Research Article

Effect of Graded Dietary Protein Levels on Body Composition Parameters of Hybrid (*Labeo rohita* ♀ and *Catla catla* ♂) from Pakistan

Rabia Iqbal, Muhammad Naeem*, Samrah Masud and Abir Ishtiaq

Institute of Pure and Applied Biology (Zoology Division), Bahauddin Zakariya University, Multan, Pakistan.

Abstract | Present study was conducted to estimate the body composition parameters in hybrid fry (*Labeo rohita* ♀ and *Catla catla* ♂) by feeding them at three graded protein feeds (15%CP, 20%CP, 25%CP). Eighteen days old fry of hybrid (*L. rohita* ♀ and *C. catla* ♂) of 0.12±0.08 average weight(g) and length(cm) 1.63±0.21 were acclimatized and shifted in hapas (8x6x3 ft.), fed at the rate of 5% of their wet body weight. Ten samples from each hapa were randomly selected at the end of three months (90 days trial) for body composition analysis. Mean percent water, ash, fat and protein contents were ranged 80.55±0.94 to 80.55±0.94; 2.23±0.13 to 2.23±0.13, 5.85±0.55 to 6.63±0.97 and 10.59±1.67 to 11.93±1.67 in wet body weight of hybrid fish (Labeo rohita ♀ and Catla catla ♂), respectively, reared under different treatments. Mean water and fat contents showed highest percent values in T1 (15%CP), while ash and protein contents were found highest in T3 (25%CP) in the hybrid fish. Protein and lipid showed inverse relation as with increasing dietary protein levels as T3 (25%) feed showed highest protein content and lowest lipid content. Inverse relation was seen between water content and fat content. % water showed relationship with % fat, % protein and % organic content in T1 feed, while T2 and T3 showed no significant correlation. All (T1, T2, T3) feeds showed no relation between % water and % ash (wet and dry). All (T1, T2, T3) feeds observed highly significant correlation between body weight and water content. Investigation of body size on total body composition parameters revealed generally in different feeds an increasing trend in total protein, total ash and total organic content, while a decreasing trend in total fat content. Total length in all (T1, T2, T3) feeds observed no influence on total water content, total protein content and total organic content, while an influence on ash content and total fat content was observed.

Received | April 10, 2019; **Accepted** | March 08, 2020; **Published** | April 28, 2020
***Correspondence** | Muhammad Naeem, Institute of Pure and Applied Biology (Zoology Division), Bahauddin Zakariya University, Multan, Pakistan; Email: dr_naeembzu@yahoo.com
Citation | Iqbal, R., M. Naeem, S. Masud and A. Ishtiaq. 2020. Effect of graded dietary protein levels on body composition parameters of hybrid (*Labeo rohita* ♀ and *Catla catla* ♂) from Pakistan. *Sarhad Journal of Agriculture,* 36(2): 548-558.
DOI | http://dx.doi.org/10.17582/journal.sja/2020/36.2.548.558
Keywords | Hybrid, Graded protein, Hapa, Body composition

Introduction

Now a days people are sensitive for healthy eating than in the past (Oriakpono et al., 2011). Due to high nutritional value, people prefer white meat like fish over the red meat (Ayisi et al., 2017). With respect to Pakistan, Aquaculture is a very new and developed activity due to rich aquatic resources and having 60,470 hectares total fish pond area (Akhtar, 2001). Similar to composition of muscle meats, fish and shell fish consists of 19% protein. Fish is also preferred due to having low fat content as compared to beef (Ndome et al., 2010; Al-Ghanim, 2016; Tsironi and Taoukis, 2017). Many diseases such as cancers, arterial hypertension and inflammatory diseases are prevented and cured by using fish poly unsaturated fatty acids (Turkmen et al., 2005)

Body value (biological and functional condition) of a fish can be determined by evaluating body composition but it is a time consuming process (Soltan and Tharwat, 2006). Different ingredients such as protein, fat, water, ash and organic content are major quantifying body composition parameters, whereas carbohydrate and non-protein compounds are usually neglected (Jakhar et al., 2012). Each content acts as an indicator for another content as water show inverse relation to lipids and protein. In other words, a low proportion of water means a greater amount of lipid as well as protein (Dempson et al., 2004).

The values of body composition parameters may vary in different environmental conditions in same fish species due to changes in water quality, sex, feeding conditions, state of maturity and by other biotic and abiotic factors such as food availability and hydrologic level (Sogard and Spencer, 2004; Blake et al., 2006; Jeyasanta and Patterson, 2014). Regional variations such as water temperature may also influence body composition parameters as rate of fish metabolism increases in summer season and decreases in winter, similarly higher level of lipid contents in eutrophic waters as compared to oligotrophic waters may cause variations in body composition parameters (Bureau et al., 2002). Water depth also has an impact on body composition parameters as increasing depth causes high protein and low fat content in fish (Drazen, 2007; Suseno et al., 2010). Due to high quality protein, fish is preferred in the world as an important food source so it is very necessary to analyze its proximate body composition before using as food source (Foran et al., 2005; Fawole, 2007).

No well-defined published literature regarding body composition of hybrid using graded protein levels is present. Hypophysation is an artificial method by which hybrids of interspecific and intergeneric nature of Indian major carps as *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* and *Labeo calbasu* can be produced (Chaudhuri, 1959; Khan et al., 1986). Growth rate of *Catla* X *Labeo* hybrids is slightly low as compared to *Catla* but hybrids showed rapid growth than *Labeo* (Natarajan et al., 1976). Hybrids possessed best characters of Catla and *Labeo* as small head and deep body with more flesh as compared to both of parents, so showed a best choice of culturing than either of parents (Basavaraju et al., 1995). Morphology of catla-labeo hybrid is in between of two parents and generally phytophagous in nutrition and gets maturity in three years (Bhowmick et al., 1981). Scales and fin color in *Catla-Labeo* hybrid is similar to male parent and pectorals in these hybrids reach near ventral base. Gill rakers are most prominent in hybrid than parents and caudal peduncle is broader as compared to both parents (Reddy et al., 1980).

