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**EFFECT OF DIFFERENT DIETARY PROTEIN LEVELS ON PROXIMATE
COMPOSITION OF *LABEO CALBASU* FROM PAKISTAN**

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ABSTRACT

Proximate body composition analysis implies percent composition of moisture, lipids and protein. The aim of the study of to evaluate the effect of the various levels of dietary protein on the body composition of *Labeo calbasu*. Fish were fed with 15%, 20% and 25% crude protein in diet in treatment 1 (T1), treatment 2 (T2) and treatment 3 (T3), respectively. At the end of feeding trial, a total of 120 samples of *Labeo calbasu*, 40 belonging to each of the three dietary protein treatments were evaluated for proximate body composition. Mean water, ash, fat and protein in wet weigh of *L. calbasu* were ranged 76.59-77.19%, 3.63-4.68%, 4.08-4.86% and 13.87-15.66%, respectively, in different treatments. Ash and fat percentages were found highest in T1, water parentage was found highest in T2, while mean protein content percentage value was found highest in T3 (25% crude protein). All the body constituents were found to be highly correlated ($P < 0.001$) with the fish size (length and weight) in T1, T2 and T3, except for ash contents in T2 ($r = 0.472$) and T3 ($r = 0.412$) showing correlation at $P < 0.01$. Slope (b value) indicated positive correlations in all the relationships between fish size and different constituents. No studies on *L. calbasu* have yet attempted to evaluate the effect of dietary protein levels on body composition and to relate the size of the fish with the parameters of whole body composition. Thus, this study will provide the first reference from Pakistan.

Key words: Carp, *Labeo calbasu*, dietary protein, Fat, Ash, Protein contents, Regression analysis, Fish size

INTRODUCTION

Fish culturing has been increased to solve industry in aquaculture not only provide problem of food shortage. Fisheries food resources but also increased chances

of livelihood and income for increasing population [1].

Water, protein, ash and lipids are the significant segments of palatable portion of fish. Proximate body composition implies the examination of protein, water, fat and ash remains of fish. The protein is the best source in fish [2].

In live fish, water is the major constituent while protein makes second important constituent of fish body. Lower percentage of water in fish may result higher level of the protein and fats and thus more noteworthy the vitality estimation of the fish [3].

The data of body composition of fish is critical for nutritionists who are keen on low fat and high protein sustenance. This data is additionally vital for researchers who are chipping away at forming these nourishments into astounding sustenance which may have high protein. This also guarantees the fine meat quality and flavour [4]. However, proximate composition may vary with size [5, 6], season [7] and dietary protein levels [8].

Labeo calbasu is an herbivorous fish and commercially important species often cultured in Pakistan, South Western China, Bangladesh, Thailand and India [1, 9].

The present work was aimed to study the effect of different dietary protein levels (15%, 20% and 25%) on the proximate composition and its relation to body size of

an economically important fish species, *Labeo calbasu*, from Pakistan.

MATERIALS AND METHODS

Total 120 specimens of *Labeo calbasu* were taken for analysis of body composition, 40 samples from each of the three treatments i.e., T1, T2 and T3 in which fish were fed with 15%, 20% and 25% dietary protein levels, respectively. The live fish were anesthetized by MS222. Weight and total length (TL) of fish was noted.

Fish samples were dried in electric oven at 70°C till constant weight. Dried samples were weighted to calculate the water contents in the fish. The dry samples were then grinded to make powder. The dry powder sample of each fish was kept in plastic bottles with proper labelling, for further analyses.

For ash analyses in fish samples, 1g powder sample was taken in preweighed heat resistant crucibles, which were subjected to 500°C in muffle furnace for 24 hr, after which these were reweighted to calculate the ash contents in *L. calbasu*.

To calculate lipids, methodology of Bligh and Dyer [10] was applied, by using a mixture of chloroform and methanol in a 1:2 ratio. For this purpose, 1g sample powder of *L. calbasu* was taken in test tube and 10 ml solution was added in it. It was remained undisturbed for whole night and then subjected to centrifugation.

Supernatant was shifted in preweighed glass bottles and kept in oven till dryness and constant weight. Glass bottles were reweighed to determine the lipid contents.

