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**PROXIMATE COMPOSITION OF FARMED WALKING CATFISH *CLARIAS*
BATRACHUS (LINNAEUS, 1758) FROM MULTAN, PAKISTAN**

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ABSTRACT

Sixty samples of *Clarias batrachus* starting from 26.6 – 48.2cm in length and 179 – 867g in weight were collected from fish form on Muzffargarh road near Multan, Pakistan. In this study the estimates of protein, fat, ash, organic contents and moisture contents were studied. Mean values of water, ash, fat, protein and organic contents were 75.75%, 2.33%, 5.44%, 16.46%, and 21.91% in wet body and in dry body these values were 75.75%, 9.75%, 22.73%, 67.52% and 90.25% respectively. Body weight and complete length shows positive co-relation with all body constituent while condition factor has least effect. Fat was found to be non-significant in relation ($p>0.05$), while protein was in significant ($p<0.001$) and ash and organic contents were highly significant ($p<0.001$) with body weight and water. Present study is the first reference on *Clarias batrachus* provided on proximate composition from the region.

Keywords: *Clarias batrachus*, proteins, fats, ash, organic contents, proximate composition, regression analysis

INTRODUCTION

Different ions are present in fish body. Fish is not only source of protein but itself
Their balanced situation is guarantee of consumes proteins and lipids from
better functioning of the body cells [1]. different terrestrial sources in the form of

diet [2]. Many researchers are working for the improvement of the fisheries industry and from last few decades the maximum works has implemented for the welfare of this industry [3]. Fish is extensively accepted due to its best taste and low cholesterol level. It has many important nutrients which are necessary for the human to intake. Fish is at a time is both type of source, most cheaply as well as the most expensive source of animal protein [4]. Proximate composition is one of the important parameters which is used to measure the fish ecology, its body functioning and feeding habits [5, 6]. Nutrients obtained from fishes like protein, unsaturated fats and lipids compounds are very easy digestible [7].

Clarias batrachus is that fish which is culturing at huge level and it is fulfilling the need of fish meat not only in Asian countries but playing vital role in worldwide need of fish [8]. Catfish are known to be omnivorous in their nourishment propensities [9]. Catfishes are likewise solid and tolerant to a wide scope of distressing natural conditions [10]. Fish meat is more recommended and eaten among the children due to its softness of meat and easily digestible and prevents the children from those diseases which are

produced in them due to lacking nutrients [11]. Quality of human life is the indicator of those animals which are living in its surrounding. More good health can cause the wellbeing for those animals living in that area [12]. Production of catfish is increasing every year than production of any other fish species. But use of poor-quality feed by the local farmers is decreasing the growth of catfish [13]. Nutrient contents of the fish have also been reduced using such substandard feed. Survival rate of fingerlings is a major factor in its growth. If the fingerlings are properly look after then the production of the fish will be good but unfortunately in many Asian countries specially in India the huge production of catfish is damaged due to non-technical handling [14].

In proximate analysis, all essential and non-essential components of the fish have been measured. Fish usually absorbs some non-essential metals from its habitat which become the part of human body, when such fishes are used by humans. So, it is necessary to ensure the dietary effects of these metals on the human body, which are usually found in the fish body [15]. Different techniques have been adopted to measure the energy contents of the fish. Some studies demonstrated that energy

density and contents of nutrients in the fish body changes with the change in fish body size, but these differences are also species dependent [16, 17]. Fatty acids of the fish are considered as important nutrient due to having its curative effects for human beings. Many fatal diseases have been recovered by using the polyunsaturated fatty acids of the fish which cannot be synthesized by human body itself [18]. The demand of fish as a source of proteinous food is increasing day by day and freshwater fish is considered more valuable due to its high nutritionist values and low fat. Food scientist not only emphasizing over the increased protein condition of the fish but they also working over the colour, texture, flavour and other nutritive values in the fish [19]. Fats and fatty acids which are received from the fish meat are very valuable because obtaining of these fats is not so easy from other food sources [8].

The growth of fish is dependent on many factors including temperature, dissolved oxygen in water, level of water, pressure of air, humidity and other environmental factors. These factors describe the developmental stages of the fish and it is noted that these developmental factors are more dependent over the size rather than age of the fish [20, 21]. The average

consumption of fish is very low in the Serbia as compared to other European countries and it is not so easy to educate the common peoples of that area about the importance of fish. That fish is the basic dietary component and the most important fatty acids can be obtained by eating fishes either of freshwater or marine [22]. In carnivorous fishes, the utilization of protein can be increased by replacing it with the lipids [23]. Generally, the digestion of lipid in the fish body is very high as compared to other basic components however it is also noted that this digestion in fish is species dependent [24].

