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Comparison of Protein Concentration in Red and White Muscles in Two Species of Bony Fish

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Abstract:

The current study deals with estimating the protein concentration and the effect of fish weight on protein concentration values in red and white muscles in two different regions (R1 : Anterior region lies 2 cm behind the head and R2: posterior region lies 2cm from caudal fin) in two types of bony fish, namely common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*). Samples were collected from Karmat Ali river- north of Basrah between October 2019 and February 2020. The protein was extracted using protein extraction buffer, the current study show that the average of protein concentration in red muscles of Nile tilapia ranged between 7.74-7.4 mg / ml and (6.8-8.85 mg / ml) in R1 and R2 region respectively, while it ranged between 173-334 mg / ml and 127-253 mg / ml in R1and R2 region in white muscles, respectively. In Carp, protein concentration for red muscles was 7.19-9.10 mg / ml and 6.87-8.41 mg / ml in R1 and R2 regions, respectively. On the other hand, protein concentration in white muscles ranged between 98.7-250.2 mg / ml and 61.5-214.1 mg / ml in both R1 and R2 regions respectively. The statistical analysis results of the protein concentration in the red and white muscles in the body regions indicated that there was a significant difference($P<0.05$) and non- significant difference ($P>0.05$) between the protein concentration in red and white muscles in the studied species ,The current study concluded that white muscles contain a higher protein concentration than red muscles and that weight gain has a significant effect on protein concentration in white muscles.

Keywords: BCA assay, Common carp, Fish muscles, Nile Tilapia, Protein concentration

Introduction:

Generally, fish is considered as a vital source to provide animal protein especially in the poor countries. With the increase in population numbers and the increase in food consumption, it became necessary to search for an alternative source of animal protein that contains all the essential amino acids, the world's attention has turned to the marine environment to obtain those important elements [1] . Fish meat type is considered as an important food source because it contains a high percentage of protein and important mineral elements such as Calcium, Iodine and Phosphorus in addition to having a high percentage of Lipids and Vitamins [2]. This study dealt with two common species of important and economically fish in Iraq, namely common carp fish,(*Cyprinus carpio* L)., which belongs to the Cyprindae. It is a widespread fish in the environment characterized by its high nutritional value and ability to with stand environmental conditions as it has a wide temperature and a high salt tolerance [3]. Nile tilapia fish ,(*Oreochromis niloticus* L.) is one of the endemic fish in Africa, but it was introduced to other countries for fish farming . These types of fish were introduced to the ponds located on Tigris river near Baghdad city. It was recorded for the first time in the Iraqi environment in Shatt Al-Arab River in Abu Al-Khaseeb District - south of Basrah [4]. Nile tilapia is characterized by having colored lines with regular shape in the caudal fin. The *O. niloticus* is stated to be the third recorded species of the Cichildea which is found in Iraq beside the other two types, *Coptodon zillii and O. aureus* [5].

Fish muscles, when analyzed chemically, consist of several components, including protein, lipid, moisture (water), ash (mineral elements) and

vitamins [6]. Fish protein is characterized by being highly nutritional value because it contains all the amino acids. That are limited in other animal and plant proteins such as methionine and lysine [7] .

Protein concentration can be estimated by using a number of modern methods, including the Lowry method, the Brad Ford method, and BCA assay,The choice of the appropriate method depends on the type of protein extracted and its content of amino acids and the components of the extraction Buffer. In this study, the BCA method was used to estimate the protein concentration in the two types of muscle. This method is advantageous in that it does not interact with as many contaminants and Buffer components as the Folin-Ciocalteu reagent, especially detergents. Components that interfere with the BCA assay either lead to the reduction of Cu2+ or copper chelators (e.g., EGTA). Generally, these are not critical components of buffers and can be easily removed or omitted prior to the assay [8]. This assay also used to estimate the least amount of protein. Therefore, the current study aims to estimate the protein concentration using the BCA assay (named after the presence of Bicinchoninic acid) and the effect of fish weight on protein concentration rates in the two muscle types in addition to studying the correlation coefficient of fish length with the amount of protein concentration in two different regions of two species of bony fish

Materials and Methods:

Sampling

Seventy-two fishes were collected from *C. carpio* and *O. niloticus* with length ranging between 150-250 mm and weight 100-300 g. The samples were collected between October 2019 and February 2020 from Karmat Ali river- north of Basrah by using gill nets with different sizes, then the samples were transported to the Chordata lab in the Department of Biology - College of Education for Pure Sciences - University of Basrah ,two different regions in studied species body were determined R1 and R2 (Figs. 1 and 2) and separated red and white muscles of each R2 and R1 region separately.

