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Effects of Dietary Protein Levels on the Growth, Feed Utilization and Haemato-Biochemical Parameters of Freshwater Fish, *Cyprinus Carpio Var. Specularis*

Imtiaz Ahmed* **and Amir Maqbool**

Fish Nutrition Research Laboratory, Department of Zoology, University of Kashmir, Hazratbal, Srinagar

***Corresponding author:** Imtiaz Ahmed, Fish Nutrition Research Laboratory, Department of Zoology, University of Kashmir, Hazratbal, Srinagar, India, Tel: +91 9419968539; Fax: 019422421357; E-mail: imtiazamu1@yahoo.com

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Abstract

An 8-week feeding trial was conducted to study the effects of dietary protein levels on the growth, feed utilization and haemato-biochemical parameters of mirror carp, *Cyprinus carpio specularis* (1.50 ± 0.02 g; 4.5 ± 0.05 cm). Six casein-gelatin based isocaloric (367 kcal 100 g^{-1}, gross energy) diets containing graded levels of dietary protein (25%-50% CP) were formulated. 20 fish were randomly stocked in triplicate groups in 75L circular trough fitted with continuous flow-through system and fed experimental diets at 4% BW/day at 0800 and 1700h. Maximum live weight gain (258%), best feed conversion ratio (FCR) (1.63) and protein efficiency ratio (PER) (1.53) were obtained in fish fed diet containing 40% dietary protein. However, quadratic regression analysis live weight gain, FCR, PER and body protein deposition (BPD) data indicated requirements for dietary protein at 43.5%, 41.6%, 34.7% and 37.3% of dry diet, respectively. Significantly higher whole body protein, low moisture and intermediate body fat contents were recorded at 40% protein containing diet (P<0.05). While minimum ash content was recorded at 25% protein level. The highest HIS value (3.39%) was observed at the lowest protein level. Significant differences were also observed in Hb, HCT and RBC values of different groups fed with varying levels of dietary protein (P<0.05). Whereas, no significant differences were observed in their WBC count except at 25% protein level, where higher WBC count was recorded (P>0.05). Based on the above results, it is recommended that 41.5% protein level would be useful for optimum growth and efficient feed utilization of this fish species.

Keywords: *Cyprinus carpio specularis*; Dietary protein requirement; Growth; Blood; Biochemical parameters

Introduction

The global contribution of fish as a source of protein is indeed high, ranging from 10% to 15% of the human food basket across the world. It is estimated that around 60% of people in many developing countries depend on fish, for over 30% of their animal protein supplies [1]. The protein content of most fishes averages 15 to 20% on wet weight basis [2]. Fish also contains significant amounts of all essential amino acids, which are not available in plant protein sources and the digestibility of fish is approximately 5-15% higher than the plant-source foods [3].

Precise information on nutritional requirements of cultured species to provide appropriate amount of nutrients for optimal growth is essential to reduce feed cost, which accounts for a significant portion of the costs of an aquaculture enterprise. The development of cost-effective feeds that provide balanced diet to maximize growth, while minimizing environmental effects, depends on knowing the species' nutritional requirements and meeting those requirements with balanced diet formulations and appropriate feeding practices [4].

Among all the nutrients required by fish for growth and maintenance, protein is one of the most important and initial constituent, which comprises about 65-70% of the dry weight of fish muscle [5], and is also metabolized as an energy source by fish. Protein plays an important role in supporting fish growth [6-8]. Fish consume protein to obtain the essential and non-essential amino acids, which are necessary for muscle formation and enzymatic function and in part provide energy for maintenance [9]. Inadequate protein in the diet results in a reduction or cessation of growth and a loss of weight due to withdrawal of protein from less vital tissues to maintain the functions of more vital organs and tissues. Whereas, diet with excessive protein contents usually leads to extra energy costs, increased nitrogenous excretions and occasionally retarded fish growth [10,11]. Since protein constitutes in fish culture the single most expensive item in artificial feeds, it is logical to incorporate only that much, which is necessary for normal maintenance demand and growth. Any excess is considered wasteful, biologically as well as economically and therefore, it is important to minimize the amount of protein used for energy [12-14]. Thus an optimum dietary protein level in the diet is important for fish growth and maintenance of good farming environments [15].

