



The Use of Crude Fish Oil (CFO) in Vannamei Shrimp (*Litopenaeus vannamei*) Feed on EPA and DHA Contents

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Abstract

Omega-3 fatty acids (Alpha-linolenic acid) and omega-6 fatty acids (Linoleic acid) are a group of essential fatty acids. Essential fatty acids are fatty acids that cannot be synthesized by the body so that must be supplied from the diet. One of the sources of essential fatty acids is derived from fish oil. This study aims to determine the effect of Crude Fish Oil (CFO) in the feed to EPA and DHA content in penaeid shrimp meat. The research method used was a completely randomized design. The treatments used are the varying content of Crude Fish Oil (CFO), which are P0 (0%), P1 (2%), P2 (4%), P3 (6%), and P4 (8%). The results of the study showed significant differences ($p < 0.05$) on the content of EPA and DHA in penaeid shrimp meat. The highest content of EPA and DHA found in P4 treatment (8%) and the lowest at P0 treatment (0%). The use of CFO in penaeid shrimp feed need further study related to the growth of shrimps and prawns reproductive cycle to increase the productivity of penaeid shrimp. CFO on feed should be used at a dose of 6%.

INTRODUCTION

Indonesian fisheries resources have enormous potential to contribute to the fulfillment of nutrition for the Indonesian people, both from capture fisheries and aquaculture resources. One source of nutrition that comes from fishery resources is shrimp. According to Oksuz *et al.* (2009), shrimp contains omega-3 and omega-6 unsaturated fatty acids.

Omega-3 fatty acids (Alpha-linolenic acid) and omega-6 fatty acids (Linoleic acid) are included in the group of essential fatty acids along with arachidonic acid. Essential fatty acids are fatty acids that cannot be synthesized by

the body themselves, so they must be obtained from the food consumed (Williams, 1999).

Omega-3 fatty acids, including EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid), are very beneficial for human health (Swanson *et al.*, 2012). Besides, they are also beneficial for the shrimp itself, namely for the growth and development of shrimp (Elovaara, 2001).

One source of omega-3 is derived from fish oil. Fish oil is different from other oils because fish oil contains various fatty acids. Fish oil derived from marine

fish is a good source of polyunsaturated fatty acids (PUFAs), especially for long-chain omega-3 fatty acids (EFSA, 2010).

The high content of omega-3 fatty acids in shrimp is thought to come from the natural feed. Apart from the feed, several other factors such as species, age, season, and habitat also play an effect on omega-3 fatty acids (Patawi, 1996). Research on the use of artificial feed added with crude fish oil as a source of EPA and DHA fatty acids so far has not been done in vannamei shrimp (Pramana *et al.*, 2019; Agustono *et al.*, 2019). The high EPA and DHA fatty acid content in crude fish oil are expected to increase the EPA and DHA content in vannamei shrimp meat.

METHODOLOGY

Place and Time

This research was conducted at the Laboratory of the Faculty of Fisheries and Marine, Airlangga University. EPA and DHA analysis were carried out at the Chemistry Laboratory of the University of Muhammadiyah Malang.

Research Material

The materials used in this study were vannamei shrimp weighing \pm 5 grams, crude fish oil, fish meal, rice bran, soybean meal, tapioca, corn flour, premix, freshwater, and seawater. The research equipment used were aquariums, aerators, thermometer, DO Test kit, pH test kit, ammonia test kit, refractometer, and gas chromatography.

Research Design

The experimental design used in this study was a completely randomized design (CRD) with Crude Fish Oil as a treatment. The treatments used were 5 treatments and 4 replications namely P0 (0%), P1 (2%), P2 (4%), P3 (6%), and P4 (8%). The experimental treatment was carried out for 21 days in an aquarium container with a density of 10

fish/aquarium and the feed given was 4% of the total biomass weight.

Work Procedures

EPA and DHA Analysis

EPA and DHA analyses were carried out using the gas chromatography (GC) method which was carried out at the Chemical Laboratory of the University of Muhammadiyah Malang.

First, the samples were prepared, then weighed 1 - 10 g depending on the assumption of fatty acid composition. If it is solid, melt it by heating. A solution consisting of 17.5 ml diethyl ether, 17.5 ml petroleum ether, 6.5 ml 95% ethanol was added and everything is then transferred into a separating funnel.

