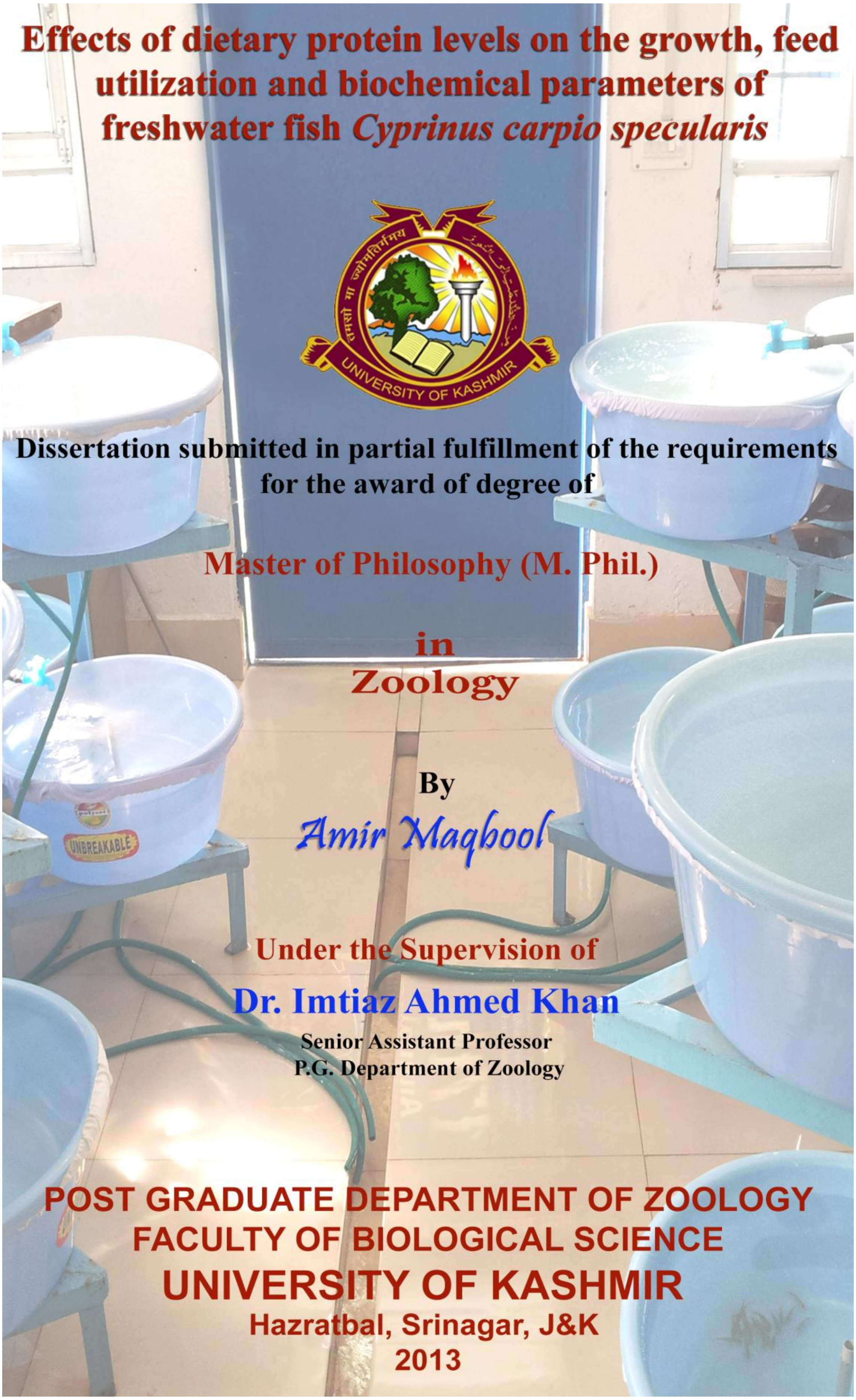

Effects of dietary protein levels on the growth, feed utilization and biochemical parameters of freshwater fish *Cyprinus carpio specularis*

Dissertation submitted in partial fulfillment of the requirements for the award of degree of

Master of Philosophy (M. Phil.)

in
Zoology

By

Amir Maqbool

Under the Supervision of

Dr. Imtiaz Ahmed Khan

Senior Assistant Professor
P.G. Department of Zoology

POST GRADUATE DEPARTMENT OF ZOOLOGY
FACULTY OF BIOLOGICAL SCIENCE
UNIVERSITY OF KASHMIR
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(Dr. M. N. Azim)
Professor & Head

CERTIFICATE

This is to certify that the Dissertation entitled **'Effects of dietary protein levels on the growth, feed utilization and biochemical parameters of freshwater fish *Cyprinus carpio specularis*'** has been completed under my supervision by **Mr. Amir Maqbool.** The work is original and independently pursued by the candidate. It embodies some interesting observations contributing to the existing knowledge on the subject.

The candidate is permitted to submit the work for the award of degree of **Master of Philosophy** in **Zoology** of the University of Kashmir, Hazratbal, Srinagar (J&K) India.

(Dr. Imtiaz Ahmed Khan)
Research Guide
Senior Assistant Professor
Department of Zoology

(Prof. M. Nayyar Azim)
Head
Department of Zoology

DEDICATED

TO

MY PARENTS

ACKNOWLEDGEMENTS

In the name of Allah, The most gracious, most merciful

On the very inception, I bow in reverence before almighty Allah and thank him whole heartedly for showering his grace on this ordinary mortal & blessing me with the honour of completing this dissertation & presenting it in its present form.

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Amir Maqbool

List of Contents

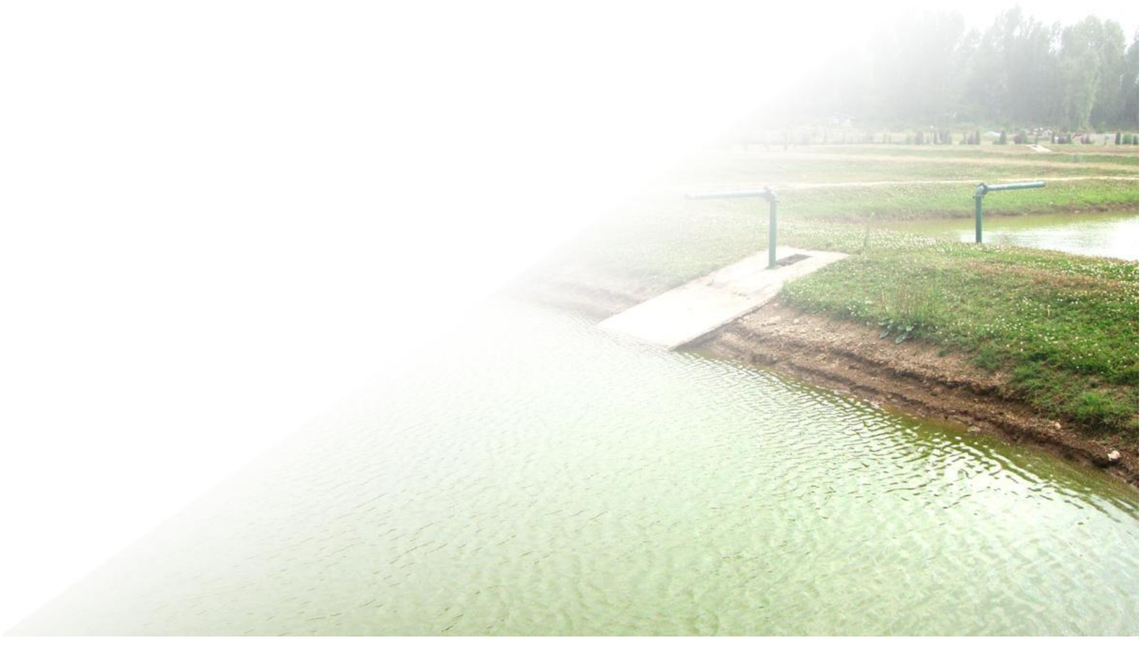
List of Plates

List of Tables

List of Figures

CHAPTER - 1

Introduction



Introduction

Today, due to enormous increase in human population, malnutrition has become a major threat to the world. Millions of people are going without proper food. The problem of feeding the teeming million mouths and maintaining self sufficiency in food by increasing the production has been considered as one of the basic point of the national policy under different plan periods. It has been remarked that science must help us to speedily improve production, the responsibilities of the scientists and technologists have therefore, increased manifolds to cope up with the situation and to develop standard technologies which can be used to exploit the natural resources in order to obtain a good quality food for the people of our nation, because natural and human resources can be profitably developed and equally shared to create more employment and reduce drudgery, to strengthen our nation and reduce vulnerability.

Among all the natural resources available for human nutrition, fish is not only a source of high quality protein and fat but it also has other qualities that make it very valuable as a nutrient. Fish is highly nutritious, tasty and easily digested. It is much sought after by a broad cross-section of the world's population, particularly in developing countries. The global contribution of fish as a source of protein is indeed high, ranging from 10% to 15% of the human food basket across the world. It is estimated that around 60 percent of people in many developing countries depend on fish, for over 30 percent of their animal protein supplies (FAO, 2005).

Fish products are comparable to meat and dairy products in nutritional quality, depending on the methods used in preservation and preparation. Proteins are important for growth and development of the body, maintenance and repairing of worn out tissues and for production of enzymes and hormones required for many bodily functions. The protein content of most fishes averages 15 to 20% on wet weight basis (Murray and Burt, 1983). Fish also contains significant amounts of all essential amino acids, which are not available in plant protein sources and the digestibility of fish is approximately 5-15% higher than the plant-source foods (WHO, 1985). Moreover, the sensory properties of an otherwise balanced diet can be enhanced through fish products, thus facilitating and contributing to greater consumption.

The contribution of fish to human nutrition and its impact on health has been examined from different aspects in both developed and developing countries. In developing countries, under nutrition and micronutrient deficiencies are the main public health problems related to nutrition. To improve nutritional status of moderately undernourished children, it is estimated that approximately one-third of protein should be provided by animal-source foods in the diet, so that the animal source foods can be fully utilized to compensate the shortage of essential nutrients especially amino acids in staple foods thereby, having a significant impact on their growth (Michaelsen *et al*., 2009). In this respect, fish is more affordable and accessible animal source food. Besides quality proteins, fishes are also rich in micronutrients, especially iron, calcium, zinc and vitamin A and D.

The diet of an average Indian is very low in calories and the animal protein component in the diet is alarmingly low. Hence, there is an urgent need to increase animal protein in the daily diet of the Indian population. In the face of ever enlarging pressure on food from a combination of factors, a compelling need has arisen to utilize all available options for bioproduction, of which one of the readily available options is aquaculture. It has high potential to provide more protein, more acceptable food, more calories and to better the feeding habit of a segment of the population. During the latter part of the 20th century, aquaculture has gained greatest attention of the whole world due to its innate advantages. New species are being cultured, new technologies for more intensive culture have been introduced, a large research base has been established and commercial investment is being attracted into aquaculture. Thus aquaculture is now recognized as a viable and profitable enterprise worldwide and will continue to grow and supply an increasingly larger percentage of fishery products consumed. This is assured because supply, price and quality of marine fish fluctuate considerably, as the ocean is inadequately managed and its yield is unpredictable. But when fish are cultured, supply can be regulated more effectively. With the present technology and research base, yield and risks for a number of aquaculture enterprises are now predictable, which makes them attractive investment opportunities.

In recent years, the aquaculture industry has grown rapidly to become an internationally important source of food and income. Aquaculture is among the food production activities with the highest sustainable growth up to 9% per year (FAO, 2010). In fact, the farming of fish and shellfish for food now supplies approximately 50% by weight of all the fish and shellfish consumed worldwide. Global aquaculture output has more than quadrupled since 1985, and more than doubled in the last decade, reaching 62.9 million tonnes in 2005 (FAO, 2007). The latest FAO report on the state of the world fisheries and aquaculture (FAO, 2010) indicates a global aquaculture production of 55.1 million tonnes.

The human population is increasing worldwide, which results in serious shortage of aquatic food and in order to maintain at least the current level of per-capita consumption of aquatic foods, the world will require an additional 23 million tonnes thereof by 2020. This additional supply will have to come from aquaculture (FAO, 2012). Global forecasts on food security indicate that aquaculture will compensate for the stagnating supply of seafood from fisheries and give an important contribution in feeding the growing human population with valuable animal proteins (Delgado *et al*., 2003; Brugere and Ridler, 2004). Demand for seafood currently exceeds from wild harvesting, but increased production from aquaculture has so far supplied the difference between demand and supply. The world seafood consumption has continued to rise in the past decade because of increased production from aquaculture.

Aquaculture production has doubled since 1990 (Aiken and Sinclair, 1995), and this remarkable trend will have to continue to meet expected future seafood demand. Annual per capita world seafood consumption is currently 16 kg, and rising incomes in developing countries will probably result in higher fish consumption in the future, thus raising world per capita consumption. Growing population will also increase the world demand for seafood. These developments will widen the gap between seafood production from capture fisheries and world seafood demand, thus increasing the demand for farmed fish. Aiken and Sinclair (1995) predict that by the year 2025, there will be a shortfall of 55 million metric tons (mmt) between seafood harvest of 60 mmt and demand for seafood of 115 mmt.

The average year to year growth rate for aquaculture production between 1970 and 2009 was 8.3%, compared to 4.9% for poultry, 2.9% for pig, 1.8% for sheep and goats, 1.4% for cattle and 1.2% for fisheries (Natale *et al*., 2013). Aquaculture is the most active food production industry in terms of growth rate with most of the production taking place in Asia. It is unequivocally agreed that global aquaculture production will continue to increase, and much of the increased production in developing countries of Asia is likely to be achieved through the expansion of intensive and semi-intensive aquaculture. Aquaculture continues to grow at a relatively high and constant rate over the past decades. Within this industry's growth, a wide variety of species are being farmed to satisfy the diverse demands both for the local consumption as well as for global seafood trade.

India produces about 6.57 million metric tons fish every year. The inland-sector, which has a growth rate of 6%, contributes around 55% of it. For aquaculture production to increase, many technological problems must be overcome including insufficient balanced feed supplies, brood stock and seed stock production and water resources. A key element that characterizes aquaculture in respect of fisheries is the higher degree of control on the production process (Natale *et al.*, 2013). However, one of the problems facing the most important aspects of fish production improvement and sustainable aquaculture involves the fish nutrition, which demands improvements in feed formulation and feed ingredient modification. Reducing the environmental effects of aquaculture through improvements in nutrient utilization by fish is critical for aquaculture production to increase. Fish nutritionists must focus on increasing the efficiency of production and on lowering environmental effects through increased nutrient retention. This will provide safe and nutritious fishery products in a sustainable and environmentally compatible fashion.

Precise information on nutritional requirements of cultured species to provide appropriate amount of nutrients for optimal growth is essential to reduce feed cost. As feed still continues to hamper the developmental activities, especially in developing countries including India, So the quest for comprehensive knowledge on the nutrient requirements of fish at various stages of life cycle and on the types of food material best suited for them is an essential prerequisite for formulating any strategy of the controlled propagation of fin fishes. Thus, nutrition plays a key role in the aquaculture industry by influencing the growth and health of fish. In addition, feed accounts for a significant portion of the costs of an aquaculture enterprise, and therefore nutrition also influences the economic returns of the industry. The development of cost-effective feeds that provide balanced diet to maximize growth, while minimizing environmental effects, depends on knowing the species' nutritional requirements and meeting those requirements with balanced diet formulations and appropriate feeding practices (National research council, 2011).

