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Effect of Body Size and Condition Factor on Proximate Composition of *Clarias batrachus* Under Different Concentration of Ammonia

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ABSTRACT

This study is focused on examining the proximate body composition (water, fats, proteins, and ash) of *Clarias batrachus,* exposed to different concentrations of ammonia. Fish were kept in different groups with ammonia concentrations of 0 mg/L (T-0), 0.25mg/L (T-I), 0.5mg/L (T-II), 0.75mg/L (T-III), and 1mg/L (T-IV) for 3 months. At the end of the trial, a total of 50 samples of *C. batrachus* (10 from each treatment group) were taken to analyze the body composition. Mean (±SE) values of water, ash, fat, and protein contents in % wet body weight of *C. batrachus* were found 77.07±0.80% to 82.23±0.55%, 2.98±0.03%to 3.34±0.17%, 3.90±0.19% to 4.58±0.30% and 10.53±0.40 to 15.36±0.56 in the different studied treatment groups. Water and protein contents were significantly ($P<0.05$) affected by the exposure of ammonia to *C. batrachus.* Significantly highest level of protein content was found in the fish group which was not treated with ammonia exposure (T-0). Whereas ash and fat contents showed no significant ($P>0.05$) difference between the ammonia-treated groups and the control group. Regression analysis showed a clear effect of fish size on water and protein contents in *C. batrachus* reared in different treatment groups. While ash was found correlated only in T-0 ($p<0.001$; $r=0.939$), and fat in T-0 ($p<0.001$; $r=0.929$) and T-III ($p<0.05$; $r=0.636$). However, the condition factor remained constant with the proximate composition of *C. batrachus.* This study would be helpful in the measurement of the body composition of fishes with the size of fishes affected by different concentrations of ammonia to analyze the nutritional value of *C. batrachus*.

Keywords: Ammonia exposure, Proximate composition, Condition factor, Predictive equation, *Clarias batrachus.*

1. INTRODUCTION

Fish is an essential component of food for consumers worldwide due to its quality nutrient profile which is imperative for proper body functioning, growth, and reproduction (Ahmed et al., 2015). A body composition indicates the quality of a healthy food (Kamal et al., 2007). The

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proximate body composition is calculated to evaluate information regarding the fish's health and physiological conditions in addition to ensuring nutritional consistency (Dempson et al., 2004). Fish water, fat, protein, and ash content analysis provides proximate body composition. However, these values vary significantly depending on size, sexual condition, feeding season, physical activity, and species (Aberoumand, 2012). A good indicator of a fish's quality, nutritional value, physiological state, and habitat is its chemical composition in its flesh (Ravichandran et al., 2011).

Fish consists primarily of water (66-81%), protein (16-21%), minerals (1.2-1.5%), fat (0.2-25%), and carbohydrates (0–0.5%) (Begum et al., 2016). Water, protein, fat, and ash make up 96% to 98% of fish's body composition. The "proximate composition" of fish is the evaluation of these components (Rani et al., 2016). In general, it has been seen that non-protein and carbohydrates compounds are typically neglected during examination, as these are accessible in somewhat level of wet mass that is normally thought to be insignificant (Petricorena, 2015). Knowing the fish's proximate composition is necessary for estimating their energy value and preparing the most effective commercial and industrial processing (Hantoush et al., 2015). Apart from this, the fish bioenergetics study and the pollutants effects have revealed the significance of proximate composition (Ahmed and Sheikh, 2017).

