



Effect of Dietary Protein Levels on Body Composition of *Catla catla* from Pakistan

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**Abstract:** Samples of major carp, *Catla catla*, were collected for the analyses of proximate body composition which were fed 15% crude protein (CP) in treatment-1 (T1), 20% CP in treatment-2 (T2), 25% CP in treatment-3 (T3) and 30% CP in treatment-4 (T4), reared under polyculture system in earthen ponds with a stocking density of 2000 fish/acre. Moisture, ash, fat, protein, organic contents and fat free dry mass were measured in fish samples of the different treatments. In the wet weight of the *Catla catla*, mean values of moisture, ash, fat and protein contents were ranged 72.93%-81.88%, 3.26%-6.03%, 3.35%-6.83% and 13.52%-15.94, respectively, in different treatments. Results also revealed a definite effect of varying dietary protein levels on the proximate body composition of *Catla catla*. Moisture and protein contents in wet weight of the fish body were found significantly higher ( $p < 0.05$ ) in the fish samples fed with 25% (T3) and 30% CP (T4) than those provided 15% (T1) and 20% CP in artificial fish feed (T2). However, percentage ash and fat contents in wet body weight of *C. catla* were not found significantly different ( $p > 0.05$ ) in different treatments. Results for regression analyses showed strong positive correlation ( $p < 0.001$ ) of percent moisture content with percent ash content while negative correlation with protein and fat percentage, in wet body weight of *Catla catla*. Negative correlation was found in %moisture and %ash content, while %fat and %protein were positively correlated with fish size (weight and length) in wet body weight for all treatments. The baseline data generated on nutritional composition of this carp would be of use for both the consumers as well as fish processors.

**Keywords:** Major carp, Artificial fish feed, Fish body contents, Fish size, Condition factor, Regression model equations

1. **INTRODUCTION**

Fish receives great consideration as a potential source of various essential nutrients and animal protein for human diets (Fawole *et al.*, 2007). In Asian and other developing countries, fish adds a valuable amount of total animal protein (Iqbal *et al.*, 2014). Along with nutritive capabilities, fish protein is also considered vital source of income for the people of these countries. Thus, fish farming is expanding in developing as well as developed countries (Louka *et al.*, 2004).

Major carp are among the fish fauna that dominates South Asia and are the main species of fish that is cultivated in both the public and private sectors.

The cyprinid species, *Catla catla* (Thailand), non-predatory major carp, is the most imperative member of existing fish farming system in Pakistan and India, because of acquiescence to rear in different ecosystems and consumer preference (Ayyappan and Jena, 1998).

Proximate body composition of fish has been of interest in health and nutrition studies (Tobin *et al.*, 2006) due to increased interest in the safety and quality of fish products (Dumas *et al.*, 2010). Information on body composition is vital for maximal utilization of fish and its products (Silva and Chamul, 2000). It is also often required to confirm that fish flesh meet the requirements of commercial specifications and food regulations (Sutharshiny and Sivashanthini, 2011). Moreover, fish body

composition is of unlimited attention in fisheries and aquaculture sector because it affects growth, appetite and food utilization efficiency of fish (Breck, 2014). It also influences on other traits of ecology and fish biology, including survival, reproduction and energy value to predators. Energy density of fish can also be estimated from the body composition. The relationships among body size, water and other body contents of the fish can also be valuable in studying different aspects of fish growth and bioenergetics (Breck, 2014). Naeem *et al.* (2011), Yousaf *et al.* (2011), Iqbal *et al.* (2014) and Khalid and Naeem (2018) have earlier studied the body contents of different fish species from the rivers, reservoir and farms of Pakistan and stated that freshwater fishes are high quality source of protein. Thus, it is imperative to determine the percentage of different body contents of fish and to define some very strong patterns and very general in body composition with fish size and condition factor. This may also help in demonstrating how body composition can be assessed by taking mass of water contents (Naeem and Ishtiaq, 2011).

Normally, body composition of whole fish consists of moisture, protein and fat percentages as 70-80%, 20-30% and 2-12%, respectively (Love, 1980). Proximate composition of fish rely on various factors including season, habitat, sex of the fish and food availability (Brett *et al.*, 1969; Oliveira *et al.*, 2003).

Intensification of fish production has led to dependence on artificial feeds. Although, protein is considered the most expensive constituent in fish

feeds but it is also the imperative aspect affecting feed cost and growth performance of fish (Luo *et al.*, 2004). Dietary protein levels significantly effects survival, growth, yield (Siddiqui and Khan, 2009) and body composition of fish (Parveen *et al.*, 2012).

Hence, the objective of this work was to study the effect of varying dietary protein levels (15%, 20%, 25%, and 30% crude protein) on proximate body composition of *Catlacatla* and to determine higher protein content percentage by feeding the diet containing minimum crude protein percentage, reared under polyculture system, from southern Punjab, Pakistan.

## 2. MATERIALS AND METHODS

Four different fish feeds, comprising 15%, 20%, 25% and 30% crude protein (CP), were formulated by using locally available fish feed ingredients like Canola Meal, Corn Gluten Meal 30% and 60%, Dicalcium Phosphate, Fishmeal, Rice Polish, Sarson (Mustard Seed) Meal, Soybean Meal, Soybean Oil, Sunflower Meal, Vitamin Premixes and Wheat Bran. Feed ingredients were ground and mixed to homogenized, and pelleted.