Supplementary feeding that caters to the nutrient need of cultured fish is one of the limiting factors for both intensive and semi-intensive fish farming system. The paucity of knowledge on basic aspects viz., nutrient requirements of cultured species of fishes, feeding habits, local availability, cost and nutritive values of feed ingredients and ability of fish to utilize nutrients are key factors that determine the growth of fish under consideration. Among all the nutrients required by fish for growth and maintenance, protein is one of the most important and initial constituent, which comprises about 65-70% of the dry weight of fish muscle (Wilson and Halver, 1986), and is also metabolized as an energy source by fish. Protein plays an important role in supporting fish growth (Lovell, 1979; Jones *et al*., 1996; Lee *et al*., 2000; Luo *et al*., 2004). Fish consume protein to obtain the essential and non-essential amino acids, which are necessary for muscle formation and enzymatic function and in part provide energy for maintenance (Yang *et al*., 2002). Protein is digested or hydrolyzed and releases free amino acids, which are absorbed from the intestinal tract and distributed by the blood to the organs and tissues. These amino acids are used by the various tissues to synthesize new protein. A regular intake of protein or amino acids is required because amino acids are used continuously by the fish, either to build new proteins (as during growth and reproduction) or to replace existing proteins (maintenance). Inadequate protein in the diet results in a reduction or cessation of growth and a loss of weight due to withdrawal of protein from less vital tissues to maintain the functions of more vital organs and tissues. On the other hand, if too much protein is supplied in the diet, only part of it will be utilized to make new proteins, and the remainder will be converted to energy. Excess protein in fish diet may be wasteful and cause diets to be unnecessarily expensive (Ahmad, 2000).

Dietary protein is one of the major determinates of fish growth, which represents the commercial success of any intensively cultured fish species that depends on market demand and production. Also the largest fraction of this production cost depends on nutritionally balanced diet, with protein comprising the most expensive component (NRC, 1993). It is therefore necessary that optimum dietary protein levels are known to establish best growth and survival at the lowest cost (Gatlin, 2010)

The capacity of the fish to synthesis protein *de nova* from carbon skeleton is limited and thus it has to be supplied in diet. Protein levels in successful feeds range from 20-60% to accommodate for differences in the physiological needs of different fish species (Hepher, 1988; Wilson, 1989). Since protein constitutes in fish culture the single most expensive item in artificial feeds, it is logical to incorporate only that much, which is necessary for normal maintenance demand and growth. Any excess is considered wasteful, biologically as well as economically and therefore, it is important to minimize the amount of protein used for energy (NRC 1993; De Silva and Anderson, 1995; Abdel-tawwab *et al*., 2010; Deng *et al*., 2011). To maximize nutrient utilization and minimize the solid and soluble waste load, it is essential to provide cultured fish with the optimum level of protein (Cho, 1993).

Generally nutrients absorbed in excess of requirements may be excreted as ammonia and urea (Kibria *et al*., 1998). The diet development for particular fish species, therefore requires a precise assessment of its protein needs which determines, to a large extend, the overall success of its production. Thus an optimum dietary protein level in the diet is important for fish growth and maintenance of good farming environments (Guo *et al*., 2012). If excessive protein is supplied in diets, only part of it will be utilized to synthesize new tissues for growth and the excess proportion will be metabolized as energy source. Whereas, diet with excessive protein contents usually leads to extra energy costs, increased nitrogenous excretions and occasionally retarded fish growth (Lee *et al*., 2001; Yang *et al*., 2002; Mohanta *et al*., 2008; Monentcham *et al*., 2009; Abdel-Tawwab *et al*., 2010). The aim of aquaculture should, therefore, be to provide sufficient and optimum amount of protein for good growth through balanced feed.

Mirror carp, *Cyprinus carpio* var. *specularis*, as a freshwater fish species, has been one of the most widely cultured species all over the world due to its fast growth rate and easy cultivation (Guler *et al*., 2008). It is also one of the most widely cultured species in India. This species is omnivorous, hardy, tolerant of wide fluctuations in environmental conditions, especially temperature and is preferred for culture by many farmers (Nandeesha *et al*., 2000). But in temperate climatic condition of the Kashmir valley, it can be culture with indigenous *Schizothorax* spp. (Snow trout) under composite fish culture and with grass carp, *Ctenopharyngodon idella* and silver carp, *Hypophthalmichthys molitrix*, under polyculture technique. These carp species cultured using diverse aquaculture techniques having the compatibility with the temperate climatic conditions of Kashmir can cater to the dietary protein needs of the people and have got the great potential of generating employment opportunities among the rural population of Kashmir valley.

Mirror carp is very popular as a food-fish in Europe and elsewhere and is well suited for rearing in fish farms, due to which it is considered as number one fish species for aquaculture industry. It is also popular as anglers' fish. The annual tonnage of common carp, not to mention the other cyprinids, produced in China alone exceeds the weight of all other fish, such as trout and salmon, produced by aquaculture worldwide. Culture of mirror carp provides the country an opportunity to reduce protein deficiency and to earn foreign currency by exporting this fish. The natural conditions that suit carp are lowland lakes and rivers where there is abundant vegetation to provide food and shelter. Mirror carp dwells in middle and lower reaches of rivers and shallow confined waters. The fish can survive cold winter periods and can also survive in a very low oxygen concentration (0.3-0.5 mg L^{-1}) as well as in supersaturation of oxygen content in the water. It is omnivorous, feeds mainly on bottom-living insect larvae, small snails, crustaceans, and some vegetable matter. Carp fry feeds on zooplankton such as rotifers and copepods, but as they grow up, they become benthic feeders, feeding on animals and other organic materials (Tamas and Horvath, 1976; Van Limborg, 1978). Since it is an omnivorous fish, it can utilize fat and carbohydrates as dietary energy sources (Yilmaz *et al*., 2005). It breeds in confined waters throughout the year except in extreme winter months due to very low temperature. Peak breeding period is during February-March and July-August.

In Kashmir mirror carp is reported locally to be very common in Lakes (Das and Subla, 1964; Fotedar and Qadri, 1974). The species was introduced in Kashmir valley in 1955-56 (Das and Subla, 1964; Fotedar and Qadri, 1974; Jhingran, 1991). *C. carpio* provides about 70-75% of the total fish catch (presumemably by weight) in the Kashmir valley (Fotedar and Qadri, 1974). However, due to increasing population and growing awareness that capture fisheries are exhaustible, attention is warranted towards finding possibilities offered by aquaculture through extensive and intensive techniques for providing alternative animal protein to people of the valley. This exotic fish is the most suitable species for culture in small seasonal water bodies and pond of different sizes. The fish has been categorized as eurythermal i.e. it can withstand wide range of temperature fluctuation, so it is well suited for culture in the climatic conditions of Kashmir valley. It is also capable of withstanding fluctuation in the water levels and other adverse environmental factors. Besides, it has an inviting appearance and a delectable taste, which imparts a remarkable market acceptance.

To overcome market saturation problems and to maintain industry profitability there is a dire need to diversify production by reducing the input cost and developing economical feeds. Mirror carp is considered as having a great potential, given its good acceptability by consumers, high market price, easy adaptation to captivity, acceptable growth and farming characteristics. Unlike rainbow trout and brown trout, which are carnivorous, mirror carp is considered omnivorous fish that feed on chironomids, tubificids, larger zooplankton and zooperiphyton (Sibbing, 1988), but the bulk of its diet consists of detritus (Chapman and Fernando, 1994; Michel and Oberdorff, 1995). Given its efficient use of carbohydrates (Stone, 2003) and fat as energy, tolerance to high levels of dietary plant protein sources, such as soybean meal and its high potential for digesting polysaccharides, it is expected that it will use commercial diets more cost-effectively than carnivorous species.

To intensify rearing and culture of this species, provision of nutritionally balanced feed becomes necessary. Therefore, it is essential to know the minimum protein requirement for optimum growth in formulating a balanced ration, since protein is an important major nutrient for growth and other metabolic activities, as well as costs more than carbohydrate and fat. Excess dietary protein not only costs more but also increases the energy cost of assimilation by increasing the specific dynamic action (LeGrow and Beamish, 1986).

Although some aspects of nutritional requirements of mirror carp have been worked out in the past by different workers, but no information related to the nutrient requirements is available for this fish species cultured in the Kashmir valley. Keeping this in view, the present investigation was designed to study the effects of dietary protein levels on growth, feed utilization, whole body composition and haematological parameters of mirror carp, in order to determine the optimum dietary protein requirement of this fish, with a view to develop a nutritionally balanced diet for optimum production of this fish species through aquaculture.

CHAPTER - 2

Review of Literature

Review of Literature

Fish feeding experiments are carried out mainly with the purpose to quantifying the gross dietary nutrient requirements of a particular fish species so as to incorporate the minimum level of each particular nutrient in the diets. The knowledge gained from these studies regarding the dietary requirements for optimum growth could be successfully incorporated in the formulation of synthetic feeds, whereby both plant as well as animal dietary sources could be incorporated for the feed formulations. This would lead to the production of nutritious and cost effective feeds, best suited for the optimum growth and increased fish production and reducing the operating expenditure involved in the fish culture system. Thus, a sound knowledge of the dietary requirements of a particular cultured fish species forms the backbone of commercially viable aquaculture system.

The dietary protein requirements of fishes are more precisely determined under defined experimental setup through feeding purified and semi-purified diets. Ingredients such as casein, gelatin, whole egg protein, egg albumin, fish protein concentrate, fish meal, soymeal, cod muscle, gelatin and amino acids are used to determine the dietary protein requirement of fishes. In order to estimate the protein requirements of fish, numerous investigators have utilized various semi-purified and purified diets. Most investigators used isoenergetic diets to determine the dietary protein requirements of the fishes.

Generally, it has been established that the protein requirements of fish are much higher than those of land animals (Lovell, 1989). It has also been reported by several workers in the past that the efficiency of protein utilization is lower in fish than in other animals. The variations in protein requirement of different fish species is due to certain factors such as size and age of fish, water temperature, different laboratory conditions, dietary protein ingredient combinations and also various statistical methods employed for the determination of dietary protein requirement of fishes. Protein requirements of fish decrease with increasing size and age. For example, the optimal dietary protein level for very young salmonids is 45 to 50% of the diet, while juveniles require 40% and yearlings require about 35% dietary protein (Hilton and Slinger, 1981; Hardy, 1989). Similarly, channel catfish, *Ictalurus punctatus* fry require about 40% protein, whereas fingerlings require 30 to 35% protein and larger fish (>110 g) require 25 to

35% protein (Page and Andrews, 1973; Wilson, 1991). Lim (1989) reported that larger fish require only 25 to 35% dietary protein, depending on the rearing conditions. Changes in water temperature have also been reported to alter the protein requirement of some fish species. For example, striped bass were found to require 47% protein at 20 ^{0}C and about 55% protein at 24 ^{0}C (Millikin, 1982; 1983). In general, the growth rate and feed intake increase as the water temperature increases, thus it is mostly believed that a change in water temperature affects feed intake much more than the protein requirement.

Delong *et al*. (1958) was first to report the dietary protein requirements of a coldwater fish, chinook salmon, *Oncorhynchus tshawytscha*. Since then extensive research has been carried out on other fish species and was reviewed in NRC, 1981; 1993. Among the warm-water fishes, research on dietary protein requirements has been carried out extensively on channel catfish, common carp, grass carp, Japanese eel, milkfish, red sea bream, striped bass, tilapia, yellowtail and mullets, and the details about the protein requirement of these fishes has been reviewed by Wilson and Halver (1986).

Cho *et al*. (1976), studied the influence of dietary protein on feed utilization by rainbow trout, *Oncorhynchus mykiss*. They used herring meal and soybean meal as the dietary protein sources. These workers suggested that an optimum level of 40% dietary protein for promoting growth in rainbow trout and also mentioned that reducing the protein level below 40% resulted in lower final body weights, higher feed: gain ratios and body composition with more protein and less fat.

In India, Sen *et al.* (1978), were first to make an attempt to understand the optimum level of protein requirements of Indian major carps, using purified test diets fortified with vitamins and minerals. Singh and Bhanot (1988) reported that the dietary protein requirements of catla, *Catla catla* fry was 47% at water temperature 20-30 ^{0}C compared to fingerling 40% reported by Mohanty *et al.* (1988).

De Silva and Perera (1985), studied the effects of dietary protein levels on growth, feed conversion, and protein utilization in young Nile tilapia, *Tilapia nilotica* and reported an optimal dietary protein level of 28-30% for its optimum growth and conversion efficiency. Two to three week old *T. nilotica* young were fed 6% of the body weight a fish meal based diet twice daily. Efficiency of feed conversion increased with dietary protein content up to 30% protein level and then decreased at higher protein containing diets.

Brown *et al*. (1992), reported that the optimum dietary protein requirement level for juvenile sunshine bass, *Morone chrysops* ♀ x *M. saxatilis* ♂ approximately 41% of dry diet, based on weight gain, relative growth and feed efficiency. They used fish meal as the main dietary protein source for their 8-week feeding trial. Further, they also mentioned that hepato-somatic index and intraperitoneal fat ratio values were also found inversely related to dietary protein levels.

A 10-week feeding trial was conducted by Tidwell *et al*. (1992), to study the growth, feed conversion, and protein utilization of female green sunfish (*Lepomis cyanellus*) x male bluegill hybrids (*Lepomis macrochirus*) fed isocaloric diets with different protein levels and recommended that higher protein feeds (35% or greater) showed improved growth performance and production potential of this hybrid fish as compared to lower protein (32%) containing diet.

Fiogbe *et al*. (1996), conducted a feeding experiment to evaluate the effects of dietary crude protein level on growth of Eurasian perch, *Perca fluviatilis* juveniles by using casein and cod meal as the dietary protein sources and fish were fed 5% of their body weight daily. They recommended an optimal dietary crude protein requirement of *P. fluviatilis* for maximum growth and excellent nutrient efficiency is within the range of 36.8 to 43.6% dietary protein of the diet.

Perez *et al.* (1997), conducted a detailed feeding trial to study the effects of varying levels of dietary protein content on growth of European sea bass, *Dicentrarchus labrax* fingerlings fed extruded based diets. Fish were fed by hand 5 times a day to apparent satiation, using fish meal and blood meal as the dietary protein sources. They suggested that the diet containing 45% dietary protein would be optimum for the best performance of European sea bass fingerlings.

Elangovan and Shim (1997), carried out an experiment to determine the dietary protein requirement of cyprinid fish, *Barbodes altus* cultured under controlled conditions. The fish were fed at 5% body weight, casein and gelatin based moist feed twice daily for 10-weeks. On the basis of quadratic regression analysis, they recommended that the optimum dietary protein requirement of *B. altus* was at 41.7% for maximum growth and efficient feed utilization.

A 7-week feeding experiment was conducted by Lazo *et al*. (1998), to study the effects of dietary protein level on growth, feed efficiency and survival of juvenile florida pompano, *Trachinotus carolinus*. Under this study, fish meal and soy meal was used as the dietary protein sources. Fish fed the highest protein containing diet had lower percentage of daily feed consumption values than did fish fed the lowest protein diet. Growth and feed efficiency ratios increased with the increase of dietary protein level and were highest for fish fed the diet containing 45% dietary protein. Their results indicated that juvenile *T. carolinus* required a minimum of 45% protein for maximum growth and feed efficiency ratio.

Shyong *et al.* (1998), studied the effects of dietary protein concentration on growth and muscle composition of juvenile, *Zacco barbata.* Fish were fed moist diet once daily for 10 weeks with white fish meal serving as the major dietary protein source. Using broken line model analysis with weight gain, they established that the optimum dietary protein level for *Z. barbata* approximately at 32.0% for maximum growth of the fish.

Al Hafedh (1999), studied the effects of dietary protein levels on growth and body composition of four sizes (0.51, 45, 96 and 264g) of Nile tilapia, *Oreochromis niloticus*. Fishes were fed commercial feed thrice a day, having graded levels of dietary protein with fish meal as the main dietary protein source and the experiments were carried out for 140 days. In all the sizes, there was a progressive increased in growth with increasing dietary protein. Whole body composition of the smaller fish was significantly influenced by dietary protein content. In larger fish, no significant influence of dietary protein level on body protein content was found. They suggested that 40% protein containing diet for fry (0.51g) and 30% protein for larger tilapia (96-264g) for their maximum growth.

Peres and Oliva-teles (1999), reported an optimum dietary protein requirement at 48% for maximum growth of juvenile European seabass, *D. labrax*. Fish were fed a fish meal based diet to satiation twice a day for 12 weeks. Specific growth rate and feed conversion efficiency were reported significantly higher at 48% and 56% protein containing diets than those fed other dietary protein levels.

Dietary protein requirement of discus, Symphysodon spp. was reported at 44.90-50.10% by Chong *et al*. (2000). Fish were fed a fish meal and casein based diet, to apparent satiation twice a day for 12 weeks. Growth rate increased significantly with the increase of protein level up to 50% diet and then decreased. These workers also mentioned that feed conversion ratio (FCR) varied inversely with growth rate.