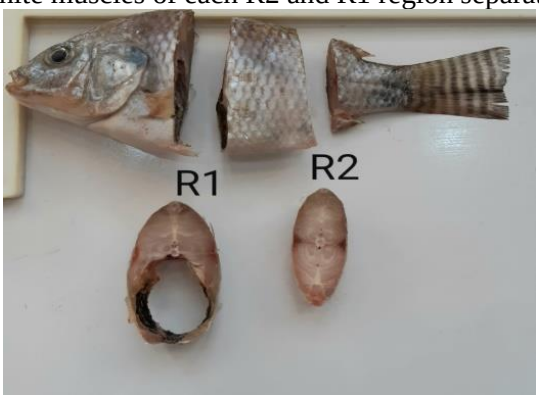

Figure 1. R1 and R2 region *in O.niloticus*

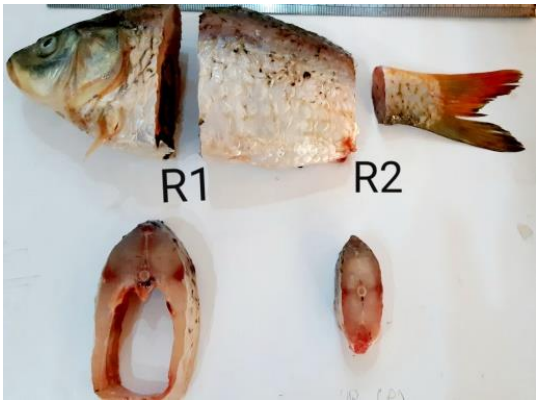

Figure 2. R1 and R2 region in *C. carpio*

Protein extraction Method

The protein was extracted using the Protein Extraction Buffer prepared by the Iraqi Company for Biotechnology. The extraction Buffer consists of 50 mM 4-(2-hydroxyethyle) -1-piperazineethanesulfonic acid (HEPES,pH 7.5), 150mM NaCl, 10mM Dithiothreitol (DTT), 1mM Polymethylsulfinyl fluoride (PMSF), 1mM Ethylenediaminetetraacetic acid (EDTA) and 1% (w/v) Sodium dodecyl sulphate(SDS).

The protein was extracted with the following steps:

1. 0.5 g weight is taken from each red and white muscles and mashed well with the addition of 1 ml of the Protein Extraction Buffer.
2. The mixture is transferred to liquid nitrogen and immersed 3 times for 5min.
3. It is let at room temperature for 20 min then put in a cooler centrifuge 18000 xg for 20 min.

Calculate of protein concentration

To calculate protein concentration a special BCA-assay kit is used from Thermo Fisher Scientific according to the following points:

1. Working Reagent (WR) was prepared by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B)
2. Different concentrations of standardized protein known as Bovin Serum Albumin are prepared
3. Adding 25µL of both the standard solution and unknown protein samples to their pits
4. Adding 200 µL working reagent to each hole and mixed well, using Plate shaker for 30 seconds.
5. The plate is covered by a plate sealer and incubated at 37oC for 30 minutes, then the plate is cooled at room temperature.
6. Measuring absorbance at a wavelength of 562 nm. Using the Eliza reader (Thermo Fisher Scientific-USA), the protein concentration of both red and white muscles is determined based on the standard curve (Fig. 3)

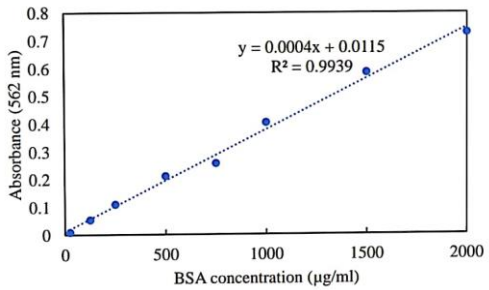

Figure 3. The standard curve for estimating protein concentration

Statistical analysis

The data was analyzed statistically by using one way ANOVA at a significant level ($p < 0.05$) according to the SPSS version 23 (Statistical Package for Social Sciences).

