

The Analysis of Protein Level and Amino Acid Profile in Eels (*Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor*) of Lake Poso

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Abstract: In this study used a sample of eel fish species *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* from Poso lake that has a high protein content and is a fish endemic to Central Sulawesi. These eels are consumed by the surrounding community of the lake, but the nutritional content remains unknown. The present research was aimed to determine the protein level and amino acid profile of *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* eels. The protein level testing used Kjeldahl method, and amino acid profile used High Performance Liquid Chromatography. The results demonstrate the protein level of the two samples *Anguilla marmorata* (Q.) Gaimard eels have a protein content of 41.84% and *Anguilla bicolor* eels at 33.75%. *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* eels contain 18 types of amino acid, comprised of 9 types of essential amino acids and 9 types of non-essential amino acids. Of the two samples of eel species *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor*, have complete protein quality because it has all kinds of essential amino acids.

Keywords: Amino acid, *Anguilla bicolor*, *Anguilla marmorata*, Protein.

INTRODUCTION

Indonesia has abundant diversity in fish species, including 8,500 and 1,300 species live in freshwater ecosystem [1]. One region in Indonesia with plenty of endemic freshwater fish species is Sulawesi. Endemic fish is fish species found in particular areas, such as rivers, lakes, islands, countries, and continents [2].

Central Sulawesi is a region with vast freshwater areas, one of which is Lake Poso. In general, there are several species of in Lake Poso, including eels (*Anguilla* sp). There are five (5) eel species found in Lake Poso, namely *Anguilla marmorata* (Q.), Gaimard, *Anguilla celebensis*, *Anguilla interioris*, *Anguilla bicolor*, dan *Anguilla borneoensis* [3]. *Anguilla marmorata* and *Anguilla bicolor* are the two species largely found and used as subsistence by the community living in banks of Lake Poso. This fish is very popular with the people due to their tastiness, and they are easy to find.

As food, fish is foodstuff with considerable protein level. The nutritional value of fresh fish consists of 17% protein, minerals and 2.5% to 4.5% vitamins. Fresh fish as protein source has advantages in comparison with protein sources from other materials; fish protein amino acid is particularly rich in lysine [4].

To date, there are no protein analysis publications or research results on the nutritional level of *Anguilla marmorata* and *Anguilla bicolor* eels from Lake Poso. The nutritional analysis of eels is highly needed, especially its protein level and its composition or amino acid profile. Based on these findings, there is a possibility of similar protein level between cultivated eels and Central Sulawesi's endemic eels. In order to prove the hypothesis, it is necessary to conduct analysis of protein level and amino acid profile in *Anguilla marmorata* and *Anguilla bicolor* eels from Lake Poso.

MATERIALS AND METHODS

Materials

Materials used in this research include: distilled water, sulfuric acid (H_2SO_4) acid, boric acid (H_3BO_3), phenolphthalein indicator, hydrochloric acid (HCl) 0.2 N, sodium chloride (NaCl), sodium hydroxide (NaOH), AccQ-Fluor Borate, Kjeldahl tablets, Fluor Reagent A, and standard solution of amino acids.

Methods

Samples Technique

The samples used were from Lake Poso, sampling was carried out using *purposive sampling* method is a sample determination technique by choosing a sample among the population according to what the researcher wanted, ie based on the type of fish [5]. The types of

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samples taken were Eel fish *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* from Poso lake.

Sample Preparation

Sample preparation stage was begun with washing the sample using running water to clear the slime, and then the internal organs (viscera and gills) were rewashed. After the sample was cleaned, filleting stage began, where stainless steel knife was used to dissect them. The objective was to separate the bones from the flesh. The next process was weeding. It was done by pulling out the bones attached to the flesh. And the last stage was done by cutting off samples into small size and it was ready for analysis [6].

Protein Analysis

The sample was weighed by 0.5 grams and subsequently inserted into a 250 ml Kjeldhal flask. The destruction stage was performed by adding concentrated 1 gram of selenium and sulfuric acid (H₂SO₄) of 12 ml heated in smoker cabinet for 2 hours at 420°C until the color changed into a clear greenish solution. And then, destruction outcome was cooled off. The destruction outcome was then added with 50 ml NaOH at 40%, and 25 ml aquadest, and was then distilled. Distillate was placed in a flask containing a mixture of 4% 10 ml boric acid (H₃BO₃) and 3 drops of phenolphthalein. Afterwards, titration was done by adding HCl 0.01 N, little by little until it returned into a clear orange color. Determination of blank is where the treatment is the same as the sample, namely destructing, distilling and titrating. However, it differs in the use of only unsampled 1.2 grams of selenium.