Chou *et al*. (2001), conducted an 8-week feeding trial to determine the dietary protein requirement of cobia, *Rachycentron canadum*. Fish meal and casein were used as the dietary protein sources. Their analysis revealed that a weight gain reached its peak at a dietary protein concentration of 44.5%, which was regarded as the most suitable level for maximum fish growth and better feed utilization efficiency.

Alvarez-Gonzalez *et al*. (2001), studied the effect of dietary protein level on growth and body composition of juvenile spotted sand bass, *Paralabrax maculatofasciatus*. Fish were fed a sardine meal based feed to apparent satiation, twice daily. These workers reported that a significant lower weight gain was observed in fish fed diet containing 40% protein compared to those fish fed at higher protein containing diets and finally they recommended that 45% protein containing diet was most appropriate for best growth rate of spotted sand bass juveniles.

The dietary protein requirement of silver perch, *Bidyanus bidyanus* fingerlings was estimated to be 28% by Allan *et al*. (2001). The experimental fishes were fed a fishmeal based diet twice daily. They reported that weight gain increased with the increase of dietary protein level. However, they also mentioned that protein above 28% did not produce significant increase in weight gain or improved FCR values. The whole body protein and moisture content also increased, whereas whole body fat content decreased with the increase of dietary protein levels.

Kim *et al*. (2001), conducted a 6-weeks feeding trial to study the effects of dietary protein levels on growth, utilization of protein and energy of juvenile haddock, *Melanogrammus aeglefinus*, by using fish meal and casein as dietary protein sources. Fish were hand-fed a dry pelleted diet, three times a day to apparent satiation. A dietary protein requirement of 53.8% was estimated on the basis of nitrogen gain. Further, it was reported that the hepatosomatic index of fish significantly decreased from 10.70% to 7.30% as dietary protein level increased. Later on Kim *et al*. (2001) also conducted another 8-week feeding trial experiment to re-evaluate the optimum dietary protein level for the maximum growth of juvenile Korean rockfish, *Sebastes schlegeli*. This time they replaced fishmeal with Rock-fish muscle as the protein source along with casein as main dietary protein source. Fish were fed 4% of body weight initially (twice daily) which was gradually decreased to 3% in the later part of the feeding trial. These workers finally recommended that the optimum dietary protein requirement for maximum growth of juvenile Korean rockfish could be equal to or greater than 48.6%, but less than 50% dietary protein.

Dietary protein requirement of juvenile giant croaker, *Nibea japonica* was reported at 45% by Lee *et al*. (2001). Fish were fed a white fish meal based diet twice daily for 6 weeks. Weight gain and feed efficiency were best in fish fed diets containing 45% protein or more. The protein efficiency ratio value was maximized in fish fed diet containing 45% protein and thereafter, a decline in PER was noted in fish fed 50% protein containing diet. They also noted that higher dietary protein content not only resulted in lower PER, but also produced a lower HSI and higher fat accumulation in the whole body of juvenile giant croaker.

The optimum dietary protein requirement of mahseer, *Tor putitora* fingerlings was reported approximately at 40% by Hossain *et al*. (2002). Fish were fed a casein-gelatin based diet to satiation, twice daily for 50 days. Weight gain and specific growth rate of fish increased proportionally with the increase in dietary protein concentration up to 40% and thereafter, a decrease in weight gain and SGR was recorded with further increase in dietary protein levels. They also observed that fish fed on diet containing 40% protein level showed the significantly highest weight gain and feed utilization. The body compositional data also revealed that the carcass protein content increased progressively with the increase of dietary protein levels, while carcass fat content showed inverse result. Where, decrease in carcass fat was reported with the increase of dietary protein levels.

Yang *et al*. (2002), studied the effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch, *B. bidyanus* by conducting an 8-week feeding trial. Fish were fed a white fish meal based dry diet, thrice a day. They observed that both percent weight gain and feed efficiency significantly increased with increasing dietary protein levels. The protein efficiency ratio, productive protein value, hepatosomatic index and intra-peritoneal fat ratios were inversely correlated with dietary protein level. Fat content decreased with increasing dietary protein levels and dry matter content also slightly decreased. They finally recommended that the optimum dietary protein requirement for the growth of juvenile silver perch was estimated at 42.15% for its maximum production.

A 20-week feeding trial was conducted by Lee *et al*. (2002), to study the effects of digestible protein levels in practical diets on growth, protein utilization and body composition of juvenile rockfish, *S. schlegeli*. Under this study, they found that weight gain and feed efficiency ratio of fish was improved as dietary protein levels increased. Further they also mentioned that the effects of dietary protein levels were also clearly seen in hepato-somatic index, visceral-somatic index, protein efficiency ratio, and protein retention efficiency. On the basis of their results they recommended that a practical diet containing 42% dietary protein as the optimum protein requirement for juvenile rockfish.

Giri *et al*. (2003), conducted a feeding trial to study the effect of dietary protein level on growth, survival, feed utilization and body composition of hybrid post-larvae of catfish, *Clarias batrachus* × *Clarias gariepinus*. Fish meal based diet was fed twice daily fixed at 10% of the BW/day for a period of 30 days. The results showed that an increase in body weight gain, SGR%, PER and decreased FCR was observed with increasing dietary protein level. The fish showed gradual increase in whole body protein content as well as ash and decrease in whole body fat content with increasing dietary protein concentration. The study indicated that for maximum growth, best feed utilization and highest survival of hybrid catfish post larvae required 35%-40% dietary protein.

The optimum dietary protein requirement for the growth of juvenile silver perch, *Spinibarbus hollandi* was estimated at 32.7% by Yang *et al*. (2003). The fish were fed a white fish meal based diet at 3% of BW/day twice daily for 10 weeks. Their results revealed that both weight gain (%) and FER increased significantly with increasing dietary protein levels, while the PER and productive fat values were inversely correlated. They mentioned that the whole body composition also showed significant results; the carcass protein of fish fed lower protein diets was significantly lower than that of the fish fed higher protein diets and the carcass fat content decreased with increasing dietary protein levels, whereas moisture was inversely related to fat content. Thus they finally concluded that varied dietary protein levels not only affected growth parameters, but also produced significant differences in whole body composition of the fish.

Meyer and Fracalossi (2004), conducted a 90 day feeding trial to study the protein requirement of jundia, *Rhamdia quelen* fingerlings. Fish were fed a casein-gelatin based diet until apparent satiation twice a day. Weight gain, SGR (%), feed efficiency, apparent net protein utilization and energy retention increased with the increase of dietary protein concentration. On their conclusion they recommended that the protein requirement values within the range of 32.6 to 37.3% considered optimum for *R. quelen* fingerlings for their maximum growth.

Islam and Tanaka (2004), reported a range of 45%-50% optimum dietary protein requirement for the endangered cyprinid, *Tor putitora*. Soybean meal and mustard oil cake were used as dietary protein sources and fish were fed 5% of BW/day daily. They observed that growth and FCR data was influenced significantly by dietary protein contents; higher growth rate and lower FCR values were obtained with increasing dietary protein content. The effects of dietary protein concentrations on whole body composition were also observed in the study. They reported that higher values of protein and ash, while lower values of moisture and fat contents were found in the same study.

Salhi *et al*. (2004), reported the dietary protein requirement of black catfish, *R. quelen* fry at 37%. Fish meal served as the main dietary protein source and fishes were fed to apparent satiation twice daily for 30 days. They also mentioned that although growth rate and feed conversion ratio was significantly improved but protein efficiency ratio could not produce any significant difference.

Skalli *et al*. (2004), investigated the effects of the dietary protein levels on growth and nutrient utilization in common dentex, *Dentex dentex* by conducting two separate feeding trials, at different growth stages in order to identify suitable protein level for the formulation of dry diet for this species. Fish were fed a fish meal based diet to satiation thrice daily for 6 weeks in first trial and 12 weeks in second trial. Fishes having an average individual weight of 10g showed an optimum growth at 52.2% protein level, while fishes having an average individual weight of 92g showed maximum growth rate and best-FCR at 43.4% dietary protein level.

Gonzalez *et al*. (2005), suggested that the dietary protein requirement of southern flounder, *Paralichthys lethostigma* is within the range of 50.3% - 50.8% for its maximum growth and efficient feed utilization. Under this study fish were fed 3% body weight twice daily for 12 weeks. Fishmeal and casein were used as dietary protein sources.

Royes *et al*. (2005), recommended 40% protein diet for juvenile African cichlid, *Pseudotropheus scolofi.* Fish were fed casein and fish meal based diet, three times daily for 10 weeks. Final weight gain was significantly different among the treatments.

Saidy *et al*. (2005), conducted a feeding trial in concrete tanks to examine the effect of two protein levels on growth performance, production traits and body composition of juvenile Nile tilapia, *O. niloticus*. Soybean meal served as the main dietary protein source along with 25% to 30% of protein from commercial ingredients. Under this study they noted that the fish did not show any significant increase in weight gain, SGR% and FCR with increasing dietary protein levels and they concluded that 25% dietary protein would be useful for adult Nile tilapia for its maximum growth and efficient feed utilization.

Li *et al*. (2006), studied the effects of dietary protein concentration and feeding regime on channel catfish, *I. punctatus* fingerlings. Soybean meal and menhaden fish meal were used as dietary protein sources. No significant differences were observed in weight gain of fish fed with diets containing various levels of dietary protein. They observed that 24% and 36% protein diet provided the same growth and feed conversion efficiency. On this observation they finally recommended that 28% protein containing diet would be useful for the growth of channel catfish fingerlings.

Okorie *et al*. (2007), reported optimum dietary protein requirement of juvenile Japanese eel, *A. Japonica* at 44.3% based on weight gain (%), SGR (%), and PER. Fish were fed 3% of BW/day at the beginning, which was reduced in the later part of feeding trial and fed 2% of BW/day twice daily for 16 weeks. These workers reported significant effects of varying levels of dietary protein on weight gain, SGR, and PER.

A 45 day feeding trial was conducted by Debnath *et al*. (2007), to study the effects of different crude protein levels on digestive enzymes and metabolic profile of Indian major carp, *Labeo rohita* fingerlings. Fish were fed a fish meal based diet, initially 5% of the BW/day and later on gradually adjusted the feeding ration on the basis of daily observation of feed consumption. They observed that crude protein content of fish was significantly affected by dietary crude protein content in the diet and showed efficient conversion and deposition of protein with the increase in dietary protein concentration. Considering the cost effectiveness of the feed and based on liver and plasma free amino acids and also plasma protein fractions, 30% crude protein in practical diet was recommended as the optimal dietary protein level for *L. rohita* fingerlings for its optimum growth and conversion efficiency.

Dietary protein requirement of juvenile black sea bass, *Centropristis striata* was reported in the range of 45-52.6% by Alam *et al.* (2008). Under this study the fish were fed to apparent satiation a herring meal diet twice a day for 8 weeks. They reported that the maximum weight gain occurred at 52.6% dietary protein level, which also resulted in best-feed conversion efficiency and protein efficiency ratio. Therefore, they finally recommended 52.5% dietary protein level as optimum level for growth and mass production of this species.

The dietary protein requirement of Malaysian mahseer, *Tor tambroides* fingerlings, was reported at 48% for maximum growth by Ng *et al.* (2008) when using a casein-gelatin based purified diet for their experiment. Growth performance and feed conversion ratio generally improved with increasing dietary protein and maximum specific growth rate was observed at 48% dietary protein while, the FCR was also reported on the same level. However, they noticed that the results related to PER and net protein utilization showed a decreasing trend of efficiency with increasing dietary protein content in the diets.

Singh *et al.* (2008), conducted a feeding experiment to study the influence of dietary protein levels and water temperature on growth, body composition and nutrient utilization of Indian major carp. *Cirrhinus mrigala* fry. Fish meal based diet was fed to the fry at 4% of body weight, twice a day for a period of 90 days. They found that 36% protein concentration in the diet yielded highest weight gain and gross conversion efficiency. Finally they concluded that diet with 36% dietary protein would be useful for the best growth and efficient feed utilization of *C. mrigala* fry. Another feeding trial experiment was conducted by Singh *et al.* (2009), to evaluate the dietary protein level for Asian catfish, *Clarias batrachus* fry using a fish meal based diet. Fish were fed at 5% of BW/day, twice daily for a period of 60 days. Fry fed with diet containing 36% protein showed the highest mean weight gain and conversion efficiency. These workers finally concluded that the diet containing 36% protein was optimum for the growth of *C. batrachus* fry.

Bicudo *et al*. (2009), conducted a 60 days feeding trial and reported that best growth performance was observed in Pacu, *Piaractus mesopotamicus* fingerlings when fed on a fish meal based diet containing 27.1% dietary protein level.

A 30% crude protein level was reported optimum for growth of Nile tilapia, *O. niloticus* juveniles (monosex) by Bahnasawy (2009). The fish were fed a fish meal based diet at a rate of 3% of body weight once a day for 180 days. Specific growth rate increased significantly with increasing dietary protein levels. They also observed that the protein efficiency ratio was inversely correlated with dietary protein levels. While, the protein content of the fish muscle increased with increasing dietary protein levels and the inverse result in body fat was reported.

Sealey *et al.* (2009), conducted a growth trial on juvenile neon tetra, *Paracheirodon innesi* to study the effects of varying dietary levels on growth. Fish were fed an Atlantic menhaden meal based diet, three times per day. Fish fed diets containing 45% to 55% crude protein had significantly greater weight gain than did fish fed 25% crude protein. Fish fed 25% crude protein had a significantly higher feed conversion ratio than did those fed 45% or 55% crude protein. Finally, these workers recommended a dietary protein level at approximately 45% for the optimum growth and efficient feed utilization.

Dietary protein requirement of juvenile tiger puffer, *Takifugu rubripes* was reported at 41% for optimum growth and physiological performances by Kim and Lee (2009). Fish were fed a white fish meal based diet at 2 to 4% of body weight, six times a day for 8 weeks. The lowest feed efficiency was found in fish groups fed 35% diet. Whole body protein content of fish fed the diets containing 50 and 55% protein was significantly higher than that of fish fed 35% protein containing diet.

Siddiqui and Khan (2009), studied the effects of dietary protein levels on growth, feed utilization, protein retention efficiency and body composition of young Indian catfish, singhi, *Heteropneustes fossilis*. They observed that the inclusion of dietary protein in the range of 40-43% was reported optimum for the growth of young *H. fossilis.* The fish were fed to apparent satiation casein-gelatin based diet twice a day for 8 weeks.

Optimum dietary protein requirement for amazonian tambaqui, *Colossoma macropomum* was reported at 30% by Oishi *et al.* (2010). Fish were fed to satiation a soybean meal based diet twice daily for 60 days. Weight gain and specific growth rate increased consistently with increasing dietary protein level. Feed intake followed the same trend resulting in best-feed efficiency in fish fed diet with 35% protein, while protein efficiency ratio decreased with increasing dietary protein levels. They also reported that carcass ash and protein had linear relationship with dietary protein levels, while the whole body fat content showed a decreasing trend, in the same study.

Liu *et al*. (2009), studied the effects of dietary protein levels on the growth performance, digestive capacity and amino acid metabolism of juvenile jian carp, *Cyprinus carpio* var. jian. Their results indicated that the dietary protein requirement was estimated at 34.1% for maximum growth of the fish. During the feeding trial, fish were hand-fed to apparent satiation, a brown fish meal based diet eight times daily for 45 days.

Ergun *et al*. (2010), suggested that blue streak hap, *Labidochromis caeruleus* requires more than 35% dietary protein for its maximum growth. Fish were fed to apparent satiation, a fish meal based diet three times a day for 8 weeks. Maximum growth of fish was observed at the 40% protein containing diet, while specific growth rate and feed efficiency ratio increased from 30% to 40% protein levels and thereafter decreased further.

A 10-weeks feeding trial was conducted by Abdel-Tawwab *et al*. (2010), to assess the interaction between dietary protein level and fish weight on the growth, feed utilization, and physiological alterations of Nile tilapia, *O. niloticus*. Fish were fed to satiation, a fish meal and soybean meal based diet twice daily. These workers concluded that the optimum dietary protein required for Nile tilapia fry (~0.50 g) was estimated at 45% protein containing diet for optimum growth; whereas, fingerling (~20 g) and advanced juvenile (~40 g) performed optimally when fed at 35% protein diet.

