COMPARATIVE EVALUATION OF
DIFFERENT FEED INGREDIENTS FOR STINGING CATFISH
HETEROPNEUSTES FOSSILIS (BLOCH)

By

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THESIS

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Dedicated To,

My sir,

Dr. C. M. Nair

&

My loving brother……

DECLARATION

I hereby declare that this thesis entitled “**Comparative evaluation of different feed ingredients for Stinging catfish, *Heteropneustes fossilis* (Bloch)**” is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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Certified that this thesis entitled "**Comparative evaluation of different feed ingredients for Stinging catfish,** *Heteropneustes fossilis* **(Bloch)"** is a record of research work done independently by Mr. Kumar Katya under my guidance and supervision and that it has not previously formed the basis for award of any degree, fellowship or associateship to him.

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Introduction

1. INTRODUCTION

Freshwater aquaculture in India is predominantly carp oriented, and many of the endemic Indian inland freshwater fishes fall outside the purview of the ongoing freshwater farming system. However taking into account the preference for carps, farmers are in search of hardy species, such as catfishes that can tolerate low oxygen conditions and adverse environment particularly in derelict waters. Many of these fish are nutritionally and medically important, thanks to their flesh quality with many unique characters.

The Indian subcontinent harbours 142 species of catfishes belonging to 43 genera. Among them the endemic Indian catfish *Heteropneustes fossilis* is of prime value. The excellent flesh quality, high protein content, physiologically available iron, essential amino acid and low fat content make *H.fossilis* commercially and nutritionally important (Alok *et al*., 1993; Ayyappan *et al*., 2001).Because of high hemoglobin content, sometimes doctors suggest the patients of anemia to have large amount of *H.fossilis* in their food menu (Bhatt,1968). Fewer intramuscular bones, tender flesh with delicious mild taste and ability to be marketed in live condition, make this species highly priced and demanded freshwater fish.

Fish farmers in India tend to prefer the exotic African catfish (*Clarias gariepinus*) compared to native catfish due to better availability of seed, their wider feeding spectrum, cheap dietary requirements, fast growth and short culture period. However, these exotic catfishes pose a heavy threat to native fish biodiversity and hence the Government of India put a ban on its culture, although farmers are still producing them due to favorable short term profits. It has become imperative to promote native catfish culture among fish farmers as an alternative to exotic fish culture for income generation and ultimately to conserve fish biodiversity.

Heteropneustes fossilis (Bloch) which used to belong to the family Heteropneustidae, very recently has been shifted to the Claridae family (Diogo, Chardon and Vandewalle, 2003). Commonly known as stinging catfish, Indian cat fish, Singhi, known as Kari in Malayalam, widely distributed in Indian subcontinent and Southeast Asian region. The range of this species encompasses India, Thailand, Bangladesh, Pakistan, Nepal, Sri Lanka, Myanmar, Indonesia, and Cambodia (Smith 1945; Burgess, 1989). In India it inhabits West Bengal, U.P, M.P, Bihar, Punjab, Haryana, Orissa, Assam, Gujarat, A.P, Tamil Nadu, Karnataka and Kerala.

Primary habitat of *Heteropneustes fossilis* includes ponds, ditches, swamps, and marshes. It can tolerate slightly brackish water, and can adapts well to hypoxic water bodies and to high stocking densities (Dehadrai *et al*., 1985). Singhi culture has gained attention because of its omnivorous feeding habit and ability to efficiently utilize feed stuffs of animal and plant origin and to withstand high ammonia and adverse environmental conditions. Maximum size of 38 cm has been recorded from wild, while in culture *H. fossilis* attain marketable size in 6 months. Singhi can also be cultured with other species but it is best suited for monoculture and an average production of 4510 kg /ha has been recorded.

However, commercial hatchery production of the seed of Singhi has not been perfected and farming is mostly dependent upon natural seed. One of the most significant limiting factors restricting the expansion of *Heteropneustes fossilis* culture is the high cost of trash fish used as feed for its culture. Trash fish supply is irregular and unreliable and now experiencing very intense competition from the fish meal industry. The feeding technique used for this species need to be revised. The trash fish has to be replaced by some equally good but inexpensive feed. Only limited nutrient requirement data are available for this species. Information on the basic nutritional requirements and feeding of *H. fossilis* is needed in view of the emphasis on catfish culture in the country (Tripathy and Das, 1976; Dehadri and Thakur, 1980). It has been reported that inclusion of dietary protein in the range of 40–43% is optimum for the growth of young *H. fossilis* (Siddiqui and Khan, 2009). Level of dietary protein requirement is of fundamental importance because it is the most important factor affecting growth performance of fish and the feed cost (Lovell, 1989; Luo *et al*., 2004), as the feed cost is obviously the largest operational cost.

The formulation of cheap diets is very important to the commercial culture of fish and shrimp. This can be achieved by utilizing and experimenting upon a variety of locally available low priced materials (Usha Goswami and Goswami, 1982). Among different feed ingredients clam meat, (Protein content varying from 38-56 %, depending upon its species), is a favourite with excellent feed attractant and growth promoting properties. Fish meal is one of the most common ingredients used in commercial fish and shrimp feed. It is well known for high protein content ranging from 60 to 80 %, is a rich source of energy and minerals and unidentified growth factor. The high cost of fish meal making it increasingly difficult to produce aqua feeds based on fish meal as the sole source of protein, and the growing environmental concerns over the indiscriminate exploitation of fish from the sea for its production, have all necessitated to investigate on alternate sources of protein in supplementary feeds meant for aquaculture. Among the ingredients being investigated as alternatives to fish meal, the products derived from soybeans (*Glycine max* L.) are some of the most promising (Lim *et al*., 1998; Hardy, 1999; Storebakken *et al*., 2000; Swick, 2002) because of the secure supply, low cost and favourable protein/amino acid composition. It has one of the best amino acid profiles of all protein rich plant feed stuffs, meeting the essential amino acid requirement of fish (N.R.C. 1983).

Therefore the present study aims at comparative evaluation of three protein source viz. clam meat, fish meal and soybean meal for the survival and growth of *Heteropneustes fossilis* fingerlings with a view to reduce the feeding costs, thereby making its culture an economic enterprise.

Review of Literature

2. REVIEW OF LITERATURE

The nutrient requirements of fishes are same as those of higher animals, as far as quality is concerned. Quantitatively, however different levels are being used to optimize production of fish subject to variation in stocking density, age and weight, water quality parameter and management practice (Hasting, 1979). Even though, artificial feeding of warm water fishes become very popular in recent years, studies on their nutritional requirement is limited compared to those of cold water fishes. Specifically the literature on catfish culture is limited and outdated and the promise of catfish culture has yet to be fulfilled. The catfish industry needs considerable research and developments input to make it popular (Haniffa, 2004). Information on the basic nutritional requirements and feeding of *H. fossilis* is needed in view of emphasis on catfish culture in the country (Tripathi and Das, 1976; Dehadrai and Thakur, 1980). Any balanced formula for fish diets must include an energy source plus sufficient indispensable amino acids, essential fatty acids, specified vitamins and minerals (Halver, 1976). Food ration level is also reported to influence fish growth, feed conversion and chemical composition of a fish (Huisaman, 1976; Reddy and Katre, 1979; Reinitz, 1983).

2.1. NUTRITIONAL REQUIREMENT OF WARM WATER FISHES WITH SPECIAL REFERENCE TO CATFISHES

2.1.1. Protein

The capacity of fish to synthesize Amino acid *de novo* from carbon skeleton is limited and most of the protein must therefore be supplied through diet (Hepher, 1988). Fish have high dietary protein requirement (Deng *et al.,* 2006). The optimum dietary level of protein required for maximum growth of farmed fishes is 50-300 % higher than that of terrestrial animals (Cowey, 1979).

The intensification of fish culture has led to dependence on artificial feeds. Protein is the most expensive component in fish feeds and also the most important factor affecting growth performance of fish and feed cost (Lovell, 1989; Luo *et* *al*., 2004). Reducing the feeding costs could be the key factor for successful development of aquaculture.

The significance of qualitative and quantitative feeds is well recognized (Jauncey, 1982; Mohanty and Samantary, 1996; Gunasekera *et. al.,* 2000; Yang *et al.,* 2002; Giri *et al.,* 2003; Deepak and Garg 2003; Yang *et. al.,* 2003; Sales *et al.,*2003; Kalla *et al.,* 2004; Islam and Tanaka, 2004; Luo *et al*., 2004; Cortes-Jacinto *et al.,* 2005; Kim and Lee, 2005; Tibbetts *et al.,* 2005; Sa *et al.,* 2006). Level of dietary protein is of fundamental importance, because it significantly influences growth, survival, and yield of fish as well as economics of a farming industry by determining the feed cost which is obviously the largest operational cost. Increase in dietary protein has often been associated with higher growth rate in many species. However, there is a certain level beyond which further growth is not supported, and may even decrease (Mohanty and Samantary, 1996; Shiau and Lan, 1996; McGoogan and Gatlin, 1999; Gunasekera *et al.,* 2000; Kim and Lall, 2001; Yang *et al*., 2002; Abbas *et al.,* 2005; Debnath *et al.,* 2007; Kvale *et al*., 2007).Considerable research effort has been expended to determine the quantity and quality of dietary protein necessary to achieve optimum performance of fish.

The protein requirement of *Heteropneustes fossilis* is 27.73-35.43 % (Akand *et al.,* 1989). Siddiqui and Khan (2009) conducted an experiment to assess the effect of dietary protein on growth, feed utilization, protein retention efficiency and body composition of young *Heteropneustes fossilis* with diets of varying levels of protein (25, 30, 35, 40, 45, and 50% of the diet) and concluded that inclusion of dietary protein in the range of 40–43% is optimum for the growth of this fish. A significant fall in growth and conversion efficiencies was noted at 50% protein of the diet indicating that 40% protein diet satisfied the requirement and is considered optimum for achieving maximum growth and excellent conversion efficiency. A similar trend has been observed in many other fish species irrespective of culture strategies (Jauncey, 1982; Cho *et al.,* 1985; Khan and Jafri, 1991; Vergara *et al.,* 1996; Bai *et al.,* 1999; Ng *et al*., 2001; Kim *et al.,* 2002; Kim and Lee, 2005; Wang *et al.,* 2006). The decline in growth performance at protein level above 40%, authors attributed to the fact that the fish body cannot utilize dietary protein once the optimum level has been reached (Phillips, 1972). The growth depressing affect of feeding fish with levels of protein higher than the requirement, is also evident in carps (Sen.*et al.,* 1978, Lim *et al.,* 1979; Singh *et al.*, 1980; Khan and Jafri., 1991). These observations can be linked to a possible reduction in dietary energy available for normal growth due to extra energy expenditure diffuse towards deamination and excretion of excessive amount of amino acid (Khan and Jafri,1991).

Chakraborty (1981) studied the growth performance of catfish *Heteropneustes fossilis* feeds prepared from such indigenous materials as frog meal, mustard cake meal, rice bran, aroid leaves and table salt and concluded that good feed must contain 40% or more of good quality protein.

Protein requirement of warm water fishes have been studied in detail (Ogino and Satio,1970; Sin,1975; Dabroski,1977; Sen *et al*.,1978; Takeuchi *et al.,*1979; Singh and Sinha,1981; Millikin 1982; Wilson,1985; Wilson and Halver,1986; Singh and Bhanot.1988; Mohanty *et al.,*1988; Khan and Jafri,1981; Pongmanural and Watanable,1991; 1993;Hassan *et al.,* 1955).

Trieu *et al,* 2002 conducted an experiment to observe the effect of different protein levels on the growth and survival of snakehead (*Channa striatus*) and concluded that formulated feed with 50% of protein level is ideal for the best growth and survival. Pandian and Vivekanandan (1976) suggested that feed application of greater than 5% of body weight for juvenile murrel could even reduce the growth, apparently due to increased surfacing and swimming activities. According to Chuapoehuk (1987) the protein requirement of walking catfish (*Clarias batrachus)* is 30%.

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

Review on the protein requirement of fishes made by Wilson and Halver (1986), emphasized that dietary protein requirement of fish is influenced by protein to energy ratio, the amino acid composition and digestibility of the protein, and the amount of non-protein source in the diet.

The level of protein for best growth rates and food conversion efficiency depends on environmental condition, age, size and genetic factors. The protein requirement of the fish decreases with increase in size and age in several warm water fish species (Page and Andrew, 1973; Barlin and Haller, 1982; Renukardhya and Varghese, 1986; Khan and Jafri, 1991).

The utilization of protein is also influenced by calorific content of the diet and growth occurs only if the rations contain energy in proper ratio (Garling and Wilson, 1976; Cowey and Sergeant, 1977). In catla the relationship between energy to protein is calculated as 8 Kcal/g protein (Singh and Bhanot, 1981).When isocalorific diets were compared in *C. mrigala* (Hassan *et al.,* 1995), better growth was recorded with increased protein levels.

Brown (1978) recommended cheap complete catfish feeds for various weight categories. According to him better feed efficiency may be obtained from a well-balanced diet containing 24% protein than from a poorly balanced diet containing 36% protein. Fed free choice and balanced in amino acids and energy, 25–30% protein is adequate for larger fish; Fingerlings respond better to higher protein levels of 30 to 36%.

Murai *et al*., 1981 and Kaushik and Dabrowski (1983) found that in carp, protein utilization was better when a diet containing whole protein was used than a mixture of amino acid. Dabrowski (1982) observed significant absorption of protein macromolecules (peptides) all along the digestive track of common carp. Mc lean and Donaldson (1990) reported that absorption of macromolecules was several times higher in stomachless carp than in fish having a functional stomach.

Digestiblity of protein from normally used practical or purified ingredients of diets is high in all fishes (Kirchgessner *et al.,* 1986, Pongmanurat and watanable, 1993).

2.1.2. Essential amino acids

Fish like other animal do not have a true protein requirement but has a requirement for a balanced mixture of essential and non essential amino acids. Essential amino acid requirement by fish has been reviewed by Wilson (1985, 1989). The fish species so far studied have been found to be supplemented with following essential amino acids viz, arginine, histidine, leucine, isoleucine, lysine, metheionine, phenylamine, threonine, tryptophan and valine. Khan and Jafri (1993) are of opinion that it is the amino acid profile of the diet which determines the biological value of its protein.

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

Borloangan and Benitez (1990) stated that the more closely the essential amino acids profile of the dietary protein resemble the amino acids requirement of the fish, the higher the nutritional value of the protein. Thus in practical feed formulation, knowledge of the quantitative amino acid requirement of the fish is of utmost importance.

Ahmed (2007) conducted an experiment to evaluate threonine requirement of fingerling Indian catfish, *Heteropneustes fossilis* using isonitrogenous and isoenergetic diets at 40% crude protein and recommended that, the diet for fingerling *H. fossilis* should contain threonine at a level of 1.27 g per 100 g of dry diet, corresponding to 3.17 g per 100 g of dietary protein for optimum growth and efficient feed utilization. Hastings (1979) recommended 0 .56% of methionine in the diet of channel cat fish, and cystine can be used to replace with methionine up to the level of 60%. According to Dupree (1971) the lysine requirement for channel catfish under experimental condition was between 1.25 and 1.75% of the dry diet.