The current study demonstrated different protein concentrations of R1 and R2 regions in the studied species. In *O.niloticus*, Protein concentrations for red muscle in R1 and R2 region ranged between 7.4- 7.74 mg/ml and 6.8-8.85 mg/m, respectively ,while it was ranged between 173-334 mg/ml and 127-253 mg/ml for white muscles in R1 and R2 regions, respectively (Table 1).

The protein concentration values in the red muscles of *C.carpio* ranged between 7.19-10.43 mg/ml and 6.87-10.14 mg/ml in R1 and R2 regions respectively ,whereas the protein concentration values in the white muscles ranged between 98.7-250.2 mg/ml and 61.5-214.1 mg/ml for R1and R2 regions respectively (Table 1)

Results:

Table 1. protein concentration values in red and white muscles in the studied species.

Fish species	Total length (mm)	Total weight (g)	Protein conc. in R1 region Mg/ ml		Protein conc. in R2 region Mg/ ml	
			Red ± SD	White ±SD	Red± SD	White± SD
O. niloticus	150	100	7.74 ±2.05	173±40.84	6.8±1.70	127.16±23.92
	200	200	6.9±0.83	233.88±24.92	7.17±0.85	164.76±26.66
	250	300	7.47±1.85	334.05±48.54	8.85±0.86	253.16±49.63
C. carpio	150	100	7.19±1.06	98.70±6.63	6.87±0.70	61.57±4.89
	200	200	10.43±1.59	199.60±13.31	10.14±1.40	147.60±25.39
	250	300	9.10±0.86	250.12±26.16	8.41±1.24	214.18±15.51

± Standard Deviation

The results of the statistical analysis of the average protein concentration in the R1 region in *O.niloticus* showed a significant increase in the protein concentration of the white muscle with the increase in weight as the increase in the weight of the fish leads to an increase in the protein content of the white muscle, while the weight gain did not show a significant effect on the protein concentration in the red muscles of the same area, in the R2 region,. It was found that there was a significant increase in the protein concentration in the white muscles at a weight of 100 grams and 300 grams only, while the weight gain did not make a significant difference in the protein concentration in the red muscles in the mentioned area (Fig 4)

Results of analysis showed statistically for the protein concentration rates in *C. carpio* fish in the R1 region showed a significant increase in the protein content of the white muscles, as the protein concentration rates increased with the increase in fish weight, while in the red muscles, significant differences were found at 100 and 200 grams weight only while the weight gain to 300 grams was non significant difference, while in the R2 region, the results showed a significant increase in the protein content in white muscles with an increase in fish weight, whereas in red muscles, significant differences were found when increasing the weight from 100 grams to 200 grams in protein content. On the other hand the weight gain to 300 grams was not a significant difference in protein content (Fig 5)

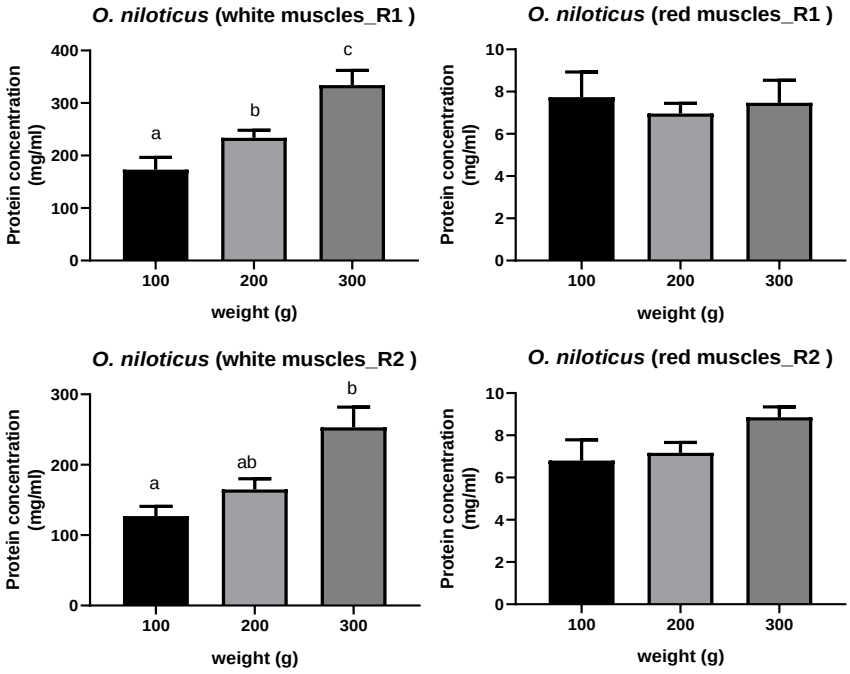

Figure 4. protein Concentration in red and white muscle in different weights of *O. niloticus*
a, b, c the different letters indicate significant differences (P <0.05) between the different groups

