Effects of dietary protein levels on the growth, feed utilization and biochemical parameters of common carp, *Cyprinus carpio* (*communis*)

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

Master of Philosophy (M. Phil.)

IN
Zoology

By

Nusrat Ara

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,
Srinagar-190006, J&K

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POST GRADUATE DEPARTMENT OF ZOOLOGY
FACULTY OF BIOLOGICAL SCIENCE
UNIVERSITY OF KASHMIR
Hazratbal, Srinagar, J&K
2013

Telephone 91-9469042084 (M)
E-mail: *mnayyarazim@yahoo.com*

POST GRADUATE DEPARTMENT OF ZOOLOGY
THE UNIVERSITY OF KASHMIR, SRINAGAR 190006
(NAAC ACCREDITED GRADE "A")

(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 common carp, *Cyprinus carpio* (*communis*)'** has been completed under my supervision by **Ms. Nusrat Ara.** 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

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Nusrat Ara.

Dedicated to

my Parents

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Introduction

A large proportion of the world population suffers from under-nourishment, both in quantity and quality. Majority of the human population is still living on a suboptimal nutritional level in the light of the dietary standards recommended by appropriate international and national bodies which results in lot of physical consequences including mortality and morbidity etc, besides, hidden consequences of inadequate micronutrient intake (FAO, 1957) (NRC, 1958). The protein-energy malnutrition acuity with respect to micronutrients have changed from a focus on the results of clinical deficiencies of micronutrients (such as anemia, goiter, and eye problems) to the invisible consequences of inadequate nutrition that compromise immune function, cognitive development, growth, reproductive performance, and work productivity.

Fishery remained an important food the world over from time immemorial and has been investigated more than any other animal group for their nutritional value, biology and many other aspects of their life history. The unique value of fish for supplementing the nutritional qualities of man's diet and also for animal feeding is well recognized. Fish is a fabulous food, versatile and packed with health benefits. It has lot of potential and can therefore be expected to provide relief from malnutrition especially in Asian countries (Ashraf *et al*., 2011). The consumption of fish per capita is expanding globally, and the pattern of fish consumption is changing. In developing countries fish is still very much an essential food component for main source of protein. More than one billion people worldwide rely on fish as an important source of animal proteins, deriving at least 20% of protein from fish and this share could exceed 25% in poor countries (FAO, 2002).

Fish are becoming an increasingly important and preferred protein source for humans. It makes a vital contribution to the survival and health of a significant portion of the World's population. Fish protein is also important in the diets of the lowest income group (Jha and Jha, 1994).

The importance of fish in providing easily digested protein of high biological value is well documented. Proteins represent the largest chemical group in the animal body, with the exception of water. The whole body fish carcass contains 75% water and about 15-20%

protein. The digestibility of fish is approximately 5-15% higher than plant-source foods (WHO, 1985). In terms of the concentrations of amino acids, the total amount of protein absorbed into the body is determined by the lowest concentration of essential amino acids.

Fish is a major source of food for human nutrition providing a significant quantity of dietary protein and lipid in many countries (Bouriga *et al*., 2010). Fish is more nutritious than staple foods, providing animal protein, essential fatty acids and micronutrients. The interventions of food-based strategies which promote production and consumption of locally available nutritious foods have utilized fish instead of supplement distribution as a sustainable way of tackling micronutrient deficiencies (Gibson and Hotz, 2001; Tontisirin *et al*., 2002). Fish is, therefore, a good source of protein; the consumption of fish deserves to be encouraged to promote the level of nutrition in the whole world, and particularly in countries consuming high-cereal and low-protein diets. 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. Often referred to as rich food for poor people, fish is a rich source of protein which is essential for healthy growth and development.

It has been suggested that societies with high fish intake have considerably lower rates of acute myocardial infractions, other heart diseases and atherosclerosis (Bang and Dyerberg, 1980; Blanchet *et al.,* 2000). It is one of the most important components of animal protein and is widely accepted as a good source of protein and other elements for maintenance of healthy body (Andrews, 2001).

Besides protein, fish is a basic contributor of polyunsaturated fatty acids for human diet, which play a very important role as cardioprotective device (Duran and Talas, 2009). Therefore, the consumption of fish is not only associated with proteins and minerals, but also largely with omega-3-poly unsaturated fatty acids (PUFA) content (Ackman, 1989). n-3 PUFA are considered to be important in human nutrition due to their therapeutic role in reducing certain cardio-vascular disorders (Lands, 1986; Stickney and Hardy, 1989). Thus, it is important for human health to increase the consumption of fish or fish products which are rich in PUFA of n-3 family and poor in PUFA of n-6 family (Burr, 1989; Sargent, 1997).

India being a developing nation, is constantly exploring ideas for an inclusive sustainable development for the people. Fisheries sector plays a very important role in the socio economic development of India. Indian fisheries has made great strides during last five decades with the production levels increasing from 7,50,000 tons of fish in 1950-51 to 6.40 million tons in 2005-2006, of which the contribution from the inland sector is around 3.30 million tons (51.60% of the total) compared to 3.10 million tons (48.4%) from the marine sector. The global contribution of India in fish production has increased from 3.26 percent in 1985 (Alagarswami, 1995) to 4.41 percent in 1997.

Recent studies in Asia suggest that low-income people consume less fish than rich people (Dey *et al.,* 2006; Hossain *et al*., 2005; Bose and Dey, 2007), but they still depend on fish as a major source of animal protein (Dey *et al*., 2005; Bose and Dey, 2007).

The role of nutrition is one of the most important factors for fish production because 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.

In culturing fish in captivity, nothing is more important than well-balanced diets and adequate feeding. If there is no utilized feed intake by the fish, then there will be no growth and death will eventually occur. An under nourished fish is never able to maintain its health and be productive, regardless of the quality of its environment. Therefore, the production of nutritionally balanced diets for fish requires efforts in research, quality control and biological evaluation of feed. Since feed is the single largest item in the recurring expenditure of a fish farm, hence, the suitability and cost-effectiveness of the feed is of paramount importance for the commercial success of aquaculture.

Artificial feed constitutes a major cost factor in the operation of an aquaculture enterprise (Villareal, 1995; Chamberlain, 1996; D’Abramo and Sheen, 1996), reaching in many cases upto 40-60% of total expenses (Akiyama and Chwang, 1989; Shiau, 1998).

Thus supplementary feeding is the highest recurring cost in intensive and semi-intensive fish culture system with protein sources accounting for a significant proportion of this cost (Wang *et al.*, 2006). The development of cost-effective feeds that provide balanced nutrition to maximize growth, while minimizing environmental effects, depends on knowing the species' nutritional requirements and meeting those requirements with balanced feed formulations and appropriate feeding practices. Aquaculture has great potential for food production and the alleviation of poverty. A balance between food security and the environmental costs of food production must be attained (Emerson, 1999). Information about the nutritional requirement of fishes provides the foundation for the development of cost effective, practical feed formulations. Therefore, precise knowledge of the nutrient composition and availability of practical feedstuffs will be necessary to ensure the exact nutrient requirement of the concerned species.

The principle goal of fish cultivation is the production of fish of commercial value in the shortest possible time and in as large a quantity 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 for increasing the output from aquaculture through intensive and semi-intensive fish farming which is gaining importance in India. As a result, use of supplementary feeds in aquaculture has been inevitable for the success of fish culture. Therefore, the precise knowledge on nutritional requirements, scientific formulations of feeds and data on on-farm feed evaluation have become necessary for the formulation of cost-effective feed of cultured fish species.

Aquaculture interventions can provide a significant contribution to improving nutritional status of households through people consuming fish produced from their own ponds, selling fish for household income to enhance their purchasing power, and by expanding wider accessibility to fish by lowering market prices (Ahmed and Lorica, 2002; Dey *et al.*, 2006; Aiga *et al*, 2009; Jahan *et al.*, 2010).

Aquaculture has increasingly played a significant role in fish production, and the remarkable trend of increased aquaculture production will have to continue to meet expected future demand. In the course of future, aquaculture has expanded from being almost negligible to fully comparable with capture production in terms of feeding people in the world. Aquaculture has also evolved in terms of technical innovation and adaptation to meet changing requirements (FAO, 2012).

Fish nutrition is one of the most important aspects of fish production improvement and sustainable aquaculture, 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 the increase of aquaculture production (Davis, 2009). Development of alternate protein sources for fish feeds which support rapid fish growth, but do not increase pollution from aquaculture will require the combined efforts of all of the major scientific disciplines that collectively constitute aquaculture.

Culture of fishes under captivity is essential because major part of world's food is being supplied from fishery sources (Tripathi *et al*., 2002). In the context of both the dietary benefits of fish consumption and the increasing contribution made to fish production by aquaculture, it is clear that aquaculture is now undergoing and will continue to undergo a revolution in quality and quantity of production similar to that which has driven the improvement in agricultural stock animals for the past few decades. It has been reported that aquaculture is the fastest growing food production sector in the world (FAO, 1999). During recent years aquaculture continued to advance more rapidly than any other field of animal production in the world, and it is expected to continue to expand to provide fish for a growing world population. Since, the aquaculture production is increasing day by day therefore, it must contend with rapidly approaching limits on key feed ingredients and on increasing sensitivity to the effects of aquaculture on the aquatic environment. Many of these effects are associated with diet, so fish nutrition research 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.

The growing demand for fish protein in India has motivated active development of aquaculture. Indian freshwater aquaculture has grown rapidly during the last three decades. The rapid growth of aquaculture has now become the major source of quality protein for human consumption and made a significant source of foreign currency to many developing countries in the international trade. Supplemental feeding has become an integral part of fish culture. The major constraint for developing a complete low cost diet is the paucity of knowledge on the nutritional requirements specifically for carp culture. Dietary nutritional requirements are more precisely determined through feeding purified diet under culture condition as the ultimate aim of artificial feeding in aquaculture is to achieve maximum protein deposition with minimum input of feeds and at a minimum cost. Fish requires diets with high level of protein and an amino acid profile similar to that of their own tissues. Protein is the main essential nutrient for maintaining life and promoting growth (Lovell, 1989). It is essential for fast growth and health, and also important for enzyme and hormone production essential for metabolism. Therefore, a liberal and continuous supply of protein is needed. Fish generally consume protein to obtain the essential and nonessential amino acids which are necessary for muscle formation and enzymatic function and in part provides energy for maintenance (Yang *et al*., 2002). The 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 continually 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 reduction or cessation of growth or loss of weight due to withdrawal of protein from less vital tissues to maintain the functions of more vital tissues and to replace dead cells. On the other hand, if too much protein is supplied in the diet, only part of it will be used 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 and also cause water pollution due to the additional nitrogen loading (Ahmad, 2000).

Dietary protein is one of the major components of fish growth. The capacity of fish to synthesize protein de nova from carbon skeleton is limited and thus it has to be incorporated in the diet. Generally 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). Excess dietary protein is used for energy and leads to deterioration of culture water quality because of an increase in ammonia excretion (Tibbetts *et al.,* 2000) and also put additional expenditure which ultimately results in low output. Because, the ultimate goal of feed formulation and production is to produce a feed that supports maximum production at the lowest possible cost, and with minimum level of protein in the diet that will supply essential amino acids to give acceptable growth of good quality fish (Webster *et al*., 1995; Halver and Hardy, 2002).