According to Fagbenro and Nwanna (1999) the tryptophan requirement of African catfish, *Clarias gariepinus* is 11 g/kg of dietary protein. Catfish fed diets containing tryptophan below 11 g/kg protein showed reduced growth response and feed utilization. Authors observed anorexia after 10 days in catfish fed diets containing 3 or 5 g tryptophan/kg protein, and slight curvature of the vertebral column was noted in catfish fed tryptophan deficient diets (3, 5, 7 g/kg protein).

Andrew (1978) suggest that, as in the case with other monogastric animals, the amino acid contents as well as protein levels have to be considered when dietary protein sources are interchanged in catfish feed formulations. Ravi and Devraj (1991) and Khan and Jafri (1993) have noticed proper growth when fed with amino acids free diets in common carp, catla and rohu respectively.

Comparison of data on the whole body EAA profile with that of the requirement profile of common carp and catla shows that these two pattern are correlated (Mambrine and Kaushik, 1993). Good correlations between dietary amino acid concentration after a meal have been observed in the carp (Zeitler,1984; Blasco *et al.,*1991).

Murai *et al.,*1984 found that when carp were fed a diet with amino acid and the sole nitrogen source, urinary excretion of amino acid was increased, representing almost 36% of total nitrogen excreted in the urine.

Increasing the feeding frequency significantly improves the time course availability of amino acids, leading to growth rates comparable to those obtained with a whole protein diet (Yamada *et al.,* 1981).

Studies also indicate that adequate treatments (protein by coating with casein or agar, pH adjustment etc.) of synthetic amino acid incorporated into diets improve amino acid utilization in common carp and several other fishes (Murai *et* *al.,* 1981; 1982; 1983). Thus essential amino acid supplementation can be efficient means of improving the essential amino acid balance or for the estimation of essential amino acid requirements.

Studies by Ravi and Devraj (1981) on the essential amino acid needs of *Catla catla* showed that it is very similar to the values recommended for common carp (Ogino, 1980). A relatively constant essential amino acid requirement profile of Indian major carp was reported by Mohanty and kaushik (1991).

In early stages the relative composition of essential amino acids profile showed little variation and the prominent amino acid detected were leucine, lysine, valine, isolucine, phenylalamine and serine (Ronnestad, 1992). The identical profile may have resulted from the hydrolysis of a common yolk protein-phosvitin-corresponding to water uptake during swelling. arginine, lysine, metheionine, tryptophan are indispensable amino acid limiting in many of the plant origin feed stuffs, generally used in fish diets (NAS-NRC 1983, Tacon and Jackson, 1985). Khan and Jafri (1993) estimated the optimum requirements of arginine, methionine, tryptophan and lysine 1%.0.2%, 0.2% and 2% respectively in *Labeo rohita* fry. Shrivandan and Varghese (1996) reported that optimum requirement of threonine for rohu as 1.7% of the dry diet which cross ponds to 4.18% of diet protein.

2.1.3. Lipids

Lipid is a major source of metabolic energy in fish. Being highly digestible, it has greater sparing action than dietary carbohydrates on protein (Ellis and Reigh, 1991). Besides playing a definitive role in feed utilization, dietary lipid level is also a dominant factor in determining the quality of the fish, it is important that appropriate amount of lipid is incorporated in the fish diet.

Dietary Lipid besides providing energy, serves as source of essential amino acid. Watanabe (1982) reviewed the role of lipid in fish nutrition pointing out the need for essential amino acid. Several studies on the influence of dietary lipids on growth and fatty acid composition of teleost fishes are available (Yingst and Stickney, 1979; Borologan and Parazo, 1991).

Lipids are almost completely digestible by fish and is even favoured over carbohydrate as an energy source (Cowey and Sargent,1977; Cho *et al.,*1985; Mukhopadhyay and Rout, 1966).Two main functions of lipid in fish body are energy provision and biochemical activity.

Andrews (1971) in his paper indicated that environmental temperature influences the level of fat in catfish; it is evident that other factors such as dietary protein and energy will also influence body composition.

Desilva *et al.,* 1991 reported no growth in fish fed a diet beyond a particular optimum protein/fat ratio. Jafri *et al.,* 1995 observed a reduction in growth in *Cirrihinus mrigala* when fed with higher levels of lipids than the optimum. Inclusion of 7 % of lipid as an energy source in 40% crude protein feed was found to be optimal for the growth of *H. fossilis* (Anwar and Jafri, 1995).

2.1.4. Essential fatty acids

Many studies reported the requirement of n-3 polyunsaturated fatty acids (PUFA) for fishes, but the extent to which n-6 series of PUFA are essential, remain to be established. Since fishes are incapable of *de-novo* synthesis of 18:2 n-6,18:3 n-3,20:5n-3 and 22:6 n-3 acids, dietary sources of these fatty acids are likely to be essential for normal growth and survival. But there was no sign of fatty acid deficiency in carps even after long term feeding with fat free diets (Kaushik, 1995).

Estimates show that dietary supply of 10% of both 18:3 n-3 and 18:2 n-6 leads to better growth in juvenile common carp (Satoh, 1991). Essential fatty acids need of grass carp estimated by Takeuchi *et al.*, 1991 was in the same range as that obtained for common carp 1% 18:2 n-6 and 0.5-1% of 18:3 n-3. But studies with common carp larvae fed on purified diets showed (a) the requirements for n-3 fatty acids are lower than the recommended one (b) the quantitative need for n-6 fatty acid is about 0.25% of the diet and (c) a dietary supply of phospholipid is beneficial (Radung -Nato, 1993).

Once essential fatty acids need are met through proper lipid source in the diet. Optimal dietary fat levels are below 12% in the practical diets of many cyprinids (Kaushik, 1995).

Stickney and Andrews (1971) noted the combined effect of dietary lipids and environmental temperature on growth, metabolism and body composition of channel catfish (*I. punctatus*). The lipid level in fish carcasses increased with temperature, up to 30°C for all dietary supplements. At the optimum temperature for growth (30°C), fish fed beef tallow contained less lipid than those fed the other supplements. The fatty acid composition of the diets was reflected in both liver and carcass lipid.

Murai *et al.,*1989 found no improvement in growth of carp beyond 5% dietary lipid level at constant protein but variable energy levels, at any given crude protein level in the diet an increase in dietary fat content was found to lead to reduced growth and decrease efficiency of protein retention in juvenile carp.

It is found that the n-3 highly unsaturated fatty acids (n3 PUFA) are required for the normal growth and survival of larval fishes, which were low in n-3 HUFA (Kanazaw, 1985 Dhert *et al.,* 1990, Koven *et al.,* 1992). It was revealed that eicosa pentaenoic acid (EPA 20:5 n-3); one of the most essential fatty acid in fish, is a constituent of the cellular membrane of several developing tissue (Kanazawa *et al.,* 1982). Kanazawa (1985) suggested that rapidly growing larval fish needs relatively large amount of exogenous EPA.

2.1.5. Phospholipids

Kanazaw (1993) and Sargent *et al.,* 1994 suggested the need for phospholipids in the diet of certain fish larval stages. Neto *et al.,* 1994 and Geurden *et al.*, 1995 observed that addition of phospholipids was important to obtain initial survival and growth of first feeding carp larvae, as also observed by Szlaminska *et al. (*1993) in gold fish. A diet with 2% of phospholipids provides better larval performance than a diet with only 1% phospholipids (Randunz - Neto *et al.,* 1995).

Phosphates are known to act as emulsifier in an aqueous environment such as the intestinal lumen. According to Kanzawa (1993) they could be essential in allowing the absorption of dietary lipids like cholesterol and triglycerids. The possible function of dietary decision as emulsifier also has been suggested by Koven *et al. (*1993).

Choline, a vitamin-like nutrient, is an important component of the phospholipids lecithin and certain other complex lipids. It serves as a source of labile methyl groups for the synthesis of various methylated metabolites and as a precursor of acetylcholine. Most animals can synthesize choline if adequate methyl donors such as methionine are present in the diet. However, the studies with fish identified choline as essential for maximal weight gain (Craig and Gatlin, 1996 ; Griffin *et al*., 1994; Hung, 1989). The quantitative requirement of choline has been studied in only a few species of fish. For example, 1,000, 400, 1700–3200, 500, 588 and 714–813 mg/kg were reported to be the amount of choline required in the diet for lake trout (Ketola, 1976), channel catfish (Wilson and Poe, 1988), white sturgeon (Hung, 1989), striped bass (Griffin *et al.,* 1994), red drum (Craig and Gatlin, 1996) and rainbow trout (Rumsey, 1991), respectively. Shiau and Pen (2000) conducted an experiment to quantify the choline requirement of tilapia and concluded that dietary choline requirement for tilapia is about 1000 mg/kg diet.

2.1.6. Carbohydrates

Carbohydrates are cheapest source of energy. The omnivorous and herbivorous fishes adapt to utilization of high carbohydrate diets (Shimeno *et al.,* 1981). Carbohydrates are used in fish diet primarily as energy source and for their binding properties. It can be added in excess of the amount that can be efficiently utilized for energy by the fish (Krodahl *et al.,* 2005). According to Furukawa and Ogasawara (1952), a 5% cellulose additive in fish diets has a favorable effect on the nitrogen retention and body growth in common carp. But Bargot (1981) and

Lessel *et al.,* 1986 found that cellulose is not digested at all by common carp and gold fish.

Furichi and Yone (1980) and Shimeno *et al.,* 1990 reported that common carp juveniles are able to utilize complex polysaccharides more effectively than simple sugars, unlike the case of salmonids and eels.

The omnivorous fish *M. montanus* needs a low amount (9.48%) of dietary carbohydrate for its maximum growth (Arockiaraj *et al.,* 2008) whereas Habib *et al*. (1994) reported a comparatively high requirement of dietary carbohydrate (30%) for maximum growth in silver barb, which may be due to its herbivorous nature. Haniffa and Arockiaraj (1999) and Arockiaraj *et al*. (1999, 2004b and 2004c) reported that 14% dietary carbohydrate level is necessary for the maximum growth of the stripped murrel *Channa striatus*. But usually herbivorous fish can metabolize carbohydrates better than carnivorous and omnivorous fish as reported by Cowey and Sargent (1979) and Furuichi and Yone (1980).

Distribution of marked amolytic activity has been studied in IMC including *Labeo rohita* (Dhage, 1968). Kawai and Ikeda (1972) pointed out that in carp; carbohydrate digesting enzymes exist in varying levels. Murai *et al.* (1983) reported a poor performance of complex carbohydrate like Dextrin and alpha starch compared to maltose or glucose in carp. This can be correlated with the presence of specific enzyme and to the overall digestibility of such carbohydrates in cyprinids. High levels of carbohydrate metabolizing enzymes and their significance have been reported in fish intestine particularly in herbivorous fish (Smith, 1989). Kheyyali *et. al.,* 1989 reported that dietary carbohydrate promoted an increase in activities of glyocolytic and lipogenic enzymes. Das and Tripathi (1991) showed in fingerlings and adult grass carp, the pattern of distribution and activity of the digestive enzymes are related to the type of diet ingested by the fish.

Erfanullah and Jafri (1995) obtained maximum growth (100%), in terms of percent live weight gain with sucrose, followed by fructose (85%), glucose (78%) and Dextrin (71%) diets in *Labeo rohita.* They also observed a relatively increased body fat deposition in rohu, with sucrose based feed, presumely due to lipogenesis from this dietary carbohydrate source.

Ufodike and Matty (1983) found that rice starch or tapioca starch (incorporation level of 45%) are well utilized by common carp. Increasing the feeding frequency from 2 to 6 times per day also improved the utilization of different sources of carbohydrate, incorporated at 30% level in common carp (Murai *et al.,* 1983).

2.1.7. Vitamins

In general fishes require four fat soluble vitamins (A, D, E, and K) and eleven water soluble vitamins. Of these thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, folic acid and vitamin B_{12} are required in small quantities and they function as co-enzyme, while inositol, choline and biotin are required in higher quantities. Yet requirement of fish vary with SPP, age, size and growth rate, physiological condition especially at the times of wound healing and stress. Some fishes have the ability to synthesize vitamins from glucose substrates or amino acids, while in some fishes intestinal micro flora can synthesize vitamin (Hepher, 1988, Halver, 1989).These sources reduce the dependence of dietary sources of vitamins in fishes.

Vitamin C is considered to be a very important component in the diet of fish especially for the air breathing fish. It has been established that vitamin C is required by all animals for body maintenance, growth and other biological performance, and the vitamin C level needed for these functions varies with the species and culture environment (Delong *et al.,* 1958; Lovell, 1972). Alam *et al.,* 2009 conducted an experiment to evaluate the vitamin C requirement of *Heteropneustes fossilis* and observed that, the fish diet containing 1200 mg/ kg of Vitamin C level has been found to be more effective for best growth of this fish with a better protein efficiency ratio than other diet with different level of vitamin C.

Murai and Andrews (1975) reported that supplemental pantothenic acid markedly improves growth rate, survival and feed conversion and prevents loss of appetite, clubbed gills and sluggishness. According to these researchers, diets fed to cat-fish fry (*Ictalurus punctatus*) should contain at least 250 mg of pantothenic acid/kg of diet. Lovell and Lie (1978) observed that channel catfish fingerlings fed semi purified diets in flowing - water aquariums for 16 WK required a dietary source of vitamin D for normal growth and bone mineralization. Wilson *et al.,* 1988 conducted two experiment to evaluate vitamin E requirement of fingerlings of channel cat fish fed with Purified diets containing 1% cod liver oil, 4% stripped lard and adequate selenium supplemented with graded levels of DL-alpha-tocopheryl acetate and obtained results of 50mg/kg of diet which differed from previous report.

Based on the weight gain and mortality data in the preliminary experiment by Dupree (1969) the requirement for vitamin E appears to be near 20 units/kg of dry feed; Dupree (1970) fed a series of 16 purified diets that contained 0–20,000 units of Vitamin A as the acetate or as beta carotene per kg of dry ingredients. Weight gain was linear with the quantity of vitamin A acetate up to 1,000 units. Andrews and Murai (1979) reported that for maximum growth, the catfish require pyridoxine, about 3 mg/kg diet, though 2.2 mg/kg prevented all other signs of deficiency.

Unlike other fish species, common carp seems to be able to oxidize gluconolatono to 2-keto-gulunolactone which subsequently forms ascorbic acid spontaneously (Yamamota *et al.,*1978).However scurvy occurred in common carp when fed with a vitamin-c depleted diet(Kitamura *et al.,*1965).Controversy exist as regards the requirement of ascorbic acid in the common carp (Dabrowski *et al.,*1988).

The optimum requirement of vitamin C is found to be about 700mg/kg for *C. mrigala* (Mahajan and Agrawal, 1980). Mahajan and Sharma (1976) reported better growth rate and survival rate in common carp and rohu, fed with Vitamin-B complex yeast combination.