Feed is the major input showing 60% of total expenses in fish culture (Li and Wang, 2004). Estimated findings of protein requirement for carnivorous fish is 400 to 550 g kg $^{-1}$ and for herbivorous and omnivorous species 250 to 350 g kg $^{-1}$ (NRC, 1983). Protein being an expensive macronutrient in fish feed has too much importance in nutritional studies (Meyer and Fracalossi, 2005) because fish weight gain is greatly affected by protein quantity present in fish feed (Martinez-Palacios et al., 2007; Zuanon et al., 2009). Cost of fish feed can be reduced by reducing dietary protein up to that level without affecting fish growth (Kim et al., 2003).

The aim of the study was to study the effect of graded dietary protein levels (15, 20 and 25% CP) on body composition of hybrid fish (*Labeo rohita* ♀ and *Catla catla* ♂) from Pakistan.

Materials and Methods

Study site and duration

Present study was conducted from June to August 2017 for 90 days at Tawakkal Fish Hatchery, Tawakkal Nagar, rural demonstration (30° 11′ 27″ N, 71° 15′ 4″ E), 18 Km Jhang road, Muzaffar Garh, Punjab, Pakistan.

Formulation of feed

Three crude protein diets [T1 (15%), T2 (20%), T3 (25%)] were prepared from cheaper and locally available feed ingredients containing soybean meal, maize gluten, fish meal, sunflower meal, rice polish and wheat bran. All ingredients were dried in oven at 60^{0}C to a constant weight. In grinder mixing of calculated quantities of dry contents was done for 40 minutes to make a paste. In feed mixing machine dried contents were mixed in powdered form and for 30 minutes they were stirred and feed was kept in polythene bags for experimental use.

Experimental layout

The hybrid fry (*Labeo rohita* ♀ and *Catla catla* ♂) were selected for this experiment. Main purpose of this experiment was to observe effect of three protein diets on growth and body composition of hybrid fry. Eighteen days old fry of the hybrid fish of average weight 0.12±0.08 (g) and average length 1.63±0.21 (cm) were kept (150 fishes per hapa) in hapas (8×6×3 ft.) in earthen ponds with stagnant water, after acclimatization of two weeks in cemented tanks. Experiment was conducted in duplicate for each treatment and then took average values of two hapa results per treatment for each body composition parameter. Fish meal @ 10% of body weight was given during acclimatization period. Pond was filled with water up to level of 3-4 feet and this level was managed during whole experiment. Three protein diets (15%, 20%, 25%) were used in this experiment. These diets were dried and in grinded powder form. Feed was given to the fish at the rate of 5% of fish body weight throughout the experimental period, and feed was given to the fishes one time in a day at 11:00 am.

Body composition analysis

At the end of 90 days feeding trial, 10 hybrids (*Catla catla*♂ × *Labeo rohita*♀) from each hapa of three protein diets were selected randomly of different body sizes ranging from 7.00 – 11.00 cm total length and 9.00- 23.50 g body weight, using a cast net and live fishes were transported to research laboratory, Institute of Pure and Applied Biology, Bahaddin Zakariya University, Multan.

Calculation of water content

Electronic digital balance (chyo-MP-3000) was used to weight fish to nearest 0.01 g and wooden measuring tray was used to measure their length to nearest 0.1 cm. Each fish was dried individually till constant weight in an electric oven at 60-65 ˚C to estimate water content. Pestle and mortar was used to crush each dried fish in powder form and processed in an electric blender (Moulinex). Plastic bottles with proper labeling were used to preserve powdered fish for further analysis. Following formulas was used to calculate water content and water percentage (%).

$$\text{Total (water content)} = \text{body (wet weight)} - \text{body (dry weight)}$$

$$\text{Water percentage (\%)} = \frac{\text{Total (water content)}}{\text{Body (wet weight)}} \times 100$$

Calculation of ash

Heat resistant china crucibles for each sample using 500-1000 mg sub-samples were used for determining ash content by placing in a muffle furnace (RJM 1.8-10, China) for 24 hours at 450-500^{0}C and after cooling reweighted each sample. Following equation was used to calculate ash content and its percentage (%).

$$\text{Total (ash content)} = \text{initial sample weight} - \text{loss of weight (during heating)}$$

$$\text{Percentage (\%) of ash in wet mass} = \frac{\text{Total ash in fish}}{\text{Wet weight of fish}} \times 100$$

$$\text{Percentage (\%) of ash in dry mass} = \frac{\text{Total ash in fish}}{\text{Dry weight of fish}} \times 100$$

Calculation of lipid

Extraction method by using 1:2 mixture of chloroform and methanol was used to determine lipid content of dry tissue (Bligh and Dyer, 1959). 1.00g sub-sample of powdered form dry tissue was mixed into 10ml of chloroform and methanol mixture, left over night and then centrifuged after which clear supernatant was removed in pr-weighed bottles. These bottles were then put on a hot plate at 40-50 ^{0}C to evaporate the solvent to dryness leaving the lipid fraction. Lipids were then weighed on electric digital balance to the nearest 0.01 g. Following equation was used to calculate lipid content and its percentage (%).

$$\text{Total (lipid content)} = \text{bottle weight having lipid} - \text{bottle weight before adding the lipid}$$

$$\text{Percentage (\%) of lipid in wet mass} = \frac{\text{Total lipid in fish}}{\text{Wet weight of fish}} \times 100$$

$$\text{Percentage (\%) of lipid in dry mass} = \frac{\text{Total lipid in fish}}{\text{Dry weight of fish}} \times 100$$

Calculation of protein

Protein content was determined by difference from mass of other major contents as ash, lipid and water (Salam and Davies, 1994). Total amount of protein in fish can be calculated as:

$$\text{Total protein content} = \text{dry weight of sample} - (\text{ash content} + \text{fat content})$$

Data analysis

Data analysis was performed by using ANOVA to determine significant differences among three treatment groups body composition parameters.

Regression analysis was used to to analyze the impact of fish body size on different body constituents and to analyze difference among different body composition parameters in three different dietary protein feeds.

Results and Discussion

Total of 10 specimens from each treatment group (T1, T2, T3) of different sizes ranging from 9.00 to 10.00 g body weight and 7.00 to 7.90 cm total length in T1 feed, 11.50 to 12.90 gm body weight and 8.20 to 9.10 cm total length in T2 feed and 12.60 to 15.50 gm body weight and 9.00 to 11.00 cm total length were selected for whole body composition.

