The Profile of Amino Acids in Sea Horse (\textit{Hippocampus sp})

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Abstract. Seahorse (\textit{Hippocampus sp}) is a marine fisheries commodity that has high economic and nutritional values. The aims of study were to determine the proximate composition and the profile of the amino acids content in seahorses. The method used refers to the AOAC (Association of Official Analytical Chemistry) for proximate analysis and a GC-MS (Gas Chromatography-Mass Spectroscopy) for the amino acid analysis. This research was done in March 2021. The sea horses were carried out from Simeulue waters, Simeulue district, Aceh Province, Indonesia. Samples were divided into 2 sizes, small size (3.00-5.00 cm) and large size ((5.01-10.00 cm). The analysis was done at laboratorium of Syiah Kuala University. The results of the study was found that the proximate content of the seahorse was water content (6.29%), ash content (25.43%), fat content (0.57%), protein content (39.32%) and carbohydrates (28.48%). The amino acid composition of large seahorses and small seahorses consists of four types of essential amino acids, namely leucine, isoleucine, histidine and valine, and six types of non-essential amino acids, namely alanine, glycine, aspartic acid, asparagine, arginine and serine.

1. Introduction

Seahorse \textit{Hippocampus spp} is a unique marine species and widespread throughout the waters of the world. Seahorses are often found in shallow waters such as sea grass ecosystems [1]. Seahorses have a unique body shape, which head like a horse and its unique way of swimming, makes this animal attractive because it is not found in other marine animals. Besides having a unique body morphology, it also has a high economic value. Seahorses are also often used as ornamental fish and souvenirs. It also used as raw materials for medicine traditional [2]. The use of seahorses in China is more widely used as traditional Chinese medicine in dry form compared to its sale as aquarium ornamental fish [3].

Seahorses are efficacious as ingredients in traditional Chinese medicine for generations and believed to strengthen stamina. Various benefits of seahorses have been carried out by several researchers. Seahorses can improve the histological changes of the seminal vesicles and prostate gland [4], increase sperm count (spermatogenesis) [5], have antibacterial activity against \textit{Klebsiella pneumoniae}, \textit{Vibrio}
cholerae and Staphylococcus aureus [6], increased blood hemoglobin levels, have anti-fatigue activity [7] and have antioxidant activity of 24.04% [8].

Seahorses can be used as antifatigue with a cell proliferation activity value of 160% [7] and able to inhibit oxidation activity with an IC_{50} value of 43.8 ppm [8]. inhibiting the growth of E. Coli bacteria with an inhibition zone of 4 mm, and inhibiting the growth of the fungus Aspergillus flavus with an inhibitory zone of 2 mm. The many benefits of seahorses are due to the presence of complex bioactive compounds. Bioactive compounds commonly found in active ingredients are secondary metabolites and primary metabolites [6]. Therefore, people often take advantage of seahorse as an alternative in natural medicine [9]. Another benefit of seahorses is as a source of animal protein for human consumption.

Proteins are needed by the body and plays a role in metabolic processes that occur in the body. Proteins are large molecules that are comprised of one or more long chains of amino acids. Amino acids are divided into two groups, namely essential and non-essential amino acids. Essential amino acids can not be synthesized by the body and must be obtained from food sources of protein. Non-essential amino acids can be produced by the human body. The quality of proteins are assessed from the ratio of the amino acids contained in the protein [10]. Therefore, the differences in the number and types of amino acids contained in seahorses play important role in providing benefits to humans. Proteins and amino acids are also nutrients that play an important role in the structure and metabolism of all aquatic organisms, and sufficient amounts are required to promote their growth [11].

In addition, the another nutritionals content of seahorses are vitamins, minerals, carbohydrates, fiber, and others. The nutritional content of the seahorse has the potential as a dietary supplement for human health [12]. Research related to proximate analysis and amino acid content in seahorses has been widely carried out. [12] stated that seahorses have a chemical content on a dry basis with a protein content of 70.70%, fat content of 1.71%, ash content of 20.92% and Zn mineral content of 38.15 (μg/g). The nutritional content of seahorses can also be influenced by their living habitat and food sources. Therefore, the purposes of this study were to determine the proximate content and the amino acid profile of seahorses that live in the waters of Simeulue Regency, Aceh Province, Indonesia.

2. Materials and Methods
2.1 Materials
This research was conducted at March 2021. The seahorse samples were obtained from the waters of Simeulue Regency, Simeulue, Aceh with size of 3-10 cm. The samples were divided into 2 size of 3-5 cm (small) and 6-10 cm (large). The chemicals used for proximate analysis were 100 ml acetone, HCL, 30% NaOH, 2% boric acid, 0.1% Bromocresol Green, 0.1% methyl red, nitrogen, selenium and aquades. N-hexane was used for the extraction of seahorse oil.