2.1.8. Minerals

The dietary mineral requirements of fishes were reviewed by Nose and Arai (1979) and other researcher. In general minerals required by fish are calcium, magnesium, Phosphorus and a number of trace elements like iron, copper, iodine, magnease, selenium, zinc, chromium, cobalt, boron and molydenum.

Fishes have the ability to absorb inorganic elements from the surrounding water as well as from the diet. This ability to exchange inorganic ions across the gill membrane and body surface makes it difficult to elucidate the nutritional formation of dietary minerals (Nose & Arai, 1979). Ions absorbed from the external medium have nutritional and osmoregulatory implications.

Ca and P are the most abundant inorganic elements in animal body and 99% of calcium and 80 % of phosphorus are located in skeletal tissues. These two inorganic elements have been demonstrated to play an important role in fish nutrients.

Muraikami (1967) showed that the cranial deformity, associated with other disorders of skeleton, occurred in hatchery reared carp fed with artificial diets and that this is prevented or healed effectively by the addition of 5% calcium mono hydrogen phosphate in the diet. Common carp was found to show good growth as purified test diet containing calcium as low as 30mg/100g, if an adequate amount of phospholipid is provided in the diet (Ogino and Kanizono, 1975).

In freshwater fish, the amount of dietary magnesium was found to have a significant effect on growth and magnesium deficiency was experimentally induced in common carp. Maximum growth was attained, when 60-70mg magnesium/100 g was added in the diet (Chiou and Ogino, 1970). For the growth, the contents of magnesium and calcium in the body, the minimum requirement for dietary magnesium was estimated as 40-50mg/100g by Chiou and Ogino (1976). They also observed that the amount of magnesium required by fresh water fishes does not vary significantly from species to species. Murray and Andrews (1979) have reported the effect of dietary salt on growth of channel catfish. They concluded that, the 0.06% sodium and 0.17% chloride already present in basal diet was sufficient for good growth and feed efficiency.

Availability of phosphorus from fish meal was found to be lower in carp than in the rainbow trout, such differences probably originating from lack of gastric (low PH) digestion in the stomach less carp (Satoh, 1983).

Nose and Arai (1979) found that increasing the available phosphorus level in the diet from 0.5 to 1 %, led to an almost two fold weight gain in common carp, but excess dietary supply of (7%) tricalcium phosphate reduced the absorption of Zinc, Magnese and of Phosphorus by nearly 50% (Satoch *et al.,*1983).

2.2. ENERGY

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

A number of studies have dealt with basal metabolic rates as affected by body weight and water temperature (Kaushik, 1995). Drawing data from such studies (Huisman, 1974; Hepher, 1988; Cui and Lui, 1990; Yamaoto, 1991; Chakraborty *et al.,* 1992), the basal metabolic rate-body weight (BW) relationship of useful carp at temperature of 23-25^{0}C was found to be well described by the following equation.

$$O_2 \text{ intake (mg/fish/d)} = 10.5 \text{ X BW}$$

Weight-specific basal (resting) metabolic rates were found to decrease with a weight component of -0.20. Similar to other teleosts, both fasting metabolic rates and maintenance energy requirement of carps are affected by water temperature. Analysis of data from the literature cited above shows that the resting metabolic rates of carp at low water temperature (10-17^{0}c) are extremely low. But Sehwarz and Kirchgessner (1984) found that the maintenance energy needs of common carp are reduced at low temperature; 19 & 45 KJ DE/Kg BW$^{0.75}$/day at 10 & 20^{0}c, respectively. Providing the optimum energy level in diets for shrimp or fish is important because an excess or deficiency of useful energy can result in reduced growth rates (NRC, 1983). For example, excess dietary energy may result in decreased nutrient intake by the fish or excessive fat deposition in the fish, whereas a low dietary energy density will result in the animal utilizing nutrients for energy provision rather than for tissue synthesis as growth (Robinson, 1989).

At present there is very little useful information on the practical dietary energy requirements of fish or shrimp; this has been due primarily to difficulties encountered with the quantitative measurement of the energy losses within the energy budget equation (Brafield, 1985). However, factors known to influence the energy requirements of fish and shrimp, include 1) water temperature (metabolic rate, and consequently maintenance energy requirements, increasing with temperature; Brett and Groves, 1979). 2) animal size (metabolic rate, and consequently maintenance energy requirements, decreasing with increasing animal size; Brett and Groves, 1979). 3) Physiological status (energy requirements increasing during periods of gonad production and reproductive activity such as spawning migration; Wooton, 1985). 4) Water flow (energy requirements for maintaining station in water increasing with increasing water flow; Brett and Groves, 1979; Knights, 1985). 5) light exposure (energy requirements for voluntary activity being less during night-time 'rest' periods) and 6) water quality and stress (pollutants, increased salinity, low dissolved oxygen concentration, and excessive crowding increasing the maintenance energy requirements; Talbot, 1985; Knights, 1985).

Hastings (1979) recommended 1,200 kcal of metabolisable energy per pound of ration but did not identify the unit (poultry, swine etc) which was used in calculating these values. The reason for such low rate at low temperature is not clear. Dietary energy also influences the carcass composition of fat (Zeitter *et al.,* 1984). A positive correlation was noted in *C.mrigala* between dietary energy level and carcass protein or ash content (Hasan & Jafri, 1996). A reduction in growth rate with dietary energy level exceeding 376 k Cal/g was also noticed by the authors.

2.3. PROTEIN SOURCES USED IN SUPPLEMENTARY FEEDING

The growth potential of fish is influenced to a great extent by the food quality (Pandian, 1967). Protein is the most expensive component in fish feeds and also the most important factor affecting growth performance of fish and feed cost (Lovell 1989; Luo *et al*., 2004). Reducing the feeding costs could be a key factor for successful development of aquaculture. Fish have high dietary protein requirement (Deng *et al.,* 2006). The main protein source selected for fish feed must have high protein content especially at the younger stages. Although, protein is the costliest item in fish feeds (Wee, 1988), various animal and plant sources have been used alone or in combination in fish feeds. Based on their origin protein sources used in feeds can be broadly divided into plant protein sources and animal protein sources.

A brief review of various protein sources used in fish feed formulation is dealt with.

2.3.1. Protein sources of Animal origin

It is well known, that fishmeal forms the principal source of protein in commercial fish diets. It is also a rich source of energy and minerals, and is highly digestible and palatable for most fishes (Lovell, 1989).

Fish meal is one of the most common ingredients used in the commercial shrimp and fish feed, possibly due to its large scale availability. Fish meal is reported to be a good source of protein with essential amino acids and high biological value. Fish meal made from good quality whole fish that is properly processed is the best quality protein source commonly available for the preparation of aqua feeds. Fish meal prepared from whole fish contains 60-80% protein and is a rich source of energy and minerals and is highly digestible and palatable for most species of shrimp and fish. It is high in available lysine and methionine, the two amino acids most deficient in plant feed stuffs.

Timbol, as early as 1969, found that in young *C. chanos*, a high protein trout feed on fishmeal gives a higher weight gain and survival rate than a diet with low protein rabbit-feed containing basically alfalfa. Milkfish fry fed on four formulated dry diets containing 40% crude protein with fish meal as the major source of protein, had significantly higher survival and weight gain, compared to those fed on *Moina* and blended water hyacinth leaves (Santiago *et al.,* 1983). A feed containing 60% fish meal and 16.9% fat was found to be the best diet among a series of dry diets tested for *Lates calcarifer* fry. However, a diet containing 20% fish meal and 13.4% fat gave practically the same result (Tucker *et al.,* 1988). Tubongbauna - Marasigne (1990) found that feed from Japanese fish meal based diets was superior to a combination of pasteurized trash fish and Japanese fish meal (1:1 ratio) or pasteurized fish meal alone or local fish meal based diets in *Lates calcarifer* fry.

But, although fish meal is a high quality protein source for fin fishes, it seems to have lower nutritional value for shrimps and prawns, especially when used as a sole protein source. Deshimaru and Shigueno (1972) attributed this to the shortage of phenylalanine and the basic amino acids (arginine, histidine and lysine) in fish meal. Generally, the essential amino acid profile of the protein of the animal’s body closely approximates its dietary requirements. Fish meal normally does not provide all the essential amino acids to the required level and is found to be generally poor in threonine, phenylalanine, arginine and histidine (Lovell, 1989).

However, in the past two decades, the replacement of fish meal protein with other alternative sources was clearly back in focus with increased pressure on diet cost (Smith, 1990) and inconsistent supply. Alternate protein sources which are cheap and locally available and can supplement fish meal without significantly reducing the production have been put to use. Blood-meal-ruminant content mixture is used as a partial or complete substitute for fish meal in commercial catfish rations (Reece *et al.,* 1975). Asgaard (1984) found that slaughter blood had no negative effect on growth, health or organoleptic characters of salmonids. Viola (1975) stated that the meal made from poultry meat and feathers can serve as a good substitute for fish meal in carp diets. However, kerns and Roelofs

(1977) found that the growth rate and feed conversion efficiency were inversely related to the level of poultry waste in common carp diets. Kumar and Singh (1983) noted that pelleted poultry litter resulted in faster growth rate in common carp under laboratory conditions than with traditional feed mixture of groundnut oilcake and rice bran. The importance of processed piggery waste as feed material for common carp has been reported by Watson (1985). Bull *et al.* (1988) found that slaughter-house waste, vegetable waste, poultry farm waste and press cake waste to be equally effective in the feed of common carp. The percentage weight gain PER, SGR, and feed conversion efficiencies were not significantly different among the diets.

The value of shrimp by-product meal as an alternate protein source in the diets of channel catfish and *Liza parsia* has been elucidated by Robinette and Dearing (1978) and Kiron (1989) respectively. However, the results indicated poor performance; the reason attributed was that shrimp by-product meal was neither digestible nor palatable as fish meal or that it was deficient in some unidentified growth factors. Contrary to this Afolabi *et al.* (1980) reported better protein efficiency ratio when shrimp and fish wastes were used. Protein digestibility of shrimp waste meal was found to be 89.68 % when fed to fish *Clarias batrachus* (Borthakur, 1998).

Jayachandran and Paulraj (1976, 1977) have shown that silkworm pupae and prawn waste can be profitably utilized as feed for common carp. Defatted silkworm pupae were a better source than non-defatted one in the diet of common carp. With a feed conversion of 2.96 (Nandeesha *et. al.,* 1989, 1990). Forster (1976), reported that prawn waste contain several essential amino acids which induce high growth rate in prawns. Amar *et al.,* 2009 experimented with prawn shell waste collected from shrimp-processing plants and subjected to fermentation using 20 chitinoclastic and proteolytic/non-proteolytic bacterial strains and conducted a feeding experiment with post larvae (PL21) of Indian white prawn, *Fenneropenaeus indicus*. Enhanced growth was observed in prawns with the fermentation products generated by using *Bacillus* spp., these products of bacterial fermentation hold promise as growth enhancers and immunostimulants in aquaculture.

Lukowicz (1978) investigated the possibility of replacing fishmeal in carp diets with krill (*Euphasia superba*) meal, it compared favorably with that of fish meal. When squid was used as food for salmonids and rainbow trout increase in growth was observed (Asgaard, 1984), Squid meal has also been used successfully to replace rice bran in shrimp feeds in Asia (Devendra, 1988). Squid, shrimp and mussel meat extracts are known to be good sources of feeding stimulants, especially for carnivorous fishes like red sea bream and sea bass (Paulraj, 1989). Fresh clam meat was conventionally used as feed for prawns by Kanazawa *et al.,* 1970, who reported that fresh short necked clam *Tapes philippinarum* gave superior growth rates for *Penaeus japonicus* compared to compounded diet. Deshimaru and Shigueno (1972) attributed the superiority of short necked clam to the similarity of its amino acid profile with that of *Penaeus japonicus*. Forster and Beard (1973) obtained similar results in *Palaemon serratus* with fresh mussel meat. Ali (1982) obtained high increase in live weight and good FCR, using clam meat powder feed for *Penaeus indicus*. He reported poor performance and heavy mortality with fresh clam meat, *Sunetta scripta* used as control diet for *Penaeus indicus*. Vellegas (1978) observed that growth and survival of *Penaeus monodon* larvae fed with *Tapes* was only next to that of compounded diet. Josekutty and Susheela (1991) obtained higher percentage weight gain, lowest FCR and 100% survival for *Penaeus monodon* fed on diet containing clam meat as major protein source.

Kumar *et.al.,* 2008 experimented with 3 different live feeds to study the growth and survival of early larvae of *Channa striatus* and observed that mosquito larvae is the most suitable feed for larval rearing of this species with a survival rate of 96.66%. Feed intake of fish depends on size of the prey and predator, quality, density, physical attractiveness and mode of presentation of food (James *et al*., 1993). The wriggling movements of large and nutritionally rich prey organisms such as *Chironomous* larvae and *Culex pipiens* larvae minimize the temporal and energy cost of feeding and maximize growth in *Cyprinus carpio* (James *et al*., 1993). Reddy and Katre (2003) studied the maximum feeding frequency required for the optimum growth of *Heteropneustes fossilis* using oligochaete worm *Tubifex tubifex* as food and concluded that, an amount of worm substance equivalent to 12.73% live body weight/fish day^{-1} represents the maximum amount.

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

2.3.2. Protein sources of Plant origin

For several years, there has been continuing interest in identifying and developing ingredients as alternatives to fish meal for use in aqua feeds (Hardy, 1995; Tacon *et al*., 1998). Recently, however, the concern raised about the negative impact of fish meal production on global fish stocks has heightened this interest (Naylor *et al.,* 2000). Andrews (1978) reported that several by-products and sources of plant protein used as donors of amino acid to cat fish have some effects on the output of catfish.

Among the ingredients being investigated as alternatives to fishmeal, the products derived from soybean (*Glycine max L.*) 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. According to Food and Agriculture Organization of the United Nations statistics, global soybean meal production has increased from about 15 million tons in 1961 to about 107 million tons in 2001, whereas fish meal production has remained fairly static at 5 to 7 million tons and is expected to remain at these levels for the foreseeable future.

Soybean meal appears to have been used widely, as it is the dominant oil seed protein available world wide and economically viable too (Anon, 1978). It has one of the best amino acid profiles of all protein rich plant feedstuffs, meeting the EAA requirements of fish (N.R.C., 1983). Preliminary results of its use in carp diets have been conflicting, as several workers have reported a reduction in both growth and feed conversion efficiency when higher levels of soybean meal are included.

Available data on use of soy in fish argued that virtually all fish could tolerate a minimum of 10-15% soybean meal in diets, but that several of the carnivorous species can not tolerate more than 20% soybean meal. The salmonids (trout, salmon and char) are one of the most sensitive species to soy in diets, tolerating no more than 25-30%, with some species tolerating no more than 15%. This is part of the reason there have been a higher number of soybean evaluations in salmonids than other species. However, it is unclear why we experience this limitation, in the expense of fish meal (Viola, 1975, Hepher *et al.,* 1979). However, its successful use as complete replacement of fish meal has been reported in *Oreochromis aureus* (Davis and Stickney, 1978). And a replacement of 25-75% dietary protein in tilapia (Jackson *et al*., 1982; Viola and Arieli, 1983; Davies *et al.,* 1989) And *C. chanos* (Shiau *et al.,* 1988).