Calculation:

$$\%N = \frac{(ml\ HCl - ml\ Blank) \times Normality \times 14,007 \times 100}{mg\ of\ sample}$$

$$\% \text{ protein: } \% N \times 6,25$$

Amino Acid Analysis

Sample Solution

The sample is weighed at 0.1 grams and then added with 5 ml of 6N HCl, and was then vortexed and hydrolyzed for 22 hours at 110°C and cooled down. It was subsequently moved into 50 ml volumetric flask and added with distilled water up to limit sign, filtered with 0.45 µm filter, pipetted by 500 mL, followed by 40 mL of *a-amino-N-butyric acid* (AABA), then added with approximately 460 mL of distilled water, pipetted with 10 µm solution, added with 70 µL AccQ-Fluor Borate,

and then vortexed. Furthermore, 20 µL reagent flour A was added and vortexed, and then was settled for 1 minute, incubated at 55°C for 10 minutes, and was injected on HPLC tool [7].

Standard Solution

Standard 40 µm amino acid mix pipette was added with 40 µL of internal standard *a-amino-n-butyric acid* (AABA), and then added with 920 µL of distilled water after being homogenized. 10 mL standard was taken and was then added with 70 µL AccQ-Fluor Borate and was then vortexed. Furthermore, 20 µL reagent flour A was added, vortexed, and then added for 1 minute. Afterwards, it was incubated at 55°C for 10 minutes and was then injected to HPLC instrument.

Calculation:

$$\frac{\text{Rationality of sampel} \times \left(C_{\text{standard}} \frac{\text{pmol}}{1000000000} \right) \times BM \times fp \times 1000}{\text{Rationality standard} \times \text{sample weight}(g)}$$

$$\text{Amino acid level (mg/ g)} = \text{amino acid level (mg/ kg)} / 1000$$

$$\text{Tryptophan level (mg/ kg)} = (\text{area-intercept}) \times fp / (\text{slope} \times \text{sample weight})$$

RESULTS

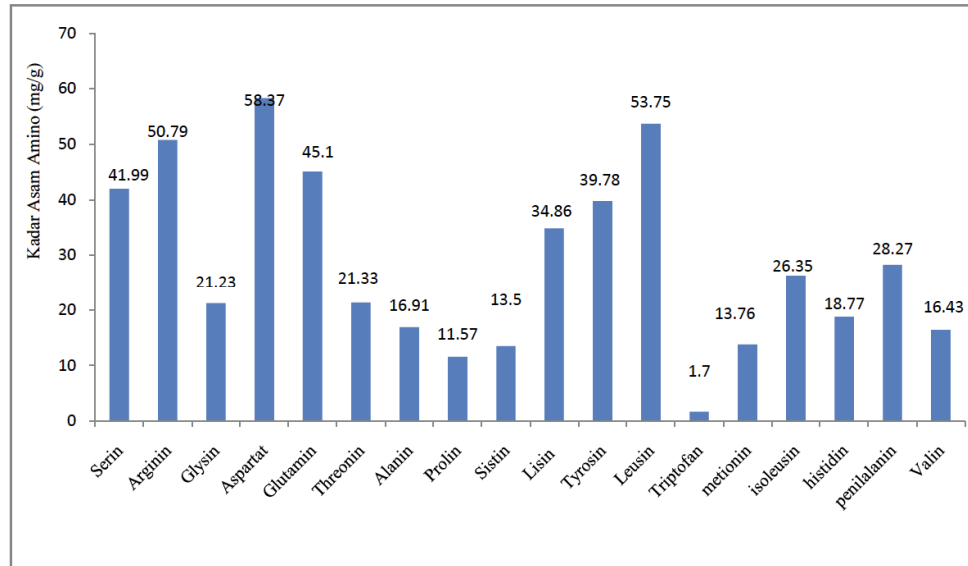
The result of an analysis of amino acid profile and eel protein content of *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* are presented in Graphic images 1, 2, and 3.

DISCUSSIONS

The present research was conducted to analyze the nutrient level, i.e. the protein level and amino acid profile of *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* eels, becoming the information on the fish's nutritional value which is also endemic fish of Central Sulawesi. *Anguilla marmorata* (Q.) Gaimard eels have a yellowish skin and black spots, whilst *Anguilla bicolor* has dark skin. This research conducted analysis of protein level and amino acid profile.

