HIGH DENSITY REARING OF *LABEO ROHITA* (HAMILTON) SPAWN INDOORS USING DIFFERENT FEEDS

By

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TO MY PARENTS

DECLA`RATION

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INTRODUCTION

1. INTRODUCTION

The world is becoming more and more dependent on commercial supply of food materials. Aquaculture with its great and as semi exploited potential is playing a key role in the total world food supply. Recently, rearing techniques for various kinds of fish, and methods for the mass production of living feeds have advanced markedly and partly as a consequence, the number of fish species in commercial production increases every year (Watanabe, *et al.*, 1983). It is estimated that more than 300 species of fish are cultured in the world. The culture of fish in freshwater carried out in inshore waters is a well understood area of aquaculture. But for the last fifteen years, almost everywhere in the world, immense progress has been made with the development of new techniques, making possible the use of new areas of water for cultivation (Barnabe, 1990). Of all the species of fin fish or shell fish used for aquaculture, carps undoubtedly have the oldest history (Pillay, 1990) and are the largest group among the farmed fish. Their production from culture was 10.37 million tonnes in 1995, with common carp contributing the major share (FAO,1997).

Carps are the mainstay of Indian freshwater aquaculture also. The rohu, *Labeo rohita* is the most widely cultured of the Indian major carps (Shivananda and Varghese , 1996). The nonavailability of adequate number of good quality seed for the growout operations, is being continued as a major problem in many countries including India. India's present production of about 14,500 million fish seed is not adequate even to stock 50% of the available fresh water resources (Basavaraja and Antony, 1997). Although the techniques in induced breeding of carps have undergone much

improvements, total seed production has not increased much, possibly because there are no corresponding improvements in the spawn rearing techniques (Nair *et al.* 1989).

Carp spawn is usually reared in outdoor nursery systems where they are fed on natural plankton and supplementary feed (ground nut oil cake and rice bran in 1:1 ratio). The survival rate from spawn to fry in the outdoor nursery systems usually range from 30-50% (Alikunhi,1952; Alikunhi, 1957; Keshavappa *et al.*, 1990). The mortality of carp spawn in nursery ponds, among other factors, is due to lack of proper food (Lal and Kapur, 1986). Being small and tender and at the same time, predatory in nature, carp larvae should be provided with sufficient population of tiny animalcules, preferably rotifers and cladocerans, for better survival and growth. Furthermore, presence of natural food organisms especially zooplankton, has been reported to increase the efficiency of even artificial feeds used for rearing carp larvae (Lubzens *et al.*, 1984).

Despite the simple characteristics of gut, the larvae require sufficient food to grow during the early period. A nutritionally adequate introductory feed holds the clue to successful larviculture. But the outdoor nursery rearing using natural plankton can no longer be considered as an ideal method of larval rearing. The presence of undesirable microbes, predatory copepods, insects and insect larvae make this method, a very risky one. So now a days the indoor rearing is being encouraged for the larval rearing of different fish species. The availability of desirable larval food at the right time, congenial water quality parameters and easy management are the advantages of indoor larval rearing. In commercial nursery operations, the ultimate objective is to promote both fast

growth and high survival rate because young fish of a particular size are usually sold or stocked based on number rather than on biomass. So, in the present indoor-larval rearing experiment, eight different larval diets are being tried for the rearing of rohu spawn to evaluate the efficiency of different diets.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Nutritional requirements of warm water finfishes with special reference to cyprinids

The nutrient requirements of fish are the 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 variations in stocking density, age and weight, water quality parameters and management practices (Hastings, 1979). Eventhough, artificial feeding of warm water fishes has become popular in recent years, studies on their nutritional requirements are limited compared to those of cold water fishes. 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 (Huisman, 1976; Reddy and Katre, 1979; Reinitz, 1983).

Nutrition studies in carp are mainly on common carp (*Cyprinus carpio*), with limited work with other cyprinids such as the grass carp (*Ctenopharyngodon idella*) or the Indian major carps, rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*) and catla *(Catla catla*) (Kaushik, 1995).

2.1.1. Protein

The capacity of fish to synthesize amino acids *de novo* from carbon skeleton is limited and most of the protein must therefore be supplied through the diet (Hepher, 1988). The optimum dietary level of protein required for maximum growth of farmed

fishes is 50-300% higher than that of terrestrial animals (Cowey, 1979).

Protein requirements of cultivable warm water fishes have been studied in detail (Ogino and Satio, 1970; Sin, 1973; Dabrowski, 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, 1991; Pongmaneerat and Watanabe, 1991; 1993; Hassan *et al.*, 1995). The optimum protein requirement of grass carp fry is 41-43% (Dabrowski, 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 balance, 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 rate and food conversion efficiency, depends on environmental conditions, age and 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 Andrews, 1973; Balarin and Haller, 1982; NAS-NRC, 1983; Renukaradhya and Varghese, 1986; Khan and Jafri, 1991).

The utilization of protein by fish is also influenced by calorific content of the diet and growth occurs only if the ration contains sufficient energy in proper ratio (Garling and Willson, 1976; Cowey and Sargent, 1977). In catla, the relationship between energy to protein is calculated as 8 Kcal/g protein (Singh and Bhanot, 1988). When isocalorific

diets were compared in *Cirrhinus mrigala* (Hassan *et al.*, 1995), better growth performance was recorded with increased protein levels.

Lee and Putnam (1973) and Cowey (1979) have stated that in fish fed at excessive protein and energy levels, surplus protein is used as energy leading to fat deposition, thereby adding to calorific value of flesh. The high calorie content in early stages may prove advantageous to fish released in the natural environment for stock replenishment (Hassan *et al.*, 1995).

Murai *et al.*(1981) and Kaushik and Dabrowski (1983) found that in carp, protein utilization was better when diets containing whole protein was used than a mixture of aminoacids. Dabrowski (1982) observed significant absorption of protein macromolecules (peptides) all along the digestive tract of common carp. Mc Lean and Donaldson (1990) reported that absorption of macro-molecules was several times higher in the stomachless carp than in fish having a functional stomach. Orally delivered peptides have been shown to be absorbed by common carp (Hertz *et al.*, 1992), although direct evidence on the *in vivo* potency of such biologically or immunologically active molecules is lacking.

Digestibility of proteins from normally used practical or purified ingredients of diets is high in all fish (Kirchgessner *et al.*, 1986; Pongmaneerat and Watanabe, 1993).

The growth depressing effect of feeding fish with levels of protein higher than the requirement, is also evident in carps (Sen *et al.*, 1978; Jayaram, 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

towards deaminaion and excretion of excessive amount of aminoacids (Khan and Jafri, 1991).

2.1.1.1. Essential amino acids

Fish, like other animals do not have a true protein requirement but has a requirement for a well 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 the following essential amino acids, viz, arginine, histidine, leucine, isoleucine, lysine, methionine, phenyl alanine, threonine, tryptophan and valine. Khan and Jafri (1993) are of the opinion that it is the amino acid profile of the diet which determines the biological value of its protein.

The importance of A/E ratios (relative proportion of Indispensable Amino Acids to that of the sum of IAA) is well established now. Even marginal deficiencies 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%.

Borlongan and Benitez (1990) stated that the more closely the essential aminoacid profile of the dietary protein resembles the amino acid 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.

Aoe *et al.* (1970), Ravi and Devaraj (1991) and Khan and Jafri (1993) have noticed poor growth when fed with amino acid free diets in common carp, catla and

rohu respectively.

Comparison of data on the whole body IAA profile with that of the requirement profile of common carp and catla shows that these two patterns are correlated (Mambrini and Kaushik, 1993). Good correlations between dietary amino acid concentrations after a meal have been observed in the carp (Zebian, 1977; Blasco *et al.*, 1991).

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

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

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

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

In early stages, the relative composition of the FAA (freely soluble amino acids)

pool showed little variation and the predominant amino acids detected were leucine, lysine, valine, isoleucine, alanine and serine (Ronnestad, 1992). The identical profiles may have resulted from the hydrolysis of a common yolk protein - phosvitin - corresponding to water uptake during swelling.

Arginine, lysine, methionine and tryptophan are the indispensable amino acids 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 as 1%, 1.2%, 0.2%, and 2% of the diet respectively in *Labeo rohita* fry. Shivananda and Varghese (1996) reported that optimum requirement of threonine for rohu as 1.71% of the dry diet which corresponds to 4.18% of the dietary protein.

2.1.2. Lipids

Lipid is a major source of metabolic energy in fish. Being highly digestible, it has greater sparing action than dietary carbohydrates or proteins (Ellis and Reigh, 1991) playing a definitive role in feed utilization. Since 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 fish diet.

