ORIGINAL ARTICLE

Estimation of amino acids concentration in red and white muscle fibers in two types of Mugilidea fish in Basrah province, Iraq

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ABSTRACT

The current study was conducted to calculate the concentrations of amino acids in two types of Mugilidae fish. They are Planiliza klunzingeri and P. subviridis in red and white muscle fibers for two regions; The first region (R1) which is 2 cm away from the operculum and the second region (R2) that lise 2 cm near the caudal fin. The results recorded 16 amino acids, Essential amino acids(EAAs) having highest concentration in fish studied in white muscle fibers in the R1 region with a value of 4665.4 (43.9%) in P. subviridis, while the lowest value was at a concentration 1427.12 (56%) in P. klunzingeri. Branched chain amino acids (BCAAs) included (valine, isoleucine and lysine) recorded the highest value in the R2 region 1624.79 (35.4%) in P. subviridis and the lowest value in white muscle fibers in R2 with a total concentration of 182.31 (6.0%). As for the Non-essential amino acids (Non –EAAs), the highest value of 5974.41 (55.9%) was recorded in the white muscle fibers in the R1 region of P. klunzingeri and the lowest value by 1000.86 (41%) in the white muscle fibers in R2 in P. subviridis.

Keywords: Amino acid, BCAAs, Mugilidea fish, White muscles, Red muscles.

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INTRODUCTION

Fish is not important only to humans because of its high nutritional value, but it is also converted into fish powder and it is one of the most important components of the diet that poultry feed on specifically and on which humans depend in part From their diet [1], fish oil and fishmeal are the main sources of fat and protein in fish feed due to their good nutritional value and high energy [2]. Proteins are formed from the union of long chains of polymers of amino acids linked with another amino acid by a covalent bond, , as there are more than 700 amino acids in nature, but 20 of them It is only the basis for building protein inside the animal cell, which can be classified into two categories: Essential and Non-essential amino acids [3] due to the presence of important minerals and essential amino acids, considering fish as a main source of protein [4]. The amount of essential amino acids (EAAs) in the muscles of freshwater fish and marine fish is an important food source and therefore its use as a source of animal protein has spread all over the world. EAA such as: Lysine. Methionine. Cysteine. Threonine and Tryptophan are necessary for building body tissues [5]. The amino acids that are included in the composition of fish tissues and muscles are more nutritional benefit compared to other sources of amino acids such as egg proteins, milk and ruminant meat. The quantity and quality of amino acids change between species and within the same species during different stages of growth, as many studies indicated the important role of acids amino acids in animal bodies, so the content of amino acids in fish muscles must be measured to determine the optimal and best type for humans [6]. [7] indicated that the amino acids present in fish muscles are one of the most effective nutritional supplements in improving health performance, especially BCAA which is a part of the Essential amino acids and constitute 30-35% Which has an important role in building protein in the muscles as it works to reduce muscle damage and reduce pain after exercise and high physical exertion [8]. International studies have proven the important role of the amino acid leucine in building muscle protein, while isoleucine and valine work in energy production and regulation of sugar level in the

body [9] and also contribute to reducing fatigue during exercise by reducing serotonin production in the brain [10]. Some quantitative differences between the types of amino acids are related to a set of changes, especially changes in the genetic information specific to the same species and on the basis of muscle proteins that are manufactured by the body itself [11].

MATERIAL AND METHODS

Sampling

72 samples were collected for fish studied with lengths ranging between 75-275 mm and weights 60-220 gm. It collected from the Al-Faw area / Kaser AL-Amwage within Iraqi territorial waters between March and June / 2022 using seine nets with a length of 73 meters, a height of 4 meters, and an eye size of 3 x 3 cm. Sampling included the *P. klunzingeri* and *P. subviridis*. Morphometric measures of total length (1 mm) and weight (0.5 gm) were taken and divided into four groups, the first group of fish varied from 75 -124 mm, while the second group from 125 - 174 mm, and the third group's from 175-224 mm, the last group from 225-275 mm in preparation for later laboratory examinations.