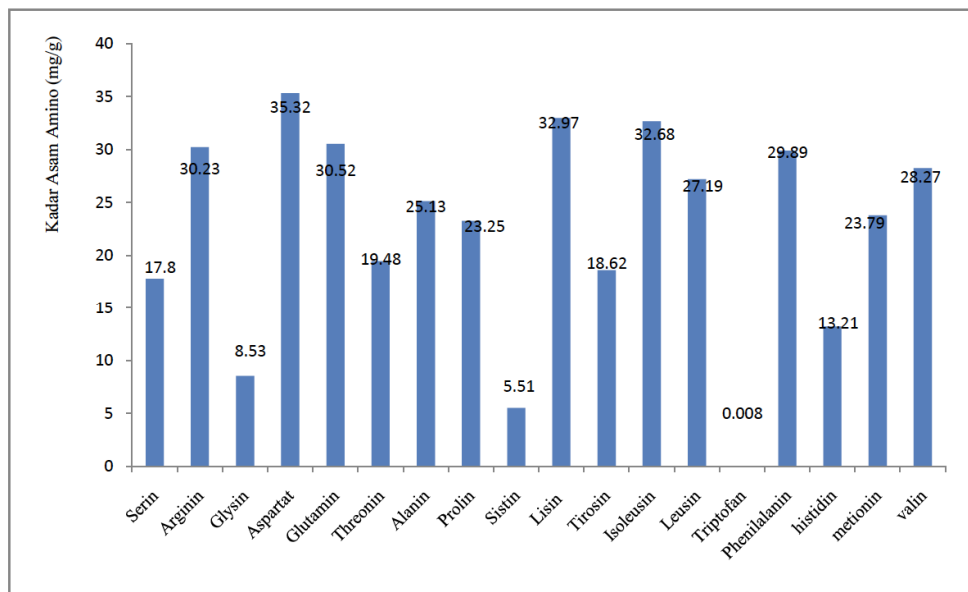
The research was conducted to analyze the nutritional content, namely the protein level and amino acid profile of *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* eels, thereby becoming information on the fish's nutritional value which is also an endemic fish of Central Sulawesi. *Anguilla marmorata* (Q.) Gaimard eels have a yellowish skin and black spots, whilst

Analysis Result on Amino Acid Level of *Anguilla marmorata* (Q.) Gaimard



Graph 1: Amino Acid Level of *Anguilla marmorata* (Q.) Gaimard.

Analysis Result on Amino Acid Level of *Anguilla bicolor*



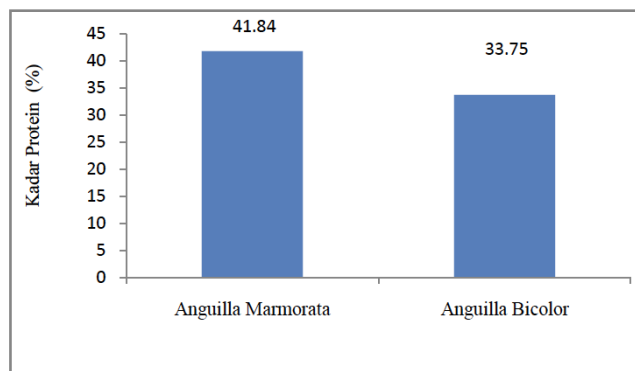
Graph 2: Amino Acid Level of *Anguilla bicolor*.

Anguilla bicolor has dark skin. This research conducted analysis of protein level and amino acid profile.

In the protein analysis stage in eels, Kjeldhal method was used. The determination using Kjeldhal method was performed by volumetric analysis with titration technique. The measurement principle of nitrogen content in food with Kejedhal method is the oxidation of organic compounds by sulfuric acid into carbon dioxide, water and nitrogen. Nitrogen compounds are released in the form of ammonia (ammonium sulfate),

meanwhile carbon dioxide and water are separated by distillation process. Ammonia reacts with excess acid, forming ammonium sulfate. The solution is made alkaline, and the ammonia is evaporated and absorbed in boric acid solution. The nitrogen in the solution is determined by acid titration of HCl 0.1 N. The total protein level is the amount of grams of protein in food, i.e. the amount of grams multiplied by a conversion factor of 6.25, based on the assumption that the protein contains 16 percent of nitrogen.

Protein Analysis



Graph 3: Protein Level.

