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Comparison of Liver Fat Content and Triglyceride of Timpakul Fish (*Periophthalmodon schlosseri*) and Snakehead Fish (*Channa striata*).

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ABSTRACT

Liver fat and Triglycerides content are important energy sources, for animals to be able to adapt with the environment. Timpakul Fish (*P.schlosseri*) live in the habitat of tidal mangrove areas and have higher adaptation activities on the land and water than *C. Srtiata*. Environmental adaptation of *P. schlosseri* will affect the adaptation toward the different structure of the anatomy, physiology, and behavior of the fish in general, especially in *C. Srtiata* fish. One of the physiology adaptation of the liver can be seen in the liver fat and Triglyceride content profile. The aim of this study is to find out the comparison of the contents of liver fat and Triglyceride in the timpakul fish (*P. schlosseri*) and snakehead fish (*C. striata*). Liver fat content was measured using Soxhlet method whereas Triglyceride content was measured using *Enzymatic-Trinder* method of *Labtest*. The results showed that *P. schlosseri* have higher liver fat and Triglyceride content than *C. striata*. The liver fat content in *P. schlosseri* fish is $51\pm 4.5\%$ and it is higher than *C. striata* which is $6.8\pm 0.84\%$. The Triglyceride content in *P. schlosseri* fish is higher than *C. striata* which is 136.67 ± 26.22 mg/dL compared to *C. striata* that is only 79.75 ± 28.5 mg/dL.

Keywords: *P.schlosseri*, *C.striata*, liver, fat content, Triglyceride

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INTRODUCTION

Fat is the main fuel or energy source for almost all organisms. Fat or triacylglycerol is an enormous energy reserves because it is in the reduced form and the anhydrous form. Perfect oxidation of fatty acids produces 9 kcal/g energy compared to carbohydrates and proteins that produce 4 kcal/g energy. The adipose cell is devoted to involve in the synthesis and to become storage of triacylglycerol as well as to mobilize the triacylglycerol into a fuel molecule that will be transferred to other tissues by blood ^[1].

Fat and oil are triglyceride compounds of glycerol. In the formation, the triglyceride is the result of the condensation of one glycerol molecule and three fatty acid molecules (generally those three fatty acids are different), which forms one triglyceride molecule and a water molecule ^[2]. If triglyceride is being hydrolysis, it will produce 3 long chain fatty acid molecules and one molecule of glycerol. The length of the fatty acid chain in Triglyceride which formed naturally may vary, but the most common lengths are 16, 18, or 20 carbon atoms. The longer the carbon chain, the more energy or ATP that is produced ^[3].

Triglyceride is the major lipid compounds contained in food ingredients and as important sources of energy, especially for the animals. In liver cells, fatty acids are degraded or synthesized into Triglyceride. Most Triglyceride is stored in adipose tissue cells. Fish habitat affects on the chemical content in the body such as proximat, amino acids, and fatty acids ^[4]. Mentioned that the fish which were included in *Gobiidae* species that live in brackish tidal habitats generally have different adaptation activities of anatomy, physiology, and behavior compared to other fish ^[5]. Those differences affect the liver structure which has a unique structure seen from its color and shape, so it is assumed that it has a fat content which is stored as a backup energy source. The differences also affect the liver physiology that has functions in the process of substances metabolism; one of them is lipid metabolism. Liver cells can be the storage of fat, carbohydrates, and proteins that can be recycled when the body lacks of energy and involve in extreme circumstances activities ^[6].

P. schlosseri can survive in hypoxic conditions that it tends to have the *air-breathing* ability. The habitat of this fish is in the mud with water which stays up at all times in the tidal areas. The water in the habitat of *P. schlosseri* tend to have low oxygen levels due to the water exchange cycle that only occurs when the water is in high tide ^[7]. *Air-breathing* ability is also owned by the snakehead fish (*Channa striata*). *Channa striata* has a high body survival against the environmental conditions of unfavorable waters. *Channa striata* can survive because it has the respiratory aid device called diverticula that can use and take advantage of the free oxygen in the air to involve in its breathing process when the condition of water lack comes ^[8]. This freshwater fish can live in several habitats such as rivers, wetlands, lakes, and fields. This fish, which is known by the local name "Haruan", has *suprabrancial* space that serves as an *air-breathing* organ that makes it unique in the *Channidae* fish species ^[9].