Knowing the nutritional values in the feed of the fish is very important for the good growth of the fish. Those fishes which feed on such feed which is highly enriched by the protein, produce good meat and growth of those fishes is also increased as compared to other fishes [25]. Plant based feed of the fish is used to produce fish feed. These sources are the continuous source as these are present more extensively than any other protein source [26]. Fish is the major source of many essential nutrients like carbohydrates, fats, proteins and minerals. These are the nutrients which required for every living

animal for its good and rapid growth and fish is also required these nutrients in their diet for good health and active physiology [27]. The information obtained from the meat composition can also be used by the ecologist to predict the changing situations of the environment [28].

MATERIALS AND METHODS

Fish specimens were collected from private fish form at head Muhammad wala, Muzaffargarh road, near Multan during the early hours of the day and packed in the already cleaned oxygen filled plastic containers and transported to the Fisheries Proximate Laboratory of the Institute of Pure and Applied Biology, (Zoology Division), Bahauddin Zakariya University, Multan. Male and female fishes were separated by recognizing their genital pore differences. All the fish samples were properly cleaned with clean water and properly weighed by using electric balance. Each specimen was properly packed in aluminum foil and was kept in dry oven at temperature of 70°C for drying. The samples were kept in the oven until the constant weight was not obtained.

When fish was properly dried then it was homogenized by using Laboratory Rotor Mill Pulverisette 14. The digestion process followed which has also been used by [29]

with minor modifications. A 10ml solution (1:5) was prepared with the combination of 65% pure nitric acid and 70% of perchloric acid and few drops of solution of sodium chloride were also added in the solution and these products were used in concentrated forms. Powdered form of fish was collected in a beaker (Teflon). The taken amount of powder was one (1.0) g which was collected in the form of fine homogenous powder and was mixed properly in the solution. The solution was gently heated at $(70 \pm 5^{\circ}\text{C})$. It was a continuous process which continued for 12 hours. This continuous heating causes the destruction of the organic matter. The destruction has become easy because of liberation of chlorine molecules and which also caused losing of the chemical bond.

1.1 Proximate analysis

The proximate analysis was made in which ash contents, moisture contents, protein contents, fat contents and organic contents were measured in this research. To measure the moisture contents the fish samples were dried in an oven with a constant temperature of 70°C . These samples were weighed on daily basis. The heating process was continued until the constant wait was not obtained. The constant dry weight of fish was used to

measure the moisture percent in the fish. Dry matter and ash contents were measured according to AOAC [30]. Percentage of nitrogen was also measured by using auto analyzer (Model # 1030, Tecator, Höganäs, Sweden). The lipids of the fish were extracted from the cleaned fish fillet. Bones, stomach, liver and viscera of the fish was cleaned properly. There are different methods, but solvent extraction method was used for the extraction of lipids, in which two solvent were used, which were chloroform and methanol [31]. Rotary pump evacuator was used to evaporate the solvent from the lipid solution. Lipids were stored at the temperature of 4 °C in the presence of inert gas before its use. Standard methods were utilised to determine the chemical and physical properties of the lipids [32].

1.2 Measurement of fatty acids and their profile

Methyl ester mixture and its preparation from lipid

A round bottom flask was taken of 125 ml and 5g of lipid was introduced in it. This was saponified with solution of alcoholic potassium hydroxide and 50 ml of it was used for this purpose. This solution was continuously refluxed on water bath. This process was continued for almost 45

minutes and in this the whole solution become very transparent. When the moisture was cooled then it was neutralised with hydrochloric acid (5N). Over the condensation bath the evaporation was being made which was used for the removal of alcohol from the neutralised solution. Ether was inserted in a new funnel. The volume of ether was 20-30 ml. This ether solution was used for the separation of the aqueous mixture (acidified). This extraction process was repeated three times for getting the best result. There were some impurities of hydrochloric acid was attached with this ether extract and it was purified by washing with water. This was a good strategy to remove the all those attached impurities. The mixture of fatty acid was also produced when the ether was separated from extort. There was the step of esterification and the mixture of fatty acid was esterified by using solution of sulfuric acid in methanolic form. This acid was 0.25 M with 5 ml/g of acid. As the esterification process was completed the whole mixture was dissolved in the 25ml solution of ether in a different funnel. This was washed with the solution of sodium carbonate (Na_2CO_3). This washing process was continued until the while solution was

purified. In last, the while solution was again purified with the water and this was dried by passing sodium sulphate Na_2SO_4 over it. There were some impurities of ether in the solution which was removed by using mixture of methyl ester $\text{C}_{14}\text{--}\text{C}_{24}$ [33].