Cyprinus carpio, as a freshwater fish species, has been one of the most widely cultured species all over the world due to its fast growth rate and easy cultivation [16]. Two varieties of common carp (*Cyprinus carpio*) viz: scale carp (*C.carpio var. communis*) and mirror carp (*C. carpio var. specularis*) are commercially cultured in Jammu and Kashmir. The mirror carp is detritus feeder feeding on decaying organic matter. This fish is herbivorous eating almost 80-85% plant food. It is column feeder [17]. The plant food consists of micro and macro phytes besides planktonic organisms. 15-20% of animal food consists of rotifers, annelids, crustaceans and insect larvae [18]. It is prolific breeder and has attained phenomenal population in all the lakes and rivers except for fast running cold hill stream. Almost 50% of fish population in valley lakes is mirror carp [19]. Mirror carp differs from other common carps in the development of back muscle (dorsal muscle) which is higher than the normal carp [20]. Due to this, mirror carp is also called as high back carp. Although some aspects of nutritional requirements of mirror carp have been worked out in the past by different workers [21-25], but no information related to the dietary protein requirement for the fingerling stage is available for this fish species. Keeping this in view, the present investigation was designed to study the effects of dietary protein levels on growth, feed utilization, whole body composition and haematological parameters of mirror carp, in order to determine the optimum dietary protein requirement of this fish, with a view to develop a nutritionally balanced diet for optimum production of this fish species through aquaculture.

Materials and Methods

Source of fish stock and acclimatization

Induced bred fingerlings of mirror carp, *Cyprinus carpio* var. *specularis* with the same batch and in apparent good health were procured from the 'State Government Fishery Department seed farm Manasbal'. The fingerlings were transported in polythene bags filled with water and oxygen and brought to the fish feeding trial laboratory (wet-lab) at the Department of Zoology, of . These fingerlings were first given a prophylactic dip in $KMnO_4$ (5 mgL^{-1}) to rule out any possible microbial infection and stocked in indoor circular aqua blue colored plastic fish tank (water volume = 600 L) for a fortnight. During this period, the fish were fed to satiation a mixture of soybean, mustard oil cake, rice bran, and wheat bran in the form of moist cake twice a day at 08:00 and 17:00 hours. These fingerlings were then acclimated for 2 weeks on H-440 diet [26] near to satiation twice a day at 08:00 and 17:00 h in the form of moist cake. A preliminary feed trial was conducted before the start of feeding trial to determine the appropriate feeding level and feeding schedule of the fish.

Preparation of experimental diets

Six casein-gelatin based isocaloric (367 kcal 100 g^{-1}, gross energy) diets containing graded levels of dietary protein (25%, 30%, 35%, 40%, 45%, and 50% crude protein) were formulated (Table 1). Diets were prepared taking into account the amount of protein contributed by casein and gelatin and made isocaloric by adjusting the amount of dextrin in the diet. Calculated quantities of dry ingredients were thoroughly mixed and stirred in a known volume of hot water (80°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°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°C until used. The composition of vitamin and mineral premixes were prepared as per Halver [26].

Diet (%)						
Ingredients (g 100g^{-1}, dry diet)	**(I)**	**(II)**	**(III)**	**(IV)**	**(V)**	**(VI)**
Casein[1]	24.4	29.2	34	38.8	43.6	48.6
Gelatin[2]	6.1	7.3	8.5	9.7	10.9	12.15
Dextrin[3]	50.62	44.24	37.87	31.5	25.13	18.49
Corn oil	6	6	6	6	6	6
Cod liver oil	3	3	3	3	3	3
Mineral mix[4]	4	4	4	4	4	4
Vitamin mix[4,5]	3	3	3	3	3	3
Carboxymethyl cellulose	2	2	2	2	2	2
Alpha cellulose	0.88	1.26	1.63	2	2.37	2.76
Total	100	100	100	100	100	100
Calculated crude protein (g 100g^{-1})	25	30	35	40	45	50
Analysed crude protein (g 100g^{-1})	24.87	29.65	34.58	40.16	44.79	50.18
Gross energy[6] (kcal g100g^{-1}, dry diet)	367	367	367	367	367	367

Table 1: Formulation and proximate composition of experimental diets used for estimating the dietary protein requirement of mirror carp, *Cyprinus carpio var. specularis* fingerlings. [1]Crude protein (80%), [2]Crude protein (93%) Loba Chemie, India; [3]Loba Chemie, India. [4]Halver 2002 mineral ($AlCl_3$. $6H_2O$, 150; $ZnSO_4$. $7H_2O$, 3000; CuCl,100; $MnSO4._4$-$6H_2O$, 800; KI,150; $CoCl_2.6H_2O$,1000 mg kg^{-1}; plus USP # 2 Ca $(H2PO_4)_2$. H_2O, 135.8; $C_6H10CaO_6$ 327.0; $C_6H_5O_7Fe.5H_2O$, 29.8; $MgSO_4.7H_2O$, 132.0; KH_2PO_4 (dibasic), 239.8; $NaH_2PO_4.2H_2O$, 87.2; NaCl, 43.5 (g kg^{-1}); [5]vitamin mix (choline chloride 5000: thiamin HCL 50; riboflavin 200; pyridoxine HCL 50; nicotinic acid 750; calcium pentothenate 500; inositol 2000; biotin 5.0; folic acid 15; ascorbic acid 1000; menadione 40; alpha-tocopheryl acetate 400; cyanocobalamine 0.1 (g kg^{-1}). [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).