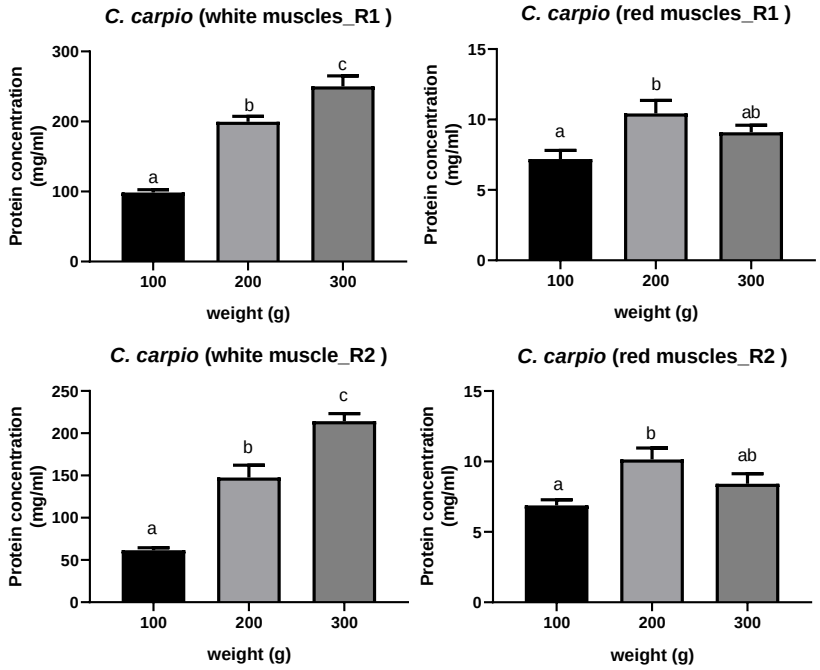

Figure 5. protein Concentration in red and white muscle in different weights of *C. carpio*
a, b, c the different letters indicate significant differences (P <0.05) between the different groups

Discussion:

The muscle tissue makes up the largest part of the body's mass in fish, there are two basic types of muscles (red and white muscles) that can be distinguished through several phenotypic and anatomical characteristics, including color, location, diameters of muscle fiber and some chemical properties. The red muscles are in the form of a superficial layer located under the skin at the side of the lateral line and is strongly associated with the dermis from through the connective tissue, it extends from the head to the caudal fin and is rich in blood supply and has a high lipid content, these

muscles used for slow and sustained swimming [9]. The white muscles occupy a space greater in the muscular tissue, low blood supply and high protein content [10]. So, the present study showed that the muscle tissue is composed mainly of red and white muscles in the studied species.

The protein distribution in fish varies by a small percentage in the head region and by an equal percentage in the dorsal and abdomen regions [11]. The protein content is very important in assessing the quality of fish muscles, as muscles with low protein tend to lose a lot of water when cooking and this spoils the texture of meat [12]. The protein content is the second highest component after moisture content [13] There are many factors that affect protein concentration in fish muscles including sex, season, nutrition, physiological condition, muscle location and type and other factors like environmental condition [14-17] so the current results show difference in protein concentration in studied body regions and also show the effect of weight on the protein content in muscles. The increase in protein concentration in white muscle was observed when fish was overweight, and this was due to the increase in muscle mass, as the study of protein content in fish is the focus of attention of many researchers, where it is possible through knowledge of protein concentration to distinguish between species and confirm their diagnosis through electrophoresis of protein extracted from the fish muscles [18]. The amount of protein extracted from the muscles depends mainly on the efficiency of the extracting solution to obtain a high percentage of protein, as the use of DTT in the extraction solutions of different types prevents the oxidation of the protein and thus ensures the acquisition of an appropriate and safe amount of protein [19]. A number of researchers also used Sodium Dodecyl Sulphate (SDS) to obtain muscle protein extract and identification of the species of fish from the separation patterns obtained on SDS polyacrylamide gel electrophoresis The electrophoretic separation patterns of the SDS extracts show that it is possible to obtain highly reproducible protein patterns which are species-specific for a wide range of commercially important species of fish, This procedure offers advantages over other methods available for the identification of the fish species [18] .The DTT and SDS combination had a clear effect on obtaining higher amounts of protein compared to use one of them in the extraction process [20].Therefore ,the present results, and through the use of the BCA assay method, showed the presence of differences between the protein concentration in the red and white muscles in the studied species, where the white muscles have a higher protein concentration than compared with the red muscles. This supports that the white muscles have a higher protein content than the red muscles, these results agree with [21]. Although the method for estimating protein varied, the protein concentration values in the red muscles ranged between 6-10 mg in studied species, these values are consistent with the study [18], which used the same method to estimate protein concentration in Twenty-five species of fish. The current results were compared with this only study because of the lack of a local symmetric study that was used BCA assay to estimate total protein concentration. For this reason, the current study is the first local study to address the BCA assay method for estimating protein concentration in red and white muscles and the effect of increasing fish weight on protein content in muscles in two types of local bony fish. Also, the results pointed that the protein concentration in the red and white muscles varies with the different studied regions, where a decrease in the protein concentration toward the caudal region in the white muscles is observed due to the decrease in the percentage of the white muscles in this region, and this is the opposite of what happens with the red muscles .The caudal region is characterized by a high percentage of red muscle and a small percentage of white muscle, muscle and less white muscle compared to the Anterior region, so the amount of fat is higher and the amount of protein is less .These results agree with many previous studies including [22-24].