Since, proteins are the structural components and energy source in diets of fish and play a key role as they are very essential for body maintenance and growth, and also one of the costliest dietary constituents in diet and generally fish requires higher levels of protein in diet (Cowey, 1975). Hence, determination of optimum protein requirements for fish is a logical first step to the development of low cost feed.

While formulating practical diets for fish, protein assumes greater importance because of the level and quality of protein greatly influencing the cost of feeds. So the protein content has to be carefully adjusted in feeds, bearing in mind that the dietary protein in excess to that required for growth is only catabolized (Cowey, 1979) and that lower protein levels invariably lead to poor growth and low feed efficiency making fish farming uneconomical.

The common carp, *Cyprinus carpio* is one of the most widely farmed fish globally. In India, it is one of the four fish species commonly farmed, either singly or in combination with the Indian major carps (IMC's), catla, *Catla catla*; rohu, *Labeo rohita* and mrigal, *Cirrhinus mrigala*. But in temperate climatic condition of Kashmir valley, it can be cultured with indigenous *Schizothorax* spps (Snow trout) under composite fish culture and with grass carp, *Ctenopharyngodon idella* and silver carp, *Hypothalmicthys molitrix* under polyculture system. These carp species cultured using diverse aquaculture techniques having the compatibility with the temperate climatic conditions of Kashmir have got the great potential for generating employment among the rural population of Kashmir valley. The species is reported locally to be very common in Lakes (Das & Subla, 1964; Fotedar & Qadri, 1974).

Cyprinus carpio was introduced in Kashmir valley in 1956 (Das and Subla, 1964; Fotedar and Qadri, 1974; Jhingran, 1991). Fotedar and Qadri (1974) reported that *C. carpio* provides about 70-75% of the total fish catch in the Kashmir valley. However, due to increasing population and growing awareness that capture fisheries are exhaustible, therefore, attention is warranted towards finding possibilities offered by aquaculture through extensive/semi-intensive techniques for providing alternative animal protein in the valley.

Common carp, an 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. Besides, common carp has an inviting appearance and a delectable taste, which imparts a remarkable market acceptance.

The natural conditions that suit carp are lowland lakes and rivers where there is abundant vegetation to provide food and shelter. Common carp is known to tolerate a wide range of temperature, readily surviving winter in frozen over ponds or lakes. However, their preferred spawning temperature is 13-18 ^{0}C (John Diggle *et al*, 2012). Common carp dwells in middle and lower reaches of rivers and shallow confined waters. The fish can survive cold winter periods with low oxygen concentration (0.3-0.5 mg L^{-1}) as well as supersaturating condition. It is omnivorous and fry feeds on zooplankton such as rotifers and copepods, but as they grow up, they become benthic feeders, feeding mainly on animals and other organic materials (Tamas and Horvath, 1976; Van Limborg, 1978). They are most active in the mornings than evenings; hence common carp is a typical diurnal eater.

Culture of *C. carpio* provides the country an opportunity to reduce protein deficiency and to earn foreign currency by exporting this fish. To intensify rearing and culture of this species, provision of nutritionally balanced feed becomes necessary. It is essential to know the minimum protein requirement for optimum growth in formulating a balanced ration, since protein is an important major nutrient component 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 several workers have reported the protein requirement of different fish species in the past, however, no systematic information regarding the nutrient requirement of common carp, *C. carpio* var. *communis* commonly called as scale carp have been reported under Kashmir waters. Therefore, the purpose of the present study was to define the dietary protein requirement of scale carp fingerling and provide basic information for formulating practical diets for culture of this species in the Kashmir valley in order to optimize the production of scale carp through aquaculture.

Review of Literature

The science of fish nutrition has advanced rapidly over the past three decades in India largely as a result of the development of commercial fish farming systems with the help of fish feeding trial experiments which are carried out mainly with the purpose of quantifying the gross dietary nutrient requirements such as carbohydrates, proteins, lipids, vitamins and minerals of a particular fish species so as to incorporate the minimum level of each particular nutrient in the diet. The ideal protein is the one that provides the exact balance of amino acids needed for optimum performance and maximum growth. Formulating a feed based on an ideal protein is an effective way of using less protein in the diet to meet amino acid requirements. Maximizing the effective use of protein and minimizing the amount needed in feeds substantially reduces production costs and increases farm profitability. A sound knowledge, therefore, of the dietary requirements of a particular cultivable fish species forms the backbone of commercially viable aquaculture system. Fish do not have a specific protein requirement but rather a definite requirement for essential amino acids that comprise proteins. In case of ideal protein there is no amino acid deficiency or excess.

In order to develop efficient and economical feed formulas for aquaculture, basic information is required on the nutrient requirements of the species cultivated; the chemical composition and organoleptic properties of feed ingredients in relation to their acceptance and the ability of fish to digest and utilize nutrients from various sources. Although the knowledge of protein requirements of fishes have been expanding steadily in the recent years and the details of some work exclusively carried out on the dietary protein requirement of fish have been listed in the present study in the form of review of literature, but no information is available on the dietary protein requirement of *C. carpio* var. *communis* under Kashmir water.

Basically the ideal protein concept in feed formulation requires that the essential amino acid requirements of fish be met using digestible amino acids rather than total amino acids because fish, like other animals are not 100% efficient in utilizing what they consume. Generally, it has been established that the protein requirements of fish are much higher than those of land animals (Lovell, 1989). Aquaculture feeds characteristically contain a higher percentage of protein usually in the range of (30-60%) on dry weight basis than feeds used in agriculture for poultry and beef.

The protein requirements of fishes are precisely estimated under defined experimental setup by feeding purified and semi-purified diets. In order to determine the protein requirements of fish, numerous investigators have utilized various semi-purified and purified diets by using various feed ingredients like casein, gelatin, whole egg protein, fish meal, soybean meal, mustard oil cake, cod oil, corn oil, linseed oil, crystalline amino acids, vitamins and minerals etc. Most investigators used isoenergetic diets to determine the dietary protein requirements of the fishes. 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 protein requirement of fishes. Generally, the 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). Further, channel catfish, *Ictalurus punctatus* fry require about 40% protein, whereas fingerlings require only 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. 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). It has been established that the growth rate and feed intake mainly depends on water temperature, the growth rate and feed intake increase as the water temperature increases, therefore, it is mostly believed that a change in water temperature affects feed intake much more than the protein requirement.

The feeding experiment related to the protein requirements of fish was conducted by Delong *et al*. (1958) and reported the dietary protein requirements of a coldwater fish, chinook salmon, *Oncorhynchus tshawytscha*. Since then a very exhaustive research work has been carried out on other fish species and was earlier reviewed in NRC, 1981; 1993. Among the warm-water fish species, research on dietary protein requirements has mostly 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 requirements of these fishes has been reviewed by Wilson and Halver (1986).

The influence of dietary protein on feed utilization by rainbow trout, *Oncorhynchus mykiss* was studied by Cho *et al*. (1976). 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.

The protein requirement study in fish was first time carried out in India by Sen *et al.* (1978) to determine the optimum dietary protein requirements of Indian major carps, using purified test diets fortified with vitamins and minerals. Later Singh and Bhanot (1988) reported that the dietary protein requirements of 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), conducted an experiment to study the effects of dietary protein levels on growth, food conversion, and protein utilization in young Nile tilapia, *Tilapia nilotica* and reported that an optimal dietary protein level of 28-30% for its optimum growth and conversion efficiency. In this study they used two to three week old *T. nilotica* young and fed 6% of the body weight a fish meal based diet twice daily. Efficiency of food conversion increased with dietary protein content up to 30% protein level and therefore decreased at higher protein containing diets.

Brown *et al*. (1992), recommended the optimum dietary protein requirement level at 41% for juvenile sunshine bass, *Morone chrysops* ♀ x *M. saxatilis* ♂, 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.

Tidwell *et al*. (1992), conducted a 10-week feeding trial 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.

Arzel *et al*. (1995), studied the protein requirement of brown trout fry, *Salmo trutta* by feeding diets containing graded levels of protein. The fish were fed for 52 days with a fixed ration which was modified every 2 weeks according to their body weight. He reported 53% dietary protein for excellent growth and feed utilization of brown trout fry, *S. trutta*.

A feeding experiment was conducted by Fiogbe *et al*, 1996 to determine 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 suggested an optimal dietary crude protein requirement of *P. fluviatilis* for maximum growth and excellent nutrient efficiency within the range of 36.80 to 43.60% dietary protein of the diet.

A detailed feeding trial was carried out by Perez *et al.* (1997) 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 handfed 5 times a day to apparent satiation, using fish meal and blood meal as the dietary protein sources. They recommended that the diet containing 45% dietary protein would be optimum for the best performance of European sea bass fingerlings.

Elangovan and Shim (1997), conducted a feeding trial 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 wet-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 41.70% for maximum growth and efficient feed utilization.

Lazo *et al*. (1998), conducted a 7-week feeding experiment 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.

The effects of dietary protein concentration on growth and muscle composition of juvenile, *Zacco barbata* were studied by Shyong *et al*. (1998). 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 the optimal dietary protein level for *Z. barbata* approximately at 32% for maximum growth of the fish.

Al Hafedh (1999), investigated 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 increase in growth with increasing dietary protein levels. 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.

Keefe and Rouse (1999), showed that 33% protein seems to be adequate for juveniles upto 1 g, using practical diets. However, nutritional requirements of each stage of development have not been defined properly.

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

Chong *et al*. (2000), suggested the dietary protein requirement of discus, Symphysodon spp. at 44.90-50.10%. In this feeding trial the 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 dietary protein level up to 50% diet and then decreased. These workers also reported that feed conversion ratio (FCR) varied inversely with growth rate.

Watanabe *et al*. (2000), conducted three feeding experiments pragmatically to determine the protein requirements of different sized yellowtail, *Seriola lalandi* (31, 94, and 506 g on average) for maximum growth and maintenance of body weight under different water temperatures (29.8, 27.1 and 18.8 ^{0}C on average) in small net cages. Fish were fed to satiation. The growth rate was highest for the satiation group and decreased proportionally to the feeding levels. These workers finally recommended that the dietary protein requirements for maximum growth were 22.50 g, 27.30 g, and 7.70 g/kg BW per day, respectively, for fish with the initial body weight of 31, 94, and 506 g; while the requirements for maintenance of body weight were 3.40 g, 3.10 g, and 2.70 g/kg BW per day, respectively. They also mentioned in the same study that these requirements decreased with increasing fish size and decreasing water temperature.

The effects of dietary protein levels on growth and body composition of juvenile spotted sand bass, *Paralabrax maculatofasciatus* were studied by Alvarez-Gonzalez *et al*. (2001). Fish were fed a sardine meal based feed to apparent satiation, twice daily. These workers reported that lower weight 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 of spotted sand bass juveniles.

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

An 8-week feeding trial was conducted by Chou *et al.* (2001) 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 weight gain reached its peak at a dietary protein concentration of 44.50%, which was regarded as the most suitable level for maximum fish growth and better feed utilization efficiency.

