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RESEARCH ARTICLE

COMPARING STUDY THE CONTENT OF OMEGA-3 AND OMEGA-6 FATTY ACIDS MEAT OF EELS (*ANGUILLA MARMORATA* (Q.) GAIMARD) *GLASS EEL, YELLOW EEL,* AND *SILVER EEL* PHASE FROM PALU RIVER AND POSO LAKE

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ABSTRACT

Eel (*Anguilla marmorata* (Q.) Gaimard) has a high fatty acid content of essentials fatty acids. This study aims to compare the levels of omega-3 fatty acids and omega-6 fish Eel phases of *glass eel, yellow eel,* and *silver eel* from the river Palu and lake Poso. The research stages include oil extraction by sokletasi method then analyzed FAME (*Fatty Acid Methyl Ester*) using *Gas Chromatography* (GC-MS). The result showed that the total fat content of *glass eel* phase from Palu (1.9939%) was higher than that of Poso lake (1.2349%), while the *yellow eel* and *silver eel* phases of total fat content from Palu (1.0933% and 0.9960%) were lower than the lake Poso (16.7766% and 19.5776%). Level of omega-3 fatty acid and omega-6 phase *glass eel* from Palu (0.201%) higher than Poso lake (0.088%), while in *yellow eel* and *silver eel* phase from Palu (0.101% and 0.059%) lower than origin of lake Poso (1.079% and 3.121%).

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INTRODUCTION

Indonesia is known to have a considerable wealth of fishery resources. About 16% of the world's fish species are estimated to live in Indonesian waters. According to the data, the total number of fish species found in Indonesian waters reaches 7,000 species. About 2,000 of them are freshwater fish. Freshwater fish is a type of fish that live and inland waters (inland water), ie waters with salinity (salinity) less than 5 per ml (0-5%) (Khairuman, 2008). One region of Indonesia that has many endemic freshwater fish is Sulawesi. Around 117 species have been identified in the territorial waters of Sulawesi (Husnah, Tahjo *et al*., 2008). According to (Ndobe, 2010), freshwater fish in Central Sulawesi recorded 62 species and 52 of them are endemic fish species. One of the fish species in Sulawesi is eel fish. Research on eel fish is still focused on Posoriver and lake, while other rivers and lakes, including Palu river, are still very limited. The Poso region has quite high eel (sogili) potential because it is supported by the deep bay of Tomini as well as the presence of the vast waters of Poso (Mc, Kinnon, 2006).

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There are 5 species of eels located in Poso lake, one of which is used in research is *Anguilla marmorata. Anguilla marmorata* species are cosmopolitan eel species (widely spread) with distribution areas throughout tropical waters. This type of fish has a black back and patterned, while the stomach is white (Sarwono, 2002). In addition, *Anguilla marmorata* is the most widely cultivated species in Indonesia (Hurricane, M. Dan Nofiandi, 2015). Eel fish have cadadromous properties ie the period before adult eel fish live in fresh water then migrate to lay eggs or breed in sea water (Affandi *et al*., 1995). The eel cycles have several stages: egg, larva (*leptopchepalus*), *glass eel* (age 4-7 months, with length 50-70mm), elver (age <1-5 years, length ≤300 mm), *yellow eel* (age> 5 years and long> 300 mm), and *silver eel* (age 10-20 years long ≤ 500 mm-1.6 m) (Aoyama, 2009). The content of omega-3 and omega-6 fatty acids present in the *glass eel, yellow eel*, and *silver eel* phases are very different, can be seen from the size and number of samples, because the standard good fat for adult eels is more than 3% (Ikenoue, 2006). Fish is one source of protein, contains unsaturated fatty acids that are beneficial to heart health, brain intelligence, and blood vessels. Two of the most potentially unsaturated fatty acids that can be used as the basic ingredients of drugs are omega-6 and omega-3 (KKP, 2011). According to (Topan, M. AndNofiandi, 2015), in eels fish body contains unsaturated fatty acids needed by the body, including omega-3 and omega-6 fatty acids.