Experimental design and feeding trial

The fishes were sorted out from the acclimatized fish lots maintained in the wet laboratory and the desired number of *C. carpio* var. *specularis* fingerlings with almost similar body weight and size (1.50 ± 0.02 g; 4.5 ± 0.05 cm) were randomly selected in triplicate groups in 75 L high-density polyvinyl circular troughs (water volume 65 L) fitted with a continuous water flow-through system at the rate of 20 fish per trough for each dietary treatment levels. The water exchange rate in each trough was maintained at 1.0-1.5 L min^{-1}. The feeding schedule and feeding levels were chosen after carefully observing the feeding behaviour of the fish and their intake. For this purpose an 8-week preliminary feeding trial was also conducted under the same experimental setup in order to determine the appropriate ration size of the fish by feeding fish at the rate of 1%, 2%, 3%, 4%, 5% and 6% BW/day, results showed that the optimum ration size of the fish is approximately 4-4.5%. As per the result obtained in the preliminary feeding trial, the experimental fish were fed test diet in the form of moist cake at the rate of 4% of the body weight six days a week twice a day at 08:00 and 17:00 h, dividing into two equal feeding. The feeding trials lasted for eight weeks. Initial and weekly weights were recorded on a top loading balance (Sartorius CPA-224S 0.1 mg sensitivity, Goettingen, Germany). Fecal matter was siphoned before feeding and the daily feed offered was recorded. The uneaten feed (if any) was collected after active feeding approximately for 40 min with the help of siphoning pipe and collection tubes. The collected feed was then oven-dried at 100°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 numbers of fish were randomly sacrificed for the assessment of whole body composition.

Water quality analysis

The physico-chemical parameters of water (temperature, dissolved oxygen, free carbon dioxide, total alkalinity and pH) were recorded daily, following the standard methods [27]. The water sample for analysis was collected early in the morning before the feeding was done. Water temperature (23.6-24.5°C) was recorded using a mercury thermometer, dissolved oxygen (6.1-6.8 mg L^{-1}) was estimated by Winkler's iodimetric test, free carbon dioxide (3.9-5.7 mg L^{-1}), total alkalinity (91-112 mg L^{-1}) by titrimetric methods, respectively. While, pH (7.2-7.6) was measured by using a digital pH meter (pH ep-HI 98107, USA).

Chemical analysis

The Proximate composition of casein, gelatin, and experimental diet, initial and final carcass was estimated using standard [28] methods for dry matter (oven drying at 105 ± 1°C for 22 h), crude protein (N-Kjeldhal X 6.25), crude lipid (solvent extraction with petroleum ether B.P 40-60°C) by using Soxtec extraction technique (FOSS Avanti automatic 2050, Sweden), and ash (oven incineration at 650°C for 2-4 h) were determined. At the end of the experiment, eight fish were randomly pooled from each replicate of dietary treatment and three sub-samples of each replicate from the pooled sample (n=3×3) were analysed for final body composition. Similarly, three fish were randomly selected from each replicate of dietary treatment for organ index estimation and blood collection.

Hematological parameters

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

Haemoglobin (Hb)

Haemoglobin content of blood was estimated by the method of Drabkin [29]. 20µl of blood was mixed with 5 ml of Drabkin solution (Loba chemie, India) and left to stand for at least 15 minutes. Haemoglobin concentration was determined by measuring the absorbance at 540 nm and compared to that of haemoglobin standard (Ranbaxy, India). Prior to reading the absorbance, hemoglobin test samples were centrifuged to remove dispersed nuclear material.

Haematocrit (HCT)

Haematocrit (HCT%) was determined on the basis of sedimentation of blood. Heparinised blood (50µl) was taken in a micro-haematocrit capillary (Na-heparinised) and spun in a micro-haematocrit centrifuge (REMI RM-12C, India) at 12,000 rpm for 5 min to obtain haematocrit value. The haematocrit value was measured using a haematocrit reader and reported as percentage [30].

Red blood cell (RBC) and white blood cell count (WBC)

For RBC and WBC count, a blood sample (20 µl) was taken with a micro pipette (Finpipette, Finland), and diluted with Natt-Herrick's [31] diluent (1:200). The diluted sample was placed in a Neubauer improved haemocytometer (Marienfeld-Superior, Lauda-Konigshofen, Germany) and then the blood cells were counted using a light microscope (Magnus-MLM, India). RBC indices viz: mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volumes (MCV) were calculated according to Dacie and Lewis [32].