A 6-week feeding trial was conducted by Kim *et al*. (2001) to study the effects of dietary protein levels on growth, utilization of protein and energy of juvenile haddock, *Melanogrammus aeglefinus*, using fish meal and casein as dietary protein sources. Fish were hand-fed a dry pelleted diet, three times a day to apparent satiation. They reported that a dietary protein requirement at 53.80% 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 rockfish muscle as the protein source along with casein as main dietary protein source. Fish were fed 4% of their body weight initially (twice daily) which was gradually decreased to 3% in the later part of the feeding trial. These workers finally concluded that the optimum dietary protein requirement for maximum growth of juvenile Korean rockfish could be equal to or greater than 48.60%, but less than 50% dietary protein.

Lee *et al*. (2001), estimated the dietary protein requirement of juvenile giant croaker, *Nibea japonica* at 45%. 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 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. These workers also noted that higher dietary protein content not only resulted in lower PER, but also produced a lower HSI value with highest fat accumulation in the whole body of juvenile giant croaker.

Tibbets *et al.* (2001), conducted an experiment to determine the optimum dietary ratio of digestible protein and energy for juvenile American eel, *Anguilla rostrata.* The fish were fed to satiation herring meal based diets. Non-linear quadratic regression of energy retention efficiency (ERE) against dietary DP/DE ratio yielded an estimated optimum DP/DE ratio of 22.1g DP MJDE $^{-1}$.

Hossain *et al*. (2002), reported 40% optimum dietary protein requirement of mahseer, *Tor putitora* fingerlings. 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), conducted an 8-week feeding trial to study the effects of dietary protein levels on growth performance, carcass composition and ammonia excretion in juvenile silver perch, *Bidyanus bidyanus*. 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, protein productive 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 suggested that the optimum dietary protein requirement for the growth of juvenile silver perch was estimated at 42.15% for its maximum growth and efficient feed utilization.

Lee *et al*. (2002), conducted a 20-week feeding trial to study the effects of digestible protein levels in practical diets on growth, protein utilization and body composition of juvenile rockfish, *Sebastes 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 concluded that a practical diet containing 42.0% dietary protein would be useful for better growth and efficient feed utilization of juvenile rock fish.

A feeding trial was conducted by Giri *et al*. (2003) 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. They mentioned that an increase in body weight gain, SGR%, PER and decreased FCR was observed with increasing dietary protein levels. The fish also showed a gradual increase in whole body protein content as well as ash and decrease in whole body fat content with increasing dietary protein concentrations. Their study revealed that for maximum growth, best feed utilization and highest survival of hybrid catfish post larvae required 35%-40% dietary protein.

Yang *et al*. (2003), recommended the optimum dietary protein requirement of juvenile silver perch, *Spinibarbus hollandi* at 32.70% for the maximum growth. The fish were fed a white fish meal based diet at 3% of BW/day twice daily for 10 weeks. Their results indicated that both weight gain (%) and FER increased significantly with increasing dietary protein levels, while the PER and productive fat value 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 the whole body composition of the fish.

A 90 days feeding trial was conducted by Meyer and Fracalossi (2004) 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 the basis of their findings, they recommended the protein requirement values within the range of 32.60 to 37.30% optimum for *R. quelen* fingerlings for their maximum growth.

The optimum dietary protein requirement of the endangered cyprinid, *Tor putitora* was recommended within the range of 45-50% by Islam and Tanaka (2004). In this study they used soya bean meal and mustard oil cake as dietary protein sources, while fish were fed 5% BW/day. 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 observed in the same study.

Ai *et al.* (2004), reported 41% dietary protein to be optimum for juvenile Japanese seabass, *Lateolabrax japonicas* by studying the effects of dietary protein level on growth and body composition.

Salhi *et al*. (2004), studied the dietary protein requirement of black catfish, *Rhamdia. quelen* fry at 37%. Fish meal served as the main dietary protein source while fishes were fed to approximate 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 differences among each dietary protein levels.

Skalli *et al*. (2004), carried out feeding experiments to study the effects of the dietary protein levels on growth and nutrient utilization in common dentex, *Dentex dentex*. These workers conducted two separate feeding trials, at different growth stages in order to identify suitable protein level for the formulation of diet for this species. Fish were fed a fish meal based diet to satiation thrice daily for 6 weeks in first feeding trial and 12 weeks in the second feeding trial. Those fishes having an average individual weight of 10g showed an optimum growth at 52.20% protein level, while those having an average individual weight of 92 g showed maximum growth rate and best-FCR at 43.40% dietary protein level.

Gonzalez *et al*. (2005), conducted a 12-week feeding trial to determine the protein requirement of southern flounder, *Paralichthys lethostigma*. They formulated isocaloric test diet with graded levels of dietary protein along with constant level of dietary lipids. On the basis of their results they mentioned that the protein requirement was determined by relating percent increase in weight gain with dietary protein levels by utilizing two statistical methodologies: least squares regression analysis (protein requirement 50.30% CP) and a four-parameter logistic growth curve (50.80% CP).

Royes *et al*. (2005), suggested 40% dietary protein containing diet for juvenile African cichlid, *Pseudotropheus scolofi.* Fish were fed casein and fish meal based diet, three times daily for 10 weeks. They concluded that the final weight gain was significantly different among each dietary treatment level.

A 28-week feeding trial was conducted out by Saidy *et al*. (2005) in concrete tanks to examine the effect of two dietary 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 observed that the fish did not show any significant increase in weight gain, SGR% and FCR with increasing dietary protein levels and finally they concluded that 25% dietary protein would be useful for adult Nile tilapia for its maximum growth and better feed utilization.

Li *et al*. (2006), conducted an experiment to examine 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. In this study these workers found that 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 containing diets provided the same growth and feed conversion efficiency. On this observation they finally recommended that 28% protein containing diet would be useful for the maximum growth of channel catfish fingerlings.

A feeding experiment was carried out by Okorie *et al*. (2007) to reevaluate the optimum dietary protein requirement of juvenile Japanese eel, *Anguilla japonica.* They recommended 44.30% protein diet 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.

Debnath *et al*. (2007), conducted a 45 day feeding trial 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. By 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 optimum dietary protein level for *L. rohita* fingerlings for its maximum growth and better feed conversion efficiency.

A growth experiment was conducted by Zhou *et al*. (2007) to determine the optimal dietary protein requirement for juvenile ivory shell, *Babylonia areolate* reared in indoor aerated aquaria. Isoenergetic experimental diets using fish meal, casein and gelatin as protein sources were formulated to contain graded levels of protein. Fish were fed twice daily for 8 weeks. On the basis of quadratic regression analysis of weight gain against dietary protein level, they recommended that diet containing 45% dietary protein would be useful for maximum growth and efficient feed utilization of juvenile ivory shell.

Alam *et al*. (2008), recommended the dietary protein requirement of juvenile black sea bass, *Centropristis striata* in the range of 45%-52.60%. 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 52.60% dietary protein level, which also resulted in best-feed conversion efficiency and protein efficiency ratio. Therefore, they finally recommended 52.50% dietary protein level as optimum level for growth and mass production of this species.

Ng *et al*. (2008), studied the dietary protein requirement of the Malaysian mahseer, *Tor tambroides* fingerlings. On the basis of their results they finally recommended that diet containing 48% dietary protein level would be useful for maximum growth of this species. A casein-gelatin based purified diet was used for the 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.

A feeding experiment was carried out by Singh *et al*. (2008) 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. In this feeding trial 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. Later on another feeding trial experiment was also conducted by Singh *et al*. (2009) to evaluate the dietary protein level for Asian catfish, *Clarias batrachus* fry using a fish meal based diet. This time fish were fed at 5% of BW/day, twice daily for a period of 60 days. They noted that 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 experiment and recommended that best growth performance was observed in Pacu, *Piaractus mesopotamicus* fingerlings when fed on a fish meal based diet containing 27.10% dietary protein level. Weight gain and SGR% were affected by protein level. Protein efficiency ratio decreased with increasing dietary protein among all levels of dietary energy.

Bahnasawy (2009), recommended 30% crude protein level is optimum for the maximum growth of monosex Nile tilapia, *O. niloticus* juveniles. 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), studied the effects of varying dietary protein levels on growth of juvenile neon tetra, *Paracheirodon innesi*. Under this study the fish were fed an Atlantic menhaden meal based diet, three times per day. The Fish fed diets containing 45% to 55% crude protein had significantly greater weight gain than did fish fed 25% crude protein. While fish fed 25% crude protein had a significantly higher feed conversion ratio than did those fed 45% or 55% crude protein. These workers finally recommended a dietary protein level at approximately 45% for the optimum growth and efficient feed utilization of neon tetra.

Kim and Lee (2009), reported 41% dietary protein optimum for growth and physiological performances for juvenile tiger puffer, *Takifugu rubripes.* 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.

The dietary protein requirement of juvenile jian carp, *Cyprinus carpio* var. jian was estimated at 34.10% by Liu *et al*. (2009). They studied the effects of dietary protein levels on the growth performance, digestive capacity and amino acid metabolism. During the feeding trial, fish were hand-fed to apparent satiety, a brown fish meal based diet eight times daily for 45 days.

The effects of dietary protein levels on growth, feed utilization, protein retention efficiency and body composition of young Indian catfish, singhi, *Heteropneustes fossilis* were studied by Siddiqui and Khan (2009). They reported that the inclusion of dietary protein in the range of 40-43% was 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.

Zakeri *et al*. (2009), found that the best-growth and spawning performance was achieved at 40% dietary protein level in yellowfin sea bream, *Acanthopagrus latus.*

Oishi *et al*. (2010), reported the optimum dietary protein requirement for amazonian tambaqui, *Colossoma macropomum* at 30%. In this study 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 containing 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.

Ergun *et al.* (2010), recommended 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.

Abdel-Tawwab *et al*. (2010), conducted a 10-week feeding trial 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 (~0.5 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 containing diet.

Veras *et al*. (2010), evaluated the growth performance and body composition of giant trahira, *Hoplias lacerdae* fingerlings. They suggested the optimum crude protein requirement at 47% for the maximum growth and better energy retention-efficiency of giant trahira.

Hossain *et al*. (2010), reported 49% dietary protein level optimum for juvenile silver pomfret, *Pampus argenteus*. 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 daily weight gain and weight gain (%) of fish increased as dietary protein level increased.

An 8-weeks feeding experiment was conducted by Zhang *et al*. (2010) to determine the optimum dietary protein requirement of juvenile black sea bream, *Sparus macrocephalus*. They suggested that 41.40% 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 along with the improvement of feed efficiency ratio as dietary protein level increased, while the maximum feed efficiency retention (FER) was reported at 41.80% protein containing diet.

The dietary protein requirement of juvenile Asian red-tailed catfish, *Hemibagrus wyckioides* was reported by Deng *et al*. (2011). Under this study the 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. They recommended that the optimum dietary protein requirement for maximum specific growth rate of juvenile *H. wyckioides* was reported at 44.12%. In the 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 requirement within the range of 35% to 41% based on growth rate, feed conversion ratio and protein utilization.