The unsaturated fatty acids included in the omega-3 group, *linolenic acid*, EPA, and DHA, are also called essential fatty acids (Bahar, 2006). Based on their chemical structure, *eicosapentanoic acid* has 20 carbon chains and 5 double bonds, *docosahexaenoic acid* has 22 carbon chains and 6 double bonds, and *α-linolenic acid* has 18 carbon chains and 3 double bonds (Collins, 2010). Omega 6 is a polyunsaturated fatty acid having its first double bond at the 6th position. Omega 6 is one of the essential fatty acids. Essential fatty acids actually consist of *linoleic acid* (AL) or *linoleic acid* (LA), *linolenic acid* (ALN) or *linolenic acid* (ALA) as well as *arachidonic acid* or *arachidonic acid* (AA). Fatty acids are distinguished according to the amount of carbon they contain: short-chain fatty acids (6 carbon atoms or less), medium chain (8 to 12 carbon), long chains (14 – 18 carbon), and very long chains (20 carbon atoms or more). Fatty acids composed of carbon chains that bind all the hydrogen they can bind to are called saturated fatty acids. Fatty acids containing one or more double bonds which can be said to be an additional hydrogen atom are called unsaturated fatty acids. Monounsaturated fatty acids contain one double bond, while the polyunsaturated fatty acids contain two or more double bonds (Almatsier, 2003).

The two most potent types of unsaturated fatty acids that can be used as medicinal substances are omega-3 and omega-6. Omega-6 fatty acids can prevent the occurrence of constriction of blood vessels caused because attached to cholesterol in the inner walls of blood vessels. While the benefits of omega-3 fatty acids for the body, among others, to improve the endurance of heart muscle cells from damage, dilute blood viscosity, lower LDL (*Low Density Lipoprotein*), and increase HDL (*High Density Lipoprotein*), as well as for bone health (Mensink & Katan, 1992). Analysis of omega-3 fatty acid composition and omega-6 fish of Sidat (*Anguilla marmorata* (Q.) Gaimard) was done qualitatively and quantitatively using gas chromatography instrument (GC). To identify the fatty acid components by equating the retention time of the sample with the fatty acid retention time of FAME standard (*Fatty Acid Methyl Ester*) Mix which is known with certainty the type of fatty acids (Panagan, A, T., Heni, Y., Mila, 2011).

MATERIALS AND METHODS

Materials: Materials used in this research include: hexane, sodium hydroxide (NaOH), methanol, boron trifluoride (BF3), saturated sodium chloride (NaCl), n-hexane (C_6H_{14}), ice cubes, and FAME (*Fatty Acid Methyl Ester*) Mix.

Sample Preparation: The samples used in this study were eel (*Anguilla marmorata* (Q.) Gaimard) *glass eel, yellow eel,* and *silver eel* phase taken from Posolake and Palu river. The fish are put into *sterofoam* containers containing ice cubes before processing to be extracted. Next, the sample was washed and drained. The drained sample is then dried using an oven at 60 ° C. Sugarfish (*Anguilla marmorata*) is dried for 24 hours. After the sample is dry, the sample is blended to a powder, then stored in room temperature (20-25 ° C) in a stainless steel container.

Fat Level Analysis

Pre Extraction Methods for Analysis of Fat Levels: Weigh the sample of eel powder (*Anguilla marmorata*) *glass eel* phase, *yellow eel*, and *silver eel* obtained from Posolake and Palu river as much as 1.5 ± 0.0005 gram (W1). Then dry soxcap capsule containing sample and aluminum cup using oven at 105 ± 2 ° C for 2 hours. After drying, weigh the aluminum cup (W2 pre). Then dried again using oven for 2 hours at 105 ± 2 ° C. Further cooled in the desiccator and mount the timble on the *soxtecextraction* unit, then remove the aluminum cup and grab the cellulose timble, then re-fabricate the aluminum cup at 105 ± 2 ° C until the solvent evaporates, then weigh the aluminum cup to determine its weight (W3 pre).