Growth parameters

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

Weight gain (%) = Final body weight-initial body weight/initial weight x 100

Specific growth rate (SGR %) = 100 x
(In final wet weight (g)-In initial wet weight g)/duration (days)

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

Body protein deposition (BPD %)=
$100 \times (BW_f \times BCP_f) - (BW_i \times BCP_i) / [TF \times CP]$

Where BWi and BWf = mean initial and final body weight (g), BCPi and BCFf = mean initial and final percentage of muscle protein, TF

=Total amount of diet consumed, and CP=Percentage of crude protein of the diet.

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

Statistical analysis

Responses of mirror carp fingerlings to graded levels of dietary protein were measured by weight gain (%) feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR %) and body composition. These response variables were subjected to one-way analysis of variance (ANOVA) [33,34]. To determine the significant differences among the treatments, Duncan's Multiple Range Test [35] was employed. To predict more accurate responses to the dietary protein intake, the optimum dietary protein level was estimated using second-degree polynomial regression analysis ($Y = ax^2 + bx + c$) as described by Zeitoun et al. [36]. Statistical analysis was done using SPSS 11.5 (SPSS Inc., Chicago, IL, USA).

Results

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

Dietary protein levels (g 100 g^{-1}, dry diet)						
	25	30	35	40	45	50
Average initial weight (g)	1.557 ± 0.04	1.592 ±0.02	1.608 ± 0.01	1.599 ± 0.02	1.605 ± 0.02	1.614 ± 0.03
Average final weight (g)	3.142 ± 0.15	3.968 ± 0.07	4.909 ± 0.07	5.731 ± 0.05	5.623 ± 0.11	5.189 ± 0.13
Live weight gain (%)	101.64 ± 5.10d	149.25 ± 7.58c	205.37 ± 6.80b	258.48 ± 8.36a	250.43 ± 8.50a	221.48 ± 5.23b
Specific growth rate (SGR)	1.25 ± 0.04^d	1.63 ± 0.04^c	1.99 ± 0.03^b	2.28 ± 0.03^a	2.24 ± 0.04^a	2.08 ± 0.02^b
Feed conversion ratio (FCR)	2.93 ± 0.06^a	2.45 ± 0.05^b	1.92 ± 0.05^d	1.63 ± 0.04^e	1.70 ± 0.05^e	2.08 ± 0.07^c
Protein efficiency ratio (PER)	1.36 ± 0.03^b	1.37 ± 0.02^b	1.49 ± 0.04^a	1.53 ± 0.04^a	1.31 ± 0.03^b	0.96 ±0.03^c
Body protein deposition (BPD)	19.46 ± 0.53^d	21.41 ± 0.44^c	24.60 ± 0.63^b	28.50 ± 0.69^a	23.86 ± 0.70^b	16.60 ± 0.69^e
HIS (%)	3.39 ± 0.05^a	3.08 ± 0.05^b	2.80 ± 0.07^c	2.41 ± 0.02^d	2.33 ± 0.04^d	2.72 ± 0.03^c
Survival (%)	100	100	100	100	100	100

Table 2: Growth, FCR, protein deposition and percentage survival of mirror carp, *Cyprinus carpio var. specularis* fingerlings fed diets containing varying levels of dietary protein for 8 weeks (mean values of 3 replicates + SEM; n=3)*. *Mean values of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different (P>0.05).

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

In order to get statistically more precise information, all the growth parameters were subjected to second-degree polynomial regression analysis. When live weight gain data (Y) and dietary protein levels (X) were analyzed using second-degree polynomial analysis, a break-point was evident at 43.5% dietary protein level (Figure 1). The relationship was described by the equation:

$$Y = - 0.4568x^2 + 39.7203x - 616.0843 \ (r = 0.969)$$

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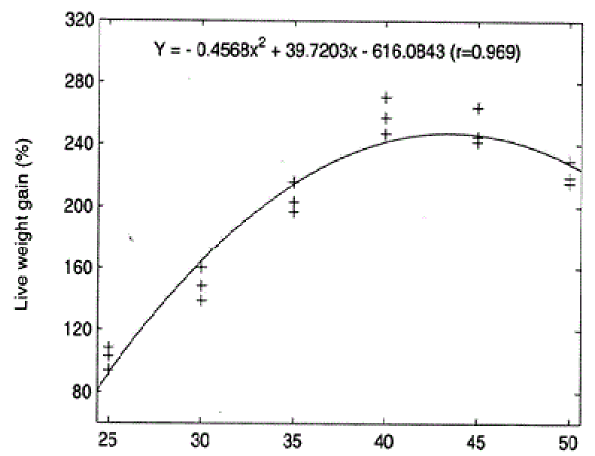

Figure 1: Dietary protein levels (g $100g^{-1}$).