Kikuchi (1999) fed juvenile Japanese flounder *Paralicthys olivaceus* a series of diets containing defatted soybean meal in replacement of fish meal of unspecified origin. In this trial, smaller amounts of other protein sources also were included with the soybean meal. The author concluded that soybean meal could effectively replace up to 45% of fish meal, provided that other protein sources were present, along with a feeding attractant (blue mussel in this trial). Earlier work with this species (Kikuchi *et al.,* 1994, cited in Kikuchi, 1999) had shown that soybean meal protein could replace up to 50% of fish meal in diets for this species, when suitable supplemental amino acids were included. Gomes *et al.* (1997) examined the use of soy protein concentrates with and without the presence of an attractant (a mixture of amino acids included at 2.5%) in diets for European sea bass in comparison with fish meal. The fish fed the fish meal diet performed the best of all the groups in this trial, but including the attractant in the soy based diet significantly improved the feed consumption and subsequent growth of the fish.

Solvent extracted cotton seed can replace 20 – 35%, rape seed 28 – 42% and sunflower 70% of fish meal in the diets of *Oreochromis mossambicus, O. niloticus and_C.carpio* (Dabrowski and Kozlowska, 1981; Viola *et al.,* 1981, 1983, Jackson *et al.,* 1982; Davies *et al.,* 1989; Shiau *et al.,* 1990). Full-fat soybean meal can replace 58% of the diet and cotton seed meal 21% of the fish meal in the diet of *Tilapia nilotica* without significant decrease in growth rate (Lovell, 1980; Viola *et al.,* 1982; Wee and Shu, 1989; Shiau *et al.,* 1990). Roasted Indian mustard seed cake replaced up to 20% fish meal and groundnut expeller cake up to 17% of fish meal in the diet of *Cyprinus carpio* (Jackson *et al.,* 1982). Autoclaved mustard oil cake improved growth performance and food utilization in carp (Hossain and Jauncey, 1990).

A base line information on the potential use of legumes viz., pigeon pea (*Cajanus cajan*). Mungo (*Phaseolus radiatus*), kidney bean (*P. vulgaris*) and soybean (*Glycine max*) as protein sources for *C. chanos* is given by Dela Pena *et al.* (1987). Of these only mungo and soyabean at 25% of dietary protein level could replace fish meal to reduce cost without affecting growth, survival and efficiency of feed conversion. Martinez-Palacois *et al.* (1988) and Desilva and Gunasekhera (1989) have tried jack bean (*Canvalia ensiformis*) and green pea (*Phaseolus aureus*) respectively, in tilapia feeds.

Water hyacinth (*Eichhornia crassipes*) has been successfully used in the feeds of *O. niloticus* (Edwards *et al.,* 1985). Variable results were obtained when *Azolla pinnata* was fed to Nile tilapia (SEAFDEC, 1984; Alamazan *et al.,* 1986).

The reports on the use of ipil-ipil (*Leucaena leucocephala*) leaf meal for rearing tilapia to marketable size are found varying (Camacho and Dureza, 1977; Pantastico and Baldia, 1980, Wee and Wang, 1987; Olvera Novao *et al.,* 1980). Cassava (*Manihot esculenta*) leaf meal has been demonstrated to be a viable partial dietary protein source for Nile tilapia (Cruz and Fabian, 1980; Ng and Wee, 1989).

Although grains and grain products are the main carbohydrate sources in the diets of cultivated fishes and other livestocks {Darunna, 2000}, an attempt to fullfill the energy requirement of livestock through the use of root and tubers could probably ameliorate the stiff competition with cereals and grains (Agbede *et. al.,* 2002). Furthermore to keep up with annual increase of fish production, research should be targeted towards the use of alternative or unconventional feed ingredients such as root and tubers which could probably improve the feed water stability and nutrient retention, increase efficiency of digestibility and reduce cost of fish feed production (Falayi, *et.al*., 2003 and 2004).

Jackson *et al.* (1982) have shown that the use of a combination of several plant protein sources is more advisable than as single form, as the protein sources have different limiting amino acids. Their experiments with Nile tilapia indicate that cotton seed, rape seed and sunflower seed promoted reasonable growth when provided at 50% of total dietary protein, while copra, soybean and groundnut might have performed more favorably in the diets, had they been supplemented with single limiting amino acids.

2.3.3. Mixed Protein sources

Deshimaru and Shigueno (1972) and Conklin *et al.,* 1977 have demonstrated that a mixture of two or more protein sources in the diet show better growth than single protein source. New (1976) reported that relatively higher amount of animal proteins than plant protein sources gave better results in the mixed diets. The improved performance of mixed diet is mainly because of the fact that neither animal protein source nor plant protein source can provide all the essential amino acids in adequate levels. While the plant protein sources are deficient in lysine, the animal protein sources are deficient in sulphur containing amino acids. The deficiency may be overcome by mixing both the sources of protein. For fast growing animals, the protein which contains EAA, in the same balance as those found in the body protein of growing animal is evaluated as high in protein quality (Nose, 1979). So usually a mixture of protein sources are used in the formulation of fish feeds.

Molla *et al.* (1973) conducted an experiment in order to develop a suitable feed for cat fish *Heteropneustes fossilis* of the six types of feeds tried, five had a common base of vegetable origin, feeds differed only in their animal protein contents and observed that feed consisting of only fish meal with no vegetable base resulted in weight reduction of the fingerlings. Otubusin (1987) reported that feed containing 10% blood meal (31.34% protein) gave the best performance in the culture of tilapia (*Oreochromis niloticus)*. Blood meal is uniquely rich in lysine, having twice the lysine content of white fish meal and almost three times the level in dehulled soybean meal (Crashaw, 1994). Allan (1998) reported that blood meal in feeds was well utilized by barramundi and Atlantic salmon which were adapted to carnivorous diets.

Mitra and Das (1965) evaluated various protein sources for Indian major and minor carp fry. Among the several sources tried, higher survival and yield of carp spawn were obtained when fed with till oil cake, Rice powder and black gram, silkworm pupae and fish meal as compared to rice bran.

Several artificial diets compounded from natural and synthetic materials have been tried for the larvae and fry of mullets with limited success (Nash and Shehadeh, 1980). Rangaswamy (1984) obtained best growth of *Liza parsia* fry with a mixture of Bengal gram, prawn head waste and sago in 2:2:1 ratio. A mixture of wheat middling, cotton seed meal, soybean meal and tuna fish meal in 4:1:1:1 ratio along with propylene glycol and vitamins is recommended for the rearing of larvae and fry of mullets by Nash and Shehadeh (1980). Studies have shown that feeding *Liza parsia* fry with wheat flour and fish meal in 1:1 ratio (Chakraborthy *et al.,* 1981) or rice bran and prawn meal in 1:2 ratio (Chakraborthy *et al.,* 1984), gave better results in fertilized ponds than conventional feeds consisting of groundnut oil cake, fish meal or rice bran mixture. Kiron (1989) found that a feed compounded from groundnut oil cake, gingerly oil cake, and coconut cake. Rice bran, mangrove leaves, fish meal and prawn head waste providing a dietary protein level of 35% to be the best food for *Liza parsia* in brackish water ponds.

Samsi (1979) attempted various feedstuffs like fish meal, meat and bone meal, shrimp head meal, copra meal and *ipil-ipil* meal as protein sources for milkfish fingerlings. Animal protein sources were better utilized than plant sources. Carreon *et al.* (1984) reported that growth of milkfish fry supplied with natural plankton were lower than those reared on artificial detritus made from rice straw, hulls and chicken manure. Diets with fish meal as major protein source (21%) and the balance protein (16%) level supplemented with animal (shrimp head meal and/or meat and bone meal) and plant (soybean meal and/or corn gluten meal) sources in a 41% protein diet promoted good growth and survival of milkfish fry.

In *Siganus canaliculatus*, a brackish water herbivore, feed formulated from fish meal, soybean meal, rice bran, corn meal, coconut oilcake, sea weed gum, fish oil and vitamin premixes was a better source than broiler chicken feed or broiler chicken feed with fish meal combination (Kungvankij *et al.,* 1990).

Supplementing the deficient amino acids, deficient in protein sources with exogenous crystalline amino acids is a means to overcome the inherent amino acid imbalances in many protein sources. Thus, Chiu *et al.* (1989a) demonstrated that addition of crystalline lysine would significantly improve the biological value of corn gluten as a protein source to milkfish fry. Shiau *et al.* (1988). Also report that 67% of fish meal in milkfish could be replaced by hexane extracted soybean meal with a methionine supplement without any adverse effect on growth and FCR.

Fish silage is a less expensive form of fish meal. Acid silages have been used in the feeds of salmonids. Silage produced from trash fish mixed with fish meal , soybean meal and compounded pellets or silage from waste grown tilapia are also tested as fish meal replacements in *Clarias* spp. (Wee *et al.,* 1989). However a negative effect of silage on growth has been reported in *Scophthalmus maximus* by Calcedo-Juanes (1989).

The use of leaf protein concentrate (LPC) potato protein concentrate (PPC), and protein hydrolysates as unconventional protein sources in fish feeds are yet to be tested in warm water fin fishes (Tacon and Jackson, 1985).

Materials and Methods

3. MATERIALS AND METHODS

The collection and rearing of fingerlings of *Heteropneustes fossilis*, their feeding regimes and other experimental details followed for determining the gain in weight and length and survival are presented sequentially. The experiment was conducted at the College of Fisheries, Panangad, Kochi for a period of 6 weeks.

3.1. EXPERIMENTAL ANIMALS

The fingerlings of *Heteropneustes fossilis* were procured from backwaters of Alappuzha district near Mancombu and transported to the college in FRP tanks. After salt treatment, four hundreds fingerlings were introduced into an oval, flat bottom fiber glass tank of 3 ton capacity, half filled with fresh water and provided with gentle aeration. The fingerlings were fed *ad libitum* with pelleted commercial feed and trash fish. No water exchange was done during acclimatization. After 14 days the fingerlings were transferred to experimental tanks.

Plate 1. Initial size of the experimental fish

3.2. EXPERIMENTAL REARING FACILITIES

The experiment was conducted under culture conditions as follow

3.2.1. Circular cement tanks

Circular, flat bottom cement tanks with the following specifications were used for the experiments to rear the animals for a further period of 6 weeks.

Capacity of the tank	-380 liters
Diameter	- 91 cm
Height	- 60 cm
Thickness of wall	- 7cm

Clear fresh water from the well was used for filling the tanks up to a height of 50 cm. 5 cm of soil base was provided at the bottom to create natural environment. Soil was washed thoroughly before use. The tanks were kept outside and covered with net to protect against insect and dust and prevent escaping of fish by jumping. Tanks were kept outside the lab and experiment was conducted outdoor.

Plate 2. Experimental Tanks

3.3. PREPARATION OF EXPERIMENTAL FEED INGREDIENTS

3.3.1. Feed ingredients

Three major feed ingredients used in the experiment were, fish meal, clam meat and soymeal. Rice bran was used as common ingredient and tapioca flour as binder as well as carbohydrate supplement. 1% of Supplevite-M, a vitamin-mineral mixture (Sarabhai chemicals, Bombay) was added to the each experimental diet. Ingredients and feeds were analyzed to identify protein and lipid concentration (A.O.A.C.1990). Pearson square method was used to get required combination of ingredients and to maintain protein concentration at 40%.

3.3.2. Processing of the major protein sources

Various protein sources used for the feed formulation were processed using the following methods.

Clam meat: Meat of the black clam *villorita cyprinoides* purchased from local market, was washed thoroughly and steamed in an autoclave at ambient pressure for 15 minutes and then dried in an electric dryer for 12 hours at 60^0

Fish meal: Fish meal purchased locally was dried for 6 hours at 60^0c in an electric dryer.

Soymeal: Fresh soymeal of required quantity was purchased from local market and dried in an electric dryer for 6 hours at 60^0C.

Rice bran: Fresh rice bran was procured locally and dried for 6 hours in an electric dryer after sieving.

The dried ingredients were powdered separately in a pulverizer and passed through a sieve of 250 microns. The powdered ingredients were packed separately in airtight plastic bottles and used immediately for feed preparation, after analysis of proximate composition.

3.3.3. Proximate composition of Feed ingredients (A.O.A.C)

Proximate compositions of all the feed ingredients were analyzed prior to feed formulation.

Crude protein content was estimated by Microkjedahl,s method (A.O.A.C,1990). Percentage of nitrogen obtained was multiplied by the factor 6.25 to get the crude protein content. Solvent extraction using petroleum ether (B.P. 40-60 ^{0}C) in a soxhlet extraction apparatus for 6 hours was carried out to estimate the crude fat content. Proximate composition of feed ingredients are given in Table -1

3.3.4. Formulation and processing of experimental feeds

Three different feeds were formulated fixing their protein concentration at 40% using Pearson square method. The feeds prepared were –

T1- Fish meal and Rice bran

T2- Clam meat and Rice bran

T3- Soymeal and Rice bran

The proportions of the ingredients used for the preparation of pelleted feeds are given in Table 2

The experimental feeds were prepared separately by mixing required quantity of ingredients. The respective ingredients were weighed accurately in an electronic balance and all the ingredients except vitamin were well mixed in a dry mortar. The dry mixture was made into dough by adding sufficient volume of water (1:1.25 W/V) and mixed well in a mortar. The dough was transferred to glass bowl and cooked in a pressure cooker for 30 minutes. The cooked dough was rapidly cooled and mixed well in a mortar along with supplevite-M.

The well mixed dough was pelletized using a hand pelletizer with a diameter of 2mm to a clean tray as a single layer and dried at 60 ^{0}c for 12 hours in an electric dryer. The dried pellets were packed in airtight container bottles separately and stored in a refrigerator.

3.3.5. Proximate composition of experimental diets

Proximate composition of the experimental diets was analyzed to evaluate the nutrient status. Methodology employed was same as that for the ingredients. Boyd's (1979) method was used to estimate the moisture content. The sample was heated to 105 ^{0}c for 30 minutes and then dried at 65^{0}c till a constant weight was obtained.

Table 1. Proximate composition of feed ingredients (A.O.A.C.1990).

Ingredients	**Protein (%)**	**Fat (%)**
Fish meal	72	6
Clam meat	46	7
Soymeal	48.3	6
Tapioca	1.27	
Rice bran	18.3	4

Table 2. The ingredient proportion (%) of the formulated feeds.

Ingredients	**T1**	**T2**	**T3**
Fish meal	53		
Clam meat		70.4	
Soymeal			65.7
Rice bran	36	18.6	23.3
Tapioca	10	10	10
Vitamin	1	1	1

Table 3. Proximate composition of formulated experimental feeds (A.O.A.C.1990).

Feeds	Protein %	Lipid %	Moisture %
Fish meal (T1)	40	7	10
Clam meat (T2)	40	7	8
Soymeal (T3)	40	7	10

Formulated Feeds

Plate 3. Fish meal

Plate 4. Clam meat

Plate 5. Soybean meal

3.4. EXPERIMENTAL DESIGN AND PROCEDURE

The acclimatized fingerlings of *Heteropneustes fossilis* were transferred to cement tanks. The tanks were stocked with 10 fingerlings of average size (11.22 ± 1.27g and 12.24 ± 0.40 cm). The fingerlings were fed with prepared feeds at the rate of 5% of biomass for 42 days. Optimum water quality parameters were maintained. Sampling was done at weekly intervals to check the water quality parameters.