Conclusion:

The study concludes that there is a variation in protein concentration rates in different body regions. It also concludes that white muscles contain a higher protein concentration than red muscles , and protein concentration in white muscle is increased when fish is overweight, these differences are due to the species, sex, growth, muscle type and its location in addition to other factors.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.

- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Basrah.

Authors' contributions statement:

NM abd-alsahb wrote a part of the manuscript and collected the samples. AJ Mansour wrote another part of manuscript and interpretation the data. SAM AL-Asadi analyzed all parameters. All Authores read the manuscript carefully and approved the final version of their MS.

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مقارنة تركيز البروتين في العضلات الحمر والبيض في نوعين من الأسماك العظمية

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الخلاصة:

تتناول الدراسة الحالية تقدير تركيز البروتين ودراسة تأثير زيادة وزن الأسماك على تركيز البروتين في العضلات الحمر والبيض في منطقتين مختلفتين (R1: المنطقة الأمامية وتبعد 2 سم خلف الرأس و R2: المنطقة الخلفية وتبعد 2 سم عن الزعنفة الذيلية) في نوعين من الأسماك العظمية ، وهما الكارب الشائع (*Cyprinus carpio*) والبلطي النيلي (*Oreochromis niloticus*) ، تم جمع العينات من نهر كرمة علي شمال البصرة في الفترة ما بين أكتوبر 2019 وفبراير 2020، وتم استخلاص البروتين بأستخدام Protein extraction buffer ، أظهرت الدراسة الحالية أن متوسط تركيز البروتين في العضلات الحمر للبلطي النيلي كان يتراوح بين(7.74-7.4)ملغم / مل و (6.8 - 8.85 ملغم / مل) في منطقة R1 و R2 على التوالي ، بينما تراوح بين (173 -334 ملغم / مل) و(127-253 ملغم / مل) في العضلات البيض في منطقة R1 و R2 على التوالي. اما في الكارب كان تركيز البروتين للعضلات الحمر بين (7.19-9.10 ملغم / مل و 6.87-8.41 ملغم / مل) في منطقتي R1 وR2 على التوالي. بينما تراوح تركيز البروتين في العضلات البيض بين 98.7-250.2 ملغم / مل)و(61.5-214.1 ملغم / مل) في منطقتي R1 و R2 على التوالي. أشارت نتائج التحليل الإحصائي لتركيز البروتين في العضلات الحمر والبيض في مناطق الجسم إلى وجود فروقاً معنوية ($P <0.05$) وفروقاً غير معنوية ($P> 0.05$) بين تركيز البروتين في العضلات الحمر والبيض في الأنواع المدروسة واستنتجت الدراسة الحالية أن العضلات البيض تحتوي على تركيز بروتيني أعلى من العضلات الحمر وأن زيادة الوزن لها تأثير معنوي على تركيز البروتين في العضلات البيض.

الكلمات المفتاحية : طريقة BCA , الكارب الأعتيادي ،عضلات الأسماك ، تركيز البروتين ، البلطي النيلي