Based on that theory, this research will continue toward its physiology that is liver fat and Triglyceride content is the source of energy reserves as a form of physiological adaptation toward its habitat especially in South Kalimantan province. There are similarities between the nature of *P. schlosseri* and *C. striata* in terms of physiological adaptation toward their habitat which is the *air-breathing* ability, so it is necessary to investigate the comparison of liver fat and Triglyceride content of *schlosseri P.* and *C. striata*.

MATERIALS AND METHODS

Material

Materials used were the hearts of 6 *schlosseri P.* and *C. striata* fish, blood samples from 6 *schlosseri P.* and *C. Striata* fish, *Ethylene Diamine Tetra Acid* (EDTA), Na_2SO_4 (which has functions to absorb/bind water that is still in the sample), the *n-hexane* solvent, *Labtest* Triglyceride reagents, standard solution of Triglyceride *Labtest*, *akuades*, filter paper, white thread, label paper, *aluminum foil*, 1 kg sugar plastic, rubber bands, paper towels, ice cubes and tap water.

Research Procedures

Sampling

The sampling of fish sample selecting was done by using *purposive sampling* based on the consideration of certain characteristics which are fundamental characteristics of the population ^[10]. *P. schlosseri* was taken from Sungai Barito's estuary of South Kalimantan province, Indonesia by fishing them using small-sized shrimp feed. The fish was lured by moving the bait feed around the mouth. The *P. schlosseri* that could capture direct feed was put in the bamboo cages. *C. striata* was obtained by buying them from traditional market of Bauntung Banjarbaru.

Sample Preparation

P. schlosseri's blood samples were directly taken on the set location while *C. striata* was immediately brought home to have blood drawn after being purchased from traditional market. The blood should be drawn by using a 3 mL syringe that has been moistened with *Ethylene Diamine Tetra Acid* (EDTA) anticoagulant. Blood can be taken on the position of: measuring a distance of about two fingers from the base of the anal fin then the syringe needle was directed about 5°-10° under *linea lateralis* until it touched the L spine. The blood was drawn slowly (about 1-1,5mL) then the syringe needle was removed and the blood sample was transferred into the blood *tube*. The available blood sample was directly brought to clinical laboratories to be measured its triglyceride levels ^[11].

Before the liver was taken, the total length (cm), standard length (cm), width (cm), and height (cm) of *P. schlosseri* was measured by using a block and ruler millimeter, then the weight (grams) of *P. schlosseri* was weighed using the analytical balance. After that, *P. schlosseri* was dissected to take its organs. Then the length (cm) and width (cm) of the liver organ was being measured by using a block and ruler millimeter. Finally, the liver was weighed (grams) by using an analytical balance. The same procedure also was done to *C. striata*.

Once the fish was dissected and taken its organs, the liver of each *P. schlosseri* was directly placed on the filter paper and spiked with Na₂SO₄ as much as 0.5 grams and then wrapped and covered with a white thread ^[12]. Then, the white thread should be left for about ± 15 cm as a handle. After that, the liver sample is inserted into a small cup and is poured with ± 50 mL *n-hexane* solvent. Finally, the cup was immediately covered with *aluminum foil* and sugar plastic, then it was tied with a rubber band and stored at the temperature room (so that the solvent did not evaporate). The same procedures was done to *C. striata*.

Measurement of Liver Fat Content

Fat content was measured by using the Soxhlet method. The dried Soxhlet flask was first weighed using analytical balance to know its weight (gram). After that, the flask was mounted on a Soxhlet extraction device. Then, it was filled with *n-hexane* solvent as much as ± 200 mL which was poured through the hood ^{hasan}. The liver sample along with the *n-hexane* solvent which was in the small cup was poured into the hood. The hood is then covered with a condenser and the appliance or device was powered by electricity for ± 3 hours (with cooling water that was tap water which was given ice cubes that continued to flow in the condenser using an aerator). *N-hexane* solvent in the Soxhlet flask will rise and fall periodically in the hood until the color changed into yellowish translucent. The Soxhlet flask containing *n-hexane* that had changed its color then was distilled at a temperature of 60°-70°C until all the solvent evaporated. After that, the Soxhlet flask was being heated-up in the oven for 1 hour at 105°C to evaporate the solvent remnants. Finally, the flask was cooled and weighed to determine the final weight (grams) by using the analytical balance. The fat content was determined by using formula ^[13].