2.2 Methods
2.2.1 Proximate analysis.
Proximate analysis was performed according to the AOAC methods [13]. Analysis of water content using an oven at 105°C for 24 hours, ash content using a furnace temperature of 600 °C, fat content using soxhlet extraction method. Analysis of protein content using the semimicro Kjeldahl method and analysis of carbohydrate content is calculated by difference. The proximate analysis of samples was done at the Environmental Quality Testing Engineering Laboratory, Faculty of Engineering and at the Agricultural Product Analysis Laboratory, Faculty of Agriculture, Syiah Kuala University.

2.2.2 The analysis of amino acids
The content of Amino acid was analysed using GC-MS equipment. Separation of amino acids using a Rxi-5ms capillary column with a length of 30 m, an outer diameter of 0.25 mm, and a liquid phase thickness of 0.25 m. The liquid phase (5% biphenyl and 95% polysiloxane) and helium as a carrier gas were added. The oven temperature was set at 75°C and kept for 5 minutes, then increased to 290°C at a rate of 10°C/min, rose again to 320°C at a rate of 20°C/min, and finally remained at that temperature.
for 5 minutes. The ejector temperature used was 260 °C. In split mode, the sample was injected at a 1:2 ratio at 260 °C, and the injection volume is 1 L. Mass spectrometry (70 eV, collision mode electrons) scan range of 35-500 m/z equals the mass scan time of 0.2 s. Column flow rate 1.5 mL/min, pressure 3000Psi, total flow rate: 34.4 mL/min. The total analytical process was 40 minutes.

2.2.3. The extraction of seahorse oil.
The seahorses were dried using oven at 105°C for 8 hours, cut into small pieces and grinded using a mortar, next the samples were weighed using an analytical balance. Then the sample was put into filter paper and extracted using a Soxhlet extractor for 5 hours by adding 200 ml of N-hexane as a solvent. The solvent started to rise to the top was repeatedly until the oil extraction started to thicken and the oil obtained was no longer contained in the solvent N-hexane.

3. Results and Discussion

3.1. Proximate composition
The result of proximate composition of the seahorse were presented in Table 1. The higher composition of seahorse was According to [12] the proximate content in the body of the seahorse can characterize the physiological and health conditions of the seahorse. In general, the body composition of the seahorse is obtained by proximate analysis, but measurements of physiological conditions can also be determined using a comparison of the standard weight and length relationship.

| No. | Composition       | Value (%) |
|-----|------------------|-----------|
| 1   | Water content    | 6.29      |
| 2   | Ash content      | 25.43     |
| 3   | Fat content      | 0.47      |
| 4   | Protein content  | 39.32     |
| 5   | Carbohydrate content | 28.48  |

The proximate composition of the seahorse was calculated based on dry weight. The water content of the sampled seahorse was 6.29%. According to [11], water is a basic component found in seahorse raw materials. Bound water is a type of water that is difficult to remove from seahorse meat even by drying. The water content contained in each type of seahorse is different. The difference in water content of seahorses is caused by several factors including the fishing season and the age of the species. [12] claimed that the average water content contained in fresh seahorses is 65-76%. Added by [14] the water content in marine biota ranges from 50-85% depending on the type of species and nutritional conditions in the body. Another low factor is the fishing season for species that are suspected to be during the reproductive process, so that the species will experience a loss of water content in their bodies.

[12] stated that seahorses have a chemical content on a dry basis with a protein content of 70.70%, fat content of 1.71%, ash content of 20.92%. The nutritional content of seahorses can also be influenced by their living habitat and food sources. [15] studied the quality of juvenile sea horse (Hippocampus barbouiri) after modification of natural feed Artemia nauplii in Phronima sp as a food source. Various proximate compositions were obtained that crude protein content of 37.12-61.47%, lipid of 3.82-21.55% and ash content of 6.27-28.12%.

The content of ash contained in the sea horse was 25.43%. The ash content of seahorses in the study was very high compared to other marine animals such as fish. According to the results of research from [16], the average value of ash content in body parts of tuna fish is 1.48%. [17] stated that the ash content in tuna is influenced by the type of food and mineral content in the fish habitat. The high and low ash content depends on the ability of the food to regulate and absorb metals.
The protein content of the seahorse was 39.32% which was obtained from mixing several sexes of the seahorse that were sampled. The difference in the chemical content of the seahorse is due to environmental conditions of the surrounding waters, type of food, habitat where it lives, gender and reproductive status. [15] studied the quality of juvenile sea horse (*Hippocampus barbouri*), which was the juveniles were fed with a modified diet, the crude protein content was 37.12-61.47%, which was higher than the value obtained in this study. The protein and amino acid content in marine animals can be increased by providing natural artificial feed. Artificial feed can be enriched in its nutritional content and also easier to digest [18]; [19]. Protein in the body of fish is a compound that has the highest content. Protein also plays an important role in body function and structure, such as growth and reproduction. Therefore, protein is the largest part of the muscles, organs and bones. Protein contains a chain of amino acids that are very important, especially for growth (anabolism) and catabolic functions [20].