Table 1: *Mean values and ranges of various body composition parameters of hybrid (Labeo rohita ♀ and Catla catla ♂) n = 10 in each case.*

Body constituents	Treatment groups	Mean ± S.D	Range
Water content (%)	T1(15%) T2(20%) T3(25%)	80.55±0.94 80.83±0.54 79.22±0.54	80-82.83 80.00-81.74 78.57-80.16
Ash content (% Wet weight)	T1(15%) T2(20%) T3(25%)	2.23±0.13 2.36±0.29 2.57±0.84	2.05-2.40 1.83-2.81 1.72-4.65
Ash content (% dry weight)	T1(15%) T2(20%) T3(25%)	11.49±0.53 12.30±1.47 12.38±4.24	10.89-12.00 9.17-14.17 8.67-23.05
Fat content (% wet weight)	T1(15%) T2(20%) T3(25%)	6.63±0.97 5.85±0.55 6.28±0.75	5.08-6.67 5.40-8.03 5.45-8.10
Fat content (% dry weight)	T1(15%) T2(20%) T3(25%)	34.33±6.42 30.51±2.65 30.26±4.03	25.86-39.84 27.27-33.33 27.21-42.86
Protein contents (% wet weight)	T1(15%) T2(20%) T3(25%)	10.59±1.67 10.96±0.51 11.93±1.67	7.75-12.15 10.24-11.74 7.48-13.14
Protein contents (% dry weight)	T1(15%) T2(20%) T3(25%)	54.18±6.46 57.19±2.21 57.36±7.52	45.14-60.73 53.33-60.30 37.11-62.64
Organic content (% wet weight)	T1(15%) T2(20%) T3(25%)	17.22±0.86 16.82±0.57 18.22±1.08	15.11-17.80 16.10-18.17 15.51-19.30
Organic content (% dry weight)	T1(15%) T2(20%) T3(25%)	88.51±0.53 87.70±1.47 87.62±4.25	88.00-89.11 85.83-90.83 76.95-91.33

S.D: Standard Deviation.

Average values and ranges of many body composition parameters of hybrid (*Catla catla* ♂ × *Labeo rohita* ♀) were given in Table 1. Percentage of water content in T1 (15%), T2 (20%) and T3 (25%) feeds was 80.55±0.94, 80.83±0.54 and 79.22±0.54 respectively. Ash content (% wet weight) in T1, T2 and T3 feeds was 2.23±0.13, 2.36±0.2 and 2.57±0.84 respectively, while ash content (% dry weight) was 11.49±0.53, 12.30±1.47 and 12.38±4.24 in T1, T2 and T3 respectively. Percentage fat wet weight in T1 (15%) was 6.63±0.97, in T2 (20%) was 5.85±0.55 and in T3 (25%) was 6.28±0.75, while fat (% dry weight) in all (T1, T2, T3) feeds was 34.33±6.42, 30.51±2.65 and 30.26±4.03 respectively. Protein (% wet weight) in T1, T2 and T3 feeds was 10.59±1.67, 10.96±0.51 and 11.93±1.67 respectively, while protein (% dry weight) was 54.18±6.46 in T1 (15%) and 57.19±2.21 in T2 (20%) feed and 57.36±7.52 in T3 (25%) feed. Organic content (% wet weight) in T1 was 17.22±0.86, in T2 was 16.82±0.57 and in T3 feed was 18.22±1.08, organic content (% dry weight) was 88.51±0.53 in T1 (15%) and 87.70±1.47 in T2 (20%) feed and 87.62±4.25 in T3 (25%) feed. ANOVA analysis confirmed highly significant difference ($P<0.001$) between % water, % organic content wet weight, significant difference ($P<0.01$) was observed between % protein (wet and dry weight), % fat (wet and dry weight) and % ash wet weight, while no significant difference ($P>0.05$) was noted between % organic content dry weight and % ash dry weight values of three treatments (T1, T2, T3) groups.

Relationship between % water and various % body contents

When % water was plotted with fat wet and dry weight, T1 (15%) feed showed positively least significant and significant correlation respectively, and value of "b" exhibited positive allometric relationship in both of these (Table 2), while T2 (20%) and T3 (25%) feeds showed non-significant results in both cases (Table 2). Relationship of % water with % protein wet and dry weight confirmed that T1 (15%) feed examined highly significant and significant inverse correlation respectively and all metrically negative relationship, while T2 (20%) and T3 (25%) feeds observed non-significant results (Table 2). % water and % ash wet and dry weight analysis showed that all (T1, T2, T3) feeds had non-significant results. Analysis of % water with organic content wet weight of T1, T2, T3 feeds showed highly significant, significant and least significant inverse correlation respectively and value of "b" showed negative allometry in all feeds when b=0 while all (T1, T2, T3) feeds observed non-significant correlation when % water was plotted against organic content dry weight (Table 2).

Relationship between wet body weight and total values of water, fat, ash, protein and organic content in log transformed data

Calculation of correlation between wet body weight and total water content confirmed that all (T1, T2, T3) feeds showed highly significant positive correlation (Table 3). When b=1, all (T1, T2, T3) feeds showed negative allometry in total water content against wet body weight (Table 3). Analysis of log transformed wet body weight with log transformed total fat content revealed that T1 (15%) showed least significant correlation negatively, and also exhibited negative allomeric correlation, while T2 (20%) and T3 (25%) have no influence (non-significant correlation) (Table 3).

Table 2: *Statistical parameters of % water content versus % body composition parameters of hybrid (Labeo rohita ♀ and Catla catla ♂) (n = 10).*

