Effect of the addition of crude fish oil (CFO) in feed to the content of EPA and DHA in mud crab (Scylla serrata)

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Abstract. Mud crabs (Scylla serrata) cannot synthesize essential fatty acids, so it needs a supply of Omega-3 fatty acids. One source of unsaturated fatty acids are Omega-3 in Crude Fish Oil (CFO). CFO is a source of essential fatty acids which contains EPA and DHA. The aim is to determine the provision of CFO on trash fish feed to the content of Omega-3 in mud crab. The method is experimentally using completely randomized design with five treatments and four replications. The number of CFO doses that added in a trash fish feed are P0 (0%), P1 (2%), P2 (4%), P3 (6%) and P4 (8%). Analysis of the content of EPA and DHA in mud crab performed using gas chromatography-mass spectrometry equipped with a column of silica and helium as a gas booster. The results showed that the addition of CFO on the feed provides a significantly different effect (P <0.05) of Omega-3 (EPA and DHA). Content of EPA and DHA in mud crab has shown the increased results when giving the smallest dose, 2% of CFO at P1 treatment, but when the dose was increased again to 4%, 6% and even 8% the results did not show any significant difference.

1. Introduction
Mud crab is one of the commodities with high economic value and interest of this crab consumption in the country or overseas continues to increase [1]. According to Kanna [2], mud crabs are commodities that are very favorable for cultivation because in addition to having a fast growth rate, mud crabs also have a high selling price. This species of crab preferred by consumers because the whole body is not soft or hard so that all parts of the crab's body can be consumed [3].

Consumption of mud crabs cannot be carried out continuously because cholesterol is high at 66.67 mg / 100mg. Limit cholesterol consumption in normal human is about 300 mg / day [4]. It is the human barrier to eating mud crab meat. Trash fish feed or artificial feed is given in the cultivation of mud crab.

The cholesterol content of crab feed can be suppressed by adding CFO, CFO or fish oil, which is one of the nutrients that contains fatty acids that are rich in benefits because it contains about 25% saturated fatty acids and 75% unsaturated fatty acids, one of which is omega-3 [5]. Omega-3 is one of
the unsaturated fatty acids that are essential for the body and especially needed for people with high cholesterol.

Unsaturated fatty acids EPA (Eicosapentaenoic Acid) and DHA (Decosahexaenoic Acid) is a type of omega-3 that be the most dominant in fish oil. These fatty acids are not produced by the fish, but rather by marine plants like algae. The content of EPA and DHA in fish caused by the fish consume algae that contain both these fatty acids [6]. Fatty acid EPA and DHA can lower the cholesterol by inhibiting the synthesis of Low Density Lipoprotein (LDL) and increase the synthesis of High Density Lipoprotein (HDL) [7].

2. Materials and methods
2.1 Place and time
This study was conducted in March 28th to April 28th 2016, at the Education Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. Fatty acid analysis performed on Unit Testing, Faculty of Pharmacy, Universitas Airlangga, Surabaya.

2.2 Tools and materials
The equipment used in this study included 60 tanks, siphon hoses, aerators, aeration hoses, aeration stones, aerators, large plastic tubs, measuring cups, analytical scales, pH meters, thermometers, DO meters and ammonia test kits.

The animals that used in the study were 60 crabs (Scylla serrata) with a size of 100-150 grams per head obtained from the lagoon fish market, Surabaya. The maintenance medium that used in this study was brackish water with a volume of 2.5 liters per aquarium measuring 25x20x30 cm3. The feed that will be used in this research is trash fish mixed with Crude fish oil.

2.3 Work procedures
Preparation of this research is by cleaning the equipment that will be used. The equipment is aquarium maintenance, plastic barrels and basins washed with soap, rinse it, then give chlorine, rinse and dry it. The dried aquarium filled with brackish water in each tank. Reservoir is filled with water and aerated, then put mud crab put the aquarium. Then the mud crabs were fasted for one day to eliminate the effect of feed that has been given before.