Farhat and Khan (2011), suggested that the inclusion of protein in the range of 34.40%-39.60% 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 were recorded in fish fed 35% protein containing diet.

The effects of dietary protein levels on growth performance, nitrogen and energy budget of juvenile hybrid sturgeon, *Acipenser baerii* ♀ x *A. gueldenstaedtii* ♂ were studied by Guo *et al*. (2012). 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 diets. While, diets containing excessive protein contents not only resulted in inferior growth performance but also utilized higher proportions of protein and energy for excretion.

Coutinho *et al*. (2012), reported the dietary protein requirement of sharp snout sea bream, *Diplodus puntazzo* juveniles at 43%. Fish were fed to apparent visual satiety a fish meal based diets 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 observed. On the basis of their findings, they recommended that the optimum dietary protein requirement of *D. puntazzo* was within the range of 42.90% to 43.80% for maximum growth and good nitrogen retention efficiency.

Recently, a 9-week feeding experiment was conducted by Liu *et al.* (2013) to estimate the optimum dietary protein requirement for juvenile tongue sole, *Cynoglossus semilaevis.* Fish were fed to apparent satiation, a fish meal based diets 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.

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

Materials and Methods

Systematic position

Experimental fish

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Cypriniformes

Family: Cyprinidae

Genus: *Cyprinus*

Species: *Cyprinus carpio*

Variety: *Cyprinus carpio communis*

Induced bred fingerlings of scale carp, *C. carpio* var. *communis* with the same batch and in apparent good health were obtained 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 ingredient based formulated feed containing 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. Prior to the start of experimental feeding trial, an 8-week preliminary feeding trial was conducted 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 (GE)) diets containing graded levels of dietary protein (25%, 30%, 35%, 40%, 45%, and 50% crude protein) were formulated (Table1). 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 known 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 first sorted out from the acclimatized fish lots maintained in the wet laboratory in polyvinyl circular tank and the desired number of *C. carpio* var. *communis* fingerlings with almost similar body weight and size (2.02±0.05g; 5.10±0.12cm) 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 behavior of the fish and their intake. For this purpose as reported earlier 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% BW/day. 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 feedings. 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 was 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, 1992). The water sample for analysis was collected early in the morning before the feeding was done. Water temperature (22.6-24.8 ^{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 (3.8- 5.8 mg L^{-1}), total alkalinity (95-115 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. The details 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 ^{0}C for 24 hours. The petri dish containing the dried sample was cooled in a desiccator for about half an hour 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 quantification 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 the volume was raised 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 initial and final 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 finely powdered sample is placed in Whatman Thimble plugged with defatted cotton on the top of the thimbles. These thimbles were 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 weighed. Then 60-70ml of petroleum ether was added 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, within stipulated time (1 hour). 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 of extaction cups along with extracted fat 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, reweighed 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.

Haematological analysis:

At the end of the feeding trial 5 fingerlings from each set were sacrificed for haematological analysis. The caudle peduncle was severed and blood samples were drawn with the help of fine capillary tubes. Blood from each test group was pooled and stored in ethylenediaminetetraacetic acid (EDTA) vials. Haematological analysis was carried out with the help of Automatic haematological analyzer (Auto Haematological Analyzer, LabLife Noble *III*, India). Following haematological parameters were analyzed in the present study; Haemoglobin (Hb), Haematocrit (HCT%), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC).

While WBC count was calculated with help of the following formulae:

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

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

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 (Ahmed *et al.*, 2003):

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 x [(BW_f x BCP_f) - (BW_i x BCP_i)] / [TF x 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 %) = $\frac{\text{Liver weight (g)}}{\text{Body weight (g)}}$ x 100

Statistical analysis

Responses of scale carp fingerlings fed graded levels of dietary protein were measured by weight gain (%) feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR%) and whole body composition data. 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. In order 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).

