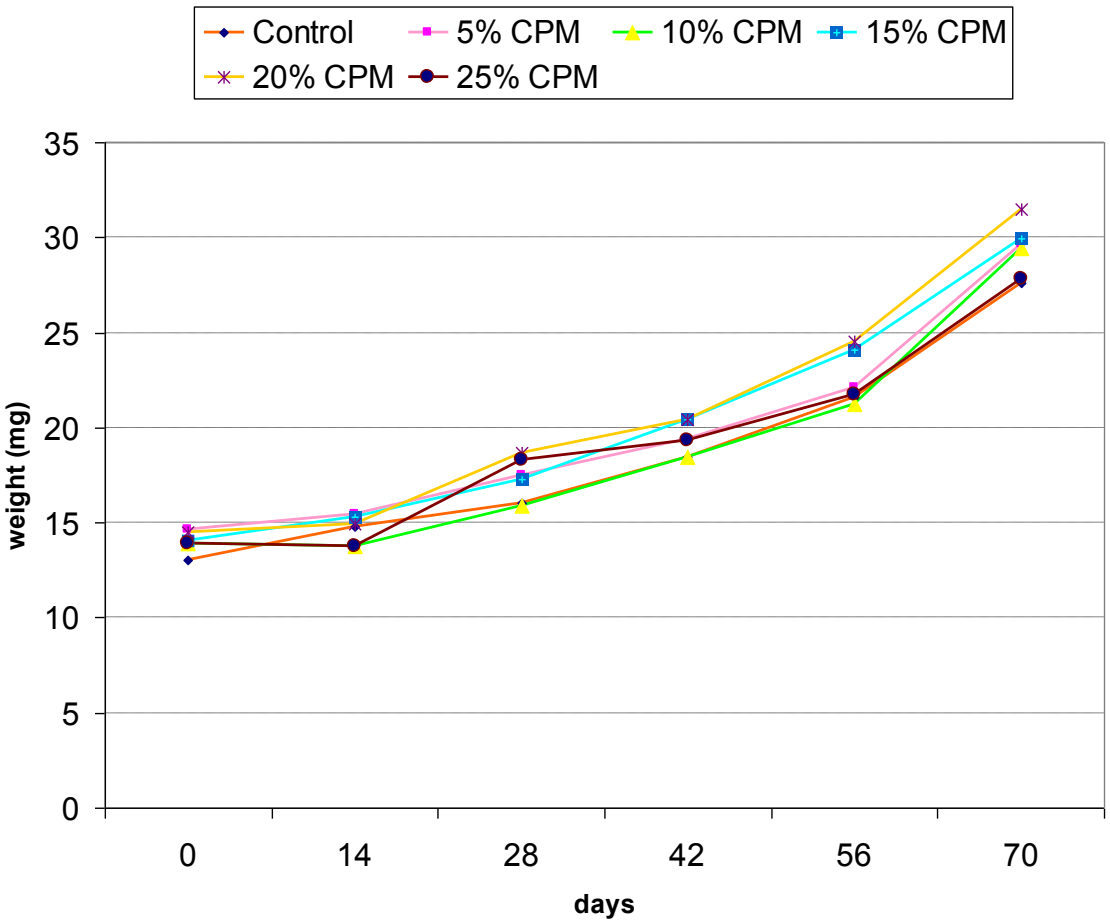

Fig. 1. Average weight (mg) attained by rohu in different treatments.

Table. 13. Weight gain of rohu in different treatments

Treatments	Replication	Average initial Weight (mg)	Average final weight (mm)	Net weight gain (mg)	Increment/ day	Percentage Weight gain
Control	1	14.3	31.9	17.6	0.25	123.08
	2	12.3	25.8	13.5	0.19	109.76
	3	12.4	25.2	12.8	0.18	103.23
	Mean ±SD			**14.63 ±2.59**	**0.21 ±0.03**	**112.02 ±10.12**
5% CPM	1	16.9	29.3	12.4	0.18	73.37
	2	12.5	29.8	17.3	0.25	138.4
	3	14.6	29.9	15.3	0.22	104.79
	Mean ±SD			**15 ±2.46**	**0.22 ±0.03**	**105.52 ±32.52**
10% CPM	1	12.8	29.5	16.7	0.24	130.47
	2	13.9	30.5	16.6	0.24	119.42
	3	15.0	28.3	13.3	0.19	88.67
	Mean ±SD			**15.53 ±1.93**	**0.22 ±0.02**	**112.85 ±21.66**
15% CPM	1	14.0	31.1	17.1	0.24	122.14
	2	14.1	27.9	13.8	0.20	97.87
	3	14.1	30.8	16.7	0.24	118.44
	Mean ±SD			**15.87 ±1.80**	**0.23 ±0.02**	**112.82 ±13.08**
20% CPM	1	14.3	29.4	15.1	0.21	105.59
	2	14.4	33.0	18.6	0.26	129.17
	3	14.7	32.1	17.4	0.25	118.37
	Mean ±SD			**17.03 ±1.78**	**0.24 ±0.02**	**117.71 ±11.80**
25% CPM		14.4	29.0	14.6	0.21	101.39
		12.7	29.0	16.3	0.23	128.35
		14.7	25.4	10.7	0.15	72.79
	Mean ±SD			**13.87 ±2.87**	**0.20 ±0.03**	**100.84 ±27.78**