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

The protein sparing effect of dietary lipid has also been investigated in several fresh water fishes (Viola and Arieli, 1982 ; 1983; Das *et al.*, 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, 1996). Two main functions of lipid in fish body are energy provision and biomembrane activity.

Berg and Storebakken (1991) and De Silva *et al.* (1991) reported no growth in fish fed a diet beyond a particular optimal protein/fat ratio. Jafri *et al.* (1995) observed a reduction in growth in *Cirrhinus mrigala* when fed with higher levels of lipid, than the optimum.

2.1.2.1. 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, remains to be established. Since fishes are incapable of *de novo* synthesis of 18:2 n-6, 18:3 n-3, 20:5 n-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 young carp 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). EFA needs 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 acids is about 0.25% of the diet and (c) a dietary supply of phospholipids is beneficial (Radunz - Neto, 1993).

Once EFA needs are met through proper lipid sources in the diet, additional fat supply increases the DE values of the diet. Optimal dietary fat levels are below 12% in the practical diets of many cyprinids (Kaushik, 1995).

Takeuchi *et al.* (1979) 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 decreased efficiency or protein retention in juvenile carp (Murai *et al.*, 1985).

Lipids are indispensable in the early stage of fish life. They are the main source of energy even from the gastrula stage in the fish embryos (Vetter *et al.*, 1983). The experimental loss in lipid reserves of larval fish on food deprivation underscores its primary role (Ehrlich, 1974; Tandler *et al.*, 1989).

It is found that the n-3 highly unsaturated fatty acids (n-3 HUFA) are required for the normal growth and survival of larval fish. Mortality and deficiency signs such as underdeveloped swim bladder have been reported in larval fish, which were low in n-3 HUFA (Kanazawa, 1985; Dhert *et al.*, 1990; Koven *et al.*, 1992). It was revealed that Eicosa Penta Enoic acid (EPA; 20:5 n-3), one of the most essential fatty acids in fish, is a constituent of the cellular membranes of several developing tissues (Kanazawa *et al.*, 1982). Kanazawa (1985) suggested that rapidly growing larval fish needs relatively large amounts of exogenous EPA.

2.1.2.2. Phospholipids

Kanazawa (1993) and Sargent *et al.* (1994) suggested the need for phospholipids in the diet of certain fish larval stages. Radunz-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% phospholipids provided better larval performance than a diet with only 1% phospholipids (Radunz -Neto *et al.* 1995) .

Phospholipids are known to act as emulsifiers in an aquaeous environment such as the intestinal lumen. According to Kanazawa (1993) they could be essential in allowing the absorption of dietary lipids like cholesterol and triglycerides. The possible function of dietary lecithin as emulsifier has also been suggested by Koven *et al.* (1993).

Kanazawa (1993) is of the opinion that fish larva has got limited ability to synthesize phospholipids at a sufficient rate to fulfil the demand for building and renewal of cellular membranes. Radunz-Neto *et al.* (1994) and Geurden *et al.* (1995) observed that larval sensitivity to phospholipid deficiency is restricted to the first 2-3 weeks of feeding only.

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

2.1.3. Carbohydrate

The carbohydrates are the cheapest source of energy. The omnivorous and herbivorous fishes adapt to utilization of high carbohydrate diets (Shimeno *et al.*, 1981). According to Furkawa and Ogasawara (1952) a 5% cellulose addition in fish diets has a favourable effect on the nitrogen retention and body growth in common carp. But Bergot (1981) and Lessel *et al.* (1986) found that cellulose is not digested at all by common carp and grass carp or gold fish.

The source and complexity of carbohydrate and the presence of carbohydrate metabolizing enzymes are known to influence carbohydrate utilization in fishes (NAS-NRC, 1983).

Furuichi and Yone (1980) and Shimeno *et al.* (1981) reported that common carp juveniles are able to utilize complex polysaccharides more efficiently than simple sugars, unlike the case of salmonids and eels.

Although some information is available on the effects of dietary carbohydrate level in Indian major carps (Sen *et al.*, 1978; Erfanullah and Jafri, 1993; 1994), the utilization of different carbohydrates by these fishes has not been investigated.

Distribution of marked amyloclistic activity has been studied in the Indian major carps, 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 carbohydrates like dextrin and alpha starch

compared to maltose or glucose in carp.This can be correlated with the presence of specific enzymes 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 herbivores (Smith, 1989). Kheyyali *et al.* (1989) reported that dietary carbohydrates promoted an increase in the activities of glycolytic and lipogenic enzymes. Das and Tripathi (1991) showed in fingerling 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, presumably 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 meals per day also improved the utilization of different sources of carbohydrates, incorporated at 30% level in common carp (Murai *et al.*, 1983).

Furuichi and Yone (1980) reported growth retardation in carp receiving 40% carbohydrate diet.

The highest weight gain obtained in *Cirrhinus mrigala* fed diet with a maximum of 34% carbohydrate indicates that this level of dietary carbohydrate inclusion is within the tolerance limit of the fish (Hassan and Jafri, 1996).

2.1.4. 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-enzymes, while myo-inositol, choline and biotin are required in higher quantities. Vitamin requirement of fish vary with species, age, size and growth rate, physiological conditions especially at times of wound healing and stress. Some fishes have the ability to synthesize vitamins from glucose substrates or aminoacids, while in some others intestinal microflora can synthesize vitamins (Hepher, 1988; Halver, 1989). These sources reduce the dependence of dietary sources for vitamins in fishes.

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

In other cyprinids, vitamin - C free diets led to retardation of growth, lordosis and scoliosis and increased mortality (Agrawal and Mahajan, 1980). Carp larvae require vitamin -C which is opposite of what has been reported for juveniles and adults of this species.

The optimum requirement of vitamin -C is found to be about 700mg/kg diet for *Cirrhinus 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.

Cowey *et al.* (1985), Satoh *et al.* (1987) and Dabrowski (1990) found a rapid drop in the ascorbic acid content during development. Dabrowski *et al.* (1988) explained that ascorbic acid is particularly essential in cyprinid larvae as yolk reserves are limited compared to salmonids.

2.1.5. Minerals

The dietary mineral requirement of fish was reviewed by Cowey and Sargent (1972) and Nose and Arai (1979). In general, minerals required by fish are calcium, magnesium, phosphorus and a number of trace elements like iron, copper, iodine, manganese, selenium, zinc, chromium, cobalt, boron and molybdenum.

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

Eventhough boron and molybdenum are shown to improve the growth in common carp (George, 1970), this species was found to be not very sensitive to the depletion of dietary mineral mixture (Ogino and Kanizono, 1975) compared to trouts.

Calcium and phosphorus 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 the skeleton, occurred in young 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 on 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 fresh water 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 60-70 mg magnesium/100g in the diet (Chiou and Ogino, 1976). For the growth, and contents of magnesium and calcium in the body, the minimum requirement for dietary magnesium was estimated as 40-50 mg /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.

Availability of phosphorus from fish meals 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 stomachless carp (Satoh, 1991).

Nose and Arai (1979) found that increasing the available phosphorus levels 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%) tri calcium phosphate reduced the absorption of zinc, manganese and of phosphorus by nearly 50% (Satoh *et al.*, 1989).

By successive deletion of several trace elements in an otherwise complete diet, Satoh *et al.* (1983) observed that deletion of manganese had a much greater growth depressing effect than of magnesium ,copper, zinc or cobalt.

Although, Mn availability from fish meals was high in carp, dietary supplementation was found to be necessary (Satoh *et al.*, 1989).

Satoh *et al.* (1983) found that Zn deficiency had much less deleterious effects in the common carp than in the rainbow trout. But excess dietary zinc will decrease the absorption of Zn by carp (Brafield and Koodie, 1991).

2.1.6. Energy

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

Precise information on the energy requirements for maintenance and growth of cyprinids is scarce. 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 Liu, 1990; Yamamoto, 1991; Chakraborti *et al.*, 1992), the basal metabolic rate- body weight (BW) relationship of unfed carp at temperatures of 23-25^{0}C was found to be well described by the following equation.

O_2 uptake (mg/fish/d)= $10.5 \times BW^{0.8}$

Weight - specific basal (resting) metabolic rates were found to decrease with a

weight exponent of – 0.20. Similar to other teleosts, both fasting metabolic rates and maintenance energy requirements of carp are affected by water temperature. Analysis of data from the literature cited above shows that the resting metabolic rates of carp at low water temperature (10-17^{0}C) are extremely low. But Schwarz and Kirchgessner (1984) found that the maintenance energy needs of common carp were reduced at low temperatures: 19 and 45 KJ DE/kg BW $^{0.75}$/ day at 10 and 20^0 C, respectively.