The analysis results on proteins found in *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* eels can be seen in Graph 3 as the result of proteins analysis histogram. The results demonstrate that the protein level of both species of Central Sulawesi *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* are different. *Anguilla marmorata* (Q.) Gaimard contains protein of 41.84 (%) and *Anguilla bicolor* of 33.75 (%). The total content owned by eels differs from the research [8], stating that the content of *Anguilla bicolor* eels is 30.50 (%). This difference is caused by several factors, such as habitat, age, and the food digested [9]. The protein level in the body functions as the builder and maintenance. High level of protein shows utilization opportunity as one source of animal protein, where the value of the protein in *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor* eels is included high quality or perfect protein.

Organic compounds serve as a component of protein are amino acids. Amino acid analysis was performed using *High Performance Liquid Chromatography* (HPLC). The column on HPLC tool used is ACCQ-Tag Ultra C₁₈ so that the reagents used are in the form of ACCQ-Tag Fluor Borate which is aimed to produce free amino acids. The detector used is PDA detector (Photodiode Array) with a wavelength of 260 nm. It can be interpreted that the detector emits at wavelength of 260 nm and captures fluorescence emitted by the sample. Therefore, KCKT method is appropriate HPLC method to be used for non-volatile compounds, stable and not thermally.

Amino acids are usually soluble in water and insoluble in non-polar organic solvents, such as ether, acetone, and chloroform [10], as based on their chemical properties, amino acid is a weak base; therefore hydrolysis is performed first, which is aimed to produce free amino acid [9].

Internal standard solution or AABA (*α-amino-N-butyric acid*) is used for correcting factor in volumetric error during sample preparation and correcting the loss of amino acid residues during the hydrolysis process that will be detected by reduction in internal standard; hence the use of internal standard solution can improve precision. Amino acid is derivatized first to form derivatives that are able to fluoresce for altering the molecular structure or analyte polarity so as to produce a better chromatogram peak.

Anguilla marmorata (Q.) Gaimard and *Anguilla bicolor* eels have considerably complete amino acid profile and consist of 9 types of essential amino acids and 9 types of non-essential amino acids. When added together, there are 18 types of amino acids as protein constituents. *Anguilla marmorata* (Q.) Gaimard contains essential amino acid. There are 9 essential amino acids in the level. The result can be seen in Graph 2 from histogram result of amino acids. Thereby, *Anguilla marmorata* (Q.) Gaimard eels have considerable essential amino acids compared with Nila fish studied with only 8 essential amino acids. Leucine has the highest level, found in *Anguilla marmorata* (Q.) Gaimard eels at 53.75 mg/g, leucine serves to stimulate brain function, increases energy levels of muscle, and helps bone healing. Leucine also has function in maintaining the immune system [11].

From the analysis of *Anguilla marmorata* (Q.) Gaimard eels, they have 9 different types of non-essential amino acids. The level of essential amino acid can be seen in Graph 2 Chromatogram Results of Amino Acid Analysis. Therefore, *Anguilla marmorata* (Q.) Gaimard has considerably complete non-essential amino acid compared with tilapia studied with only 6 types of non-essential amino acids. Aspartic acid is the highest type of amino acids with a total 58.37 mg/g. Aspartic acid is a component playing a role in the biosynthesis of urea, precursor pyrimidine. In addition, aspartic acid is useful for the treatment of chronic fatigue and increased energy [12].

Meanwhile, *Anguilla bicolor* eels contain 10 essential amino acids. The level of it can be seen in Graph 2. Chromatogram Results of Amino Acid Analysis. Therefore, *Anguilla bicolor* eels have considerably complete essential amino acids compared with tilapia studied with only 9 types of essential amino acids. Tyrosine has the highest level, as found in *Anguilla bicolor* eels, equals to 39.78 mg/g. Tyrosine has several benefits that can reduce stress, anti-depressant, and drug and cocaine detoxification [12]. From the analysis of *Anguilla bicolor* eels, they have 9

different types of non-essential amino acids. The essential amino acid level can be seen in Graph 2. Chromatogram Results of Amino Acid Analysis. Thereby, *Anguilla bicolor* eels have has a non-essential amino acid that is considerably complete, compared with tilapia studied with only 6 types of non-essential amino acids. Aspartic acid is highest type of amino acid playing a role in urea biosynthesis, a pyrimidine precursor. Besides, aspartic acid is useful for the treatment of chronic fatigue and increased energy [12].