After 42 days of experiment, fishes were harvested with the help of scoop net after complete draining of tanks. The experiment was conducted in a Completely Randomized Design with 3 treatments and 5 replications; the three treatments being fish meal (T1), clam meat (T2) and soymeal (T3).

The experimental fishes were acclimatized for 2 weeks prior to stocking. After acclimatization period the initial length and weight measurement of individual fish were recorded.

The initial average and weight of fishes in fish meal, clam meat and soymeal were 10.2g, 10.88g and 11.63g and length 12.35cm, 12.14cm and 12.24cm respectively.

Each treatment group of animals were fed at the rate of 5% biomass for the experiment period of 42 days, twice a day. Water quality was maintained uniformly in all treatments tanks, to compensate evaporation loss water was filled weekly.

After 42 days, the harvesting was done after noting the final length and weight and survival ascertained.

3.5. WATER QUALITY PARAMETERS

During the experiment, the water quality parameters such as temperature, pH, Dissolved oxygen, ammonia and alkalinity were measured at weekly interval by using the following methods.

Temperature: By using mercury thermometer of 0.1ºC pH: By using universal indicator solution

Dissolved oxygen: By using DO meter

Ammonia: By using Ammonia testing kit (BIOSOL)

Alkalinity: By using Alkalinity testing kit (BIOSOL)

3.6. EVALUTION CRITERIA

The parameters used for evaluation were growth (Average gain in weight), average gain in length, average percentage weight gain, Specific growth rate (SGR), Percentage survival, Food Conversion Ratio (FCR) and Protein efficiency ratio (PER).

3.6.1. Average gain in weight

It gives the increase in weight of the animals during the experimental period. It was calculated using the formula.

Average gain in wt. (g) = Average Final wt. (g) – Average Initial wt. (g)

3.6.2. Average gain in length

This gives the increase in standard length during the experiment period. It was calculated applying the formula.

Average gain in length (cm) =Average Final length (cm) – Average Initial length (cm)

3.6.3. Average percentage weight gain.

Percentage gain in weight of the animal was calculated using following formula.

$$\text{Weight gain (\%)} = \frac{\text{Final Wt.(g)} - \text{Initial Wt.(g)}}{\text{Initial Wt.(g)}} \times 100$$

3.6.4. Specific growth rate

In the present study, growth performance was also measured in terms of specific growth rate (SGR) since it is more effective growth index than absolute weight gain or percentage growth rate (Hepher, 1988). In the present study, SGR was calculated using the following formula.

$$\text{SGR (\%)} = \frac{\ln(\text{W2}) - \ln(\text{W1})}{\text{Time interval in days}} \times 100$$

Where,

W1= Initial weight of animal (g)

W2= Final weight of animal (g)

The calculated value gives the average percentage increase in the body weight per day over a period of 42 days.

3.6.5. Survival rate

The survival rate of fishes is expressed in terms of percentage. This was calculated as follows:

$$\text{Survival (\%)} = \frac{\text{Final Number}}{\text{Initial Number}} \times 100$$

3.6.6. Food conversion ratio

Food conversion ratio (FCR) is the ability with which an animal can convert the feed consumed into edible and other products (Devendra, 1989). FCR gives an idea about the amount of feed required to produce unit increase in weight of animals. It is the commonly used index to measure the efficiency of a diet used in the experiment. FCR of the experimental diet was calculated using following formula.

$$\text{FCR} = \frac{\text{Average weight of food consumed in dry weight}}{\text{Average live weight gain}}$$

3.6.7. Protein efficiency ratio

Protein efficiency ratio is defined as the weight gain per unit intake of protein (Paulraj, 1982). It was calculated by employing the formula of Hepher (1988).

$$PER = \frac{\text{Wet weight gain of fish (g)}}{\text{Crude protein consumed (g)}}$$

3.7. STATISTICAL ANALYSIS

The experiment was carried out by using the Completely Randomized Design (CRD). The data pertaining to biological parameters were statistically analyzed. The means of the three treatments T1 (Fish meal), T2 (Clam meat) and T3 (Soymeal) were compared using One-Way ANOVA. Multiple comparisons of mean values were carried out using Tukey's HSD test. The means of T1 (fish meal) and T2 (clam meat) and T3 (soymeal) were compared using two sample Student's t-test (Rangaswami, 2002).

Results

4. RESULTS

The growth and survival of *Heteropneustes fossilis* in three different treatments were evaluated. The details of the observations made during the study period are presented below. The fish meal, clam meat and soymeal denoted as T1, T2 and T3, respectively.

4.1. BIOLOGICAL PARAMETERS

4.1.1. Gain in weight

The average live weight gain and percentage weight gain of fishes in different treatments presented in Table 4.

The mean weight gain of *H. fossilis* in the three treatments T1, T2 and T3 was found to be 8.56g, 5.68g and 6.09g respectively. The highest average live weight gain was obtained in treatment T1 i.e., fish meal and lowest observed in treatment T2 i.e., clam meat.

Analysis of variance of the data (Table 4a) showed gain in weight significantly ($P>0.05$) different among the three treatments. Multiple comparisons as per Tukey's HSD test (Table 4b) revealed that weight gain was highest for T1 (fish meal) followed by T3 (soymeal) and T2 (clam meat). T1 showed significantly higher weight gain than T2 but on par with T3. There was no significant difference between T2 and T3.

The growth observed in different treatments graphically presented in Fig 1a and Fig 1b.

The average percentage weight gain of fishes from their initial size in treatments T1, T2 and T3 was 85.63%, 52.54% and 48.32% respectively. The highest percentage gain was obtained in treatment T1 with 85.63% and lowest in treatment T3 with 48.32%.

4.1.2. Gain in length

The average gain in total length of fishes in different treatments presented in Table 5.

Initial and final length of fishes in the three treatments is shown in plate 1 and 6a, 6b, 6c respectively. The average gain in length of *Heteropneustes fossilis* fishes in the three treatments T1, T2 and T3 was found to be 1.09 cm, 1.20 cm and 1.11 cm respectively. The highest average gain in length was obtained in treatment T2 i.e., clam meat and lowest in treatment T1 i.e., fish meal.

Analysis of variance of the data (Table 5a) showed no significant ($P<0.01$) difference in the average gain in total length, among the three treatments. All the treatments have same effects on the length gain, as there is no significant different among treatments, multiple comparisons as per Tukey's HSD test can not be applied.

Table 4. Analysis of gain in weight of *Heteropneustes fossilis* in different treatments.

Treatment	Repli-cation	Average initial weight (g)	Average final weight (g)	Gain in weight (g)	Average live weight gain (g) (Mean±SD)	Percentage weight gain	Average percentage weight gain (Mean±SD)
T1	1	10.6	16.2	5.4	8.56 ± 2.40	50	85.636 ± 30.023
	2	8.9	19.6	10.7		120.22	
	3	11.2	17.8	6.6		58.92	
	4	10	20.4	10.4		104	
	5	10.1	19.7	9.7		95.04	
T2	1	9.7	16.3	6.6	5.68 ± 0.72	68.04	52.542 ± 9.025
	2	10.8	15.9	5.1		47.22	
	3	11.8	18	6.2		52.54	
	4	11.4	17	5.6		49.12	
	5	10.7	15.6	4.9		45.79	
T3	1	12.25	20.6	8.35	6.09 ± 1.40	68.16	48.326 ± 12.41
	2	13.2	17.8	4.6		34.84	
	3	12.9	19.1	6.2		48.06	
	4	12.85	18.25	5.45		42.02	
	5	11.95	17.8	5.85		48.55	

Table 4a. ANOVA Table.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F Value	P value
Treatment	24.272	2	12.136	4.418*	0.037
Error	32.967	12	2.747		
Total	57.239	14			

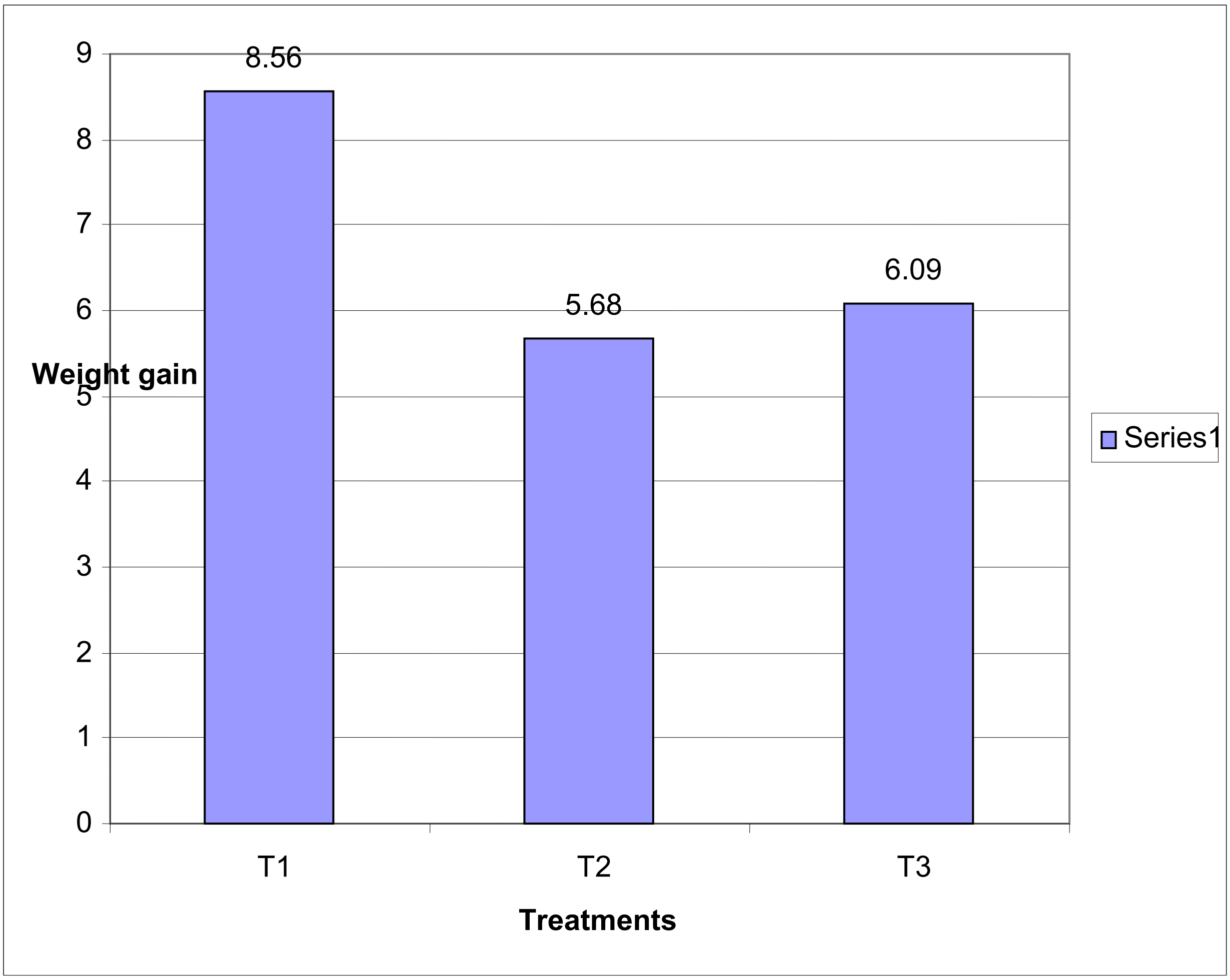
**Significant at 5 % level

Table 4b. Tukey's HSD test.

Treatment	Average gain in weight(g)
T1	8.56^{b}
T2	5.68^{a}
T3	6.09^{ab}

Means with common subscripts are statistically not different (P> 0.05).

Fig 1a. Total weight gain of *Heteropneustes fossilis* in different treatments.

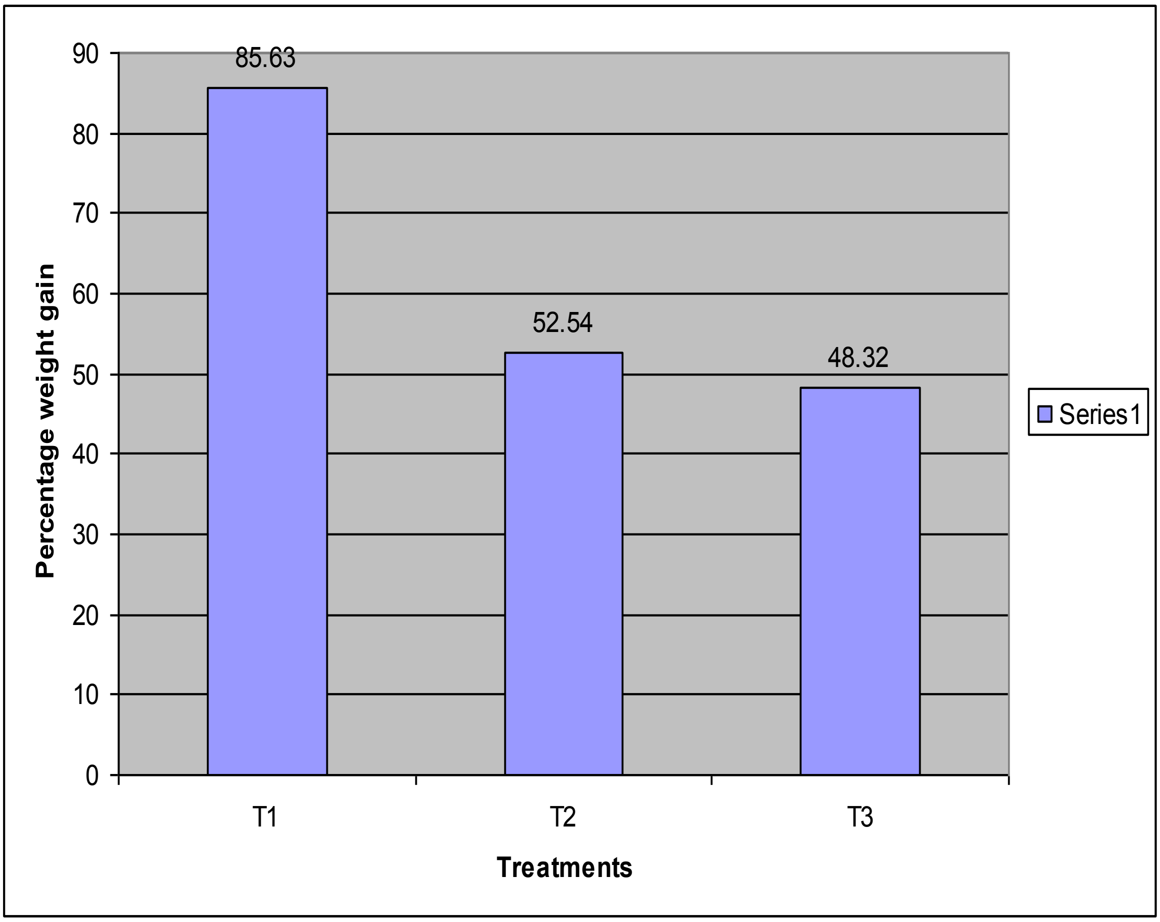


T1- Fish meal

T2- Clam meat

T3- Soymeal

Fig 1b. Percentage weight gain of *Heteropneustes fossilis* in three treatments



T1- Fish meal

T2- Clam meat

T3- Soymeal

Gain in weight of *Heteropneustes fossilis* in different treatments.

