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# Changes in Amino Acids Content in Muscle of European Eel (*Anguilla anguilla*) in Relation to Body Size

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***Abstract*—**European eels (*Anguilla anguilla*) belong to Anguilliformes order and Anguillidae family. They are generally classified as warm-water fish. Eels have a great commercial value in Europe and Asian countries. Eels can reach high weights, although their commercial size is relatively low in some countries. The capture of larger eels would facilitate the recovery of the species, as well as having a greater number of either glass eels or elvers for aquaculture. In the last years, the demand and the price of eels have increased significantly. However, European eel is considered critically endangered by the International Union for the Conservation of Nature (IUCN) Red List. The biochemical composition of fishes is an important aspect of quality and affects the nutritional value and consumption quality of fish. In addition, knowing this composition can help predict an individual's condition for their recovery. Fish is known to be important source of protein rich in essential amino acids. However, there is very little information about changes in amino acids composition of European eels with increase in size. The aim of this study was to evaluate the effect of two different weight categories on the amino acids content in muscle tissue of wild European eels. European eels were caught in River Ulla (Galicia, NW Spain), during winter. The eels were slaughtered in ice water immersion. Then, they were purchased and transferred to the laboratory. The eels were subdivided into two groups, according to the weight. The samples were kept frozen (-20 °C) until their analysis. Frozen eels were defrosted and the white muscle between the head and the anal hole. was extracted, in order to obtain amino acids composition. Thirty eels for each group were used. Liquid chromatography was used for separation and quantification of amino a cids. The results conclude that the eels are rich in glutamic acid, leucine, lysine, threonine, valine, isoleucine and phenylalanine. The analysis showed that there are significant differences ($p < 0.05$) among the eels with different sizes. Histidine, threonine, lysine, hydroxyproline, serine, glycine, arginine, alanine and proline were higher in small eels. European eels muscle presents between 45 and 46% of essential amino acids in the total amino acids. European eels have a well-balanced and high quality protein source in the respect of E/NE ratio. However, eels with higher weight showed a better ratio of essential and non-essential amino acid.

***Keywords*—**European eels, amino acids, HPLC, body size.

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## I. Introduction

THE European eel is a species considered catadromous, it spends part of its life in rivers, in a stage of growth and another, in the sea, where they migrate to spawn [1]. Eels have a great commercial value in Europe (mainly Spain, Portugal, Italy and Netherlands), and Asian countries (mainly Japan, China, Korea and Taiwan,). The female can reach maximum size of 137 cm and maximum weight of 9 kg, while the males are smaller, reaching maximum size of 51 cm and maximum weight of 2.8 kg. However, commercial size is usually between 150 and 250 g, depending on the country [23]. The most required specimens are those of small or medium size.

The population of eels has fallen drastically, due to the different risks that threaten their survival, being, the main ones, the increase of the contamination of human origin, the environmental impacts by means of the construction of diverse obstacles in the rivers such as the hydroelectric dams that prevent that they manage to overcome rivers and overfishing, among others [2]. European eel can be found in all the watersheds from the Iberian Peninsula; however, the presence of obstacles caused that the distribution of the eel is reduced to the coastal regions.

The nutritional composition in European eels thus provides important insights into the physiological and energetic status of fish, which can help predicting an individual's condition for their recovery. European eels are an important source of proteins, fat, vitamins, and mineral elements.

The composition of fish varies depending on various factors such as age, sex, species, environment, feeding, season and migratory behavior that occur during the time of capture [3]. Fat is the most variable compound; however, protein is usually a most constant constituent [4].

Fish proteins have a high biological value because their content in essential amino acids, which must be provided in the diet because of humans are not proficient of producing them [5], [6]. Proteins are very important in biological systems since they are present in diverse forms such as structural elements, enzymes, antibodies, etc. and they are essential in specific biological functions such as the provision of essential amino acids.

Amino acids are a heterogeneous group of biomolecules that have common structural and functional characteristics. They are also precursors for synthesis of important substances such as peptide, nucleotides hormones, and neurotransmitters.

