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The potential addition of lemuru oil to commercial feed to increase the content of EPA and DHA in eels (*Monopterus albus*)

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Abstract. Eels are a freshwater fish commodity that has a high economic value, particularly in Indonesia. Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) are a type of omega-3 which is the most dominant in fish oil. EPA and DHA are not produced by the fish's body itself, but rather, it is produced by marine plants like algae. EPA and DHA have an important role in the growth and function of the brain of the organism, including eels. This study aimed to improve the content of EPA and DHA in eels. This research used an experimental method with a randomized complete design consisting of five treatments with four replications. The analysis of the data was conducted using the Variant Analysis of Variance (ANOVA) test. If there were any differences among the treatments, then the analysis would be continued with Duncan's Multiple Range Test. The results showed that the EPA and DHA content of the meat increased with the addition of lemuru oil. The DHA content in eel meat in this study was directly proportional to the content of DHA in the formula feed. The addition of lemuru fish oil by 6% could increase the EPA content up to 2.8709%. It could also increase the DHA content up to 1.2951%.

1. Introduction

Eel (*Monopterus albus*) is a freshwater fish that is exported continuously. The export needs can reach up to 1,765 tons per week [1]. Eel also contains fatty acids which are needed by humans [2]. The cholesterol content of eels reaches up to 145.35-154 mg / 100g [3] and the content of EPA and DHA in eels is 7.16% [4]. Eicosapentanoic Acid and Docosahexaenoic Acid are the most dominant types of omega-3 in fish oil. They have an important role in eels, especially for brain growth and function [5]. EPA and DHA are not produced by the fish's bodies, but by marine plants such as algae. In this study, we added lemuru fish oil into eel fish to increase its EPA-DHA.

Lemuru fish are marine fish. They have a higher content of EPA and DHA compared to carnivorous fish such as sharks, tuna, and sailfish [6]. Research on the addition of lemuru fish oil to commercial feed is expected to increase the EPA and DHA content in eels.



2. Materials and methods

2.1 Materials

The materials used in this study consisted of 20 aquariums of 40 x 30 x 30 cm³, aerators, hoses, aeration stones, large plastic tubs, measuring cups, digital scales, pH meters, a DO meter and ammonia test kits. Eels weighing 25-30 grams were used in this study. The eels were obtained from eel farmers in Kandangan, Kediri, East Java. The materials used consisted of lemuru fish oil from PT. Maya, Muncar-Banyuwangi, and the Feng Li brand of commercial feed.

2.2 Method

2.2.1 Preparation

The first step was preparing the aquariums (40 x 30 x 30 cm³). 100 eels were measured in length and weight and 5 fish were put into each aquarium.

2.2.2 Feed

The feed used for this study was commercial feed with a size of 2 mm. The feed was mixed with tapioca flour and lemuru fish oil. The doses of lemuru fish oil were in accordance with the treatment. The mixed feed was added to warm water and stirred evenly until the paste was shaped. The treatment feed had been previously analyzed in advance to determine the nutritional content in the feed. The results of the proximate analysis of the feed ingredients can be found in Table 1.

Table 1. Feed Ingredients Analysis

Contains	Commercial Feed ^{*a}	Tapioca Flour ^{*a}	Lemuru Fish Oil ^{*b}
Dry Ingredients	91,77 %	89,5 %	91,19 %
Ash	12,05 %	0,20 %	-
Crude Protein	36,32 %	1,74 %	3,74 %
Crude Fat	5,64 %	1,52 %	70,4 %
Crude Fiber	5,32 %	1,45 %	0,75 %
Water	8,23 %	10,50 %	8,81 %
Nitrogen Free Extract	32,42 %	84,57 %	16,61 %
Energy Metabolism (kcal/kg)	3282,07	2822,17	8280

Source: a) Unit of Laboratory Inspection Service, Consultation and Training, Faculty of Veterinary Medicine, Universitas Airlangga, 2016.

b) [7]

2.2.3 Feeding

Feeding the eels was carried out to total as much as 3-5% of the eel's weight with the eels being fed 3 times a day (7:00 a.m., 12:00 p.m. and 17:00 p.m.). The study was carried out after the feed adaptation process.

2.2.4 Parameter

The main parameters in this study were the levels of EPA and DHA in the eel meat at the Unit of Laboratory Inspection, Universitas Airlangga, Surabaya. The supporting parameters observed were the water quality parameters such as temperature, DO, pH and ammonia.

2.2.5 Data Analysis

The data obtained was analyzed using the ANOVA (Analysis of Variance) statistical test to determine whether there were differences between treatments. This will be followed by Duncan's Multiple Range Test with a 5% significance level to find out the best treatment [8].

