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Lemuru Fish (*Sardinella Lemuru*) Oil effects on Body Weight of Wistar Rats Induced by Trans Fatty Acids

Abdurrahman Ali[1]✉, Annis Catur Adi[2], Sri Adiningsih[3]
[1-3] Faculty of Public Health, Universitas Airlangga, Indonesia
✉abdurrahmanali27@gmail.com,Phone: +6281290015808

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Abstrak

The content of EPA and DHA in lemuru fish oil can be used to prevent obesity risk factors. This study aims to determine the effect of purified lemuru fish oil on the weight of Wistar rats induced by trans fatty acids. This research is an experimental study using a randomized block design with a post test only control group. The research sample was 30 male Wistar rats aged two to three months, grouped into two control groups (K) which were given standard feed (K0) and those given standard feed after two weeks of trans fatty acid induction (K1) and three treatment groups (P).) with lemuru fish oil intervention as much as 0.34 ml (P1), 0.67 ml (P2), and 1.34 ml (P3). The induction material was 2.3 g of liquid margarine, containing 3.8% trans fatty acids. The intervention material was lemuru fish oil from the purification process containing 18.05% EPA and 9.37% DHA. Induction of margarine for two weeks made the body weight of the rats increased by 27.90% (P2) compared to the control (K0) 16.06%. After the intervention period of lemuru fish oil for three weeks, the weight of the treatment group P1 (18.53%), P2 (14.74%), and P3 (13.19%) was lower than the control group K0 (22.79%) and K1 (25.31%).

Keywords: Lemuru Fish Oil; Body Weight

PENDAHULUAN

Hyperlipidemia is closely related to the risk of obesity. Obesity and overweight globally are the fifth leading cause of death with 2.8 million adults occurring each year (Mohammadnezhad et al., 2017). This condition arises due to various conditions that can support the occurrence of the potential for the disease or are called factors, one of which is diet. Changes in people's current habits and dietary patterns can affect the type of food and the daily nutritional content consumed. Fried food and snacks in the form of cakes, as well as other fast food have become one of the foods that are often consumed in Indonesia. Without realizing it, it is closely related to the nutrients contained in it (Almatsier, 2011). It is known that as many as 40.7% (≥1 time per day) of the Indonesian population have the behavior of fried foods, contain fat, and cholesterol (Riskesdas, 2013)

The risk of the habit of consuming fried food, which contains fat and cholesterol. The potential content of trans saturated fatty acids which have a negative effect on health if the processing is carried out repeatedly (Chen et al., 2014). Generally in the use of vegetable oils and margarine in food processing can be found the content of trans fatty acids. However, solid margarine products are known to have a higher content of trans fatty acids. The content of trans fatty acids in various baked goods such as crackers, pies, biscuits, and wafers) is in the range of 1% to 30% of total fat (Boahen et al., 2012).

Excessive food consumption behavior is estimated to be the cause of obesity (Al-Qahtani, 2019). The habit of consuming fatty foods (40.7%), consuming sweet foods (53.1%), lack of consumption of vegetables and fruit (93.5%), and less physical activity (26.1%). increased incidence of obesity (Hermina & Prihatini, 2016). If these things are reduced, then the indications of obesity can be controlled.

Indonesia as a maritime country rich in various types of pelagic fish has the potential as a world fish oil producer. According to BPS (2020), the estimated potential of small pelagic fish resources is 294,092 thousand tons/year. Lemuru fish is one of the many pelagic fish that can be found around the Bali and Banyuwangi Straits (BPS, 2020; Maulana et al., 2014). Generally, lemuru fish oil is a by-product of flour processing or fish canning. It is known that lemuru fish oil has a high omega-3 composition (Rubio-Rodríguez et al., 2010). The content of unsaturated fatty acids in lemuru fish oil can be used as an additional intake of nutrients for the body (Andhikawati et al., 2020).

It was recorded that 29.68% of the total omega-3 content was found in the fatty acid composition of lemuru fish oil resulting from the canning process (Ibrahim et al., 2015). Research by Maulana et al. (2014), found as much as 15.53% of the total levels of omega-3 (EPA + DHA) in lemuru fish oil (Maulana et al., 2014).

Various studies show the omega-3 content of fish oil is beneficial for health. Studies using experimental animals in the form of mice have shown beneficial effects of omega-3s even in obese conditions (Flachs et al., 2014). The intervention study of administering oil containing PUFA from fish oil explained that short-term dietary supplementation with fish oil could increase biomarkers associated with metabolic syndrome (Lee et al., 2014). This is because the high content of omega-3 can be used for human health, especially for body weight which needs to be further investigated through research activities.