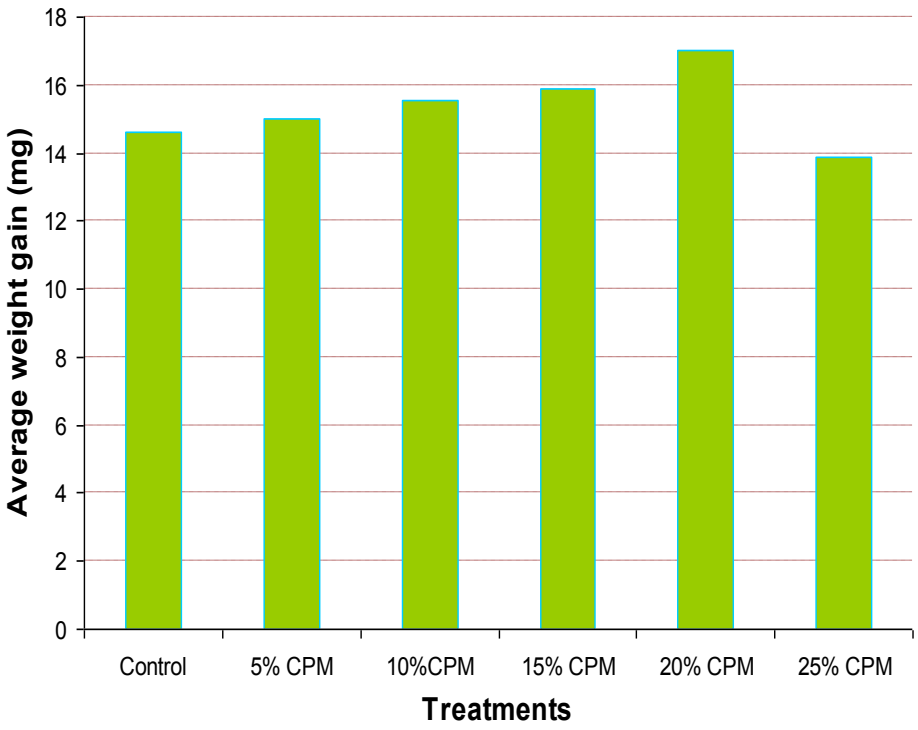

Fig. 2. Average weight gain of rohu in different treatments

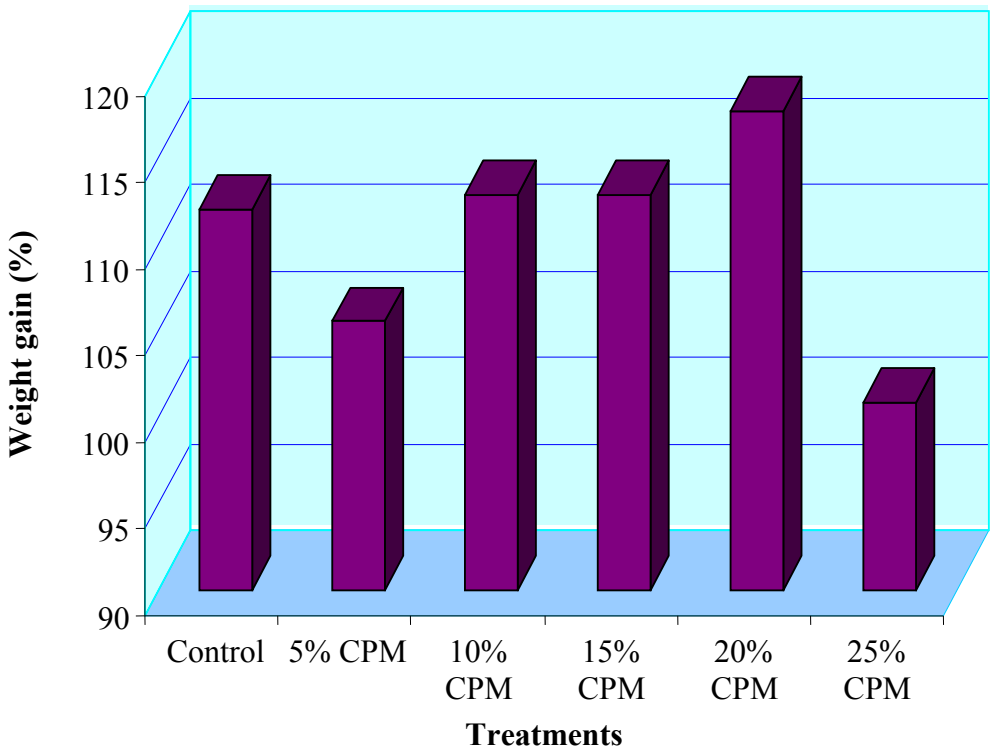

Fig. 3. **Percentage weight gain of rohu in different treatments**

Table.14. Analysis of variance of growth data (weight).

Sources of Variation	**Sum of Squares**	**Degrees of freedom**	**Mean sum of squares**	**F**	**P-value**	**F critical**
Between treatments	1953.94	9	217.1044	3.006682*	0.002866	1.958764
Between days	3221.744	5	644.3487	8.923595*	3.14E-07	2.289852
Interaction	220.2312	45	4.894026	0.067777	1	1.474058
Within Treatments	8664.876	120	72.2073			
Total	14060.79	179				

**Significance at 5%

Table15. **Analysis of variance of the data on percentage gain in weight of rohu.**

Sources of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	P-value	F critical
Between treatments	549.0236	5	109.8047	0.242986*	0.935495	3.105875
Within treatments	5422.763	12	451.8969			
Total	5971.786	17				

*Not significant at 5% level of α

Table. 16. Growth in length (mm) of rohu in different treatments.

Treatments	Days / Replications	0	14	28	42	56	70
Control	1	45.8	46.7	50.3	52.1	55.4	61.1
	2	47.9	51.7	53.1	55.0	58.5	63.9
	3	50.0	50.3	51.6	54.6	57.0	61.5
	Average	**47.9**	**49.57**	**51.67**	**53.9**	**56.97**	**62.17**
	±SD	**1.71**	**2.17**	**1.14**	**1.28**	**1.27**	**1.24**
5% CPM	1	48.2	49.8	49.1	56.9	60.6	64.6
	2	49.7	52.4	55.4	57.8	61.2	66.3
	3	50.6	51.0	54.0	60.3	61.7	67.6
	Average	**49.5**	**51.07**	**52.83**	**58.33**	**61.17**	**66.17**
	±SD	**0.98**	**1.06**	**2.70**	**1.44**	**0.45**	**1.23**
10% CPM	1	49.6	51.5	54.7	56	58.1	63.8
	2	47.3	48.1	49.9	53.7	55.7	72.5
	3	48.1	49.8	53	54.3	57.6	62.4
	Average	**48.33**	**49.8**	**52.53**	**54.67**	**57.13**	**66.23**
	±SD	**0.95**	**1.39**	**1.99**	**0.97**	**1.03**	**4.47**
15% CPM	1	49.8	49.3	53.6	56.7	62.1	69.3
	2	48.6	49.2	55	58.1	59.8	64.1
	3	51.2	50.6	53.1	55.6	60.2	64.8
	Average	**49.87**	**49.7**	**53.9**	**56.8**	**60.7**	**66.07**
	±SD	**1.06**	**0.64**	**0.80**	**1.02**	**1.00**	**2.30**
20% CPM	1	42.4	48.9	51.5	58.9	61.2	65.6
	2	45.9	48.4	52.4	52.6	55.9	63
	3	49.3	49.7	53.2	54.6	56.7	61
	Average	**45.87**	**49.0**	**52.37**	**55.37**	**57.93**	**63.2**
	±SD	**2.82**	**0.54**	**0.69**	**2.63**	**2.33**	**1.88**
25% CPM	1	49.8	51.5	52.2	55.6	57.4	64.4
	2	50.1	50.4	54.9	58.1	58.3	62.9
	3	50.1	52.1	54.7	55.6	56.6	61.5
	Average	**50.0**	**51.33**	**53.93**	**56.43**	**57.43**	**62.93**
	±SD	**0.14**	**0.70**	**1.23**	**1.18**	**0.69**	**1.18**