3. Results

3.1 EPA

Data from the calculation of the EPA content in the eels showed there to be a significant difference. The average EPA content in the eels ranged from 1.3596% to 2.8913%. The data on the average DHA content in the eel meat can be seen in Table 2.

Table 2. Average EPA content in the eels.

Treatment	EPA + SD ($\sqrt{y + 0,5}$) $\pm$ SD (%)	Transformation
P0 (0%)	1,3596 ^b $\pm$ 0,63382	1,3505 $\pm$ 0,2189
P1 (2%)	1,4046 ^b $\pm$ 0,19672	1,3786 $\pm$ 0,0723
P2 (4%)	1,5760 ^b $\pm$ 0,18257	1,4399 $\pm$ 0,0623
P3 (6%)	2,8709 ^a $\pm$ 0,81774	1,8254 $\pm$ 0,2280
P4 (8%)	2,8913 ^a $\pm$ 0,23010	1,8407 $\pm$ 0,0633

Note: * The superscripts that were different in the same column show significant differences ($p < 0.05$)

* The same superscript in the same column showed no difference ($p > 0.05$)

Based on the results of the Analysis of Variants (ANOVA), it was found that the addition of lemuru fish oil to commercial feed showed significant differences ($p < 0.05$) in each treatment related to the EPA content found in the eel's meat.

Based on the results of Duncan's Multiple Distance Test, there were significant differences between the treatments. The highest EPA content was obtained from the P4 treatment but this was not significantly different from the P3 treatment. P4 was significantly different from the treatments of P2, P1 and P0. The P2 treatment was not significantly different from P0 and P1.

3.2 DHA

Data from the calculation of the DHA content in the eel's meat (*M. albus*) showed significant differences. The average EPA content in the eels ranged between 0.3822% and 1.4574%. The average data for DHA can be seen in Table 3.

Table 3. Average DHA in the eel's meat

Treatment	Content DHA + SD ($\sqrt{y + 0,5}$) $\pm$ SD (%)	Transformation
P0 (0%)	0,3822 ^b $\pm$ 0,07898	0,9386 $\pm$ 0,04130
P1 (2%)	0,6203 ^b $\pm$ 0,04847	1,0583 $\pm$ 0,02273
P2 (4%)	0,7956 ^b $\pm$ 0,37607	1,1299 $\pm$ 0,15873
P3 (6%)	1,2951 ^a $\pm$ 0,43093	1,3324 $\pm$ 0,16177
P4 (8%)	1,4574 ^a $\pm$ 0,38483	1,6361 $\pm$ 0,59413

Remarks: * The superscripts that were different in the same column show significant differences ($p < 0.05$)

* The same superscript in the same column showed no difference ($p > 0.05$)

Based on the results of the Analysis of Variants (ANOVA), it was found that the addition of lemuru fish oil showed significant differences ($p < 0.05$) in each treatment regarding the EPA content of the eel's meat.

The highest EPA content was obtained from the P4 treatment but this was not significantly different from the P3 treatment. P4 was significantly different from the treatments of P2, P1 and P0. The treatment of P2 was not significantly different from the treatments of P0 and P1. Based on the analysis of variance on the content of EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid), this study showed that feed containing lemuru fish oil showed significantly different effects (p

< 0.05). The EPA content of the meat was increasing along with the increasing addition of lemuru fish oil in the feed.

3.3 Water quality

Good water quality in the maintenance medium would greatly support the growth of the fish being maintained. The temperature during the study ranged from 27 to 28 °C. This temperature range was the optimal temperature for the growth of the eels. According to [9], eels live in a temperature range of 28 to 32°C. During the study, the pH was in the range of 7.3 to 7.7. The ammonia levels during the study were 0.5-1 mg / L. The dissolved oxygen (DO) during the study was 4 mg / L.

4. Discussion

The increase in the EPA and DHA fatty acid content in the eel meat was influenced by the level of lemuru fish oil in the feed formula. The growth of the rice eels was influenced by the feed that was related to their eating habits and the type of feed given [10]. The feeding was adjusted according to the nature of the eating habits as a driver of growth and to increase production. Based on nutritional needs, some of the substances that must be fulfilled to support the metabolic processes of the body of the eel include carbohydrates, proteins, fats, vitamins and minerals.

The content of EPA and DHA in the eels in this study were directly proportional to the content of EPA and DHA in the feed formulas. This means that the increase in EPA and DHA in the feed would be accompanied by an increase in the EPA and DHA content in the rice eel meat. The content of fatty acids contained in the eel meat reflects the fatty acids consumed by the eel. The fatty acids contained in eel meat were derived from the fatty acids consumed by the eel [11].