The reasons for such low rate at low temperatures are not clear. Dietary energy also influences the carcass composition of fat (Zeitler *et al.*, 1984). A positive correlation was noted in *Cirrhinus mrigala* between dietary energy level and carcass fat content and an inverse relationship between the calorie density and carcass protein or ash content (Hassan and Jafri, 1996). A reduction in growth rate with dietary energy level exceeding 367 K Cal/g was also noticed.

2.2. Early biology of fish related to feeding

The initial nourishment to the developing fish egg is drawn from the egg yolk. The nutrient content of the eggs vary with species, but the dynamics of yolk absorption are similar among groups. Embryonic growth in fish depends on yolk composition, its digestion by the syncytium or analogous tissue, the absorption and transport of yolk nutrients to developing tissues for somatic organization and metabolic demands for survival. An increase in the protein component with growth was observed in all the three species of Indian major carps until 48 hrs., which may be due to the morphogenetic processes undergone by the larvae (Heming and Buddington,1988). Love (1980) suggested that the increase in the levels of free amino acids during the early development

reflects the protein synthetic activity. Jurca *et al.* (1975) also observed quantitative changes in the soluble proteins during early development in three species of carps.

The decline in lipids levels with development observed in all the three species suggest that lipids are utilized to meet the energy needs of the growing larvae (Watanabe and Kiron,1994).

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

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

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

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

2.2.1. Larval nutrition in cyprinids

Potential specific growth rates and protein synthesis rates are much higher in larvae than in juveniles or adult carp (Fauconneau, 1984). Recently, multidisciplinary approaches (enzymatic activities, histocytology of the liver, DNA-RNA ratio etc.) have been developed parallel to zootechnical performance (survival, growth, food conversion ratio etc.) to assess the quality of larval feeds (Kawai and Ikeda, 1973; Dabrowski and Glogowski, 1977; Dabrowski, 1982; Storch *et al.*, 1983).

It is accepted that proteolytic activity has an influence on larval growth (Dabrowski, 1982; Ueberschar, 1988). The proteolytic activity depends on the nature of the food, the developmental stage and the species concerned. According to Dabrowski and Glogowski (1977) and Lauff and Hofer (1984), the use of natural food to start larval rearing could contribute upto 80% of the total proteolytic activity, due to proteases in the natural food.

Bryant and Matty (1981) found that common carp larvae with an initial body weight $\geq$ 9.5 mg have better ability to utilize and survive on artificial diet alone than smaller larvae. But Dabrowski (1984) observed that the larvae of cyprinids could be transferred directly to a dry diet even at a lower size (wet weight 5-6 mg).

2.3. Diets for larval fish

The scarcity of suitable planktonic prey organisms when the larvae commences exogenous nutrition, will result in body tissue autolysis and eventual death (Bagarinao, 1986). The natural diets include different types of phytoplankton, small zooplankters and invertebrate larvae. These are considered as the live food organisms for the larviculture, while the other non living larval feed stuffs are listed as the artificial feeds. The criteria in deciding the food value are basically its physical qualities such as purity, availability and nutrient/energy obtainable from it (Leger *et al.*, 1987). In addition, it should be easily procurable and economical.

2.3.1. Live feeds

The low survival of fry fed on commercial dry diet may be due to rapid degradation of the left over feed, resulting in a subsequent increase in ammonia in the rearing water. The growth of pathogenic microbes could also be enhanced in the presence of excess food (Charlon and Bergot, 1984).

A majority of cultured carps initially require a micro-zooplankton diet, then progressively feed on larger and different items as they grow in size and make a gradual transition to adult feeding habits (Jhingran and Pullin,1985).

The success of fish hatchery operation all over the world is intricately linked to the ready availability and supply of natural food; notably zooplanktonic organisms (Uhling, 1980; Dhert *et al.*,1990; Ovie *et al.*, 1993). One serious problem in nursery operations is the shortage or nonavailability of live food organisms when needed (Baldia

et al., 1985;Santiago and Reyes, 1989).

Big head and silver carp larvae start to take food when the density of living prey in the water maintained is 500 individuals/m^3 with the optimal density of zooplankton, 1000 individuals/m^3 (Szlaminska and Przybyl, 1986).

Coregonid larvae (*Coregonus schinzi*) show a certain reluctance to accept a dry diet compared with live zooplankton (Dabrowski *et al.*, 1984).

The reason why certain type of fish larvae can be reared only when fed with live plankton may be due to the fact that they depend on the digestive engymes of their prey (Dabrowski and Glogowski , 1977). According to Dabrowski (1982) many small fish larvae do not have enzymes for digesting non-living diets and initial digestion in these fish larvae may be carried out by enzymes present in their live prey. Kainz (1976) and others maintain that inadequate proteolytic enzyme activity is the cause of the feeding difficulties shown by cyprinid fish larvae with processed diets.

Several hypotheses have been proposed to explain the low effectiveness of the dry diet as the sole food supply for fish larvae. Fluchter (1982) and Abi-Ayad and Kestemont (1994) said that the different larval stages have specific nutritional needs. But according to Dabrowski and Kaushik (1982) pyrimidines present in the live food may be essential for larval fish.

It is considered that dry diets are inadequate to nourish small fish larvae during the first stages of feeding, and that such diets would be used successfully only after the larvae had been fed on live food for some time (Bryant and Matty, 1981; Charlon and Bergot, 1984).

2.3.1.1. *Artemia*

The discovery by Seale (1933) and Rollefsen (1939) that the larvae of *Artemia*, the brine shrimp, are an excellent food for fish fry represented an important stage in the development of aquaculture. This live food can be easily produced from the cysts, commercially available. The nauplii have got very good nutritional qualities. In addition, they could be used as carriers to deliver essential nutrients, pigments, prophylactics and therapeutics to fish larvae (Leger *et al.*, 1987). Considerable progress has been made on technical aspects related to its propagation and high quality cysts are readily available in the market. Since the early nauplii have the maximum food value, loosing upto 30% with age (Benjits *et al.*, 1976), they are to be judiciously utilized as food for the particular stage of the larval fish.

Bryant and Matty (1981) observed that the growth rate of *Artemia* nauplii fed carp larvae is double than that of the commercial diet-fed carp larvae . Fluchter (1980) found that protein digestion enzymes in live *Artemia* nauplii are responsible for successful rearing of white fish *(Coregonus lavaretus)* larvae. Fluchter (1982) reported that *Artemia* nauplii contain the necessary substances for the metamorphosis of the fish larvae.

Besides their high nutritional value, live *Artemia* nauplii can actively swim for up to 5 hrs. in fresh water before sinking to the bottom and dying (Hoff and Snell, 1989; Quin *et al.*, 1997).

The dead *Artemia* nauplii are eaten as readily as living ones by the fish larvae (Fluchter, 1980). This may be due to a strong element of smell, and perhaps of taste

involved in the food intake mechanism of the fish larvae.

Artemia-fed post larvae weighing between 15 and 150 mg showed optimal growth for feeding rates ranging from 100 to 150% of body weight per day, corresponding to a dry matter intake of approximately 10-15% per day (Bryant and Matty, 1980).

2.3.1.2. *Moina*

The fresh water cladoceran, *Moina* spp can be mass cultured (Ang, 1973; Ventura and Enderez, 1980; Lee *et al.*, 1983; Shim, 1988 a) and has often been used as live food for a large variety of fish larvae (Watanabe *et al.*, 1983; Buddington and Doroshov, 1984; Fermin and Recometa, 1988;Alam *et al.*,1991; Alam *et al.*,1993). *Moina macrocopa* was shown to be a suitable live food for *Lates calcarifer* fry(NICA, 1986; Fermin, 1991; Fermin and Bolivar,1994; Ganzon-Nart and Fermin, 1994). Besides having a high reproductive rate (Baldia, 1984) and high nutritive value (Watanabe, *et al.*,1983), *Moina* is also known to be a suitable live food for rearing larvae to adult tropical aquarium fish (Shim, 1988b). *Moina* is easy to propagate using animal wastes as culture medium (Ventura and Enderez, 1980; Shim, 1988a).

The content of n-3 HUFAs in the lipid of *Moina* is significantly affected by the culture medium. *Moina* cultured with poultry manure had high contents of 20:5 n-3, indicating the high nutritional value as a live food for fish (Watanabe, *et al.*, 1983). *Moina* is also found to take up emulsified lipids, making the nutritional upgrading, easy. They are also reported to form dormant cysts (ephippia) in unfavourable conditions enabling the easy propagation of the animal from dry cysts (Thressiamma *et al.*, 1991).

2.3.2. Artificial larval diets

Two decades of research on the formulation of micro diets to replace biofood in larviculture met with only limited success (Kanazawa, 1992).

Several attempts were made to develop artificial diets with a view to replace live food in the rearing of fish post-larvae (Chow, 1980; Bryant and Matty, 1981; UNDP/FAO,1983; Dabrowski and Kaushik, 1985; Csengeri and Petitjean, 1987; Swamy *et al.*, 1988; Alami.- Durante *et al.*, 1991; Jafri *et al.*,1991).