RESULTS

Mean values of all body constituents regarding to their proximate studies was measured. It was found that 75% fish body is composed on water contents. All parameters of the proximate studies including different organic contents, total protein contents in the body, complete ash profile and total fat were found in greater quantity in the dry matter of the fish as compared to wet weight of specimen's body. Ash contents were found in least range 2.334 for body weight (wet) and 9.75 for body weight (dry). The protein stuffing was also found in the greater quantity in the fish after water (Table 1). The regression parameters were measured between the water contents and body constituents of the *Clarias batrachus*. The student's t-test values explained that the slope (b) of regression lines were different statistically in all cases when water contents were correlated with other body constituents (Table 2). Fat showed non-

significant relationship in wet body weight with total water percentage in water ($p>0.05$; $r=0.2314$). While, fat in dry weight and ash in wet body weight was found to be least significant ($p<0.05$; $r=0.3092$; $r=0.2950$) respectively. The protein in dry body weight was found to be in significant ($p<0.001$; $r=0.4007$) co-relation with total water percentage in the fish body. Protein in wet body weight ($r=0.8601$), Ash in dry body weight ($r=0.4816$) and organic contents in both wet and dry body weight (0.9927 and 0.4816) was all found to be highly significant ($p<0.001$) with the total water percentage in the fish body (Table 2). These showed the major differences of slope (b) values among the wet and dry body constituents.

When all the log values of these parameters were co-related with the log values of the wet body weight, all relations were highly significant ($p<0.001$) with each other. In these only water contents were found to be in isometric relationship with total weight of body while protein contents and organic stuffing in the fish was found to be in positive allometry and fat and ash both contents are found to be in negative allometry. All of these are in positive co-relation (Table 3). The

statistical parameters of total length (Log) and all parameters of the body was found to be highly significant in which only organic contents are found to be in isometric relationship while all other proximate parameters of the fish body like water contents, fat contents and ash contents were found to be in negative allometry while only protein is the constituent that found to be in positive allometric relationship with positive co-relation (Table 4).

When co-related with the condition factor then all the body proximate parameters found in non-significant relationship except only protein which was found to be in significant ($p < 0.01$) relationship. With reference to b value the water and fat are in positive co-relation while protein, fat and organic contents are found to be in negative co-relation in which no isometric relationship was seen but water and fat

showed the positive allometry while protein, ash and organic stuffing were in negative allometric relationship with condition factor (Table 5). Ash percentages shown very negligible correlation ($r > 0.079$) with the condition factor (k) and protein was highly significant ($r > 0.345351$; $p < 0.001$) with the condition factor while other components were also related significantly with the condition factor (Table 5). Multiple regression shows similar least significant ($p < 0.05$; $r = 0.307899$; $r = 0.313184$ respectively) relationship among water and fat with body weight, condition factor (K) and ash while the protein was the parameter which was with high significant ($r = 0.486687$; $p < 0.001$) relationship while the level with ash was significant only ($r = 0.344161$; $p < 0.01$) relationship (Table 6).

Table 1: Ranges of nutrients found in body of *Clarias batrachus* (n= 60)

Body constituents	Mean $\pm$ S.D	Limit
Moisture content (in Percentage)	75.755 $\pm$ 3.342	68.808 – 83.847
Ash content (wet) (in Percentage)	02.334 $\pm$ 0.410	01.386 – 03.307
Ash content (dry) (in Percentage)	9.75 $\pm$ 1.901	6 – 14
Fat content (wet) (in Percentage)	5.444 $\pm$ 1.632	1.731 – 12.627
Fat content (dry) (in Percentage)	22.733 $\pm$ 6.625	7 – 42
Protein contents (wet) (in Percentage)	16.467 $\pm$ 3.306	9.369 – 23.388
Protein contents (dry) (in Percentage)	67.517 $\pm$ 7.398	47 – 83
Organic contents(wet) (in Percentage)	21.911 $\pm$ 3.245	14.053 – 29.009
Organic contents(wet) (in Percentage)	90.25 $\pm$ 1.901	86 – 94

Table 2: Statistical parameters of % water content versus % body constituents of *Clarias batrachus*