The specific growth rate of mirror carp fed varied levels of dietary protein also produced somewhat similar trends as obtained in the growth rate. The SGR (Y) to dietary protein level (X) was also analyzed by using second-degree polynomial regression analysis (Figure 2) and the break point was evident at 43.22% protein level. The mathematical equation was:

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

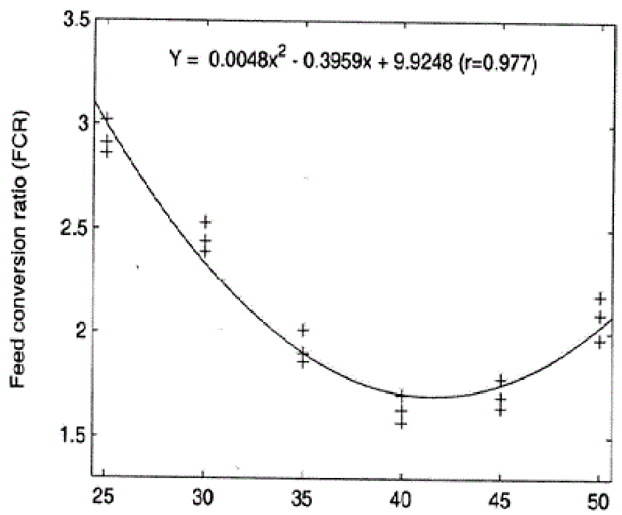

Figure 2: Dietary protein levels (g $100g^{-1}$).

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

$$Y = 0.0048x^2 - 0.3959x + 9.9248 \ (r = 0.977)$$

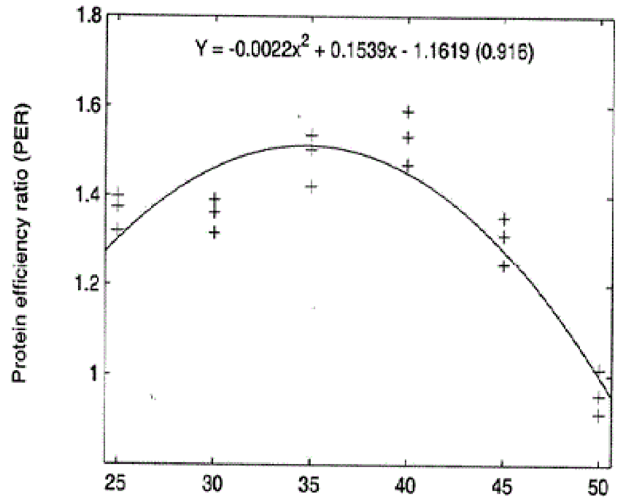

Figure 3: Dietary protein levels (g $100g^{-1}$).

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

$$Y = - 0.0022x^2 + 0.1538x - 1.1618 \ (r = 0.916)$$

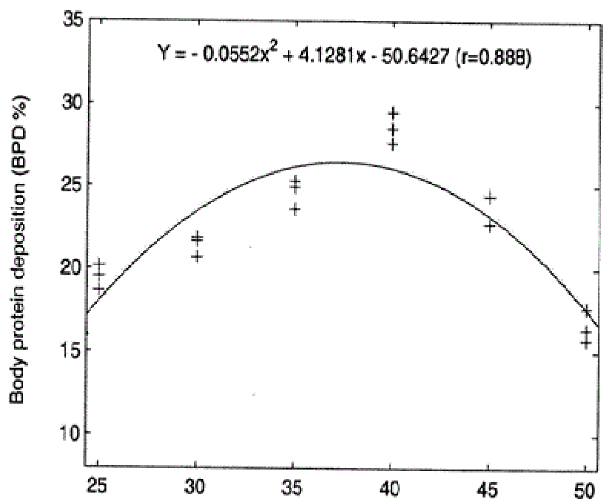

Figure 4: Dietary protein levels (g $100g^{-1}$).

Based on the above polynomial equations the FCR and PER indicated that the optimum dietary protein requirement of mirror carp was estimated to be at 41.6% and 34.7%, respectively.

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

	Dietary protein levels (g 100 g^{-1}, dry diet)						
	Initial	25	30	35	40	45	50
Moisture (%)	80.86 ± 0.44	77.49 ± 0.26^a	75.66 ± 0.040^b	74.34 ± 0.22^c	72.57 ± 0.17^d	72.25 ± 0.20^d	73.75 ± 0.14^c
Protein (%)	12.01 ± 0.15	13.15 ± 0.06^f	14.27 ± 0.05^e	15.06 ± 0.08^d	16.77 ± 0.07^a	16.49 ± 0.05^b	15.62 ± 0.06^c
Fat (%)	3.37 ± 0.13	4.81 ± 0.09^f	5.41 ± 0.11^e	6.13 ± 0.07^d	6.72 ± 0.08^b	7.61 ± 0.09^a	6.43 ± 0.07^c
Ash (%)	2.66 ± 0.05	3.16 ± 0.04^a	2.90 ± 0.02^b	2.61 ± 0.02^e	2.72 ± 0.04^d	2.65 ± 0.03d,^{e}	2.82 ± 0.02^c

Table 3: Whole body composition of fingerling, *Cyprinus carpio var. specularis* fed diets containing graded levels of dietary protein for 8 weeks (mean values of 3 replicates + SEM; n=3) [*]. [*]Mean values of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different (P>0.05).