Relationships		r	a	b	S. E. (b)	t value when b=0
% Water (x)	T1(15%)	0.683*	-50.22	0.7058	0.267	2.646
%Fat wet weight (y)	T2(20%)	0.363ns	35.59	-0.3679	0.334	-1.100
	T3(25%)	0.321ns	-29.61	0.4530	0.472	0.960
% Water (x)	T1(15%)	0.833**	-422.71	5.6742	1.330	4.266
%Fat dry weight (y)	T2(20%)	0.070ns	58.18	-0.3423	1.730	-0.198
	T3(25%)	0.488ns	-260.24	3.6670	2.319	1.581
% Water (x)	T1(15%)	0.913***	140.90	-1.6178	0.256	-6.325
%Protein wet weight (y)	T2(20%)	0.571ns	54.84	-0.5428	0.276	-1.966
	T3(25%)	0.564ns	151.43	-1.7609	0.912	-1.931
% Water (x)	T1(15%)	0.851**	523.64	-5.8284	1.272	-4.582
%Protein dry weight(y)	T2(20%)	0.049ns	41.06	0.1995	1.439	0.139
	T3(25%)	0.410 ns	511.51	-5.7329	4.514	-1.270
%Water (x)	T1(15%)	0.631ns	9.32	-0.0880	0.038	-2.303
%Ash wet weight (y)	T2(20%)	0.165 ns	9.57	-0.0893	0.189	-0.473
	T3(25%)	0.195ns	-21.82	0.3078	0.546	0.563
% Water (x)	T1(15%)	0.270ns	-0.93	0.1542	0.195	0.792
%Ash dry weight (y)	T2(20%)	0.053 ns	0.76	0.1428	0.959	0.149
	T3(25%)	0.261 ns	-151.27	2.0659	2.699	0.765
% Water (x)	T1(15%)	0.993***	90.68	-0.9121	0.038	-23.871
% Organic contents wet	T2(20%)	0.863**	90.43	-0.9107	0.189	-4.825
weight (y)	T3(25%)	0.646*	121.82	-1.3078	0.546	-2.394
% Water (x)	T1(15%)	0.270 ns	100.93	-0.1542	0.195	-0.792
% Organic contents dry	T2(20%)	0.053ns	99.24	-0.1428	0.959	-0.149
weight (y)	T3(25%)	0.261 ns	251.27	-2.0659	2.699	-0.765

*r: Correlation Coefficient; a: Intercept; b: slope; S.E: Standard Error; *** P<0.001; ** P<0.01; * P<0.05; n.s p > 0.005*

Table 3: *Statistical parameters of log wet body weight (g) versus total log body composition parameters (g) of hybrid (Labeo rohita ♀ and Catla catla ♂) (n = 10).*

Relationships		r	a	b	S. E. (b)	t value when b=1
Log body weight (x)	T1(15%)	0.969***	-0.02	0.9217	0.083	11.078
Log water content (y)	T2(20%)	0.982***	-0.03	0.9423	0.064	14.838
	T3(25%)	0.995***	-0.05	0.9560	0.032	29.568
Log body weight, g (x)	T1(15%)	0.754*	1.48	-1.6931	0.521	-3.251
Log fat content (y)	T2(20%)	0.619 ns	-2.25	1.9433	0.871	2.231
	T3(25%)	0.346 ns	-0.77	0.6147	0.589	1.043
Log body weight (x)	T1(15%)	0.761*	-3.23	3.2768	0.989	3.314
Log protein content (y)	T2(20%)	0.462 ns	-0.60	0.6624	0.450	1.472
	T3(25%)	0.610 ns	-1.87	1.8239	0.838	2.176
Log body weight (x)	T1(15%)	0.796**	-2.16	1.5105	0.406	3.724
Log ash content (y)	T2(20%)	0.550 ns	-2.94	2.2180	1.191	1.862
	T3(25%)	0.146 ns	-1.19	0.6293	1.509	0.417
Log body weight (x)	T1(15%)	0.766**	-1.05	1.2913	0.383	3.376
Log organic contents (y)	T2(20%)	0.764*	-0.89	1.1057	0.330	3.346
	T3(25%)	0.826**	-1.09	1.3059	0.315	4.151

*r: Correlation Coefficient; a: Intercept; b: slope; S.E: Standard Error; *** P<0.001; ** P<0.01; * P<0.05; n.s p > 0.005*

When log body weight was analyzed with log total protein, T1 (15%) feed observed least significant positive allometric correlation, while T2 (20%) observed non-significant correlation and T3 (25%) revealed allometrically negative and non-significant correlation (Table 3). Correlation between log transformed wet body weight and log transformed total ash content showed that T1 (15%) has significant allometrically positive correlation (Table 3), while T2 (20%) and T3 (25%) has non-significant correlation

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(Table 3). Analysis of results between log wet body weight and log total organic content revealed that T1 (15%) and T3 (25%) showed positive significant correlation, while T2 (20%) observed least significant positive correlation (Table 3). All (T1, T2, T3) feeds exhibited positive allometry in log transformed total organic content against wet body weight in all (T1, T2, T3) feeds (Table 3).

Relationship between total length and total values of water, fat, ash, protein and organic content in log transformed data

Analysis of correlation between log transformed total length and total value of log transformed water revealed that T1 (15%) observed least significant positive correlation and exhibited negative allometry when b=3, while, T2 (20%) and T3 (25%) feeds showed non-significant correlation (Table 4). Analysis of log transformed total length with log transformed total fat content revealed that T1 (15%) showed negatively allometric and non-significant correlation, while T2 (20%) have no influence (non-significant correlation) and T3 (25%) showed highly significant positive relation but allometrically negative correlation (Table 4). When log total length was analyzed with log total protein, all (T1, T2, T3) feeds showed non-significant correlation (Table 4). Correlation between log transformed total length and log transformed total ash content showed that T1 (15%) and T2 (20%) has non-significant correlation while, T3 (25%) showed least significant and allometrically negative correlation (Table 4). Analysis of results between log total length and log total organic content revealed that all (T1, T2, T3) feeds showed non-significant correlation (Table 4).

Protein is the most expensive and most important content in fish feed that should be supplied in right amount to support good health and minimum cost (Zehra and Khan, 2011). For better understanding of nutritional requirement, preparation of dietary feeds is a first step. Current study was also conducted to understand dietary requirement of hybrid of *Catla catla* and *Labeo rohita* and its impact on body composition parameters. Overall mean values of all body composition parameters (water, fat, protein, ash, organic content) in all (T1, T2, T3) feeds analyzed in whole wet body weight of hybrid are similar to the findings of many investigators (Naeem and Salam, 2010; Naeem et al., 2011a; Daudpota et al., 2016; Khalid and Naeem, 2018) but contrary to the investigation of many researchers (Salam et al., 2000; Mohamed, 2009; Mohamed et al., 2014; Naeem et al., 2017), values of present study are more or less when compared to above mentioned researchers.