Study conducted on major nutrition in four eel fish aquaculture from Namwon, South Korea ie (*Anguilla japonica*, *Anguilla rostrata*, *Anguilla bicolor* and *Anguilla marmorata*), with the Kjeldahl and HPLC methods founded a total protein total in *Anguilla marmorata* (17,7%), *Anguilla bicolor* (17,4%), *Anguilla rostrata* (16,3%), and *Anguilla japonica* (15,8%). The Amino acid profiles result obtained 15 types of amino acids, where in the four species the highest amino acid content was glutamic acid respectively (2.44%, 2.5%, 2.39%, and 2.23%) and the lowest was tyrosine respectively (0.54%, 0.57%, 0.51%, and 0.52%) [13].

Another study on the comparison of the fatty acid profile of eel fish (*Anguilla marmorata* (Q.) Gaimard) in the yellow eel from Palu River and Poso Lake using the gas chromatography method. The result of fatty acid analysis of eel from Palu River and Poso Lake showed saturated fatty acid content of 2.766 g/100 g and 0.275 g/100 g; monounsaturated fatty acids of 4.029 g/100 g and 0.276 g/100 g; and polyunsaturated fatty acids of 0.541 g /100 g and 0.102 g /100 g, respectively. The fatty acid compositions of yellow eel meat from Palu River are 23 species and from Poso Lake are 18 species. The fatty acid composition in yellow eel meat from Palu River and not found in Lake Poso are Cis-11,14,17-eicosatrienoic, gamma-linoleic acid, myristoleic acid, Cis -10-pentadecenoate, and heneicosanoic acid [14].

It is known that eel fish have a complete amino acid composition, nearly similar with amino acid composition of other freshwater fish. From the amino acid composition, a calculation is done to chemical score. The calculation result shows the essential amino acid level in *Anguilla marmorata* (Q.) Gaimard eels have the highest chemistry score, at 77.87 mg/g. And in bicolor eels, the highest chemical score is isoleucine at 81.75 mg/g. According Acton dan Rudd (1987), fish meat has a chemical score between 57-85 mg/ g. From the research, it can be seen that the chemical score of fish vary based on species, age, anatomy, type of food, and geographical location.

The highest chemical score is chicken eggs, i.e. 100. Therefore they are used as referal protein. From Graph 1, it can be seen that in *Anguilla marmorata* (Q.) Gaimard eels, tryptophan amino acid chemical score is at the lowest, at 1.7 mg/g, below the standard protein at 10 mg/ g. It shows that the amino acid of tryptophan is a borderline amino acid in *Anguilla marmorata* (Q.) Gaimard eels. And in *bicolor* eels, the lowest chemical score of tryptophan amino acid is 0.08 mg/ g of protein, below the standard protein at 10 mg/g, which is also a borderline amino acid.

The amino acid level in fish is not the same. Each species has varying physiological processes. It also distinguishes the nutritional content of each fish species [15].

CONCLUSION

Of the two samples of eel species *Anguilla marmorata* (Q.) Gaimard and *Anguilla bicolor*, have complete protein quality because it has all kinds of essential amino acids. The protein content of *Anguilla marmorata* (Q.) Gaimard species is 41.48% and *Anguilla bicolor* is 33.75%. While the result of amino acid profile analysis from both samples of fish obtained 18 types of protein amino acid composed of 9 types of essential amino acids in *Anguilla marmorata* (Q.) Gaimard species (leucine 53.75 mg/g, lysine 34.86 mg/g, cystine 13, 50 mg/g, tyrosine 39,78 mg/g, threonine 21,33 mg/g, valine 16,43 mg/g, tryptophan 1,7 mg/g, isoleucine 26,35 mg/g, phenylalanine 28,27 mg/g), *Anguilla bicolor* species (isoleucine 32.68 mg/g, leucine 27.19 mg/g, lysine 32.97 mg/g, cystine 5.1 mg/g, phenylalanine 29.89 mg/g, tyrosine 18,62 mg/g, threonine 19.48 mg/g, tryptophan 0.08 mg/g, valine 28.27 mg/g) and 9 types of non-essential amino acids in *Anguilla marmorata* (Q.) Gaimard species (glycine 21.23 mg/g, Glutamine 45,10 mg/g, serine 41,99 mg/g, alanine 16,91 mg/g, arginine 50,79 mg/g, proline 11,57 mg/g, histidine 18,77 mg/g, metionin 13, 76 mg/g, aspartic acid 58.37 mg/g), *Anguilla bicolor* species (glycine 8,53 mg/g, glutamine 30,52 mg/g, serine 17,80 mg/g, alanine 25,13 mg/g, arginine 30,23 mg/g, proline 23,25 mg/g, histidine 13, 21 mg/g, methionine 23.79 mg/g, aspartic acid 35.32 mg/g).

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