Young *Catlacatla* were procured from a public sector hatchery and were reared in earthen ponds of southern Punjab, Pakistan, after acclimatization for one week by feeding diet containing 30% CP, under polyculture system with a stocking density of 2000 fish/acre for 180 days, during July to December, 2014. At the start of feeding trial, mean±SE total length and body weight of fish was recorded as 11.12±1.64 cm and 44.63±16.77g, respectively. Fish were fed with diets comprising 15% CP in treatment-1 (T1), 20% CP in treatment-2 (T2), 25% CP in treatment-3 (T3) and 30% CP in treatment-4 (T4), in triplicate, at the rate of 4% of body weight of the fish. Water quality parameters were monitored and kept in suitable ranges for the fish growth, during the feeding trial. Water depth was maintained upto 1.5 meter in each pond throughout the feeding trial.

At the end of feeding trial, 30 samples from each treatment, and a total of 120 samples of *Catlacatla* were collected with the help of drag net, for analyses of body composition.

Collected fish samples were anaesthetized using MS222 (Sandoz). After blotting dry, fish specimens were measured for total length (TL) and weighed to the nearest 0.1 cm and 0.01 g, respectively, and condition factor was also calculated (Weatherley and Gill, 1987). Moisture content was determined by oven drying method. Each fish sample was kept into the oven (Incucell, MMM Medcenter Einrichtungen GmbH, MMM-Group) by placing in the aluminum foil tray till constant weight. Dry matter of each fish specimen was finely ground and homogenized for further analyses.

Total ash content of the fish was determined by burning dry powder of sample at 550°C for 24 h in muffle furnace (RJM-1.8-10A). Fat content was extracted in chloroform and methanol mixture (ratio 1:2 v/v) following the method of Bligh and Dyer (1959), and Salam and Davies (1994). In this process, weighted powder of each fish sample was taken in test tube, mixed with solution of above solvents, stirred and then covered with aluminum foil. It was left over night and then centrifuged. The clear supernatant of each sample was removed carefully into pre-weighed small glass bottles and placed in oven that evaporated the solvent to dryness and leaving the lipid fraction. Protein contents of the fish were assessed by difference from weight of other leading contents *i.e.*, moisture, ash and fat, following Weatherley and Gill (1987), Salam and Davies (1994) and Naeem and Ishtiaq (2011). Organic contents were calculated ultimately, by difference from ash content.

Percentages of various body contents obtained were statistically analyzed by using One-way analysis of variance (ANOVA) to determine the effects of dietary crude protein level on the body composition of the fish. Differences among treatment means were determined by Duncan's Multiple Range Test at a  $p < 0.05$  level of significance. Statistical analyses were performed by using Mini Tab and SPSS (IBM, version 23). Correlation coefficients for regression analyses were considered significant at  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.05$  (Zar, 1996). Variance inflation factor was also calculated for multiple regression analyses.

## 3. RESULTS

Total length (TL), wet body weight (WW) and condition factor (K) of *Catlacatla* from different treatments are given in **Table 1**. While, various constituents in percentage (%) of wet (WW) and dry weight (DW) for *C. catla* from different treatments are provided in **Table 2**.

### 3.1 Moisture Content in *Catlacatla*

Overall moisture content in all four groups ranged from 72.93% to 81.88% of wet body weight of *Catlacatla*. Mean (±SE) values of moisture content in the wet body weight of *C. catla* was found 76.73% (±0.35), 77.05% (±0.37), 74.82% (±0.14) in T1, T2 and T3, while 75.12% (±0.17) in T4, respectively, when reared under polyculture system (**Table 2**).

Results for analysis of variance (ANOVA) for moisture in percentage (%) of WW and DW in different treatments for *C. catla* indicated that moisture content contents in whole fish body was affected by dietary protein level. Results of Duncan's multiple range test for percentage moisture content in the body of *C. catla* indicated that mean moisture content (%) in T1 and T2 was found significantly higher than T3 and T4 group in the body of *C. catla*.

### 3.2 Ash Contents in *Catla*

Percentage ash content (WW) of *C. catla* was not found significantly different ( $p>0.05$ ) among treatments for *C. catla*. Ash content (DW) of *C. catla*

in T1 (15%CP) was found significantly highest ( $p<0.05$ ) and was found significantly lowest in the fish reared in T3 (25%CP).

**Table 1: Descriptive statistics of size and condition factor for farmed *Catla***

	Total Length (cm)		Wet Body Weight (g)		Condition Factor	
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE
Treatment 1(15%CP)	35.00 - 41.90	38.57 $\pm$ 0.36	560.55 - 1003.94	775.60 $\pm$ 20.71	1.29 - 1.41	1.34 $\pm$ 0.01
Treatment 2(20%CP)	35.00 - 41.70	38.82 $\pm$ 0.41	553.48 - 999.04	794.76 $\pm$ 24.40	1.27 - 1.42	1.35 $\pm$ 0.01
Treatment 3(25%CP)	37.10 - 42.00	40.48 $\pm$ 0.32	681.75 - 1105.77	911.92 $\pm$ 23.42	1.28 - 1.50	1.37 $\pm$ 0.01
Treatment 4 (30%CP)	36.80-42.00	40.28 $\pm$ 0.32	675.31 - 1091.90	901.48 $\pm$ 23.00	1.28 - 1.51	1.37 $\pm$ 0.06