Estimation of amino acids

Amino acids estimation in red and white muscles in regions R1 and R2 shown(Fig.4).fish cut into two regions and separating red muscle from white muscles in each region and for each studied fish species. 20 gm of each muscle type was taken in both regions, they were defatted and kept in airtight plastic bottles at -20°C for 24 hours [12] for conducting chemical analyzes.

Amino acid extraction process

Amino acids were extracted according to the method presented by [13]:-

1-weighing 3 gm of the sample and placing it in a 10 ml volumetric vial.

2- adding 3 ml of 6M Hydrochloric acid (HCL) with 0.1% phenol (W/V).

3- placing in a thermal oven at 105°C ± 5 °C for a period 22 hours.

4- 3 ml of sodium hydroxide and 0.1 mg of Tartaric acid were added to the sample to prevent the oxidation process in the reaction.

5- the mixture was mixed well for 15 minutes and the sample was filtered using a 0.45 um plastic filter.

6-transferred for the injection process by a High-Speed Amino Acid Analyzer LA8080 manufactured by Hitachi High Technologies.

Amino acid deriving process

1 ml of the extracted amino acids was taken, and 200 microliters of Orthophthalein aldehyde was added to it at a concentration of 5%. followed by [14] and [15].



Fig.1: sampling area

RESULTS

Results of estimating the concentration and proportions of amino acids in red and white muscles in the studied body regions (R1 and R2) in both types indicated that 16 amino acids (nine essential amino acids and seven non-essential amino acids) were recorded. The essential amino acids were Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, and Valine, whereas the non-essential amino acids were Alanine, Cysteine, Glutamic acid, Glycine, Proline, Serine, and Tyrosine.The result

revealed differences in the average values of the concentrations and percentages of amino acids in both skeletal muscles in regions R1 and R2 For the two studied types, Glutamic acid recorded the highest concentration in red muscles in the region of R1 at 1023.27 (23.4%) mg/100 g, while Leucine and Threonine recorded the lowest concentrations at 0.0 (0.0%) mg/100 g in *P. klunzingeri* (Table1).Serine and Tyrosine acids were found to have the highest concentrations, and the lowest values were 1585.01 (30.9%) and 96.50 (1.9%) mg/100 g in *P. klunzingeri*, respectively. In contrast, Valine and Arginine were found to have the highest and lowest concentrations in*P. subviridis*, with values of 1023.44 (13.7%) and 55.60 (1.2%) mg/100 g, respectively.

The highest and lowest concentrations of amino acids in white muscle were found in the R1 region, with Serine acid recording the highest concentration at 2144.99 (20.2%) mg/100 g and Phenylalanine recording the lowest concentration at 255.34 (2.4%) mg/100 g in *P.klunzingeri* (Table 1) and Fig (5), while Glutamic acid and Threonine recorded the highest and lowest concentrations at 3838.64 (43.5%) and (Fig. 6),While Arginine 104.04 (4.3%) mg/100 gm and Tyrosine 409.16 (16.8%) mg/100 gm, respectively in *P. subviridis* fish (Table 1). In the second region R2, the white muscles had the highest and lowest concentration of Methionine 850.26 (28.1%) mg / 100 g and Glycine at a concentration of 49.36 (1.6%) mg / 100 g in *P. klunzingeri* (Table 1), while Tyrosine was recorded as 409.16 (16.8%) mg/100 gm and Arginine 104.04 (4.3%) mg/100 gm in *P. subviridis*.