Plate 1. Manasbal fish seed farm

Plate 2. Netting of fingerlings

Plate 3. Transport of fingerlings

Plate 4. Fingerlings stocking tank

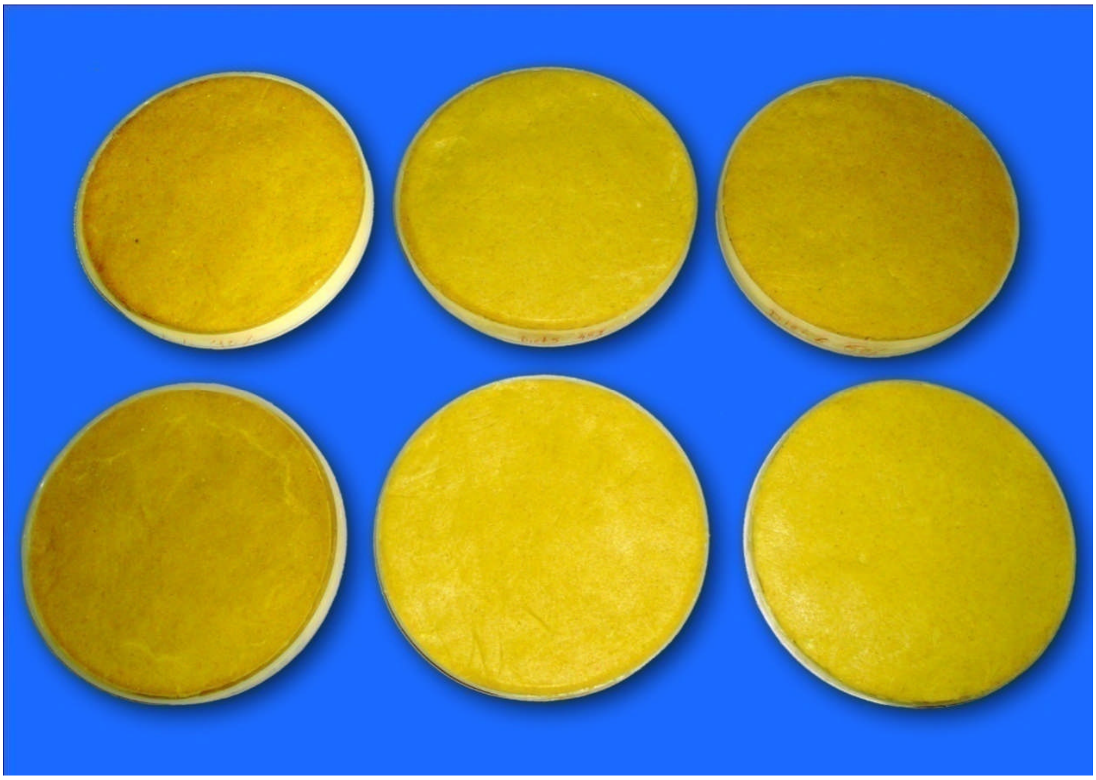

Plate 5. Moist cake experimental diets

Plate 6. Feed packed for storage

Plate 7. Wet lab flow-through system

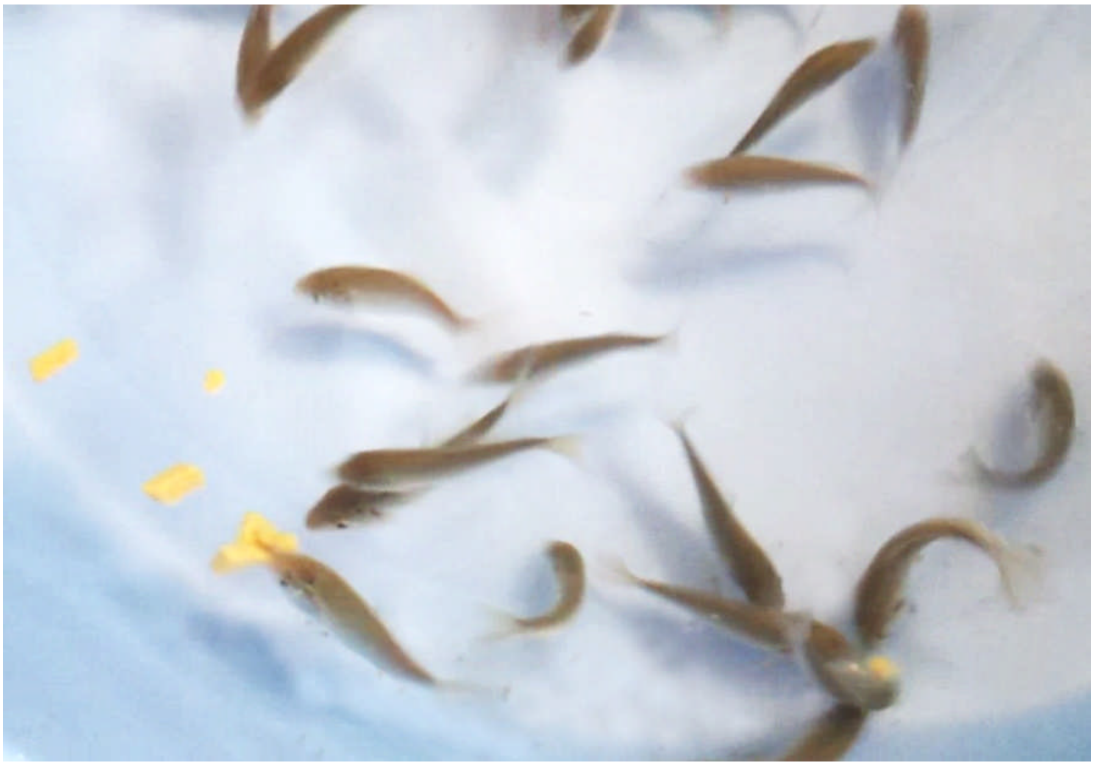

Plate 8. Fingerlings feeding

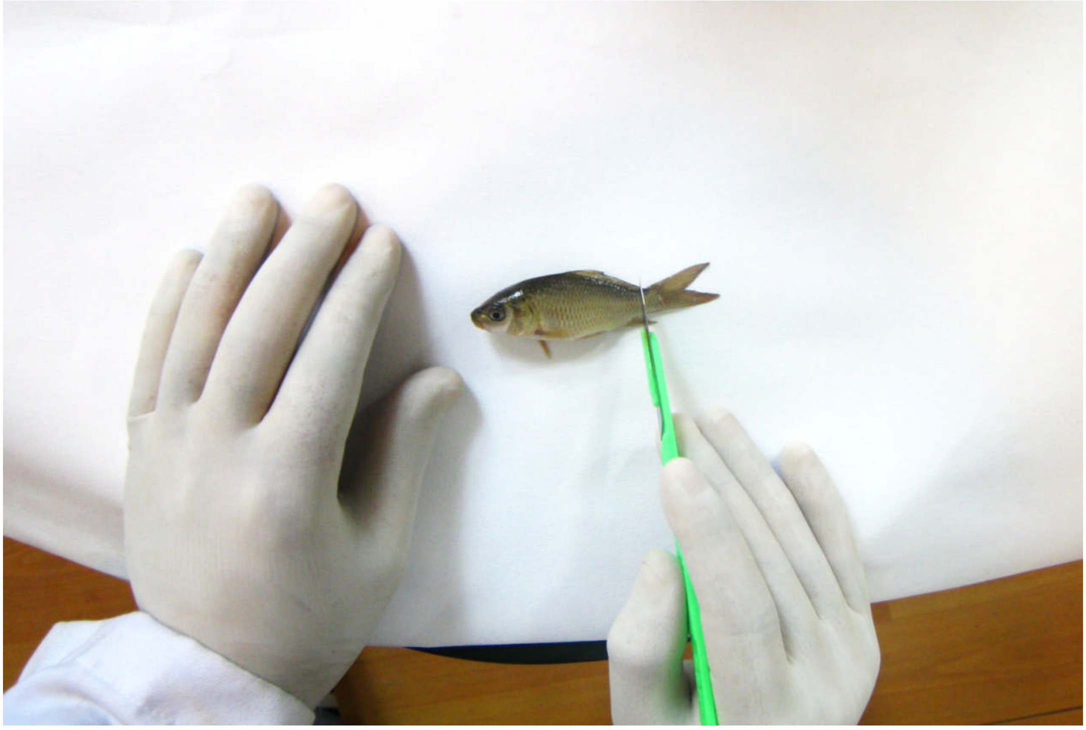

Plate 9. Severing the caudal peduncle for blood sample

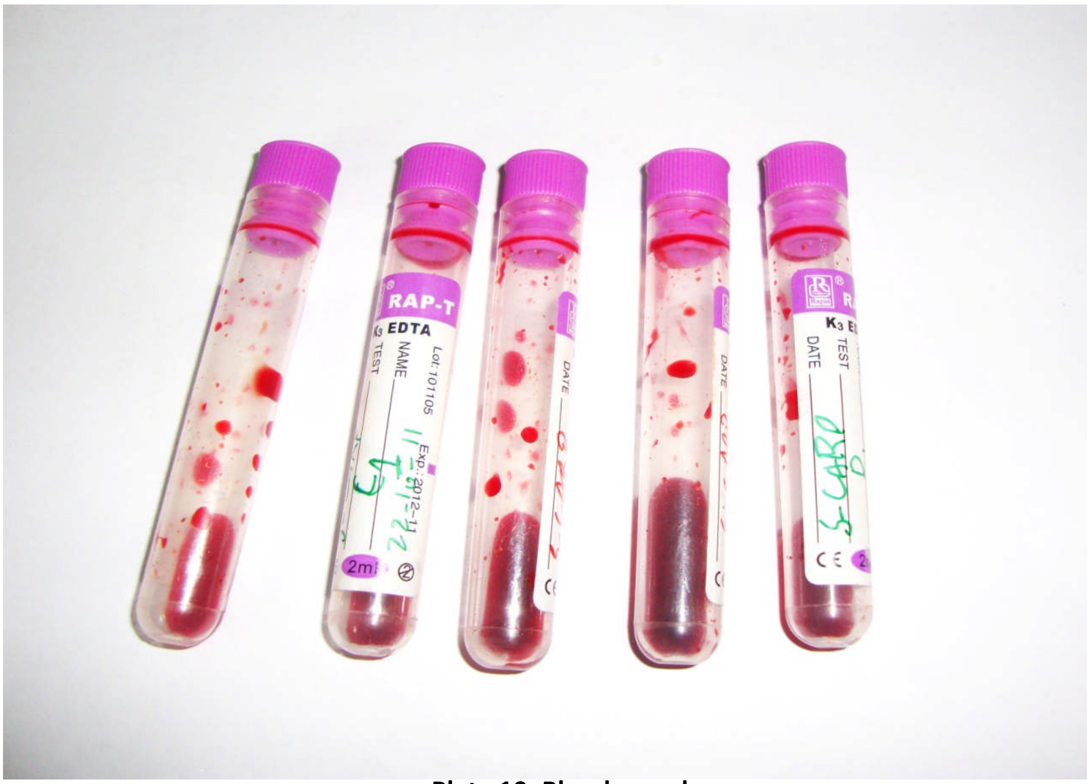

Plate 10. Blood samples

Plate 11. Automatic haematological analyzer

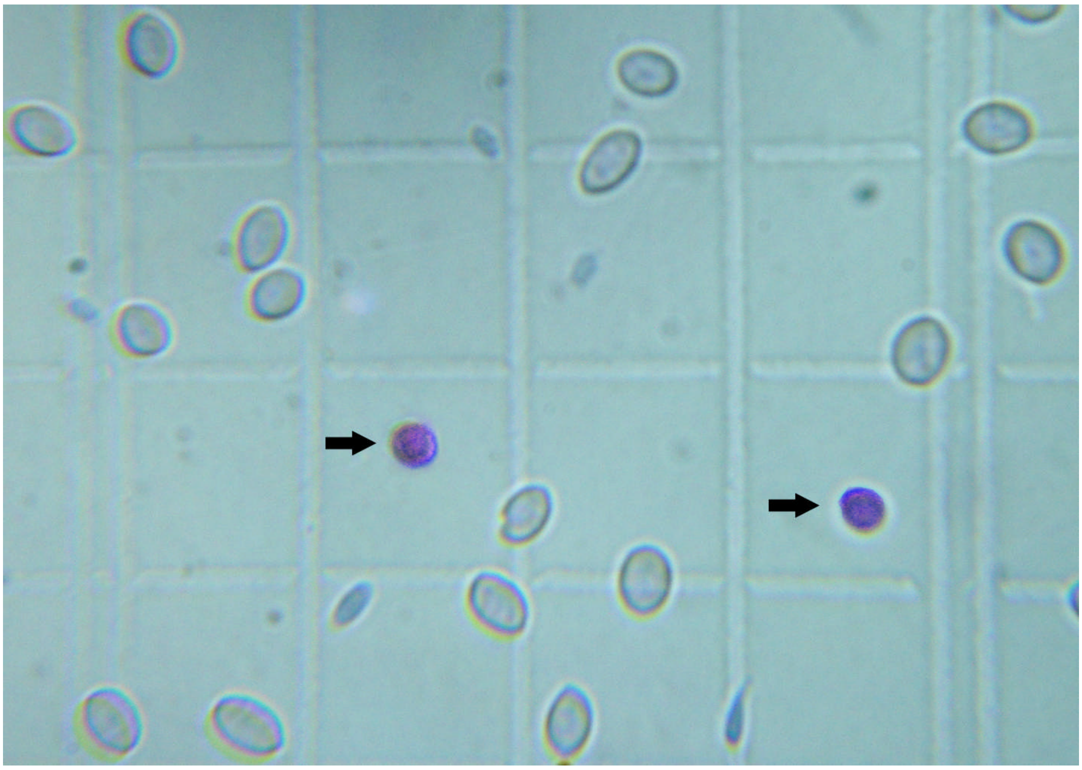

Plate 12. WBC counting in haemocytometer

Plate 13. Wet samples

Plate 14. Fingerlings drying for mass weight

Plate 15. Dry sample for biochemical analysis

Plate 16. Sample grinding

Plate 17. Nesseler's reagent

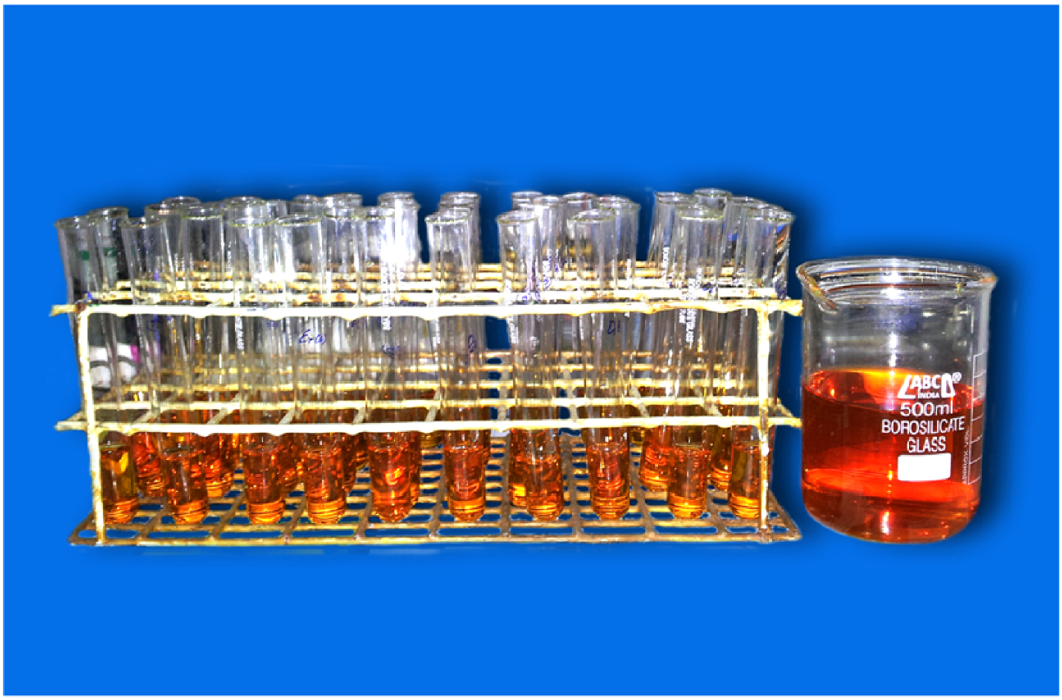

Plate 18. Protein reagents

Plate 19. Soxtec automatic fat extraction analyzer

Plate 20. Extraction cups containing fat

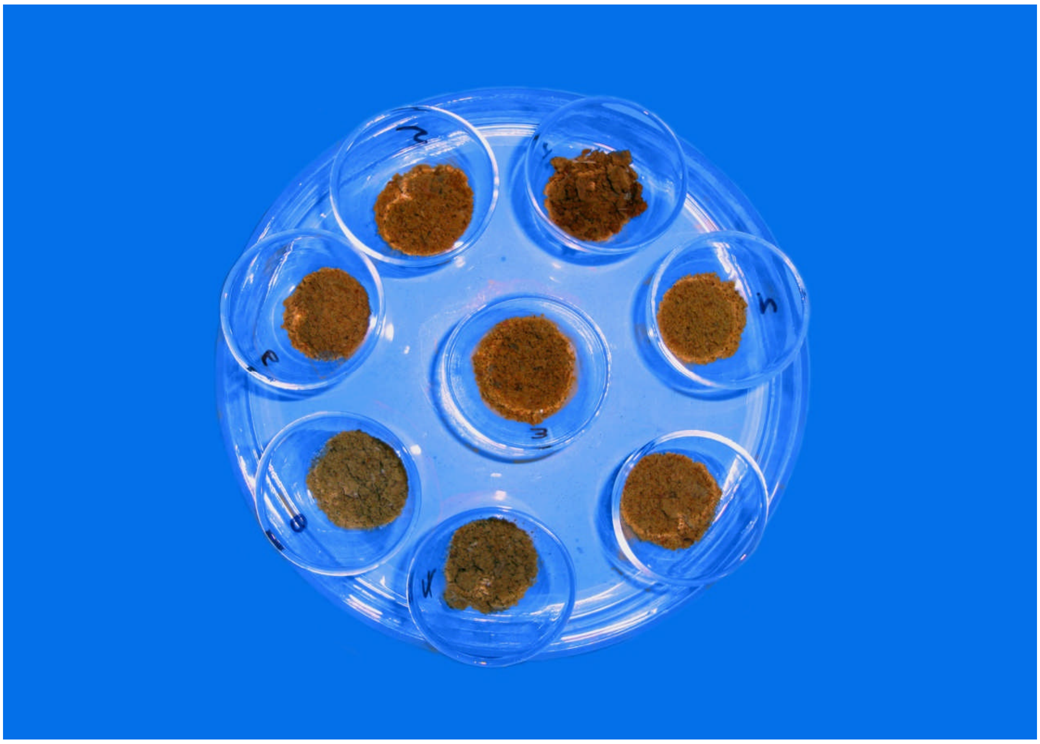

Plate 21. Samples before ashing

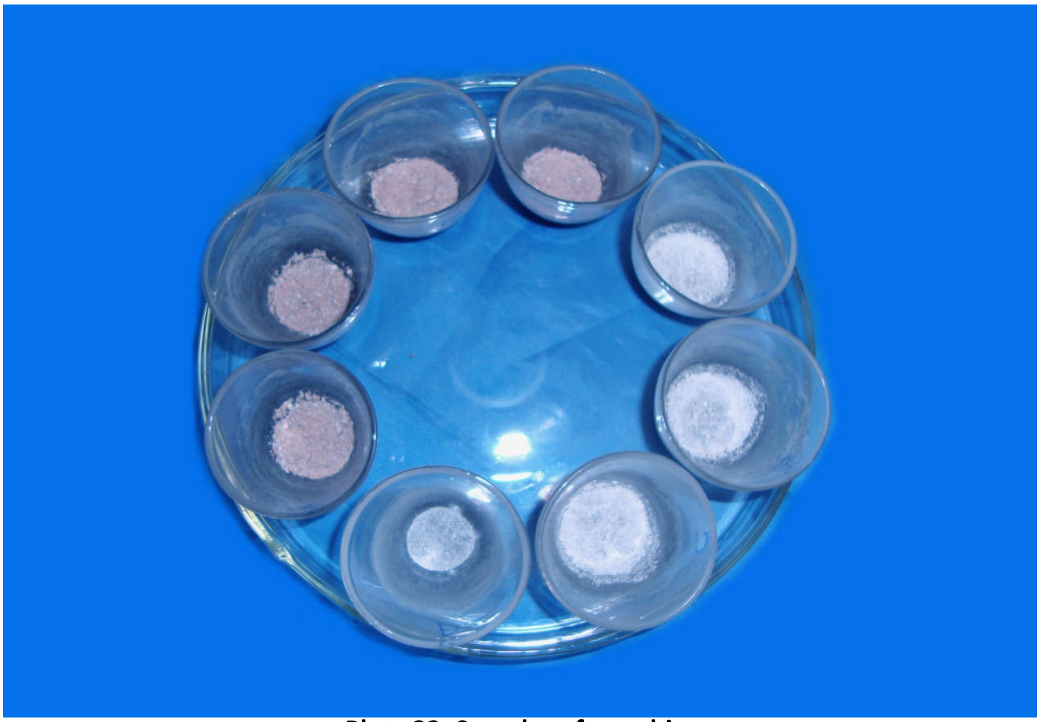

Plate 22. Samples after ashing

Results

Over the 8-week growth trial, dietary protein levels did not have a significant effect on the survival of fingerling scale carp, *C. carpio* var. *communis.* Significant (P<0.05) differences were observed in growth data of fish fed graded levels of dietary protein (Table 2). Live weight gain (LWG %), specific growth rate (SGR%), feed conversion ratio (FCR), protein efficiency ratio (PER) values for fish fed various levels of dietary protein were significantly (P<0.05) affected with increasing levels of dietary protein content. There was a linear relationship between the dietary protein levels in the diet and the increase in weight gain up to 45% protein inclusion in the diet, further increase in protein level in the diet could not produce any significant increase in weight gain as observed in the present study. Although the maximum live weight gain (246%) was achieved at 45% protein containing diet, however, this live weight gain was not significantly (P>0.05) different from those group that fed 40% protein containing diet, though a numerically lower value of weight gain (238%) was reported at 40% protein containing diet. Whereas fish fed the remaining diets containing 25%, 30%, 35% and 50% protein had significantly (P<0.05) lower weight gains than those fed 40% and 45% protein diets.

An increasing dietary protein level from 25% to 45% caused significantly (P<0.05) increased specific growth rate (SGR%) of scale carp, but a further elevation to 50% could not produce any additional promoting effects.

Feed conversion ratio (FCR) decreased progressively with linearly increasing dietary protein levels and was found to differ significantly (P<0.05) with each dietary protein level. The best-FCR (1.67) was recorded in fish fed diet containing 40% dietary protein level, which was not significantly (P>0.05) different to the group that fed 45% dietary protein containing diet (1.73), while poorest-FCR (3.18) was obtained for fish fed the lowest protein containing diets compared to all the dietary treatments, and intermediate FCR values were reported in the remaining dietary protein containing diets.

The protein efficiency ratio (PER) in fish fed various dietary protein levels produced significant (P<0.05) differences among each other. The PER showed a steady improvement as dietary protein increased from 25-45% with the significantly (P<0.05) highest PER value (1.49) achieved in fish fed diet with 40% protein containing diet, whereas significant decline in PER was noted in fish fed >40% protein containing diets (diet V and VI) and <40% protein containing diets (diet I, II and III). The heptosomatic index (HSI%) data of fish fed different levels of dietary protein also produced significant differences among each other with maximum HSI% values were observed in those groups where protein concentrations in the diets were lowest at 25% and 30% protein containing diets where, the highest HSI content i.e. 3.97% and 3.42%, respectively was noted. Moreover, the fish fed remaining dietary protein levels produced slightly lower values of HSI contents.

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 45.17% dietary protein level (Fig. 1). The relationship was described by the equation:

$$Y = - 0.3652x^2 + 32.9801x - 510.4343 \ (r = 0.950)$$

The specific growth rate of scale carp fed varied levels of dietary protein also produced somewhat similar trends as obtained in the live weight gain data. 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 44.88% protein level. The mathematical equation was:

$$Y = - 0.0026x^2 + 0.2334x - 3.040 \ (r = 0.977)$$

The FCR of scale carp fed 40% protein diet was significantly lower compared to the fish fed all other dietary levels, except at 45% dietary protein level where no significant difference between 40% and 45% were observed. The FCR (Y) to dietary protein levels (X) relationship was also best described by using a second-degree polynomial regression analysis (Fig. 3). The relationship being:

$$Y = 0.0046x^2 - 0.3993x + 10.4243 \ (r = 0.927)$$

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 by using second-degree polynomial regression analysis (Fig. 4). The mathematical equation being:

$$Y = - 0.0018x^2 + 0.1307x – 0.9514 \ (r = 0.610)$$