Optimum dietary protein level for juvenile silver pomfret, *Pampus argenteus* was reported at 49% by Hossain *et al*. (2010). Fish meal served as the primary dietary protein source. Fishes were fed to apparent satiation a moist feed twice daily for 6 weeks. They found that the weight gain (%) of fish increased as dietary protein level increased.

Zhang *et al*. (2010), conducted an 8-week feeding experiment to determine the optimum protein requirement of juvenile black sea bream, *Sparus macrocephalus* and suggested that 41.4% dietary protein level would be useful for its maximum growth and efficient feed utilization. Fish were fed to apparent satiation, a white fish meal based diet twice daily. They mentioned that although, protein efficiency ratio declined, but weight gain and specific growth rate increased alongwith the improvement of feed efficiency ratio as dietary protein level increased, while the maximum FER reported at 41.8% protein containing diet.

Deng *et al*. (2011), conducted a feeding trial to determine the dietary protein requirement of juvenile Asian red-tailed catfish, *Hemibagrus wyckioides.* Fish were hand-fed a fish meal based feed, to apparent satiation twice daily for 8 weeks. Feed conversion ratio decreased steadily, whereas protein efficiency ratio increased with increasing dietary protein levels. The optimum dietary protein requirement for maximum specific growth rate of juvenile *H. wyckioides* was reported at 44.12%. Same year, another group of researchers (Deng *et al*., 2011) conducted a feeding trial to determine the dietary protein requirement of juvenile Pacific threadfin, *Polydactylus sexfilis*. These workers suggested that the optimum dietary protein level within the range of 35-41% based on growth rate, feed conversion ratio and protein utilization.

Farhat and Khan (2011), recommended the inclusion of dietary protein in the range of 34.4-39.6% as optimum for maximizing growth potential, feed conversion, and nutrient retention in African catfish, *Clarias gariepinus* fingerling. Fish were fed to apparent satiation a casein-gelatin based diet, twice daily for 8 weeks. Maximum LWG %, PER, highest PRE, ERE, best-FCR and maximum body protein was recorded in fish fed diet containing 35% protein.

Guo *et al*. (2012), studied the effects of dietary protein levels on growth performance, nitrogen and energy budget of juvenile hybrid sturgeon, *Acipenser baerii* ♀ x *A. gueldenstaedtii* ♂. Fish were fed to apparent satiation a fishmeal based diet twice a day for 8 weeks. These workers observed that the specific growth rate increased with increasing dietary protein levels. The highest protein retention efficiency was found in the group that fed 25-30% protein containing diet. While, diets containing excessive protein contents not only resulted in inferior growth performance but also utilized higher proportions of protein and energy for excretion.

Dietary protein requirement of sharp snout sea bream, *Diplodus puntazzo* juveniles was reported at 43% by Coutinho *et al*. (2012). Fish were fed to apparent satiation, a fish meal based diet twice a day for 11 weeks. They also reported that the whole body protein content increased with the increase of dietary protein content, but no other relevant differences in body composition were noticed. On the basis of their findings, they recommended that the optimum dietary protein requirement of *D. puntazzo* was within the range of 42.9-43.8% for maximum growth and nitrogen retention.

More recently, the protein requirement of juvenile dwarf gourami, *Trichogaster lalius* was reported to be at 36.62 % by Zuanon *et al.* (2013). Fish were fed, a fish meal based diet three times a day until satiation for 90 days. These workers observed a positive linear effect of dietary protein levels on protein efficiency ratio and specific growth rate, while a negative linear effect was observed on feed conversion ratio.

Liu *et al.* (2013), conducted a 9-week feeding experiment to estimate the optimum dietary protein requirement for juvenile tongue sole, *Cynoglossus semilaevis.* Fish were fed to apparent satiation, a fish meal based diet twice daily. Weight gain (%), feed efficiency ratio and protein efficiency ratio were significantly affected by the varying dietary protein levels. They finally suggested that 55% protein containing diet would provide maximum growth and efficient feed utilization for *C. semilaevis* juveniles.

CHAPTER - 3

Materials and Methods

Materials and Methods

Experimental fish

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Cypriniformes

Family: Cyprinidae

Genus: *Cyprinus*

Species: *Cyprinus carpio*

Variety: *Cyprinus carpio specularis*

Source of fish stock and acclimation

Induced bred fingerlings of mirror carp, *Cyprinus carpio* var. *specularis* with the same batch and in apparent good health were procured from the 'State Government Fishery Department seed farm Manasbal'. The fingerlings were transported in polythene bags filled with water and oxygen and brought to the fish feeding trial laboratory (wet-lab) at the Department of Zoology, University of Kashmir. These fingerlings were first given a prophylactic dip in $KMnO_4$ (5 mg L^{-1}) to rule out any possible microbial infection and stocked in indoor circular aqua blue colored plastic fish tank (water volume = 600 L) for a fortnight. During this period, the fish were fed to satiation a mixture of soybean, mustard oil cake, rice bran, and wheat bran in the form of moist cake twice a day at 08:00 and 17:00 hours. These fingerlings were then acclimated for 2 weeks on synthetic diet (Halver, 2002) near to satiation twice a day at 08:00 and 17:00 h in the form of moist cake. A preliminary feed trial was conducted before the start of feeding trial to determine the appropriate feeding level and feeding schedule of the fish.

Preparation of experimental diets

Six casein-gelatin based isocaloric (367 kcal 100g $^{-1}$, gross energy) diets containing graded levels of dietary protein (25%, 30%, 35%, 40%, 45%, and 50% crude protein) were formulated (Table 1.). Diets were prepared taking into account the amount of protein contributed by casein and gelatin and made isocaloric by adjusting the amount of dextrin in the diet. Calculated quantities of dry ingredients were thoroughly mixed and stirred in a know volume of hot water (80 ^{0}C) in a steel bowl attached to a Hobart electric mixer. Gelatin powder was dissolved separately in a known volume of water with constant heating and stirring and then transferred to the above mixture. Other dry ingredients and oil premix, except carboxymethyl cellulose (CMC), were added to the lukewarm bowl one by one with constant mixing at 40 ^{0}C temperature. Carboxymethyl cellulose was added in last and the speed of the blender was gradually increased as the diet started to harden. The final diet, with the consistency of bread dough was poured into plastic Petri dishes and placed in a refrigerator to gel. The prepared diets were in the form of semi-moist cake from which cubes were cut and packed in sealed polythene bags and then stored at -4 ^{0}C until used.

Experimental design and feeding trial

The fishes were sorted out from the acclimatized fish lots maintained in the wet laboratory and the desired number of *C. carpio var. specularis* fingerlings with almost similar body weight and size (1.50±0.02g; 4.5±0.05cm) were randomly selected in triplicate groups in 75 L high-density polyvinyl circular troughs (water volume 65 L) fitted with a continuous water flow-through system at the rate of 20 fish per trough for each dietary treatment levels. The water exchange rate in each trough was maintained at 1.0-1.5 L min^{-1}. The feeding schedule and feeding levels were chosen after carefully observing the feeding behaviour of the fish and their intake. For this purpose an 8-week preliminary feeding trial was also conducted under the same experimental setup in order to determine the appropriate ration size of the fish by feeding fish at the rate of 1%, 2%, 3%, 4%, 5% and 6% BW/day, results showed that the optimum ration size of the fish is approximately 4-4.5%. As per the result obtained in the preliminary feeding trial, the experimental fish were fed test diet in the form of moist cake at the rate of 4% of the body weight six days a week twice a day at 08:00 and 17:00 h, dividing into two equal feeding. The feeding trials lasted for eight weeks. Initial and weekly weights were recorded on a top loading balance (Sartorius CPA-224S 0.1 mg sensitivity, Goettingen, Germany). Fecal matter was siphoned before feeding and the daily feed offered was recorded. The uneaten feed (if any) was collected after active feeding approximately for 40 min with the help of siphoning pipe and collection tubes. The collected feed was then oven-dried at 100 ^{0}C to calculate the final feed conversion ratio (FCR). No feed was offered to the fish on the day of weekly measurement. At the end of the experimental trial, desired number of fishes were randomly sacrificed for the assessment of whole body composition.

Water quality analysis

The physico-chemical parameters of water (temperature, dissolved oxygen, free carbon dioxide, total alkalinity and pH) were recorded daily, following the standard methods (APHA 1998). The water sample for analysis was collected early in the morning before the feeding was done. Water temperature (23.6-24.5 ^{0}C) was recorded using a mercury thermometer, dissolved oxygen (6.1-6.8 mg L^{-1}) was estimated by Winkler's iodimetric test, free carbon dioxide (3.9- 5.7 mg L^{-1}), total alkalinity (91-112 mg L^{-1}) by titrimetric methods, respectively. While, pH (7.2-7.6) was measured by using a digital pH meter (pHep-HI 98107, USA).

Chemical analysis

The proximate composition of experimental diet, initial and final whole body composition was estimated by using standard AOAC (1995) methods and the details of these have been given below:

Methods used for determination of moisture content

The moisture content of experimental diet, initial and final whole body fish samples was determined in the present study by using digital hot air oven (Bells, India). Known quantity of sample was taken in a pre-weighed petri dish and placed in hot air oven at 105±1 ^{0}C for 24 hours. The petri dish containing the dried sample was cooled in a desiccator and reweighed to ensure that the sample had become completely dried. The loss in weight gives an index of water from which its percentage was calculated and expressed as percentage moisture content of the sample.

The percentage of moisture content was calculated by using the following formulae:

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample (g) - Dry weight of sample (g)}}{\text{Wet weight of sample (g)}} \times 100$$

The moisture free dried feed and fish samples were grinded and finely powdered with the help of mortar and pestle for converting samples into fine powder which was used for the analysis of other parameters, such as protein, fat and ash contents.

Protein

The technique employed for the estimation of crude protein content was based on slightly modifying micro-Kjeldahl’s method (Jafri *et al*., 1964; Ahmed *et al*., 2003). Approximately, 0.1-0.5gm of sample was digested with 1:1 sulphuric acid in presence of potassium persulphate as an oxidizing agent. After complete digestion the sample was transferred in 50 ml volumetric flask and raised the volume upto 50 ml by adding double distilled water. 0.5 ml of aliquot was then taken in a test tube with Nesseler’s reagent, after 10 minutes the colour developed was read on spectrophotometer at

480nm. The optical density (OD) obtained is used for estimating the crude protein (N×6.25) content of the sample.

Fat

The crude fat content of feed ingredients, experimental diets and whole body fish samples were determined by using solvent extraction technique with petroleum ether (B.P.= 40-60 ^{0}C) by using Soxtec (Foss Avanti Automatic 2050, Swedan). Briefly 1-5 gm of dried fined powdered sample is placed in Whatman Thimble and defatted cotton is plugged on the top of the thimbles. These thimbles then put into the thimble holder and placed inside the machine i.e. attached with condenser. The aluminum made extraction cups were first dried and weighted. Then added 60-70ml of petroleum ether and finally attached with thimbles already placed inside the machine. After full programming the extraction process gets started and then completing the whole extraction process, the equipment display a message that extraction is completed. Then the extraction cup containing fat content was removed from the extraction unit and placed in digital oven for about 40-60 minutes at 60 ^{0}C for the complete evaporation of petroleum ether, later on the aluminum cups containing samples were placed in desiccators for complete coolness and finally the weight was taken.

The total fat was calculated by using following formulae:

$$\text{Total fat (\%)} = \frac{\text{Weight of fat (g)}}{\text{Weight of sample (g)}} \times 100$$

Weight of fat = Weight of extraction cup with fat - Weight of empty extraction cup.

Total Ash

The ash content of the sample is the residue left after complete ashing. The fine powdered moisture free samples were taken in clean pre-weighted silica crucibles and weighted again along with samples. The crucibles containing samples was then placed in a muffle furnace at 650 ^{0}C for about 4-6 hours or till the residue became completely white. The samples were then allowed to cool in desiccators for about 20-30 minutes, reweighted and the amount of ash was calculated as the difference in weight.

The percentage of ash was obtained by using the following formulae:

$$\text{Total ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Weight of ash= weight of crucible with ashed sample -weight of empty crucible.

Hematological parameters

At the termination of feeding trial, blood samples for analysis were collected in heparinized (Na-heparinised) capillary tubes from the haemal arch after severing the caudal peduncle. Blood was pooled from each test group and stored in heparin coated vaccutainer plastic tubes for future tests. All the hematological analysis was carried out within 2 hours after each extraction.

Haemoglobin (Hb)

Haemoglobin content of blood was estimated by the method of Drabkin (1946). 20 µl of blood was mixed with 5 ml of Drabkin solution (Loba chemie, India) and left to stand for at least 15 minutes. Haemoglobin concentration was determined by measuring the absorbance at 540 nm and compared to that of haemoglobin standard (Ranbaxy, India). Prior to reading the absorbance, hemoglobin test samples were centrifuged to remove dispersed nuclear material.

The Hb content of the sample was calculated with the following formulae:

$$\text{Hb (g dl}^{-1}\text{)} = \frac{\text{A}_{540}\text{ test sample} \times \text{concentration of standard} \times 0.25}{\text{A}_{540}\text{ standard}}$$

Haematocrit (HCT)

Haematocrit (HCT %) was determined on the basis of sedimentation of blood. Heparinised blood (50 µl) was taken in a micro-haematocrit capillary (Na-heparinised) and spun in a micro-haematocrit centrifuge (REMI RM-12C, India) at 12,000 rpm for 5 min to obtain haematocrit value. The haematocrit value was measured using a haematocrit reader and reported as percentage (Del Rio-Zaragoza *et al*., 2008).

Total erythrocyte count (RBC) and total leucocyte count (WBC)

For RBC and WBC count a blood sample (20µl) was taken with a micro pipette (Finpipette, Finland), and diluted with Natt-Herrick's (1952) diluent (1:200). The diluted sample was placed in a Neubauer improved haemocytometer (Marienfeld-Superior, Lauda-Konigshofen, Germany) and then the blood cells were counted using a light microscope (Magnus MLM, India).

The following formulae were used to calculate these parameters:

$$\text{RBC count} = \frac{\text{Number of cells counted x dilution factor x depth of chamber}}{\text{Area counted}}$$

Where dilution factor is one in 200, depth is 1/10mm and area counted = 80/400 =1/5 sq.

$$\text{RBC count} = \frac{\text{Number of cells counted x 200 x 10}}{1/5}$$

$$\text{RBC /mm}^3 = \text{number of cells counted x 10,000}$$

$$\text{WBC count} = \frac{\text{Total leucocytes counted x blood dilution x chamber depth}}{\text{Number of chambers counted}}$$

$$\text{WBC/mm}^3 = \frac{\text{Total leucocytes in 9 squares x 200 x 10}}{9}$$

Erythrocyte indices viz: mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volumes (MCV) were calculated according to Dacie and Lewis (1991).

Growth Parameters

Growth performance of the fish fed diets with different protein levels was calculated as a function of the weight gain by using the following formulae:

Weight gain (%)

= Final body weight–initial body weight/initial weight x 100

Specific growth rate (SGR %)

=100 x (In final wet weight (g)-In initial wet weight g)/duration (days)

Feed conversion ratio (FCR)

= Dry weight of feed consumed / Wet weight gain

Protein efficiency ratio (PER)

=Wet weight gain (g) / Protein consumed (g, dry weight basis)

Body protein deposition (BPD %)

$=100 \times (BW_f \times BCP_f) - (BW_i \times BCP_i) / [TF \times CP]$

Where BW_i and BW_f = mean initial and final body weight (g), BCP_i and BCF_f = mean initial and final percentage of muscle protein, TF =Total amount of diet consumed, and CP=Percentage of crude protein of the diet.

Hepatosomatic index (HSI %) = $\dfrac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$

Statistical analysis

Responses of mirror carp fingerlings to graded levels of dietary protein were measured by weight gain (%) feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR %) and body composition. These response variables were subjected to one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1967; Sokal and Rohlf, 1981). To determine the significant differences among the treatments, Duncan’s Multiple Range Test (Duncan, 1955) was employed. To predict more accurate responses to the dietary protein intake, the optimum dietary protein level was estimated using second-degree polynomial regression analysis ($Y = ax^2 + bx + c$) as described by Zeitoun *et al*. (1976). Statistical analysis was done using Minitab (version 16.0) and SPSS (version 20.0).