The level and balance between protein and energy in the diet are important considerations for larval diet as they influence feed consumption, digestibility, growth and nutrient utilization (Page and Andrews, 1973),and also the optimum energy level in diet as it spares proteins for growth (Prather and Lovell,1973).

Addition of Bioboost-Forte to animal protein and starch in an experimental diet enhanced its efficiency, for the fish larval growth (Mohanty *et al.*, 1996). Appreciable growth rate and 97% surival were obtained by them.

The beneficial effects of probiotics in larval rearing have been demonstrated in fishes also (Charlon and Bergot,1984; Dabrowski and Kaushik,1985; Bergot *et al.*,1986; Alami-Durante *et al.*, 1991). Probiotics stimulate digestion and alter the metabolic activities to depress methane production and ammonia level which are toxic to fish larvae.

No larval diet is commercially available for the Indian major carp spawn except the report on egg micro-encapsulated diet (Chow, 1980; UNDP/FAO,1983)which have

shown inconsistent results (Mohanty *et al.*, 1993).

Using a Swedish diet Kowtal *et al.* (1982) obtained cent percent surival and an absolute growth of 24mg, 104mg and 116mg for catla, rohu and mrigal respectively in 16 days rearing at 29-32^0 C.

Jena *et al.* (1996) obtained a surival rate of 67.8% for rohu spawn and 41.6% for catla spawn in a 16 day rearing experiment, with artificial diet having 45% protein-level. They obtained a growth of 24.4mm (152.4mg weight) for rohu and 24.6mm (133.1mg weight) for catla.

Mohanty *et al.* (1993) reported that it is possible to wean rohu spawn on a liver-based artificial diet.

Lakshmanan *et al.* (1967) revealed that rearing of carp fry on protein-rich supplementary feeds yielded a surival rate of 74.37% and 53.8% in catla and rohu respectively.

The importance of protein-rich diet in carp nutrition at different stages of growth for increase in yield was reported by Hepher and Chervinski (1965). Acceptance of inert diet particles does not constitute a problem for carp larvae (Anward *et al.*, 1976; Appelbaum, 1976; Grudnieswski *et al.*, 1979).

Jirasek (1976) reported that adaptation of carp fry to a commercial diet could only be accomplished from an initial body weight of 30mg although Anward *et al.* (1976) were able to wean carp larvae on to a dry compounded feed stuff after 3 days feeding on live food and 1 day of mixed administration.

No simple explanation of the inability of the experimental diet to support growth and surival at lower initial body weight is available (Bryant and Matty, 1981). Dabrowski *et al.* (1978) postulated that exogenous proteolytic enzymes may play a role in diet assimilation and reported a slight increase in surival of carp larvae fed diets containing bovine trypsin.

Nose *et al.* (1974) showed that diet pH can affect absorption of nutrients in juvenile carp. The fatty acid composition of larval foods is yet another scrutiny (Watanabe *et al.*, 1978).

Appelbaum (1977; 1979), using an artificial diet based largely on the yeast *Candide lipolytica*, succeeded in rearing larval common carp and grass carp without additional live food .

The importance of feeding rate in larval diet was well explained by Huisman (1979). Bryant and Matty (1981) suggested feeding rates between 15 and 17.5% for the optimum growth and feed conversion in the carp post larvae. Feeding rates of 50% per day was suggested by Anward *et al.* (1976) for carp post larvae fed on an artificial dry diet. Husiman (1979) suggested that feeding rates of 400% of body weight per day are necessary for larval grass carp, for the optimum growth and surival. Anward *et al.* (1976), Appelbaum (1977) and Dabrowski *et al.*(1978) have attempted to feed relatively elaborate diets to larvae immediately after hatching, with varying degrees of success. Girin (1979), Bromley (1979) and Beck and Bengston (1979) on the other hand, attempted by variation of diet presentation and formulation., to progressively reduce the age at which larvae can be successfully weaned on to artificial diets.

Inclusion of animal matter found to improve the quality of fish diets (Lakshmanan *et al.*, 1967; Chakraborty *et al.*, 1971;1973; Devaraj and Keshavappa, 1980; Singh *et al.*, 1980).

Csengeri *et al.* (1995) obtained 50% higher growth, than that of the zooplankton fed spawn when carp spawn was fed with frozen moistured liver power. But compared to *Artemia* nauplii, this diet gave lesser growth . Csengeri and Petitjean (1987) have successfully used pig liver powder as a starter feed for carp larvae.

Various artificial diets have been tested to partially or completely replace live foods for larvae of different species of carp (Appelbaum and Dor, 1978; Appelbaum and Uland, 1979; Dabrowski *et al.*, 1983; Szlaminska and Przylbyl, 1986; Csengeri and Petitjean, 1987; Escaffre and Kaushik, 1995).

In contrast with marine fish larvae, which are normally reared only with live food during the initial stage, carp larvae can be reared exclusively on dry feed with high surival and acceptable growth rates (Charlon and Bergot , 1984; Charlon *et al.*, 1986; Alami-Durante *et al.*, 1991).

2.3.3. Mixed diets

Live food either alone or in combination with artificial diets, consistently enhances growth and survival during the early larval phase in comparison with artificial diets(Imam and Habashi, 1972; Anward *et al.*, 1976; Jirasek, 1976; Dabrowski *et al.*, 1978; Dabrowska *et al.*,1979). The study of Lubzens *et al.* (1984) showed that common carp larvae raised on a combination of a commercial dry food and rotifers (*Brachionus plicatilis*) grew three times faster than those on artificial feed alone for 16 days .

Dabrowski *et al.* (1978) found that growth and surival of carp spawn substantially increased by feeding with zooplankton for the first 3 days , and then with formulated feed for the subsequent days.

The seven days experiment, by Szlaminska and Przybyl (1986) found better growth of common carp fry fed with a combination of encapsulated dry diet and zooplankton of mixed species, compared to those fed with the encapsulated diet alone or zooplankton alone.

Wolnicki and Gorni (1995) reported that for satisfactory larval growth of the ide (*Leuciscus idus* L.) and barbel larvae *(Barbus barbus* L.) at a high surival rate, a supply of live food in combination with a dry feed may be indispensable.

2.4. Changes in body composition

Changes in crude chemical composition - an important aspect of fish quality - result from stimulation or alteration of the turn over and the retention of the chemical components: proteins, lipids, carbohydrates and minerals with normal and altered development of the specific tissues (Fauconneau *et al.*, 1995). Body compositional changes over a growth period are partly attributed to body size and growth rates and partly to dietary factors. In general, protein content of fish (%wet matter) and aminoacid profiles (g/16g N) show little variation and are little affected by size of fish or by nutritional factors. Data obtained with carp (Schwarz and Kirchgessner, 1988; Focken and Becker, 1993) confirm this general observation.

Similarly in well growing carp , the mineral composition of the whole body is also relatively stable and little affected by dietary changes in protein and energy levels (Kirchgessner and Schwarz, 1986). On the other hand , fat content increases with increasing size as well as growth rates as affected by dietary factors and is inversely related to water content (Zeitler *et al.*, 1984; Focken and Becker, 1993). An excess supply of non-protein energy appears to induce a greater decomposition of fat even in fingerlings of common carp (Murai *et al*., 1985).

Improvement in growth and feed utilization can only be acheived through quantitative knowledge on digestibility, absorption, biological value and anti-nutritional factors of the potential ingredients, besides the recognition of possible nutrient interactions (Kaushik, 1995).

Diet has often been shown to have profound influence on body composition of fish (Brett *et al.*, 1969; Buckley and Groves, 1979; Huisman *et al.*, 1979).

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

and protein or ash.

Buckley and Groves (1979) reported that changes in level of dietary nutrients do not affect body protein and ash because their levels in body tissue are specified by the genetic code.

Jafri *et al.* (1995) observed highest protein deposition (31%) in *Cirrhinus mrigala* when fed with 7% dietary lipid. The gross energy retention increased with increasing dietary lipid levels, the maximum (78%) being in fish fed a diet containing 13% lipid (Jafri *et al.*, 1995).

The inverse relationship between body fat and moisture noted in mrigal fingerlings fed variable levels of dietary lipid was similarly reported in carp (Takeuchi *et al.*,1979; Viola *et al.*, 1988). Body constituents were found to be affected by dietary energy but influence of dietary protein was not seen (Hassan *et al.*, 1995). They further observed the negative correlation with dietary energy, irrespective of protein level in the diet. The whole body moisture followed a pattern of changes similar to that of body protein, in relation to body fat. An inverse relationship also existed between body fat and ash.The moisture levels of final fish were lower and the fat levels higher as compared with those of the initial fish. Crude fat content showed a slightly increasing trend while ash content exhibited a slightly decreasing trend (Yamamoto *et al.*, 1996).