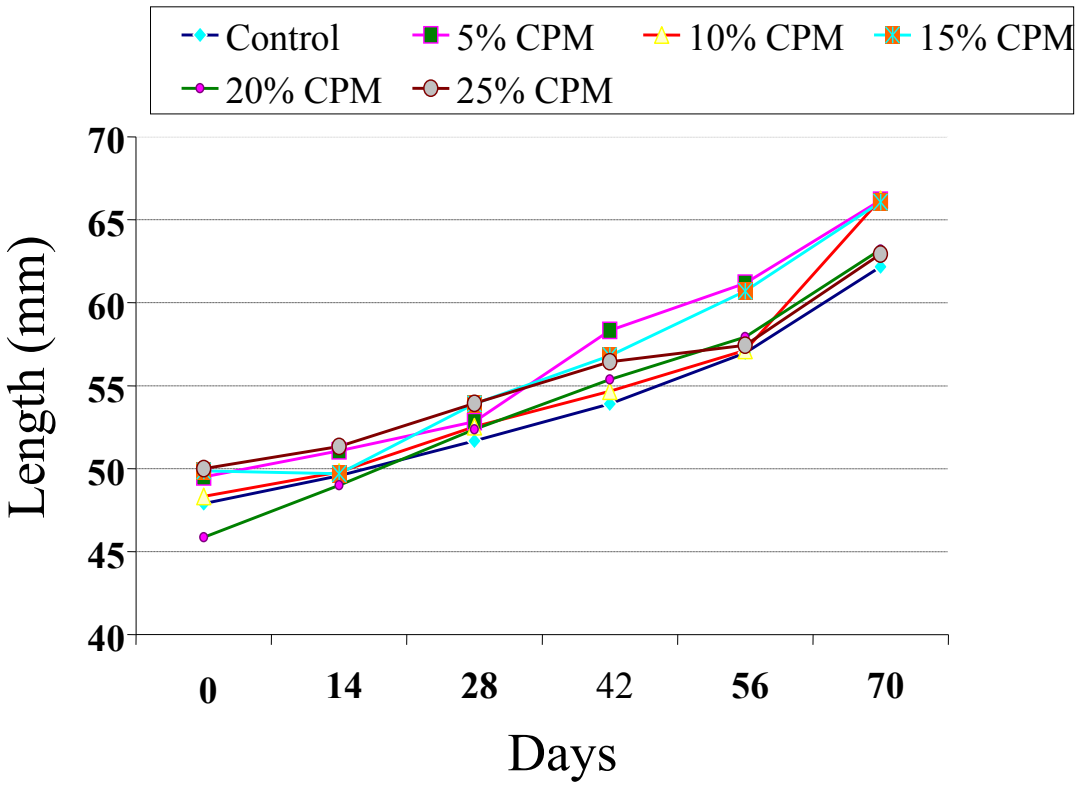

Fig.4. Average length (mm) attained by rohu in different treatments

Table. 17. Gain in length of rohu in different dietary treatments

Treatments	Days / Replications	Average initial length (mm)	Average final length (mm)	Gain in length (mm)	% length gain
Control	1	42.4	65.6	23.2	54.72
	2	45.9	63.0	17.1	37.26
	3	49.3	61.0	11.7	23.73
	Mean			**17.33**	**38.57**
	±SD			**±5.75**	**±15.54**
T1	1	45.8	61.1	15.3	33.41
	2	47.9	63.9	16.0	33.4
	3	50.0	61.5	11.5	23.0
	Mean			**14.27**	**29.97**
	±SD			**±2.42**	**±6.01**
T2	1	49.6	63.8	14.2	28.63
	2	47.3	72.5	25.2	53.28
	3	48.1	62.4	14.3	29.73
	Mean			**17.9**	**37.21**
	±SD			**±6.32**	**±13.93**
T3	1	49.8	64.4	14.6	29.32
	2	50.1	62.9	12.8	25.55
	3	50.1	61.5	11.4	22.76
	Mean			**12.93**	**25.87**
	±SD			**±1.60**	**±3.29**
T4	1	48.2	64.6	16.4	34.02
	2	49.7	66.3	16.6	33.4
	3	50.6	67.6	17.0	33.59
	Mean			**16.67**	**33.67**
	±SD			**±0.31**	**±0.32**
T5	1	49.8	69.3	19.5	39.16
	2	48.6	64.1	15.5	31.89
	3	51.2	64.8	13.6	26.56
	Mean			**16.2**	**32.57**
	±SD			**±3.01**	**±6.32**