**Table 2: Mean values ( $\pm$  Standard Error) and ranges of various constituents in percentage (%) of wet weight (WW) and dry weight (DW) for *C. catla*.**

Constituents	Treatment 1 (15%CP)		Treatment 2 (20%CP)		Treatment 3 (25%CP)		Treatment 4 (30%CP)		
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	
Moisture	74.44-81.88	76.73 $\pm$ 0.35 <sup>a</sup>	73.79-81.34	77.05 $\pm$ 0.37 <sup>a</sup>	73.63-76.53	74.82 $\pm$ 0.14 <sup>b</sup>	72.93-76.56	75.12 $\pm$ 0.17 <sup>b</sup>	
Ash	% WW	3.32-6.03	4.51 $\pm$ 0.13 <sup>a</sup>	3.26-5.97	4.25 $\pm$ 0.15 <sup>a</sup>	3.26-5.57	4.15 $\pm$ 0.13 <sup>a</sup>	3.32-5.52	4.18 $\pm$ 0.12 <sup>a</sup>
	% DW	13.04-33.28	19.70 $\pm$ 0.87 <sup>a</sup>	12.97-32.01	18.98 $\pm$ 0.99 <sup>ab</sup>	12.42-23.73	16.58 $\pm$ 0.60 <sup>c</sup>	13.18-23.19	16.88 $\pm$ 0.50 <sup>bc</sup>
Fat	% WW	4.20-5.89	5.12 $\pm$ 0.09 <sup>a</sup>	3.66-6.83	5.18 $\pm$ 0.17 <sup>a</sup>	4.13-5.90	5.10 $\pm$ 0.10 <sup>a</sup>	3.35-6.06	5.18 $\pm$ 0.13 <sup>a</sup>
	% DW	19.51-25.99	22.02 $\pm$ 0.29 <sup>a</sup>	18.39-26.97	22.44 $\pm$ 0.48 <sup>a</sup>	17.37-22.39	20.20 $\pm$ 0.31 <sup>b</sup>	14.27-24.08	20.75 $\pm$ 0.44 <sup>b</sup>
Protein	% WW	7.71-16.27	13.65 $\pm$ 0.39 <sup>b</sup>	9.02-16.30	13.52 $\pm$ 0.37 <sup>b</sup>	13.77-17.26	15.94 $\pm$ 0.17 <sup>a</sup>	13.22-17.47	15.53 $\pm$ 0.18 <sup>a</sup>
	% DW	42.55-63.88	58.27 $\pm$ 0.89 <sup>b</sup>	47.57-63.94	58.58 $\pm$ 0.77 <sup>b</sup>	58.69-65.90	63.22 $\pm$ 0.33 <sup>a</sup>	54.79-68.17	62.36 $\pm$ 0.46 <sup>a</sup>
Organic content	% WW	12.09-22.14	18.76 $\pm$ 0.45 <sup>b</sup>	12.69-22.76	18.70 $\pm$ 0.51 <sup>b</sup>	17.90-23.04	21.03 $\pm$ 0.26 <sup>a</sup>	18.30-23.50	20.70 $\pm$ 0.25 <sup>a</sup>
	% DW	66.72-86.96	80.30 $\pm$ 0.87 <sup>c</sup>	67.99-87.03	81.02 $\pm$ 0.99 <sup>bc</sup>	76.28-87.58	83.42 $\pm$ 0.60 <sup>a</sup>	76.81-86.82	83.12 $\pm$ 0.55 <sup>ab</sup>
Fat free dry mass	% WW	13.74-20.57	18.16 $\pm$ 0.30 <sup>b</sup>	14.82-19.87	17.77 $\pm$ 0.25 <sup>b</sup>	19.34-20.54	20.09 $\pm$ 0.06 <sup>a</sup>	18.47-21.04	19.71 $\pm$ 0.12 <sup>a</sup>
	% DW	74.01-80.49	77.98 $\pm$ 0.29 <sup>b</sup>	73.03-81.61	77.56 $\pm$ 0.48 <sup>b</sup>	77.61-82.63	79.80 $\pm$ 0.31 <sup>a</sup>	75.92-85.73	79.25 $\pm$ 0.44 <sup>a</sup>

Mean values sharing same superscript are not significantly different ( $p>0.05$ )

### 3.3 Fat Contents in *Catla*

Percentage fat content in wet weight of *C. catla* was not significantly affected ( $p>0.05$ ), when fed with various levels of protein diets (15%, 20%, 25% and 30% CP). However, fish fed 15% and 20% protein diets had significantly higher ( $p<0.05$ ) fat content (DW) of *C. catla* than those fed higher protein diets (25% and 30%).

### 3.4 Protein Contents in *Catla*

Fish fed a fish feed containing 15% crude protein (CP) in T1 and 20% CP in T2 had significantly lower ( $p<0.05$ ) body protein than those fed higher protein diets, 25% CP in T3 and 30% in T4. Significant difference was also indicated for protein content (DW) of *C. catla* reared in different treatments. Lower protein content percentage (DW) of *C. catla* was found in the fish samples collected from T1 and T2 than T3 and T4.