Protein contents in *L. calbasu* samples were assessed by subtracting the weight of other major contents (ash, water, fat) from total weight of fish, following [5,6].

Statistical analysis including regression evaluations were carried out by using computer programmes, MS-Excel and MiniTab.

RESULTS

A total of 120 samples of *Labeo calbasu*, 40 belonging to each of the three treatments T1, T2 and T3 (T1 with 15% dietary protein level, T2 with 20% dietary protein level and T3 with 25% dietary protein level), were evaluated for proximate body composition. Total length was measured to 11.89 ± 1.79 cm, weight was found 22.59 ± 9.98 g, while condition factor was recorded as 1.27 ± 0.17 .

Water content in *Labeo calbasu* were found highest in treatment 2 (T2), in which fish were fed with 20% dietary protein, followed by treatment 1 (T1, 15% dietary protein) and treatment 3 (T3, 25% dietary protein). The highest mean values of ash and fat contents were found in T1, while these were the lowest in T3, for both wet and dry body weight of *L. calbasu*. The results shows that ash and fat content values decrease with an increase in dietary

protein level from 15% to 25%. Mean value of protein content was found the highest in T3, followed by T2 and T1, for wet and dry body weight of *L. calbasu*, indicating the increase in protein contents by feeding the fish with higher dietary protein level (Table 1).

Ash and fat percentages were found highest in T1, water parentage was found highest in T2, while mean protein content percentage value was found highest in T3 (25% crude protein) (Table 2).

All the body constituents were found to be highly correlated ($P < 0.001$; $r = 0.643$ to 0.998) with weight of *L. calbasu* in T1, T2 and T3, except for ash contents in T2 ($r = 0.472$) and T3 ($r = 0.412$) showing correlation at $P < 0.01$. Slope (b value) indicated positive correlations. B-value also indicated isometry (b being close to 1.00) for water contents in T1 and fat in T2 with weight of *L. calbasu*. Results showed positive allometry ($b > 3$) in the relationships between protein contents and body weight of *L. calbasu*, from all the treatments. However, ash content in all treatments, water in T2 and T3, and fat in T1 and T3 showed negative allometry with increase in the fish body weight (Table 3).

TL of *L. calbasu* represented significant positive correlation. Protein content in T2 and T3 represented positive allometry ($b > 3$), while water and ash contents showed negative allometry with TL (Table 4).

Results of Table 5 showed that all the body constituents remained constant with condition factor in all treatments, except for

water in T2, and fat and ash in T1, which showed significant (P<0.05) relationships with the condition factor in *L. calbasu*.

Table 1: Percent constituents of *L. calbasu* from different treatments

Body constituents	T1		T2		T3	
	Mean ± S.D	Range	Mean ± S.D	Range	Mean ± S.D	Range
Water content (%)	76.59 ±2.77	68.59-81.21	77.19±2.40	72.73-82.03	76.64±2.64	72.36-83.16
Ash content (%Wet weight)	4.68 ±1.82	2.15-8.18	3.72±1.52	2.01-7.73	3.63±1.50	2.01-7.60
Ash content (%dry weight)	19.82±6.62	8.59-33.28	16.44±6.53	8.07-30.22	15.71±6.59	7.97-31.47
Fat content (%wet weight)	4.86±1.53	2.19-7.75	4.40±4.40	2.16-7.60	4.08±1.14	2.15-6.43
Fat content (% dry weight)	20.72 ±5.93	8.58-32.71	19.31±6.30	9.62-31.25	17.60±5.05	8.78-27.22
Protein contents (%wet weight)	13.87±2.35	11.16-20.11	14.68±2.59	11.12-20.41	15.66±3.36	10.67-20.49
Protein contents (%dry weight)	59.45±8.55	45.49-79.55	64.25±8.27	43.75-79.18	66.69±10.03	44.00-81.47