Based on the above polynomial equations the FCR and PER indicated that the optimum dietary protein requirement of scale carp was estimated to be at 43.40% and 36.30%, respectively.

The whole body composition data at the end of the feeding trial produced some significant differences (P<0.05) among all the dietary treatment levels (Table 3). Generally, whole body composition was affected with the increase of each dietary protein level. The whole body moisture content decreased significantly (P<0.05) with each incremental protein level in the diet up to 45% protein containing diet. However, at 45% protein diet significantly (P<0.05) lowest whole body moisture content was recorded, whereas highest moisture content was noted at the lowest protein containing diet (25%), followed by those groups that fed 30% protein containing diet, while slightly lower moisture values were reported in fish fed the remaining dietary protein levels. Significantly (P<0.05) highest whole body protein content was recorded in fish fed 45% protein containing diet followed by 40% and 50% protein containing diets, respectively. The whole body fat content gradually and significantly increased with each dietary protein incremental level and was found to be significantly highest in those group that fed 45% and 50% protein containing diets. However, no significant difference (P>0.05) in the whole body fat content was noted between these two groups.

Fish offered diets containing 25%, 30% and 35% protein produced significantly (P<0.05) more whole body ash content than fish fed diets containing 40%, 45% and 50% protein. Overall the highest whole body ash content was recorded in the group that fed the lowest protein containing diet i.e. 25%. Significantly (P<0.05) highest whole body protein deposition (BPD%) was also recorded in those fish group that fed 40% protein containing diet followed by those receiving 45% protein containing diet, which remained significantly

(P<0.05) highest compared to all the dietary groups, whereas intermediate protein deposition values were recorded in the remaining dietary treatment levels. The second-degree polynomial regression analysis was also employed between dietary protein level (X) and body protein deposition data (Y) and the break point was obtained at 38.71% protein level (Fig. 5). The mathematical relationship was described by the equation:

$$Y= - 0.0435x^2 + 3.3681x - 41.18 \ (r = 0.595)$$

Besides biochemical composition, the haematological parameters of scale carp fed varied levels of dietary protein were also analyzed in the present study. These parameters also produced some significant (P<0.05) differences (Table 4). The haemoglobin (Hb) content gradually and significantly increased with the increase of dietary protein level in the diets. Significantly (P<0.05) highest Hb content (10.96g dl^{-1}) was recorded in fish group fed 45% protein level, followed by 40% protein diet (10.81g dl^{-1}), while intermediate Hb values were noted at 50% and 35% protein containing diets which were significantly (P<0.05) different among each other. Whereas the lowest Hb content (6.96g dl^{-1}) was recorded at the lowest level of protein containing diet i.e. 25%. Haematocrit (HCT) values also responded to dietary protein levels in a dose dependent manner up to 45% protein level and thereafter, tended to decrease. However, significantly (P<0.05) highest HCT value (40.48%) was recorded for fish fed 45% protein containing diet, followed by those receiving 40% protein diet (38.86%). Similar to Hb the lowest HCT value (25.21%) was noted at the lowest protein containing diet (25%).

Red blood cell counts (RBC) in fish fed graded levels of dietary protein also produced significant differences among each dietary level with significantly (P<0.05) highest RBC count value (2.04×10^6 mm^{-3}) noted at 45% protein containing diet, followed by those group of fish which received 40% protein diet (1.84×10^6 mm^{-3}). The intermediate RBC count values were obtained from 50% and 35% protein containing diets, respectively. While significantly (P<0.05) lower values of RBC count were noted in lower protein doses i.e. 25% and 30% diets, respectively.

In contrary to RBC counts, the fish fed various levels of dietary protein could not produce any clear results in their leukocyte (WBC) counts, except at lowest dietary protein were significantly ($P<0.05$) highest value of WBC count was recorded, whereas the remaining dietary levels showed lower WBC count values. However, these levels produced slight numerical differences among each other. No significant ($P>0.05$) differences in mean corpuscular value (MCV) were observed in the present study, in scale carp fed various dietary protein levels, except at the lowest protein containing diet i.e. 25%, where significantly lower MCV value (184.32 fl) was recorded. Significantly ($P<0.05$) higher mean corpuscular haemoglobin (MCH) values were recorded in fish fed 35%, 40% and 50% protein containing diets. However, the MCH values of these groups were insignificantly ($P>0.05$) different among each other, whereas the remaining dietary groups produced an insignificantly ($P>0.05$) lower MCH values, except some minor differences. The mean corpuscular haemoglobin concentration (MCHC) data could not produce any clear significant differences among each dietary treatment level. Although some marginal significant differences were observed, however these differences presumably might not be related to the protein content of the diet.