In the present study % protein level increased when dietary protein level was increased from 15% to 25% similar to study of Kim et al. (2003), Tidwell et al. (2005), Ahmad et al. (2012) and Mohamed et al., 2014. Similar to the findings of Daudpota et al. (2014), protein is increasing and lipid level is decreasing in dry matter of fish in the present study with increasing dietary protein levels, while ash content is increasing with increasing dietary protein levels (Daudpotta et al., 2014). In the present study inverse relationship was found between water content and fat.

Results of regression analysis revealed that % water has significant inverse relation with % protein, % ash and % organic content in T1 (15%) feed similar to the findings of many investigators (Salam et al., 1991; Salam and Davies, 1994; Naeem and Salam, 2010; Naeem and Ishtiaq, 2011; Naeem et al., 2017), while T2 (20%) and T3 (25%) feeds showed non-significant results similar to Naeem et al. (2011a) and Naeem et al. (2011b). All feeds (T1, T2, T3) exhibited highly significant correlation between log body weight and log water content similar to the findings of Naeem and Salam (2010) in bighead carp; Naeem et al. (2011a) in hybrid; Naeem and Ishtiaq (2011) in *Mystus bleekri*. The relationship between log transformed data of total length and total water content showed that water content in hybrid fed with feed T1 increased least significantly against total length. This observation coincides with the findings of Naeem and Salam (2010) and Naeem et al. (2011a), however T2 and T3 fed fish showed non-significant correlation contrary to the above mentioned researchers.

In the present study, fat content showed decline with increasing dietary protein level similar to the observations of many researchers such as in sea bass (Ballestrazzi et al., 1994) in tilapia (Daudpota et al., 2014) in grass carp (Dabrowski, 1977) and in guppy (Fah and Leng, 1986). More or less an inverse relationship between fat and water content was observed in present study similar to the findings of Daudpota et al. (2014), which seem to maintain fish tissue volume. Fat and water are alternate for each other, as with increasing fat water content is decreasing (Shimma, 1986).

Table 4: *Statistical parameters of log total length (cm) versus log total body composition parameters (g) of hybrid (Labeo rohita ♀ and Catla catla ♂) (n = 10).*

Relationships		r	a	b	S. E. (b)	t value when b=3
Log total length (x)	T1(15%)	0.728*	0.66	0.2725	0.091	3.000
Log water content (y)	T2(20%)	0.594 ns	0.45	0.5693	0.273	2.087
	T3(25%)	0.578 ns	0.579	0.4588	0.229	2.006
Log total length (x)	T1(15%)	0.389 ns	0.10	-0.3435	0.288	-1.194
Log fat content (y)	T2(20%)	0.399 ns	-1.34	1.2509	1.017	1.230
	T3(25%)	0.873***	-1.35	1.2807	0.253	5.056
Log total length (x)	T1(15%)	0.359 ns	-0.52	0.6089	0.560	1.088
Log protein content (y)	T2(20%)	0.241 ns	-0.21	0.3463	0.492	0.704
	T3(25%)	0.225 ns	0.77	-0.5553	0.851	-0.652
Log total length (x)	T1(15%)	0.443 ns	-0.95	0.3311	0.237	1.399
Log ash content (y)	T2(20%)	0.036 ns	-0.42	-0.1453	1.424	-0.102
	T3(25%)	0.649*	-2.78	2.3136	0.958	2.415
Log total length (x)	T1(15%)	0.363 ns	0.01	0.2410	0.218	1.103
Log organic contents (y)	T2(20%)	0.460 ns	-0.32	0.6660	0.454	1.466
	T3(25%)	0.121 ns	0.24	0.1585	0.4581	0.346

*r: Correlation Coefficient; a: Intercept; b: slope; S.E: Standard Error; *** P<0.001; ** P<0.01; * P<0.05; n.s p > 0.005*

Present study showed a decrease in lipid content with increasing dietary protein level and fish size similar to the study of Hussain et al. (2016) on *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* but many studies have reported opposite trend that fish size has no influence on fat and protein deposition but other factors like season, breeding period, metabolism and feeding show impact on lipid deposition (Shulman et al., 2005; Moss et al., 2009).

Feeding frequency did not affect protein deposition in fish body (Hung and Lutes, 1987; Cho et al., 2003). In the present study protein content in wet body weight (T1=10.59, T2=10.96, T3=11.93) was increasing with increasing dietary protein levels similar to the findings of many researchers (Mazumder et al., 2008; Begum et al., 2012) but contrary to the findings of Soltan et al. (2002).

There may be some other factors which can cause variation in protein content as age and size of fish and seasonal variation (Levesque et al., 2002). Size of fish has a great influence on protein content as small sized fish has lower protein content than large sized fishes (Ashraf et al., 2011). Dietary protein level for fry must be high and decreased with increase in fish size and age (Wilson, 1989; Pillay, 1990; El-Sayed and Teshima,1991).

With increasing dietary protein levels, ash content is also increasing in present study similar to the findings of Daudpotaet al. (2014) on Nile tilapia and Naeem et al. (2017) on *Cirrhinus mrigala*.

In present study, when examined the influence of body size (wet body weight and total length) on total body constituents, it was observed that all feeds (T1, T2, T3) showed inverse correlation between body size and total fat, however observed positive correlation with total protein, total ash and total organic content. These observations are in line with those of Naeem and Salam (2010) in bighead carp, Naeem et al. (2011a) in hybrid, Naeem and Ishtiaq (2011) in *Mystus bleekri* and Naeem et al. (2017) on *Cirrhinus mrigala*. Variations in body composition parameters in different sized fish may be due to different factors like feeding, habitat or sampling season (Naeem et al., 2011a). This difference in body constituents could be due to the difference in capacity of absorption and assimilation of nutrients from the water and diet, by fish (Fawole et al., 2007).

Value of slope "b" indicated isometric condition when compared it with b=1, in log transformed values of wet body weight against total water, fat, protein, ash and organic content. It was noted that more or less all feeds (T1, T2, T3) showed positive allometry (value greater than 1) in total protein, ash and organic content, these are increasing with wet body weight. While total water and total fat exhibited negative allometry (value less than 1) showing decrease with increasing wet body weight. Although, negative allometry (b<3) was found between log transformed data of total length and all the body constituents.