Table 2: Regression of % water against % body constituents of *L. calbasu*

	Treatment	value of "r"	value of "a"	value of "b"	value of "S.E of b"	value of "t"
% Water (x) %Fat wet weight (y)	T1	0.396*	21.639	-0.219	0.082	-0.219
	T2	0.309 ^{ns}	19.206	-0.192	0.096	-0.192
	T3	0.129 ^{ns}	8.345	-0.056	0.070	-0.056
% Water (x) %Fat dry weight (y)	T1	0.232 ^{ns}	62.210	-0.553	0.377	-0.553
	T2	0.002 ^{ns}	19.636	-0.004	0.425	-0.004
	T3	0.275 ^{ns}	-22.653	0.525	0.298	0.525
% Water (x) %Protein wet weight (y)	T1	0.513***	47.269	-0.436	0.118	-0.436
	T2	0.675***	70.896	-0.728	0.129	-0.728
	T3	0.737***	87.508	-0.938	0.140	-0.938
% Water (x) %Protein dry weight(y)	T1	0.205 ^{ns}	10.992	0.633	0.490	0.633
	T2	0.127 ^{ns}	98.053	-0.438	0.554	-0.438
	T3	0.296 ^{ns}	152.641	-1.122	0.588	-1.122
%Water (x) %Ash wet weight (y)	T1	0.526***	31.093	-0.345	0.090	-0.345
	T2	0.126 ^{ns}	9.898	-0.080	0.102	-0.08
	T3	0.012 ^{ns}	4.147	-0.007	0.092	-0.007
% Water (x) %Ash dry weight (y)	T1	0.232 ^{ns}	62.210	-0.553	0.377	-0.553
	T2	0.163 ^{ns}	-17.689	0.442	0.435	0.442
	T3	0.239 ^{ns}	-29.988	0.596	0.393	0.596

Table 3: regression results log fish weight against different body parameters in wet (log) in *L. calbasu* (n = 40)

Relationships	Treatment	Value of “r”	Value of “a”	Value of “b”	Value of “b” (S.E)	Value of “t” (b=1)
Log fish weight=x Log water =y	T1	0.998***	-0.133	1.014	0.011	-89.8951
	T2	0.997***	-0.082	0.977	0.012	-82.3563
	T3	0.996***	-0.066	0.963	0.014	-70.4656
Log fish weight=x Log fat=y	T1	0.798***	-1.087	0.796	0.097	-9.51328
	T2	0.764***	-1.461	1.059	0.145	-5.83755
	T3	0.681***	-0.992	0.693	0.121	-7.57146
Log fish weight=x Log protein =y	T1	0.975***	-1.048	1.150	0.042	-22.6595
	T2	0.960***	-1.183	1.254	0.060	-15.4127
	T3	0.951***	-1.384	1.420	0.075	-11.9133
Log fish weight=x Log ash =y	T1	0.643***	-0.672	0.437	0.084	-11.4678
	T2	0.472**	-0.653	0.403	0.122	-7.79372
	T3	0.412**	-0.659	0.399	0.143	-6.59401

Table 4: Regression of total length against total values of different body contents in *L. calbasu* (n = 40)

Relationships	Treatment	Value of “r”	Value of “a”	Value of “b”	Value of “b” (SE)	Value of “t” (b=3)
Log fish length=x Log water=y	T1	0.947***	-1.689	2.663	0.146	-17.8849
	T2	0.983***	-1.880	2.890	0.088	-31.2009
	T3	0.974***	-1.806	2.821	0.107	-25.2164
Log fish length =x Log fat =y	T1	0.829***	-2.516	2.289	0.250	-9.711
	T2	0.732***	-3.313	3.042	0.459	-3.49395
	T3	0.668***	-2.252	2.037	0.368	-6.11517
Log fish length=x Log protein=y	T1	0.929***	-2.824	3.032	0.195	-12.3526
	T2	0.925***	-3.401	3.625	0.241	-8.82313
	T3	0.930***	-3.949	4.158	0.266	-7.1202
Log fish length=x Log ash =y	T1	0.543***	-1.207	1.019	0.256	-10.6998
	T2	0.436**	-1.314	1.117	0.374	-6.90439
	T3	0.432**	-1.472	1.253	0.424	-5.82247

Table 5: Regression results of condition factor (K) against different body contents in wet fish weight of *L. calbasu*