In recent times, a new concept was proposed, functional amino acids, which participate and regulate metabolic

pathways for maintenance, improvement of health, growth and reproduction. Within these would be arginine, cysteine, leucine, methionine, tryptophan, tyrosine, aspartate, glutamic acid, glycine, proline and taurine [7]. Amino acids are mainly obtained from proteins in diet. They can be classified as non-essential (NSA), if they are synthesized by our organism, or as essential (AE) if they must be contributed through the diet [8].

Fish is a source of protein rich in essential amino acids, principally lysine, which is in small amounts in other foods such as cereals. On the other hand, sensory properties of fish meat are in part depended on the amino acid distribution. Therefore, it is important to know the protein and amino acid content of fish.

In general, amino acid composition of the fish shows important differences among fish species, sexes, sizes, seasons, and geographical localities. However, scientific reports about variations in amino acids composition of European eels with increase in size. The aim of this study was to evaluate the effect of two different size categories on the amino acids content in muscle tissue of wild European eels.

## II. Methodology

### A. Samples

European eels were caught in River Ulla (Galicia, NW Spain), during the authorized fishing season (winter). The eels were transported to tanks that were connected to freshwater recirculation modules until their slaughter. The eels were slaughter in ice water immersion. Then, they were obtained and transported to the laboratory.

The eels were classified based on weight into two different groups: between 10 and 200 g (A) and more than 200 g (B). The fishes were eviscerated, washed, packed in vacuum bags, and stored at -20 °C until their analysis. Frozen eels were defrosted at temperature of 4 ± 2 °C in a refrigerator. The head, the skin, and the thorn were removed for the analysis. The portion used was the muscle tissue between the head and the anal hole.

### B. Amino Acid Analysis

The hydrolysis of the proteins was carried out by Franco et al. [9] method. The identification and quantification of amino acids were carried out by HPLC, using the conditions described by Alonso, Alvarez, and Zapico [10], with some minor modifications. The liquid chromatography equipment consisted of Termo Finnigan chromatograph with UV/VISIBLE detector by photodiode matrix Spectrasystem UV6000LP. The column used was a reversed phase $C_{18}$ Ultrasphere 5–ODS (diameter of 4.6 mm and 25 cm of length) (Beckman, Fullerton, EE.UU). The temperature of the column was controlled to 50 ± 1 ºC with a column heater (Spectrasystem 3000). The wavelength of the detector was at 254 nm. Standards of the 17 different amino acids were supplied by Sigma Chemical Co. (St Louis, MO): alanine (Ala), arginine (Arg), aspartic acid (Asp), cystine (Cys), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), valine (Val), hydroxyproline (Hyp).

The separation chromatogram of the amino acids is presented in Fig. 1.

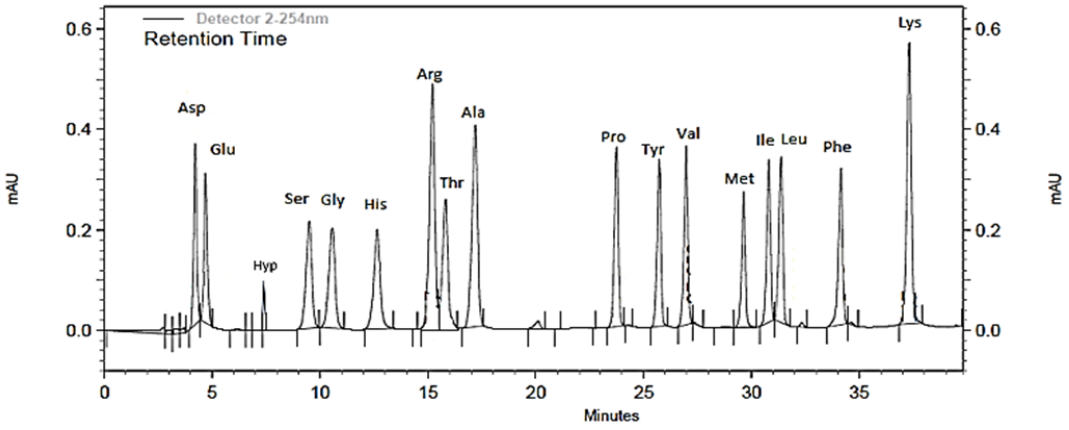


Fig. 1 Chromatogram of standards amino acids

All the samples and standards were injected at least in duplicate.