Calculation :

$$\% \text{ fat level} = \frac{W_3\ pre - W_2\ pre}{W_1}\ x\ 100\%$$

Hydrolysis Methods (Extraction) Sample for Analysis of Fat Levels: Weigh the sample of eel powder (*Anguilla marmorata*) *glass eel* phase, *yellow eel*, and *silver eel* obtained from lake Poso and river Palu as much as 1.5 ± 0.0010 gram (W1). Then enter into soxcap*capsule* that already contains the filter. Then dry the *capsule*soxcap containing the sample and the aluminum cup using the oven at 105 ± 2 ° C for 2 hours. After drying, weigh the aluminum cup (W2 hid). Then place the capsule on the *carousel* and insert the HCl 3M into the beaker until the 'R' mark. Next, place the *extraction vessel* on the hot plate and condenser on the beaker lid. Then heat to boiling (full heat), lower the heat regulator to 7.5 when the acid reagent has boiled, then heat for 1 hour. When the hydrolysis process is complete, turn off the heat regulator and remove the acid solution inside the beaker. After the acid solution runs out, enter double RO water, then wash the *carousel* in double RO water up to 3 times until the pH is about 6.7-7. Then lift the *carousel stand*, grab the *carousel stand capsule* and move it to the tissue to remove the remaining water. Move the *capsule* to drying stand and dry on microwave oven with DEFROST temperature for 15 minutes. Lift the *capsule* out of the microwave oven and plug the timble on the bottom of the *capsule*. Then push the filter until the position of the filter is tilted or perpendicular. Then attach the adapter to the *capsule*, then put the *capsule* on the *soxtec extraction* unit. When finished, remove the aluminum cup and dry it at 105 ± 2 ° C. Then cool on desiccator, then weigh aluminum cup (W3 hid).

Calculation:

$$\% \text{ fat level} = \frac{W_3\ hid - W_2\ hid}{W_1}\ x\ 100\%$$

Analysis of Fatty Acids Sample: Samples obtained from the previous stage were derivatized into fatty acid methyl esters. The methylation stage is intended to form the derived compounds from fatty acids to their methyl esters. The fatty acids are converted into other methyl or alkyl esters before being injected into gas chromatography. The fat extract was weighed 0.0375 grams and added 2 ml of 0.5 M NaOH (0.5 g of NaOH dissolved in 25 ml of methanol), then heated in a water bath at 100 ° C for 20 min. Further cooled and then added 14% BF3 in methanol (14 grams of BF3 added methanol to 100 ml) and reheated at 100 ° C for 20 min. Chill and shake until 30 ° C then add 2 ml of saturated NaCl, then divortek for ± 2 minutes and add n-heksan, then divortek back for ± 2 minutes. Further stuck at room temperature. Take a layer of n-hexane methyl ester, transfer it into a 10 ml measuring flask and dilute it and squeeze it with n-hexane. Prior to injecting methyl esters into gas chromatographic tools for the analysis of fatty acid compositions of the samples, the standard FAME (*Fatty Acid Methyl Ester*) standard solution Mix is diluted and

nitrated to n-hexane in a 10 ml measuring flask containing 500 µl of standard solution. The fatty acids present in the methyl esters will be identified by the FID (*Flame Ionization Detector*) detector or the flame ionization detector and the existing response will be recorded through the chromatogram. The fatty acid identification was performed by injecting 1µl standard solution then 1 µl of the sample solution in the gas chromatographic tool.

Calculation:

$$\% \text{ fatty acids} = \frac{\text{total area per fatty acid component x total fat}}{\text{total area of total fatty acids}}$$

Data Analysis: The analysis used is independent sample T Test, it is said there is significant difference of fatty acid value if T test result is P <0.05

RESULTS

The result of total fat content of the fish meat samples using the soxhlet extraction method was performed, where the samples were tested by 1 test in each sample to obtain total fat content. Graph of total fat content of extraction results can be seen in Figure 1 (legends) and the analysis of omega-3 and omega-6 fatty acid compositions was performed using the Gas Chromatography (GC) tool Shimadzu GC-2010 Plus to know the composition of omega-3 fatty acids and omega-6 fish flesh of *glass eel, yellow eel,* and *silver eel*. Analysis of the composition of omega-3 and omega-6 fatty acids in the sample was classified as polyunsaturated acid. The results of omega-3 and omega-6 fatty acid content analysis can be seen in Table1 (legends).