Plate 6a. T1-Fish meal

Plate 6b. T2-Clam meat

Plate 6c. T3-Soymeal

T3 is as good as T1, but T1 is superior to T2 in weight gain. The highest weight gain was observed in T1.

The growth in length observed in different treatments graphically presented in Fig 2.

4.1.3. Specific growth rate (SGR)

Specific growth rate of fishes under various treatments is given in Table 6.

The maximum SGR was recorded in T1 i.e., fish meal and minimum in T3 i.e., soymeal. The average SGR values in the three treatments T1, T2 and T3 was found to be 0.628%, 0.432% and 0.405% respectively.

Analysis of the data on specific growth rate using ANOVA (Table 6a) revealed that three treatments T2 and T3 are not significantly different. Multiple comparisons as per Tukey's HSD test (Table 6b) showed that the mean SGR of treatment T1 is significantly better than T2 and T3.

The SGR of the *H.fossilis* in different treatments graphically presented in Fig 3.

4.1.4. Percentage survival

The percentage survival of *H.fossilis* in three different treatments is given in Table 7. 100% survival was recorded from all treatments, there was no mortality in any treatment.

Table 5. Gain in total length of *Heteropneustes fossilis* in different treatments.

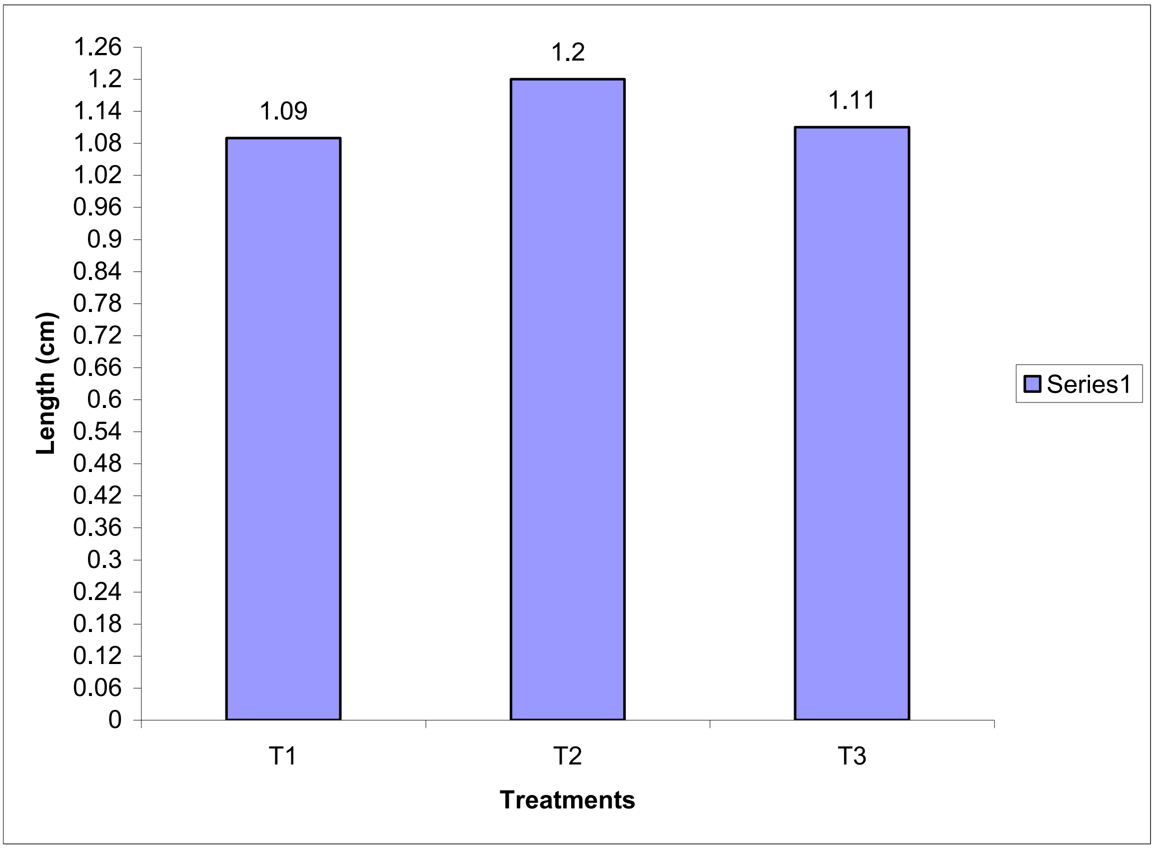
Treatment	Replication	Average initial length (cm)	Average final length (cm)	Gain in length (cm)	Average gain in length (cm) (Mean±SD)	Percentage length gain	Average percentage length gain (Mean±SD)
T1	1	12.44	13.02	0.58	1.086 ± 0.75	4.6623	8.79 ± 4.06
	2	11.63	13.75	2.12		18.22872	
	3	12.88	13.54	0.66		5.124224	
	4	12.06	13.72	1.66		13.76451	
	5	12.74	13.15	0.41		3.21821	
T2	1	11.73	13.1	1.37	1.196 ± 0.46	11.67945	9.84 ± 4.25
	2	12.52	13.12	0.6		4.792332	
	3	12.62	13.48	0.86		6.81458	
	4	12.06	13.46	1.4		11.60862	
	5	11.81	13.56	1.75		14.81795	
T3	1	12.35	13.47	1.12	1.11 ± 0.75	9.068826	9.06 ± 3.90
	2	12.56	13.05	0.49		3.901274	
	3	11.83	13.98	2.15		18.17413	
	4	12.52	12.81	0.29		2.316294	
	5	11.95	13.45	1.5		12.5523	

Table 5a. ANOVA Table.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F Value	P value
Treatment	0.033	2	0.017	0.037 (NS)	0.964
Error	5.431	12	0.453		
Total	5.464	14			

NS: Not significant

Figure 2. Gain in length of *Heteropneustes fossilis* in different treatments.



T1- Fish meal T2- Clam meat T3- Soymeal

Table 6. Specific growth rate of *Heteropneustes fossilis* in different treatments.

Treatment	Replication	Average initial weight (g)	Average final weight (g)	Specific growth rate (%)	Average specific growth rate(%) (Mean±SD)
T1	1	10.8	16.2	0.4192	0.62830 ± 0.17
	2	8.9	19.6	0.8163	
	3	11.2	17.8	0.479	
	4	10	20.4	0.737	
	5	10.1	19.7	0.69	
T2	1	9.7	16.3	0.536	0.4328 ± 0.61
	2	10.8	15.9	0.399	
	3	11.8	18	0.436	
	4	11.4	17	0.413	
	5	10.7	15.6	0.38	
T3	1	12.25	20.6	0.537	0.40500 ± 0.84
	2	13.2	17.8	0.309	
	3	12.9	19.1	0.405	
	4	12.85	18.25	0.362	
	5	11.95	17.8	0.412	

Table 6a. ANOVA Table.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F value	P value
Treatment	0.148	2	0.074	5.536*	0.020
Error	0.161	12	0.013		
Total	0.309	14			

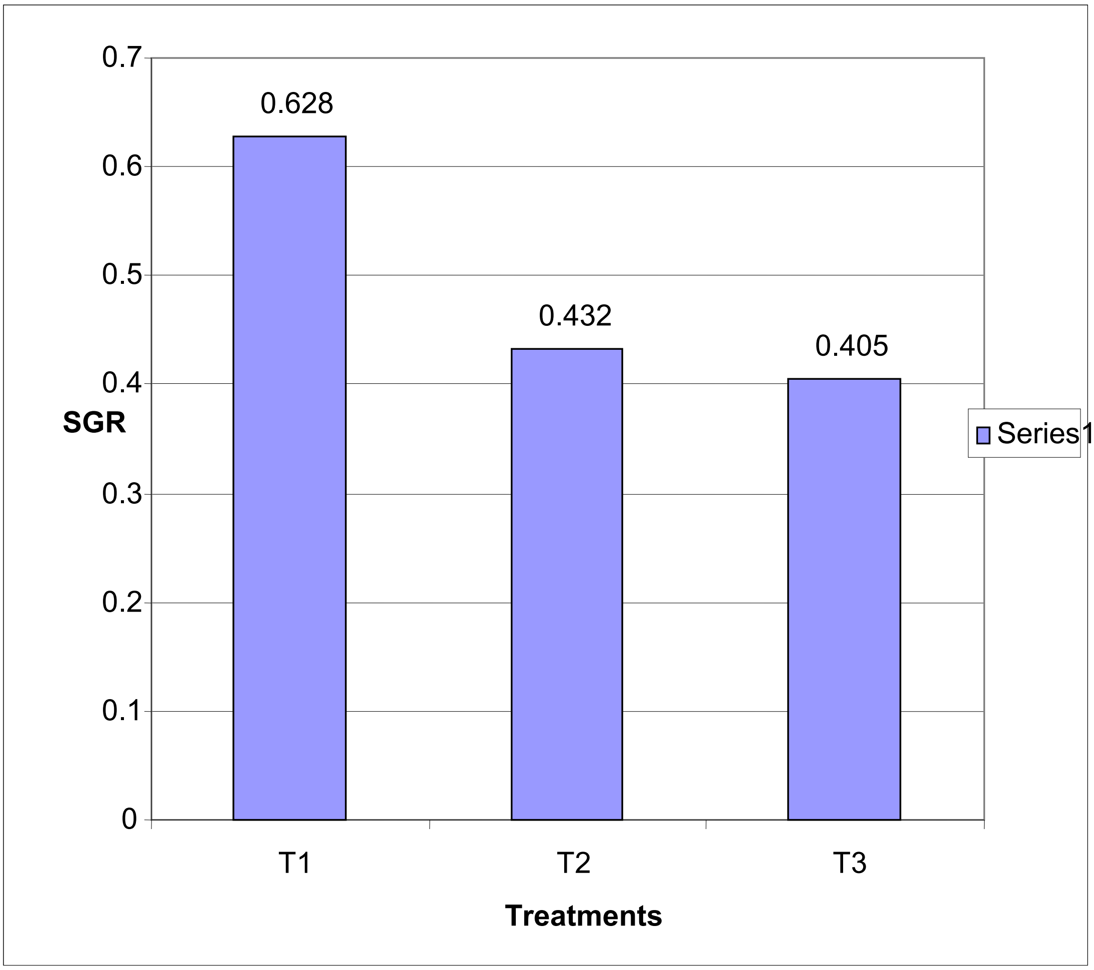
* Significant at 5% level

Table 6b. Tukey's HSD test.

Treatment	Average specific growth rate (%)
T1	.628300[a]
T2	.432800[b]
T3	.405000[b]

Values with different superscripts are significantly different ($P < 0.05$).

Fig 3. Specific growth rate (SGR) of *Heteropneustes fossilis* in different treatments.



T1- Fish meal

T2- Clam meat

T3- Soymeal

Table 7. Percentage survival of *Heteropneustes fossilis* in different treatments.

Treatment	Replication	Initial stocking no.	Final survival	Survival (%)	Average percentage survival (Mean±SD)
T1	1	10	10	100.0	100 ±0.0
	2	10	10	100.0	
	3	10	10	100.0	
	4	10	10	100.0	
	5	10	10	100.0	
T2	1	10	10	100.0	100 ±0.0
	2	10	10	100.0	
	3	10	10	100.0	
	4	10	10	100.0	
	5	10	10	100.0	
T3	1	10	10	100.0	100 ±0.0
	2	10	10	100.0	
	3	10	10	100.0	
	4	10	10	100.0	
	5	10	10	100.0	

4.1.5. Food conversion ratio

The food conversion ratios (FCR) of *H.fossilis* in various treatments given in Table 8.

The mean FCR values in the three treatments T1, T2 and T3 were 2.74, 4.07 and 4.53 respectively. The best FCR was obtained in treatment T1 (2.74) i.e., fish meal and poorest in T3 (4.35) i.e., soymeal.

Analysis of variance of FCR values showed no significant difference between treatments T1 and T2 similarly no significant difference between T2 and T3 (Table 8a). Multiple comparisons using Tukey's HSD test (Table 8b) showed that T1 and T2 are equally good but T1 is better than T3.

Graphical presentation of FCR values in various treatments is given in Fig 4.

4.1.6. Protein efficiency ratio

The protein efficiency ratio of the different treatments is given in Table 9.

The average PER values obtained in treatments T1, T2 and T3 were 1.01, 0.62 and 0.54 respectively. The highest PER value was recorded in T1 (1.01) and the lowest in T3 (0.54).

Analysis of variance (Table 9a) of the data on PER values showed no significant difference between T2 and T3 but T1 is better than T2 and T3. Multiple comparisons as per Tukey's HSD test (Table 9b) placed T2 and T3 in one group and T1 in other group.

The PER values of the different treatments graphically presented in the Fig 5.

Table 8. Food conversion ratio of *Heteropneustes fossilis* in different treatments.