In a fish body, the percentage of water is an ideal pointer of protein content and comparative fat. The percentage of water in fish body is an ideal indicator of protein and fat content. It is inversely proportional to the fat and protein content (Zehra and Khan, 2013). Many specialists discovered the proximate composition of fish which is affected by its condition factor (Azam and Naeem, 2022). The fat substance of fish sample is measured regularly, based on the relationship between water volume and lipid levels (Salam and Davies, 1994). Examining the fish's body moisture content is one of the first and most fundamental methods for determining its nutrient content. A good indicator of a food's calorie, protein, and fat content is its moisture content (Barua et al., 2012). The calorie density of fish with less moisture is higher, as is its fat and protein content. The species, size, sexual status, and season all have significant effects on these values. Due to its high digestibility and essential amino acids (EAA) presence, which fish require for growth, reproduction, maintenance, and vitamin synthesis, Protein comes in second place in fish's proximate composition after moisture (Ahmed and Sheikh, 2017). Like muscle meats composition, fish and shellfish comprise 19% protein. Additionally, fish is chosen because it contains less fat than beef (Al-Ghanim, 2016; Tsironi and Taoukis, 2017). The water proportion in it is a good indicator of its relative energy, protein, and lipid content. Fish's energy content as well as lipid and protein content would be more important at lower water percentage. Additionally, the water in which fish live and their feed intake are strongly correlated with variations in proximate composition (Dempson et al., 2004). Finding best practices for reducing consumption of fish meal and increasing fish protein utilization has emerged as an environmental and financial objective for the sustainable growth of the aquaculture feed industry over the past few decades (Dong, 2011). As a result, numerous studies have been conducted to determine how fish diets can increase the protein-sparing potential of lipids and carbohydrates (Watanabe, 2002; Fan et al., 2015).

Fish growth, development, and normal physiological metabolism are all dependent on lipids. As a result, the growth of fish is affected by the level of lipids in their diet. Accordingly, dietary lipid levels and the lipid content of fish body and muscle are positively correlated in many fish growth (Jiang et al., 2015; Huang et al., 2016). Likewise dietary lipid levels will affect moisture, ash, protein content of fish body and muscle, and particularly impacts on protein content additionally mirror the saving capability of lipid to protein from the side (Song et al., 2010). Additionally, a recruitment of lipids rather than carbohydrates as a substituted source has been shown to be a significantly more effective method of preserving the dietary protein because fish acquire a lower capacity to utilize carbohydrates (Stone, 2003). The goal is to boost growth performance by providing high-quality feeds and the right amount of essential nutrients (Kiron, 2012). The aim of study was to find out the effect of ammonia in varying concentration (0, 0.25, 0.5, 0.75, and 1mg/L) on proximate composition of *Clarias batrachus.*

2. MATERIALS AND METHODS

2.1. Ethics Statement

The Ethics Approval Committee at Quaid-i-Azam University, Islamabad, Pakistan, approved all experiments and animal-handling procedures. Experiments are performed in strict accordance with internationally accepted standards and regulations with the approval of Institutional Animal Care and institutional ethics committee.

2.2. Specimen Collection

Fish samples of *Clarias batrachus* were obtained from Al-Raheem Fish Hatchery, Muradabad, Muzaffargarh, Pakistan (Latitude 30° 20'0" N; Longitude 71° 5'0" E) and transported to Animal House, Bahauddin Zakariya University Multan (Latitude 30° 16'02.19" N; Longitude 71° 30'05.76" E).

2.3. Experimental Design

Fish were held in a hauler for 2 weeks to acclimatize at room temperature before experiment. The fish were fed a diet comprising 35% crude protein with 4% of body weight of the fish, twice a day at optimum water quality parameters during experiment with mean value of Dissolve oxygen (5.38±0.38), Temperature (27.7±0.15), pH (7.1±0.08), Chlorides (mg/L) (94.4±16.46), Light Penetration (cm) (7±0.82), Electrical Conductivity (688±50.1), Total Alkalinity (mg/l) (266±8.94), Total Hardness (mg/l) (215.4±3.58), Free Carbon Dioxide (ppm) (6.8±0.84), and Total Dissolve Solid (ppm) (343.5±24.55). Ammonia exposure took place in a 50-L plastic Hauler containing 30 fish per treatment group (T-0, T-I, T-II, T-III, and T-IV) with the concentrations 0, 0.25, 0.5, 0.75, and 1mgL-1, respectively, for 3 months (March to May 2021). Ammonium chloride (NH_4Cl) (Merck Germany) was dissolved in the plastic Hauler to make the respective concentrations. The plastic Hauler water was thoroughly exchanged for 15 days and made the same concentration in the respective plastic hauler.