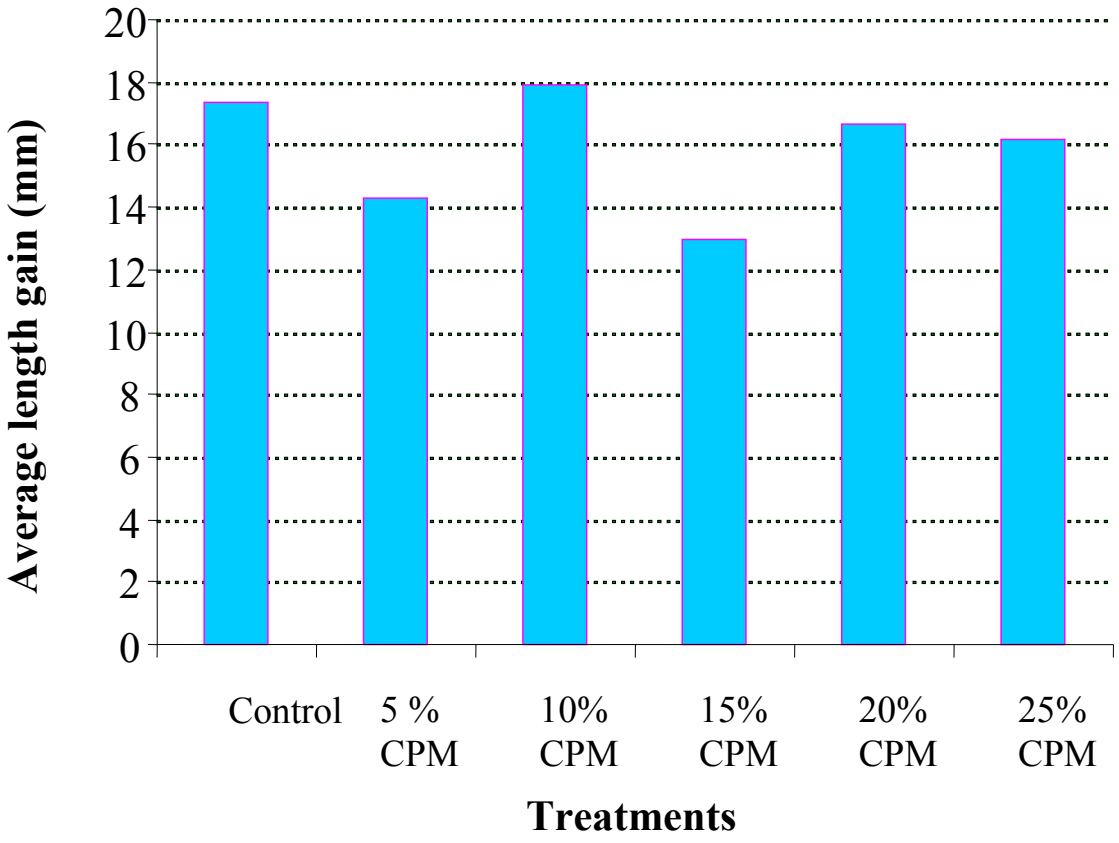

Fig. 5. **Average length gain of rohu in different treatment**.

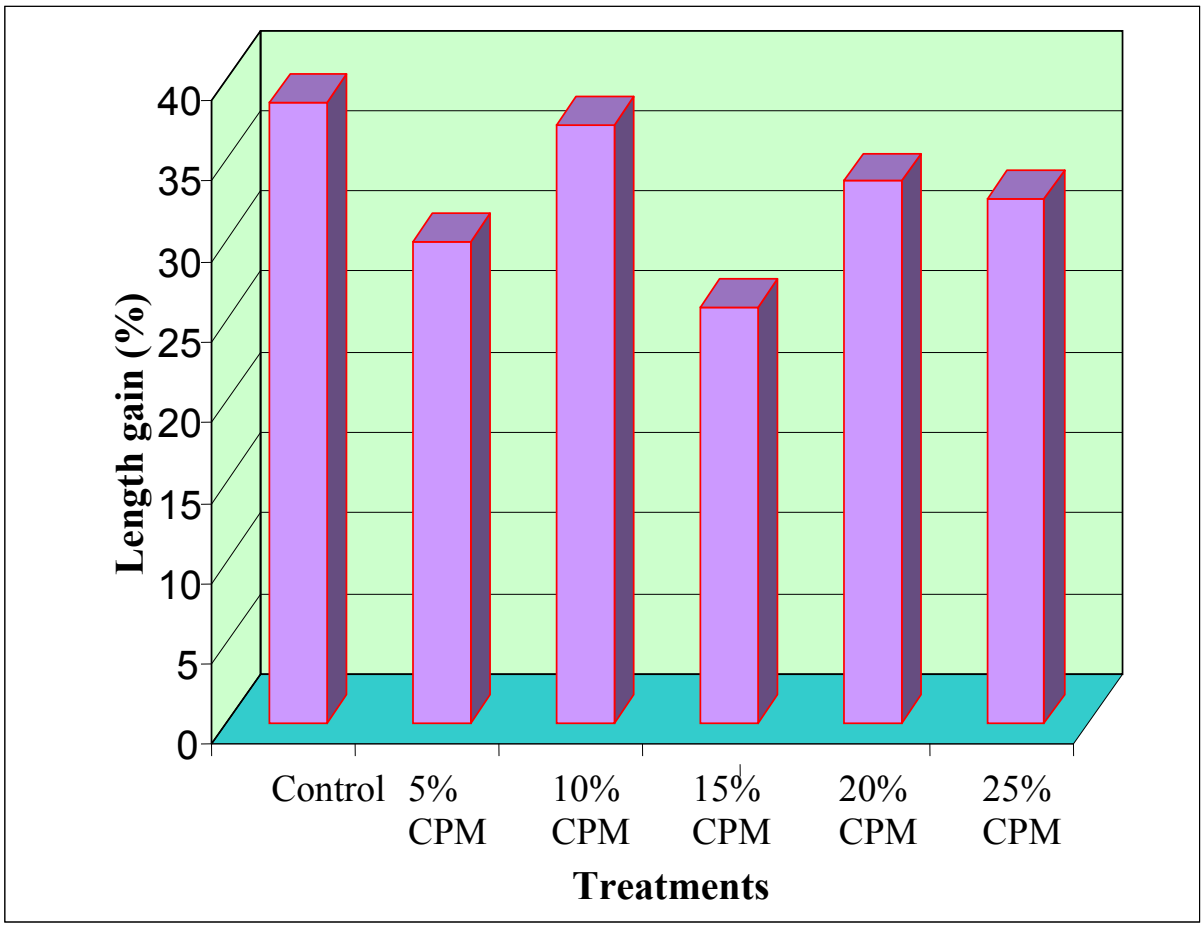

Fig. 6. Percentage length gain of rohu in different dietary treatments.

Table. 18. **Analysis of variance of growth data (length)**

Sources of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- Value	P-value	F critical
Between treatments	14201.4	9	1577.934	2.709625**	0.006582	1.958764
Between days	3394.015	5	678.8031	1.16564*	0.330111	2.289852
Interaction	247.8175	45	5.507056	0.009457	1	1.474058
Within	69881.26	120	582.3438			
Total	87724.5	179				

** Significance at 5%

* Not significant at 5%

Table. 19. **Analysis of variance on percentage gain in length of rohu in different treatments.**

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	P-value	F critical
Between treatments	328.6803	5	65.73606	0.755126*	0.598443	3.105875
Within treatments	1044.638	12	87.05316			
Total	1373.318	17				

* Not significant at 5%

4.6. Specific Growth Rate (%/Day).

The SGR values for different treatments are presented in Table. 20. Maximum SGR value is recorded for 20% CPM followed by 10%, 15%, control, 5% and 25% CPM treatments.

4.7. Survival.

Fish fed actively and appeared healthy in all treatments. The highest survival was recorded in 5% CPM treatment (93.33%) and all other treatments showed survival around 80% on termination.(Table. 21).

4.8. Feed assimilation (%).

Feed assimilation was maximum in control diet (89.34%) and minimum for 5% CPM (80.71%) diet (Table. 22). Data subjected to one-way analysis of variance showed significant difference between the treatments (Table. 23).

4.9. Feed conversion efficiency (%).

Highest FCE was observed in 10% CPM followed by 20%, 15%, control, 25% and 5% CPM dietary treatments (Table. 22).