Conclusions and Recommendations

From present study, dietary protein levels requirement and basic information about body composition of hybrid (*Labeo rohita* ♀ and *Catla catla* ♂) is provided. Major body composition parameters percentages were similar to many important commercial fishes of Pakistan. Present study concluded a linear increase in protein content with increasing dietary protein level, with a decrease trend in lipid content, So T3 (25%) fed fishes showed higher protein content and less lipid content than T1 (15%) and T2 (20%) fed fishes. Present study also confirmed that different dietary protein levels and body size influenced body composition parameters. Finally, it is concluded that 25% dietary protein level is best for good body composition in hybrid fish (*L. rohita* ♀ and *C. catla* ♂).

Novelty Statement

This study will be helpful to propagate the culture of hybrid fish (*Labeo rohita* ♀ and *Catla catla* ♂). Data will also be useful for fish feeding industries and other researcher working on the fish feeding.

Author's Contribution

Rabia Iqbal conducted her research experiment, collected the data, did statistical analysis and wrote the paper. This manuscript is part of her Ph. D research work. Muhammad Naeem supervisor has provided guideline to complete research experiment and resources for completion of research and also helped in reviewing the manuscript. Samrah Masud helped in data analysis and writing of the manuscript. Abir Ishtiaq helped in statistical analysis and reviewing of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ahmad, M., T.A, Qureshi, A.B. Singh, M. Susan, B. Kamlesh and R.C. Salman. 2012. Effect of dietary protein, lipid and carbohydrate contents on growth, feed efficiency and carcass composition of *Cyprinus carpio* communis fingerlings. Int. J. Fish. Aquacult. 4(3): 30-40.

Akhtar, N., 2001. Strategic planning for inland fisheries and aquaculture sector in Pakistan. Proc. Nat. Semin. Strateg. Plan. Fish. Aquacult. Face Challenges new Millennium. Karachi - Pakistan.

Al-Ghanim, K.A. 2016. Effect of different storage temperatures on chemical composition and sensory attributes of the flesh of Cyprinus carpio and Clarias gariepinus. Pakistan J. Zool. 48: 305-310.

Ashraf, M., A. Zafar and M. Naeem. 2011. Comparative studies on the seasonal variations in the nutritional values of three carnivorous fish species. Int. J. Agric. Biol. 13: 701-706.

Ayisi, C.L., J. Zhao and E.J. Rupia. 2017. Growth performance, feed utilization, body and fatty acid composition of Nile tilapia (*Oreochromis niloticus*) fed diets containing elevated levels of palm oil. Aquacult. Fish. 2(2): 67-77. https://doi.org/10.1016/j.aaf.2017.02.001

Ballestrazzi, R., D. Lanari, E. D. Agaro and A. Mion, 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrarchus labrax*). Aquacult. 127: 197–206. https://doi.org/10.1016/0044-8486(94)90426-X

Basavaraju, Y., K.V. Devaraj and S.P. Ayyar. 1995. Comparative growth of reciprocal carp hybrids between *Catlacatla*and *Labeo rohita* in contrast to intraspecific data collected on *D. melano-fimbriatus*. Aquaculture. 129: 187-191. https://doi.org/10.1016/0044-8486(94)00246-K

Begum, M., T. Akter and M.H. Minar. 2012. Analysis of the proximate composition of domesticated stock of pangas (*Pangasianodon hypophthalmus in* n laboratory condition. J. Environ. Sci. N. Resour. 5 (1): 69-74. https://doi.org/10.3329/jesnr.v5i1.11555

Bhowmick, R.M., R.K. Janaa, S.D. Gupta, G.V. Kowtala and M. Rout. 1981. Studies on some aspects of biology and morphometery of the intergeneric hybrid, *Catla catla* (Hamilton)× *Labeo rohita* (Hamilton) produced by hypophysation. Aquaculture. 23: 367-371. https://doi.org/10.1016/0044-8486(81)90030-2

Blake, R.W., S.D. Inglis and K.H.S. Chan. 2006. Growth, carcass composition and plasma growth hormone levels in cyclically fed rainbow trout. J. Fish Biol. 69: 807-817. https://doi.org/10.1111/j.1095-8649.2006.01150.x

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917. https://doi.org/10.1139/y59-099

Bureau, D.P., S.J. Kaushik and C.Y. Cho. 2002. Bioenergetics. In: Halver, J.E., Hardy, R.W. (eds). Fish Nutrition, 3rd edition. Acad. Press: USA, pp. 2-54.

Chaudhuri, H., 1959. Experiments on hybridization of Indian carps. Proc. Indian Sci. Cong. 45(4): 20.

Cho, S.H., Y.S. Lim, J.H. Lee, J.K. Lee and S. Park. 2003. Effects of feeding rate and feeding frequency on survival, growth, and body composition of post-larvae *Plecoglossusaltivelis*. J. World Aquacult. Soc. 34: 85–91. https://doi.org/10.1111/j.1749-7345.2003.tb00042.x

Dabrowski, K., 1977. Protein requirments of grass carp fry (*Ctenopharyngodon idella* Val.). Aquacult. 12: 663–673. https://doi.org/10.1016/0044-8486(77)90047-3

Daudpota, A.M., G. Abbas, I.B. Kalhoro, S.S.A. Shah, H. Kalhoro, M. Hafeez-ur-Rehman and A. Ghaffar. 2016. Effect of feeding frequency on growth performance, feed utilization and body composition of juvenile Nile Tilapia, *Oreochromisniloticus*(L.) Reared in Low Salinity Water. Pakistan. J. Zool. 48(1): 171-177.

Daudpota, A.M., P.J.A. Siddiqui, G. Abbas, N.T. Narejo, S.S.A. Shah, N. Khan and G. Dastagir. 2014. Effect of dietary protein level on growth performance, protein utilization and body composition of Nile tilapia cultured in low salinity water. Int. J. Interdiscip. Multidiscip. Stud. 2(2): 135-147.

Dempson, I.B., C.J. Schwarz, M. Shears and G. Furey. 2004. Comparative proximate body composition of Atlantic salmon with emphasis on parr from fluvial and lacustrine habitats. J. Fish Biol. 64: 1257-1271. https://doi.org/10.1111/j.0022-1112.2004.00389.x

Drazen, J.C., 2007. Depth related trends in proximate composition of demersal fishes in the eastern North Pacific. Deep-see Res. I. 54: 203-219. https://doi.org/10.1016/j.dsr.2006.10.007

El-Sayed, A.F.M. and S. Teshima. 1991. Tilapia nutrition in aquaculture. Rev. Aquat. Sci. 5: 247-265.