Relationships	Treatment	Value of "r"	Value of "a"	Value of "b"	Value of "b" (SE)	Value of "t" (b=0)
Condition factor =x % Water=y	T1	0.030 ^{ns}	76.190	0.335	1.799	0.335
	T2	0.393*	89.538	-9.474	3.601	-9.474
	T3	0.033 ^{ns}	75.431	0.922	4.467	0.922
Condition factor =x % Fat =y	T1	0.313*	7.169	-1.922	0.945	-1.922
	T2	0.124 ^{ns}	1.981	1.860	2.410	1.86
	T3	0.031 ^{ns}	4.553	-0.365	1.934	-0.365
Condition factor =x % Protein=y	T1	0.078 ^{ns}	14.759	-0.740	1.525	-0.74
	T2	0.277 ^{ns}	5.284	7.209	4.059	7.209
	T3	0.060 ^{ns}	12.912	2.094	5.676	2.094
Condition factor=x % Ash =y	T1	0.320*	1.881	2.327	1.118	2.327
	T2	0.026 ^{ns}	3.197	0.405	2.482	0.405
	T3	0.170 ^{ns}	7.104	-2.652	2.499	-2.652

DISCUSSION

Results of the present study represented the lowest water contents and the highest protein contents in T3, when fish were fed a diet containing 25% crude protein in diet. The results are similar to those reported by Ishtiaq and Naeem [8] who had also studied the effect of dietary protein levels on body composition of another carp *Catla catla*, and found significantly higher protein percentage and lower water percentage in the fish samples, fed upon a diet having 25% CP. Kim *et al.* [11] fed parrot fish (*Oplegnathus fasciatus*) with different protein levels, and also reported that protein and lipid increases with dietary protein. Satpathy *et al.* [12] had evaluated the effects of dietary protein on body composition in, *Labeo rohita*, fingerlings

and also reported that body protein and lipid contents increased significantly ($P < 0.05$) with dietary protein level as well as under the influence of the interactive effect of dietary protein levels.

In the present study, whole body protein content of *L. calbasu* increased with the increasing dietary protein levels which agree with the results found by Kim *et al.* [11] and Ishtiaq and Naeem [8]. Proximate composition of *L. calbasu* in terms of fat was not affected by dietary protein levels, which are in accordance with Kim *et al.* [11]. However, generally body lipid content increases with the increase in dietary protein levels as reported by Bai *et al.* [13] for yellow puffer. Ash contents in the whole body of the fish decreases with an increase in dietary protein level, in the

present study. On the contrary, Kim et al. [11] for juvenile parrot fish and Okorie et al. [14] for juvenile Japanese eel had reported that ash contents of fish fed with the experimental diets was not significantly affected by dietary protein levels.

Percentage of the water content in fish body generally influences the percentage of other body contents as reported by various authors [8, 15]. However, in the present study, percent water remained constant in many relations with different body constituents, but found significant with protein contents of the *L. calbasu* in all three treatments. Naeem et al. [16] also reported same results in wild *Notopterus notopterus*.

Body size of *L. calbasu* showed definite effect on the various body constituents in the fish, collected from different treatments which were fed various dietary protein levels (Table 3 and 4). When the allometric approach was applied on log-transformed regression relationships between total body constituents and body weight or length by comparing with $b = 1$ or $b = 3$ (an isometric slope), following Weatherley and Gill [17] and Salam and Davies [5], results showed negative allometry in ash while positive allometry in protein content with the fish size. It indicated that protein contents in the fish body increase at an accelerating rate, defying the cube law (positive allometry, $b > 3$), which is in general agreement with

the studies reported by Salam and Davies [5] and Khalid and Naeem [18]. While, Salam and Davies [5] found that ash was isometric with fish body weight and positive allometric with total length of the fish. The variation in the results might be due to difference in fish body size.

Most of the regression relationships between condition factor and various body constituents showed non significant correlation, the results are in accordance with those reported by Naeem and Ishtiaq [19] for wild *Mystus bleekeri*.

It is concluded that various levels of dietary protein have definite effect on the body composition. Moreover, size of fish also influences on the percentage of various body constituents of *Labeo calbasu*. Hence different body contents can be estimated from fish length or weight.

Conflict of Interest: No conflict of interest associated with this work.

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