4.10. Protein efficiency ratio.

PER values were highest in 10% CPM followed by 20%, 15%, 25%, control and 10% dietary treatments (Table. 22).

4.11. Apparent digestibility coefficient.

ADC which denotes the quality of protein is presented in Table.26. ADC values are found to be higher in 15% and 20% CPM diets and lowest in 25% CPM diets.

4.12. Statistical analysis.

Growth of rohu in terms of weight and length were subjected to two-way analysis of variance. Both the parameters showed significant difference between the treatments (P>0.05) (Table.14 and 15.).Percentage gain in length and weight data subjected to one-way analysis of variance showed that the treatments did not vary significantly (P>0.05) (Table.18 and 19.). Similarly the assimilation (%), FCE (%) and PER values did not vary significantly among the treatments (Table.23, 24 and 25).

Table.20. Specific Growth Rate (SGR) of rohu in different treatments over the experimental period.

Treatments	Days / Replications	0-14	15-28	29-42	43-56	57-70	Average SGR
Control	1	0.34	0.19	2.18	1.22	1.79	1.07 ±0.38
	2	1.61	0.09	0.36	1.41	1.83	
	3	0.87	1.47	0.32	0.79	1.62	
	Mean	**0.94**	**0.58**	**0.95**	**1.14**	**1.74**	
	±SD	**0.52**	**0.62**	**0.86**	**0.25**	**0.09**	
5% CPM	1	0.35	1.59	0.14	0.82	1.73	1.06 ±0.53
	2	0.76	0.59	1.37	0.71	2.78	
	3	0.83	0.34	0.90	1.24	1.81	
	Mean	**0.64**	**0.84**	**0.80**	**0.92**	**2.10**	
	±SD	**0.21**	**0.54**	**0.50**	**0.22**	**0.47**	
10% CPM	1	0.11	1.97	0.31	1.99	2.43	1.18 ±0.51
	2	0.40	1.12	1.11	0.55	2.43	
	3	0.54	0.05	2.40	0.60	2.13	
	Mean	**0.35**	**1.04**	**1.27**	**1.04**	**2.33**	
	±SD	**0.17**	**0.78**	**0.86**	**0.66**	**0.14**	
15% CPM	1	0.30	1.29	1.20	1.20	1.70	1.16 ±0.62
	2	0.44	1.50	1.46	0.19	1.29	
	3	1.04	0.18	0.85	2.21	0.12	
	Mean	**0.59**	**0.99**	**1.17**	**1.20**	**1.03**	
	±SD	**0.32**	**0.57**	**0.24**	**0.82**	**0.66**	
20% CPM	1	0.31	1.91	1.04	0.78	1.72	1.20 ±0.64
	2	0.75	1.34	0.26	2.19	1.89	
	3	0.19	1.60	1.05	0.97	1.76	
	Mean	**0.41**	**1.61**	**0.78**	**1.31**	**1.79**	
	±SD	**0.24**	**0.23**	**0.37**	**0.62**	**0.07**	
25% CPM	1	0.57	1.96	0.92	0.97	1.72	0.99 ± 0.22
	2	0.90	1.94	0.33	0.59	2.14	
	3	0.61	2.25	0.12	0.99	1.39	
	Mean	**0.69**	**2.05**	**0.45**	**0.85**	**1.75**	
	±SD	**0.14**	**0.14**	**0.33**	**0.18**	**0.30**	

Table. 21. Survival (%) of rohu in different dietary treatments

Treatment	Replications	Percentage survival
Control	1	90
	2	80
	3	80
	Mean	**83.33**
	±SD	**5.77**
5% CPM	1	80
	2	100
	3	100
	Mean	**93.33**
	±SD	**11.55**
10% CPM	1	80
	2	80
	3	80
	Mean	**80.00**
	±SD	**0.00**
15% CPM	1	90
	2	80
	3	80
	Mean	**83.33**
	±SD	**5.77**
20% CPM	1	80
	2	100
	3	70
	Mean	**83.33**
	±SD	**15.27**
25% CPM	1	80
	2	80
	3	80
	Mean	**80.00**
	±SD	**0.00**

Table.22 Assimilation (%), FCE (Feed Conversion Efficiency) (%) and PER (Protein Efficiency Ratio) of rohu fry in different treatments

Treatments	Replication	Assimilation(%)	FCE (%)	PER
Control	1	89.43	25.25	0.82
	2	89.31	22.88	0.76
	3	89.28	21.69	0.72
	Mean	**89.34**	**23.27**	**0.77**
	±SD	**±0.08**	**±1.81**	**±0.05**
5% CPM	1	80.68	17.83	0.58
	2	80.75	26.88	0.87
	3	80.71	20.58	0.67
	Mean	**80.71**	**21.76**	**0.71**
	±SD	**±0.03**	**±4.64**	**±0.15**
10% CPM	1	83.03	29.63	0.98
	2	83.15	26.81	0.88
	3	83.58	21.84	0.72
	Mean	**83.25**	**26.09**	**0.86**
	±SD	**±0.29**	**±3.94**	**±0.13**
15% CPM	1	83.32	25.18	0.84
	2	83.11	20.97	0.69
	3	83.76	25.93	0.87
	Mean	**83.39**	**24.03**	**0.80**
	±SD	**±0.33**	**±2.67**	**±0.09**
20% CPM	1	82.57	24.18	0.79
	2	82.39	24.38	0.82
	3	82.21	28.25	0.94
	Mean	**82.39**	**25.60**	**0.85**
	±SD	**±0.18**	**±2.29**	**±0.08**
25% CPM	1	82.36	23.43	0.78
	2	82.97	26.46	0.89
	3	82.65	17.17	0.58
	Mean	**82.66**	**22.35**	**0.75**
	±SD	**±0.30**	**±4.74**	**±0.16**

Table. 23. **Analysis of variance of feed assimilation by rohu in different treatments.**

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- value	P-value	F critical
Between treatments	131.3572	5	26.27145	482.4876**	2.14E-13	3.105875
Within treatments	0.6534	12	0.05445			
Total	132.0106	17				

** Significant at 5% level

Table. 24. **Analysis of variance on FCE (%) of rohu**.