The feed used in this research was trash fish, due to its low price, large amounts, and content of low cholesterol. The trash fish that be used is turmeric fish (Upeneus sulphureus) which the fish meat is taken and cut into small pieces using a chopper. Furthermore, Crude Fish Oil and a binder in the form of tapioca flour are added at a dose according to the treatment into trash fish feed. Then, the trash fish feed that has been added with Crude Fish Oil is aerated for about 5-10 minutes before being given to the mud crabs.

After maintenance for 30 days, then the crab meat was taken and analyzed the content of EPA and DHA with gas chromatography-mass spectrometry, or GC-MS using a gas chromatograph Shimadzu QP2010 equipped with a column of silica DB-5 ms and helium as a gas booster.

2.4 Research parameter
The main parameters in this study were content of Omega-3 in particular mud crab meat that content of EPA and DHA. The content of omega-3 can be determined using gas chromatography-mass spectrometry, or GC-MS at the beginning and end of the study. For the supporting parameters, there are temperature, salinity, oxygen, ammonia, and pH of the water.

2.5 Data analysis
The design that used in this study is completely randomized design (CRD), which carried out experimentally. The study consisted of five treatments with four replications. Data were analyzed using Analysis of Varian (ANOVA) followed by further trials using Duncan's multiple range test
(Duncan’s multiple range test) to determine the difference between the one treatment with the other treatments. Statistical calculations using SPSS version 23.

3. Result and discussion

3.1 Eicosapentaenoic acid (EPA)

The result of the EPA content in mud crab (Scylla serrata) meat showed significant differences. Calculation of the EPA content in mud crab meat can be seen in Table 1.

Table 1. The average content of EPA (Eicosapentaenoic Acid) in mud crab meat.

| Treatment | EPA content (mg / g) ± SD | Transformation (√Y) ± SD |
|-----------|--------------------------|--------------------------|
| P0        | 1.82a ± 0.891            | 1:32 ± 0.207             |
| P1        | 9.62b ± 3.108            | 3:07 ± 0.487             |
| P2        | 9.77b ± 2.611            | 3:11 ± 0.417             |
| P3        | 9.79b ± 1.649            | 3:12 ± 0.277             |
| P4        | 7.08b ± 2.614            | 2:62 ± 0.520             |

Information: P0: Control, P1: Addition of CFO 2%, P2: Addition of CFO 4%, P3: Addition of CFO 6%, P4: Addition of CFO 8%. Notation of different superscript letters in the same column shows the comparison between treatments there is significant difference (P <0.05)

The Analysis of Varian (ANOVA) result showed significant differences among treatments (P <0.05). Based on the results of Duncan's Multiple Range Test (Duncan's Multiple Range Test) showed significant differences. A high EPA content obtained at treatment P1, P2, P3 and P4 are significantly different from P0 treatment.

The lowest EPA content was 1.82% found in the P0 treatment with no Crude Fish Oil content in the feed which was significantly different from other treatments, but the addition of CFO treatment showed results that were not significantly different between treatments for the addition of CFO (P1, P2, P3 and P4). The EPA content of mud crab meat in this study has shown an increase when giving the smallest dose of P1 treatment of 2% Crude Fish Oil. After adding more, the CFO content in P2 treatment was 4%, which increased but was not significantly different when calculated by ANOVA. This is the same as the P3 treatment where the addition of CFO by 6% shows an increase in the EPA content but not significantly different from the P1 and P2 treatments. The highest addition of CFO with a dose of 8% CFO actually showed a decrease in EPA of mud crabs.

The EPA content in the mud crab meat did not increase with the addition of the Crude Fish Oil dosage in the feed, it can be seen in the results of the Duncan Multiple Range Test that the P1 treatment was not significantly different from P2, P3 and P4 treatments.

3.2 Docosahexaenoic acid (DHA)

The calculation result of DHA content in mud crab meat shows the differences in DHA. Calculation of DHA content in mud crab meat can be seen in Table 2.