### 3.5 Organic Content in *Catla*

Percentage organic content (wet body weight) of *Catla* fed 15% (T1) and 20% (T2) protein in artificial feed was found significantly lower than those fed 25% (T3) and 30% (T4) crude protein in artificial fish feed. *C. catla* reared in T1 had significantly lowest organic content (dry weight), while highest in the fish reared in T3 (25%CP).

### 3.6 Fat Free Dry Mass in *Catla*

Results of ANOVA and Duncan tests indicated that fat free dry mass (WW, DW) of *C. catla* in T3 and T4 were significantly higher than that of T1 and T2.

### 3.7 Correlation between Moisture Content and Various Body Constituents

Regression analyses and statistical parameters of percentage moisture(%M) content versus percentage (%) body constituents (WW, DW) of *C. catla*, in treatments 1 (T1), treatments 2 (T2), treatments 3 (T3) and treatment 4 (T4), respectively, are represented in (Table 3).

Strong correlation ( $p<0.001$ ) were found in all relationships between percent moisture and different body constituents with correlation coefficient (r) value range 0.605 to 0.992, except for % fat that was found significantly correlated ( $p<0.01$ ) with % moisture content in T2 and T4, while % protein and fat contents (DW) of *C. catla* were found insignificantly correlated in T1 (15% CP) and T4 (30% CP). All the regression analyses of studied treatments showed positive correlation in the relationships between % moisture and % ash content (WW, DW) with slope (b) value range 0.262 to 4.185.

While all other body constituents (fat and protein contents) percentage were observed negative correlation with percentage of moisture content in farmed *C. catla* (Table 3).

### Effect of Dietary Protein Levels on Body Composition of *C. catla*: Correlation between Body Size and Percentage of Body Constituents

Regression equations were also developed to analyze the relationship of WW and TL with percentage of each body constituent, and the different regression parameters are presented in (Table 4) and (Table 5), respectively. WW and TL of *C. catla* were found strongly correlated ( $p<0.001$ ) with all studied body constituents (%) of the fish in all treatments,

however, %fat (DW) was significantly correlated ( $p < 0.01$ ) with WW ( $r = 0.523$ ) and TL ( $r = 0.500$ ) in

**Table 3: Regression analyses and statistical parameters of percentage moisture (%M) content versus % body constituents in wet (WW) and dry body weight (DW) of *C. catla* reared under polyculture system in different treatments.**

Equation	Treatment	r	a	b	SE of b	t-Stat
%A(WW)=a + b %M	T1	0.681***	-15.569	0.262	0.053	4.920
	T2	0.897***	-23.288	0.357	0.033	10.710
	T3	0.956***	-61.065	0.872	0.050	17.308
	T4	0.605***	-27.502	0.422	0.105	4.019
%A(DW)=a + b %M	T1	0.866***	-145.317	2.151	0.234	9.174
	T2	0.945***	-175.463	2.524	0.166	15.238
	T3	0.966***	-296.545	4.185	0.210	19.885
	T4	0.719***	-160.662	2.364	0.432	5.467
%F (WW)=a + b %M	T1	0.678***	17.805	-0.165	0.034	-4.883
	T2	0.826***	34.020	-0.374	0.048	-7.769
	T3	0.935***	56.313	-0.685	0.049	-13.951
	T4	0.724***	47.450	-0.563	0.101	-5.559
%F (DW)=a + b %M	T1	0.330 <sup>ns</sup>	1.029	0.274	0.148	1.852
	T2	0.514**	73.888	-0.668	0.211	-3.170
	T3	0.882***	165.381	-1.941	0.196	-9.884
	T4	0.547**	129.624	-1.449	0.419	-3.462
% P(WW)=a + b %M	T1	0.990***	97.763	-1.096	0.030	-36.825
	T2	0.981***	89.268	-0.983	0.037	-26.659
	T3	0.987***	104.752	-1.187	0.036	-32.832
	T4	0.807***	80.051	-0.859	0.119	-7.221
% P(DW)=a + b %M	T1	0.956***	244.287	-2.424	0.141	-17.210
	T2	0.890***	201.575	-1.856	0.180	-10.325
	T3	0.942***	231.165	-2.245	0.151	-14.870
	T4	0.332 <sup>ns</sup>	131.037	-0.914	0.491	-1.861

%M=Percentage of Moisture; A=Ash; F=Fat; P=Protein; WW=wet weight; DW=dry weight;  
r=Correlation coefficient; a=Intercept; b=Slope; S.E=Standard Error; \*\*\*=  $p < 0.001$ ; \*\* =  $p < 0.01$ ; <sup>ns</sup> > 0.05

**Table 4: Regression analyses of wet body weight (W, g) of fish versus percentage (%) of body constituents in wet weight (WW) and dry weight (DW) of *C. catla* reared under polyculture system in different treatments.**