Table 1. Formulation and proximate composition of experimental diets used for estimating the dietary protein requirement of mirror carp, *Cyprinus carpio* var. *specularis* fingerlings.

	Diet (%)					
Ingredients (g 100g^{-1}, dry diet)	**(I)**	**(II)**	**(III)**	**(IV)**	**(V)**	**(VI)**
Casein[1]	24.40	29.20	34.0	38.80	43.60	48.60
Gelatin[2]	6.10	7.30	8.50	9.70	10.90	12.15
Dextrin[3]	50.62	44.24	37.87	31.50	25.13	18.49
Corn oil	6.00	6.00	6.00	6.00	6.00	6.00
Cod liver oil	3.00	3.00	3.00	3.00	3.00	3.00
Mineral mix[4]	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix[4,5]	3.00	3.00	3.00	3.00	3.00	3.00
Carboxymethyl cellulose	2.00	2.00	2.00	2.00	2.00	2.00
Alpha cellulose	0.88	1.26	1.63	2.00	2.37	2.76
Total	100	100	100	100	100	100
Calculated crude protein (g 100g^{-1})	25.0	30.0	35.0	40.0	45.0	50.0
Analysed crude protein (g 100g^{-1})	24.87	29.65	34.58	40.16	44.79	50.18
Gross energy[6] (kcal g 100g^{-1}, dry diet)	367.0	367.0	367.0	367.0	367.0	367.0

[1] Crude protein (80%), Loba Chemie, India

[2] Crude protein (93%), Loba Chemie, India;

[3] Loba Chemie, India.

[4] Ahmed (2010)

[5] Ahmed (2010)

[6] Calculated on the basis of physiological fuel values 4.5, 3.5 and 8.5 kcal g^{-1} for protein, carbohydrate and fat, respectively (Jauncey, 1982).

Plate 1. Netting done at Manasbal fish seed farm

Plate 2. Transport of fingerlings

Plate 3. Wet-lab flow-through system

Plate 4. Fingerling stock tank

Plate 5. Feed ingredients

Plate 6. Preparation of vitamins and minerals premix.

Plate 7. Moist cake experimental diets

Plate 8. Feed packed for storage

Plate 9. Fingerling during mass weight

Plate 10. Feeding trial setup

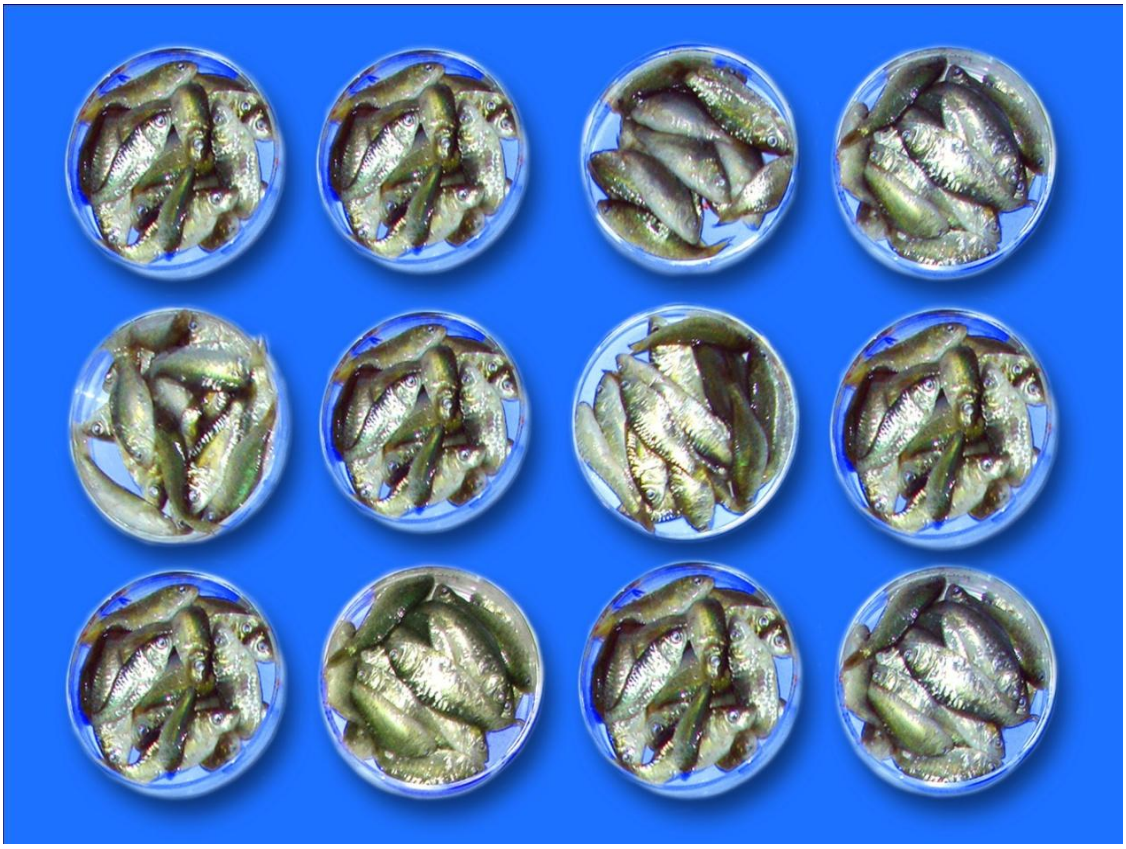


Plate 11. Final wet samples

Plate 12. Final dry sample

Plate 13. Soxtec automatic fat analyzer

Plate 14. Extraction cups containing fat

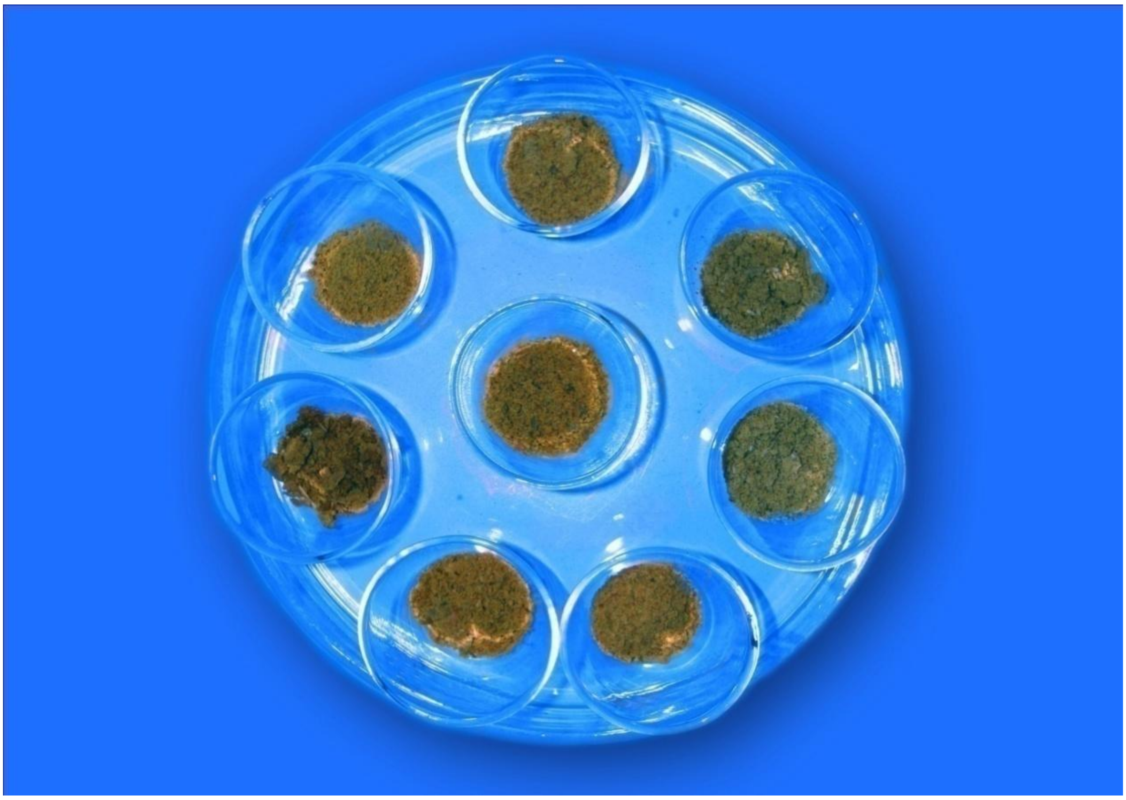


Plate 15. Samples before ashing

Plate 16. Samples after ashing

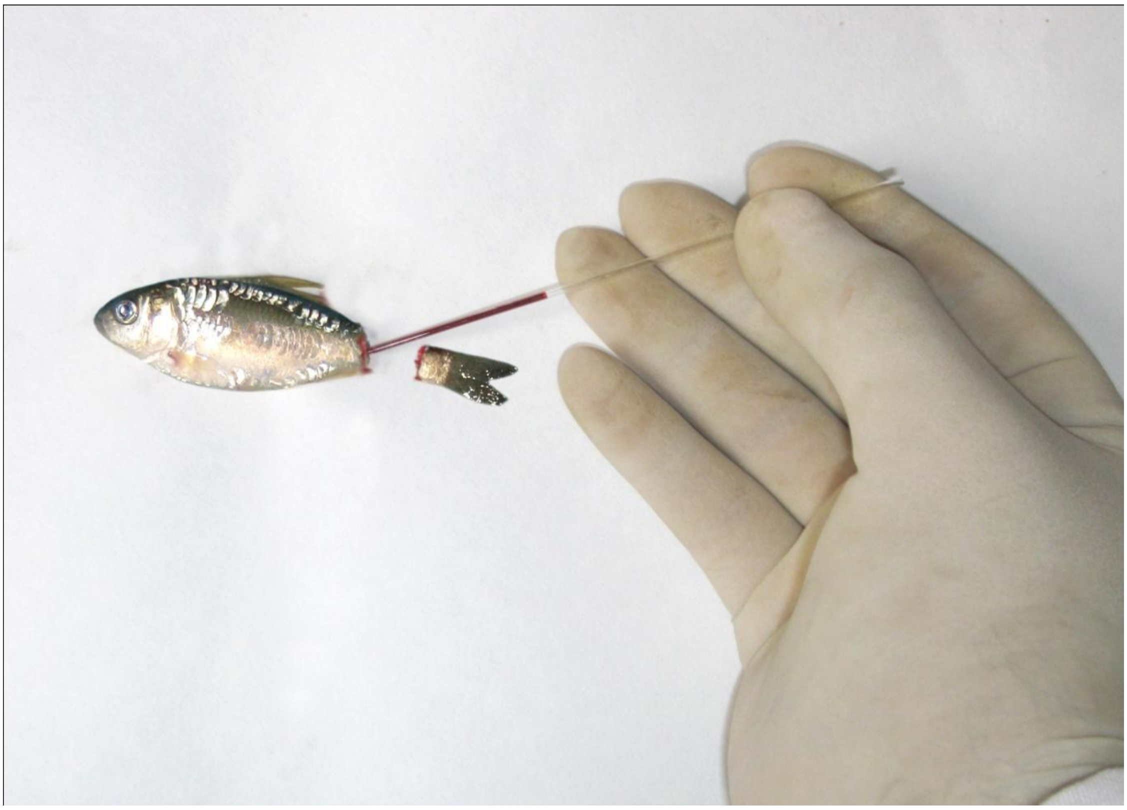


Plate 17. Collection of blood with Na-Heparinized capillary

Plate 18. Centrifugation for micro-haematocrit

Plate 19. Haemocytometer loading

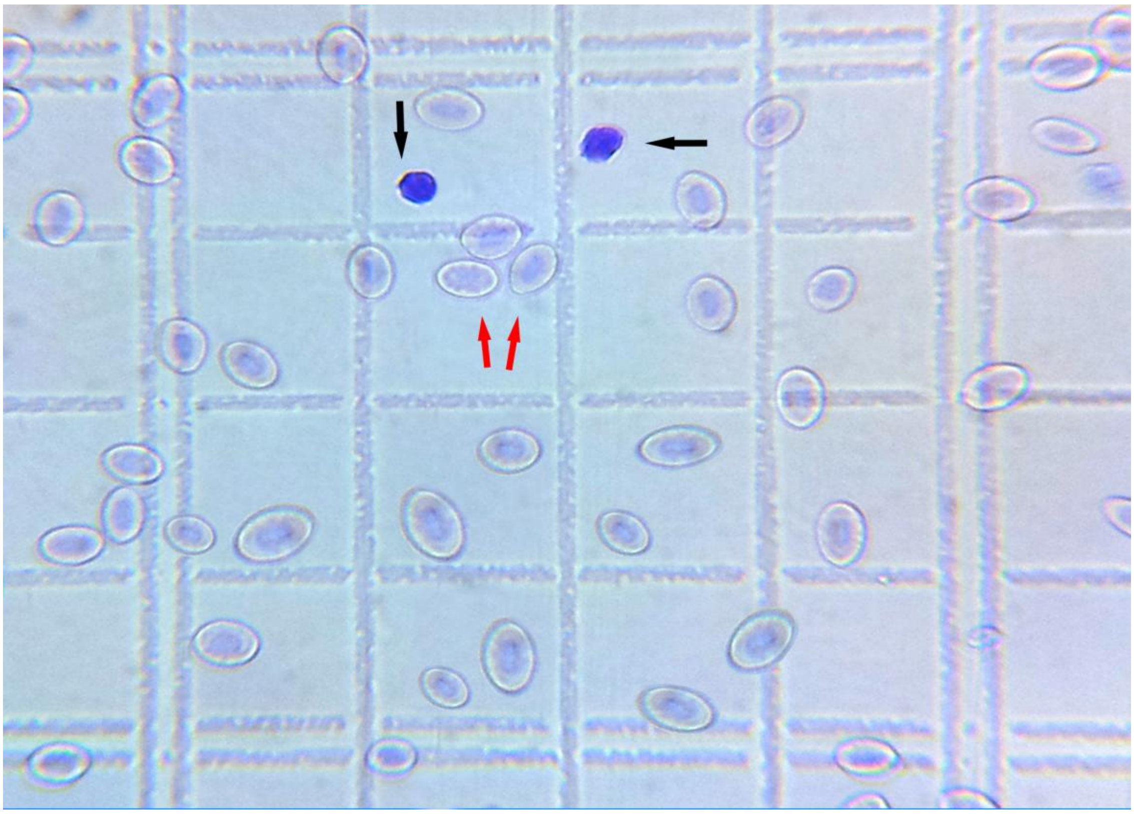


Plate 20. RBC’s (Red arrow) and WBC’s (Black arrow) in Haemocytometer counting chamber

CHAPTER - 4

Results

Results

Growth performance of mirror carp, *Cyprinus carpio* var. *specularis* fed diets containing graded levels of protein over the 8-week feeding trial are presented in Table 2. No mortality was observed among all the dietary treatment levels during the entire length of feeding trial. Live weight gain (LWG %), specific growth rate (SGR %), feed conversion ratio (FCR) and protein efficiency ratio (PER) were found to be significantly ($P<0.05$) affected with the increase of dietary protein level in the diets. A linear relationship between the percentage of protein content in the diet and the increase in weight gain up to an incorporation rate of 40% was noted. The maximum weight gain (258%) for mirror carp was obtained with the diet containing 40% dietary protein level, although it was not significantly different from that achieved by the fish fed a 45% protein diet. However, an intermediate value of growth rate was observed in fish fed diet containing lower level of dietary protein i.e. <40% and higher level of dietary protein (>45%) diets, while the poorest growth rate was recorded for fish receiving diet with 25% protein followed by those receiving diet containing 30% protein in the diet, respectively. Feed conversion ratio decreased progressively with linearly increasing dietary protein level and was found to differ significantly ($P<0.05$) among each dietary protein level. The best-FCR (1.63) was recorded with fish receiving diet at 40% dietary protein level, which was not significantly ($P>0.05$) different to group that fed at 45% protein containing diet.