DISCUSSION

The present research was conducted to compare the amount of omega-3 fatty acids and omega-6 fish meat of Sidat (*Anguilla marmorata* (Q.) Gaimard) *glass eel, yellow eel*, and *silver eel* phases of Palu and Poso lakes. In the body eel contains unsaturated fatty acids needed by the body, including omega fatty acids. Fat or fish oil has special features in terms of fatty acid composition. Fish fats contain polyunsaturated fatty acid polyunsaturated fatty acids (PUFAs) which include omega-3 and omega-6 fatty acids which are essential fatty acids the body needs to maintain optimal health (Sunarya, 1993). Omega-3 fatty acids are one of the unsaturated fatty acids that can not be converted into cholesterol in the body, so it can be said omega-3 lowers blood cholesterol levels (Suptijah, 1999). Omega-6 fatty acids are polyunsaturated fatty acids that have their first double bond at the 6th position. Omega-6 is one of the essential fatty acids. Omega-6 deficiency can cause hair loss, skin disorders such as eczema, and liver fat infiltration (Diana, 2012). The needs of fatty acids can be met by consuming animal fats such as sea fish and fresh fish. Fish samples used in this research are eel fish taken from Paluriver and Poso lake, sampling technique in Palu River and Poso lake is done by using purposive sampling method (Soewarno, 1987). Eel fish (*Anguilla marmorata* (Q.) Gaimard) used in this research are *glass eel* phase (50-60 mm), *yellow eel* (300-400 mm), and *silver eel* (500-600 mm). Captured fish are stored in *sterofoam* containers containing ice cubes as a temporary preservative for the fish not to rot easily before the next process (Indrawati *et al*, 2016). This study was conducted to analyze the composition of omega-3 and omega-6 fatty acids using gas chromatography tool, which was previously done by using oil socculation. Selection of soxhletasi method is based on the advantages of this method when viewed from the time as well as the solvent used. The time used is relatively short and the solvent used is relatively small, when compared with other extraction methods (Nurhasnawati, 2017). In addition, the method of socletation is also the most effective for extracting oil because with this method almost 99% oil in the sample can be extracted. Therefore, the removal of the oil component is carried out by the method of socletation (Fessenden, 1991). The use of gas chromatography in fatty acid composition analysis is because it is suitable for separating the components of fatty chemical compounds (Rubiyanto, 2017), where fat has different vapor points based on the fatty acid composition contained therein. This is in accordance with the working principle of a gas chromatographic tool which separates the compounds based on different vapor points (Gandjar and Rohman, 2010). Oil is known to have a high vapor point due to its constituent substance in the form of triacylglycerol. Therefore, before being analyzed by gas chromatography, each sample of transesterified fish oil forms a unit of methyl ester fatty acid known as FAME (*Fatty Acid Methyl Ester*) which has stable and volatile properties (Gandjar and Rohman, 2010).