Equation	Treatment	r	a	b	S. E. (b)	t value when b=0
%M =a + b WW	T1	0.672***	85.546	-0.011	0.002	-4.797
	T2	0.899***	87.885	-0.014	0.001	-10.875
	T3	0.962***	80.042	-0.006	0.0003	-18.734
	T4	0.592***	78.977	-0.004	0.001	-3.889
%A (WW)=a + b WW	T1	0.988***	9.493	-0.006	0.0002	-33.954
	T2	0.987***	8.997	-0.006	0.0002	-32.461
	T3	0.992***	9.062	-0.005	0.0001	-41.853
	T4	0.965***	8.570	-0.005	0.0002	-19.519
%A (DW)=a + b WW	T1	0.930***	50.027	-0.039	0.003	-13.433
	T2	0.966***	50.081	-0.039	0.002	-19.842
	T3	0.989***	39.849	-0.026	0.001	-36.218
	T4	0.951***	37.278	-0.023	0.001	-16.281
%F (WW)=a + b WW	T1	0.922***	2.163	0.004	0.0003	12.609
	T2	0.934***	0.078	0.006	0.0005	13.844
	T3	0.973***	1.229	0.004	0.0002	22.423
	T4	0.599***	2.140	0.003	0.001	3.962
%F (DW)=a + b WW	T1	0.375 <sup>ns</sup>	17.940	0.005	0.002	2.144
	T2	0.752***	10.658	0.015	0.002	6.046
	T3	0.946***	8.892	0.012	0.001	15.452
	T4	0.523**	11.724	0.010	0.003	3.249
%P (WW)=a + b WW	T1	0.746***	2.798	0.014	0.002	5.932
	T2	0.868***	3.040	0.013	0.001	9.234
	T3	0.960***	9.667	0.007	0.0004	18.091
	T4	0.751***	10.313	0.006	0.001	6.016
%P (DW)=a + b WW	T1	0.788***	32.033	0.034	0.005	6.776
	T2	0.769***	39.261	0.024	0.004	6.364
	T3	0.925***	51.259	0.013	0.001	12.845
	T4	0.633***	50.998	0.013	0.003	4.321

A. ISHTIAQ, M. NAEEM

**Table 5: Regression analyses of total length (TL, cm) versus % body constituents of *C. catla*, reared under polyculture system in different treatments.**

Equation	Treatment	r	a	b	S. E.(b)	t value whenb=0
%M =a + b TL	T1	0.665***	101.820	-0.651	0.138	-4.706
	T2	0.883***	108.024	-0.798	0.080	-9.962
	T3	0.903***	90.831	-0.396	0.036	-11.109
	T4	0.546**	86.675	-0.287	0.083	-3.444
%A (WW)=a + b TL	T1	0.985***	18.805	-0.371	0.012	-30.502
	T2	0.978***	17.938	-0.352	0.014	-25.069
	T3	0.974***	19.908	-0.389	0.017	-22.946
	T4	0.940***	18.066	-0.345	0.024	-14.522
%A (DW)=a + b TL	T1	0.928***	106.689	-2.255	0.171	-13.169
	T2	0.957***	108.681	-2.311	0.132	-17.542
	T3	0.971***	91.213	-1.844	0.085	-21.672
	T4	0.924***	81.269	-1.599	0.125	-12.765
% F (WW)=a + b TL	T1	0.941***	-3.550	0.225	0.015	14.709
	T2	0.912***	-9.314	0.373	0.032	11.774
	T3	0.945***	-7.176	0.303	0.020	15.303
	T4	0.566**	-4.142	0.231	0.064	3.633
% F (DW)=a + b TL	T1	0.410*	9.190	0.333	0.140	2.381
	T2	0.739***	-11.251	0.868	0.149	5.810
	T3	0.935***	-16.316	0.902	0.065	13.972
	T4	0.500**	-7.301	0.697	0.228	3.056
% P (WW)=a + b TL	T1	0.735***	-17.075	0.797	0.139	5.730
	T2	0.858***	-16.649	0.777	0.088	8.845
	T3	0.914***	-3.563	0.482	0.040	11.923
	T4	0.715***	-0.598	0.400	0.074	5.405
% P (DW)=a + b TL	T1	0.774***	-15.879	1.923	0.297	6.475
	T2	0.766***	2.570	1.443	0.229	6.302
	T3	0.902***	25.102	0.942	0.085	11.048
	T4	0.622***	26.032	0.902	0.215	4.205

%M=Percentage of Moisture; A=Ash; F=Fat; P=Protein; WW= Wet Weight; DW= Dry Weight; TL= Total Length; \*\*\*=  $p < 0.001$ ; \*\* =  $p < 0.01$ ; \* =  $p < 0.05$

**Table 6: Multiple regression relationships among percentages (%) of body constituents (Moisture, Ash, Fat, Protein) in wet body weight (WW, g), wet body weight (WW, g), total length (TL, cm) and condition factor (K) for *C. catla*.**