The chemical composition of flesh mainly depends on the composition of diets and digestibility of nutrients (Dabrowski and Kozak, 1979; Jayaram and Shetty, 1980).

An inverse relationship between moisture and protein is observed in catla (Nandeesha *et al.*, 1989) and between moisture and fat in tilapia (Jauncey, 1982; Edwards *et al.*, 1985).

During ontogeny of carp, there is an early increase in the protein content of the whole body, carcass and muscle (Takeuchi *et al.*, 1979; Hossain and Jauncey, 1989). Only minor changes are observed in that component even if carp are fasted (Shcherbina and Griyayev, 1990; Shimeno *et al.*, 1990) or fed a deficient or imbalanced diet (Zeitler *et al.*, 1984; Venugopal and Keshavanath, 1984; D' Mellow *et al.*, 1989; Viola *et al.*, 1992).

The amino acid composition of protein synthesized at different ages had been found to be very similar by Zeitler, *et al.* (1984).

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

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

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

Senappa and Devaraj (1995) observed a protein loss in the body carcass with

different diets, however percentage loss decreased with increase in dietary protein level. Ash accretion increased in the carcass, in all the diets.

Santiago and Reyes (1991) reported increased moisture in the body carcass of big head carp fed diets with higher protein and no clear variation in fat and ash accretion was associated with the diets.

The moisture content in the body carcass of catla showed no change from initial values in different diets. Only protein loss was significant and weight gain was mainly associated with increased ash and fat in the body carcass (Senappa and Devaraj, 1995).

The fatty acid composition of the fillet is determined by dietary fatty acids (Takeuchi and Watanabe, 1977; Kanazawa *et al.*, 1980; Mukhopadhyaya and Rout, 1996).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The experiment was designed to evaluate the efficiency of different diets and their combinations on the growth and survival of *Labeo rohita* spawn, indoors under high stocking density.

3.1. Materials

3.1.1. Experimental animals

Three day old rohu spawn collected from the jar hatchery of the College of Fisheries, Panangad with an average length of 6 mm (weight 3.5mg) were used for the experiment. Healthy spawn of uniform size were selected for the experiment.

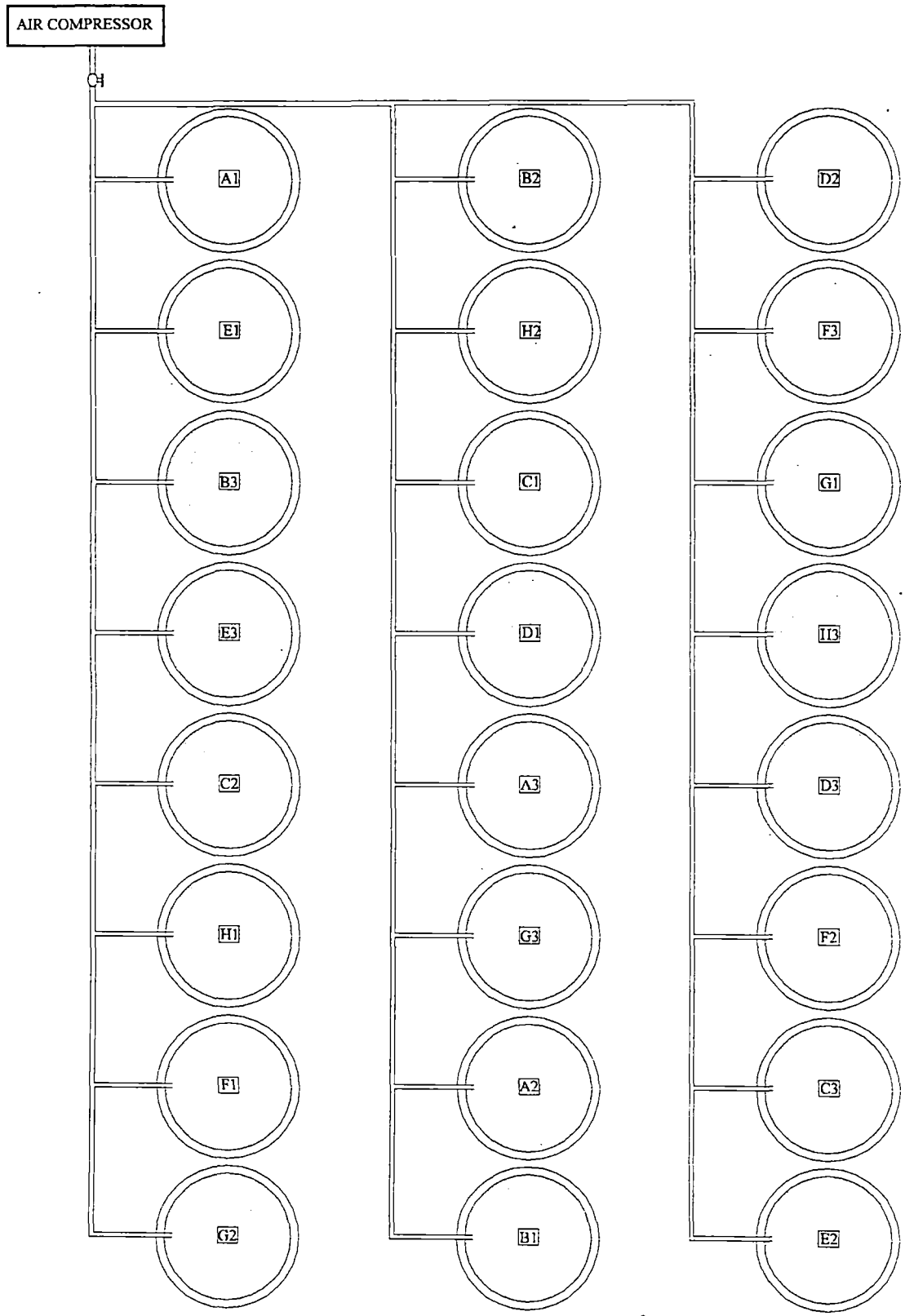
3.1.2. Experimental tanks

Circular FRP tanks of 83 l capacity with 0.54 m diameter and 0.34m height filled to a height of 0.18m so as to get an effective water volume of 40 l were used for the experiment. Air from an air compressor channelled through P.V.C. pipes and diffused using tubings and air stones was used for continuous aeration of the water. Air regulators were used to control the air flow in the tanks. The lay-out of the experimental tanks is given in the Figure 1.

3.1.3. Live feeds

The live feeds used for the experiment were newly hatched *Artemia* nauplii, mass cultured *Moina micrura* and mixed zooplankton.

Figure 1. Lay- out of the experimental set up



3.1.3.1. *Artemia* nauplii

Bio marine brand Artemia cyst was used. The cyst was incubated and hatched to nauplii in 20 ppt saline water in cylindroconical FRP tanks. Nauplii were harvested, after 18-24 hours,using standard techniques (Sorgeloos, 1976).

3.1.3.2. *Moina micrura* Kurz

Moina was cultured in outdoor concrete tanks with a size of 1m diameter and 1m height. The original stock for inoculation was isolated from a fresh water pond, and culture carried out using technique developed by Thressiamma *et al.* (1991).

Periodic harvesting was done with a No.8 plankton net and the freshly harvested *Moina* passed through a 300 micron mesh sieve to get animals of smaller size only.

3.1.3.3. Conventional Feed

A feed containing powdered GOC and rice bran was used in one treatment together with mixed zooplankton, containing *Daphnia, Moina,* Copepods and Calanoids (conventional method). The mixed zooplankton collected from a pond prepared and manured in the usual conventional way for nurseries (Jhingran,1991), using the same No.8 plankton net, was quantified qualitatively and quantitatively before being fed to the experimental animals.

The different diets used for the experiment are listed in the Table 1.

3.2. Methods

3.2.1. Formulated feed

A micro-bound diet which was successfully tried for rohu spawn rearing by Nair *et al.* (1989) was used in the present experiment.

3.2.1.1. Feed ingredients

The necessary ingredients viz. clam meat, tapioca and GOC were purchased from the local market and powdered after sufficient drying. They were then passed through a 50 micron sieve and stored in air tight plastic containers for use.

3.2.1.2. Proximate composition of feed ingredients

Proximate composition of all the feed ingredients except the vitamin mixture and the antibiotic was analysed prior to feed formulation. Estimation of the moisture level was done by Boyd's (1979) method. The crude protein content was estimated by Microkjeldahl's method. (AOAC, 1990). The nitrogen content was then multiplied by the factor 6.25, to arrive at the crude protein content. Crude fat was extracted using petroleum ether (B.P. 40-60^{0}C) in a Soxhlet extraction apparatus for 16 hours. Method of Pearson (1977) was used to estimate the crude fibre. The ash content was estimated by burning the sample at 550^{0}C ± 10^{0}C for 6 hours in a muffle furnace. The carbohydrate content was found out by Hastings' (1979) difference method as Nitrogen Free Extract, using the following formula.