Source of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F- value	P-value	F critical
Between treatments	45.19391	5	9.038782	0.720953*	0.620311	3.105875
Within treatments	150.4472	12	12.53727			
Total	195.6411	17				

* Not significant at 5% level

Table. 25. **Analysis of variance on PER of diets.**

Sources of Variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value	P-value	F critical
Between treatments	0.053044	5	0.010609	0.77563*	0.58557	3.105875
Within treatments	0.164133	12	0.013678			
Total	0.217178	17				

* Not significant at 5% level

Table. 26. Apparent digestibillity coefficient of different diets fed to rohu

Treatment	Replication	ADC
Control	1	32.89
	2	32.46
	3	32.77
	Mean	**32.70**
	±SD	**0.18**
5% CPM	1	31.73
	2	31.69
	3	31.81
	Mean	**31.74**
	±SD	**0.04**
10% CPM	1	32.2
	2	31.65
	3	32.13
	Mean	**31.99**
	±SD	**0.24**
15% CPM	1	34.71
	2	34.86
	3	33.62
	Mean	**34.39**
	±SD	**0.55**
20% CPM	1	34.05
	2	33.82
	3	35.15
	Mean	**34.34**
	±SD	**0.58**
25% CPM	1	29.15
	2	29.76
	3	29.08
	Mean	**29.33**
	±SD	**0.30**

Discussion

5. Discussion

Aiming at nutritional and economic benefits, there have been attempts, in recent times, to increase the use of under-utililised plant materials to replace conventional feed ingredients like groundnut oil cake and fishmeal, in livestock and fish production (Falaye *et al*., 1999). Emerging human food industries related to plants and fruits are generating increased amounts of by-products, that are largely unexplored as potential feed ingredients. In selecting an alternate feed ingredient the most important criterion is its availability. The feed ingredient selected in the present study is a by-product of cocoa, an economic cash crop in many parts of Kerala. Cocoa pod husk, a by-product of chocolate manufacturing industry, is available in bulk throughout the year. Moreover, in India, very few studies have been conducted utilising locally available feed ingredients in carps (Jayaram and Shetty, 1980; Nandeesha *et al*., 1991; Ray and Das, 1994; Mukhopadhyay and Ray, 1997).

There are reports on the use of cocoa pod husk as an energy source in pig and cow rations. The potential of cocoa pod husk as a feed ingredient has been tested in tilapia (*Oreochromis niloticus*) and catfish (*Clarias isheriensis*) (Fagbenro, 1992; Falaye et al., 1999). Defatted cocoa cake, a by-product of cocoa bean fermentation process was evaluated as a direct feed in *Tilapia guineensis* (Fagbenro, 1988b). These trials formed the basis for the present study on Indian major carp, *Labeo rohita*. A 70 day study was conducted with five cocoa pod husk based diets and a control diet based on fishmeal. The results of the experiment are discussed in the following pages.

5.1. Proximate composition of feed ingredients.

No single feed stuff can supply all the nutrients and the energy required for optimum growth of fish (Lovell, 1989). Hence, commercial fish feeds are comprised of a mixture of feed stuffs and vitamin and mineral premixes that provide adequate amounts of essential nutrients as well as the energy necessary for their utilisation. In the present study, fishmeal and groundnut oil cake served as the protein sources and wheat bran, tapioca flour and cocoa pod husk as energy supplements. Since cocoa pod husk meal has low protein content (7.35%) (Table. 2) it cannot be considered as a protein source because

those sources having protein content above 20% are considered as protein sources (Lovell, 1989).

Although protein is used as an energy source for fish, a number of studies have pointed out the importance of using less expensive energy, in the form of lipid and carbohydrate, in order to save protein. Carbohydrate not only supplies the necessary energy but also has protein sparing effect in fish diet (Shimeno *et al*., 1981; Furuichi and Yone, 1982; Lovell, 1989). In addition, research is directed at investigating the use of plant products and other materials as an energy source in preference to proteins (Wee and Ng, 1986).

Cocoa pod husk is a very good source of carbobydrate (42.35%, Table.2). Although some information is available on the effects of dietary carbohydrate level in Indian major carps (Sen *et al*., 1978; Erfanullah and Jafri, 1993), the utilisation of different carbohydrates by these fish has not been investigated (Erfanullah and Jafri, 1995). Common carp is found to utilise complex polysaccharides more efficiently than simple sugars (Furuichi and Yone, 1982; Shimeno *et al.*, 1981).

The species selected for the present study, rohu, is a herbivore and a column feeder and prefers to feed on plant matter, including decaying vegetation (Jhingran, 1991). It can accept plant protein and mixed diets with rich carbohydrate (Haniffa *et al*., 1987). Relatively high levels of amylase, cellulase, protease and lipase activity have been reported in the gut and hepatopancreas of Indian major carps (Kawai and Ikeda, 1971; Das and Tripathi, 1991; Erfanullah and Jafri, 1998).

Traditionally, fish meal has been utilised as a main component in balanced diets for fish. However, its high cost and sometimes poor quality have limited its potential for use in commercial fish diets (Martinez *et al*., 1988). In the absence of a single fish meal analogue, it is important to formulate diets based on a mixture of such ingredients which can collectively replace dietary fish meal to an extent and serve as a good source of dietary protein and energy (Gomes *et al.,* 1993). In the present investigation, diets are formulated with a combination of ingredients like cocoa pod husk, groundnut oil cake, wheat bran and tapioca flour to replace the fishmeal content as much as possible.

All the ingredients used were of desirable quality and the results of proximate composition analysis (Table.2) were comparable to that given by Lovell (1989).

5.2. Proximate composition of formulated diets.

Diets were designed with a view to develop isonitrogenous feeds with 30% crude protein. The crude protein content was fixed at 30% as specified by FAO (1983) as the minimum protein(%) requirement of Indian major carp fry and fingerlings. This is in close agreement with the optimum protein requirement worked out by Renukaradhya and Varghese (1986) for Indian major carps. Studies by Jeyachandran and Paulraj (1976) reported that protein levels higher than 12-15% give excellent growth in Indian major carps. In contradiction to this Sen *et al*. (1978) stated that the protein requirement of spawn and fry range from 35 to 45%. However, the protein requirement decreases with age.

All diets were isonitrogenous (29.93±0.36% crude protein content) and isocaloric (397.84±9.22KJ/100g) (Table.3). The dietary carbohydrate levels are above 30% in all the diets. Work done by Erfanullah and Jafri (1998) reveals that Indian major carps thrive well on diets containing relatively higher levels of dietary carbohydrate (more than 36%). This is consistent with the results of an earlier study which reported increased fish growth of *Labeo rohita* fingerlings fed increased carbohydrate (35%) (Erfanullah and Jafri, 1995).

In carps, high levels of rice and cassava in diet (40%) promoted protein digestion (Ufodike and Matty, 1983). However, the energy digestibility of various carbohydrate sources in Indian major carps seems to be more related to the source, complexity and degree of hydrolysis through starch gelatinization of carbohydrate. This is because nitrogen-free extract contains both highly digestible carbohydrates as well as large amounts of indigestible lignin (Erfanullah and Jafri, 1998).

