Comparative Study of Fish Eel Amino Acid Profile (*Anguilla marmorata* (Q.) Gaimard) on *Silver eel* Phase from Palu River and Poso Lake

Jamaluddin*, Cindra Rusli, Yonelian Yuyun and Agustinus Widodo

Department of Pharmacy, Mathematics and Natural Science of Faculty, Tadulako University, Indonesia

Abstract: Amino acid is an organic component containing amine and carboxyl groups. Amino acids are needed by the human body. One animal that has the amino acid content is eel (*Anguilla marmorata* (Q.) Gaimard) from Palu River and Poso Lake in Central Sulawesi which are endemic fish. This study aims to determine the comparison of amino acid profile in eel (*Anguilla marmorata* (Q.) Gaimard) on *silver eel* phase. Testing amino acid profile using *High Performance Liquid Chromatography* (HPLC). The results showed that the eel (*Anguilla marmorata* (Q.) Gaimard) on phase of *silver eel* from the Palu River and Poso Lake contained 18 kinds of amino acid consisting of 9 kinds of essential amino acids and 9 types of non-essential amino acids. Data comparison shows a significant difference in glycine P = 0.000 and has no significant differences in the valine at P = 0.132.

Keywords: Amino acid, *Anguilla marmorata, silver eel*.

INTRODUCTION

Fishing is one sector that is relied upon for the future development of Indonesia because it has the potential to contribute to the fulfillment of public nutrition Indonesia [1]. Indonesia is one of the countries that has abundant eels resources scattered along the south east coast of the island of Java, Sumatra island's west coast, the east coast of the island of Borneo, around the coast of Sulawesi Island, and the northern coast of Papua [2]. Eel in Indonesia has not been used optimally because not widely known by the public. Indonesia has become one of the countries that has the potential to develop the production of eels in local and international markets [3].

Sulawesi is one of the islands which is found species of eel. Some studies indicate that there are eels and river shore in Sulawesi, including South Sulawesi, North Sulawesi and West Sulawesi. In Sulawesi waters there are several kinds of eel namely, *Anguilla marmorata* (Q.) Gaimard*, Anguilla bicolor Pacifica, Anguilla bicolor bicolor,* and *Anguilla celebensis*.

Research in the central region of Indonesia included samples of two estuarine area in Central Sulawesi, the Palu River (representing the Makassar Strait) and Poso River (representing Tomini) (Department of Marine and Fisheries in Central Sulawesi) [4]. In Central Sulawesi eel fish populations are found in rivers, lakes, and estuaries of Palu. Many researches tend to focus on Poso river basins. Availability of data is lacking on the river or lake, including Palu River [5].

Eel (*Anguilla* spp.) is a fish out of order *Anguilliformes* belonging katadromus fish. Katadromus fish are fish that migrate from freshwater to marine waters. Eels spawn in the ocean, producing larvae (*Leptocephalus*)*,* and carried away by the current turbulence in the direction of the waterfront [6].

Silver eel (mature gonads) are the final stages of the life cycle of eels, which are migratory to the sea to spawn. According to Saso, *et al* (2007) [7] eel consumption size is 120 grams. In other words, the phase *silver eel* of eels can be processed and consumed, whereby the weight ofeel phase *silver eel* is 250g-22kg [8]. Almost no research about the phases of *silver eel* on a tropical species of eel (*Anguillasp.*) [9].

The advantages of eel apart economically is seen from the quality of the eel itself ie nutritional content [10]. Eel is a kind of fish that has a very high nutritional value, rich in protein vitamins D and E, as well as having mucoprotein, amino acid and ribonucleic acid [11].

Amino acids are needed by the human body. These amino acids function of repairing damaged tissue after injury, protect the liver from toxic substances, lowering blood pressure, regulate cholesterol metabolism, promote the secretion of growth hormone and reduces levels of ammonia in the blood [12].