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Amino acids	Red muscle fibers in R1		Red muscle fibers in R2		White muscle fibers in R1		White muscle fibers in R2	
	mg∖ml	%	mg∖ml	%	mg∖ml	%	mg∖ml	%
			Essential am	ino acids				
Arginine(Arg)	263.05 ± 9.2	5.3	55.60 ± 11.2	1.2	494.70 ± 9.1	5.6	104.04±5.6	4.3
Histidine(His)	0.00	0.0	0.00	0.0	560.00±28.3	6.3	185.31±14.1	4.6
Isoleucine(Ile)	0.00	0.0	274.82±20.3	6.1	487.33±12.0	5.5	157.73±5.8	6.5
Leucine(Leu)	335.25 ± 20.1	6.7	321.53 ± 21.2	7.0	0.00	0.0	145.67±2.4	6.0
Lysine(Lys)	637.54 ± 15.2	12.8	0.00	0.0	0.00	0.0	0.00	0.0
Methionine(Met)	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
Phenylalanine(Phe)	175.77 ± 9.5	3.5	809.92±17.2	17.6	1151.82±32.2	13.1	318.25±6.2	13.1
Threonine(Thr)	250.04 ± 7.3	5.0	123.56±11.4	2.7	239.84 ± 6.7	2.7	113.14±7.1	4.6
Valine(Val)	680.39 ± 22.7	13.7	1028.44±33.1	22.3	356.77 ± 4.9	4.0	402.98±3.9	16.5
$\sum EAA$	2092	47	2613.87	56.9	3290.46	37.2	1427.12	55.6
$\sum BCAAs$	1015.64 2	20.4	1624.79	35.4	844.1	9.5	706.38	29
	Non - Essential am	nino acid	ls					
Alanine(Ala)	377.72 ± 13.2	7.6	157.57±13.8	3.4	0.00	0.0	0.00	0.0
Cystine(Cys)	589.93 ± 7.3	11.6	805.78 ± 5.1	17.5	461.15±14.2	5.2	379.63±13.	15.6
Glutamic acid(Glu)	316.77 ± 11.6	6.4	219.61 ± 9.2	4.8	3838.69±33.	43.5	212.07±9.3	9.1
Glysine(Gly)	590.64 ± 4.5	11.9	263.88±11.9	5.7	0.00	0.0	0.00	0.0
Proline(Pro)	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
Serine(Ser)	515.29 ± 10.5	10.3	245.83±13.8	5.3	0.00	0.0	0.00	0.0
Tyrosine(Tyr)	246.69 ± 5.1 5	5.0	295.35 ± 4.2	6.4	602.65±15.1	6.8	409.16±2.8	16.8
$\sum Non-EAA$	2637.04 5	52.8	1988.02	43.1	4902.49	55.5	1000.86	41.5

Table (1): Concentration and percentages of amino acids in red and white muscle fibers in R1 and R2 in *P. klunzingeri*

±.....SD

 Σ EAA: refers to the sum of the concentrations of essential amino acids.

 $\overline{\Sigma}$ BCAAs: Refer to the sum of the essential branched chain amino acids (Isoleucine, Leucine, Valine). Σ Non-EAA: refers to the sum of non-essential amino acids.

Current results showed the difference in the values of the concentrations of (BCAAs), which included each of the amino acids (Isoleucine, Leucine and Valine) in red and white muscles in both regions R1 and R2 and for the two studied species, BCAAs recorded a total concentration of 496.97 (9.9%) mg\100 g and 1037.34 (20.2%) mg\100 gm in red muscles in R1 and R2 in red muscles in *P. klunzingeri*, (Table 1), while its total concentration was in white muscles For regions R1 and R2 it is 644.75 (6.1%) and 182.31 (6.0%) mg / 100 g, respectively (Table 1), whereas in *P. subviridis* the BCCAs recorded a total concentration of 1015.64 (20.4%) and 1624.79 (35.4%)) mg / 100 gm in the red muscles in regions R1 and R2 respectively , whilst it recorded a total concentration of 844.1 (9.5%) and 706.38 (29%) mg / 100 gm in the white muscles and for the regions R1 and R2 respectively (Table 2). Results showed that the Non-essential amino acids had higher total concentrations in *P. klunzingeri* compared to their total concentrations in *P. subviridis* fish for the red and white muscle types and for the two studied body