Fah, S.K. and C.Y. Leng. 1986. Some studies on the protein requirement of the guppy, *Poecilia retculata* (peters). J. Aquacult. Aquat. Sci. 44: 1–12.

Fawole, O.O., M.A. Ogundiran, T.A. Ayandiran and O.F. Olagunju. 2007. Proximate and Mineral Composition in Some Selected Fresh Water Fishes in Nigeria, Internet J. Food Saf. 9: 52-55.

Foran, J.A., D.O. Carpenter, M.C. Hamilton, B.A. Knuth and J. Schwager. 2005. Riskbased consumption advice for farmed Atlantic and wild pacific salmon contaminated with dioxins anddioxinlike compounds. Environ. Hlth. Perspect. 33: 552-556. https://doi.org/10.1289/ehp.7626

Hung, S.S.O. and P.B. Lutes. 1987. Optimum feeding rate of hatchery-product juvenile white sturgeon (*Acipensertransmontanus*) at 20°C. Aquaculture. 65: 307–317. https://doi.org/10.1016/0044-8486(87)90243-2

Hussain, M.Z., A.N. Asiya, W.A. Shahzadah, L. Abdul, S. Hussain, R. Iqbal and M. Ali. 2016. Effect of Feeding Habit, Size and Season on Proximate Composition of Commercially Important Fishes from Lentic Water Bodies of Indus River at Ghazi Ghat, Pakistan. Pak. J. Zool. 48(6): 1877-1884.

Jakhar, J.K., A.K. Pal, A.D. Reddy, N.P. Sahu, G. Venkateshwarlu and H.K. Vardia. 2012. Fatty acid composition of some selected Indian fishes. Afr. J. Basic App. Sci. 4(5): 155-160.

Jeyasanta, K.I. and J. Patterson. 2014. Nutritive evaluation of trash fishes in Tuticorin (India). World J. Fish Mar. Sci. 6(3): 275-288.

Khalid, M. and M. Naeem. 2018. Proximate analysis of grass carp (*Ctenopharyngodon idella*) from Southern Punjab, Pakistan. Sarhad J. Agric. 34(3): 632-639. https://doi.org/10.17582/journal.sja/2018/34.3.632.639

Khan, H.A., S.D. Gupta, P.V.G. Reddy and S.K. Sahoo. 1986. Use of milk, urea, sodium sulphite and human urine for degumming fertilized eggs of common carp, *Cyprinuccarpio* L., Aquact. Hung. (Szarvas). 5: 47–54.

Kim, K., X. Wang and S.C. Bai. 2003. Reevaluation of the dietary protein requirement of Japanese flounder (*Parlichthysolivaceus*). J. World. Aquacult. Soc. 34(2): 133-139. https://doi.org/10.1111/j.1749-7345.2003.tb00049.x

Levesque, H.M., T.W. Moon, P.G.C. Campbelland A. Hontela. 2002. Seasonal variation in carbohydrate and lipidmetabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. Aquat. Toxic. 60: 257-267. https://doi.org/10.1016/S0166-445X(02)00012-7

Li, P. and X. Wang. 2004. Nutritional value of fisheries by-catch and byproduct meals in the diet of red drum *(Sciaenops ocellatus).* Aquacult. 236: 485–496. https://doi.org/10.1016/j.aquaculture.2004.02.010

Martinez-Palacios, C., M.G. Ríos-Durán, L. Ambriz-Cervantes, K.J. Jauncey and L.G. Ross. 2007. Dietary protein requirement of juvenile Mexican Silverside (*Menidiaestor* Jordan, 1879), a stomachlesszooplanktophagus fish. Aquacult. Nutr. 13: 304-310. https://doi.org/10.1111/j.1365-2095.2007.00479.x

Mazumder, M.S.A., M.M. Rahman, A.T.A. Ahmed, M. Begum and M.A. Hossain. 2008. Proximate composition of some small indigenous fish species (SIS) in Bangladesh. Int. J. Sustain. Crop. Prod. 3 (4): 18-23.

Meyer, G., and D.M. Fracalossi. 2005. Estimation of Jundià (*Rhamdiaquelen*) dietary amino acid requirements based on muscle amino composition. Sci. Agric. 62(4): 401-405. https://doi.org/10.1590/S0103-90162005000400015

Mohamed, H.B., 2009. Effect of dietery protein levels on growth performance and body composition of monosexnile tilapia, *Oreochromus niloticus* L. reared in fertilized tanks. Pak. J. Nutr. 8(5): 674-678. https://doi.org/10.3923/pjn.2009.674.678

Mohamed, H.E., R. Al-Maqbaly and H.M. Mansour. 2014. Proximate composition, amino acid and mineral contents of five commercial Nile fishes in Sudan. Afr. J. Food. Sci. 4: 640–654.

Moss, J.H., E.V. Farley, A.M. Feldman and J.N. Ianelli. 2009. Spatial distribution, energetic status, and food habits of eastern Bering Sea age-0 walleye pollock. Trans. Am. Fish Soc. 138: 497-505. https://doi.org/10.1577/T08-126.1

Naeem, M., A. Rasul, A. Salam, S. Iqbal, A. Ishtiaq and M. Athar. 2011b. Proximate analysis of female population of wild featherback fish (*Notopterus notopterus*) in relation to body size and condition factor. Afr. J. Biotechnol. 10(19): 3867-3871.

Naeem, M. and A. Salam. 2010. Proximate composition of fresh water bighead carp, *Aristichthys nobilis*, in relation to body size and condition factor from Islamabad, Pakistan. Afr. J. Biotechnol. 9: 8687-8692.

Naeem, M., A. Salam, M.A. Bashir, A. Ishtiaq, Q. Gillani and A. Salam. 2011a. Effect of body size and condition factor on whole body composition of hybrid (*Catla catla* ♂ × *Labeo rohita* ♀) from Pakistan. Int. J. Bioeng. Life. Sci. 5 (11): 823-826.

Naeem, M., A. Salam, R. Baby, A. Ishtiaq and S.A. Rasool. 2011. Study of body composition of female population of farmed *Oreochromis mossambicus*in relation to body size and condition factor from Pakistan. Proc. 2011 International Conference on Bioscience, Biochemistry and Bioinformatics (ICBBB 2011). pp. 360-363.