Address correspondence to this article at the Department of Pharmacy, Mathematics and Natural Science of Faculty, Tadulako University, Palu, Central Sulawesi, (Postal: 94118), Indonesia; Tel: +6281355198799;
E-mail: jamal_farmasi02@yahoo.co.id

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Based on the description of the background of the above, it has not been found or publication of data analysis of the amino acid profile of eel (*Anguilla marmorata*) on phase of *silver eel* originating from Poso Lake and Central Sulawesi Palu River. Seeing the importance of amino acids for humans and eel is one of the endemic fish in Central Sulawesi which contains amino acids, it is necessary to do a comparative analysis of the amino acid profile of *Anguilla marmorata* eel from Palu River and Poso Lake to have comparison of amino acid profiles of eel species of *Anguilla marmorata* from both sites.

MATERIAL AND METHODS

Material

Base used in this study is eel species of *Anguilla marmorata* (Q.) Gaimard weighing 2.7 to 3.5 kg and a length of 1.3-1.5 m size ranges from the Palu river and Poso lake, as well as other materials used for the analysis of amino acid profile that is distilled (H_2O), Hydrochloric acid (HCl), AccQ-Fluor Borate, Fluor Reagent A, a standard solution of amino acids.

Methods

Sample Preparation

The samples used in this study were eel fish (*Anguilla marmorata* (Q.) Gaimard) on phase of *silver eel* from the Palu River and Poso Lake. Eel was washed and gutted and drained. Samples were drained and then dried using an oven at a temperature of 60℃. Eel fish was dried for 26 hours. Once the dried sample then it is blended up into powder and stored at room temperature (20-25 ˚C) in *stainleess steel* container.

Analysis of Amino Acid

Preparation of Sample

Sample was weighed 0.1 g then was add 5 ml of 6 N HCl then was used vortex, after it was hydrolyzed for 22 hours at a temperature of 110 ˚C and then cooled, and then moved to the flask 50 ml then added distilled water up to the mark then filtered by filter 0.45 µm and 500 pipette µl filter, add 40 µl *a-amino-N-butyric acid* (AABA), then added distilled water approximately 460 µl, then pipette 10 µl solution, then added 70 µl AccQ-Fluor Borate, then vortex. After it was added 20 µl reagent flour A, then vortex then let it stand for 1 minute and then incubated at 55 ˚C for 10 minutes, after which it was injected into the HPLC [13].

Preparation of Standard Solutions

Pipette 40 µm standard amino acid mix was then added 40 mL of internal standard *a-amino-n-butyric acid* (AABA) then added 920 mL of distilled water after it was homogeneous. Taken standard 10 mL and then added 70 mL *AccQ-Fluor Borate* and vortex. Then added fluorine of 20 mL reagent A, then vortex for 1 minute. After it was incubated at 55 °C subs 10 minutes and then injected on the HPLC instrument [14].

Calculation of:

a) amino acid levels (mg/kg)

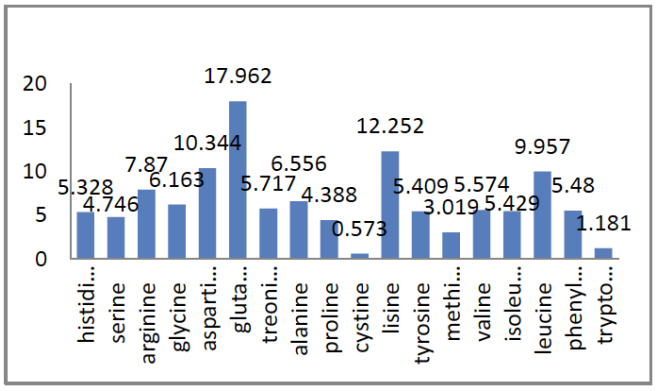
$$\frac{sample\ analytic\ ratio\ xC\ standard\ \frac{(pmol}{}1000000000)xBMxfpx\ 100}{standard\ analytic\ ratio\ x\ sample\quad weight(g)}$$

b) Calculation of the amino acid profile comparable data were analyzed using SPSS statistical test of the Independent samples t-test. Interpretation comparison of the amino acid that is meaningful or significant when P <0.01.