The lipid content of diets was above 4% (Table.3). According to FAO (1983) the minimum lipid requirement of carp fry is 8%. But on the basis of weight gain, feed conversion, nutrient retention, and body composition, diets containing 27-36% carbohydrate, 4-8% lipid, carbohydrate to lipid ratios of 3.39-8.93 were optimal for *Catla*

catla, L. rohita and *C. mrigala* (Erfanullah and Jafri, 1995). Lipids are found to have more protein sparing effect than carbohydrates. The findings relating to carps, tilapia and catfish shows that they can utilise carbohydrate effectively as an energy source than dietary lipid (Ogino *et al*., 1976, Sethuramanlingam and Haniffa, 2001b).

Fibre content of different diets in the study ranged from 6 – 15% (Table. 3). Lovell (1989) found no benefit in increasing fibre in practical catfish diets above the basal content of 2.8% and that increasing fibre beyond 14% reduced growth rate, probably by diluting nutrients in the diet. The fibre fraction of the feed stuffs contain compounds such as cellulose, pentosan and hemicellulose, which are reportedly indigestible in carp (Kirchgessner *et al*., 1986; Ding, 1991).In the present study lowest growth rate at 25% CPM diet (Table.16) can be attributed to the presence of a high fibre content (15.42%). The moisture content of the diets were less than 10% (Table.3) and in accordance with dry pellet specifications given by Lovell (1989).

5.3. Water stability of pelleted feeds.

Carps being slow feeders require feeds which remain stable in water without much disintegration, for atleast one hour (Das *et al*., 1994). Therefore, water stability is an important criterion in assessing its efficiency. The results of the water stability test of diets revealed that 10% CPM diet has higher water stability and control diet has the least (Table. 5). The stability of pellets is influenced by different factors like feed composition, nature of ingredients, type of processing and moisture content (Hastings, 1976). The best water stability of 10% CPM diet can be attributed to the high fat content in the diet. Pellets of higher fat content retained compactness for longer period as fat prevents water penetration (Jayaram and Shetty, 1981, Jayadev and Vishwanath, 2000). Inspite of higher fat content, control diet showed least water stability. This can be caused due to the poor gelatinization during steam conditioning. The degree of stability of a diet is also dependent on the extent of gelatinisation during steam pelleting. Among the cocoa pod husk meal diets, water stability was found to decrease with the increase in inclusion levels of cocoa pod husk meal. The high percentage of crude fibre content in these feeds must have resulted in poor gelatinisation. Similar findings have been reported by Das *et al*., 1994 in diets containing leaf powders of *Eichhornia, Colocasia* and *Gliricidia.*

5.4. Water quality parameters.

The values of different hydrobiological parameters did not show any distinct trend between the treatments as also at different samplings. The recorded values were: water temperature 22-27ºC; pH 6.5-7.5; dissolved oxygen 3.78-5.28 mg/l; total alkalinity 67.24-126.91 mg/l and ammonia 0.021-0.084 ppm, with the water quality parameters being within optimum ranges throughout the culture period (Table. 7-11).

Since the water was filtered through a close meshed bolting cloth before filling the tanks plankton content was meager. Moreover, excess feeds and faecal matter were removed daily by siphoning three-fourth of water out and refilling with filtered freshwater. During sampling, tanks were scrubbed and thoroughly washed to avoid any plankton growth.

5.4.1. Temperature

The range of temperature (18.6 – 30.7ºC) is favourable for Indian major carp growth (Jhingran, 1991). Hence the observed values of temperature were within the desirable range.

5.4.2. pH.

pH in a range of 7.1 -7.7 is most suitable for fish production (Boyd, 1982). The observed range of pH (6.5-7.5) did not vary much from this range.

5.4.3. Dissolved oxygen.

Dissolved oxygen concentration (3.78 – 5.28 mg/l) in various treatments was also within the suitable range for carp growth (2.4 – 14.0) (Parameswaran *et al*., 1971). Carps are capable of tolerating low oxygen (up to 3.0 mg/l) eventhough about 6 mg/l of dissolved oxygen is required for better growth (Huet, 1972). Jena *et al*. (1998) opined that rohu and catla being surface dwellers were more sensitive to oxygen depletion.

5.4.4. Total Alkalinity.

Total alkalinity is a measure of productivity and in higher productive waters, the alkalinity ought to be over 100 mg/l (Alikunhi, 1957; Jhingran, 1991). The range of total alkalinity permissible for fish ponds ranges from 20-200mg/l (Huet, 1972). The observed range of alkalinity is therefore within the permissible range (Table.10).

5.4.5. Ammonia –nitrogen value.

Ammonia should be maintained below 0.1 ppm in fish ponds (Huet, 1972). The observed values however, fall under this range (Table.11).

Hence it can be concluded that addition of cocoa pod meal in diets did not alter the water quality. The difference in the growth performance of fish reported in this investigation can therefore not be attributed to poor water quality.

5.5. Fish growth.

One of the most common difficulties observed when alternative sources of feed stuffs are used in fish diets is acceptability by fish, which is evidently related to palatability as reported by Rodriguez *et al*. (1996). In this study, the acceptance of the experimental diets was low during the first week, perhaps due to the flavour, but the problem disappeared after the fish adapted to the taste of the experimental diets. Feed enhancers were not added to the diets in the present study since this work is the first one of its kind in Indian major carps.

The results of the present study showed that cocoa pod husk meal inclusion in rohu diets at a 20% level seems to have no deleterious effects on the growth. The growth rates of rohu were 0.21 g, 0.22 g, 0.22 g, 0.23 g, 0.24 g and 0.20 g/day with control, 5% CPM, 10% CPM, 15% CPM, 20% CPM and 25% CPM respectively. The lower growth rates in the present study may be due to small size of the FRP tanks and short experimental duration. Similar growth rates were reported when vermicompost was used as organic manure for fish production (Deolalikar and Mitra, 2004).

The superiority of 20% cocoa pod husk diets over 25% cocoa pod husk diets with regard to fish weight gain, growth performance and yield was attributed to the relatively low fibre content of 20% cocoa pod husk diets. Work on the use of cocoa pod meal in fish feeds has been limited to a few species *viz*; *Clarias isheriensis* and *Oreochromis niloticus* where 10% inclusion level is found to give best growth performance (Fagbenro, 1992; Falaye *et al*., 1999). The level of tolerance appears to vary between species. Chinook salmon and coho salmon have been reported to tolerate diets containing up to 34 and 22% cottonseed meal, respectively (Fowler, 1980) where as in *Labeo rohita* 6.5% level of cotton seed meal, containing 0.036% gossypol significantly affected the growth and conversion efficiencies (Usmani *et al*., 1997).