At the end of the trial, 10 fish samples from each group and a total 50 samples of *Clarias batrachus* were collected for proximate analyses. The oven-drying method was used to determine the amount of water. All samples were wrapped in aluminum foil and dried in a calibrated oven at 70-80°C until the weight becomes constant. The difference between the oven-dried fish sample and the wet body weight of each sample was used to calculate the total water.

$$\text{Water (\%)} = \frac{Weight\,Loss\,during\,Oven\,Drying}{Original\,Wet\,Body\,Wieght\,of\;Sample\,Taken} X100$$

The dried fish specimen was gently grinded through a grinder until became homogenized. Dry samples of powder were stored in tiny plastic bottles with suitable labeling for further analysis. The ash content of the fish was evaluated by burning the 1 gram of sample in a muffle furnace for 24 hours at 550^{o}C.

The following calculations were used to calculate the amount of ash:

Ash content=Initial weight of sample Weight loss during incineration

$$Ash\ (\%) = \frac{Incinerated\ Sample\ Weight}{Sample\ Weight} X100$$

Fat content was extracted using a chloroform and methanol mixture (1:2 vol/vol ratio). 1 gram sample was taken in a test tube, with a 10ml solution of solvents, stirred, and then covered with aluminum foil overnight. Removed the supernatant carefully in pre-weighed glass bottles and were placed in an oven for evaporation for 2 to 3 days until the weight of the bottle become constant. The percentage of fat content in the sample was computed using the formula below.

$$\text{Fat (\%)} = \frac{Weight\ of Residue\ left\ in\ Bottle}{Sample\ weight}\ x100$$

The weight of leading constituents, such as water, ash, and fat, was subtracted from wet body weight of fish to determine the protein content. Analysis of variance by the difference in mean values and Duncan’s Multiple Range Test were performed on SPSS (Ver. 17), while other statistical analysis including regression analyses were carried out by using MS Excel package.

3. RESULTS

Mean (±SE) weight of *Clarias batrachus* was found 22.95±1.12, 18.02±0.94, 21.24 ±0.87, 24.84 ±1.10 and 26.94 ±1.38 g, with mean (±SE) values of condition factor 0.76±0.01, 0.74 ±0.01, 0.74 ±0.02, 0.72 ±0.02 and 0.69 ±0.01 in T-0, T-I, T-II, T-III, and T-IV, respectively.

Mean values of different body constituents in percentage (%) of wet and dry weight of *Clarias batrachus* are provided in table 1. Water content (%) of *Clarias batrachus* was found significantly (P<0.05) higher in T-III (81.12±0.55%) and T-IV (82.23±0.55%) than T-I, T-II and control groups, representing maximum water percentage in the treatment groups comprising higher ammonium concentration. Ash contents (%) in wet body weight of *C. batrachus* showed no significant (P>0.05) difference among various treatment groups including control group. Ash contents (%) in dry body weight of the fish showed higher mean values in T-III (17.19±1.07%) and T-IV (18.80±0.80) than T-I, T-II, and control groups. Mean fat contents (wet weight) were ranged 3.90-4.58% in different treatments, however no significant (P>0.05) difference was found in fat contents (wet and dry body weight) of *C. batrachus* among ammonia treatment groups and control group. Significantly highest (P<0.05) value of mean protein content (15.36±0.56%) in the wet body of *C. batrachus* was observed in control group, while it was significantly lowest

(P<0.05) in T-III (11.53±0.47%) and T-IV (10.53±0.40%). Protein content (dry body weight) remained significantly higher (P<0.05) in T-0, T-I and T-II than T-III and T-IV. Results are described in table 1.

Table 1. Mean (±SE) values of various body constituents in percentage (%) of wet and dry weight of Clarias batrachus.