12.5 ml of 1% Na_2CO_3 solution was added to the separating funnel containing the sample and solution, mixed for 30 seconds until homogeneous. The sample is left to stand until the two liquids separate (water at the bottom and the ether solvent at the top). The lower liquid is separated and collected, the remaining portion of the ether is washed again with 1.5 ml of 95% ethanol and 7.5 ml of 1% Na_2CO_3 solution. Mix and let it sit. Separate the bottom and combine it again with the collected water.

The remaining portion of the ether is washed again with 6.5 ml water. Once again separate the water and combine it with the water that has been collected. After all the solvent has been evaporated, the remaining oil in the form of triglycerides is used for further analysis.

Fatty acid salt collected in the water (from 4 times collection) is freed by the addition of 1.5 ml 10% H_2SO_4 . Mix until evenly distributed in the separating funnel then add 12.5 ml of the diethyl ether: petroleum ether = 1: 1 mixture. Mix and let it sit until the two liquids separate.

The ether (top) part is separated and collected. The remaining part of the water that remains is washed again with the solvent mixture up to three times and collect the ether solution obtained. 1 g Na_2SO_4 is added to the ether solution to bind the remaining water.

The ether liquid is filtered through filter paper, washed with a mixture of ether to wash the fatty acids that stick to the container and filter paper. The filtrate obtained was dried with N₂ gas. After all the ether has evaporated, it can be stored for fatty acid analysis using the GC method.

Esterification of Fatty Acid Sample

For free fatty acids to evaporate more easily so it is suitable for separation via GC, it is necessary for fatty acids esterification into their esters. Add 2 ml of the diazomethane solution to the free fatty acid sample in the test tube. To speed up the reaction, add 2-5 ml of 10% methanol in diethyl ether. If diazomethane is sufficient, the yellow color will not

disappear after the air bubbles that occur during the reaction stop (within 15 minutes at room temperature). After the air bubbles stop forming, dry the excess diazomethane and ether solvent with N₂ gas. Add diethyl ether solvent in a known volume (for example 10 ml) to obtain a solution of fatty acid ester with a certain concentration. The solution is ready to be injected into the GC.

Feed Composition

The calculated ransom composition in feed is displayed in the following Table 1. The calculated Digestible Energy (DE) was obtained by subtracting the total gross energy and the obtained value was 3292,07 Kkal/kg.

Table 1. Calculation of feed ransom composition.

Ingredient	%	Gram	% Protein	% Fat	% Ash	% Crude Fiber	% NFE	Gross Energy (Kkal/kg)
Fish flour	47	435	21.39	3.89	11.45	2.75	5.59	4118.70
Tapioca flour	5	46	0.13	0.055	0.01	0.04	3.97	3793.03
Fine Bran	3	28	0.34	0.39	0.28	0.25	1.36	3999.46
Cornstarch	10	93	0.91	0.379	0.13	0.26	7.09	4077.76
Soybean Meal	32	296	12.16	1.72	2.62	1.54	10.56	4305.44
Crude Fish Oil	8	74	0.09	4.02	0	0	0	5166.9
Premix	3	28						
Total	108	1000	35.02	10.45	14.4929	4.85	28.58	4115.09

Data Analysis

The data obtained from the experiment were analyzed using descriptive analysis. Once the results obtained, the data were presented in tables.

RESULTS AND DISCUSSION

EPA and DHA Content

The results of the calculation of EPA and DHA content in vannamei shrimp (*Litopenaeus vannamei*) showed that there were differences in EPA and DHA content. The average EPA and DHA content of vannamei shrimp can be seen in Table 2.

Table 2. Average and standard deviation of EPA and DHA in vannamei shrimp meat.

Treatment	EPA Content ± SD (mg/g)	DHA Content ± SD (mg/g)
P0 (0%)	0.38 ^a ± 0.018	0.12 ^a ± 0.027
P1 (2%)	0.47 ^b ± 0.014	0.17 ^b ± 0.007
P2 (4%)	0.52 ^c ± 0.004	0.20 ^c ± 0.001
P3 (6%)	0.54 ^{cd} ± 0.005	0.21 ^{cd} ± 0.002
P4 (8%)	0.56 ^d ± 0.003	0.22 ^d ± 0.001

Note: Different superscripts in the same column show significant differences (p < 0.05).