The value of fat content in seahorses was 0.47%. According to the results of research from [21], it is stated that the average value of fat content in the tuna meat is 0.51%. Fat is one of the excess energy stored by animals so that the amount of fat in the animal's body that is used as food is determined by the energy balance of the animal. Carbohydrates are one of the most important nutritional components because they act as a source of energy. Determination of carbohydrate content obtained from the reduction of the number 100 with the percentage of other components (water, ash, protein and fat). The carbohydrate content in seahorses is 28.48%. The high levels of carbohydrates in the seahorse indicate that the seahorse is very good as a source of food.

### 3.2. Amino acids

The protein quality of a material can be determined from the amino acid content that composes it. Amino acid analysis was used to determine the amount of amino acid content and the types of amino acids contained in autistic horses that live in the waters of Simeulue district. Amino acids are needed by the human body. Some of the functions of amino acids are repairing damaged tissue after injury, protecting the liver from various toxic substances, lowering blood pressure, regulating cholesterol metabolism, encouraging growth hormone secretion, and reducing ammonia levels in the blood [22]. The content of amino acids essential and non-essential was presented at Figure 1 dan Table 2, respectively.

The results of the amino acid analysis in the seahorse showed that there were four types of essential amino acids contained in the seahorse, namely leucine, isoleucine, histidine, and valine. According to [22], leucine was used to maintain immune balance in the body. Meanwhile, Leucine can stimulate brain function, increase muscle energy levels, help reduce excessive blood sugar levels, and help heal bones, muscle tissue and skin (especially to accelerate wound healing after surgery). Histidine has a function to promote growth and repair damaged body tissues. Isoleucine functions to assist in repairing damaged tissue, developing intelligence, maintaining the body's nitrogen balance, forming hemoglobin and stabilizing blood sugar levels. Isoleucine deficiency can cause symptoms of hypoglycemia.

![Figure 1](image_url) **Figure 1.** The content of amino acids essential in seahorses from Simeulue district.
According to the results of research from [21], it is stated that the value of the amino acid content contained in gunshot fish has the lowest value, namely valine 0.80% in male gun fish and 0.71% in female gun fish. Meanwhile, according to Putra (2018), gamat derived from sea cucumbers contains essential amino acids which can also be used as live food for the development of Artemia [23]. Valine is an amino acid that has branched chains that function as an increase in body power and increase energy, valine is very important for increasing growth, lowering blood sugar levels and maintaining muscle tissue can also stimulate intelligence, strengthen muscles, help repair damaged tissue. and maintain nitrogen balance. Valine deficiency can cause muscle coordination and the body to be very sensitive to pain, heat and cold [22]. If two proteins that have different types of amino acids are consumed together, the deficiency of amino acids from one protein can be covered by an excess of similar amino acids in other proteins. The two proteins support each other so that the nutritional quality of the mixture is higher.

Table 2. The content of non-essential amino acids (%).

| No. | Types of amino acids | Big seahorse | Small seahorse |
|-----|----------------------|--------------|---------------|
| 1   | Alanine              | 0.05±0.01    | 0.03±0.01     |
| 2   | Glycine              | 2.09±0.06    | 0.02±0.03     |
| 3   | Aspartic Acid        | -            | 0.11±0.09     |
| 4   | Asparagine           | 0.08±0.01    | -             |
| 5   | Arginine             | 0.11±0.01    | 0.09±0.05     |
| 6   | serine               | 0.03±0.02    | -             |

The content of non-essential amino acids found in seahorses are alanine, glycine, aspartic acid, asparagine, arginine, and serine. The highest value of non-essential amino acid content was found in large seahorse samples, namely glycine 2.13%. Glycine is an amino acid that can inhibit the formation process in the brain that can make movements stiffer as in multiple sclerosis (nerve disorders in the brain, eyes, and spine). The lowest amino acid content in the large and small seahorse samples was serine with a value of 0.01%.

4. Conclusion
This research can be concluded that the sea horse samples contain nutritional components in the form of fat, protein, and carbohydrates. The amino acid composition of large and small seahorses consists of four types of essential amino acids, namely (isoleucine, leucine, histidine, and valine) and six types of non-essential amino acids (aspartic acid, alanine, arginine, serine, glycine and asparagine).

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