Naeem, M. and I. Abir. 2011. Proximate composition of *Mystusbleekeri*in relation to body size and condition factor from Nala Daik, Sialkot, Pakistan. Afr. J. Biotech. 10(52): 10765-10763. https://doi.org/10.5897/AJB10.2339

Naeem, M., I. Abir and N. Saba. 2017. Influence of dietary protein on proximate composition of Mori, *Cirrhinus mrigala (*Hamilton). Matrix Sci. Pharm. 1(2): 1-5. https://doi.org/10.26480/msp.02.2017.01.05

Natarajan, A.V., V.R. Desai and D.N. Mishra. 1976. On the account of inter-generic Catla-Rohu Hybrid in Rih and (Uttar Pradesh) with an account of its potential role in reservoir fisheries development in India. J. Inland Fish Soc. India. 8: 83-90.

Ndome, C., O. Oriakpono and O. Agnes, 2010. Proximate composition and nutritional value of some commonly consumed fishes in Calabar. J. Trop. Freshw. Biol. 19: 11-18.

NRC, 1983. Nutrient requirements of warm water fishes and shellfishes, Washington, DC, USA, National Academic Press, Washington, DC, USA. pp. 102.

Oriakpono, O., N. Frank–Peterside and C. Ndome. 2011. Microbiological assessment of stored Tilapia guineensis. Afr. J. Food Sci. 5: 242-247.

Pillay, T.V.R., 1990. Aquaculture: Principles and practices. Fishing news book. Blackwell Sci. Publ., Ltd., Oxford, UK. pp. 575.

Reddy, P., Konda and T.J. Varghese. 1980. On the taxonomic characters of two major carp hybrids, catla - rohuand rohu – catla. Proc. Indian Acad. Sci. (Anim. Sci.). 89: 419–429. https://doi.org/10.1007/BF03179128

Salam, A., M. Ali and S. Masud. 2000. Effect of various food deprivation regimes on body composition dynamics of thaila, *Catla catla.* Journalof Research (Science) Bahauddin Zakariya Univ., Multan, Pakistan. 11(1): 26-32.

Salam, A., M.N. Khan and M. Naeem. 1991. Effect of body size and condition factor on body composition of an exotic fish, *Tilapia nilotica.* Proc. Pak. Cong. Zool. 11: 191-200.

Salam, A. and P.M.C, Davies. 1994. Body composition of Northern Pike (*Esoxlucius* L.) in relation to body size and condition factor. J. Fish Res. 19: 193-204. https://doi.org/10.1016/0165-7836(94)90038-8

Salam, A. and P.M.C. Davies. 1997. Energetics of body composition of gold fish, *Carassius auratus* L. in relation to body size and condition factor. Acta Sci. 7: 1-6.

Shimma, H., 1986. Growth and proximate composition of two races, Yamato and Mirror of carp *Cyprinuscarpio*. Bull. Nat. Res. Inst. Aquacult. 9: 15–20.

Shulman, G.E., V.N. Nikolsky, T.V. Yuneva, G.S. Minyuk, V.Y. Shchepkin, A.M. Shchepkina, E.V. Ivleva, O.A. Yunev, I.S. Dobrovolov, F. Bingel and A.E. Kideys. 2005. Fat content in Black Sea sprat as an indicator of fish food supply and ecosystem condition. Mar. Ecol. Prog. Ser. 293: 201-212. https://doi.org/10.3354/meps293201

Sogard, S.M. and M.L. Spencer. 2004. Energy allocation in juvenile sablefish: effects of temperature ration and body size. J. Fish Biol. 64: 726-738. https://doi.org/10.1111/j.1095-8649.2004.00342.x

Soltan, M.A., A.A. Radwan and I.M. Samra. 2002. Effect of varying protein, energy and protein to energy ratio on growth, feed efficiency and body composition of nile tilapia, *Oreochromis niloticus* by the 1st annual conference of the egyptian aquaculture society, Al-Aresh, North Sinai, Egypt.

Soltan, M.A. and A.A. Tharwat. 2006. Use of fish silage for partial or complete replacement of fish meal in diets of Nile tilapia, *Oreochromis niloticus* and African catfish, *Clarias gariepinus*. Egypt J. Nutr. Feeds. 9(2): 299-314.

Suseno, S.H., A.Y. Tajul, W.A. Nadiah, Hamidah, Asti and S. Ali. 2010. Proximate, fatty acid and mineral composition of selected deep sea fish species from southern java ocean and western Sumatra ocean, Indonesia. Int. Food Res. J. 17: 905-914.

Tidwell, J.H., S.D. Coyle, L.A. Bright and D. Yasharian. 2005. Evaluation of plant and animal source proteins for replacement of fish meal in practical diets for the largemouth bass, *Micropterus salmoides*. J. World. Aquacult. Soc. 36: 454-463. https://doi.org/10.1111/j.1749-7345.2005.tb00393.x

Tsironi, T. and P.S. Taoukis. 2017. Effect of storage temperature and osmotic pre-treatment with alternative solutes on the shelf-life of gilthead seabream (*Sparus aurata*) fillets. Aquacul. Fish. 2(1): 39-47. http://dx.doi.org/10.1016/j.aaf.2016.10.003

Turkmen, A., T. Aro, T. Nurmi and H. Kallio. 2005. Heavy metals in three commercially valuable fish species from Iskenderun Bay. Northern east Mediterranean Sea, Turkey. Food Chem. 91: 167-172. https://doi.org/10.1016/j.foodchem.2004.08.008

Wilson, R.P., 1989. Protein and amino acid requirements of fishes. *In*: S. Shiau (ed.). Prog. Fish Nutr., Nat. Taiwan Ocean Univ., Keelung, Taiwan. pp. 51-76.

Zehra, S. and M.A. Khan. 2011. Dietary protein requirement for fingerling *Channa punctatus* (Bloch), based on growth, feed conversion, protein retention and biochemical composition. Aquacult. Int. 20(2): 383-395. https://doi.org/10.1007/s10499-011-9470-8

Zuanon, J.A., A.L. Salaro, S.S. Moraes, L.M. Alves, E.M. Balbino and E.S. Araujo. 2009. Dietary protein and energy requirements of juvenile freshwater angle fish. R. Bras. Zootec. 38(6): 989-993. https://doi.org/10.1590/S1516-35982009000600003