The results of the analysis of variance (ANOVA) for EPA showed a significant difference ($p < 0.05$). The Duncan Multiple Range test also showed significant differences between treatments. Treatments P0, P1, P2, and P4 differed between treatments, but in P3 treatment did not differ from treatment P2 and P4. The highest EPA content was obtained from treatment P4 but not significantly different from treatment P3.

The results of the ANOVA for DHA showed a significant difference ($p < 0.05$). Duncan's Multiple Range test shows a significant difference. Treatment P0, P1, P2, and P4 differed between treatments, but in P3 treatment did not differ from treatment P2 and P4. The highest DHA content was obtained from treatment P4 but not significantly different from treatment P3.

The increase in EPA and DHA content in vannamei shrimp meat is affected by the level of Crude Fish Oil in the feed formula. This is following the statement of Patawi (1996) that the composition of shrimp meat varies greatly depending on the shrimp feed, besides the effect of species, age, season, and habitat.

The EPA and DHA contents in vannamei shrimp meat in this study are directly proportional to the EPA and DHA content in the feed formula, which means an increase in EPA and DHA in the feed will be accompanied by an increase in vannamei shrimp meat. This is consistent with the statement of Cuzon *et al.* (2004), which states that the fatty acid content contained in shrimp meat reflects the fatty acids consumed by the shrimp. However, the same type of feed given cannot guarantee the same content and quality of fatty acids consumed by the shrimp. This is due to the nature of fatty acids, especially unsaturated fatty acids, which are easily oxidized. The existence of different ways of handling feed (e.g. processing, storage, and transportation) can cause the composition and quality of fatty acids in feed to change (Patawi, 1996).

The producers of omega-3 fatty acids are not fish, shrimp, or shellfish because the synthesis of EPA and DHA in these animals is low. EPA and DHA in fish, shrimp, and shellfish are obtained from marine microorganisms they feed. Marine microorganisms that are the main producers of omega-3 fatty acids are *Chlorella*, Diatom, and Dinoflagellates which are phytoplankton. These organisms synthesize omega-3 fatty acids very efficiently because they have a relatively simple food chain cycle. Algae families that contain high omega-3 fatty acids include *Chlorella minutissima*, *Euglena gracilis*, and *Hitchia closetrium*, which are around 20-40% of the total fatty acid content (Patawi, 1996). In this study, the source of omega-3 fatty acids EPA and DHA given through feed to shrimp is CFO, where the EPA content in CFO is 10.7173% and DHA is 7.0108% so that if the feed formula containing Crude Fish Oil is consumed by the shrimp, the omega-3 EPA and DHA fatty acids will be absorbed by the shrimp.

In the shrimp body, fat metabolism takes place in the hepatopancreas (Ceccaldi, 1989). Furthermore, EPA and DHA will be stored in phospholipids and triacylglycerols, according to the statement of Halver (1989) which states that phospholipids and triacylglycerols contain PUFAs but the PUFA content in phospholipids is higher than triacylglycerols. Fish phospholipids contain a high level of omega-3 and omega-6. Omega-3 fatty acids that are high in shrimp meat can lower cholesterol in the blood. Omega-3 fatty acids can inhibit the synthesis of Very Low-Density Lipoprotein (VLDL) so that the production of Low-Density Lipoprotein (LDL) is reduced. The high levels of VLDL and LDL are what can cause cholesterol deposits in the blood (Patawi, 1996).

Shrimp itself has a limited ability to elongate and desaturate linolenic acid into EPA and DHA (Felix and Velazquez, 2002) so it can be said that the synthesis of EPA and DHA in shrimp is low. Therefore, EPA and DHA are essential fatty acids for

shrimp because they are important for maintaining cellular function (Halver, 1989), so shrimp must get a supply of EPA and DHA fatty acids from the food they consume.

The average water quality during the study is presented in the following Table 3.

Table 3. Average water quality.