Repeatability tests were performed by injecting a standard and a sample consecutively six times in a day. Reproducibility tests were also carried out by injecting the standard and the sample twice a day for three days under the same experimental conditions. Significant differences ($P < 0.05$) were not found between the results obtained in these tests.

### C. Statistical Analysis

All analyses have been done at least in triplicate. The statistical treatment of the data was carried out by analysis of variance (ANOVA). The least squares test (LSD) was applied for a 95% confidence interval ($p \leq 0.05$) for the comparison of the mean values, using the statistical software Statistica version 7.1 of Statsoft © Inc. (Tulsa, OK, USA). Mean values and standard deviations were used.

## III. Results

The method used in this study allowed the analysis of 17 amino acids. Fig. 2 showed the chromatogram of the amino acids in eels. Amino acids composition of the eels samples are listed on Fig. 3.

Glutamic acid was the most abundant amino acid, compared to other amino acids, following by arginine, alanine and aspartic acid, with similar values. There was not variation in the content of these amino acids of eels with different size.

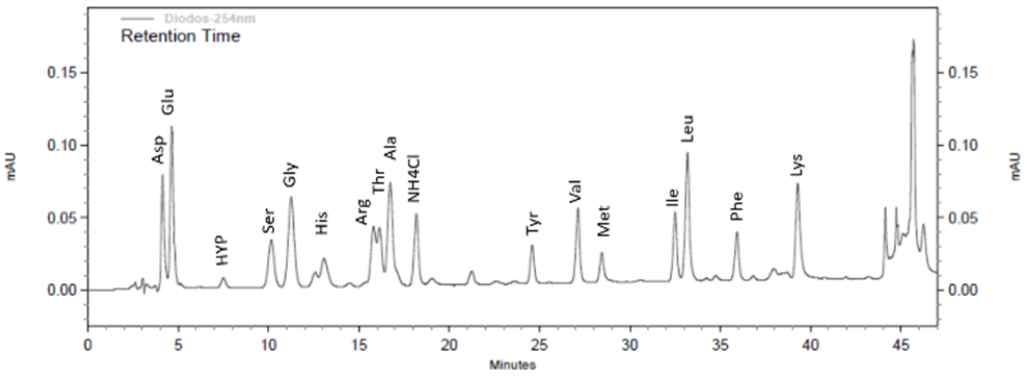


Fig. 2 Chromatogram amino acid content in muscle eel.

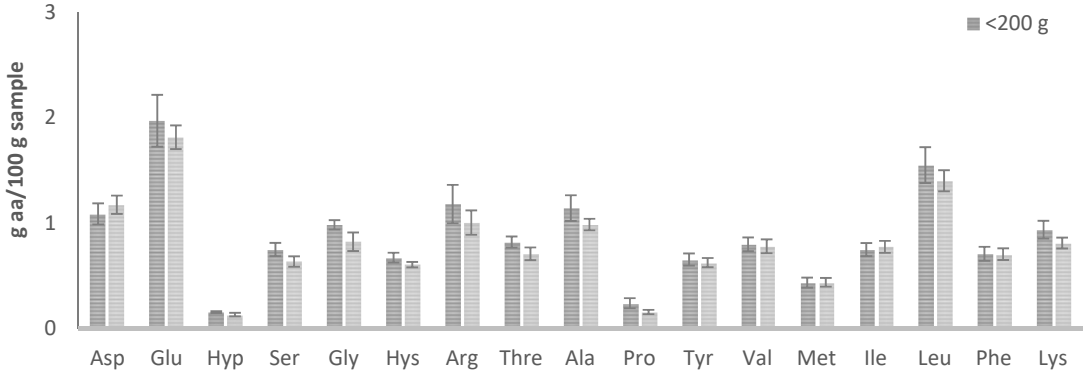


Fig. 3 Amino acid content in eels with different size

Proline (0.15-0.24 g/100 g sample) and hydroxyproline (0.13-0.15 g/100 g sample) were the lowest in eels.