regions R1 and R2 as shown in tables (1,2), which recorded a total concentration of 2497.06 (52.5%) and 3179.54 (62.2%) mg / 100 g in red muscles in the regions R1 and R2 in *P. klunzingeri* (Table 1), while it was 2637.04 (52.8%) and 1988.02 (43.1%) mg / 100 g in the two regions R1 and R2 in P. subviridis respectively (Table 21), while total concentrations were recorded in white muscle and R1 and R2 regions in P. klunzingeri, which were estimated at 5974.41 (55.9%) and 1393.63 (46.1%) mg/100 g, respectively, while values of 4902.49 (55.5%) mg / 100 gm in R1 and 1000.86 (41.5%) mg / 100 gm in R2 were recorded in *P. subviridis* (Table 2). The statistical results of the differences recorded between the concentrations of essential, nonessential and branched amino acids in the red and white muscles and for the regions R1 and R2 and for the two studied species, the statistical results gave significant differences P<0.05 and in-significant differences P>0.05 when the results were statistically analyzed, significant differences were recorded P< 0.05 when analyzing the results of the concentrations of EAAs in white muscle in R1 and R2 and their concentrations in red and white muscle in R1 region (Table 3), and concentrations of Non-EAAs in white muscle in R1 and R2 in *P. klunzingeri* (Table 4), While no significant differences (P>0.05) were recorded when analyzing the results of the concentrations of EAAs, Non-EAAs and BCAAs in red muscle fibers in P. klunzingeri and red and white muscle fibers in P. subviridis and for the region R1 and R2 as shown in Tables (3,4,5,6,7,8).

Table (2): Concentration and percentages of amino acids in red and white muscle fibers in R1 and
R2 in <i>P. subviridis</i>

Amino acids Red muscle fib R1			Red muscle fib R2	ers in	White muscle fibers in R1		White muscle fibers in R2	
	mg∖ml	%	mg∖ml	%	mg∖ml	%	mg∖ml	%
	Essential amino	acids						
Arginine(Arg)	709.50±22.3	14.1	178.33±11.4	3.5	510.81±1.9	4.8	164.69±10.5	5.4
Histidine(His)	605.67±20.4	12.1	367.65 ± 3.5	7.2	1173.50±3.4	11.0	95.23 ± 4.5	3.1
Isoleucine(Ile)	87.34 ± 6.2	1.7	181.86 ±1.5	3.5	296.46±1.5	2.8	0.00	0.0
Leucine(Leu)	0.00	0.0	147.48 ± 2.3	2.9	348.29±5.8	3.3	182.31 ± 3.1	6.0
Lysine(Lys)	205.72 ± 8.2	4.1	212.42 ± 4.9	4.1	1335.41±11.1	12.6	219.46 ± 6.7	7.3
Methionine(Met)	420.18 ± 1.5	8.4	0.00	0.0	745.59±21.7	7.0	850.26 ± 4.2	28.1
henylalanine(Phe)	100.06 ± 4.8	2.0	158.50 ± 3.9	3.1	255.34±10.5	2.4	120.61 ± 1.0	4.0
Threonine(Thr)	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
Valine(Val)	409.63 ± 8.1	8.2	708.06 ± 7.2	13.8	0.00	0.0	0.00	0.0
$\sum EAA$	2538.1	50.6	1954.3	38.1	4665.4	43.9	1632.56	53.9
$\sum BCAAs$	496.97	9.9	1037.34	20.2	644.75	6.1	182.31	6.0
	Non - Essential an	nino ac	ids					
Alanine(Ala)	427.07±27.4	8.5	305.51 ± 1.5	5.9	434.56±16.3	4.1	74.40 ± 3.8	2.5
Cystine(Cys)	375.34±17.2	7.5	410.59 ± 1.8	8.1	1577.52±28.3	14.5	505.95±32.0	16.7
Glutamic acid(Glu)	1023.27±33.5	23.4	626.33 ± 2.0	12.4	370.13 ± 8.1	3.5	483.82 ± 4.9	16.1
Glysine(Gly)	0.00	0.0	155.60 ± 1.9	3.0	885.44 ± 5.4	8.3	49.36 ± 5.2	1.6
Proline(Pro)	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0
Serine(Ser)	493.96 ± 8.1	9.6	1585.01±2.2	30.9	2144.99±22.	20.2	206.42 ± 1.7	6.8
Tyrosine(Tyr)	177.42 ± 9.2	3.5	96.50 ± 5.5	1.9	561.77 ± 3.4	5.3	73.68 ± 1.0	2.4
$\sum Non - EAA$	2497.06	52.5	3179.54	62.2	5974.41	55.9	1393.63	46.1