Whole body ash content was found to be significantly higher at lower dietary protein containing diets i.e. 25% and 30%, whereas significantly lower ash content values were observed in fish fed the remaining dietary protein levels (P<0.05). Also fish fed diet containing 40% protein resulted in highest whole body protein deposition (BPD %), which was significantly highest among all the dietary groups. Second-degree polynomial regression analysis was also employed between the body protein deposition (Y) to dietary protein level (X) and a break-point was obtained at 37.3% protein level. The mathematical equation was:

$$Y = - 0.0552x^2 + 4.1281x - 50.6427 \ (r = 0.888)$$

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

	Dietary protein levels (g 100g-1, dry diet)					
	25	30	35	40	45	50
Hb (gdl^{-1})[1]	6.86 ± 0.07^e	7.77 ± 0.08^d	9.27 ± 0.07^c	10.78 ± 0.10^a	10.92 ± 0.15^a	10.24 ± 0.07^b
HCT (%)[2]	22.19 ± 0.69^e	26.403 ± 0.83^d	30.83 ± 0.90^c	36.77 ± 0.72^a	38.26 ± 0.56^a	33.58 ± 0.50^b
RBC (×106/mm^3)[3]	1.23 ± 0.02^e	1.34 ± 0.03^d	1.52 ± 0.03^c	1.78 ± 0.04^b	1.90 ± 0.05^a	1.62 ± 0.04^c
WBC (×104/mm^3)[4]	2.42 ± 0.04^a	2.36 ± 0.05^{ab}	2.33 ± 0.03^{ab}	2.28 ± 0.04^b	2.30 ± 0.06^{ab}	2.24 ± 0.05^b
MCV (fl)[5]	180.33 ± 5.61^b	196.74 ± 8.74^a	202.62 ± 6.55^a	208.61 ± 4.73^a	201.78 ± 2.83^a	207.93 ± 6.38^a
MCH (pg)[6]	55.71 ± 0.53^d	57.86 ± 0.81^c	60.91 ± 1.02^{ab}	60.58 ± 0.83^b	57.60 ± 0.91^c	63.21 ± 0.13^a
MCHC (gdl^{-1})[7]	30.93 ± 0.98^a	29.46 ± 0.90^{ab}	30.10 ± 1.03^{ab}	29.34 ± 0.55^{ab}	28.94 ± 0.44^b	30.42 ± 0.64^{ab}

Table 4: Effect of experimental diets on hematological parameters of mirror carp, *Cyprinus carpio var. specularis* fingerlings for 8 weeks (mean values of 3 replicates + SEM; n=3)[*]. [*]Mean values of 3 replicates ± SEM; Mean values sharing the same superscript are not significantly different (P>0.05). [1]Haemoglobin concentration; [2]Haematocrit; [3]Red blood cell count; [4]White blood cell count; [5]Mean corpuscular volume; [6]Mean corpuscular haemoglobin; [7]Mean corpuscular haemoglobin concentration.

Red blood cell counts (RBC) in fish fed various dietary protein levels also produced significant differences. Significantly highest RBC value (1.9×106 mm^{-3}) was noted at 45% protein diet, followed by those receiving diet at 40% protein diet (P<0.05). Intermediate RBC values were recorded in fish fed other dietary protein levels, except those fed 25% and 30% protein diets, where significantly lowest RBC count values were obtained (P<0.05). Whereas, the fish fed varied levels of dietary protein could not produce any significant difference in their leukocyte (WBC) counts, except at lowest levels where slightly higher WBC counts were recorded.

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

Discussion

Understanding the dietary protein requirement of fingerling stage of mirror carp becomes a pre-requisite for the development of nutritionally balanced, efficient and cost effective feed for culturing practice. In the present study, graded levels of dietary protein content had a significant effect on the growth rate, feed conversion ratio, protein efficiency ratio and specific growth rate. The growth and conversion efficiencies gradually increased with the increase of dietary protein levels from 25% to 45% protein containing diet. Although the maximum growth parameters were obtained when fish were fed at 45% protein containing diet, however, this growth rate was not significantly different to those groups that were fed at 40% protein diet. Whereas, the best-FCR, PER, SGR and BPD was recorded with fish fed 40% protein diet. Therefore, inclusion of 40% protein in the diet for fingerling mirror carp is more appropriate and economical. Also the growth rate significantly fell beyond the requirement level, especially at 50% protein diet, indicating that 40% protein diet (Diet IV) satisfied the protein requirement of the fish and is considered optimum for achieving maximum growth and efficient nutrient conversion efficiency. The decrease in growth rate at protein levels above the optimum requirement may be attributed to the fact that the fish body cannot utilize the dietary protein once after reaching the optimum protein level [37]. The excessive protein content in the diet could reduce the growth performance of fish due to higher energy requirement for catabolism rather than for protein deposition. The decrease in weight gain, when the fish were fed excess level of dietary protein may also be because of a reduction in available energy for growth and due to inadequate non-protein energy necessary to deaminate the high protein feed [38,39]. The reduced growth rate and decreased protein utilization beyond requirement of dietary protein level is well documented in the past by several workers [39-46].