Table 2. The average content of DHA (docosahexaenoic acid) in mud crab.

| Treatment | DHA (mg / g) ± SD | Transformation (√Y) ± SD |
|-----------|-------------------|--------------------------|
| P0        | 1.19a ± 0.636     | 1:06 ± 0.295             |
| P1        | 4.63b ± 1.068     | 2:14 ± 0.255             |
| P2        | 4.66b ± 0.861     | 2:16 ± 0.205             |
| P3        | 6.39b ± 0.959     | 2:52 ± 0.197             |
| P4        | 4.77b ± 1.658     | 2:17 ± 0.368             |
Information : P0: Control, P1: Addition of CFO 2%, Q2: Addition of CFO 4%, P3: Addition of CFO 6%, P4: Addition of CFO 8%. Notation of different superscript letters in the same column shows the comparison between treatments there is significant difference (P <0.05)

The results of the analysis of variance (ANOVA) showed a very significant difference between treatments (P <0.01). Based on the results of Duncan's Multiple Range Test, it shows a real difference. High EPA content was obtained in treatment P1, P2, P3 and P4 which were significantly different from treatment P0.

The lowest DHA content was 1.19% in the P0 treatment with no Crude Fish Oil content in the feed which was significantly different from other treatments, but the addition of CFO showed results that were not significantly different between treatments for the addition of CFO (P1, P2, P3 and P4). DHA content of mud crab meat in this study has shown an increase when giving the smallest dose of P1 treatment of 2% Crude Fish Oil. After adding the CFO content in P2 treatment by 4%, it increased but was not significantly different when calculated by ANOVA. This is as same as the P3 treatment where the addition of CFO by 6% showed an increase in DHA content but not significantly different from treatment P1 and P2. The highest addition of CFO with a dose of 8% CFO actually showed a decrease in DHA of mangrove crabs. The DHA content in the mangrove crab meat did not increase with the addition of the Crude Fish Oil dosage in the feed, it can be seen in the results of the Duncan Multiple Range Test that the P1 treatment was not significantly different from P2, P3 and P4 treatments.

3.3 Discussion eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

The EPA and DHA content in the mud crab meat in this study increased after adding Crude fish oil to the treatment. A significant increase can be seen in the first treatment with the addition of a 2% dose of Crude fish oil. This is in accordance with Rustan and Drevon [8] which states that the addition of Crude fish oil can affect fatty acids and cholesterol content.

The addition of fish oil is expected to increase the content of omega-3 or the content of EPA and DHA in this study to produce positive results where there is an increase in the content of EPA and DHA when the dose of fish oil is added by 2% or in the first treatment, but when the dose is increased again to 4%, 6% and even 8% of the results showed no significant difference. This means that there is no significant addition to the EPA and DHA content of mangrove crabs when the 4%, 6% and 8% doses are added. This is because the need for essential fatty acids for marine fish products ranges from 0.5-2% of dry feed weight [9]. This need is also very dependent on the ability of fish to naturally break down essential fatty acids both anabolically and catabolically [10].

The addition of CFO by 8% shows a decrease in the EPA and DHA content of the mangrove crab meat, this happens because there is too much energy content in the feed caused by the addition of CFO, resulting in reduced feed consumption so that the absorption of omega-3 fatty acids in the body is low. Fatty acids in feed affect metabolism and thus affect digestibility. Fat has a large energy content compared to protein and carbohydrates [11]. Increasing EPA and DHA in mud crab meat is most appropriate to use a dose of 2%.

EPA and DHA fatty acids in the control group were shown to be much lower than the EPA and DHA content in the treatment group. The lenolenic acid content in the mud crab meat from the control treatment was insufficient in the process of alternating and desaturating elongation metabolism resulting in other omega-3 derivatives such as Stearidonic acid (SDA) and Docosapentaenoic acid (DPA). According to Linder [12], the competition for the use of the Δ6 desaturase enzyme in the desaturation process of two groups of essential fatty acids, such as omega-3 and omega-6 fatty acids, where the competition will be won by a larger group.

The content of omega-3 contained in CFO is used as a source of energy for growth and reproduction, besides omega-3 is not able to inhibit cholesterol synthesis because omega-3 cannot limit enzymes for cholesterol biosynthesis [12]. Piliang and Djiojosebagio [13] state that omega-3 fatty acids play a role in regulating cholesterol metabolism which includes cholesterol transport and excretion. The clinical effect of omega-3 fatty acids in lowering cholesterol levels is thought to be due
to their effect on the production mechanism of transport lipoproteins in the liver secreted into the blood.