The protein efficiency ratio in fish fed varying dietary protein levels differed significantly ($P<0.05$) and showed an increasing tendency with increasing dietary protein level, which increased from 1.35 to 1.53 for fish fed 20% and 40% dietary protein, respectively. Whereas, a significant decline was observed in PER for fish fed 45% and 50% protein diets, with the lowest (0.96) PER being noted at 50% dietary protein level. Overall significantly ($P<0.05$) highest PER (1.53) was recorded when fish were fed a diet containing 40% protein. The hepatosomatic index (HSI) value of mirror carp in the present study also showed some significant differences, with maximum values observed with fish fed at lowest protein containing diet.

In order to get statistically more precise information, all, the growth parameters were subjected to second-degree polynomial regression analysis. When live weight gain data (Y) and dietary protein levels (X) were analyzed using second-degree polynomial analysis, a break-point was evident at 43.47% dietary protein level (Fig. 1). The relationship was described by the equation:

$$Y = - 0.4568x^2 + 39.7199x - 616.0786 \ (r = 0.959)$$

The specific growth rate of mirror carp fed varied levels of dietary protein also produced somewhat similar trends as obtained in the growth rate. The SGR (Y) to dietary protein level (X) was also analyzed by using second-degree polynomial regression analysis (Fig. 2) and the break point was evident at 42.93% protein level. The mathematical equation was:

$$Y = - 0.0031x^2 + 0.2662x - 3.5271 \ (r = 0.975)$$

The FCR of mirror carp fed 40% and 45% dietary protein was significantly lower than those fed other dietary protein levels. The FCR (Y) to dietary protein levels (X) relationship was also best described using a second-degree polynomial regression analysis (Fig. 3). The relationship being:

$$Y = 0.0048x^2 - 0.3977x + 9.9543 \ (r = 0.975)$$

Significantly ($P<0.05$) highest PER was recorded with fish fed at 40% protein containing diet. The PER (Y) to dietary protein level (X) was also best described using second-degree polynomial regression analysis (Fig. 4). The equation being as:

$$Y = - 0.0023x^2 + 0.1590x - 1.2586 \ (r = 0.896)$$

Based on the above polynomial equations the FCR and PER indicated that the optimum dietary protein requirement of mirror carp was estimated to be at 41.42% and 34.56%, respectively.

At the end of feeding trial, significant differences ($P<0.05$) in whole body composition were observed among all the dietary groups (Table 3). Generally, body composition was affected by increasing dietary protein levels. Whole body moisture content gradually decreased with the increase in the dietary protein content of the diet up to 45%. However, fish fed 45% protein containing diet produced significantly ($P<0.05$) lowest whole body moisture content, which was insignificantly ($P>0.05$) different to the group that fed 40% protein diet. Whole body protein content was significantly ($P<0.05$) higher in fish fed diet containing 40% protein followed by those receiving diet containing 45% and 50%, respectively. Whole body fat content gradually increased with the increase of dietary protein level and significantly ($P<0.05$) highest body fat content was recorded with fish group that were fed 45% protein diet, followed by those fed at 40% protein diet, while intermediate whole body fat values were recorded in those groups that fed 35% and 50% protein diets, respectively.

Whole body ash content was found to be significantly higher ($P<0.05$) at lower dietary protein containing diets i.e. 25% and 30% , whereas significantly lower ash content values were observed in fish fed the remaining dietary protein levels. Also fish fed diet containing 40% protein resulted in highest whole body protein deposition (BPD%), which was significantly highest among all the dietary groups. Second-degree polynomial regression analysis (Fig. 5) was also employed between the body protein deposition (Y) to dietary protein level (X) and a break-point was obtained at 37.31% protein level. The mathematical equation was:

$$Y = - 0.0553x^2 + 4.1274x - 50.6271 \quad (r = 0.816)$$

The haematological parameters of mirror carp fed diets containing varied dietary protein levels also produced some significant differences (Table 4). The fish fed diet containing 40% and 45% protein diets had significantly ($P<0.05$) highest haemoglobin (Hb) content, followed by those receiving 50% protein diet. Whereas, intermediate values of Hb content was recorded at 35% protein diet, while poorest Hb content was estimated at lowest level of protein diet i.e. 25%. Haematocrit (HCT) values increased significantly ($P<0.05$) with the increase in dietary protein levels from 25% to 45% protein containing diets. However, higher HCT value (38.26%) was recorded for fish fed 45% protein diet, while the lowest HCT value (22.19%) was noted at the lowest protein level (25%).

Red blood cell counts (RBC) in fish fed various dietary protein levels also produced significant differences. Significantly ($P<0.05$) highest RBC value (1.9×10^6 mm^{-3}) was noted at 45% protein diet, followed by those receiving diet at 40% protein diet. Intermediate RBC values were recorded in fish fed other dietary protein levels, except those fed 25% and 30% protein diets, where significantly ($P< 0.05$) lowest RBC count values were obtained. Whereas, the fish fed varied levels of dietary protein could not produce any significant difference in their leukocyte (WBC) counts, except at lowest levels where slightly higher WBC counts were recorded.

No significant ($P>0.05$) differences in mean corpuscular volume (MCV) values were observed in the present study, when fish were fed varied levels of dietary protein diets, except at lowest protein containing diet i.e. 25% where, significantly ($P<0.05$) lowest MCV (180.33fl) value was noted. Similar trends were also observed in mean corpuscular haemoglobin concentration (MCHC) in the present study, when fish were fed varied levels of dietary protein content, while mean corpuscular haemoglobin (MCH) data in the present study showed significant differences. The highest MCH values were noted at 50% and 35% protein containing diets, which were not significantly different among each other. Whereas, the intermediate values of MCH were recorded in other dietary groups.

Table 2. Growth, FCR, protein deposition and percentage survival of mirror carp, *Cyprinus carpio* var. *specularis* fingerlings fed diets containing varying levels of dietary protein for 8 weeks*.

	Dietary protein levels (g 100g^{-1}, dry diet)					
	25.0	**30.0**	**35.0**	**40.0**	**45.0**	**50.0**
Average initial weight (g)	1.557 ± 0.04	1.592 ± 0.02	1.608 ± 0.01	1.599 ± 0.02	1.605 ± 0.02	1.614 ± 0.03
Average final weight (g)	3.142 ± 0.15	3.968 ± 0.07	4.909 ± 0.07	5.731 ± 0.05	5.623 ± 0.11	5.189 ± 0.13
Live weight gain (%)	101.64 ± 5.10^{d}	149.25 ± 7.58^{c}	205.37 ± 6.80^{b}	258.48 ± 8.36^{a}	250.43 ± 8.50^{a}	221.48 ± 5.23^{b}
Specific growth rate (SGR)	1.25 ± 0.04^{d}	1.63 ± 0.04^{c}	1.99 ± 0.03^{b}	2.28 ± 0.03^{a}	2.24 ± 0.04^{a}	2.08 ± 0.02^{b}
Feed conversion ratio (FCR)	2.93 ± 0.06^{a}	2.45 ± 0.05^{b}	1.92 ± 0.05^{d}	1.63 ± 0.04^{e}	1.70 ± 0.05^{e}	2.08 ± 0.07^{c}
Protein efficiency ratio (PER)	1.35 ± 0.03^{b}	1.36 ± 0.02^{b}	1.49 ± 0.04^{a}	1.53 ± 0.04^{a}	1.30 ± 0.03^{b}	0.96 ± 0.03^{c}
Body protein deposition (BPD)	19.46 ± 0.53^{d}	21.41 ± 0.44^{c}	24.60 ± 0.63^{b}	28.50 ± 0.69^{a}	23.86 ± 0.70^{b}	16.60 ± 0.69^{e}
Hepatosomatic index (HSI)	3.39 ± 0.05^{a}	3.08 ± 0.05^{b}	2.80 ± 0.07^{c}	2.41 ± 0.02^{d}	2.33 ± 0.04^{d}	2.72 ± 0.03^{c}
Survival (%)	100	100	100	100	100	100

*Mean values of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different ($P > 0.05$).

Table 3. Whole body composition of fingerling, *Cyprinus carpio* var. *specularis* fed diets containing graded levels of dietary protein for 8 weeks[*].

		Dietary protein levels (g 100g^{-1}, dry diet)					
	Initial	**25.0**	**30.0**	**35.0**	**40.0**	**45.0**	**50.0**
Moisture (%)	80.86 ± 0.44	77.49 ± 0.26^{a}	75.66 ± 0.040^{b}	74.34 ± 0.22^{c}	72.57 ± 0.17^{d}	72.25 ± 0.20^{d}	73.75 ± 0.14^{c}
Protein (%)	12.01 ± 0.15	13.15 ± 0.06^{f}	14.27 ± 0.05^{e}	15.06 ± 0.08^{d}	16.77 ± 0.07^{a}	16.49 ± 0.05^{b}	15.62 ± 0.06^{c}
Fat (%)	3.37 ± 0.13	4.81 ± 0.09^{f}	5.41 ± 0.11^{e}	6.13 ± 0.07^{d}	6.72 ± 0.08^{b}	7.61 ± 0.09^{a}	6.43 ± 0.07^{c}
Ash (%)	2.66 ± 0.05	3.16 ± 0.04^{a}	2.90 ± 0.02^{b}	2.61 ± 0.02^{e}	2.72 ± 0.04^{d}	2.65 ± 0.03$^{d, e}$	2.82 ± 0.02^{c}

[*] Mean values of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different (*P*>0.05).

Table 4. Effect of experimental diets on hematological parameters of mirror carp, *Cyprinus carpio* var. *specularis* fingerlings[*].

	Dietary protein levels (g 100g^{-1}, dry diet)					
	25.0	**30.0**	**35.0**	**40.0**	**45.0**	**50.0**
Hb (g dl^{-1})[1]	6.856 ±0.07^{e}	7.770 ± 0.08^{d}	9.270 ±0.07^{c}	10.780 ±0.10^{a}	10.920 ±0.15^{a}	10.240±0.07^{b}
HCT (%)[2]	22.190 ± 0.69^{e}	26.403 ±0.83^{d}	30.830 ± 0.90^{c}	36.770 ±0.72^{a}	38.260 ±0.56^{a}	33.580 ±0.50^{b}
RBC (x10^{6}/mm^{3})[3]	1.231 ± 0.02^{e}	1.343 ±0.03^{d}	1.522 ±0.03^{c}	1.780 ±0.04^{b}	1.897 ± 0.05^{a}	1.621 ±0.04^{c}
WBC (x10^{4}/mm^{3})[4]	2.422 ±0.04^{a}	2.361 ±0.05^{ab}	2.327 ±0.03^{ab}	2.275 ±0.04^{b}	2.303 ± 0.06^{ab}	2.244 ± 0.05^{b}
MCV (fl)[5]	180.33 ±5.61^{b}	196.736 ± 8.73^{a}	202.622 ± 6.55^{a}	208.610 ± 4.73^{a}	201.780 ± 2.83^{a}	207.930 ±6.38^{a}
MCH (pg)[6]	55.71 ± 0.53^{d}	57.86 ±0.81^{c}	60.91 ±1.02^{a,b}	60.58 ±0.83^{b}	57.60 ± 0.91^{c}	63.21 ± 0.13^{a}
MCHC (g dl^{-1})[7]	30.93 ±0.98^{a}	29.46 ± 0.90^{a,b}	30.10 ±1.03^{a,b}	29.34 ±0.55^{a,b}	28.94 ± 0.44^{b}	30.42 ±0.64^{a,b}

[*] Mean values of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different ($P > 0.05$).

[1] Haemoglobin concentration; [2] Haematocrit; [3] Red blood cell count; [4] White blood cell count; [5] Mean corpuscular volume; [6] Mean corpuscular haemoglobin; [7] Mean corpuscular haemoglobin concentration.

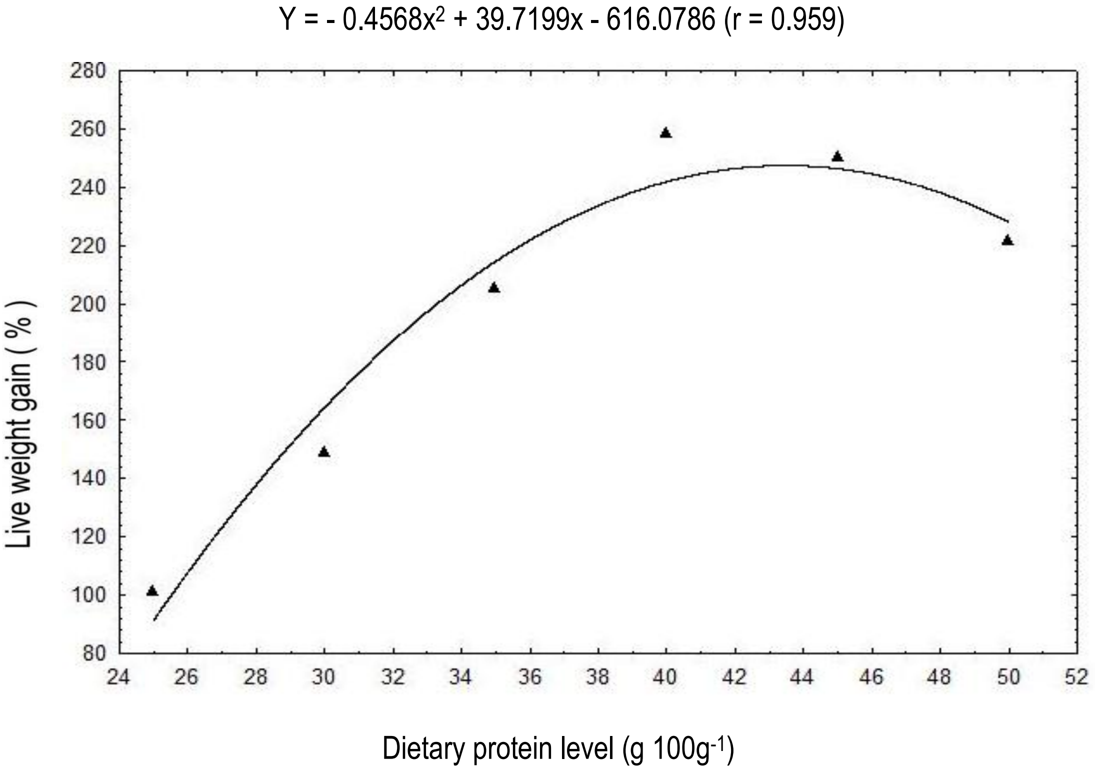


Fig. 1. Second-order polynomial relationship between live weight gain (%) and dietary protein levels of mirror carp, *Cyprinus carpio* var. *specularis* fingerlings fed with experimental diets for 8 weeks (*n* = 3).

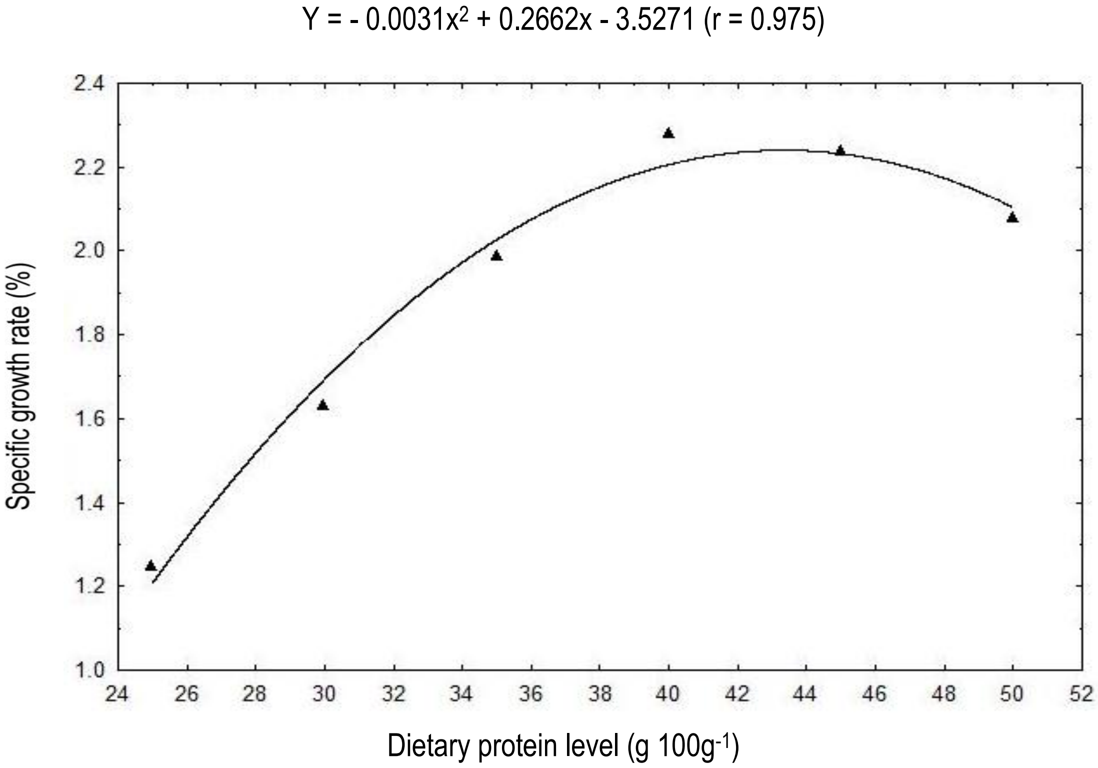


Fig. 2. Second-order polynomial relationship between dietary protein levels and specific growth rate (SGR%) of mirror carp, *Cyprinus carpio* var. *specularis* fingerlings fed with experimental diets for 8 weeks (*n* = 3).