Therefore, this study aims to determine the effect of giving lemur fish oil on body weight of rats that have been conditioned by hyperlipidemia through induction using a diet high in trans fat content..

METHOD

Design, place, and time

. This study uses a randomized post-test design with control group with a layout using a randomized block design (RAK). The study began in August to September 2021, for six weeks consisting of one week of adaptation, two weeks of induction, and three weeks of intervention.

Experimental animal research was conducted at the Biochemistry Laboratory, FK UNAIR. Analysis of the trans fatty acid content of margarine samples was carried out at the Health Nutrition Laboratory, Department of Health Nutrition, FKM UNAIR. Tests for the profile of fatty acids and peroxides of lemurukas fish oil were carried out at the Integrated Laboratory of IPB. Mechanical purification analysis, fatty acid profile testing, peroxide, and lemuru fish oil toxicity test were carried out at the Health Nutrition Laboratory, Department of Health Nutrition FKM UNAIR. This research has obtained ethical clearance from the Health Research Ethics Commission (KEPK) FKG UNAIR No. 410/HRECC.FODM/VII/2021.

Materials and Tools

The materials used consisted of 30-month-old free-range Wistar-grooved rats with an average weight of 100-200 grams, standard feed BRAVO 512, coarse lemuru fish oil, aquades, and heated margarine. Fatty acid profile analysis of samples of margarine and lemuru fish oil used standard fat solution, 0.5 N NaOH solution in methanol, 16% BF3 solution, saturated NaCl solution, hexane, and anhydrous Na2SO4. Mechanical purification of lemuru fish oil using bentonite and 2% w/v activated charcoal. Testing the peroxide value using 0.1 ml of ammonium thiocyanate solution and 0.1 ml of ferrochloride solution. Toxicity test of lemuru fish oil used Artemia salina larvae, tween 20, and sea water with 65%, 40%, and 35% salinities.

Preparation of experimental animals

Experimental rats were divided into five groups, namely the control group was given standard feed BR-512 (K0), the control group was given standard feed BR-512 after being induced with margarine (K1), the lemuru fish oil treatment group was 0.34 ml (P1), the lemuru fish oil treatment group was 0.67 ml (P2), and the lemuru fish oil treatment group was 1.34 ml (P3). Wistar strain white rats used in the experiment were bovine-free 2-3 months old with an average weight of 100-200 g, and according to the criteria for healthy mice.

The calculation of the number of experimental mice in this study was calculated using the Federer formula (1991) in (Maryanto, 2013)

The number of experimental animal groups used were five groups (two controls and three treatments). Furthermore, according to the formula, the minimum sample for each group of rats is five. In this study, six rats were used in each group, so the population in this study was 30 rats.

Research stages

This study began with an adaptation period of 1 day with the aim of the mice being able to adapt to environmental conditions during the experimental period. Mice were fed the standard BR-512 diet (Table 1). Feeding and drinking water is done ad libitum. Experimental mice were weighed after the adaptation period and divided into five groups. The body weight between groups of experimental mice was tried to be homogeneous (<20%), so that it was able to give a relatively similar treatment effect (Muchtadi, 1989).

After going through the adaptation period, the induction period was continued for 14 days. After that, the intervention period was continued for 21 days. The stages of the research carried out during the induction and intervention periods were feed preparation, feeding, weighing once every three days, analysis of trans margarine fat content, lipid profile analysis, peroxide value analysis, and lemuru fish oil toxicity test

Tabel 1 Composition

Feed Nutrition	Compostion[1]	Composition[2]
Water	Maks 12%	10,49%
Crut Protein	19,5-21,5%	20,67%
Crut Fat	Min 5%	7,6%
Coarse Fiber	Maks 5%	1,94%
Ash	Maks 7%	-
Calsium	0,9-1,1%	-
Fosfor	0,6-0,9%	-
M.E	3125 kkal/kg	-

[1]Packaging Comfeed rat feed PT. Charoen Pokphand Indonesia
[2]Non-Ruminant Nutrition Laboratory Faculty of Animal Husbandry Unand (Luthfiyah & Widjajanto 2011***)***

Experimental feeding. During the one week adaptation period, rats were fed BR-512 and drinking water ad libitum. Feeding was adjusted to the needs of rats of 20 g, in the form of pellets (Dewi & Hernowati, 2008). The weight of the feed given and the remaining feed were weighed every day.

During the induction period, control rats (K1) and treatment groups (P1, P2, and P3) were fed BR-512 and heated liquid margarine. The provision of liquid margarine is carried out with disonde to ensure that the induction material is consumed as a whole. The amount of feed given to experimental rats during the induction period is presented in Table 2.