Significant differences ($p < 0.05$) were obtained in the quantities of some amino acids, in dependence on fish weights. Comparing small (< 200 g) and large eels (> 200 g), the small eels contain higher levels of histidine, threonine, lysine, hydroxyproline, serine, glycine, arginine, alanine and proline. Eels with higher weight showed lower levels of total essential and non essential amino acids than smaller eels.

Total amino acids (AA), essential (EA) and non-essential (NEA) amino acid of the eel samples are recorded in Fig. 4.

The total essential amino acids ranged from 6.23 to 6.65 %, while total non-essential amino acids content ranged from 7.38 to 8.16 %.

The ratio between essential and non-essential amino acids (EA/NEA) (Fig. 4) is an index to define the quality of the protein. The EA/NEA ratio has been found to be between 0.81 and 0.84.

The present study shows the presence of a better amount of essential and non-essential amino acids in the longer eel profile. These results obtained showed that European eels have a well-balanced and high quality protein source in the respect of E/NE ratio.

The different amino acids patterns in eels with different sizes suggest different amino acid nutrition requirements. This is important to detect quality and quantity of amino acids in diets in cultivated European eels.

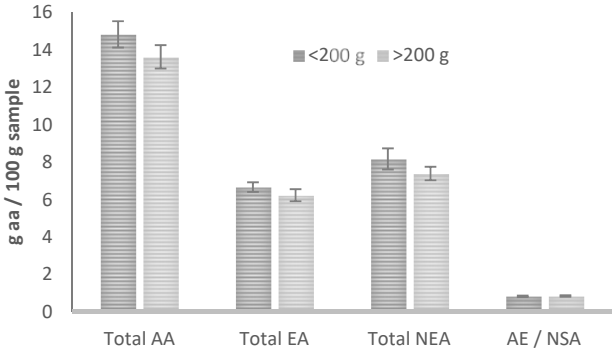


Fig. 4 Total essential and non-essential amino acid of eels

Many investigators have published analysis of fish composition, but few have examined the changes in amino

acids composition in relation to weight.

These results are in agreement with the finding of other studies on different fish species [11]-[19], and with the data given in different databases for the eel (Table I).

Many investigators have published analysis of fish composition but few have examined the changes in amino acids composition in relation to size. Some authors have observed different amino acids profiles between juvenile and adult fish. For example, Ng and Hung [20] point out that different amino acids in the whole body of white sturgeon differed among different sizes. Toppe et al. [21] detected differences between small and large herring (large herring contain lower levels of protein and different amino acids than small fish). Sankar et al. [22] observed that protein content was high in anchovy with medium sized fish, and that essential amino acid content was higher in anchovy with small and medium size compared to larger fish.

TABLE I
AMINO ACID CONTENT OF THE EEL (G/100 G) ACCORDING TO DIFFERENT DATABASE

| | USDA | BDA |
|---|---|---|
| Arg | 1.10 | 0.71 |
| His | 0.54 | 0.35 |
| Ile | 0,85 | 0.54 |
| Leu | 1.50 | 0.96 |
| Lys | 1.69 | 1.08 |
| Met | 0.55 | 0.35 |
| Phe | 0.72 | 0.46 |
| Thr | 0.81 | 0.52 |
| Tyr | 0.62 | 0.4 |
| Val | 0.95 | 0.61 |
| Trp | 0.21 | 0.13 |
| Cys | 0.20 | 0.13 |
| Glu | 2.75 | 1.76 |
| Gly | 0.89 | 0.57 |
| Pro | 0.65 | 0.42 |
| Ala | 1.12 | 0.72 |
| Asp | 1.89 | 1.21 |
| Ser | 0.75 | 0.48 |

USDA: United States Department of Agriculture. BDA: Banca Dati di Composizione, Italy.

## IV. CONCLUSION

The findings of our research showed that European eels are a source of high quality protein, with a well-balanced composition of amino acids. Body size influences amino acid content in European eels.

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