Treatment	Repli-cation	Average initial weight (g)	Average final weight (g)	Average live weight gain(g)	Average of feed consumed (g)	FCR	Average FCR Mean±SD
T1	1	10.8	16.2	5.4	22.68	4.20	2.74 ± 1.07
	2	8.9	19.6	10.7	18.69	1.74	
	3	11.2	17.8	6.6	23.52	3.56	
	4	10	20.4	10.4	21.0	2.01	
	5	10.1	19.7	9.6	21.21	2.20	
T2	1	9.7	16.3	6.6	20.37	3.08	4.07 ± 0.59
	2	10.8	15.9	5.1	22.68	4.44	
	3	11.8	18	6.2	24.78	3.99	
	4	11.4	17	5.6	23.94	4.27	
	5	10.7	15.6	4.9	22.47	4.58	
T3	1	12.25	20.6	8.35	25.75	3.08	4.53 ± 1.07
	2	13.2	17.8	4.6	27.72	6.02	
	3	12.9	19.1	6.2	27.09	4.36	
	4	12.85	18.25	5.45	26.98	4.95	
	5	11.95	17.8	5.85	25.09	4.28	

Table 8a. ANOVA Table.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F Value	P value
Treatment	8.686	2	4.343	4.897*	0.028
Error	10.642	12	0.887		
Total	19.328	14			

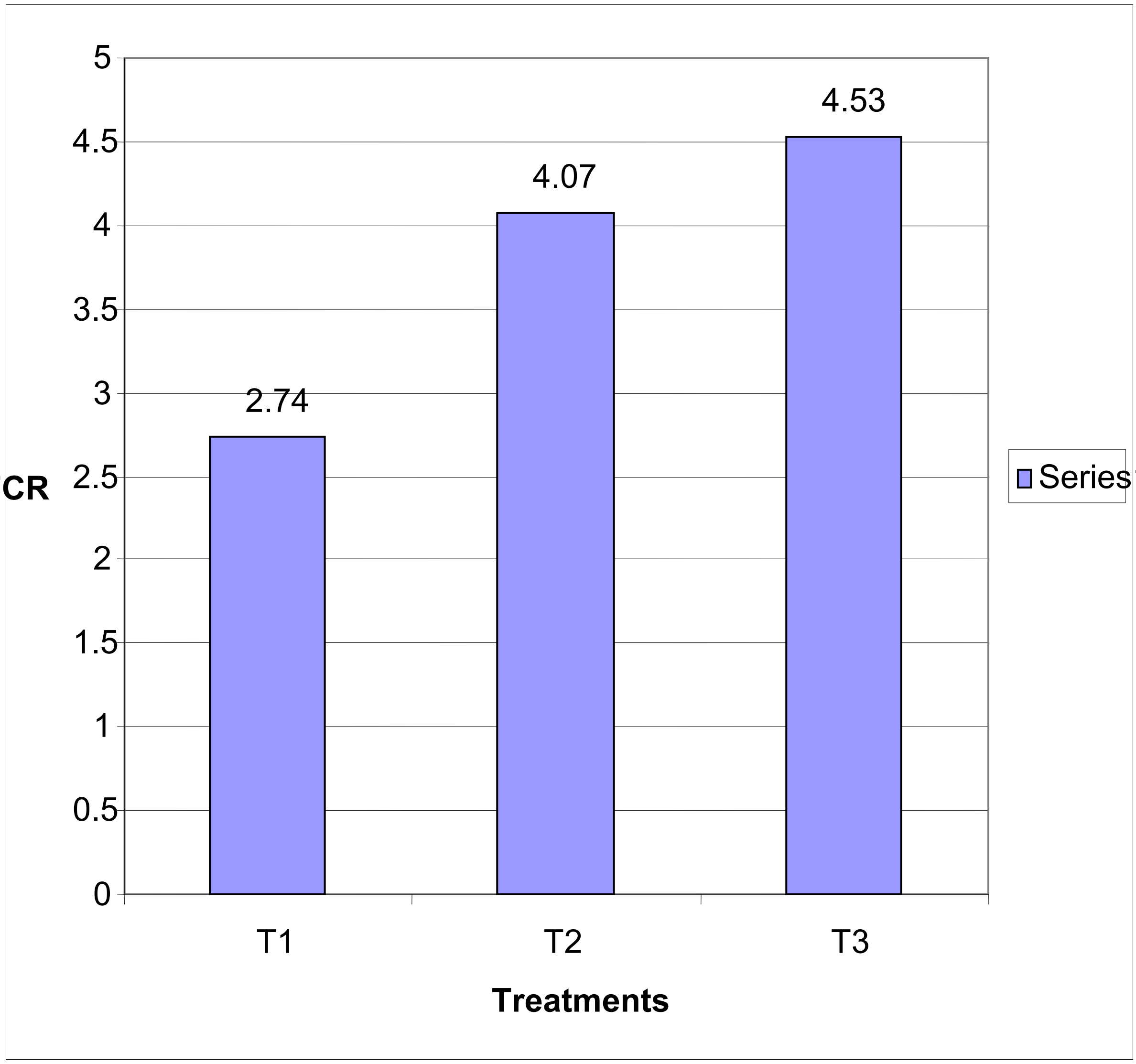
**Significant at 5% level

Table 8b. Tukey's HSD test.

Treatment	Average FCR
T1	2.74^{a}
T2	4.07^{ab}
T3	4.53^{b}

Values with common superscripts are not significantly different (P> 0.05).

Fig 4. FCR of *Heteropneustes fossilis* in different treatments.



T1- Fish meal

T2- Clam meat

T3- soymeal

Table 9. Protein Efficiency Ratio (PER) of *Heteropneustes fossilis* in different treatments

Treatment	Repli-cation	Average initial weight (g)	Average final weight (g)	Average live weight gain (g)	Average of protein consumed (g)	PER	Average PER (Mean±SD)
T1	1	10.8	16.2	5.4	9.07	0.59	1.00 ± 0.35
	2	8.9	19.6	10.7	7.47	1.43	
	3	11.2	17.8	6.6	9.40	0.70	
	4	10	20.4	10.4	8.4	1.23	
	5	10.1	19.7	9.7	8.48	1.14	
T2	1	9.7	16.3	6.6	8.14	0.81	0.62 ± 0.10
	2	10.8	15.9	5.1	9.07	0.56	
	3	11.8	18	6.2	9.91	0.62	
	4	11.4	17	5.6	9.57	0.58	
	5	10.7	15.6	4.9	8.98	0.54	
T3	1	12.25	20.6	8.35	10.81	0.77	0.54 ± 0.14
	2	13.2	17.8	4.6	11.64	0.39	
	3	12.9	19.1	6.2	11.37	0.54	
	4	12.85	18.25	5.45	11.33	0.48	
	5	11.95	17.8	5.85	10.53	0.55	

Table 9a. ANOVA Table.

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F value	P value
Treatment	0.657	2	0.329	6.147*	0.015
Error	0.641	12	0.053		
Total	1.299	14			

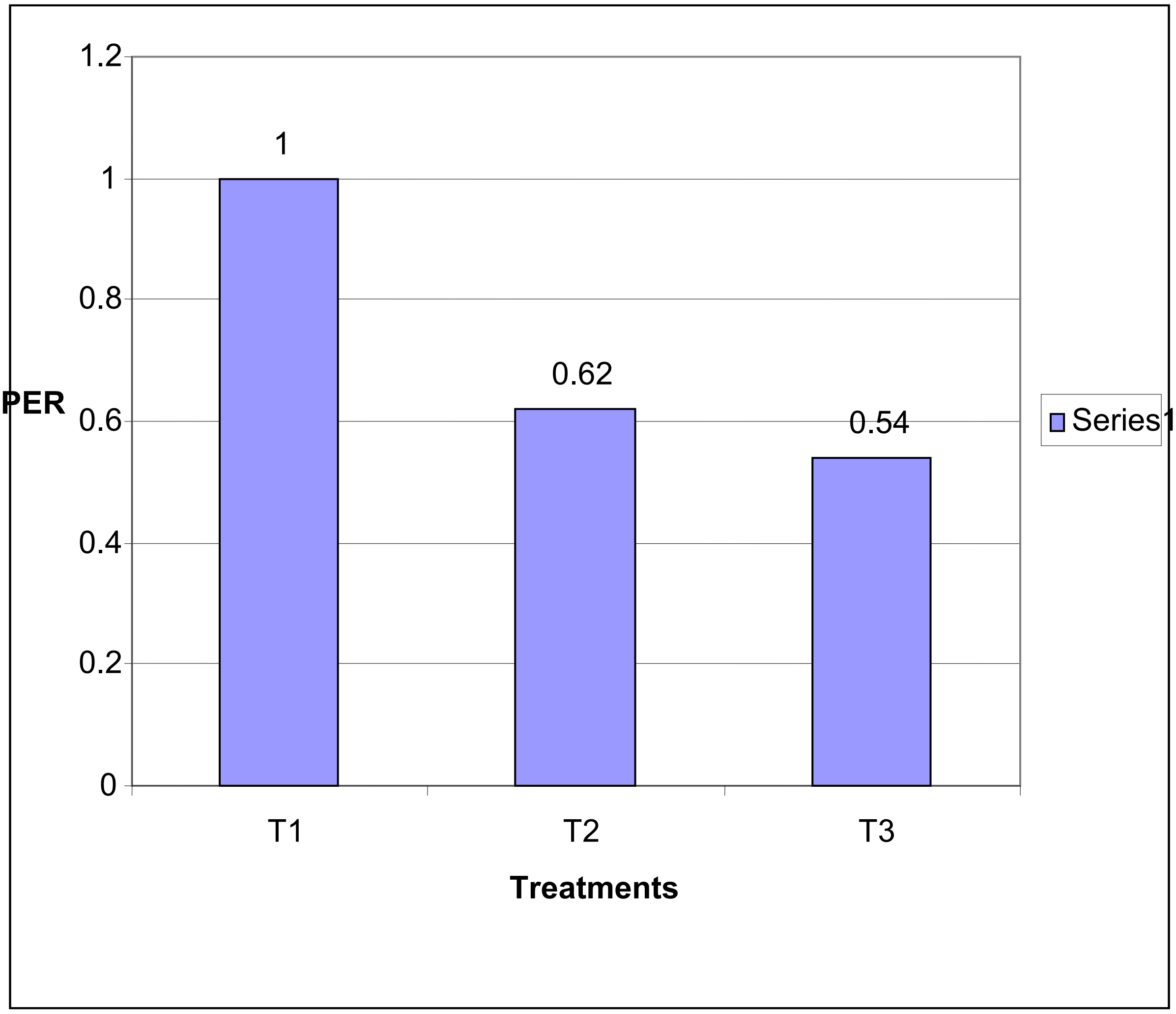
*Significant at 5% level ($p<0.05$)

Table 9b.Tukey's HSD test.

Treatment	Average PER
T1	1.00[a]
T2	0.62[b]
T3	0.54[b]

Values with common superscripts are not significantly different ($P>0.05$).

Fig 5. Protein efficiency ratio of *Heteropneustes fossilis* in different treatments.



T1- Fish meal

T2- Clam meat

T3- Soymeal

4.2. WATER QUALITY PARAMETERS

4.2.1. Water temperature

The summary statistics of water temperature in the experimental tanks during the study period is given in Table 10. Minimum temperature recorded was 27.8ºC and maximum temperature was 32.1ºC. Weekly mean temperature values ranged from 28.4 to 30.6ºC.

4.2.2. pH

The summary statistics of pH in the experimental tanks is given in the Table 12. PH values observed during the study period in treatment T1, T2 and T3 were varying from 7.33 to 7.58, 7.25 to 7.66 and 7.08 to 7.41 respectively.

4.2.3. Dissolved oxygen

The summary statistics of dissolved oxygen (D.O) in the experimental tanks is given in Table 14. DO values observed during the study period in treatment T1, T2 and T3 were varying from 5.41 to 6.33, 6.75 to 7.0 and 6.83 to 7.5 respectively. Lowest DO was recorded in T1 and highest in T3.

4.2.4. Total alkalinity

The summary statistics of total alkalinity in the experimental tanks is given in Table 13. Mean average recorded alkalinity in treatments T1, T2 and T3 were 190.33, 190.33 and 186.66 respectively. Highest alkalinity was recorded in T1 and lowest in T3.

4.2.5. Ammonia

The summary statistics of ammonia in the experimental tanks is given in Table 11. The ammonia concentration varying in treatments T1, T2 and T3 were 0.338±.399, 0.326±.296 and 0.19±.262 respectively. Highest ammonia was recorded during the study period from the treatment T1 and lowest from T3.

Table 10. Water temperature (C^o) in the experimental tanks during the study period.

Temp.	Weeks					
	1	2	3	4	5	6
Mean SD	28.6 0.00	30.3 0.15	30.4 0.17	30.6 0.48	28.4 0.42	28.4 0.41
Range	28.6-28.6	30.1-30.6	30.1-30.6	30.1-32.1	27.8-28.9	27.8-29.1

Table 11. Ammonia concentration in different treatments during study period.

Treatments	1st	2nd	3rd	4th	5th	6th	Mean	Std.dev
T1	0	0.11	0.15	0.33	0.33	1.11	.338	.399
T2	0	0.8	0.15	0.15	0.32	0.54	.326	.296
T3	0	0	0.7	0.11	0.12	0.23	.19	.262

Table 12. PH in different treatments during study period.

Treatment	Repli-cation	Weeks 1st	2nd	3rd	4th	5th	6th	Avg mean ± SD
T1	1	7	7.5	7	7	7.5	8	7.33± 0.408
	2	7	7.5	7.5	7.5	7	7.5	7.33±0.25
	3	7.5	7.5	7.5	7	7	7.5	7.33±0.25
	4	7.5	8	7	7	7	7.5	7.33±0.25
	5	7	8	7.5	7.5	7.5	8	7.58±0.37
T2	1	8	7	7	7	7.5	7	7.25±0.41
	2	8	8	7.5	7.5	7.5	7.5	7.66±0.25
	3	8	8	7	7	7.5	8	7.58±0.5
	4	7	7.5	7	8	7	7.5	7.33±0.40
	5	7.5	7	7.5	7.5	7.5	7	7.33±0.40
T3	1	7	7	7	7	7.5	7	7.08±.20
	2	7.5	7.5	7	7.5	7.5	7.5	7.41±0.20
	3	8	7	7.5	7.5	7	7.5	7.41±0.37
	4	7.5	7.5	7.5	7	7	7	7.25±0.27
	5	7	7.5	7.5	7	7.5	7	7.25±0.27

Table 13. Alkalinity in different treatments during study period.

Treatment	Repli-cation	1st	2nd	3rd	4th	5th	6th	Avg mean± SD
T1	1	180	200	180	200	180	200	190.33±10.66
	2	200	200	180	180	200	200	
	3	200	180	180	180	200	180	
	4	180	200	200	170	200	200	
	5	200	180	180	200	180	200	
T2	1	200	200	200	180	180	200	190.33±10.66
	2	180	180	180	200	200	180	
	3	200	200	200	180	180	200	
	4	180	170	180	180	200	200	
	5	200	180	200	200	180	200	
T3	1	180	200	180	180	200	180	186.66±10.61
	2	200	180	200	200	180	180	
	3	180	200	180	180	200	170	
	4	200	180	200	200	180	170	
	5	180	200	180	180	180	180	

Table 14. DO in different treatments during study period.

Treatment	Repli-cation	1st	2nd	3rd	4th	5th	6th	Avg mean± SD
T1	1	6	7	7.5	6	6	5	5.41±0.88
	2	6	7	7.5	6	6	5	5.41±0.88
	3	6.5	7	7.5	5.5	5	4.7	6.03±1.13
	4	6	7.5	7	6.5	6.5	4.5	6.33±1.03
	5	7	8	7.5	7	4	4.5	6.33±1.66
T2	1	6	7	7.5	6	7.5	6.5	6.75±0.68
	2	6.5	7	7.5	6.5	7	7	6.91±0.37
	3	7	7	7.5	5	7.5	6.5	6.75±0.93
	4	6.5	7.5	7.5	7	7	6.5	7.0±0.44
	5	7	8	7	6.5	7	6.5	7.0±0.54
T3	1	6.5	6.5	7	7	7	7	6.83±0.25
	2	7	7.5	6	7.5	7.5	7.5	7.16±0.60
	3	6.5	8.5	7.5	7.5	8	7	7.5±0.70
	4	7	7.5	7	8	8	6.5	7.33±0.61
	5	7	7	7.5	7	8	7	7.25±0.41

Discussion

5. DISCUSSION

In view of the scarcity of fish and limited water areas available, a search for additional species besides carp fishes was started in the early seventies (Kulkarni, 1971; Dehadrai, 1972). There were two criteria for selecting the new species for pisciculture: first, the economic viability of such a venture, and second, the suitability of fish for a given body of water. Air breathing fishes, by virtue of their hardy nature, their capacity to breathe atmospheric oxygen and tolerance to adverse environmental conditions, permit very high stocking density. Therefore, these fishes have been found to be an ideal group for fish culture (Tripathi, 1976; Nurugesan and Kumariah, 1976; and Pathak *et al.,* 1976).