Equations	Treatment	r	a	b <sub>1</sub> ±S.E	b <sub>2</sub> ±S.E	VIF
%M=a+b <sub>1</sub> TL+b <sub>2</sub> WW	T1	0.411*	89.02	-0.137±0.737	-0.009±0.013	1.825
	T2	0.795***	89.257	-0.053±0.377	-0.013±0.006	5.236
	T3	0.923***	78.184	0.065±0.074	-0.007±0.001	13.889
	T4	0.305 <sup>ns</sup>	76.795	0.076±0.253	-0.005±0.003	1.546
%A=a+b <sub>1</sub> TL+b <sub>2</sub> WW	T1	0.981***	13.513	-0.158±0.050	-0.004±0.0008	58.824
	T2	0.976***	11.675	-0.104±0.052	-0.004±0.0009	43.478
	T3	0.994***	12.704	-0.127±0.020	-0.004±0.0002	142.857
	T4	0.933***	11.184	-0.091±0.055	-0.003±0.0007	16.129
%F=a+b <sub>1</sub> TL+b <sub>2</sub> WW	T1	0.877***	-3.852	0.237±0.082	-0.0002±0.001	8.772
	T2	0.863***	0.806	-0.028±0.139	0.007±0.002	7.874
	T3	0.947***	-0.569	0.063±0.045	0.003±0.0006	20.408
	T4	0.312 <sup>ns</sup>	2.290	-0.005±0.195	0.003±0.002	1.560
%P=a + b <sub>1</sub> TL+b <sub>2</sub> WW	T1	0.524**	1.32	0.058±0.734	0.013±0.013	2.257
	T2	0.736***	-1.74	0.186±0.428	0.010±0.007	4.082
	T3	0.915***	9.680	-0.0004±0.093	0.007±0.001	12.658
	T4	0.532**	9.731	0.020±0.220	0.005±0.003	2.294
%M=a + b <sub>1</sub> WW+b <sub>2</sub> K	T1	0.414*	89.62	-0.011±0.002	-2.990±7.849	1.832
	T2	0.794***	88.483	-0.013±0.001	-0.454±3.801	5.236
	T3	0.924***	81.034	-0.006±0.0004	-0.857±0.842	14.085
	T4	0.304 <sup>ns</sup>	79.585	-0.004±0.001	-0.525±2.787	1.543
%A=a + b <sub>1</sub> WW+b <sub>2</sub> K	T1	0.976***	8.421	-0.006±0.0001	0.788±0.610	45.455
	T2	0.972***	8.679	-0.006±0.0002	0.241±0.556	0.026
	T3	0.991***	7.571	-0.006±0.0001	1.288±0.262	0.008
	T4	0.932***	7.494	-0.005±0.0002	0.929±0.605	0.063
%F=a + b <sub>1</sub> WW+b <sub>2</sub> K	T1	0.875***	5.490	0.004±0.0003	-2.444±0.885	0.117
	T2	0.864***	0.769	0.006±0.0004	-0.523±1.402	0.127
	T3	0.947***	2.048	0.004±0.0002	-0.708±0.512	0.049
	T4	0.312 <sup>ns</sup>	2.386	0.003±0.001	-0.212±2.152	0.641
Effect of Dietary Protein Levels on Body Composition	T1	0.530**	-3.53	0.014±0.002	4.646±7.781	0.437
	T2	0.735***	2.068	0.013±0.001	0.737±4.328	0.247
	T3	0.916***	9.347	0.007±0.0004	0.277±1.064	0.079
	T4	0.532**	10.536	0.006±0.001	-0.192±2.434	0.436

T4 (CP-30) and non-significantly correlated ( $p > 0.05$ ;  $r = 0.410$ ) with TL of *C. catla* in T1 which was fed an  $r = 0.375$ ) with WW while least significant ( $p < 0.05$ ; artificial fish feed containing 15% crude protein level.

Furthermore, %fat (WW) was found significant ( $p < 0.01$ ;  $r = 0.566$ ) in T4 which was fed 30% CP level in artificial fish feed. % Moisture and ash (in both WW and DW) showed negative correlation while %fat and protein contents (WW, DW) displayed positive correlations with an increase in size of the *C. catla*.

### 3.9 Multiple Regression Analyses (MRA)

Table 6 shows results multiple regression analyses (MRA) among percentages (%) of body constituents (moisture, ash, fat and protein) in wet weight (WW, g) total length (TL, cm) and condition factor (K) for *C. catla*, fed with 15% (T1), 20% (T2), 25% (T3) and 30% (T4) crude protein in artificial fish feed.

Percentages of moisture (%M), ash (%A), fat (%F) and protein (%P) contents in wet weight (WW) of *Catlacatla* were observed strongly correlated ( $p < 0.001$ ) with multiple correlation coefficient ( $r$ ) value ranged from 0.736 to 0.994 in all treatments (T1-T4), except for %P which was found significantly correlated in T1 ( $r = 0.524$ ) and T4 ( $r = 0.532$ ), % M which was found least significantly correlated ( $p < 0.05$ ) in T1 ( $r = 0.411$ ), and %M and %F which showed insignificant correlations ( $p > 0.05$ ) with total length (TL) and wet weight (WW) of *Catlacatla*.

Similarly, %M, %A, %F and %P (WW) of *Catlacatla* were found highly correlated ( $p < 0.001$ ) with correlation coefficient ranged from 0.735 to 0.991 in all treatments (T1-T4), except for %P which was found significantly correlated in T1 ( $r = 0.530$ ) and T4 ( $r = 0.532$ ), %M which was found least significantly correlated ( $p < 0.05$ ) in T1 ( $r = 0.414$ ), while % M and % F which showed insignificant correlations ( $p > 0.05$ ) with wet weight and condition factor (K) of *C. catla*.