Table 2 The amount of feed given during the induction period

Feed Type	K0	K1	P1	P2	P3
BR-512 (g)	20	20	20	20	20
Melted Margarin (g)	0	2,3	2,3	2,3	2,3

The reference for the induction period is a high-fat diet (>30% energy requirements). According to the feed label BR-512, there is an energy of 62.5 kcal in 20 g of feed. Meanwhile, according to Table 1, the fat content of BR-512 feed is 7.6%, so there is 1.52 g of fat which is equivalent to 13.68 kcal. This amount meets 21.9% of the total daily energy needs of rats of 62.5 kcal.

This study uses a high-fat diet >40% of energy requirements to achieve hypertriglyceridemia. To meet this requirement, at least 18.1% fat must be added from margarine. The amount of margarine added is 2.3 g. It is known that there are 20 g of fat in a 25 g serving of margarine. Therefore, in 2.3 grams of margarine, the fat content is 1.84 grams or the equivalent of 16.56 kcal. This amount meets 26.5% of the total daily energy needs of rats. Thus, the total fat energy in the rat's diet is 30.24 kcal or 48.4% of the daily energy requirement.

During the intervention period, lemuru fish oil was added to the treatment rat group (P1, P2, and P3). Meanwhile, the control group (K0 and K1) were only given standard BR-512 feed. The amount of feed given to rats during the intervention period can be seen in Table 3.

Table 3 Amount of feed given during the intervention period

Feed Type	K0	K1	P1	P2	P3
BR-512 (g)	20	20	20	20	20
Fish Oil (ml)	0	0	0,34	0,67	1,34

The need for omega-3 fish oil in the healthy adult group (>18 years) is 1.1-1.6 g (RDA, 2019). The use of the dose refers to the study (Nisa et al., 2017) in patients with risk factors for heart disease with each treatment group that has been modified, namely 3.4 g (P1), 6.8 g (P2), and 13.6 g (P3). Next, the human dose conversion (70 kg) was carried out in white rats (P3). 200 g) in milliliters (Kurniawan, 2011)

Weighing of experimental mice. Rat body weight was weighed every three days using a digital scale and recorded. This is to see the effect of trans fatty acid induction and lemur fish oil intervention on body weight of experimental rats. Weight was averaged per group from the adaptation period to the end of the intervention period**.**

Trans fat analysis of margarine samples. Trans fatty acid data for margarine samples were obtained from the analysis at the Health Nutrition Laboratory, FKM UNAIR. Analysis of margarine samples using the Gas Chromatography (GC) method.

Analysis of the quality and quality of lemuru fish oil. Data on the fatty acid profile of lemurukasardi oil was obtained from the Integrated Laboratory, IPB. The results of the analysis of physical characteristics, purification, fatty acid profile, peroxide number, and toxicity test of purified lemuru fish oil were obtained from the analysis at the Health Nutrition Laboratory, FKM UNAIR.

Processing and data analysis

Processing of data in the form of body weight of experimental rats made the average, percentage increase, and made a statistical graph using Microsoft Excel.

RESULTS AND DISCUSSION

Trans fatty acids in margarine samples

The results of the analysis of trans fatty acids in the margarine sample were 3.8% w/w (Table 4). The results have exceeded the consumption threshold value. Recommendations from the American Heart Association (AHA) in 2017 and the European Food Safety Association (EFSA) in 2018, consume as little trans fatty acids as possible (0%) (EFSA, 2018; Sacks et al., 2017).