Constituents		**To (Control)**	**T-I (0.25mg/L)**	**T-II (0.5mg/L)**	**T-III (0.75mg/L)**	**T-IV (1mg/L)**	**Sig.**
Water (%)		77.07±0.80[b]	77.69±0.34[b]	78.73±0.43[b]	81.12±0.55[a]	82.23±0.55[a]	.000
Ash	Wet Wt.	2.98±0.03[ns]	3.11±0.26[ns]	3.13±0.19[ns]	3.23±0.20[ns]	3.34±0.17[ns]	.721
	Dry Wt.	13.15±0.48[b]	13.84±1.01[b]	14.65±0.69[b]	17.19±1.07[a]	18.80±0.80[a]	.000
Fat	Wet Wt.	4.58±0.30[ns]	4.45±0.31[ns]	4.31±0.22[ns]	4.12±0.16[ns]	3.90±0.19[ns]	.317
	Dry Wt.	19.84±0.78[ns]	19.93±1.32[ns]	20.24±0.79[ns]	21.80±0.47[ns]	21.95±0.72[ns]	.235
Protein	Wet Wt.	15.36±0.56[a]	14.75±0.41[ab]	13.83±0.27[b]	11.53±0.47[c]	10.53±0.40[c]	.000
	Dry Wt.	67.00±0.64[a]	66.23±1.98[a]	65.11±1.20[a]	61.01±1.28[b]	59.24±1.36[b]	.000

Note: Sig.=Significant; n.s=non-significant.

Table 2. Statistical regression parameters of percentage water content versus % body constituent in wet and dry weight of C. batrachus.

Equation	***Treatment***	***r***	***a***	***b***	***SE of b***	***t-Stat***
%Ash Wet Wt. = *a* + *b*% Water	T-o	-0.135[ns]	3.382	-0.005	0.014	-0.363
	T-I	-0.761*	48.598	-0.585	0.187	-3.128
	T-II	-0.742*	29.052	-0.329	0.112	-2.952
	T-III	-0.110[ns]	6.486	-0.040	0.136	-0.295
	T-IV	-0.577[ns]	17.573	-0.173	0.092	-1.884
%Fat Wet Wt. = *a* + *b* % Water	T-o	-0.870**	29.543	-0.324	0.069	-4.701
	T-I	-0.280[ns]	24.564	-0.259	0.332	-0.779
	T-II	-0.661*	30.921	-0.338	0.144	-2.348
	T-III	-0.822**	23.800	-0.243	0.063	-3.855
	T-IV	-0.712*	23.791	-0.242	0.089	-2.704
%Protein Wet Wt. = *a* + *b* % Water	T-o	-0.961***	67.075	-0.671	0.072	-9.321
	T-I	-0.127[ns]	26.838	-0.156	0.457	-0.340
	T-II	-0.528[ns]	40.027	-0.333	0.201	-1.657
	T-III	-0.840**	69.715	-0.717	0.173	-4.136
	T-IV	-0.804**	58.636	-0.585	0.162	-3.611

Note: r=Correlation Coefficient; a=Intercept; b=Slope; S.E=Standard Error; ***= p<0.001; ** = p< 0.01; * = p< 0.05; [ns]> 0.05.

Percent water content in the wet body weight of *Clarias batrachus* showed significant negative correlation (*p*<0.05) with % ash contents of the fish reared in T-I and T-II. It was inversely correlated with fat contents of *C. batrachus* in all the studied groups except for T-I. %Protein content was also showed negative correlation with % water content of the studied fish reared in T-0 (p<0.001, r=-0.961), T-III (p<0.01, r=-0.840-) and T-IV(p<0.001, r=-0.804-), as described in table 2.

Fish size (body weight and total length) showed significant positive strong correlation (p<0.001) in T-0, T-I and T-IV, while significant positive correlation (p<0.01) in T-II and T-III with total water content in *C. batrachus*. Ash content found positively correlated (p<0.001) only in control group (T-0) with the fish size. Fat contents in the fish showed significant positive strong correlation (p<0.001) in control group and least significant (p<0.05) in T-III with body size of *C. batrachus*. Protein contents also represented highly significant positive correlation (p<0.001) in T-0 and T-I, significant (p<0.01) in T-II and T-III and least significant (p<0.05) in T-IV with both body weight and total length of *C. batrachus* (Tables 3 & 4).