Relationships	r	a	b	S. E. (b)	t value when b=0
% Water (x) %Fat wet weight (y)	0.231413	14.00317	-0.11298	0.062366	2.961114
% Water (x) %Fat dry weight (y)	0.309253	-23.711	0.613089	0.247552	2.476607
% Water (x) %Protein wet weight (y)	0.860185	80.92163	-0.85083	0.066236	-12.8455
% Water (x) %Protein dry weight(y)	0.400731	134.7178	-0.88709	0.266311	-3.33103
%Water (x) %Ash wet weight (y)	0.29501	5.075203	-0.03619	0.01539	-2.35138
% Water (x) %Ash dry weight (y)	0.481641	-11.0068	0.274	0.065463	4.185536
% Water (x) % Organic contents wet weight (y)	0.992687	94.9248	-0.96381	0.01539	-62.6262
% Water (x) % Organic contents dry weight (y)	0.481641	111.0068	-0.274	0.065463	-4.18554

r = Correlation Coefficient; a = Intercept; b = slope; S.E= Standard Error; *** P<0.001

Table 3: Regression analysis in weight (whole body) (wet) in Log form V/S proximate constituents of body (%) (Log) in *Clarias batrachus* (n = 60)

Correlations	r(regression)	(Intercept)a	(Slope)b	S. E. (b)	t (b=0)
Body Weight (Log) water contents (Log)	0.9950***	-0.05505	0.974962	0.012813	-1.95411
Weight (Whole body) (Log) fat contents (Log)	0.7637***	-0.79042	0.812861	0.090227	-2.07041
Weight (Whole body) (Log) protein contents (Log)	0.9376***	-1.36123	1.215729	0.059197	3.64425
Weight (Whole body) (Log) ash contents (Log)	0.9112***	-1.3738	0.899388	0.053383	-1.88472
Weight (Whole body) (Log) organic stuffing(Log)	0.9566***	-0.95502	1.110362	0.044426	2.48417

Table 4:Regression analysis of complete length (TL, cm) in Log form V/S body's proximate constituents (%) -(Log) in *Clarias batrachus* (n = 60).

Correlations	r(regression)	(Intercept)a	(Slope)b	S. Error (b)	t (slope=0)
complete length (Log) water contents (Log)	0.960424***	-1.55177	2.579476	0.09823	-4.28101
complete length (Log) fat contents (Log)	0.711446***	-1.9202	2.075663	0.269213	-3.43348
complete length (Log) protein stuffing (Log)	0.932666***	-3.38251	3.314799	0.16835	1.86991
complete length (Log) ash stuffing (Log)	0.90024***	-2.84276	2.435534	0.15467	-3.64949
complete length (Log) organic stuffing (Log)	0.940035***	-2.74346	2.990918	0.142496	-0.06373

Table 5: Regression analysis of (K) Condition factor V/S body's proximate constituents (Percent)) (weight, g) (wet) of *Clarias batrachus* (n = 60).

Correlations	r(regression)	(Intercept)a	(Slope)b	S. E. (b)	t (b=0)
Condition factor (K) Water(in percent)	0.235408 ^{ns}	69.23258	8.032135	4.354284	1.844651
Condition factor(K) Fat (in percent)	0.237504 ^{ns}	2.231869	3.956347	2.124715	1.86206
Condition factor (K) Protein (in percent)	0.345351 ^{**}	25.9312	-11.6553	4.158831	-2.80255
Condition factor (K) Ash (in percent)	0.079602 ^{ns}	2.604354	-0.33316	0.547821	-0.60816
Condition factor (K) Organic stuffing(in percent)	0.232403 ^{ns}	28.16307	-7.69897	4.230774	-1.81976

Table 6: Multiple regression b/w all body's proximate constituents (in percentage) with condition factor (K); and complete body weight (W) g of *Clarias batrachus* (n=60)

Relationship	r	a	b ₁ ± S.E	b ₂ ± S.E	r ²
Water(Percent) = a+b ₁ W+ b ₂ K	0.307899	72.04375	-.00361 ± .002293	6.660933 ± 4.387042	0.094802
Fat(Percent) = a+b ₁ W+b ₂ K	0.313184	3.643682	-.00181 ± .001118	3.267708 ± 2.137937	0.098084
Protein(Percent) = a+b ₁ W+b ₂ K	0.486687	21.12642	0.006173 ± 0.002083	-9.3117 ± 3.984312	0.236864
Ash(Percent) = a+b ₁ W+b ₂ K	0.344161	3.186145	-.00075 ± .000278	-0.61694 ± 0.531064	0.118447