5.6. Specific growth rate.

Despite the inferior growth produced by the high level (25%) cocoa husk diets as compared to control, the other diets with lower levels of cocoa husk compared favourably with latter in terms of weight gain, SGR and FCE, with no significant differences ($P>0.05$). Average SGR values obtained in the present study were comparable to that obtained in studies with mango seeds and green gram (Omoregie, 2001; DeSilva and Gunasekhara, 1989).

5.7. Protein efficiency ratio.

The PER values attained were lower in all the diets due to reduced growth rate. These values were lower than those reported by Falaye et *al*. (1999) when cocoa pod husk diets were fed to *Oreochromis niloticus* or by Omoregie (2001) when mango seed and palm kernel meal was fed to *Labeo senegalensis*. But results are comparable to those obtained when Fagbenro (1992) fed cocoa husk diets to *Clarias isheriensis* or when cotton seed meals were tried in *Labeo rohita* fry diets (Usmani *et al*., 1997).

5.8. Apparent digestibility co-efficient.

It is generally recognised that digestibility data are useful only when ingredients do not contain any anti-nutritional factors like gossypol, tannins, complex polysaccharides, anti-trypsin and other interfering substances which influence the digestibility of various nutrients in the diet and give erroneous results (Lall, 1991). In the

present study ADC values of 15% and 20% cocoa pod husk diets were superior to other diets. However, these values were much lower compared to those obtained in *Clarias isheriensis* fed cocoa husk diets (Fagbenro, 1992). One of the possible explanations for poor digestibility may be due to high fibre content in the diets. Lovell (1989) reported that at higher inclusion levels of fibrous feedstuffs, excessive dietary fibre causes the dilution of nutrients, especially proteins, which is primarily used for fish growth. Fibre content interfering with protein digestibility has been reported by Erfanullah and Jafri (1998) when Bengal gram dust, soybean husk and rice bran were included in the diets of Indian major carps. Omoregie (2001) reported poor protein digestibility of mango seed and palm kernel meal in *Labeo senegalensis* due to high fibre content in diets.

5.9. Biochemical composition of rohu muscle.

During the present study, a decrease in muscle protein and fat content was noticed in rohu fingerlings fed higher levels of cocoa pod husk meal.Similar trends in muscle protein and fat levels were reported in *Oreochromis niloticus* (Keembiyehetty and Desilva, 1993), rainbow trout (Gomes *et al*., 1993) fed higher levels of cowpea and black gram meal, and pea and rapeseed meals, respectively. Saha and Ray (1998a) also reported a same trend when chuni (low cost cattle fodder) was included at higher levels (above 20%) in the diets of *Labeo rohita* fingerlings. DeSilva and Gunasekhara (1989) reported a decrease in muscle lipid, and an increase in the moisture and ash content of *O. niloticus* fed diets containing higher levels of green gram. Moisture and lipid level in fish muscle appeared to be inversely related during the present investigation, which is in close agreement with the findings of the previous workers (Ray and Das, 1992; Saha and Ray, 1998a).

In conclusion, results obtained from this investigation showed that for the levels studied, 20% cocoa pod husk meal can be incorporated into the diets of *Labeo rohita* without significant depression in growth and deleterious effects on health of the fish. The depressed growth rates at higher inclusion levels can be due to high fibre content or alkaloid theobromine content in cocoa products. There is paucity of information on effects of theobromine on fish physiology but toxic levels of theobromine have been defined for other animals. Although the present results provide little evidence of toxicity

of dietary cocoa pod husk on *L. rohita,* further toxicological studies on possible long-term effects of theobromine on fish are warranted.

Summary

6. Summary

The present study was taken up to elucidate the utility of cocoa pod husk meal as a feed ingredient for *Labeo rohita* fingerlings. The methodology, results and the conclusions of the study are as follows.

1. The diets were formultated according to the methods of Olvera *et al*., (1990) with an overall protein content of 30%. A fishmeal based diet devoid of cocoa pod husk meal served as the control. The test diets contained cocoa pod meal incorporated at 5%, 10%, 15%, 20%, 25% levels of inclusion.
2. Fry of rohu were fed with experimental diets at 5% level for 70 days.
3. Prepared feeds were analysed for proximate composition and water stability. Protein was 30% on an average, while fat content ranged from 4.46% (20% CPM) to 4.84% (control) in diets.
4. The feeds were found to be of good nutritional quality at the end of four months of storage.
5. Water quality parameters recorded were within the range favourable for fish growth. Plankton production was meagre in the tanks.
6. Fish fed 20% CPM diet recorded the highest growth of rohu followed by 15%, 10%, 5%, control and 25% respectively.
7. Statistical analysis of growth data employing ANOVA revealed the existence of significant difference between different treatments at 5% level of significance.
8. The overall survival was high above 80% in all the dietary treatments.
9. Fish fed 15% and 20% CPM diets recorded highest FCE and PER values respectively.
10. Apparent protein digestibility was high in 15% and 20% CPM diets with no significant differences among the dietary treatments.

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UTILISATION OF COCOA POD HUSK AS A FEED INGREDIENT FOR *LABEO ROHITA* (HAMILTON) FINGERLINGS

By

R. NAVYA

THESIS

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Faculty of Fisheries

Kerala Agricultural University

DEPARTMENT OF AQUACULTURE

COLLEGE OF FISHERIES

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Abstract

A 70 day study was undertaken to evaluate the use of cocoa-pod husk meal as a dietary ingredient for rohu, *Labeo rohita* (Hamilton) fingerlings. Six iso-nitrogenous diets (30% crude protein) were formulated. The five test diets had cocoa pod husk meal incorporated at 5, 10, 15, 20 and 25% inclusion levels. A fish meal-based diet devoid of cocoa pod husk meal served as the control. Feeding was done at 5% once daily. The test diets were acceptable to the fish and gave positive feed consumption and growth. Optimum performance in terms of weight gain was achieved by fish fed a diet with 20% inclusion level of cocoa pod husk meal. However, FCE, fish weight gain and SGR showed a decline in fish fed the test diet, particularly so in the 25% cocoa pod husk meal diet. The reduced fish growth was caused by the high fibre content of cocoa husk which resulted in low protein digestibilities during the study. No direct toxic effect of theobromine and other purine alkaloids in cocoa products were reflected in the study, cocoa pod husk can thus be safely recommended as a feed ingredient in supplementary diets for *Labeo rohita,* up to an inclusion level of 20%.