Measurement of Triglyceride Content

Triglyceride was measured by using *Enzymatic-Trinder* method of *Labtest*. The blood sample which was in the blood tube was firstly being *centrifuged* for 10 minutes at a speed of 5000 rpm. Having separated particles (blood cells sediment at the bottom, *buffycoat* in the middle, and serum on the top), the sample was placed in a test tube rack. Reagents and standard solutions were then prepared in other test tubes. A total of 500 µL reagents was incorporated into the test tube and was added with standard solution of Triglyceride as

much as 5 μ L and then was shaken and incubated for 10 minutes at 37 °C using a *stopwatch*. A total of 500 μ L reagent was incorporated into other test tubes then was added with 5 μ L of sample blood serum and was shaken as well as incubated for 10 minutes at 37 °C using a *stopwatch*. The standard values were measured by using a spectrophotometer with 490-520 nm wave length, so that it can obtain 200 mg/dL score. Then, the spectrophotometer was washed by using *akuades*. After that, the sample was measured by using a spectrophotometer with 490-520 nm wave length. To obtain the Triglyceride value, the calculation used was the sample absorbance score multiplied by the specified standard absorbance score and then it was divided by the standard score which was previously measured (200 mg / dL) to obtain the final result.

Data analysis

The data were analyzed by using SPSS Statistics program (version 21.0). Homogeneity test was performed to see the data diversity by using Levene test with 5% real level then it continued with T Independent Samples test by using 5% real level to know the difference of the mean fat content of *P. schlosseri* with *C. striata* and the mean of triglyceride content *P. schlosseri* with *C. striata*.

RESULTS AND DISCUSSION

Heart Fat Content

The mean of body weight, weight of liver, and liver fat content from 6 samples of *P. schlosseri* and 6 samples of *C. striata* that have been used in this research can be seen in the table 1.

Table 1. The mean of body weight, weight of liver, and liver fat content of *P. schlosseri* and *C. striata*

Ikan	Berat tubuh (g) (mean±stdev)	Berat hati (g) (mean±stdev)	Kadar lemak hati (%) (mean±stdev)
<i>P. schlosseri</i>	153,56±27,75	3,38±0,96	51±4,5
<i>C. striata</i>	168,9±24,96	1,14±0,24	6,8±0,84

Inf: sig. value is <0,05, it can be concluded that there is diversity (variation) of liver fat content in both of fish species

Homogeneity test was performed to see the diversity of liver fat content in both fish species by using Levene test with 5% real level. The obtained sig. value is <0,05, it can be concluded that there is diversity (variation) of liver fat content in both of fish species. Furthermore, T Independent Samples test was conducted by using a real level of 5% in order to determine whether there was a difference of mean liver fat content in both fish species or not. The obtained sig. value is <0,05, it can be concluded that there is different mean of the liver fat content in both of fish species.

The mean calculation of the result in Table 1 showed that the body weight of *P. schlosseri* is lower than *C. striata* but it has a liver weight that \pm 3 times higher, while the body weight *C. striata* higher than *P. schlosseri* but has lower heavy heart. The data from the measurements of the two fish samples showed that the body weight of the fish, the higher fat content of the liver. Stated that the lipid content in the fish liver showed a tendency to increase with the increasing body weight of the fish [12]. The female fish has a correlation between the mass of the heart and the mass of the gonads. It can be seen during the dissection of *P. schlosseri*. Some *P. schlosseri* have large ovaries followed by a large liver as well as other *P. schlosseri* whose ovaries are immature or *P. schlosseri* has no ovary [14]. Affirmed that the fat content of the fish is not always the same at all times. The maximum fat content is usually achieved when the fish has reached to spawn. Conversely, after the spawning period is over, the fat content decreased rapidly and reached the lowest level [15].

The results showed that *P. schlosseri* had higher liver fat levels than *C. striata*. The mean value of liver fat content in *P. schlosseri* according to that mentioned that the fish liver may contain 30% -70% fat [16]. Based on study that measured the liver fat content of *silver hake fish (Merluccius bilinearis)* and *red hake fish (Urophycis chuss)*, it has vary value from low to high. *M. bilinearis* has a value of liver fat content between 1.7% -78.7% with an average of 24.7%, whereas *U. chuss* has a liver fat content value of 8.8% -47% with an average of 30, 2% [17]. However, in the study of that measured the levels of liver fat *Pleurogrammus azonus*, the value ranged from 1.1% to 7.7% ^{vdovin}, and in the study that measured *Catla catla* liver fat content, is only between 3.32% -5.91% [12]. The substantial variation in liver fat levels are due to several factors, such as individual

differences, age variation, and gonadal maturity ^[17]. The variation of chemical composition can occur between the species and between the individuals in one species ^[18].