RESULTS

Analysis Amino Acid

Analysis results of amino acid profile of *Anguilla marmorata* (Q.) Gaimard eel on phase of *silver eel* from the river Palu can be seen in the Graph **1** below:

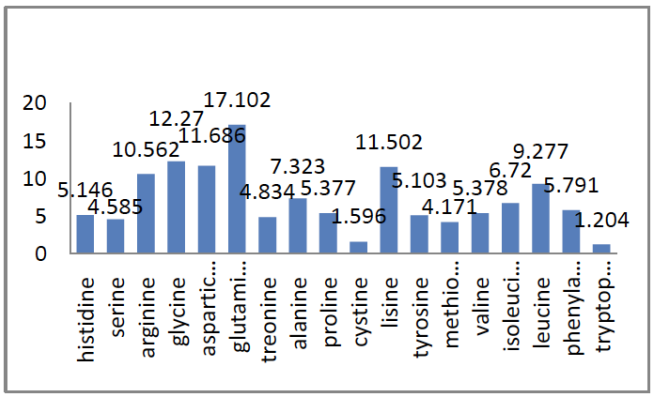


Graph 1: Levels of Amino Acids in Palu's eel.

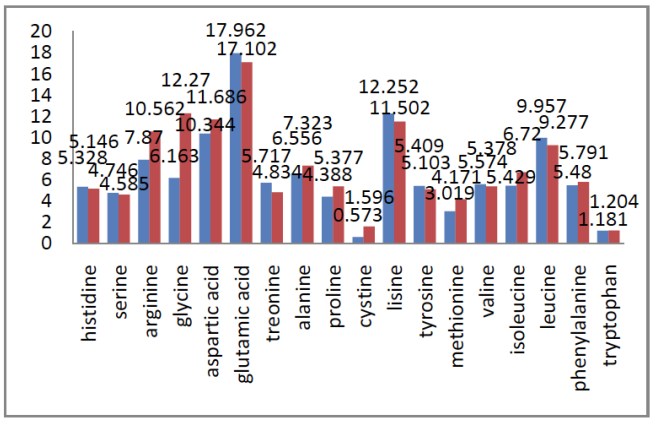
Analysis results of amino acid profile of *Anguilla marmorata* (Q.) Gaimard eel on phase of *silver eel* from lake Poso can be seen in the Graph **2** below:

Results Comparison Profile Amino

Results of the comparison amino acid profile in *Anguilla marmorata* (Q.) Gaimard eel on phase of *silver eel* from the Palu River and Poso Lake can be seen in the Graph **3** below:



Graph 2: Levels of Amino Acids in Poso's eel.



Graph 3: The comparison of eel Content.

DISCUSSION

This study was conducted to obtain comparison of amino acid profile of eel species *Anguilla marmorata* (Q.) Gaimard on phase *of silver eel* from Palu River and Poso Lake in Central Sulawesi that are endemic fish, so it can be information on the nutritional value of fish. The fish of this species has a characteristic yellowish color with black spots. In this research, the analysis is the content of the amino acid profile.

At this stage of eel amino acid analysis was performed using *High Performance Liquid Chromatography* (HPLC). The use of HPLC columns at this time tool used is ACCQ-Tag Ultra C18 so that *the reagents* used in the form of ACCQ-Tag Flour Borate which aims to produce free amino acids. The detector used is a PDA detector (*Phaotodiode Array*) with a wave length of 260 nm and emission capture fluorescence emitted by the sample. HPLC method is ideal to use for compounds that are not easily evaporate.