NFE = (100 - % crude protein on dry weight basis + % crude fat on dry weight basis + % crude fibre on dry weight basis + % ash).

3.2.1.3. Formulation and preparation of test diet

The test diet was formulated by keeping its protein level constant at 40%. The proportion of various ingredients used for the formulation of the test diet is given in Table 2.

The ingredients (except vitamin mix and the antibiotic) for the test diet were mixed thoroughly and the mixture then hand kneaded using water (1:1.25 W/V) to get a soft dough. It was autoclaved at ambient pressure for 30 minutes, cooled, and then the vitamin mixture to a level of 1.5 % and the antibiotic to a level of 0.15% were added. The dough was again mixed thoroughly, and then pelletized and dried in hot air oven at 60 °C, till the moisture content became less than 10% (over night). The pellets were then ground and passed through a 300 miron mesh seive. It was then kept in an air tight plastic container and stored free from moisture and sunlight. The size of the formulated feed particles ranged from 50-300 microns.

3.2.1.4. Proximate composition of the formulated feed

Immediately after the preparation of the feed, its proximate composition was analysed, using techniques similar to that used for the ingredients.

3.2.1.5. Keeping quality of formulated feed

The proximate composition of the feed was again analysed after 21 days storage.

3.3. Study to evaluate the efficacy of different feeds

The total length of the rohu spawn was measured to the nearest millimetre from tip of the snout to the tip of the tail. The spawn were then blotted dry carefully between

folds of filter paper and weighed in a Shimadzu, Japan electronic balance having a sensitivity of 0.01 mg. A lot of 490 numbers of spawn were collectively weighed and dried in an oven at 40^0C for 48 hours, to carry out the initial biochemical composition.

400 numbers of rohu spawn were then introduced into each tank at a stocking density of 10,000/m^3. They were given eight different types of diets, as mentioned in the Table 1. Each feed was replicated thrice. In total, there were 24 tanks; 3 replications and 8 treatments.

Feeding was done *ad libitum* at 6 hourly intervals daily. When the combination of live feed and artificial feed was given, the feeds were alternated starting with live feed at 6 AM. The left over feed and the excreta were siphoned off from the bottom every day,just before the first feeding by using a PVC tube of 4 mm. diameter, after stopping the aeration and allowing the waste to settle down at the bottom.

3.4. Water quality management

Water temperature of the experimental tanks was monitored daily in morning and evening hours using a mercury thermometer of accuracy of 0.1^0C. The pH of the water was measured once in a day, with indicator solution. The dissolved oxygen content (by Winkler's method), Ammonia nitrogen content (by Phenol-hypochlorite spectrphotometric method) and total hardness (by EDTA-Eriochrome black-T indicator method) of the water were also determined at intervals of 5 day.

3.5. Recording of observations

The observations were recorded and the survival rate, specific growth rate and normalised biomass index (NBI) determined as shown below.

Table 1. Different diets used for the experiment

1.	Rice bran and G.O.C in 1:1 Ratio + mixed zooplankton	**(A)**
2.	Size graded *Moina micrura*	**(B)**
3.	Newly hatched *Artemia* nauplii	**(C)**
4.	Formulated feed	**(D)**
5.	*Moina* + formulated feed	**(E)**
6.	*Artemia* + formulated feed	**(F)**
7.	First three days *Moina* and formulated feed later on	**(G)**
8.	First three days *Artemia* nauplii and formulated feed later on	**(H)**

Table 2. Proportion of various ingredients in the formulated feed

Ingredients	Weight (g/100g feed)
Clam meat	56
Egg	9
G.O.C.	30
Tapioca powder	5

3.5.1. Survival rate

$$\% \text{ survival} = \frac{\text{Initial number-final number}}{\text{Initial number}} \times 100$$

3.5.2. Growth

$$\text{Growth } \% = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \times 100$$

3.5.3. Specific growth rate (SGR)

$$SGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \times 100$$

Where W_1 = weight at time t_1; W_2=weight at time t_2.

It gives the average percentage increase in body weight per day of fry over the experimental period.

3.5.4. Normalised biomass index (NBI)

Normalized biomass index was calculated using the formula of Beck (1979) to get the combined effect of growth and survival.

$$NBI = \frac{(Wf \times Nf) - (Wi \times Ni)}{100}$$

Wf = Final av.weight in mg

Nf = final number

Wi = Initial av.weight in mg

Ni = Initial number

3.6. Biochemical analysis of carcass

Proximate composition of the carcass of fry was done after 21 days of growth, to determine the best diet, using the standard procedures, as described by Nair and Sherief (1993).

3.7 Statistical Analysis

The experiment was conducted using the Completely Randomized Design with three replications for each of the eight tretments. The results were analysed using the Analysis of Variance technique. Wherever necessary Arc sine transformations were made before analysing the data. Pair wise comparisons were also made using the Critical Difference method (Snedecor and Cochran, 1968).

RESULTS

4. RESULTS

4.1. Proximate composition of feed ingredients and experimental diets

4.1.1. Feed ingredients

The proximate composition of ingredients used in the formulation of the artificial diet is given in Table 3.

4.1.2. Experimental diets

The proximate composition of the different diets is given in the Table 4.

No significant loss of nutrients was observed in the case of formulated feed, after the storage of 21 days.

Table 3. P roximate composition of feed ingredients (on dry weight basis)

Ingredients	Crude% protein	Fat%	Ash%	Fibre%	N.F.E.%
Clam meat	55.85	10.57	8.72	2.95	21.91
Egg	26.35	67.83	2.75	-	3.07
G.O.C	35.72	7.78	6.42	3.92	46.16
Tapioca	7.67	0.99	6.14	2.39	82.81

Table 4. Proximate composition of the test diets

Contents %	✡ *Artemia* nauplii	✡ *Moina*	Formulated feed
Crude Protein	60.9	68.75	40.29
Crude lipid	19.09	22.6	12.97
Carbohydrate	-	-	28.55
Crude fibre	-	-	2.15
Ash	11.5	1.48	6.64
Moisture	-	-	9.4

✡ Source: Cresswell, R.L. 1993

4.2. Water quality management

4.2.1. Temperature

The water temperature ranged from 27.1 to 31.8^{0}C. There was no significant variation in temperatures of different treatments.

4.2.2. pH

The pH of the water fluctuated from 6.5 to 7.5, during the study. There was no significant difference in pH among the experimental tanks.

4.2.3. Dissolved oxygen

The dissolved oxygen content of water varied from 9.8 to 10.9 ppm. There was no significant difference in dissolved oxygen content among tanks, as continuous aeration was provided.

4.2.4. Total hardness

The total hardness of water recorded was between 40-60 ppm. This range was more or less the same in different tanks.

4.2.5. Ammonia

The ammonia content of water varied from 0.24 to 0.44 ppm. There was no significant difference in ammonia content among different tanks.

4.3. Efficiency of different diets

4.3.1. % Survival

The % survival of the spawn fed with different diets are given in Table 5. Analysis of variance of the data is given in Table 9.