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

Ingredients (g $100g^{-1}$, dry diet)	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).

Table 2. Growth, FCR, protein deposition and percentage survival of scale carp, *Cyprinus carpio* var. *communis* 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)	1.966 ±0.03	2.010 ±0.02	2.017 ±0.03	1.961 ±0.02	2.036 ±0.03	1.994 ±0.04
Average final weight (g)	3.842 ±0.12	4.809 ±0.11	5.681 ±0.14	6.631 ±0.13	7.056 ±0.22	6.294 ±0.16
Live weight gain (%)	95.45 ±5.11^{e}	139.28 ±5.89^{d}	181.74 ±7.01^{c}	238.22 ±6.39^{a}	246.53 ±7.33^{a}	2.15.42 ± 5.80^{b}
Specific growth rate (SGR)	1.20 ±0.05^{e}	1.57 ±0.04^{d}	1.85 ±0.04^{c}	2.18 ±0.03^{a}	2.22 ±0.03^{a}	2.05 ± 0.04^{b}
Feed conversion ratio (FCR)	3.18 ±0.06^{a}	2.85 ±0.07^{b}	2.18 ±0.04^{c}	1.67 ±0.05^{d}	1.73 ±0.06^{d}	2.11 ± 0.04^{c}
Protein efficiency ratio (PER)	1.27 ±0.01^{b}	1.18 ±0.02^{c}	1.31 ±0.03^{b}	1.49 ±0.04^{a}	1.28 ±0.04^{b}	0.95 ± 0.02^{d}
Body protein deposition (BPD)	17.64 ±0.71^{c}	18.77 ±0.73^{c}	20.32 ±0.77^{b}	27.09 ±1.14^{a}	24.27 ±0.85^{b}	16.72 ± 0.20^{c}
Hepatosomatic index (HSI)	3.97 ± 0.12^{a}	3.42 ± 0.06^{b}	2.89 ± 0.04^{c}	2.53 ± 0.03^{d}	2.62 ± 0.02^{d}	2.96 ± 0.05^{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 fingerlings, *Cyprinus carpio* var. *communis* fed diets containing varying 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 (%)	80.76 ±0.35	77.57 ±0.32 ^{a}	75.75 ±0.24 ^{b}	74.38 ±0.16 ^{c}	73.37 ±0.20 ^{d}	72.17 ±0.26 ^{e}	73.12 ±0.15 ^{d}
Protein (%)	11.64 ±0.17	12.74 ±0.19 ^{f}	14.19 ±0.08 ^{e}	15.10 ±0.10 ^{d}	16.20 ±0.09 ^{b}	16.78 ±0.06 ^{a}	15.73 ±0.07 ^{c}
Fat (%)	3.52 ±0.07	4.76 ±0.13 ^{e}	5.33 ±0.50 ^{d}	6.24 ±0.07 ^{c}	6.69 ±0.10 ^{b}	7.04 ±0.06 ^{a}	7.22 ±0.07 ^{a}
Ash (%)	2.78 ±0.03	3.24 ±0.05 ^{a}	3.02 ±0.09 ^{b}	2.87 ±0.06 ^{b}	2.53 ±0.05 ^{d}	2.66 ±0.03 ^{c,d}	2.71 ±0.03 ^{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 haematological parameters of scale carp, *Cyprinus carpio* var. *communis* 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]	6.96 ±0.06^{f}	7.90 ±0.03^{e}	9.55 ±0.05^{d}	10.81 ±0.02^{b}	10.96 ±0.05^{a}	10.14±0.04^{c}
HCT (%)[2]	25.21 ±0.38^{f}	29.33 ±0.35^{e}	33.74 ±0.26^{d}	38.86 ±0.45^{b}	40.48 ±0.31^{a}	35.42 ±0.12^{c}
RBC (x10^{6}mm^{-3})[3]	1.37 ±0.03^{e}	1.49 ±0.02^{d}	1.65 ±0.03^{c}	1.84 ±0.02^{b}	2.04 ±0.05^{a}	1.73 ±0.03^{c}
WBC (x10^{3}mm^{-3})[4]	2.48 ±0.04^{a}	2.35 ±0.05^{b}	2.31 ±0.04^{b,c}	2.13 ±0.04^{d}	2.24 ±0.02^{c}	2.13 ±0.06^{d}
MCV (fl)[5]	184.32 ±7.08^{c}	197.26 ±5.46^{b}	204.64 ±3.82^{a,b}	211.04 ±4.46^{a}	198.66 ±4.17^{a,b}	205.37 ±3.76^{a,b}
MCH (pg)[6]	50.86 ±0.94^{c}	53.14 ±0.98^{b,c}	57.96 ±1.30^{a}	58.69 ±0.47^{a}	53.85 ±1.10^{b}	58.79 ±1.08^{a}
MCHC (g dl^{-1})[7]	27.62 ±0.64^{a,b,c}	26.95 ±0.34^{c}	28.32 ±0.27^{a,b}	27.52 ±0.50^{b,c}	27.08 ±0.12^{c}	28.63 ±0.15^{a}

*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 concentrate.

Discussion

In practical diet formulations for fish, protein constitutes one of the major costs in terms of nutrients and ingredients. To minimize feed costs, it is necessary to optimize dietary protein level as well as the exact utilization of feed. Increases in dietary protein have often been associated with higher growth rate in majority of the fish species as protein is one of the most vital components which provide the essential amino acids building block for protein synthesis. However, it is generally assumed that at a particular protein level the fish gain maximum growth rate and beyond that further increase in protein cannot support growth, and may even decrease growth rate as reported in *Barbodes altus* (Elangovan and Shim, 1997).

Hence a clear understanding of the requirement of major nutrients especially protein for different stages of cultivated fish is a pre-requisite to develop nutritionally balanced and cost-effective feed which would meet the demand for their growth. Such an assessment can be made using purified and semi-purified diets. Generally fishes require a high protein containing diet for maximum growth compared to terrestrial animals (Lovell, 1989). However, from nutritional and environmental protection stand points, high protein diet may not essentially be appropriate, for reasons of protein being the costliest component and the excretion of nitrogenous waste increases the oxygen demand polluting the water medium. Thus one of the challenges faced by fish nutritionist is to determine the exact and appropriate protein requirement of the fish.

The dietary protein intake is related to nutritional status, health and growth of fish. Efficient utilization in the form of better growth and conversion efficiency of protein, however, depends mainly on the availability of other nutrients like carbohydrates and fats in appropriate quantities (Wilson and Halver, 1986; NRC, 1993). On the other hand inadequate protein content in the diet results in loss of weight and also causes lot of nutritional deficiency related diseases. While lack of essential amino acids or its improper balance in diet often becomes a limiting factor for normal fish growth; besides excess amino acids if supplied in the diet, only part of it used for protein synthesis, and reminder will be converted into energy (Ahmed *et al.,* 2003). Therefore, precise information about the protein requirement of concerned fish species is a pre-requisite for the formulation of nutritionally adequate balanced fish feed for the commercial success of aquaculture.

Hence, determining the dietary protein requirement of fingerling stage of scale carp becomes an initial and essential step 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.

In the present study, incremental levels of dietary protein content had a significant effect on the growth rate, feed conversion ratio, protein efficiency ratio and specific growth rate, which itself showed the essentiality of appropriate amount of protein content for fish growth. The growth and conversion efficiencies, dose dependently increased with the increase of dietary protein levels from 25% to 45% protein containing diet and decreased thereafter, with further increase in dietary protein content. Although the maximum growth rate (246%) was achieved 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 (238%). Whereas, the best-FCR, PER and BPD% was recorded with fish fed 40% protein diet. Therefore, inclusion of 40% protein in the diet for fingerling scale 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 same trend was observed for pomfret juveniles, probably because fish WG is directly associated with the dietary protein content and also because protein is the preferential energy source in fish metabolic routes (Hepher, 1988).

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. It has also been reported in the past that increases in dietary protein level have often associated with higher growth rates in many species. However, there is a protein level beyond which further growth is not supported, and may even decrease (Shiau and Lan, 1996; Gunasekara, *et al.*, 2000; Kim and Lall, 2001). There have been many studies showing that the excess protein in the diet can result in additional energy costs in many fish by deamination and consequent reduction to energy for growth (Shiau and Huang, 1989; Vergara *et al*., 1996). In the case of excessive dietary protein, the growth of fish remains constant or decreases because dietary protein is used to metabolize excess amino acids absorbed (Jauncey, 1982). The reduced growth rate and decreased protein utilization beyond requirement of dietary protein level has been 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, the poor feed conversion ratio was recorded in fish fed lower protein containing diets. However, improvement in FCR was noticed with increasing incorporation of dietary protein levels. The FCR values were reported in the range from 1.67-3.18 with the best-FCR values (1.67) recorded with fish fed at 40% protein containing diet. However, the best-FCR value achieved at 40% protein diet was not significantly different to that fed at 45% protein containing diet (1.73). Deng *et al*. (2011) reported that FCR values decreased with increasing dietary protein level up to the requirement level, which can be attributed to the increased protein content of the diet with resultant effect of enhanced weight gain. Improved growth and feed conversion efficiency with increasing dietary protein levels has been described in many other species (Bai *et al*.,1999; Peres and Oliva-Teles, 1999; Lee *et al*., 2001; Lee *et al*., 2006; Kim and Lee, 2009). The specific growth rate (SGR%) values also showed somewhat similar trends in the present study as the results obtained in the live weight gain of scale carp. Significantly highest protein efficiency ratio (PER) was recorded in fish fed 40% protein containing diet, followed by 35% and 45% protein containing diet, however, no significant difference was observed between these two protein levels. Whereas the remaining dietary levels could not produce any significance differences among each other and usually showed marginally lower PER values. The result in the present study related to highest PER reported at the requirement level is in agreement with the findings of Deng *et al*. (2011), where he mentioned that the maximum PER values occurred at the requirement level, while lower values of PER at the highest protein containing diet i.e. more than the requirement level indicates that excess protein was used for metabolic processes other than growth purpose. Similar trends in PER were also reported by other workers (Lee *et al.*, 2002; Siddiqui & Khan, 2009; Abbas & Siddqui, 2013).

The BPD 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 BPD were also reported by other workers (Lee *et al.*, 2002).