Table 3. Statistical regression parameters of log transformed body weight (g) versus log transformed total body constituents in wet and dry weight of C. batrachus.

Equation	***Treatment***	***r***	***a***	***b***	***S. E. (b)***	***t value when b=1***
Water =*a* + *b* Wet Weight	T-o	0.975***	0.293	0.066	0.006	-177.98
	T-I	0.977***	0.074	0.080	0.007	-153.51
	T-II	0.813**	0.135	0.076	0.020	-48.79
	T-III	0.812**	0.323	0.065	0.017	-57.14
	T-IV	0.949***	0.082	0.080	0.010	-100.28
Ash =*a* + *b* Wet Weight	T-o	0.939***	-1.341	0.081	0.011	-89.44
	T-I	-0.187^{ns}	0.006	-0.021	0.041	-24.49
	T-II	0.269^{ns}	-0.564	0.026	0.036	-28.13
	T-III	0.249^{ns}	-0.371	0.017	0.026	-39.12
	T-IV	-0.053^{ns}	-0.027	-0.002	0.013	-74.64
Fat = *a* + *b* Wet Weight	T-o	0.929***	-2.394	0.166	0.025	-40.09
	T-I	-0.284^{ns}	0.078	-0.014	0.018	-56.31
	T-II	0.245^{ns}	-0.245	0.014	0.021	-48.21
	T-III	0.636*	-0.610	0.041	0.018	-54.02
	T-IV	0.449^{ns}	-0.385	0.025	0.019	-53.13
Protein = *a* + *b* Wet Weight	T-o	0.980***	-1.419	0.136	0.010	-97.85
	T-I	0.949***	-1.099	0.113	0.014	-70.85
	T-II	0.865**	-0.735	0.084	0.018	-54.37
	T-III	0.690*	-0.680	0.075	0.029	-33.85
	T-IV	0.744*	-0.693	0.072	0.024	-41.03

Note: ***= p<0.001; ** = p< 0.01; * = p< 0.05; ^{ns}> 0.05.

Table 4. Statistical regression parameters of log transformed total length (cm) versus log transformed total body constituents in wet and dry weight of C. batrachus.

Equation	***Treatment***	***R***	***a***	***b***	***S. E. (b)***	***t value when b=3***
Water =*a* + *b* Total length	T-o	0.978***	-1.256	2.156	0.171	-15.42
	T-I	0.978***	-1.661	2.487	0.200	-12.49
	T-II	0.816**	-1.719	2.549	0.676	-1.89
	T-III	0.801**	-1.304	2.211	0.619	-2.64
	T-IV	0.951***	-2.127	2.899	0.352	-5.63
Ash =*a* + *b* Total length	T-o	0.946***	-3.267	2.672	0.344	-6.05
	T-I	-0.170 ^{ns}	0.391	-0.588	1.280	-2.93
	T-II	0.261 ^{ns}	-1.174	0.856	1.185	-1.68
	T-III	0.268 ^{ns}	-0.873	0.650	0.878	-2.76
	T-IV	-0.018 ^{ns}	-0.028	-0.024	0.485	-6.21
Fat = *a* + *b* Total length	T-o	0.935***	-6.332	5.471	0.780	1.63
	T-I	-0.286 ^{ns}	0.386	-0.441	0.555	-5.85
	T-II	0.239 ^{ns}	-0.570	0.454	0.692	-3.88
	T-III	0.633*	-1.643	1.397	0.642	-3.28
	T-IV	0.450 ^{ns}	-1.081	0.914	0.680	-3.50
Protein = *a* + *b* Total length	T-o	0.982***	-4.609	4.443	0.317	-5.02
	T-I	0.950***	-3.567	3.537	0.435	-3.36
	T-II	0.867**	-2.781	2.815	0.606	-2.14
	T-III	0.683*	-2.568	2.562	1.029	-0.35
	T-IV	0.743*	-2.677	2.610	0.881	-0.79

Note: ***= $p<0.001$; ** = $p< 0.01$; * = $p< 0.05$; ^{ns}> 0.05.