Parameter	Average
DO (mg/l)	4.8
pH	7
Ammonia (mg/l)	0.25
Salinity (ppt)	10-11

During the research process, the water temperature in the aquarium ranged from 27-29°C. This is following the statement of Wyban and Sweeney (1991), that the adequate water temperature for the maintenance of vannamei shrimp ranging from 23-30°C, meanwhile according to Elovaara (2001) the optimum water temperature for the growth of vannamei shrimp is 28.5°C. During the research process, the aquarium was closed using a tarpaulin plastic so the water temperature remains relatively stable. Controlling water temperature during the shrimp rearing process is quite important because the temperature is an environmental factor that affects the metabolism, growth, and survival of cultured shrimp (Gunarto and Hendrajat, 2008).

CONCLUSION

The addition of Crude Fish Oil to the vannamei shrimp feed formula can increase the EPA and DHA content in vannamei shrimp meat. The optimum increase in EPA and DHA is obtained from the addition of 6% Crude Fish Oil.

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REFERENCES

Agustono, A., Al Arif, M.A. and Dewi, F.S., 2019. Pemanfaatan tepung keong mas (*Pomacea canaliculata*) sebagai substitusi tepung ikan pada pakan udang vannamei (*Litopenaeus*

vannamei) terhadap nilai pencernaan serat kasar dan bahan ekstrak tanpa nitrogen (BETN). *Journal of Aquaculture and Fish Health*, 5(1), pp. 36-42. <http://dx.doi.org/10.20473/jafh.v5i1.11320>

Ceccaldi, H., 1989. Anatomy and physiology of digestive tract of Crustaceans decapods reared in aquaculture. *Advances in Tropical Aquaculture, Workshop at Tahiti, French Polynesia, 20 Feb-4 Mar 1989*. <https://archimer.ifremer.fr/doc/00000/1486/>

Cuzon, G., Lawrence, A., Gaxiola, G., Rosas, C. and Guillaume, J., 2004. Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. *Aquaculture*, 235(1-4), pp. 513-551. <https://doi.org/10.1016/j.aquaculture.2003.12.022>

EFSA Panel on Biological Hazards (BIOHAZ), 2010. Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. *EFSA Journal*, 8(10), p. 1874. <https://doi.org/10.2903/j.efsa.2010.1874>

Elovaara, A.K., 2001. *Shrimp farming manual: practical technology for intensive commercial shrimp production*. Aquaculture. cx.

Gunarto, G. and Hendrajat, E.A., 2008. Budidaya udang vanamei, *Litopenaeus vannamei* pola semi-intensif dengan aplikasi beberapa jenis probiotik komersial. *Jurnal Riset Akuakultur*, 3(3), pp. 339-349.

- <http://ejournal-balitbang.kkp.go.id/index.php/jra/article/view/2564>
- Halver, J.E., 1989. *Fish nutrition*. Second Edition. Academic Press, Inc. San Diego. California.
- Oksuz, A., Ozyilmaz, A., Aktas, M., Gercek, G. and Motte, J., 2009. A comparative study on proximate, mineral and fatty acid compositions of deep seawater rose shrimp (*Parapenaeus longirostris*, Lucas 1846) and red shrimp (*Plesionika martia*, A. Milne-Edwards, 1883). *Journal of Animal and Veterinary Advances*, 8(1), pp. 183-189. <https://medwelljournals.com/abstract/?doi=javaa.2009.183.189>
- Patawi, A., 1996. *Pengaruh habitat terhadap kandungan asam lemak omega-3 dan kolesterol pada udang windu (Penaeus monodon Fab)*. Skripsi. Program Studi Teknologi Hasil Perikanan. Fakultas Perikanan Institiut Pertanian Bogor.
- Pramana, A., Agustono, A. and Nurhajati, T., 2019. Penambahan lisin pada pakan komersial terhadap laju pertumbuhan, rasio konversi pakan dan efisiensi pakan udang galah (*Macrobrachium rosenbergii*). *Journal of Aquaculture and Fish Health*, 7(1), pp.18-24. <http://dx.doi.org/10.20473/jafh.v7i1.11226>
- Swanson, D., Block, R. and Mousa, S.A., 2012. Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Advances in nutrition*, 3(1), pp.1-7. <https://doi.org/10.3945/an.111.000893>
- Williams, R.S., 1999. *Essentials of nutrition and diet therapy*. Seventh Edition. Mosby, Inc. United States of America.
- Wyban, J. and Sweeney, J.N., 1991. *Intensive shrimp production technology: the oceanic institute shrimp manual*. The Institute. <http://agris.fao.org/agris-search/search.do?recordID=US9536517>