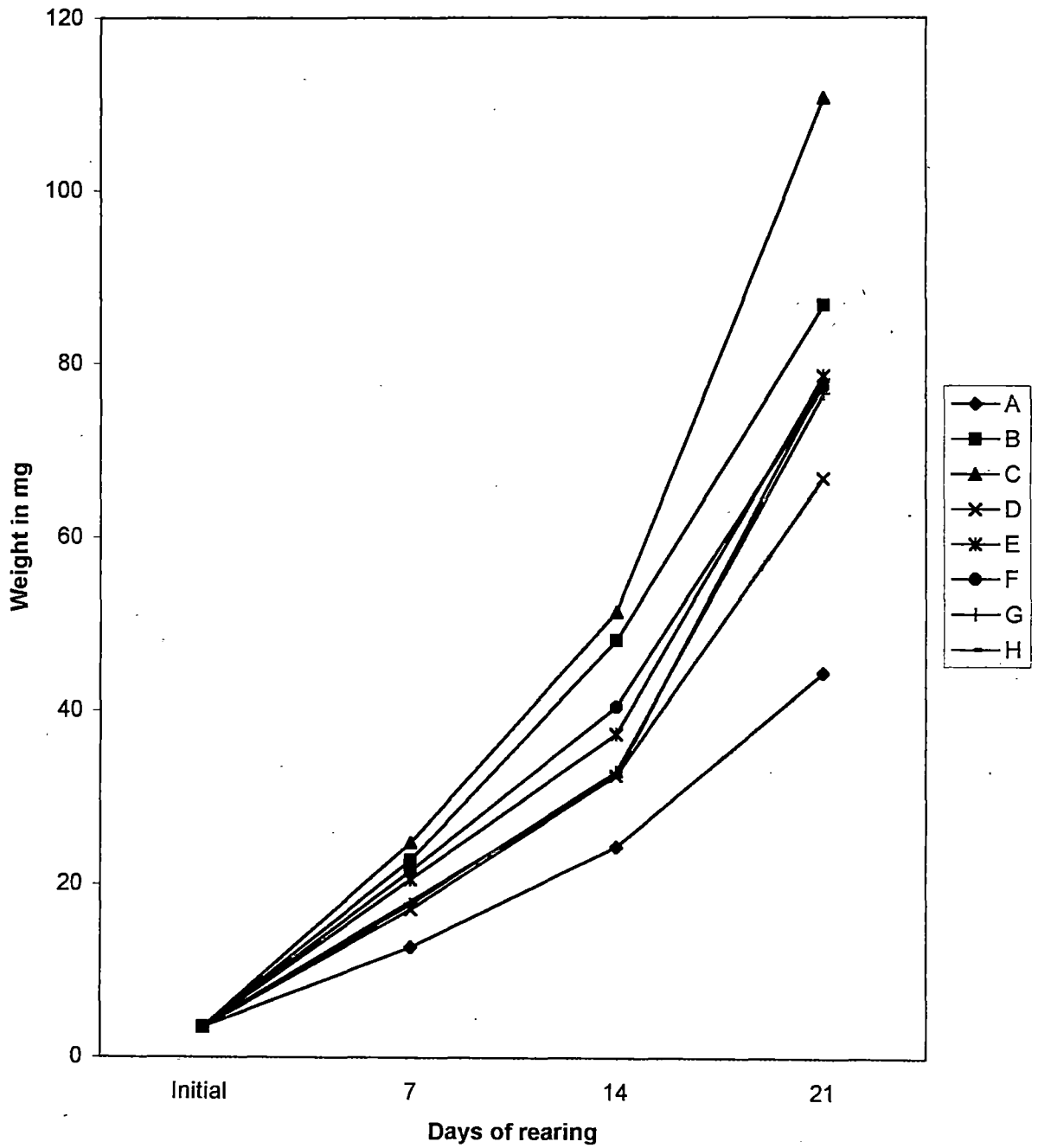
4.3.2. Specific growth rate

The mean specific growth rates of the spawn fed with different diets are given in Table 6. Analysis of variance of the data is given in Table 10. The increase in weight of the spawn fed with different diets are graphically represented in Figure.2.

4.3.3. Normalized biomass index

The NBI, which gives the combined effect of survival rate and growth of the spawn fed with different diets are given in Table 7. Analysis of variance of the data is given in Table 11.

Figure 2. Effect of different diets on the growth rate of the rohu spawn



4.4. Biochemical composition of the carcass

The biochemical composition of fish fed with different diets are given in Table 8.

Table 5. Percentage Survival of the spawn fed with different diets

Diets	Replication	% Survival
(A)	1	46
	2	48
	3	47
(B))	1	78
	2	84
	3	81
(C)	1	80
	2	83
	3	85
(D)	1	77
	2	75
	3	79
(E)	1	88
	2	85
	3	93
(F)	1	89
	2	90
	3	86
(G)	1	81
	2	83
	3	80
(H)	1	82
	2	80
	3	80

Table 6. SGR of the spawn fed with different diets

Diets	Replication	SGR
(A)	1	12.0436
	2	12.4189
	3	11.8215
(B)	1	15.2842
	2	15.2622
	3	15.3497
(C)	1	16.4694
	2	16.4565
	3	16.4220
(D)	1	14.0426
	2	14.1344
	3	13.9780
(E)	1	14.8111
	2	14.8474
	3	14.8232
(F)	1	14.7378
	2	14.8353
	3	14.6758
(G)	1	14.7131
	2	14.7254
	3	14.6758
(H)	1	14.8232
	2	14.8474
	3	14.7131

Table 7 . NBI of the fry fed with different diets

Diets	Initial No.	Final No.	Initial av. wt . (mg)	Final av.wt. (mg)	NBI	Mean NBI
	400	160	3.5	43.9	56.2400	
A	400	192	3.5	47.5	77.2000	66.0707
	400	188	3.5	41.9	64.7720	
	400	312	3.5	86.7	256.5040	
B	400	366	3.5	86.3	275.9680	267.7560
	400	324	3.5	87.9	270.7960	
	400	320	3.5	111.2	341.8400	
C	400	332	3.5	110.9	354.1880	352.1227
	400	340	3.5	110.1	360.3400	
	400	308	3.5	66.8	191.7440	
D	400	300	3.5	68.1	190.3000	192.0960
	400	316	3.5	65.9	194.2440	
	400	352	3.5	78.5	262.3200	
E	400	340	3.5	79.1	254.9400	265.3413
	400	372	3.5	78.7	278.7640	
	400	356	3.5	77.3	261.1880	
F	400	360	3.5	78.9	270.0400	259.9000
	400	344	3.5	76.3	248.4720	
	400	324	3.5	76.9	235.1560	
G	400	332	3.5	77.1	241.9720	235.7627
	400	320	3.5	76.3	230.1600	
	400	328	3.5	78.7	244.1360	
H	400	320	3.5	79.1	239.1200	238.4453
	400	320	3.5	76.9	232.0800	

Table 8. Carcass composition of *Labeo rohita* fry fed with different diets

	Initial	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)
% Moisture	86.70	84.61	81.02	80.13	82.78	81.26	81.32	82.18	82.29
* % Crude Protein	64.18	66.12	67.27	67.28	66.11	67.20	67.31	67.01	66.18
* % Crude Fat	17.43	18.30	20.98	21.01	20.05	20.84	20.90	20.32	20.28
* % ash	15.13	11.65	9.52	9.31	10.47	9.64	9.38	9.29	9.41

* On dry weight basis

Table 9. Analysis of variance of the data of % survival (Arc sine – transformed values) of the spawn fed with different dieats

Source of Variation	d.f.	S.S.	M.S.S.	F - ratio
Diets	7	1525.6242	217.9463	
Error	16	60.6138	3.7884	57.5299 **
Total	23	1586.238		

Comparison of Treatment Means (Critical Difference : 3.369)

Treatments	Mean
(A)	43.28
(D)	61.36
(H)	63.92
(B)	64.20
(G)	64.41
(C)	65.43
(F)	70.07
(E)	70.53

Treatments connected with lines are not significantly different.

**Significant at 1% level

Table 10. Analysis of variance of the data of SGR of the spawn fed with different diets

Source of Variation	d.f.	S.S.	M.S.S.	F-ratio
Diets	7	31.8141	4.5449	
Error	16	0.2252	0.0141	322.33**
Total	23	32.0393		

Comparison of Treatment Means (Critical Difference : 0.2055)

Treatments	Mean
(A)	12.09
(D)	14.05
(G)	14.70
(F)	14.75
(H)	14.79
(E)	14.83
(B)	15.30
(C)	16.45

Treatments connected with lines are not significantly different.

**Significant at 1% level

Table.11. Analysis of variance of the data of mean NBI of the spawn fed with different diets

Source of Variation	d.f.	S.S.	M.S.S.	F-ratio
Diets	7	140162.5	20023.214	
Error	16	1287.2	80.95	248.89 **
Total	23	141449.7		

Comparison of Treatment Means (Critical Difference : 15.53)

Treatments	Mean
(A)	66.07
(D)	192.10
(G)	235.76
(H)	238.45
(F)	259.90
(E)	265.34
(B)	267.76
(C)	352.12

Treatments connected with lines are not significantly different.

**Significant at 1% level

DISCUSSION

5. DISCUSSION

5.1. Protein requirement in carps

The optimum protein requirement for catla fry was found to be 30% by Renukaradhya and Varghese (1986) and Senappa and Devaraj (1995). Khan and Jafri (1991) found that catla requires an optimum protein content of 40%. The same value was suggested by Swamy and Mohanty (1988), Mohanty *et al.* (1988) and Dabrowski and Kozak (1979) for the fry of rohu, mrigal and grass carp respectively. The optimum protein requirement for common carp and rohu was found to be 45% by Sen *et al.*(1978), Singh *et al.* (1980) and Jena *et al.* (1996). Mohanty *et al.* (1990) found that a level of 45% protein in the diet inhibited growth in rohu. In the present study, the formulated feed was prepared with 40% crude protein which was found successful for the rohu spawn rearing by Nair *et al.* (1989).