±.....SD

 \sum EAA: refers to the sum of the concentrations of essential amino acids.

 Σ BCAAs: Refer to the sum of the essential branched chain amino acids (Isoleucine, Leucine, Valine). Σ Non-EAA: refers to the sum of non-essential amino acids.

Table (3): The concentration of EAAs in red and white muscle fibers in R1 and R2 in
P.klunzingeri

The characteristics researched	T-test	Sig
Concentrations of amino acids in red muscle fibers in R1 and R2	-0.50	0.961
Concentrations of amino acids in white muscle fibers in R1 and R2	2.877	0.007*
Concentrations of amino acids in red and white muscle fibers in R1	-2.047	0.049*
Concentrations of amino acids in red and white muscle fibers in R2	1.145	0.261

*: Indicates that there are significant differences

Table (4): The concentration of Non-EAAs in red and white muscle fibers in R1 and R2 in*P.klunzingeri*

The characteristics researched	T-test	Sig
Concentrations of amino acids in red muscle fibers in R1 and R2	-0.298	0.780
Concentrations of amino acids in white muscle fibers in R1 and R2	1.490	0.211
Concentrations of amino acids in red and white muscle fibers in R1	-0.298	0.780
Concentrations of amino acids in red and white muscle fibers in R2	1.490	0.211

Table (5): The concentration of BCAAs in red and white muscle fibers in R1 and R2 in *P.klunzingeri*

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The characteristics researched	T-test	Sig
Concentrations of amino acids in red muscle fibers in R1 and R2	-0.298	0.780
Concentrations of amino acids in white muscle fibers in R1 and R2	1.490	0.211
Concentrations of amino acids in red and white muscle fibers in R1	-0.298	0.780
Concentrations of amino acids in red and white muscle fibers in R2	1.490	0.211

Table (6): The concentration of EAAs in red and white muscle fibers in R1 and R2 in *P. subviridis*

The characteristics researched	T-test	Sig
Concentrations of amino acids in red muscle fibers in R1 and R2	0.236	0.815
Concentrations of amino acids in white muscle fibers in R1 and R2	1.504	0.143
Concentrations of amino acids in red and white muscle fibers in R1	-0.823	0.417
Concentrations of amino acids in red and white muscle fibers in R2	1.534	0.136

Table (7): The concentration of Non-EAAs in red and white muscle fibers in R1 and R2 in *P. subviridis*

The characteristics researched	T-test	Sig
Concentrations of amino acids in red muscle fibers in R1 and R2	0.747	0.470
Concentrations of amino acids in white muscle fibers in R1 and R2	1.039	0.319
Concentrations of amino acids in red and white muscle fibers in R1	-0.602	0.559
Concentrations of amino acids in red and white muscle fibers in R2	1.191	0.257

Table (8): The concentration of BCAAs in red and white muscle fibers in R1 and R2 in *P. subviridis*

The characteristics researched	T-test	Sig
Concentrations of amino acids in red muscle fibers in R1 and R2	0.552	-0.649
Concentrations of amino acids in white muscle fibers in R1 and R2	0.273	0.798
Concentrations of amino acids in red and white muscle fibers in R1	0.234	0.827
Concentrations of amino acids in red and white muscle fibers in R2	1.187	0.301