The fish of Gobiidae tribe that live in tidal habitat of sea water have activity of anatomy adaptation, physiology, and different behavior to the tother fish in general ^[5]. Liver has a uniqueness, such as the color and shape of the liver, so that it is suspected to have fat content stored as a source of energy reserves. It also affects the liver physiology that has functions in the process of metabolism substances, which one is lipid metabolism. Liver cells can be the storage of fat, carbohydrates, and proteins that can be recycled when the body lacks of energy and activity in extreme circumstances ^[6].

Based on the results of the research about microscopic observation of blood smear liver fish *P. schlosseri*, where the hepatocyte cells in his liver have a core that is pressed into the side of hepatocyte cells. It is thought to be due to the accumulation of fat in the liver *P. schlosseri* is useful as energy reserves associated with their physiological adaptations for activities on the water and the land ^[6].

High levels of liver fat may be caused by the fish eating habits ^[12]. In the nature, the fish should search out the organisms that have lots of fat as a food ,and it will be deposited in the body as energy reserves ^[14]. The lipid content in the fish body is also influenced by the environment and nutritional conditions ^[19]. The higher levels of *P. schlosseri* liver fat than *C. striata* are related with the metabolism of those fishes to adapt in their habitat, where *P. schlosseri* required more energy supply than *C. Striata*, because most of the time of *P. schlosseri* is spent on the land such as swimming, walking and jumping over the mud, air-breathing, feeding, and digging holes that is used as the nest ^[20]. Stated that the anatomical and behavioral adaptations of mudskipper helped these fish to live effectively on land and in water. These fish have many physiological, morphological, and behavioral specialties for amphibian life, because most of their time is spent on land. In contrast, *C. striata* has lower activities, such as swimming, air-breathing, feeding, and foaming nest among the vegetation ^[20].

The habitat of these fishes is different, because the tidal areas of seawater which are the habitat of *P. schlosseri* have extreme environment, such as high pH, uncertain environmental temperature, very low oxygen content, and high heavy metal content compared to *C. striata* habitats, such as river, swamp, or rice field. *C. striata* generally found in shallow waters, such as rivers and swamps with a depth is 40 cm and tend to choose a dark place, muddy, calm, or rocky areas to hide. Moreover, this species is also found in lakes as well as water channels down to the rice fields. *C. striata* are able to survive in swamp water conditions with low dissolved oxygen content and pH range 4.5-6 ^[21].

Triglycerides Contents

The mean of body weight, weight of liver, and triglyceride contents from 6 samples of *P. schlosseri* and 6 samples of *C. striata* that have been used in this research can be seen in the table 2.

Table 2. The mean of body weight, weight of liver, and triglyceride contents of *P. schlosseri* and *C. striata*

Ikkan	Berat tubuh (g) (mean±stdev)	Berat hati (g) (mean±stdev)	Kadar lemak hati (%) (mean±stdev)
<i>P. schlosseri</i>	121,83±11,73	1,06±0,24	136,67±26,22
<i>C. striata</i>	168,9±24,96	1,14±0,24	79,75±28,5

Inf: sig. value is <0,05, it can be concluded that there is diversity (variation) of liver fat content in both of fish species

Homogeneity test was performed to see the diversity of triglyceride contents in both fish species by using Levene test with 5% significance level. The obtained sig. value is <0,05, it can be concluded that there is variety of triglyceride contents in both of fish species. Furthermore, T Independent Samples test was conducted by using a real level of 5% in order to determine whether there was a difference of triglyceride contents mean in both of fish species or not. The obtained sig. value is <0,05, it can be concluded that there is difference of mean of triglyceride content in both of fish species.

The mean calculation of the result in Table 2 showed that the body weight of *P. schlosseri* is lower than *C. striata*, and the *P. schlosseri*'s liver weight is lower than *C. striata*. The differences in body size and weight of liver between the *P. schlosseri* samples that used for the measurement of liver fat and triglyceride

fat contents because of the differences in taking the sample. The sample is used for the measurement of liver fat content that was taken during the rainy season, where the rainy season is the time that *P. Schlosseri* used to reproduce, while the sample that is used for measuring triglyceride contents is taken during the dry season [22].