Fatty acids are formed in the liver, with dietary glucose as the main source of carbon. Through glycolysis, glucose is converted into pyruvate which enters the mitochondria and forms acetyl CoA and oxaloacetate. These two compounds combine to form citrate. Citrate is lifted into the cytosol, where it is broken down to form acetyl CoA, the carbon source for the reactions that occur in the fatty acid synthase complex. The main regulatory enzyme for this process, acetyl coAcarboxylase, forms melanyl CoA from acetyl CoA [14].

Fatty acid synthesis is not the reverse of its breakdown pathway. Fatty acid synthesis is more of a set of reactions, demonstrating the principle that the synthesis and breakdown pathways in biological systems are usually different. The pathway for fatty acid biosynthesis takes place outside the mitochondria, oxidation occurs in the mitochondrial matrix [15]. The formation of double bonds to form HUFA, EPA and DHA is very important for metabolic functions and components in cell membranes [16].

The essential fatty acids needed for optimum growth of mud crabs are eicosapenta-noic acid (EPA) (20: 4n-6) and docose-hexaenoic acid (DHA) (22: 6n-3). Omega-3 EPA and DHA are needed for cell membrane function, while DHA is essential for cell membranes of neural tissue and as a precursor for the formation of eicosanoates, for example for several hormones. Lack of essential fatty acids will cause health problems in fish including reduced fecundity and ability to form embryos, larvae death and abnormal growth, wrong pigmentation, impaired vision, inability to eat at low light intensity, abnormal behavior and decreased membrane function at low temperatures [17].

The low quality of fish oil makes the need for essential fatty acids not fulfilled due to the high amount of saturated fatty acids in the feed formulation. Fish oil content that is high in saturated fatty acids includes myristic acid, palmitic and stearic acids but the highest content is palmitic acid [18].

The high saturated palmitic fatty acid should be processed in the refining stage of the unsaturated fatty acid and trans fatty acid content. Quality fish oil is fish oil which is rich in fatty acids that are beneficial for health. Omega-3 is one of the unsaturated fatty acids that are essential for the body and especially needed for people with high cholesterol [18].

According to Bimbo (1998) [19], to get fish oil that is accepted as feed (edible oil), the non-oil fraction must be removed from the oil. According to Hodgum [20], alkaline purification removes several types of impurities in oil such as phosphatides, protein, or protein fragments. Alkaline purification almost completely removes free fatty acids that are converted into oil-insoluble soap. According to Pigott [21], purification with alkalis plays a role in removing impurities by the following mechanism, the soaped fraction absorbs alkalis and is coagulated by the hydration process, insoluble materials are trapped in coagulated materials, alkalis with free fatty acids form water insoluble compounds and some large pigments are degraded or absorbed by the sapodised fraction.

Fish oil damage can occur due to hydrolysis and oxidation processes. This damage causes an increase in the volatile odor and taste components, discoloration and decreased nutritional value of fish oil as a result of the damage process. Trans fatty acid or elaidic acid is an omega 9 oleic acid isomer which has a cis configuration to change to a stable trans [22]. This has resulted in deterioration of the quality of fish oil.

4. Conclusion
The conclusion obtained from the discussion above that the addition of Crude Fish Oil to the feed to the content of omega-3 EPA and DHA of mud crab meat is that the provision of feed with the addition of Crude Fish Oil at a dose of 2-8% can increase the content of Eicosapentaenoic Acid (EPA) in mud crab meat. Feeding with the addition of Crude Fish Oil at a dose of 2-8% can increase the content of Docosahexaenoic Acid (DHA) for mud crab meat. Based on the results, it is recommended to use the addition of 2% Crude Fish Oil to produce an increase in the EPA and DHA content of mud crab meat. It is necessary to do further research on the effect of increased levels of EPA and DHA on growth.
rates, survival rates, content of cholesterol ratios or similar studies aimed at increasing crab productivity.

5. References

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