The dietary protein levels significantly affected the whole body composition of scale carp. The whole body moisture content gradually decreased with the increase of dietary protein levels, with minimum moisture content recorded at 45% protein diet, followed by those receiving 40% and 50% protein containing diets, respectively, which is in accordance with the results for freshwater catfish, *Mystus nemurus* (Khan *et al*., 1993). While whole body protein content linearly increased with the increase of dietary protein level up to 45% and thereafter, a significant decline in the whole body protein content was noted. The highest protein content obtained in the present study, when fish fed at 45% 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 for other fish species (Khan *et al*., 1993; Bai *et al*., 1999; Kim *et al.*, 2002; Kim and Lee, 2009). 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. Similar results were also reported by other workers in the past (Shiau and Lan, 1996). The whole body ash did not show any significant difference at the higher treatment levels where body ash content slightly lower as compared to the lower protein containing diets i.e. 25% and 30% protein levels where significantly higher body ash content was recorded. The fish fed varied levels of dietary protein produced some significant differences in HSI values. The highest HSI values were observed in fish fed lower doses of dietary protein containing diets i.e. 25% and 30%, while fish fed 40% and 45% dietary protein containing diets registered lower HSI values. 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; Guo *et al*., 2012). Abdel-Tawwab *et al*. (2010), reported that hepatosomatic index was inversely affected by dietary protein levels in the diets due to the increased fish weight at higher protein levels, liver to body weight ratio decreased, similar findings were also reported by Gallagher (1999).

The assessment of hematological parameters along with biochemical composition of fish can provide valuable information for evaluating the health status of fish (Coles, 1986). Therefore, the sound knowledge of the hematological parameters provides an important guideline that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. The role of fish hematology nowadays has also proven to be a valuable modern diagnostic tool to analyze the initial health status of fish and these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before clinical symptoms appear (Bahmani *et al.*, 2001).

In the present study besides biochemical analysis, haematological analysis was also carried out 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).

Change in Hb, RBC and HCT were significantly affected by dietary protein, fish weigh etc. (Abdel-Tawwab *et al*., 2010). In the present study, significant differences were observed in Hb, RBC and HCT values of different groups fed with varied levels of dietary protein, showing a general trend of linear increase with the increase of dietary protein levels up to the requirement level, where maximum growth rate was also achieved. 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; Lundstedt *et al*., 2004; Moraes and Bidinotto, 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 (10.96 g dl^{-1}) and HCT values (40.48%) were estimated for fish fed 45% protein diet. The maximum RBC count values (2.04 x 10^6 mm^{-3}) was evident in fish fed 45% protein containing diet, followed by those receiving 40% protein diet. While intermediate RBC count values were reported in fish fed remaining dietary protein levels, excepting at 25% protein diet where the lowest RBC count was noted. The increase in RBC count with the increase of dietary protein levels reported in the present study occurred might be 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.

The mean corpuscular value (MCV) could not produce any significant differences among each other in scale carp fed various dietary protein levels, except at the lowest protein containing diet i.e 25% where significantly lower MCV value (184.32 fl) was recorded. Whereas significantly higher mean corpuscular haemoglobin (MCH) value were recorded in fish fed 35%, 40% and 50% protein containing diets. While, the MCH values among these groups were not different compared to each other. However, the remaining dietary groups produced lower MCH values, except some marginal differences. The mean corpuscular heamoglobin concentration (MCHC) data could not produce any clear significant differences among each dietary treatment level. Although some marginal significant differences were observed, however these differences presumably might not be related to the protein content of the diet.

On the basis of second-degree polynomial regression analysis of growth parameters and body composition data, the optimum dietary protein level for growth of scale carp, *C. carpio* var. *communis* fingerling is recommended to be at 42.80%. The protein requirement of scale 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, *O. niloticus*, 30 (Bahnasawy, 2009), juvenile silver perch, *Bidyanus bidyanus*, 31 (Yang *et al*., 2002), juvenile freshwater crayfish, *Cherax quadricarinatus*, 31 (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, 34.10 (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), silver perch, *Bidyanus bidyanus*, 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, *Tor 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 (Siddiqui and Khan, 2009), Mexican silverside, *Menidia estor,* 40.90 (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*, 41.40 (Zhang *et al*., 2010) and Malaysian catfish, *M. nemurus*; sharpsnout sea bream, *Diplodus puntazzo*, 42 and is almost similar to the requirement values reported for Malaysian catfish, *Mystus numerus,* 42 (Khan *et al*., 1993)**,** 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).

While the requirement recommended in the present study for scale carp is lower than the protein requirement values reported for other fish species such as 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), 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*, 49.90 (Kim *et al*., 2001), discus, *Symphysodon* spp., 44.9-50.10 (Chong *et al*., 2000), mahseer, *Tor putitora*, 45-50 (Islam and Tanaka, 2004), juvenile olive flounder, *Paralichthys olivaceus,* 46.4-51.20 (Kim *et al*., 2002), juvenile haddock, *M. aeglefinus*, 54.60 (Tibbetts *et al*., 2005), juvenile turbot, *Scophthalmus maximus*, 55 (Cho *et al*., 2005), tongue sole, *Cynoglossus semilaevis,* 55 (Ai *et al*., 2004), brown trout, *Salmo trutta*, 57 (Arzel *et al*., 1995).

The protein requirement of fish varies from species to species (NRC, 1993). The differences in results obtained in different studies could be attributed to factors such as fish size and species, feed composition and preparation method, feeding habit, experimental design, rearing method, as well as water temperature and salinity (Tuncer *et al*., 1990; Takeuchi *et al*., 1992; Keembiyehetty and Wilson, 1998). 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 juvenile carp and rainbow trout from 60-65% to as low as 30-32% occurred 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 42.80% dietary protein in the diet is optimum for the growth, efficient feed utilization of scale carp, *C. carpio* var. *communis* 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.

Summary and Conclusion

Aquaculture feeds characteristically contain a high percentage of protein. In most aquaculture operations feed costs are the major operational expense. Formulating a feed based on an ideal protein is an efficient way of using less protein in the diet to meet amino acid requirements. Maximizing the effective use of protein and minimizing the amount needed in feeds can substantially reduce production costs and increase farm profitability. In order to enhance profitability and reduce pollution, the ideal situation would be to quantify the protein requirement of fish which was the main focus of this study.

The present study was conducted with the aim of analyzing the effects of dietary protein level on the growth, feed utilization and haemato-biochemical parameters of scale carp, *Cyprinus* var. *carpio communis* fingerlings, which forms the major proportion of the fisheries production in Kashmir.

An 8-week feeding trial was conducted to investigate the influence of dietary protein levels on growth performance, whole body biochemical composition and haematological parameters of fingerling scale carp (2.02±0.05gm; 5.10±0.12cm). Six isocaloric (367 kcal g^{-1}, GE) diets containing casein and gelatin with varying levels of protein (25%, 30%, 35%, 40%, 45% and 50%) were formulated. 20 fish were randomly stocked in triplicate groups, in 75L circular trough with continuous flow through system and fed experimental diets at 4% BW/day twice daily, at 08:00 and 17:00 hours. The water exchange rate in each trough was maintained at 1.0-1.5 $Lmin^{-1}$. Maximum live weight gain (246%) was achieved at 45% protein containing diet, which was insignificantly (P>0.05) different to the fish group that fed at 40% protein diet. The specific growth rate (SGR%) of fish fed various levels of dietary protein also produced similar results as achieved in live weight gain in the present study. However, best-feed conversion ratio (1.67) occurred in fish feed diet containing 40% protein diet which was not significantly (P>0.05) different to the fish fed at 45% protein diet, while poor-FCR values were recorded in lower protein containing diets. Whereas, protein efficiency ratio of fish fed various levels of dietary protein also produced significant (*P*<0.05) differences among each dietary level, with highest PER value (1.49) was achieved at 45% protein containing diets. Significantly (*P*<0.05) highest whole body protein deposition (27.09%) was observed in fish fed 40% protein containing diets followed by those receiving 45% protein diet, which remained insignificantly (*P*>0.05) different among each other. However, second-degree polynomial regression analysis of live weight gain, feed conversion ratio (FCR), specific growth rate (SGR%) and body protein deposition data (BPD%) indicated requirement for protein at 45.17, 43.40, 44.88 and 38.71% of the diet, respectively.

The whole body composition data at the end of experimental trial produced some significant (*P*<0.05) differences among all the dietary treatment levels. The whole body moisture content decreased gradually with the increase of dietary protein levels up to 45% protein containing diet, thereafter, a slight increase in whole body moisture was recorded. The minimum moisture (72.17%) content was recorded at 45% protein containing diet, whole maximum moisture content (77.57%) was observed at the lowest protein containing diet i.e. 25%. The whole bole body protein content was significantly (*P*<0.05) highest (16.78%) in fish fed 45% protein diet, followed by those receiving 40% and 50% protein diets, respectively, while minimum whole body protein content (12.74%) was noticed at the lowest protein containing diet. The whole body fat content gradually increased with the increase of each dietary protein level. Significantly (*P*<0.05) highest whole body fat content (7.22%) was recorded in fish fed highest i.e 50% protein containing diet, while minimum whole body fat (4.76%) content was noted in fish fed lowest i.e 25% protein diet. Whole body ash content was significantly (*P*<0.05) highest in fish fed lower doses of protein diets with highest body ash (3.24%) content recorded at 25% protein containing diet, whereas an insignificantly (*P*>0.05) lower values of body ash content were recorded at higher protein containing diets.

In the present study the haematological parameters of scale carp were also studied and these parameters also produced some interesting and significant differences between various protein levels. The haemoglobin (Hb) content (10.96g dl^{-1}) was found to be significantly (*P*<0.05) higher at 45% protein diet, followed by those receiving 40% protein diet (10.81g dl^{-1}), while intermediate Hb values were recorded at 50% and 35% protein containing diets. Similarly, haematocrit (HCT%) values also produced significantly (P<0.05) among each protein incremental level with maximum (40.48%) HCT value noted in fish group that fed 45% protein diet, while lowest HCT (25.21%) observed at the lowest protein containing diet (25%). Red blood cell count (RBC) also produced significant values among each protein level. Significantly (P<0.05) highest RBC count (2.04×10^6 mm^{-3}) was recorded at 45% protein containing diet, followed by the group receiving 40% protein diet (1.73×10^6 mm^{-3}), whereas lowest RBC count was noted in first two dietary protein levels i.e. (25% and 30%). No significant differences in WBC count were recorded in fish fed various levels of dietary protein, excepting the lower dietary protein containing diet, where significantly (P<0.05) highest WBC count was recorded.

Mean corpuscular volume (MCV) could not produce significant (P<0.05) differences among each dietary protein level, except at the first protein level (25%) where significantly (P<0.05) lower MCV value (184.32fl) was recorded. While significantly (P<0.05) highest mean corpuscular haemoglobin (MCH) value were recorded in fish feed 35%, 40% and 50% protein containing diets compared to other dietary levels. Moreover, the mean corpuscular haemoglobin concentration (MCHC) data could not produce any significant (P<0.05) differences among each dietary protein level.

Based on the above results, it is recommended that the diet for fingerling scale carp, *C. carpio communis* should contain protein at 42.80% for optimum growth and efficient feed utilization.

Taking the above facts into consideration, the following list of conclusions and recommendations may be summarized in the present study:

- Significant differences in growth and haemato-biochemical composition of scale carp were observed at varying dietary protein levels.
- Based on the findings of the present study the optimum dietary protein requirement of scale carp is recommended at 42.80% protein level.

- The present study will contribute to the development of nutritionally balanced diets aimed at optimizing the production of scale carp through aquaculture.
- 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 wide variation observed in the protein requirement of scale 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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