In general, both, feed conversion ratio and protein efficiency ratio were poor in lower protein containing diets. However, improvement in FCR and PER was noticed with increasing incorporation of dietary protein levels. The best-FCR and highest PER values were recorded with fish fed at 40% protein containing diet. The BPD and PER increased with the increase in dietary protein content up to 40% and thereafter, a significant decrease was recorded with further elevation of dietary protein level i.e. 45% and 50% protein containing diets (Diet V and Diet VI). Similar trends in PER and BPD were also reported by other workers [47].

The whole body composition data showed that whole body moisture content gradually increased with the increase of dietary protein levels, with minimum moisture content was recorded at 40% protein diet. Whole body protein content linearly increased with the increase of dietary protein level up to 40% and thereafter, a decline in body protein content was noted. The highest protein content obtained in the present study, when fish fed at 40% protein diet could be due to the fact that at this particular level fish utilized the available protein content for growth more efficiently than those fed other dietary protein levels. Similar results on body protein content have also been reported by Kim et al. [39]. Kim and Lee [48] further reported that body protein content responded to dietary protein levels in a dose dependent manner and exhibited maximum protein content on that dietary protein level where maximum growth rate was also achieved.

Whole body fat content gradually increased with the increase of dietary protein levels and maximum body fat content was recorded at higher dietary protein containing diet (Diet VI). The higher whole body fat content beyond the optimum protein requirement level in the diet may be due to the fact that the excess dietary protein content in these diets gets deaminated and stored as body fat. The whole body ash did not show any significant difference among the treatment levels, except at lower protein containing diets where high body ash content was recorded. The fish fed varied levels of dietary protein produced some significant differences in HSI values. The highest HSI value (3.39%) was observed at the lowest dietary protein level, which was significantly higher compared to all the dietary groups. Higher values of HSI in lower protein diet could be due to the poor growth and health of the fish [49-51] and also due to more fat accumulations in the liver [11,15,47,52].

Besides biochemical analysis, haematological analysis was also carried out in the present study in order to find out the effects of dietary protein levels on these parameters, which are recognized as valuable tools for monitoring fish health, physiological responses, assessment of feed composition and nutritional status in relation to environmental stress [53-56].

In the present study, significant differences were observed in Hb, HCT and RBC values of different groups fed with varying levels of dietary protein, showing a general trend of linear increase with the increase of dietary protein levels. However, the haematological values obtained in the present study were within acceptable limits as reported by Svobodova et al. [56], for common carp. Fishes alter their metabolic profile to cope up with the different dietary conditions [57,58]. Hb and HCT values significantly increased with the increase of dietary protein levels from 25% - 45% protein containing diets. However, highest Hb (10.92 gdl^{-1}) and HCT values (38.26%) were recorded for fish fed 45% protein diet. An increase in RBC count was evident with the increase in dietary protein levels, which may have occurred due to its early release from the storage pool in the spleen [59,60], thus, causing a change in MCH values as well.

On the basis of second-degree polynomial regression analysis of growth parameters and body composition data, the optimum dietary protein level for optimum growth of mirror carp, *C. carpio var. specularis* fingerling is recommended to be at 41.50%. The protein requirement of fish varies from species to species and is reported with in the renge from 30 to 56% [12,13]. The protein requirement of mirror carp estimated during the present study in terms of percentage is comparable with the requirements reported for other fish species (Table 5). The differences in protein requirement among the fish species may be due to different dietary formulations, fish size and different methodologies adopted [61,62]. 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 [63]. Moreover, the 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%, when feeding rate was increased from 2-4% body weight^{-1} [12].