5.2. Water quality parameters

Labeo rohita can tolerate reasonably wide ranges of water quality parameters. The fluctuations of temperature, pH, Dissolved oxygen, Ammonia and total hardness were within the optimum limits of the species. The water quality parameters prevailed in the present experiment were more or less the same which prevailed in the study by Nair *et al.* (1989). So these rearing conditions did not seem to affect the growth of the fish.

5.3. Evaluation of different feeds

5.3.1. Based on growth

Maximum mean weight gain was observed for the fry fed on *Artemia* nauplii alone, followed by fry fed on *Moina* alone (110.7 and 86.69 mg respectively). The efficiency of *Artemia* nauplii as a spawn feed was already established by Anward *et al.* (1976).

The mean weight gain of spawn fed with formulated feed alone was 66.9 mg. Better mean weight gains were observed when *Artemia* nauplii and *Moina* were incorporated in the diet with the formulated feed (77.5mg with *Artemia* nauplii and 78.7mg with *Moina*). The corollary for this information appeared in the works by Anward *et al.* (1976); Jirasek (1976); Dabrowski *et al.* (1978); Dabrowska *et al.* (1979); Lubzens *et al.* (1984) and Szlaminska and Przybyl (1986). Significantly similar mean weight gains were observed when the spawn were fed with live food for the first three days and with formulated feed later (76.7mg for *Moina* + formulated feed and 78.2mg for *Artemia* nauplii + formulated feed). This is in conformity with Dabrowski *et al.*(1978), in common carp larvae. But the growth observed by Mohanty *et al.* (1993) for rohu spawn fed on an artificial feed alone could not be realised with any diet, in the present experiment. They got an average wet weight of 133.33 mg, with a formulated feed, in 21 days of rearing. The lowest weight gain in the present experiment was noticed in the fry fed by conventional method (44.4 mg).

5.3.2. Based on SGR

Maximum mean SGR was noticed in the fry fed with *Artemia* nauplii,followed by *Moina* (16.45 and 15.30 respectively). All other treatments except the conventional method have given significantly similar SGR of about 14. The SGR noticed by Mohanty *et al.* (1996) was a little higher (17.19) than the presently observed maximum mean SGR. Jena *et al.* (1996) could realise a still higher SGR of 27.52 for this species.

5.3.3. Based on survival

Maximum mean % survival could be realised for the spawn fed on a mixed diet of *Moina* and formulated feed and *Artemia* and formulated feed (88.6 and 88.3% respectively). The *Artemia* nauplii fed spawn has given a mean % survival of 82.6, while *Moina*-fed ones have given 81. The mean % survivals were 81.3 and 80.6 when the fry were fed with *Moina* for first three days and formulated feed later, and with *Artemia* nauplii for first three days and formulated feed later, respectively. The fry fed with formulated feed alone, has given a mean % survival of 77. This survival rate is higher than the rate which was reported by Nair *et al.* (1989) with the same feed (72.5%). In the conventional method of rearing, the mean % surival was only 47%. In earlier works the mean % survivals of 100 (for rohu, by Mohanty *et al.*, 1993), 98-100 (for common carp, by Basavaraja and Antony, 1997), 87 (for catla, by Sinha and Ramachandran (1985), and 73.4 (for common carp, by Jain, 1989). Jhingran *et al.* (1979) reported 84-96% survival for the carp larvae in recirculatory filtering ponds.

5.3.4. Based on NBI

Maximum mean NBI could be obtained for the spawn fed on *Artemia* nauplii alone followed by the *Moina* fed ones(352.12 and 267.76 respectively). Statistically similar mean NBI values were recorded for the spawn fed with the live feed + formulated feed. A mean NBI of 192.09 was obtained with the formulated feed alone. Lowest value was noted for the spawn reared by the conventional method.

5.3.5. Based on carcass composition

When carcass of the fry fed with different diets was analysed, it was seen that maximum fat deposition occurred in those fed with *Artemia* nauplii followed by the spawn fed with *Moina* (21.01 and 20.98% respectively). Increased fat deposition was noted in all the treatments, than the initial value, the minimum increment being in the case of conventional method. Crude protein content also showed an increasing trend in all the treatments, the maximum being 67.31% (for spawn fed with *Artemia* nauplii + formulated feed) and the minimum being 66.12% (for conventional method). The moisture content and the ash content showed a declining trend in all the treatments. A negative correlation was observed with the moisture and ash contents with the crude fat content of the carcass.

Hashim *et al.* (1992) observed higher carcass protein and fat,when high protein food was offered, to the fish. This fact was proved in the present study also. Decrease in body moisture and ash with inoreasing dietary energy, noticed in the present study confirms with the result in tilapia, cat fish and carp (Winfre and Stickney, 1981; Garling and Wilson, 1976 and Zeitler *et al.*, 1984).

In nursery rearing both growth and survival are important. In the present experiment, maximum mean weight gain was obtained when the spawn was fed with live feed alone, followed by the ones fed with a combination of live feed and artificial feed. But maximum mean % survival was obtained with the spawn fed with a mixed diet of live feed and formulated feed. The higher growth obtained for the spawn fed with live feed alone may be due to the comparative low survival rates in these tanks than the mixed diet - treatment tanks.

SUMMARY

6. SUMMARY

The objective of the present study is to develop a reliable technique for the rearing of rohu spawn under controlled conditions. The methodology, results and conclusions of the study are given below.

1. Three day old rohu spawn collected from the hatchery of the College of Fisheries, Panangad was used for the experiment.

2. Spawn rearing was carried out in round fibre glass tanks of 83 l capacity with a stocking density of 10,000/m^3.

3. Different diets viz. newly hatched *Artemia* nauplii, size graded *Moina micrura*, formulated feed, *Artemia* nauplii + formulated feed, *Moina micura*+ formulated feed, first 3 days *Artemia* nauplii and formulated feed later on, first 3 days *Moina micrura* and formulated feed later on and mixed zooplankton+ricebran and GOC in 1:1 ratio (conventional method), were given to the spawn.

4. The formulated diet had in its composition 40.29% protein, 12.975% fat, 6.64% ash, 2.155 % fibre, 28.55 % NFE as carbohydrate and 9.4% moisture.

5. Each test diet was replicated thrice.

6. The duration of the experiment was 21 days, after which survival rate, specific growth rate and normalized biomass index were worked out. Biochemical analysis of the carcass was also carried out.

7. The experiment was conducted using CRD and results were analysed by

ANOVA technique.

8. Maximum mean SGR of 16.45 was recorded for the spawn fed with *Artemia* nauplii alone, followed by 15.30 with *Moina* alone.

9. All other treatments except the conventional method (12.09) have given significantly similar SGR of about 14 .

10. Maximum mean survival of 88.6% was obtained with *Moina*+formulated feed closely followed by 88.3% with *Artemia*+formulated feed.

11. The *Artemia* nauplii and *Moina* fed spawn has given mean % survival of 82.6 and 81 respectively. The mean % survivals were 81.3 and 80.6 when fed with *Moina* for first three days and formulated feed later, and with *Artemia* nauplii for first three days and formulated feed later, respectively.

12. The fry fed with formulated feed alone has given a mean % survival of 77.

13. Maximum mean NBI of 352.12 was obtaind with *Artemia* followed by 267.76 with *Moina.* Statistically similar mean NBI values were observed for the spawn fed with live feed + formulated feed.

14. Maximum fat deposition (21.01%) was noted in the spawn fed on *Artemia* nauplii followed by the spawn fed with *Moina* (20.98%).

15. The fat deposition in other treatments were more or less the same (20.05 to 20.90%) except in the case of conventional method (18.3%).

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ABSTRACT

HIGH DENSITY REARING OF *LABEO ROHITA* (HAMILTON) SPAWN INDOORS USING DIFFERENT FEEDS

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Three day old rohu spawn was reared in indoor tanks with eight different feeds. The diets tried were newly hatched *Artemia* nauplii, size graded *Moina micrura*, formulated feed, *Artemia* nauplii + formulated feed, *Moina micura*+ formulated feed, first 3 days *Artemia* nauplii and formulated feed later on, first 3 days *Moina micrura* and formulated feed later on and mixed zooplankton + ricebran and GOC in 1:1 ratio (conventional method). The experiment was carried out in circular FRP tanks of 83 l capacity with a stocking density of 10,000/m^3. The duration of the experiment was 21 days. The diets of *Artemia* nauplii and *Moina* have given higher mean SGR (16.45 and 15.3 respectively) and NBI (352.12 and 267.76 respectively) without any significant statistical difference, while *Moina*+Formulated feed and *Artemia*+formulated feed have given higher mean survival rates (88.6 and 88.3% respectively). Highest fat deposition was noticed in *Artemia* fed spawn (21.01%) followed by *Moina* fed ones (20.98%). The conventional method of spawn rearing was found to be highly ineffective.