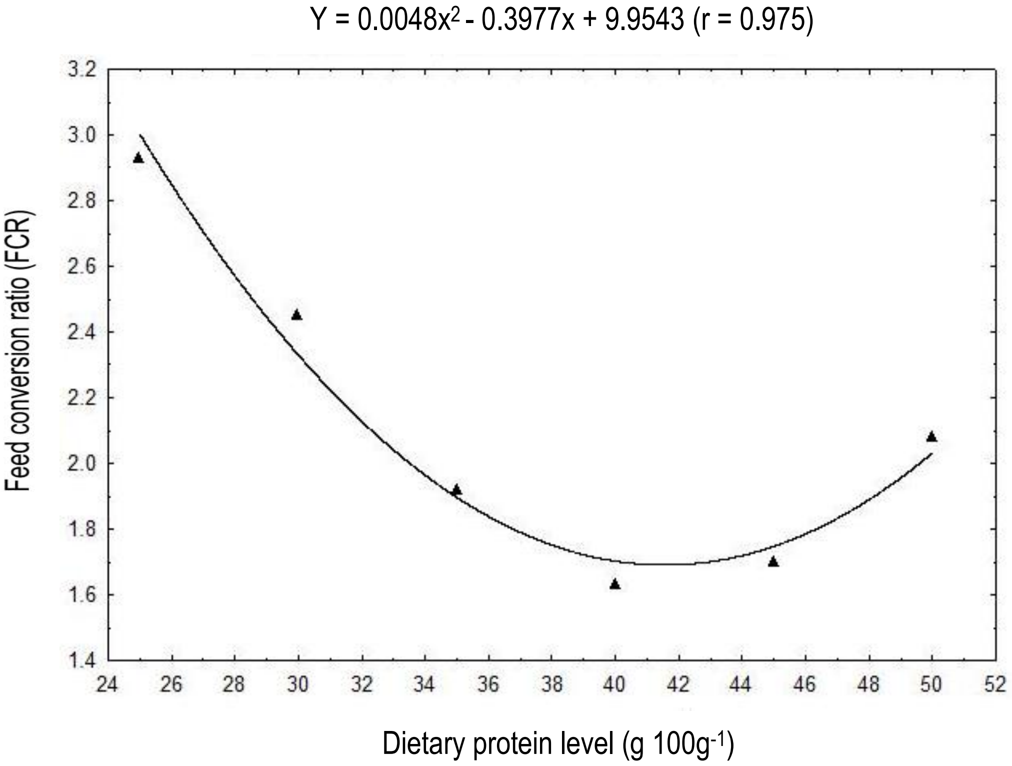


Fig. 3. Second-order polynomial relationship between dietary protein levels and feed conversion ratio (FCR) of mirror carp, *Cyprinus carpio* var. *specularis* fingerlings fed with experimental diets for 8 weeks (*n* = 3).

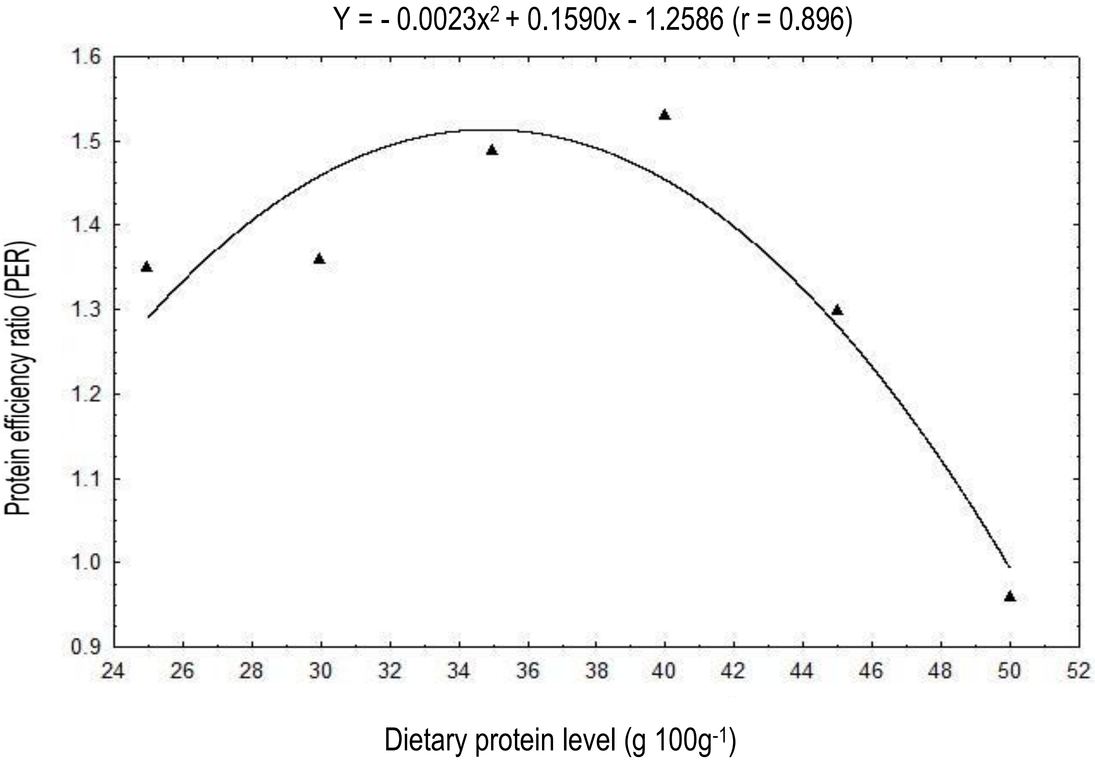


Fig. 4. Second-order polynomial relationship between dietary protein levels and protein efficiency ratio (PER) of mirror carp, *Cyprinus carpio* var. *specularis* fingerlings fed with experimental diets for 8 weeks (*n* = 3).

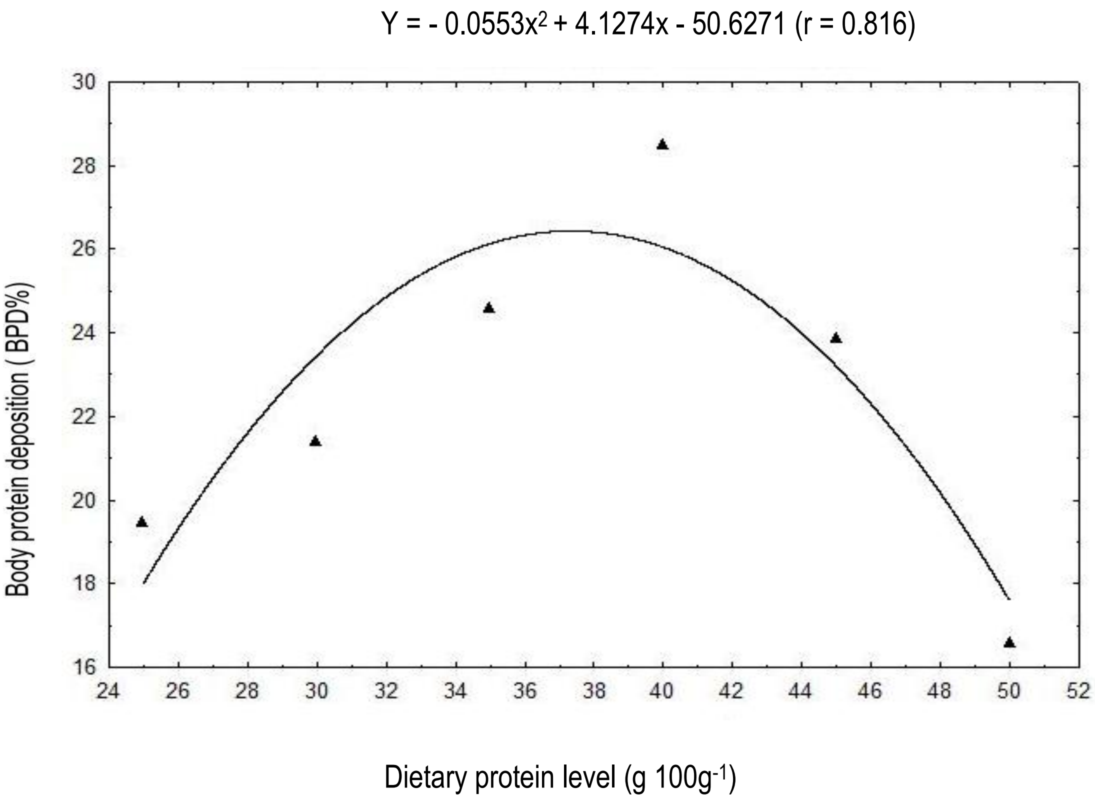
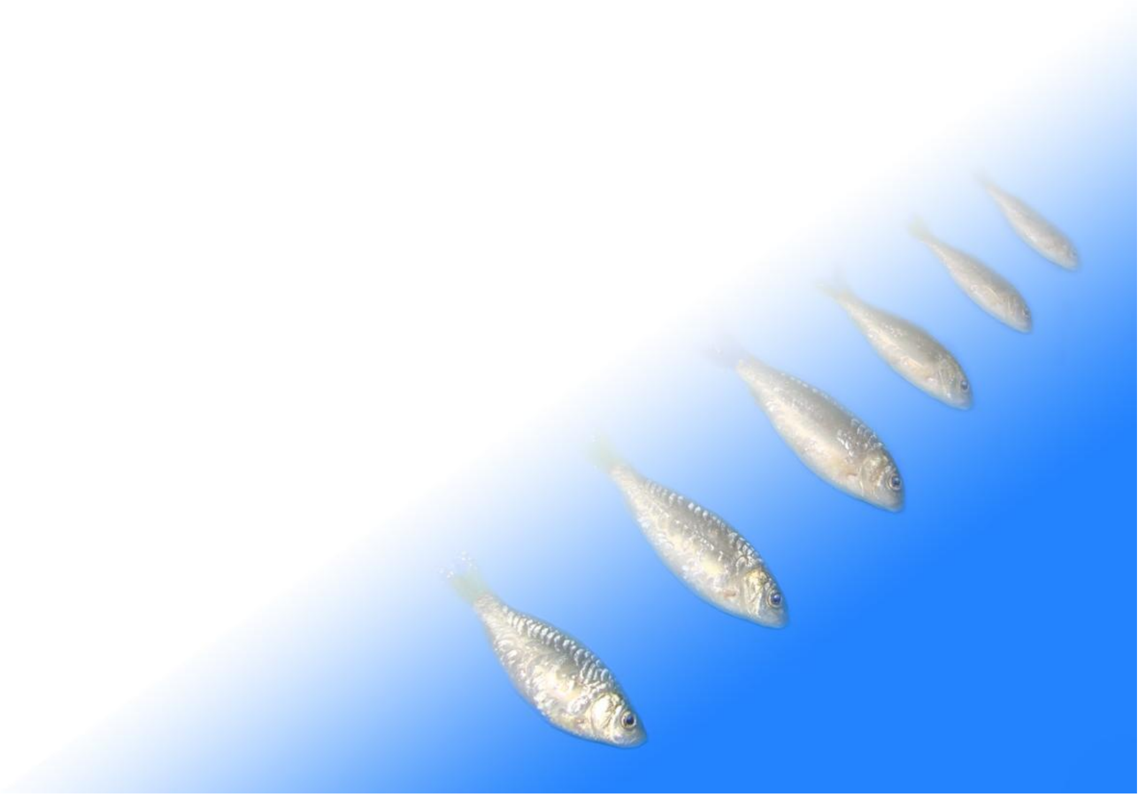


Fig. 5. Second-order polynomial relationship between dietary protein levels and body protein deposition (BPD%) of mirror carp, *Cyprinus carpio* var. *specularis* fingerlings fed with experimental diets for 8 weeks ($n = 3$).

CHAPTER - 5

Discussion



Discussion

Dietary protein intake is related to nutritional status and growth of fish. Efficient utilization of protein however depends on the availability of other nutrients like carbohydrates and fats in appropriate quantity (Wilson and Halver, 1986). Inadequate protein content in the diet results in loss of weight, also lack of essential amino acids or its improper balance in diet often becomes a limiting factor for normal fish growth; whereas excess protein in the diet also affects growth rate and nitrogen loading in the water which causes water pollution (Ahmed *et al.,* 2003).

Understanding the dietary protein requirement of fingerling stage of mirror carp becomes a pre-requisite for the development of nutritionally balanced, efficient and cost effective feed for culturing practice. Protein requirements are more precisely determined through feeding purified and semi-purified diets under defined experimental setup. Generally, optimum nutrient requirements for fish are determined through ‘dose-response’ study considering percent increase in weight gain, feed conversion ratio, protein efficiency ratio and nutrient accretion in the whole body carcass.

Increase in dietary protein content in the diets has often been associated with higher growth rates in many fish species as this component supplies the significant proportion of essential amino acids which are the building blocks of protein synthesis. However, it is a well known fact that there is a protein level beyond which further growth is not supported, and may even put adverse effect on growth.

In the present study, graded levels of dietary protein content had a significant effect on the growth rate, feed conversion ratio, protein efficiency ratio and specific growth rate. The growth and conversion efficiencies gradually increased with the increase of dietary protein levels from 25% to 45% protein containing diet. Although the maximum growth parameters were obtained when fish were fed at 45% protein containing diet, however, this growth rate was not significantly different to those groups that were fed at 40% protein diet. Whereas, the best-FCR, PER, SGR and BPD was recorded with fish fed 40% protein diet. Therefore, inclusion of 40% protein in the diet for fingerling mirror carp is more appropriate and economical. Also the growth rate significantly fell beyond the requirement level, especially at 50% protein diet, indicating that 40% protein diet (Diet IV) satisfied the protein requirement of the fish and is considered optimum for achieving maximum growth and efficient nutrient conversion efficiency. The decrease in growth rate at protein levels above the optimum requirement may be attributed to the fact that the fish body cannot utilize the dietary protein once after reaching the optimum protein level (Phillips, 1972). The excessive protein content in the diet could reduce the growth performance of fish due to higher energy requirement for catabolism rather than for protein deposition. The decrease in weight gain, when the fish were fed excess level of dietary protein may also be because of a reduction in available energy for growth and due to inadequate non-protein energy necessary to deaminate the high protein feed (Jauncy, 1982; Kim *et al.*, 2002). The reduced growth rate and decreased protein utilization beyond requirement of dietary protein level is well documented in the past by several workers (Jobling and Wandshik, 1983; Tibbetts *et al.*, 2000; Kim *et al.*, 2002; Sales *et al.*, 2003; Kalla *et al.*, 2004; Cho *et al.*, 2005; Kim and Lee, 2005 and Sa *et al.*, 2006).

In general, both, feed conversion ratio and protein efficiency ratio were poor in lower protein containing diets. However, improvement in FCR and PER was noticed with increasing incorporation of dietary protein levels. The best-FCR and highest PER values were recorded with fish fed at 40% protein containing diet. The BPD and PER increased with the increase in dietary protein content up to 40% and thereafter, a significant decrease was recorded with further elevation of dietary protein level i.e. 45% and 50% protein containing diets (Diet V and Diet VI). Similar trends in PER and BPD were also reported by other workers (Lee *et al.*, 2002).