DISCUSSION

Amino acids are defined as biologically important organic compounds that are obtained mainly from proteins in the diet, as they are considered one of the most important types of natural compounds for their participation in many basic physiological processes in which more than 20 types of amino acids participate, and one of these processes is building Peptides and proteins are also essential for fish as energy sources, endogenous protein synthesis and regulation of metabolic pathways [16]. Dietary protein quality is assessed from the ratio of essential amino acids to the ratio of non-essential amino acids, however the concept of functional amino acids (FAAs) has been proposed which are those amino acids that participate and regulate key metabolic pathways to improve health, survival, growth and muscular development for the body of The organism, which works to reduce metabolic diseases (such as obesity, diabetes and cardiovascular disorders) [17]. The main and important sources of essential amino acids include animal proteins like eggs, fish meat and poultry meat [18]. Many previous studies indicated the difference in the concentrations of amino acids in the muscles and organs of the fish body such as study [19] on 12 types of freshwater fish, in which the presence of 21 amino acids was recorded, and study [20] on the Cyprinus carpio in which 19 types of amino acids were recorded and study [21] on Cyprinus carpio, as the results of the current study agree with previous studies in the different concentrations of amino acids in The two types of muscle for the two species studied, as the results of the current study indicated the presence of 16 amino acids (nine essential amino acids and seven non-essential amino acids) in the

muscle fibers red and white and for the two studied species, which differ in the importance and role of each amino acid from the other amino acid depending on the degree of its solubility and type Electric charge [22] ,This difference is attributed to the great role of high concentration acids and their role in muscle fibers, the difference in type, protein levels, functional and physiological status of fish, genetic status, and external factors represented by feeding habits, salinity and pH [20].

BCAAs are part of the essential amino acids, that prevent Muscle damage and pain reduction after exercise and high physical exertion, as it is believed to play a key role in the production of energy in the muscles, as previous studies showed that the amino acid (Valine) has a significant role in muscle growth and giving muscles (fish meat) a distinctive flavor and addition taste for human consumption [23]. the current results showed an increase in the value of the total concentration of (BCAAs) in red muscle fibers in the R2 region and highly for the two studied species and this increase is due to the increase in the values of the concentrations of (BCAAs) in one region and its decrease in the region that can be attributed to many factors, the most important of which are differences in genus, muscle function, protein intake, feeding habits, food quality, environmental conditions and health status. In addition, the protein concentration plays a key role in the different values of (BCAAs), as this increase in the values of the concentrations of (BCAAs) in the R1 region in white muscles is due to the high amount of protein, [24] indicated that the increase in protein concentration results from an increase in the size and area of muscle fibers, while the concentration of (EAAs) decreases in the R2 region and the concentration of (BCAAs) increases, which reflects the importance of these (BCAAs) in providing the muscles with the necessary energy and compensating muscle fibers Damaged and reduce fatigue and effort to enable the fish to achieve its vital activities easily [25], as a result of the fish's dependence entirely on red muscle fibers in the production of slow and continuous movements for long periods during swimming [26]. Nonessential amino acids are considered part of amino acids that the human body cannot manufacture, but rather are formed from essential amino acids from food or as a result of the process of breaking down proteins [27], as the results of the current study showed an increase in the concentration of non-essential amino acids. The main reason is in the white muscle fibers in the R1 region for both species of study is due to the high amount of protein, and since the non-essential amino acids result from the breakdown and decomposition of proteins, so the reason for its rise in this region is due to the functional role of white muscles, [28] referred to the functional role of white muscle fibers in producing fast and sudden movements that require high energy, while the value of the total concentration in red muscle fibers decreases as a result of the over powering of (BCAAs)[9].

CONCLUSIONS

- 1. Through the results of amino acid concentration, we can conclude that white muscle fibers have greater concentrations compared to red muscle fibers in the two types studied.
- 2. Through the functional role of the (BCAAs) (valine, isoleucine and leucine) they had a great role in the growth process of both types of muscle fibers.

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