Variance Inflation Factor was also calculated for multiple regression analyses. %M, %F and %P showed high multicollinearity ( $VIF > 10$ ) with TL and WW of *C. catla* in T1, T2 and T4. While except %M in T3 and %A in T1, all other analyses of treatments for multiple correlation of percent body constituents (%M, %A, %F and %P) with wet weight and condition factor showed high multicollinearity ( $VIF > 10$ ) with variance inflation factor values ranged from 0.026 to 5.236.

## 4. DISCUSSION

Many researcher have studied the effect of different protein levels on growth performance of different carp species, for example, Dars *et al.* (2010) have carried out experiments to evaluate the effect of different diets containing 30%, 35% and 40% CP on the growth and survival of *Catlacatla* in glass aquaria and stated that diet comprising 35% CP can be

appropriate for the better growth and production of *Catlacatla*. There are also many data in the literature for proximate body composition of different carps when feeding diets containing various levels of protein in tanks, indoor cemented ponds and glass aquaria (Singh *et al.*, 2006; Siddiqui and Khan, 2009; Jiang *et al.*, 2015 Guy *et al.*, 2018), however references concerning the proximate body composition of *Catlacatla* when feeding diets containing various levels of protein and reared under polyculture system in earthen ponds, are limited and insufficient. These results revealed that proximate body composition was definitely effected by various crude protein level in fish feed. The body composition of grow-out fish under experimental feeds provided in T1 and T2 (15% and 20% crude protein, respectively) showed significant differences ( $p \leq 0.05$ ) with experimental feeds given to the fish in T3 and T4 (25% and 30% crude protein, respectively).

Different body contents, examined in the whole body of farmed *Catlacatla* from different treatments, fed with artificial diet comprising 15% CP, 20% CP, 25% CP and 30% CP in T1, T2, T3 and T4, have comparable values to those reported by Khan *et al.* (2012) and Pradhan *et al.* (2014) for the whole body composition of different fish species from wild of farming system.

The percentage of moisture is a good indicator of its protein and lipid (Yeannes and Almandos, 2003). Moisture content of *Catlacatla* in all the four treatments was found within the acceptable level (70-80%) as documented by Adewumi *et al.* (2014). Moisture content in *Catlacatla* agreed with observation of Zehra and Khan (2013) and Hasan *et al.* (2015), who have reported moisture content 75.27-78.15% by feeding diets (33%CP) containing varying levels of dietary arginine and 72.53-76.65%, collected from local retail markets, wholesale markets and rearing ponds, respectively, in the body of the same species.

Fat content in fish muscles determines the quality of fish meat (Love, 1980). From the results obtained, the mean lipid contents in different treatments (5.10%-5.18%) for *Catlacatla* indicated medium fat fish. As, Ackman (1989) categorized fish into lean (< 2 %), low fat (2 to 4 %), medium fat (4 to 8%), and high fat fish (> 8%) according to their fat content.

Khan *et al.* (2012) reported 5.4% fat content in *Catlacatla* under monoculture system by feeding 35% protein in diet, while Zehra and Khan (2013) stated 4.97% fat content in the fingerlings *Catlacatla*. These observations are in conformity with the results of the present study. However, on the other hand, Khan *et al.* (2012) also documented 3.0% fat content in *C. catla* under polyculture system by feeding 35% protein in diet. It is further inferred that dietary

protein levels in categorically affects the fat content of *Catlacatla*.

According to Ahmed and Maqbool (2017), body composition of fish is affected by the dietary protein levels. However, Jiang *et al.* (2015) evaluated the effects of dietary protein and lipid levels on body compositions of hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀) juveniles and found that dietary protein levels had no significant effect on body contents of this fish. In the present study, fish fed with 25% CP in T3 and 30% CP in T4 had significantly higher crude protein content than fish fed with 15% CP in T1 and 20% CP in T2. The higher protein content in the body of *Catlacatla* in T3 and T4 might be due to the consumption of fish feed with higher crude protein. Mean percent protein was found 13.65±0.39, 13.52±0.37, 15.94±0.17 and 15.53±0.18 in WW of *C. catla* in T1, T2 and T3 and T4, respectively. On the other hand, Zehra and Khan (2013) reported protein percentage 15.43% in the carcass of fingerling *Catlacatla* by feeding 33% crude protein level in its diet. While Khan *et al.* (2012) have reported 11.9% and 13.9% protein in the whole body of this species when fed with 35% protein diet reared under poly- and monoculture system, respectively. Ahmed and Maqbool (2017) studied proximate composition of *Cyprinus carpio specularis*, by feeding diets with different dietary protein levels (25%-50% CP) and reported moisture, ash, fat and protein contents as 77.49%, 3.16%, 4.81% and 13.15% by feeding 25% dietary protein and 75.66%, 2.90%, 5.41% and 14.27% by feeding 30% dietary protein, respectively. These observations are in conformity with the results of the present study. On the other hand, some researches contradict from these findings. As Hasan *et al.* (2015) have reported a higher protein content (19.54%), while Khan *et al.* (2012) reported a lower protein percentage (11.9%) in the body of *Catlacatla*. Guyet *et al.* (2018) Have found 72.4%, 9.9%, 8.7% and 15.4% moisture, ash, fat and protein contents, respectively, in Black Buffalo (*Ictiobus niger*) by feeding 30% protein levels in feed. Moreover, Khalid and Naeem (2018) have reported lower protein content (11.53±4.18%) in the body of *Ctenopharyngodonidella*. The reason of this variation might be certain factors such as season, spawning effects or food availability, as reported by Abdullahiet *al.* (2001). Regardless of the difference, protein values in various treatments indicate that *C. catla* is a good source of protein for consumers.