The whole body composition data showed that whole body moisture content gradually increased with the increase of dietary protein levels, with minimum moisture content was recorded at 40% protein diet. Whole body protein content linearly increased with the increase of dietary protein level up to 40% and thereafter, a decline in body protein content was noted. The highest protein content obtained in the present study, when fish fed at 40% protein diet could be due to the fact that at this particular level fish utilized the available protein content for growth more efficiently than those fed other dietary protein levels. Similar results on body protein content have also been reported by Kim *et al.* (2002). Kim and Lee (2009) further reported that body protein content responded to dietary protein levels in a dose dependent manner and exhibited maximum protein content on that dietary protein level where maximum growth rate was also achieved.

Whole body fat content gradually increased with the increase of dietary protein levels and maximum body fat content was recorded at higher dietary protein containing diet (Diet VI). The higher whole body fat content beyond the optimum protein requirement level in the diet may be due to the fact that the excess dietary protein content in these diets gets deaminated and stored as body fat. The whole body ash did not show any significant difference among the treatment levels, except at lower protein containing diets where high body ash content was recorded. The fish fed varied levels of dietary protein produced some significant differences in HSI values. The highest HSI values (3.39%) were observed at the lowest dietary protein level, which was significantly higher compared to all the dietary groups. Higher values of HSI in lower protein diets could be due to the poor growth and health of the fish (Brauge *et al*., 1994; Hamre *et al*., 2003; Moreira *et al*., 2008), and also due to more fat accumulations in the liver (Lee *et al*., 2002; Yang *et al*., 2003; Abdel-Tawwab *et al*., 2010; Guo *et al*., 2012).

Besides biochemical analysis, haematological analysis was also carried out in the present study in order to find out the effects of dietary protein levels on these parameters, which are recognized as valuable tools for monitoring fish health and physiological responses to environmental stress (Bhaskar and Rao, 1984; Schuett *et al*., 1997; Jawad *et al.*, 2004). Fish haematology is also considered a useful tool in the assessment of feed composition and nutritional status in relation to environmental conditions affecting fish (Svobodova *et al.,* 2005). Besides these, the haematological indices are also a reflection of effects of many factors, which include species, size, age, physiological status, environmental conditions and dietary regime, e.g. quality and quantity of food, dietary ingredients, protein sources and vitamins (Houston, 1997; Lim *et al*., 2000; Irianto and Austin, 2002; Brunt and Austin, 2005; Osuigwe *et al*., 2005).

In the present study, significant differences were observed in Hb, HCT and RBC values of different groups fed with varying levels of dietary protein, showing a general trend of linear increase with the increase of dietary protein levels. However, the haematological values obtained in the present study were within acceptable limits as reported by *Svobodova et al.* (2005), for common carp. Fishes alter their metabolic profile to cope up with the different dietary conditions (Bidinotto and Moraes, 2000; Moraes and Bidinotto, 2004; Lundstedt *et al*., 2004; Melo *et al*., 2006). Hb and HCT values increased significantly with the increase of dietary protein levels from 25%-45% protein containing diets. However, highest Hb (10.92 g dl^{-1}) and HCT values

(38.26%) were recorded for fish fed 45% protein diet. An increase in RBC count was evident with the increase in dietary protein levels, which may have occurred due to its early release from the storage pool in the spleen (Vijayan and Leatherland, 1989; Pulsford *et al*., 1994). This, consequently, changed the MCH value at the same time.

On the basis of second-degree polynomial regression analysis of growth parameters and body composition data, the optimum dietary protein level for growth of mirror carp, *Cyprinus carpio* var. *specularis* fingerling is recommended to be at 41.50%. The protein requirement of mirror carp estimated during the present study in terms of percentage is higher than the values reported for young grey mullet, *Mugil capito*, 24 (Papaparaskera-Papoutsoglou and Alexis, 1986), Nile tilapia, *Oreochromis niloticus*, 25 (El-Saidy and Gaber, 2005), rohu, *Labeo rohita*, 25 (Khan *et al*., 2005), juvenile greenlip abalone, *Haliotis laevigata*, 27 (Coote *et al*., 2000), young tilapia, *Oreochromis mossambicus*, 28 (De Silva *et al*., 1989), channel catfish, *Ictalurus punctatus*, 28 (LI *et al*., 2006), golden shiner, *Notemigonus crysoleucas*, 29 and goldfish, *Carassius auratus*, 32 (Lochmann and Phillips, 1994), walking catfish, *Clarias batrachus*, 30 (Chuapoehuk, 1987). Nile tilapia, *O. niloticus*, 30 (Siddiqui *et al*., 1988), *labeo rohita*, 30 (Debnath *et al*., 2007), African catfish, *Clarias gariepinus*, 34.4-39.6 (Farhat and Khan, 2011), Shingi, *H. fossilis*, 27.73–35.43 (Akand *et al*., 1989), big head carp, *Aristichthys nobilis*, 30 (Santiago and Reyes, 1991), amazonian tambaqui, *Colossoma macropomum*, 30 (Oishi *et al*., 2010), Nile tilapia, *Oreochromis niloticus*, 30 (Bahnasawy, 2009), juvenile silver perch, *Bidynus bidynus*, 31 (Yang *et al*., 2002), juvenile freshwater crayfish, *Cherax quadricarinatus*, (Cortes-Jacinto *et al*., 2003), juvenile silver perch, *Spinibarbus hollandi*, 32.7 (Yang *et al*., 2003), catla, *Catla catla*, 30–35 (Seenappa and Devaraj,1995), Jian carp, *C. carpio* var. Jian, 34.1 (Liu *et al*., 2009), Nile tilapia, *O. niloticus*, 35 (Abdel-tawwab *et al*., 2010), blue streak hap, *Labidochromis caeruleus* 35 Ergun *et al*., 2010), South African abalone, *Haliotis midae*, 35.87 (Sales *et al*., 2003), rohu, *L. rohita*, 35 (Satpathy *et al*., 2003), Asian catfish, *C. batrachus*, 36 (Singh *et al*., 2009), Indian major carp, *Cirrhinus mrigala*, 36 Singh *et al*., (2008), black catfish, *Rhamdia quelen*, 37 (Salhi *et al*., 2004), lower than the values reported for silver perch, *Bidyanus bidyanus*, 42.15 (Yang *et al*., 2002), sharpsnout sea bream, *Diplodus puntazzo*, 43 (Coutinho *et al*., 2012), pike perch, *Sander lucioperca*, 43 (Nyina-wamwiza *et al*., 2005), African catfish, *C. gariepinus*, 43 (Ali and Jauncey, 2005), bagrid catfish, *Mystus nemurus*, 44 (Ng *et al*., 2001), grouper, *Epinephelus malabaricus*, 44 (Shiau and Lan, 1996), Asian red-tailed catfish, *Hemibagrus wyckioides,* 44.12 (Deng *et al*., 2011), Catla, Nile tilapia, *O. niloticus*, 45 (El-Sayed and Teshima, 1992), juvenile Florida pompano, *Trachinotus carolinus*, 45 (Lazo *et al*., 1998), juvenile spotted sand bass, *Paralabrax maculatofascinatus*, 45 (Alvarez-Gonzalez *et al*., 2001), black sea bass, *Centropristis striata*, 45-52 (Alam *et al*., 2008), *C. catla*, 47 (Singh and Bhanot, 1988), American eel, *Anguilla rostrata*, 47 (Tibbetts *et al*., 2000), Malaysian mahseer, *Tor tambroides,* 48 (Ng *et al*., 2008), silver pomfret, *Pampus argenteus*, 49 (Hossain *et al*., 2010), juvenile haddock, *Melanogrammus aeglefinus*, 49.9 (Kim *et al*., 2001), discus, *Symphysodon* spp., 44.9–50.1 (Chong *et al*., 2000), mahseer, *Tor putitora*, 45–50 (Islam and Tanaka, 2004), juvenile olive flounder, *Paralichthys olivaceus*, 46.4–51.2 (Kim *et al*.,2002), juvenile haddock, *M. aeglefinus*, 54.6 (Tibbetts *et al*., 2005), juvenile turbot, *Scophthalmus maximus*, 55 (Cho *et al*., 2005), tongue sole, *Cynoglossus semilaevis*, 55 (Ai *et al*., 2013), brown trout, *Salmo trutta*, 57 (Arzel *et al*., 1995) and is almost similar to the requirement reported for African catfish, *C. gariepinus*, 40 (Degani *et al*., 1989), *C. batrachus*, 40 (Khan and Jafri,1990), *C. catla*, 40 (Khan and Jafri, 1991), mangrove red snapper, *Lutjanus argentimaculatus*, 40 (Catacutan *et al*., 2001), juvenile masu salmon, *Oncorhynchus masuo*, 40 (Lee and Kim, 2001), Mahseer, *T. putitora* , 40 (Hossain *et al*., 2002), juvenile blackspot sea- bream, juvenile blackspot sea- bream, *Pagellus bogaraveo, 40* (Silva *et al*., *2006)*, African Cichlid, *Pseudotropheus socolofi*, 40 (Royes And Murie, 2005), cuneate drum, *Nibea miichthioides*, 40 (Wang *et al*., 2006), milkfish, *Chanos chanos*, 40 (Jana *et al*., 2006), Persian sturgeon, *Acipenser persicus*, 40 (Mohseni *et al*., 2007), catfish, *Heteropneustes fossilis* ,40 (Qamar and Khan, 2009), comparable to the requirement values reported for Mexican silverside, *Menidia estor*, 40.9 (Martinez-Palacios *et al*., 2007), juvenile sunshine bass, *Morone chrysops* x *M. saxatilis*, 41 (Webster *et al*., 1995), Pacific threadfin , *Polydactylus sexfilis*, 41 (Deng *et al*., 2011), Tiger puffer, *Takifugu rubripes*, 41 (Kim and Lee, 2009) black sea bream, *Sparus macrocephalus*, 41.4 (Shao *et al*., 2010) and Malaysian catfish, *M. nemurus*,42 (Khan *et al*., 1993).

The protein requirement of fish varies from species to species (NRC, 1993). The differences in protein requirement among the fish species may be due to different dietary formulations, fish size and different methodologies (Tibbetts *et al*., 2005; Sa *et al*., 2006). The variations may also be attributed to different lab conditions, experimental design e.g. feeding level and frequency, water quality, water flow rate, stocking density and protein sources in the diet (Kim *et al.,* 1992). Protein requirement of fish may also vary with the feeding rate adopted. It has been reported that a decrease in the dietary protein requirement of juvinile carp and rainbow trout from 60-65% to as low as 30-32%, when feeding rate was increased from 2-4% body weight^{-1} (NRC, 1993).

The present study indicates that the dietary protein levels influences fish growth, conversion ratio and haemato-biochemical composition and therefore, it is recommended that the inclusion of 41.50% dietary protein in the diet is optimum for the growth, efficient feed utilization of mirror carp, *C. carpio* var. *specularis* fingerling. Data generated in the present study would be useful in developing nutritionally balanced diets for the intensive and semi-intensive culture of this fish species.

CHAPTER - 6

Summary and Conclusion

Summary and Conclusion

The principal goal of fish cultivation is the production of fish of commercial value in the shortest possible time and in as large a quality per unit area as possible. Irrespective of whether the culture operation is aimed at quality, quantity or economic yield, both biological and non biological means are resorted to increase the output from aquaculture. Now, the biological approaches among other include pond management measures, such as improvement and maintenance of water quality, fertilization, artificial feeding, while the biological means incorporate choice of species, their size, stocking density, proportions and health management measures.

Recognizing the importance of dietary protein in fish culture, from a biological and economical point of view, the present work focused on to quantify the optimum dietary protein requirement and most appropriate use of feed for best-feed conversion efficiency and to reduce the cost of feed. The work presented here attempts to address several aspects of the nutrition of mirror carp, *C. carpio* var. *specularis*.

Based on the results obtained in the present dissertation, it is clear that optimization of dietary protein intake is one of the most important factors with respect to growth performance, body composition, and haematological profile in mirror carp, *C. carpio* var. *specularis*.

Lack of knowledge on the optimum requirements about the major nutrients mainly protein, amino acids, carbohydrates and their interaction has hindered the formulation of nutritionally balanced fish feed in the country. Therefore, there is an urgent need to understand the optimum proportions of these nutrients in the feed for fry and fingerlings of carp to formulate least expensive and nutritionally balanced feeds, because carps form the major proportion of the fisheries production in India including Kashmir. Keeping these objectives in view, attempts were made to understand the optimum requirement of protein for early stage of mirror carp, *C. carpio* var. *specularis* using semi-purified diets.

The present study was conducted with an aim to analyze the effects of dietary protein level on the growth, feed utilization and haemato-biochemical parameters of mirror carp, *Cyprinus carpio* var. *specularis* fingerlings. Six isocaloric (367 kcal $100g^{-1}$, gross energy) diets containing graded levels of protein (25%, 30%, 35%, 40%, 45% and 50%) were formulated, using casein-gelatin based diets. *C. carpio* *var*. *specularis* fingerlings with almost similar body weight and size (1.50g 0.02; 4.50cm±0.05) were randomly selected in triplicate groups in 75 L high-density polyvinyl circular troughs (water volume 60 L) fitted with a continuous water flow-through system at the rate of 20 fish per trough for each dietary treatment levels. Fish were fed test diet in the form of moist cake at the rate of 4% of the body weight six days a week, twice a day at 08:00 and 17:00 h, dividing into two equal feeding. The water exchange rate in each trough was maintained at 1.0-1.5 min L^{-1}.

At the end of the feeding trial, the results showed that the growth performance, whole body composition and hematological parameters were significantly affected by the varying dietary protein levels. Live weight gain (LWG%), specific growth rate (SGR %), feed conversion ratio (FCR), protein efficiency ratio (PER) and body protein deposition (BPD%) were analyzed to determine the optimum dietary protein requirement of mirror carp. Weight gain increased with increasing level of dietary protein up to 40% and showed a declining tendency thereafter. Maximum LWG (258%), SGR (2.28%), best-FCR (1.63), PER (1.53) and BPD (28.50%) were observed in the group fed 40% protein containing diet. The hepatosomatic index (HSI%) values in the present study also showed significant differences with varying dietary protein level. The maximum HSI (3.39%) was observed in group fed 25% protein containing diet and the minimum value (2.33) was observed in the group fed with 40% protein diet. Significant differences ($P<0.05$) in haematological parameters were also observed among the groups fed varying dietary protein levels. Haematological indices such as haemoglobin (Hb), haematocrit (HCT %) and red blood cell count (RBC) showed an increasing trend with the increase in dietary protein level.

The study carried out with fingerlings of mirror carp revealed that significantly ($P<0.05$) highest weight gain was observed in fish fed 40% protein diet. However, the second-degree polynomial regression analysis of growth parameters and body protein deposition data revealed that the optimum dietary protein requirement for maximum growth and efficient feed utilization of mirror carp, *C. carpio* var. *specularis* fingerling to be at 41.50%.

Based on the findings of the present research a list of conclusions and recommendations may be summarized as follows:

- At varying dietary protein levels under a constant feeding regime *C. carpio* var. *specularis* displayed significant growth differences and the same was observed in haemato-biochemical composition also.
- Based on the results of the present study carried out to determine the protein requirement of mirror carp, *C. carpio* var. *specularis* fingerlings, it is recommended that 41.5% protein level would be useful for optimum growth and efficient feed utilization of this fish species under extensive, intensive and semi-intensive culture.
- The information generated from the present study would be quite useful in developing a nutritionally balanced and cost effective feed, which would in turn lead to the sustainable increase in the production of mirror carp through aquacultural practices.
- The data generated would also be useful in setting up of a baseline for the quantification of remaining dietary nutrient requirements of this species.
- The variation found in the protein requirement of mirror carp in the present study compared with the protein requirements of other fish species reported in the past by several workers are interpreted mainly as a consequence of the use of different dietary formulations, fish size and different methodologies for analyzing experimental data. The variations may also be attributed to different lab conditions, experimental design e.g., feeding level and frequency, water quality, water flow rate, stocking density and sources of protein in the diet.

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