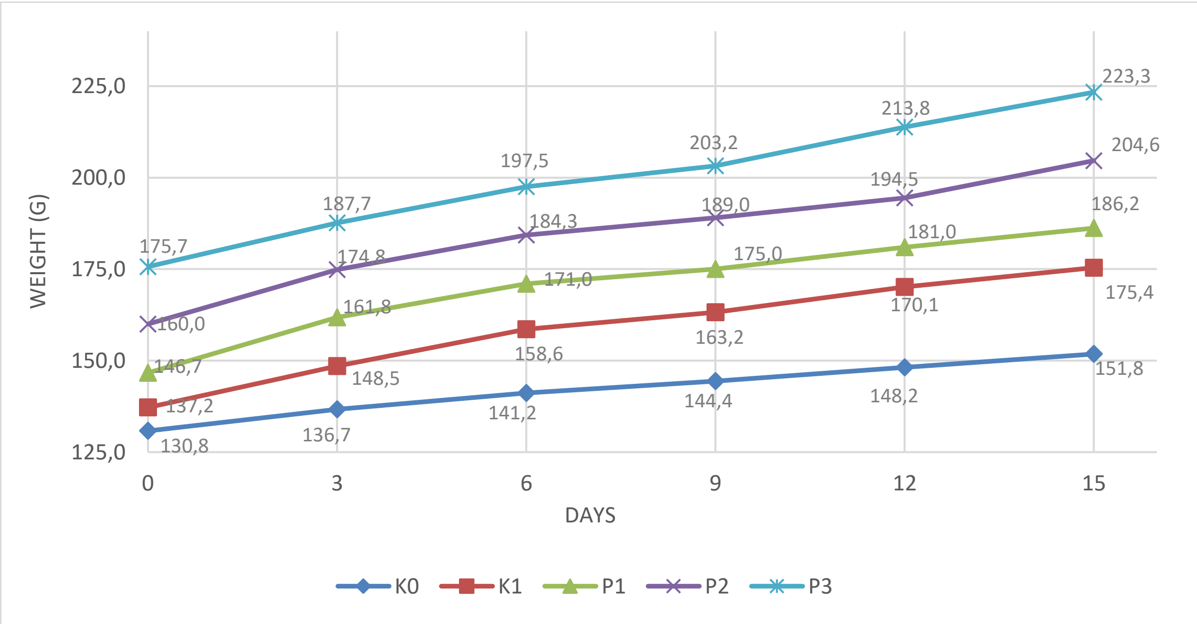
Table 4 Comparison of the results of the analysis of trans fat content of heated margarine

Repetition	Trans fat content (%)
0[a]	0,68
3[b]	1,58
6	3,8

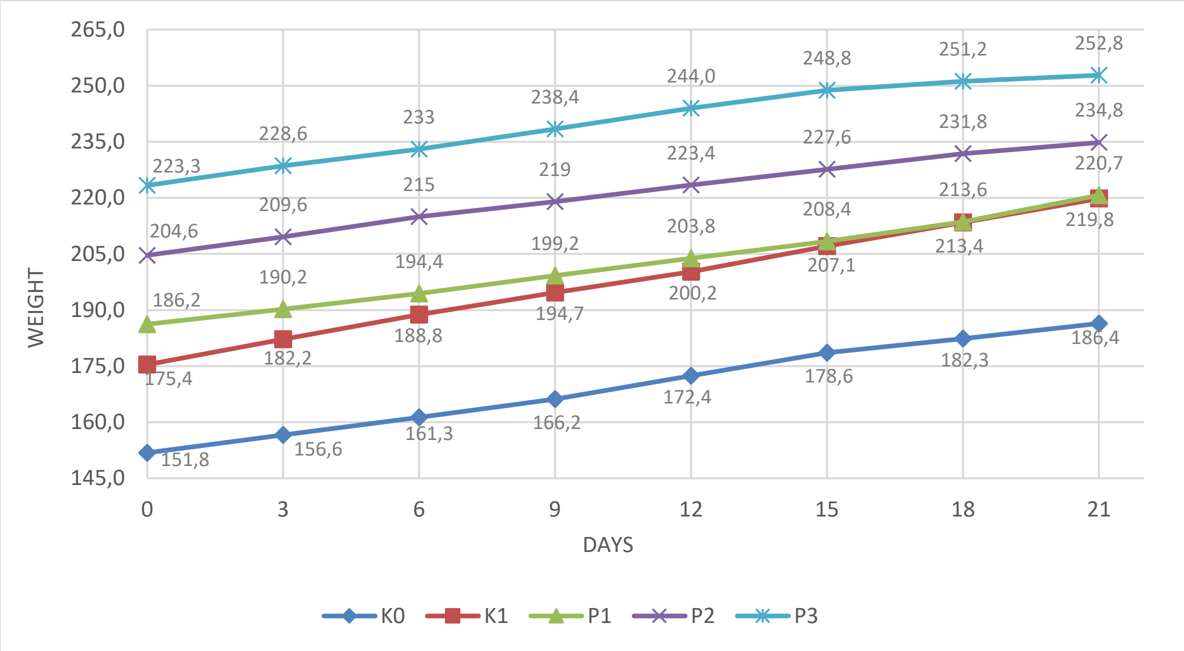
Description :
[a] Research from Airlangga University (Larasati et al., 2017)
[b] Research from Bogor Agricultural University (Ali et al., 2015)

