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Effect of Administration of Mackerel (*Rastrelliger* sp.) Oil and Omega 3 in Pregnant Female Rat (*Rattus norvegicus*) to Maternal Body Weight, Fetal Body Weight, Fetal Body Length, and Fetal Brain Weight

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Abstract

Consumption of balanced nutrition is very important for the growth of the fetus and pregnant women. Mackerel is a nutrient-rich food source that is high in protein and omega 3. Omega 3 is an important element to compose fetal brain cells, which plays a role in the stages of neurulation. This study aims to determine the effect of administration of mackerel oil and omega 3 to maternal body weight, fetal body weight, fetal body length and fetal brain weight. This study was a laboratory experimental study with a post-test only control group design. The research sample consisted of 30 pregnant rats divided into 3 research groups consisting of control, T1 (mackerel oil at a dose of 3.24 mg/day) and T2 (omega 3 at a dose of 3.24 mg/day). Oral administration was done from day 0 to day 17 of gestation using gavage-feeding tubes. The dissection on rats was carried out on the 18th day for fetal sampling, measurements were taken on the maternal body weight, fetal body weight, fetal body length and fetal brain weight. The data on maternal body weight, fetal weight, fetal body length and fetal brain weight were analyzed using non-parametric Kruskal Wallis test. The results showed that the administration of mackerel oil and omega 3 did not cause a significant increase in maternal body weight and fetal brain between groups ($p > 0.05$), but caused a significant increase in fetal weight and length ($p < 0.05$). Extract of mackerel (*Rastrelliger* sp.) oil contains more complex nutrients so that it can be used as a source of nutrition for pregnant women.

Keywords: Mackerel Oil, Omega 3, Gestation, Fetus, Prenatal Nutrition

INTRODUCTION

Consumption of balanced nutrition is very important for pregnant women and the growth of the fetus. Pregnant women consume nutrition in accordance with the nutritional adequacy rate (RDA). RDA is useful in meeting the needs for fetal growth, causing the birth of a healthy baby, can be used as a nutritional reserve for postpartum mothers, as well as ensuring sufficient milk production to meet the needs of babies in early life [1]. Research shows that malnutrition frequently occurs during pregnancy. Lack of nutrition in pregnant women can cause anemia. The prevalence of anemia in pregnant women in Asia is 39.3% [2]. Anemia in pregnant women causes bleeding and death. In addition, lack of nutrition also causes babies to be born with low birth weight (LBW). The low birth weight of the baby causes stunting and obstacles to the development of intelligence [3,4]. Beal [5] stated that one out of five pregnant women in Indonesia had chronic malnutrition. The existence of health risks for pregnant women and the fetus due to nutritional deficiencies makes the fulfillment of balanced nutrition one of the main focuses of the Sustainable Development Goals (SDG's) for 2016-2030 [6]. Important nutrients needed by pregnant women and the fetus include energy, protein, fat (omega 3 and 6), carbohydrates, fiber, water, vitamins and minerals.

The need for protein in pregnant women in the first to third three-semester has increased from 1 gram, 10 grams to 30 grams [7]. One of the foods that have a high protein content is mackerel. Mackerel is known to have high protein content and contains omega 3, which is an unsaturated fatty acid [8]. The need for omega 3 in pregnant women according to the RDA is 0.3 grams per day. Omega 3 is an element to compose neuron cell walls in brain formation [9].

Consumption of mackerel is expected to meet the needs of protein and omega 3 to ensure the mother's health and children's growth. The criteria for maternal and child health commonly observed are an increase in maternal body weight, fetal body weight, fetal body length and fetal brain weight. Based on the aforementioned background, the study

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was conducted on the effect of administration of mackerel oil and omega 3 in pregnant rats to maternal body weight, fetal body weight fetal body length and fetal brain weight.

MATERIALS AND METHODS

This research has received ethical approval No. 2.KE.033.04.2020 from the ethics commission of the Faculty of Veterinary Medicine, Universitas Airlangga. The type of research used was a laboratory experimental research with a post-test only control group design. This study used 30 rats *(Rattus norvegicus)* divided into 3 groups and each group consisted of 10 rats. The control group (C) and the two treatment groups, which were administered mackerel oil (T1) and omega 3 (T2). In both treatment groups, mackerel oil and omega 3 were administered from the first day to the 17th day of gestation with a dose of 3.24 mg/day each. The oral administration was conducted at 10h Western Indonesian Time, using a feeding tube. On the 18 day of gestation period of *Rattus norvegicus*, then cesarean section was performed.

Experimental Animal Insemination

Thirty (30) experimental female animals were synchronized with the heat cycle using PMSG and hCG. Superovulation was carried out on the 8th day with an injection of Pregnant Mare Serum Gonadhotropin (Folligon, MSD Animal Health, USA) at a dose of 10 IU/rat and 48 h later with an injection of Human Chorionic Gonadhotropin (Chorulon, MSD Animal Health, USA) at a dose of 10 IU/rat. Females were mated with 30 male rats aged 12 weeks by placing them in the same cage (monomating) for 17 h. After mating vaginal plug was observed (1st day of gestation).