Table 5. Statistical regression parameters of condition factor (K) versus percentages (%) of body constituents (wet mass, g) for C. batrachus reared in different treatments.

Equation	***Treatment***	***R***	***a***	***b***	***S. E. (b)***	***t value when b=1***
Water = *a* + *b* Condition Factor	T-o	0.562^{ns}	35.766	54.522	30.128	1.810
	T-I	-0.331 ^{ns}	84.749	-9.491	10.141	-0.936
	T-II	0.640*	66.807	16.214	7.298	2.222
	T-III	0.291 ^{ns}	75.381	7.932	9.767	0.812
	T-IV	-0.090 ^{ns}	85.257	-4.405	18.265	-0.241
Ash = *a* + *b* Condition Factor	T-o	0.614 ^{ns}	1.249	2.288	28.750	0.080
	T-I	0.614 ^{ns}	-6.955	13.538	8.484	1.596
	T-II	-0.404 ^{ns}	6.471	-4.544	8.688	-0.523
	T-III	-0.453 ^{ns}	6.494	-4.514	9.101	-0.496
	T-IV	0.143 ^{ns}	1.897	2.096	18.152	0.115
Fat = *a* + *b* Condition Factor	T-o	-0.558 ^{ns}	19.852	-20.154	30.227	-0.667
	T-I	0.392 ^{ns}	-3.263	10.372	9.888	1.049
	T-II	-0.402 ^{ns}	8.141	-5.205	8.698	-0.598
	T-III	-0.353 ^{ns}	6.173	-2.838	9.552	-0.297
	T-IV	0.014 ^{ns}	3.747	0.229	18.338	0.012
Protein = *a* + *b* Condition Factor	T-o	-0.541 ^{ns}	43.132	-36.656	30.623	-1.197
	T-I	-0.409 ^{ns}	25.469	-14.419	9.806	-1.470
	T-II	-0.405 ^{ns}	18.580	-6.465	8.686	-0.744
	T-III	-0.025 ^{ns}	11.951	-0.580	10.207	-0.057
	T-IV	0.058 ^{ns}	9.098	2.080	18.308	0.114

Note: * = $p< 0.05$; $^{ns}p> 0.05$.

Condition factor remained constant with all the body constituents of *C. batrachus* in all the ammonia treated and control group showing insignificant correlation (p<0.05) except for water content in T-II representing least significant correlation (p<0.05, r=0.640) (Table 5).

4. DISCUSSION

In the present study, proximate body composition of different body constituents (water, ash, fat, and protein) was analyzed. Results of the present study showed that mean (±SE) values of body constituent (water, ash, fat, and protein) in % wet body weight of *C. batrachus* were found 77.07±0.80% to 82.23±0.55%, 2.98±0.03%to 3.34±0.17%, 3.90±0.19% to 4.58±0.30% and 10.53±0.40 to 15.36±0.56 in the different studied treatment groups. The water content of *C. batrachus* in all groups was viewed as inside the acceptable level, which may be because of the steady water levels at various study locations, additionally revealed by Adewumi et al. (2014). Moisture content according with the study of Zehra and Khan (2013) and Hasan et al. (2015). Results of the proximate composition of *C. batrachus* in the present study were found very similar to those reported by Olaniyi et al. (2017), who have documented water, ash (dry weight), fat (dry weight) and protein content (dry weight), 73.67 ± 2.08, 14.06 ± 8.69, 25.60 ± 4.93 and 60.38 ± 1.75%, respectively, in *Clarias gariepinus*. Lal and Naeem (2021) have also observed water (73.22%), fat (3.45%) and protein contents (16.62%) in the whole wet weight of body of *Terapon jarbua*, being very close to that found in the *C. batrachus* of present study, reared in control group.