The content of omega-3 derivatives found in the eel meat were caused by most of the unsaturated fatty acids stored in the phospholipids from the cell membranes. Besides that, the type of feed that was given could not guarantee the same content and quality of the fatty acids consumed [12].

EPA and DHA are one component of Omega-3 that functions in helping the formation of blood cells, stabilizing the circulatory system by encouraging blood circulation. In general, EPA and DHA are beneficial for the growth of brain cells, visual organs and bones, as well as maintaining blood vessels and heart cells. EPA and DHA are needed for the growth and development of the brain's nerve cells to be optimal. A lack of this substance causes the nerve cells in the brain to lack the energy needed for the brain development process which can interfere with brain function drastically. Not only for the brain, but EPA and DHA also play an important role in the visual organs and bones [5].

EPA and Docosahexaenoic Acid (DHA) are competitive inhibitors with Arachidonic Acid for cyclooxygenase pathways. Cyclooxygenase enzyme, also called Prostaglandin H2 synthase (PGHS), consists of isoenzymes that catalyze the conversion of arachidonic acid into highly active lipid mediators, namely prostaglandins and thromboxane. Prostaglandins and thromboxane are involved in various pathophysiological processes, including the induction of vascular inflammatory responses (which are a response to tissue damage or infection), homeostasis, the cytoprotection of the gastric mucosa and renal regulation. Prostaglandins are present in all tissues and organs. Prostaglandins work locally and are rapidly metabolized by the body. Prostaglandins are produced after the activation of mast cells, basophils and macrophages. Prostaglandins can also be synthesized by neutrophils and eosinophils. Prostaglandin is the main mediator, which is increased by cyclooxygenases in inflammatory reactions. Prostaglandins provide effects similar to histamine, including vasodilation, bronchoconstriction, and neutrophil chemotaxis [13]. In this study, the sources of omega-3 fatty acids EPA and DHA given through the feed to the eels was found in lemuru fish oil. As the formula feed containing lemuru fish oil was consumed by the rice eels, the omega-3 fatty acids EPA and DHA contained in the lemuru fish oil were absorbed by the rice eels.

In the eel's body, the absorption of fatty acids starts from the stomach where the fatty acids are derived from the lemuru fish oil mixed with commercial feed. Lemuru fish oil consists of units of fatty acids, namely saturated fatty acids and unsaturated fatty acids. Unsaturated fatty acid content in

the lemuru fish oil is higher than the saturated fatty acid content by 2:1. The oil is rich in omega-3 fatty acids such as EPA and DHA [14].

Unsaturated fatty acids, especially EPA and DHA, are catalyzed by the lingual lipase released from the gland behind the tongue, after which it was degraded by the gastric lipase secreted by the gastric mucosa. This digestion continued into the small intestine, where there was the emulsification of fat assisted by bile salts, after which the fat would be esterified by pancreatic lipase and forms micelles so then it can be easily absorbed by the intestinal wall through enterocytes (mucosal cells). Micelles are packaged into fat droplet particles surrounded by a thin layer formed from phospholipids and apolipoprotein, after which they are released in the form of chylomicrons which are secreted into the lymphatic system. In the form of chylomicrons, omega-3 fatty acids (EPA and DHA) are taken into the blood and tissues. Kilomicrons experience an endogenous cycle which could inhibit VLDL so that it could increase HDL and reduce LDL [15].

The eels lived in water with a pH of 5 to 7 and with an oxygen saturation level of 3 to 7 ppm [9]. Dissolved oxygen is good for eel growth and survival and in this study it was between 5 to 7 mg / l and at pH 7. A pH value that exceeds or is less than the optimum range can reduce growth, and in extreme conditions, interfere with the eel's health [16].

The pH range was included in the optimal pH range of the eel aquaculture. The optimum pH value in rice eel cultivation ranges from 7-8 [17]. PH values that exceed or are less than the optimal range can reduce growth, and in extreme conditions, this can interfere with the metabolism and health of the fish. Dissolved oxygen is good for eel growth and survival between 5 to 7 mg / L [16]. The measured ammonia levels, which can cause death, were more than 1 mg / L. Ammonia sources in the water can come from any remaining food and fish feces [18]. The high content of ammonia in the water could cause stress in the eels which could reduce the consumption of the eel feed [19].

5. Conclusions

The addition of lemuru fish oil to commercial feed could increase the content of EPA and DHA in the eels. The results confirming the increased levels of EPA and DHA were obtained successfully due to the addition of lemuru fish oil (6%).

6. References

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