Indian fish farmers often prefer the exotic catfish viz: the African catfish *(Clarius gariepinus)* due to better supply of seed, their wider feeding spectrum, cheap dietary requirements, fast growth and short culture period. These exotic catfish pose a heavy threat to native fish biodiversity and hence the Government of India put a ban on them, although farmers are still producing them due to favorable short term profits. It has become imperative to promote native catfish culture among fish farmers as an alternative to exotic fish culture for income generation and ultimately to conserve fish biodiversity.

The Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar and many other researchers have succeeded in breeding and hatchery management of *Singhi*. However, commercial hatchery production of the seed of *Singhi* has not been perfected and farming is mostly dependant upon natural seed. Commonly used feed for the culture of this species are trash fish, rice bran and chicken intestine, supply of these feeds ingredients are not regular and reliable. The formulation of cheap diets is very important to the commercial culture of fish and shrimp. Feed is undoubtedly the single largest operating cost item in intensive fish culture especially using catfish, which needs a high protein (Webber and Hugenin, 1979; Cho *et al*., 1985; de Silva, 1989; Otubusin *et al*, 2007).This can be achieved by utilizing and experimenting upon a variety of locally available low priced materials (Usha

Goswami and Goswami, 1982). The present study was conducted to evaluate the efficiency of fish meal, clam meat and Soymeal for the growth and survival of Indian catfish (*Heteropneustes fossilis)*.

Only limited nutrient requirement data are available for this species. Information on the basic nutritional requirements and feeding of *H. fossilis* is needed in view of emphasis on catfish culture in the country (Tripathy and Das, 1976; Dehdri and Thakur, 1980). Earlier studies suggested that a crude protein level of 40% was the requirement of the *H. fossilis* (Niamat, 1985). Akand *et al.,* 1989 concluded that the protein requirement of *H.fossilis* at an average temperature of 29^{0}C was 27.23-35.43%, when casein was the source of protein. Recent studies conducted by Siddiqui and Khan (2009) suggest that, inclusion of protein in the range of 40-43% is optimal for the growth of this species. In the present studies protein concentration in different feeds was maintained at 40%. Inclusion of 7 % of lipid as an energy source in 40% crude protein feed was found to be optimal for the growth of *H. fossilis* (Anwar and Jafri, 1995). In all treatments fat concentration was maintained at 7%.

Alam *et al.,* 2009 conducted an experiment to evaluate the vitamin C requirement of *Heteropneustes fossilis* and observed that, the fish diet containing 1200 mg/ kg of Vitamin C level has been found to be more effective for best growth of this fish with a better protein efficiency ratio than other diet with different level of vitamin C. According to Mohamed and Ibrahim (2001) niacin requirement of *Heteropneustes fossilis* is 20 mg niacin kg^{-1}. In the present study, 1% of Supplevite-M, a vitamin-mineral mixture (Sarabhai chemicals, Bombay) was added to the each experimental diet.

A culture experiment of *H. fossilis* conducted by Pathak *et al.,* 1979 in Assam with a heavy stocking density 3lakhs fingerlings per hectare, and in a period of 5 months 6946.6kg/ha of production was obtained. While Khan *et al.,* 2003 conducted an experiment for a period of four months to see the effect of different stocking densities on production of Stinging catfish (*Heteropneustes fossilis)* in earthen ponds, and concluded that a stocking density of 60,000 individual ha- would be best recommendation for farmers. In the present study fishes were stocked at 10 numbers per tank. In during the acclimatization heavy mortality was recorded in the fiber tank, different-different methods like salt treatment, antibiotic, application of weeds on the surface of water etc. were applied to check the mortalities but after they were shifted to soil base tanks, no mortalities was recorded thereafter. So it can be concluded that soil base is essential for the culture of *H. fossilis.*

Highest weight gain obtained in the present study from fish meal. Review of literature on *H. fossilis* reveal an average growth rate for this species is 5gm/month. From the 42days of present study average weight gain, 8.56gm±2.4 obtained from the fish meal is satisfactory. Siddiqui and Khan 2009 obtained an average weight gain of 16.87± .006 gm from his 8 weeks of experiment, when 40% of protein feed was used. While Anwar and Jafri (1995) studied the effect of different lipid level on the growth of *H. fossilis,* authors obtained a mean growth of 6.13gm at 7% of lipid level, as an energy source in a 40% crude protein diet.

Undoubtedly the fish fed with fish meal – based feed performed best considering the final weigh at harvest, but in the past two decades, the replacement of fish meal protein with other alternative sources was clearly back in focus with increased pressure on diet cost (Smith, 1990) and inconsistent supply. Alternate protein sources which are cheap and locally available and can supplement fish meal without significantly reducing the production have been put to use. Among the ingredients being investigated as alternatives to fishmeal, the products derived from soybeans (*Glycine max L.*) 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. According to Food and Agriculture Organization of the United Nations statistics, global soybean meal production has increased from about 15 million tons in 1961 to about 107 million tons in 2001, whereas fish meal production has remained fairly static at 5 to 7 million tons and is expected to remain at these levels for the foreseeable future.

In the present study statistical analysis of weight gain revealed that, soybean meal is equally good as fish meal. Average weight gain obtained in present experiment from soybean meal treatment 6.09±1.40 is also satisfactory, when comparing with the experimental result of above authors for *H.fossilis.* As there is no significant difference between the fish meal and soymeal, soymeal can be used to replace fish meal in the feed of *Heteropneustes fossilis.* Use of soymeal as complete replacement of fish meal has been reported in *Oreochromis aureus* (Davis and Stickney, 1978), and a replacement of 25-75% dietary protein in tilapia (Jackson *et al*., 1982; Viola and Arieli, 1983; Davies *et al.,* 1989) and *C. chanos* (Shiau *et al.,* 1988). 100% replacement of fish meal by Soymeal has been reported in the feed of channel cat fish, but literatures reveal that supplementing soymeal with lysine is required for the catfish culture. Growth obtained from the clam meat in the present study, was lowest and the cost of feed was highest, so it is not beneficial to culture *Heteropneustes fossilis* using clam meat.

Specific growth rate obtained in the present study was highest 0.62±0.171 in fish meal treatment. But while comparing with the SGR obtained by Siddiqui and Khan (2009) from casein-gelatin based diets for this species was 1.76±0.05 for *H.fossilis* it seems to be poor. Anwar and Jafri (2005) prepared feed from casein gelatin based protein and cord and cod-liver oil and obtained SGR 2.57 at 40% protein and 7% lipid level.

None of the researcher has discussed about the length gain analysis of this species, as objective of fish culture is mainly related to weight gain. In the present study all the feeds had same effect on the length gain, there was no any statistical difference among treatments on the average length gain of *Heteropneustes fossilis.*

Narasimha Raju (2005) studied the seed production and farming of *Heteropneustes fossilis* and stated that average food conversion ratio of cat fishes are 10:1, as they consume more feed and grow less as compared to other warm water fishes. Highest FCR obtained in the present study from the fish meal (2.74±1.07) is encouraging. Alam *et al.,* obtained lowest FCR of 1.56±0.10, while evaluating the effects of different vitamin C levels on the body composition, growth performance and feed utilization efficiencies in stinging catfish (*H. fossilis)* but did not mention about the feed ingredients used during study. At the 40% protein level Siddiqui and Khan (2009), while studying the effect of different dietary protein level on growth, feed, utilization, protein retention efficiency and body composition of young *H. fossilis* got FCR 1.89±0.01 from casein-gelatin based diets.

PER obtained by Siddiqui and Khan (2009) from casein-gelatin based diets for this species was 1.75±0.24. While Anwar and Jafri (2005) at 40% protein and 7% lipid level got PER 1.76 for *H. fossilis*. From the fish meal PER obtained in the present study 1.00 is satisfactory in comparison with result of other research study for *H. fossilis.*

5.1. WATER QUALITY PARAMETER

During the present study, water quality parameters were closely monitered. Water temperature is considered to have a direct influence on growth, feeding rate, behavior and metabolism (Jobling, 1994). Table showed the results of water temperature in 3 treatments of the study. The ranges of water temperature among treatments were varying from 27.8-32.1 ^{0}C and it was within suitable range for fish growth.

pH values observed during the study period in treatment T1, T2 and T3 were varying from 7.33 to 7.58, 7.25 to 7.66 and 7.08 to 7.41 respectively. With the increase in ammonia, p^H also increases. In treatments T1 and T2 p^H was higher than T3. But it was in the suitable range required for fish growth.

Dissolved oxygen strongly influences fish metabolism. Oxygen concentration fluctuation in different treatments was presented in Table 4. Swingle (1969) indicated that suitable dissolved oxygen for fish growth was above 5 ppm. However, high oxygen concentration had negative effects on fish growth because of gas bubble disease causing block on blood vessel lead to death (Brown, 1980). In this study, oxygen concentration ranged from 4.0-8.5 ppm. Although this was not an ideal level for fish growth, but it might not affect the stinging catfish, as it is an air-breathing fish.

Heteropneustes fossilis has exceptional tolerance to high ammonia and low oxygen for several months, inhabits derelict and stagnant, slow-flowing, water bodies, agricultural fields, or swamps and wetlands (Saha and Ratha, 2007). In the present study highest ammonia concentration (1.11) was recorded in T1 (fish meal) followed by T2 (clam meat) and T3 (Soymeal). In the present study for T2 and T3 there is rise in ammonia concentration in first and second week and declined suddenly and rises gradually afterwards. The reason attributed to this decline in ammonia perhaps is the soil base, transforming ammonia into nitrite and nitrate. Inspite of highest ammonia concentration, best growth was obtained in fish meal (T1). Probably better growth would have been obtained in fish meal (T1), if ammonia concentration would have been minimized by water exchange or any other method. It has to be noted that while culturing this fish on animal protein feed, water quality parameters need to be more closely controlled than when fed on plant protein feed.

Summary

6. SUMMARY

1. Exotic catfishes pose a heavy threat to native fish biodiversity, and it has become imperative to promote native catfish culture among fish farmers as an economic alternative to exotic fish culture for income generation and ultimately to conserve fish biodiversity.

2. Indian catfish *Heteropneustes fossilis* is commercially and nutritionally important, because of its excellent flesh quality, high protein content, physiologically available iron, essential amino acid and low fat content. It fetches very high price in various parts of India.

3. The present research was performed at the College of Fisheries, Kochi, Kerala. The fingerlings of *Heteropneustes fossilis* were procured from backwaters of Alappuzha District near Mancombu and transported to the College in FRP tanks. After salt treatment, four hundred fingerlings were introduced into an oval, flat bottom fiber glass tank of 3 ton capacity, half filled with freshwater and provided with gentle aeration.

4. After two weeks of acclimatization fishes were stocked in 380L capacity cement cisterns with soil substratum at a density of 10 number per tank after recording the initial size and weight. For each treatment five replications were used.

5. Three feeds i.e. Fish meal, clam meat and soybean meal were prepared in college nutrition lab. Rice bran was used as common ingredient and tapioca flour was used as binder and carbohydrate supplement. Protein concentration was maintained at 40% and 1% of vitamin mixture was added in all three feeds.

6. To compensate the evaporation loss water was filled weekly in all tanks, no water exchange nor aeration was used during the experiment.

7. Physico-chemical parameters in the water were monitored weekly throughout the experiment. Feed remnants and excreta were removed daily before the next feeding.

8. Upon completion of the experiment, performance under each treatment was analyzed with regard to average gain in weight, average gain in standard length, specific growth rate (SGR), percentage survival, food conversion ratio (FCR) and protein efficiency ratio (PER).

9. Cent percent survival of *Heteropneustes fossilis* fingerlings was recorded in all feeds.

10. Final mean weight gain obtained from fish meal (8.56± 2.40gm) was significantly higher than that of clam meat (5.68±0.72gm). Fish meal and Soymeal (6.09±1.40gm) were equally good. Lowest FCR was obtained from the fish meal (2.74) followed by clam meat (4.07) and Soymeal (4.35) All the three feeds had same effect on length gain.

11. Results of the present study demonstrate that fish meal is still the best protein source for the culture of *Heteropneustes fossilis*, while Soymeal can be used to replace fish meal. Culture of this species on clam meat is not viable.

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ABSTRACT

Stinging catfish *Heteropneustes fossilis* (Bloch) is commercially and nutritionally important in India, fetching high price locally and enjoying good potential for aquaculture. One of the major limiting factors restricting the expansion of *H. fossilis* culture is the high cost of trash fish used as feed for its grow out. Trash fish supply is irregular and unreliable and not always recommended in fish culture. In the present study an attempt was made to evaluate different feed combination as replacement diet for trash fish in the growout of *H. fossilis* using three sources of protein. Three isonitrogenous diets containing 40% of protein viz. fish meal (FM), clam meat (CM) and soymeal (SM) as protein sources were tested, with five replications for each treatment, and fed to the fingerlings of *H. fossilis* of initial size.(11.22 ± 1.27g and 12.24 ± 0.40 cm), collected from the wild and acclimated for two weeks in freshwater and stocked in 380L capacity cement cisterns at a density of 10 numbers per tank, provided with soil substratum. Tapioca flour and vitamin mixture were added at the rate of 10% and 1% respectively in all the feeds, while rice bran constituted 36%, 18.6% and 23.3% in FM, CM and SM feeds respectively. The test feeds were fed to the fingerlings at the rate of 5% of body weight, twice daily for duration of 42 days. At the end of the trial, final length and weight measurement of each juvenile in treatments were made, and the data statistically analyzed, using One-Way ANOVA. Multiple comparisons of mean values were carried out using Tukey's HSD test, and the means were compared using two sample Student's t-test. Water quality parameters in the experimental tanks were monitored weekly. All the treatments recorded cent percent survival, and there was no significant difference in length gain among the three feeds. Although the highest weight gain was obtained from the FM (8.56 ± 2.40g), followed by SM (6.09 ±1.40g) and CM (5.68 ± 0.72g) respectively, statistical analysis revealed that there was no significant difference between FM and SM treatments, indicating that soymeal is equally good as fish meal. However the lowest food conversion ratio (FCR) of (2.74) for FM, followed by CM (4.07), and SM (4.53), which suggest that fish meal is the best source of protein in the feed for the culture of *H. fossilis*, but soymeal could also be used to replace fish meal, upon further refinement of growth trials. Highest ammonia was recorded in FM tanks, followed by CM and SM, which indicate that water quality needs to be closely maintained, when sources of animal protein are used in the feed of *H. fossilis*.

Additional research is recommended, supplementing the deficient amino acid lysine in soymeal, which might perhaps improve the weight gain and FCR of *H. fossilis* fingerlings.

COMPARATIVE EVALUATION OF
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HETEROPNEUSTES FOSSILIS (BLOCH)

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