Solution total fat content of fish meat of Sidat (*Anguilla marmorata* (Q.) Gaimard) phase of *glass eel, yellow eel*, and *silver eel* from river Palu and lake Poso showed a very significant difference can be seen in Figure 1, total fat content of fish fishSidat phase *yellow eel* and *silver eel* from Poso lake is higher than the origin of Palu river, whereas total fat content of fish flesh of *glass eel* phase from lake poso is lower than the origin of river hammer. Differences in total fat content of fish can be affected by several factors including feed type, species, body size, fishing location, water temperature, and season (Visentainer *et al*, 2007). According to Ngadiarti *et al* (2013), the difference in total fat content due to chemical physics factors can be influential. In the physics process is affected by heating, because heating damages the structure of the network so that the oil glands are released. For chemical factors, it is affected by the solvent used during the extraction of the material. The oil content increases with the increase of distillation operating temperature and because of the higher temperature the water movement is bigger, so that all of the oil contained in the tissue will be extracted in even greater quantities. According to (Stansby, M, E., and Olcott, H, 1963), fish can be classified into several classes based on the composition of fatty acids, one of which is low fat content if it contains <5% fat. The results of Eel, *glass eel, yellow eel*, and *silver eel* are 0.088-0.201g / 100g, 0.101-1.079g / 100g, 0.059-3.121g / 100g, which can be classified as low fat content fish.

The results of omega-3 fatty acid analysis of fish meat of Sidat (*Anguilla marmorata* (Q.) Gaimard) phase of *glass eel, yellow eel*, and *silver eel* from Paluriver can be seen in Table 1. From the results obtained it is known that fish flesh of *glass eel* phase Eel from Palu river contains omega-3 fatty acid compared to Poso, while *yellow eel* fish and eel *silver eel* from Palu river contains fewer omega- 3 compared to the origin of Lake Poso. Omega-3 fatty acids are polyunsaturated fatty acids consisting of 18 carbons with 3 double bonds on the 3rd carbon chain of metal (CH3). Including omega-3 fatty acids are *linolenic acid, eicosapentaenoic acid* (EPA), and *docosahexaenoic acid* (DHA) (Devi, 2010). Omega-3 fatty acids can lower blood cholesterol in various lipoprotein

fractions, including cholesterol in LDL (*low density lipoprotein*) cholesterol (Khomsan, A., Anwar, 2008). Benefits of omega-3 for the body, namely the growth of brain cells. When the lack of omega-3 fatty acids, the nerves in the brain will lack energy for growth and development process so that it can interfere with the work and brain function. Another benefit is the vision organs and bones. Omega-3 deficiency in a person can cause the eyes become blurred and disturbing vision. Another benefit is to keep blood vessels and heart cells, overcome joint diseases, maintain and maintain skin health. The body needs about 300 mg of omega-3 per day (Graha, C, 2010). The results of omega-6 fatty acid analysis of fish meat of Sidat (*Anguilla marmorata* (Q.) Gaimard) phase of *glass eel, yellow eel*, and *silver eel* from Paluriver can be seen in Table 1. From the results obtained it is known that fish flesh of *glass eel* phase Eel from Palu river contains omega-6 fatty acid compared to Poso lake, while fish of *yellow eel* and *silver eel* from Palu river contain fewer omega-6 fatty acid compared to origin of Poso lakes. Omega-6 fatty acids are polyunsaturated fatty acids consisting of 18 carbons with 2 double bonds on the 6th carbon chain of metal (CH3). Including omega-6 fatty acids are *linoleic acid*, and *arachidonic acid* (Devi, 2010). Omega-6 function for the body is to maintain blood viscosity so that when injured does not cause bleeding, supports kidney performance, fight inflammation caused by pathogen infection, support smooth muscle performance, maintain skin integrity (Lingga, 2012).