Water and protein contents were significantly affected by the exposure of ammonia to *C. batrachus*. An increasing trend in water content while decreasing trend in protein level was observed in the body of *C. batrachus* with an increase in concentration of ammonia treatment. Highest level of protein content with satisfactory amount of fat and ash contents were found in the fish group which was not treated with ammonia exposure (T-0). While the poor percentage of protein in the fish groups which were treated 0.75mg/L and 1mg/L concentration of ammonia might be due to demand for energy to detoxicate the ammonia which effects the drop in the energy for growth (Clearwater et al., 2002). Hence the results of the present study indicated negative effect of ammonia exposure on proximate composition of *C. batrachus*. The findings are in general agreement with that reported by Shin et al. (2016) who have stated that the ammonia depending on water temperature negatively influence the fish.

A good indicator of its protein and lipid content is its moisture content (Yeannes and Almandos, 2003; Naeem and Salam, 2010). Body composition can also be predicted by using percent water and by developing correlations with other body constituents. So, proximate composition can be calculated even devoid of time cost or expertise necessary for study at most of the labs (Hartman and Margraf, 2008). Numerous studies have concluded that body composition of a fish can be assessed from a volume of water by applying regression formulae (Salam and Davies, 1994; Naeem et al., 2011; Lal and Naeem, 2021). Percent water showed an inverse relationship with percentages of ash, fat and protein of *C. batrachus*, in this study. These results are comparable with those reported by Lal and Naeem, (2021). However, Ishtiaq and Naeem (2019) have found negative correlation of %water with fat and ash only in *Catla catla*. On the other hand, some non-significant correlation of %water with body constituents were found in general agreement with Iqbal et al. (2020).

Pradhan et al. (2014) describe the biochemical composition of *Catla catla* muscle and liver in India's tropical climate. There was a strong negative correlation ($P \leq 0.01$) between protein and lipid content and moisture content in their study. As a matter of fact, the utilization of lipids and protein for metabolic activities during spawning and non-feeding conditions causes fish to have higher moisture content, whereas an increase in lipids and a decrease in moisture content indicate good health. However, a few researchers (Naeem et al., 2011; Breck, 2014) reported virtually that no functional relationship between body lipids and body water was found, which is inconsistent with these findings.

Findings of the Naeem and Salam (2010) of *Aristichthys nobilis*, Bano et al. (2019) of *Labeo calbasu* and Lal and Naeem (2021) of *Terapon jarbua* show that proximate composition vary with body size (length and weight) of fish. Results of the present study also showed a definite effect of fish size on water and protein content in *C. batrachus* reared for all treatment groups. On the other hand, ash was found correlated only in T-0, while fat in T-0 and T-III. According to Kalay et al. (2008) fat content rises with fish size. Due to ageing, older animals have more fat tissue than younger ones (Yousaf et al., 2011). The difference in results for fat contents may be attributed to the difference in experimental conditions mainly ammonia levels which may cause stress on fishes.

A substantial relationship between several muscle and liver body contents (ash, lipid, protein, and moisture) and condition factors (K) (Table 5). Pradhan et al. (2014) showed parameters in *Catla catla*, Naeem et al. (2010) in *Tor putitora*, *Wallago attu* by Yousaf et al. (2011). Present work also found that the relationship between the K-value and the percentage of the body's contents were not significant, indicating that the condition factor had no effect on the percentages of water, ash, fat, and protein in *C. batrachus.*

5. CONCLUSION

Considering the results of this study, ammonia exposure to *Clarias batrachus* induced significant decrease in protein contents. Fish body size of represented clear influence on the various body constituents, however, condition factor remained constant with the proximate composition of *C. batrachus.* Further investigations on the effect of ammonia exposure on proximate composition of various fish species are needed to make the study more comprehensible.

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7. CONFLICT OF INTERESTS

No conflict of interests.

8. REFERENCE

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