Fish Species	Protein requirement (%)	References
Mirror carp, *C. carpio var. specularis*	41.5	Present study
Indian major carp, *Cattla catla*	40-47	Khan and Jafri [63], Singh and Bhanot [64]
Indian major carp, *Cirrhinus mrigala*,	36	Singh et al. [65]
Jian carp, *Cyprinus carpio*	34.1	Liu et al. [66]
Indian major carp, rohu, *Labeo rohita*	25-35	Satpathy et al. [67], Khan et al. [68] Debnath et al. [69]
Big head carp, *Aristichthys nobilis*	30	Santiago and Reyes [70]
African catfish, *Clarias. gariepinus,*	40-43	Degani et al. [71], Ali and Jauncey [72], Farhat and Khan [73]
Magur, *Clarias batrachus*	40	Khan and Jafri [74]
Malaysian catfish, *M. nemurus*	42	Khan et al. [75]
Juvenile sunshine bass, *M. chrysops x M. saxatilis*	41	Webster et al. [76]
Mangrove red snapper, *Lutjanus argentimaculatus*,	40	Catacutan et al. [77]
Juvenile masu salmon, *Oncorhynchus masuo*	40	Lee and Kim [78]
Mahseer, *Tor putitora*,	40	Hossain et al. [79]
African Cichlid, *Pseudotropheus socolofi*	40	Royes and Murie [80]
Milkfish, *Chanos chanos*	40	Jana et al. [81]
Juvenile blackspot sea bream, *Pagellus bogaraveo*,	40	Silva et al. [82]
Cuneate drum, *Nibea miichthioides*	40	Wang et al. [83]
Persian sturgeon, *Acipenser persicus*	40	Mohseni et al. [84]
Mexican silverside, *Menidia estor*	40.9	Martinez-Palacios et al. [85]
Tiger puffer, *Takifugu rubripes*	41	Kim and Lee [47]
Singhi, *Heteropnestus fossilis*	35-40	Akand et al. [86], Qamar and Khan [87]
Black sea bream, *Sparus macrocephalus*	41.4	Zhang et al. [88]
Pacific threadfin, *Polydactylus sexfilis*	41	Deng et al. [14]
Grey mullet, *Mugil capito*	24	Papaparaskera-Papoutsoglou and Alexis [89]
Nile tilapia, *Oreochromis niloticus*	25-45	Abdel-tawwab et al. [11], Siddiqui et al. [90], El-Saidy and Gaber [91]
Tilapia, *O. mossambicus*	28	De Silva et al. [92]
Juvenile silver perch, *Bidyanus bidyanus*,	42.15	Yang et al. [9]
Juvenile, *Spinibarbus hollandi*,	32.7	Yang et al. [51]
Black catfish, *Rhamdia quelen*	37	Salhi et al. [93]
Channel catfish*, Ictalurus punctatus*	28	Li et al. [94]
Golden shiner, *Notemigonus crysoleucas*	29	Lochmann and Phillips [95]
Blue streak hap, *Labidochromis caeruleus*	35	Ergun et al. [96]

Amazonian tambaqui, *Colossoma macropomum*	30	Oishi et al. [97]
Brown trout, *Salmo trutta*	57	Arzel et al. [98]
Grouper, *Epinephelus malabaricus*	44	Shiau and Lan [99]
Juvenile Florida pompano, *Trachinotus carolinus*,	45	Lazo et al. [100]
Discus, *Symphysodon spp*.	44.9-50.1	Chong et al. [101]
American eel, *Anguilla rostrata*,	47	Tibbetts et al. [40]
Spotted sand bass, *Paralabrax maculatofascinatus*	45	Alvarez-Gonzalez et al. [102]
Juvenile haddock, *Melanogrammus aeglefinus*,	49.9-54.6	Tibbetts et al. [60], Kim et al. [103]
Bagrid catfish, *Mystus nemurus*	44	Ng et al. [104]
Juvenile olive flounder, *Paralichthys olivaceus*,	46.4-51.2	Kim et al. [38]
Mahseer, *Tor putitora*,	45-50	Islam and Tanaka [105]
Juvenile turbot, *Scophthalmus maximus*	55	Cho et al. [106]
Pike perch, *Sander lucioperca*	43	Nyina-wamwiza et al. [107]
Black sea bass, *Centropristis striata*	45-52	Alam et al. [108]
Malaysian mahseer, *Tor tambroides,*	48	Ng et al. [109]
Silver pomfret, *Pampus argenteus,*	49	Hossain et al. [110]
Asian red-tailed catfish, *Hemibagrus wyckioides*	44.12	Deng et al. [111]
Sharpsnout sea bream, *Diplodus puntazzo*	43	Coutinho et al. [112]
Tongue sole, *Cynoglossus semilaevis*	55	Liu et al. [113]

Table 5: Dietary protein requirements of various cultivated fish species compared with *C. carpio var. specularis*.

The present study indicates that the dietary protein level influences fish growth, feed conversion ratio and haemato-biochemical composition of fish and therefore, it is recommended that the inclusion of 41.50% dietary protein in the diet is optimum for the growth, efficient feed utilization of mirror carp, *C. carpio* var. *specularis* fingerling. Data generated in the present study would be useful in developing nutritionally balanced diets for the intensive and semi-intensive culture of this fish species [64-113].

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