The composition of omega-3 fatty acids and omega-6 fish flesh of g*lass eel, yellow eel*, and *silver eel* from Palu and Poso lakes are found in Table 1, where fish flesh of *glass eel* phase phases the Palu contains more omega-3 and omega-6 fatty acids than Poso origin, whereas in the *yellow eel* and *silver eel* phases the Palu origin contains fewer omega-3 and omega-6 fatty acids than originally from Poso. The differences in the results of omega-3 and omega-6 fatty acids obtained from the sample can be influenced by the composition of the fat types consumed from the environment (Lebcanc, *et al*., 2006). Differences can also be caused because the nutrient content in each fish varies depending on internal and external factors. Internal factors such as species or species of fish, age and reproductive phases in fish. External factors, in the form of factors that exist in the living environment of fish in the form of habitat, availability of feed and quality of waters where live fish (Aziz, *et al*., 2013). Sidat (*Anguilla marmorata*(Q.) Gaimard) origin of the Paluriver in the *glass eel* phase contains 3 types of omega-3 fatty acids and 2 types of omega-6 fatty acids. The *yellow eel* phase contains 1 type of omega-3 fatty acids and 2 types of omega-6 fatty acids. The *silver eel* phase contains 2 types of omega-6 fatty acids and contains no omega-3 fatty acids. While the origin of lakePoso, *glass eel* phase contains 3 types of omega-3 fatty acids and 2 types of omega-6 fatty acids. The *yellow eel* phase contains 1 type of omega-3 fatty acids and 3 types of omega-6 fatty acids. The *silver eel* phase contains 2 types of omega-3 fatty acids and 3 types of omega-6 fatty acids. Each of them has a different concentration ratio although most are found with the same type of omega-3 and omega-6 fatty acids. Differences in the profile of omega-3 and omega-6 fatty acids from each fish can be influenced by several factors, ie species, geographic feed and the age and size of the fish (Ozogul, Y., Simsek, A. Balikci, E., Kenar, 2012). In the sample of fish flesh of *glass eel* phase, omega-3 and omega-6 are dominant are *linoleic acid, docosahexaenoic acid*, and *eicosapentaenoic acid*. In the *yellow ee*l phase, omega-3 and omega-6 fatty acids are the dominant ones are *linoleic acid, arachidonic acid*, and *linolenic acid*. In the *silver eel* phase, omega-3 and omega-6 fatty acids are the dominant *linoleic acid, gamma-linolenic acid*, and *arachidonic acid*. *Eicosapentaenoic acid* (EPA) is an omega-3 fatty acid group that can reduce the risk of coronary heart disease. *Arachidonic acid* is an eicosanoid in the body that is the ingredient for the formation of prostaglandins as an inflammatory agent (Ginanjar, R. G., Indra, T. M and Reza, 2015). The result of statistical data of T test is not paired, it is said there is difference of fatty acid if anova test result is P <0.05. The content of omega-3 and omega-6 fatty acids in the fish flesh of *glass eel* phase from Poso and Palu river showed significant differences, ie*eicosapentaenoic acid*, *docosahexaenoic acid*, and *linoleic acid*, while those showed no significant differences, *linolenic acid* and *arachidonic acid*. In the *yellow eel* phase, which showed significant differences, ie*docosahexaenoic acid, linoleic acid, gamma-linolenic acid*, and *arachidonic acid*, whereas no significant difference was found, *linolenic acid*. In the *silver eel* phase, there were significant differences, namely *linolenic acid, linoleic acid, gamma-linolenic acid*, and *arachidonic acid*, whereas there was no significant difference, ie*docosahexaenoic acid*. Fish containing protein, vitamins, minerals, carbohydrates and fats can be a source of nutrients for the body. According to ((FAO), 2010), recommended the consumption of fat PUFA (*polyunsaturated fatty acid*) per day can be given up to 6-11g / 100g of total energy intake. Needs of fatty acids per day can be obtained by eating fish that have a variety of Sidat type fatty acids.

Conclusion

The total fat content of eel meat has a difference which in the *glass ee*l phase total fat content of eel meat from Palu (1.9939% w / w) is higher than Poso lake (1.2349% w / w), while in *yellow eel* and *silver eel*phase total fat content from Palu (1.0933% w / w and 0.9960% w / w) was lower than that of Poso lake (16.777% w / w and 19.578% w / w). Fatty acid content contained in fish flesh of *glass eel, yellow eel*, and *silver eel* from Palu and Poso lakes are different, wherein omega-3 and omega-6 fatty acids in *glass eel* phase from Palu (0.201%) more high compared to the origin of lake Poso (0.088%), while in the *yellow eel* and *silver eel* phases from Palu (0.101% and 0.059%) lower than the origin of Poso lakes (1.079% and 3.121%).

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