DISCUSSION

Present study was based on the complete proximate analysis of *Clarias batrachus*. All studied proximate parameters were found in different ratios of significant and non-significant correlations with total length and total body weight of the species. The growth rate of *Clarias batrachus* is very good in this study and it was also declared as the best specie to feed on external diet [34]. Mean moisture content in the present study was found as 75.7 % and the same percentage (74.99%) was also reported in the genus *Clarias* and *Micromesistius* [35]. High contents of

moisture in the fish is not good as it increases its susceptibility to the microorganisms and if it is preserved for longer time it reduces the taste quality of fish [36]. The mean ash values in the present studies (2.334) showed that the minerals were present in massive quantity in the *Clarias batrachus* as compared to the other fishes as in previous it was noted as 1.7 [35]. The percentage of crude protein in *Clarias gariepinus* was recorded as 18.88 while the percentages of crude fat, ash, and moisture was recorded as 0.83, 0.93 and 76.85 respectively [37]. There was found maximum fat contents in the

Parachanna obscura and least was found in the *Hepsetus* spp. The usual ranges in fishes is as follows; water is found from range 70% to 85% while, protein was found in percentage of 20 to 30 and least percentage was found in lipid, which was in range of 2 to 12 [38].

Higher values of protein were reported in *Clarias* species by Mohammed *et al.*, [39] and same values were also mentioned by Effiong and Fakunle [40]. Protein is the major component of muscles of fish and the amount of protein in different species of the fish didn't show much deviation from each other [41]. Ackman [42] categorized the fishes into four categories according to the fats contents. If the fats concentration is < 2% then it is considered as lean fish, 2-4% is considered as low-fat contented fish, 4-8% is considered as medium fat contented fish and > 8% is considered as high fat contented fish. When *Clarias gariepinus* was feed on commercial feed than the concentration of Moisture, Ash, Crude protein and Crude Fats was found as 7.5%, 20.5%, 23.05% and 10.37 % respectively [43]. Commercially the protein requirement in the fish is influenced by many factors which include its age, temperature, its feed and the quality and composition of the

food as well and these all things constitute the protein contents of the fish [44]. In present studies the range of water in the specimens were in the standard permissible range. These were found in percentage of starting from 75 to 85. This is the indication that fish collection was from the stable source of water [45, 46]. The weight and size of the fish was increased with the time. It was found that all proximate parameters including water, fat, ash and organic contents also changed with this change in the fish. The similar increased variation was found in *Oncorhynchus mykiss* [7]. Some researchers reported the decreased values of the moisture and water contents in the fishes. The percentage of these were in range of 44 to 75 [47]. Condition factor is the tool to measure the degree of fatness and health of fish and in this study the it is found that the condition factor has negative influence over the %protein, %ash and %organic contents while it has positive effect the %water and % fat (Table 7). The same results were found for the proximate analysis of *Notopterus notpterus* [48] and *Colisa lalia* [49]. Least significant negative correlation existed between the percent of protein and condition factor and percent of organic content and condition factor. While percent

of ash and condition factor also showed least non-significant negative correlation, but it is less than of former mentioned parameters (Table 4).

The protein contents in the present study are found in the significant relationship. The similar concentrations of the protein's contents were found in the study of Rathore [26]. It is also considered that the amount of protein in the fish body is also dependent on the amount of protein which was utilized for the formation of fish feed [50]. The moisture content of the fish was 75.577 percent and the same moisture contents were found in the study of Toutou *et al.*, [51]. The good values of lipids and proteins indicate that there was not any stress for the fish. Some studies showed the lesser values for the organic contents and they were found that the reason was due to the stressful situation for the fish. Concentration of fat was increased in this study while it was reported reduced in the previous studies and the concentration of ash was reduced in this study while it was greater in other studies [52]. The knowing of nutritional values of the fish is used to identify the species on behalf of these organic contents of the fish. There is need of knowing the detail profile of the all proteins, lipids, carbohydrates and

minerals for this purpose [53]. Health of the fish must be good for showing the best nutritive values because the diseased fish will not take the proper diet and its weight is reduced and it affects not only the weight of the fish, but overall growth of the fish is affected. The organic contents in the present studied fish are found in good quantity but these can be improved by maintaining the diet of the fish. Natural food supplements are the only source of the fish feed which having not any negative effect over the growth of the fish [54]. Complete proximate information including all nutritious contents of the *Clarias batrachus* has been provided in this study which is very informative for the nutritive point of view.

CONCLUSION

The findings of these studies showed that *Clarias batrachus* is a very nutritious fish which is very good for the human use to fulfill its nutritional and protenious need. It is recommended to promote growth of *Clarias batrachus* in Pakistan.

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

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