The results showed that *P. schlosseri* had higher triglyceride contents than *C. Striata*, although has lower body weight. The higher levels of triglyceride *P. schlosseri* may be caused by eating habits. The fish should search out the organisms that have lots of fat as a food, and it will be deposited in the body as energy reserves. *P. schlosseri* has a high activities in order to survive in its habitat, so that it required triglycerides that are constantly and actively synthesized in the liver for the body tissues that performed physiological activity adaptation [12]. The lipid content in the fish body is also influenced by the environment and nutritional conditions [19].

The difference environment between *P. schlosseri* and *C. striata* also caused different types of food, so that it may be the cause of different triglyceride levels between *P. schlosseri* and *C. striata*. On the results of research stated that the main food of *C. striata* with a total length range between 12.6-26.3 cm that is observed in Sabuah Lake, Central Kalimantan was fish (44.6%) [23]. The other food is a piece of aquatic animals, water slugs, Rotifera, and Rhizopoda, in contrast, on the results of research mentioned that the main food of *P. schlosseri* is *Uca sp.* (crab) and *Oryzias sp.* (medaka fish) [24], and the results of research mentioned that the main food of *Periophthalmus sp.* (one family with *P. schlosseri*) is crustaceans, and the complementary foods are Zooplankton, Phytoplankton, Polychaeta, and the supplementary food is Hexapoda [25].

Crabs and shrimp are classified to these crustaceans, Crabs and shrimp contained monounsaturated fats that are one of the triglyceride types (monounsaturated fats), which can be consumed but only in limited amounts [26]. Crabs and shrimp included as the type of food that is needed to be considered for consumption, because of high fat content (crabs contained 150 while shrimp contained 160 in units of mg/10 gr and included in the category of "caution"). This may explain that the levels of triglycerides in the blood are influenced by the fat content that contained in the digested food.

The triglycerides contents in the blood can be influenced by several causes, such as food substances in the food, genetic factors, the increasing age cause the decline in various organs of the body, so that the balance of content Blood triglycerides are difficult to achieve, consequently, triglyceride contents tend to increase more easily [27], and the decreased lipoprotein lipase (LPL) enzyme activity that can cause Very Low Density Lipoprotein (VLDL) to Intermediate Density Lipoprotein (IDL) to be inhibited, so that the VLDL will settle in the liver and cause fatty liver in the form of fat accumulation in the sinusoids and around the liver cells [28].

In general, the liver has two functions: storage of lipids and blood sheds [14]. Lipid in fish is an important source of energy that used in various activities of life [29]. Triglycerides are the main lipid compounds that contained in foodstuffs and as an important source of energy, especially for animals. Fatty acid degraded or synthesized into triglycerides in liver cells. Most of the triglycerides are stored in adipose tissue cells. The triglycerides are degraded and constantly resorbed [30]. Triglycerides are present in the blood as macromolecules that form complexes with certain proteins (apoproteins) to form lipoproteins [31]. The triglycerides are packed into VLDL by hepatocyte and released into the blood in order to supply other tissues [30].

The metabolism of triglycerides in the body mainly occurs in the liver. The path of triglyceride metabolism is divided into 2 ways, they are exogenous pathways and endogenous pathways. In exogenous paths, triglycerides that derived from the food in the gut are packed as kilomikrons. These kilomikrons will be transported in the blood through the ductus thoracicus. In fatty tissue, triglycerides and kilomikrons undergo hydrolysis by lipoprotein lipase enzymes that are found on the surface of endothelial cells. As a result, this hydrolysis will form fatty acids and kilomikron remnan. Free fatty acids will penetrate the endothelial and enter into fatty tissue or muscle cells to be converted into triglycerides back or oxidized, while in endogenous pathways, the triglycerides that are synthesized by the liver are transported endogenously in the form of Very Low Density Lipoprotein (VLDL) that have a lot of triglycerides and hydrolysis in circulation by the lipoprotein lipase enzyme that also hydrolyzes kilomycrons into smaller lipoprotein particles that are Intermediate Density Lipoprotein (IDL) and Low Density Lipoprotein (LDL). LDL is the most cholesterol-containing lipoprotein (60-70%) [32].

CONCLUSION

Based on the results of the study it can be concluded that *P. schlosseri* has higher fat content and triglyceride contents than *C. striata*.

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