Amino acid is an organic component containing carboxyl amino group [12]. Amino acids are usually soluble in water and insoluble in non-polar organic solvents are ether, acetone, and chloroform [15]. Based on the chemical properties of the amino acid is a weak base, so the first hydrolyzed aims to generate free amino acids [12].

A standard solution or AABA (*a-amino-N-butyricacid*) are used for the purpose of correction factor error volumetric during sample preparation and correcting the loss of amino acid residues during the process of hydrolysis to be detected with reduced internal standard, so the use of a solution of an internal standard can improve the precision. Derivatized amino acids first form a derivative which can be florescence in order to alter the molecular structure or polarity of the analyte so that it will produce a better chromatogram peak.

Eel species of *Anguilla marmorata* (Q.) Gaimard phase of *silver eel* from the Palu river and Poso lake has the amino acid profile is complete and consists of 10 types of essential amino acids and eight kinds of non-essential amino acids, if added together, the results there are 18 kinds of amino acids making up the protein. Eel of *Anguilla marmorata* (Q.) Gaimard on phase *silver eel* of Palu River has a number of essential amino acids that are 9 types. For the results of the levels can be seen in Graph **3**. Eel Fish of *Anguilla marmorata* (Q.) Gaimard on phase *silver eel* of Palu river can be quite complete when compared with cork fish (*Channa striata*) studied by Asfar *et al.* (2014) [16] which only has 7 kinds of essential amino acids. Lysine has the highest levels were found in *Anguilla marmorata* (Q.) Gaimard eel on phase *silver eel* of Palu river that is equal to 12.252 mg / g. Lysine serves as a basic ingredient of blood antibodies, strengthens circulatory system, maintain the growth of normal cells along with proline and vitamin C to form collagen, prevent insomnia (to help hyperactive children), as well as the release of growth hormone [17].

The results of the analysis of *Anguilla marmorata* (Q.) Gaimard eel on phase of *silver eel* from Palu river has 9 types of non-essential amino acid, levels of non-essential amino acid can be seen in Graph **1**. Fish eel *Anguilla marmorata* (Q.) Gaimard phase of *silver eel* from the river Palu can be said to have a non essential amino acid that is quite complete compared with tilapia studied [18] which only had 6 types of non-essential amino acids. Glutamic acid is a type of non-essential amino acids the highest, amounting to 17.962 mg / g. Glutamic acid is useful to restrain excessive alcohol consumption, accelerating wound healing in the gut, improve mental health and reduce depression [19].

Anguilla marmorata (Q.) Gaimard eel on phase of *silver eel* from Lake Poso contains amino acids numbered 9 essential amino acids, for the results of the levels can be seen in Graph **2**. *Anguilla marmorata* (Q.) Gaimard eel on phase of *silver eel* from Lake Poso has the essential amino acids that are complete enough when compared with tilapia studied [18] which only has 8 essential amino acids. Lysine is an highest amino acid for *Anguilla marmorata* (Q.) Gaimard eel on phase *silver eel* of the Lake Poso that is equal to 11.502 mg / g. Llisin serves as a basic ingredient of blood antibodies, strengthens circulatory system, maintain the growth of normal cells along with proline and vitamin C to form collagen, prevent insomnia (to help hyperactive children), as well as the release of growth hormone [17].

The results of the analysis of eel *Anguilla marmorata* (Q.) Gaimard phase of *silver eel* from Lake Poso has 9 types of non essential amino acid, the amino acid non-essential can be seen in Graph **2**. *Anguilla marmorata* (Q.) Gaimard eel on phase of *silver eel* from Poso Lake can be said to have an amino acid that is quite complete when compared with milkfish (*Chanos chanos*) studied by Hafiludin (2015) [20] which only had 6 types of non-essential amino acids. Glutamic acid is a type of non-essential amino acids the highest, amounting to 17.102 mg / g. Glutamic acid is useful to restrain excessive alcohol consumption, accelerating wound healing in the gut, improve mental health and reduce depression [19].