Trans fatty acids that are given in excess amounts during induction can affect the increase in body fat reserves so that it affects weight gain. Observations of experimental rat weight during the induction period are presented in Figure 1. The control group (K0) had an increase of <20% initial body weight, while the group given liquid margarine induction (K1, P1, P2, and P3) had an increase of >20% initial body weight (Table 5). The experimental group of rats that had an average percentage of weight gain >20% were classified as obese (Trisviana, 2012). This is in line with research which states that the experimental rat group that was given a mixture of margarine as much as 1.7 grams in standard feed with a trans fatty acid content of 1.58% had an average percentage increase in body weight of 32.1% in two weeks and as much as 54% in four weeks of treatment (Ali et al., 2015)

The addition of lemuru fish oil during the intervention is expected to reduce the accumulation of fat reserves so that it can inhibit weight gain. Observation of body weight of mice during the intervention period is presented in Figure 2. During the intervention period, the average percentage of weight gain in the control group (K0 and K1) > 20% (Table 5) so that it can be categorized as obese (Trisviana, 2012). The control group (K0) experienced an increase in body weight percentage from 16.06% during the induction period, to 22.79% after the intervention period. The mean percentage of weight gain in the control group (K1) decreased slightly from 27.84% during the induction period, to 25.31% after the intervention period.



Picture 1 Observation of the average body weight of rats during the induction period



Picture 2 Observation of the average body weight of mice during the intervention period

Table 5 Percentage weight gain of mice during induction and intervention periode

Group	Percentage	
	Induction (%)	Intervention(%)
K0	16,06	22,79
K1	27,84	25,31
P1	26,95	18,53
P2	27,90	14,74
P3	27,13	13,19

Description : K0: Control (Given standard feed); K1: Control (Given with margarine until week 2; given standard feed from week 3); P1: Given margarine until the 2nd week; Given lemuru fish oil (0.34ml) from the 3rd week; P2: Given margarine until the 2nd week; Given lemuru fish oil (0.67ml) from the 3rd week; P3: Given margarine until the 2nd week; Given lemuru fish oil (1.34 ml) from the 3rd week.

Giving lemuru fish oil to the treatment group also gave the effect of changing the percentage of average body weight of the experimental rats. Gradually, the lowest dose of lemuru fish oil was 0.34 ml, giving the effect of reducing the percentage of body weight by 8.42% in the P1 group. The fish oil dose of 0.67 ml gave the effect of reducing the percentage of body weight as much as 13.16% in the P2 group. Meanwhile, the administration of lemuru fish oil with the highest dose of 1.34 ml gave the effect of reducing the percentage of body weight by 13.94% in the P3 group. Overall, the treatment groups (P1, P2, and P3) had a mean percentage change in body weight <20% of initial body weight, so they were not categorized as obese (Trisviana, 2012).

Obesity is a condition that arises due to the accumulation of fat which is characterized by an increase in body weight (Cahyaningrum, 2015). This condition is one of a collection of symptoms of metabolic disorders of the body (metabolic syndrome) which not only occurs at an adult age, but is also at risk of occurring at a young age (Christijani, 2019). Research states that water has an anti-inflammatory effect from consuming omega-3 and significantly reduces the risk of metabolic syndrome (Poudyal et al., 2011). Omega-3 consumption is also known to prevent weight gain (Buckley & Howe, 2010; Ryan & Seeley, 2013).

The provision of omega-3 in the diet has an inflammation-reducing effect by reducing the activity of nuclear receptors such as peroxisome proliferator (PPAR) which plays a role in lipid balance through regulation of gene transcription (van Dijk et al., 2009). When PPAR activity is high, lipid stores in adipose tissue and brain increase and cause hyperphagia. Both are factors that are interconnected with an increase in body fat (Lu et al., 2011; Ryan et al., 2011). Based on these factors, low PPAR activity causes less fat storage in adipose tissue and the brain, resulting in reduced stimulation to consume high-fat foods (Lu et al., 2011; Ryan et al., 2011; Ryan & Seeley, 2013).

The limitation of this study is that the lemuru fish oil used in this study is more suitable for secondary prevention in patients with metabolic syndrome. The results of weighing rats every three days need to be compared with weighing using a different day frequency. These various limitations are listed in order to obtain better research results on the same topic.

CONCLUSION

The abundant potential of processed lemuru fish products in Indonesia in the form of fish oil must be processed properly. In the study, lemuru fish oil was added to the diet of obese rats with the highest average weight loss in the P3 group, which was 13.94% during the intervention period. The content of unsaturated fatty acids in lemuru fish oil has benefits for human health. Omega-3 (EPA and DHA) which is high in lemur fish oil has been shown to be able to inhibit weight gain in experimental rats. Lemuru fish oil can be used as a prevention of obesity risk factors.

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