Mineral content of food item is measured as ash content. The range of ash in the present study proposes that *Catlacatla* is also a good source of minerals even fed with low protein diet (15 and 20% crude protein).

Predictive regression equations were established in the present work, as various authors (Salam and Davies, 1994; Yeannes and Almandos, 2003; Breck, 2014; Naeem *et al.*, 2016; Khalid and Naeem, 2018) have reported precision by adopting these equations. Result of regression models to estimate various body contents (%) as a function of moisture content (%) for *Catlacatla*, when fed with 15%, 20%, 25%, 30% crude protein in different treatments reared under polyculture system, indicated strong correlation ( $p < 0.001$ ) in all relationships between percent moisture and different body constituents ( $r = 0.605$  to  $0.992$ ), except for % fat that was found significantly correlated ( $p < 0.01$ ) with % moisture content in T2 and T4, while % protein and fat contents (DW) of *C. catla* were found insignificantly correlated in T1 (15% CP) and T4 (30% CP). Yeannes and Almandos (2003) also estimated percentage of ash, fat and protein contents as a function of percentage of moisture content by regression equations and reported significant correlation between percent moisture and body contents (ash, fat and protein).

Moreover, proximate composition of fish can be estimated from moisture content with the help of predictive regression equations. Hence, evaluating moisture content allows to predict other body contents (ash, fat and protein), with the corresponding reduction of costs when performing one, instead of many different analyses. These observations made in various studies (Yeannes and Almandos, 2003; Naeem *et al.*, 2016) are in conformity with the findings of the present work.

Significant correlation between the fish size and body constituents in the present study also revealed that the body constituents value can be predicted by just knowing fish size, without scarifying the fish, as also reported by Khalid and Naeem (2018). Furthermore, the findings of the present work showed strong relationship between moisture and protein content, indicated that the amount of moisture per unit protein decrease in larger fish. Similar regressions also noted by Naeem and Salam (2010) and Naeem and Ishtiaq (2011) in *Aristichthys nobilis* and *Mystus bleekeri*, respectively. Strength of this correlation and its incidence in various fish species indicate the biochemical or physiological source, as revealed by Breck (2014). Similarly, strong correlation was observed between moisture and ash content percentage, with the moisture content per unit ash decreasing in larger fish, also reported by Naeem and Salam (2010) and Breck (2014). Hence, calculated moisture content can be used to predict other body contents of the fish.

Present study indicated strong inverse correlation between fat and moisture content in *Catlacatla*, revealed that fat contents decreases in the fish body with the increase in moisture content. Evidence to

support this is available in other studies (Naeem and Salam, 2010; Naeem and Ishtiaq, 2011) showing similar results. Pradhan *et al.* (2014) reported biochemical composition of liver and muscle of *Catlacatla* and reported strong significant ( $p \leq 0.01$ ) inverse correlation of moisture with percent protein and lipid.

In fact, an increase in lipid and decrease in moisture content is attributed with a good condition. However, moisture content increases during spawning and non-feeding conditions due to consumption of lipid and protein for metabolic activities (Pradhan *et al.*, 2014). On the other hand, some researches (Naeem *et al.*, 2011; Breck, 2014) contradict from these findings and reported virtually no significant relationship between moisture and fat contents in fish body.

Variations in the body composition of fish, especially lipid content, are related with differences in fish growth (Cui and Wootton, 1988), appetite (Jobling and Miglavs, 1993), reproduction (Thorpe *et al.*, 1998), and survival rate (Sogard and Olla, 2000). A study on *Catlacatla* has also revealed that body conditions and biochemical composition varied significantly among sex and season (Pradhan *et al.*, 2014).

All the studied multiple regressions analyses (MRA) indicated the presence of statistically significant relationship of WW, TL and K with percentage ash (%A) and protein (%P) content, while percent moisture (%M) and fat (%F) content were found insignificant with WW and K. Thus, in the present work MRA makes an argument that there is a strong impact of WW, TL and K on %A and %P, also reported by Naeem and Ishtiaq (2011). Moreover, VIF value  $> 10$  in the present study indicating high multicollinearity and confirms the reliable and precise estimates.

## 5. CONCLUSION

From the results of the present work, it is concluded that dietary protein levels have significant effect on the proximate composition of *Catlacatla*. Higher protein content in the body of *C. catla* can be attained by feeding a diet containing 25% and 30% crude protein in polyculture system. The high percentage protein in the fish could be attributed to the fact that they are good sources of protein for human consumption as well as other predators. As the results shown that body composition of *C. catla* of treatments having 25% CP and 30% CP were significantly similar, hence feed containing 25% CP is recommended for feeding the *C. catla* to obtain high protein contents in this fish species. Moreover, predictive regression equations in the present study demonstrate that body size significantly influence the proximate body composition of *C. catla*. However,

body composition of *C. catla* does not evidently influence by the condition factor.

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