The results of analysis of meat eels have a complete amino acid composition which is almost the same as the amino acid composition of other freshwater fish

Amino acid content in fish is not the same. Depending on each species has a physiological process that is different. This also distinguishes the nutritional content of each type of fish [21].

Based on the research results, the comparison amino acid levels can be seen in Graph **3**. Comparison of the amino acid profile eel *Anguilla marmorata* (Q.) Gaimard of between river Palu and Poso lake with their respective histidine comparison value 5.326 mg / g - 5.148 mg / g. Serin 4.746 mg / g - 4.858 mg / g. Arganin 7.87 mg / g - 10.562 mg / g. Glycine 6.163 mg / g - 12.27 mg / g. Aspartic acid 10.344 mg / g - 11.686 mg / g. Glutamic acid 17.962 mg / g - 17.102 mg / g. Threonine 5.717 mg / g - 4.834 mg / g. Alanine 6.556 mg / g - 7.323 mg / g. Proline 4.388 mg / g - 5.377 mg / g. Cystine 0.573 mg / g - 1.596 mg / g. Lysine 12.252 mg / g - 11.502 mg / g. Tyrosine 5.409 mg / g - 5.104 mg / g. Methionine 3.019 mg / g - 4.171 mg / g. Valine 5,574 mg / g - 5.378 mg / g. Isoleucine 5.290 mg / g - 6.72 mg / g. Leucine 9.957 mg / g, - 9.277 mg / g. Phenylalanine 5.480 mg / g - 5.791 mg / g. Tryptophan 1.181 mg / g - 1.204 mg / g.

The test results comparing the amino acid profile were carried out using SPSS statistical test Independent samples t-test of *Anguilla marmorata* (Q.) Gaimard eel on phase of *silver eels* from the Palu River and Poso Lake found their comparative value meaningful or significant in the histidine, arganin, glycine, aspartate, glutamate, threonine, alanine, cysteine, lysine, tyrosine methionine, isoleucine, leucine and phenylalanine. The value of the comparison of the respective amino acid indicates the value of P <0.01. While the comparison obtained meaningless or insignificant shown in valine, serine and proline. with a P value> 0.01.

CONCLUSIONS

The results of the analysis of the amino acid profile of the two samples of *Anguilla marmorata* (Q.) Gaimard eel on phase of *silver eel* from Palu River and Lake Poso obtained 18 kinds of amino acids making up the protein consists of nine essential amino acids in the species *Anguilla marmorata* (Q.) Gaimard of Palu River (isoleucine, leucine, lysine, tyrosine, phenilalanin, threonine, tryptophan, valine, cystine), the species *Anguilla marmorata* (Q.) Gaimard Lake Poso (isoleucine, leucine, lysine, tyrosine, phenylalanine, threonine, tryptophan, valine, cystine) and 9 types of non essential amino acid in the species *Anguilla marmorata* (Q.) Gaimard of Palu River (histidine, serine, arganin, glycine, aspartic acid, acid glutamate, alanine, proline), the species *Anguilla marmorata* (Q.) Gaimard Poso Lake (histidine, serine, arginine, glycine, aspartic acid, glutamic acid, alanine, proline).

Independent test results for comparison with samples t-test is obtained some meaningful or significant differences in *Anguilla marmorata* (Q.) Gaimard eel on phase *silver eel* of Palu River and Lake Poso. Amino acids have a significant difference or significant namely histidine P = 0.003, arginine P = 0.001, glycine P = 0.000, aspartic P = 0.001, glutamine P = 0.002, threonine P = 0.001, alanine P = 0.001, cysteine P = 0.001, P = 0.003 lysine, tyrosine P = 0.002, P = 0.006 methionine, isoleucine P = 0.004, P = 0.001 leucine, phenylalanine P = 0.006. As for the amino acid that may otherwise have a significant

difference or not significant with a P value> 0.01 is serine P = 0.044, P = 0.013 proline, valine P = 0.132.

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