Preparation and Dosage of Mackerel Oil

The mackerel oil was obtained by extracting approximately 70 grams of dry mackerel powder (14.29% moisture content), extracted by socklet using 300 ml of chloroform solvent for 2 h and 10 min or 8 times circulation. The solvent was separated by a Buchi-rotary evaporator to obtain chloroform-free mackerel oil. The mackerel oil was further characterized by its density, viscosity, saponation number and acid number, and its toxicity was tested using the Brine Shrimp Lethality Test (BSLT) method. The composition of mackerel oil was determined using Gas Chromatography-Mass Spectrometry (GC-MS) equipped with a 30 meters long RTX-5MS type column and was operated at a column temperature of 100-280°C.

Types and Dosages of Omega 3

The type of omega 3 used in this study was omega 3 (Omeheart, RPI Santa Anna, USA). The calculation of the dose administered to the experimental animals was as much as 3.24 mg/rat/ day.

Treatment of Experimental Animals

Mackerel oil and omega 3 were administered orally using a gavage feeding tube once a day at 10h Western Indonesian Time.

Dissection of Experimental Animals

The female *Rattus norvegicus* were sedated with ketamine and acepromazine-xylazine, and then were slaughtered by cervical dislocation. Fetus born by cesarean section were weighed, and from each female rat, 3 baby rats were selected to obtain the heaviest, medium, and lightest weight.

Measurement of Maternal Body Weight, Fetal Body Weight, Fetal Body Length and Fetal Brain Weight

The body weight was measured using manual scales. The maternal body weight was measured at the beginning and end of the study that is the 0 and 17th day of gestation. The fetal body weight, body length and brain weight were measured on the 18th day of gestation. The fetal body length was measured by placing the fetus on the block millimeter paper and measured from the head to the tip of the tail.

Data Analysis

The data in this study was the data on the rat's maternal body weight, fetal body weight, fetal body length and fetal brain weight analyzed using the non-parametric Kruskal Wallis test using SPSS version 25.

RESULTS AND DISCUSSION

1. Maternal and Fetal Weight Gain

Based on the results of Kruskal Wallis test, it was known that there was no significant difference in maternal weight gain between the groups of pregnant rats administered mackerel oil extract at a dose of 3.24 mg/day, omega 3 at a dose of 3.24 mg/day and the control **(Table 1)**. Although it did not give significant results, the extract of mackerel oil at a dose of 3.24 mg/day in rats could cause weight gain because in addition to omega 3, mackerel oil extract also contains calories (40 calories/5 grams). Maternal nutritional requirement at gestation is 10-15% higher compared to normal condition [10]. Most of these nutrients are used for fetal metabolism and development so that they do not have a significant effect on maternal weight gain [11].

Table 1. Characteristics of Maternal and Fetal Body Weight.

Group	Maternal Body Weight Gain (g) Mean ± SD
C	107.10±44.53^{a}
T1	126.7±94.21^{a}
T2	114.4±53.79^{a}

Note: C: Negative control group given standard feed during gestation; T1: Treatment group administered mackerel oil with a dose of 3.24 mg/day; T2: Positive control group administered omega 3 with a dose of 3.24 mg/day

Characteristics of Fetal's Body Length and Brain Weight

Based on the results of the Mann Whitney test it was found that administration of mackerel oil extract gave a significant difference in fetal weight gain when compared to the control but did not cause a significant body weight gain when compared to the group administered omega 3 **(Table 2)**. According to Vinding [12] administration of fish oil supplements to pregnant women can increase fetal birth weight, prolong gestational age and increase the size of the gestational sac. Fulfillment of nutritional consumption in pregnant women comes from the sea such as fish, which can increase fetal length and weight [13]. Fish oil contains fatty acid compounds of omega 3 and 6. Omega 3 fatty acids are known to increase fetal weight and length by increasing the ratio of prostacyclin to thromboxane. Prostacyclin is a prostaglandin produced by endothelium, which has a vasodilating effect on blood vessels, while thromboxane has a vasoconstrictive effect. An increase in the prostacyclin thromboxane ratio causes an increase in mother's blood flow to the fetus so that the supply of oxygen and fetal nutrition can be fulfilled to support fetal growth [14].

Table 2. Fetal Body Weight, Fetal Body Length and Fetal Brain Weight from Treated Female Rats during Gestation.

Group	Fetal Body Weight (g) Mean ± SD	Fetal Body Length (cm) Mean ± SD	Fetal Brain Weight (g) Mean ± SD
C	2.78±1.26^{a}	3.55±0.71^{c}	0.85±0.26^{a}
T1	4.27±1.29^{b}	4.59±0.41^{a}	1.22±0.40^{a}
T2	3.66±1.27^{ab}	3.88±0.72^{bc}	1.01±0.41^{a}

Note: C: Negative control group given standard feed during gestation; T1: Treatment group administered mackerel oil with a dose of 3.24 mg/day; T2: Positive control group administered omega 3 with a dose of 3.24 mg/day

Based on the results of Mann Whitney test, it was known that there was a significant difference between fetal length in the control group and the group administered mackerel oil extract, but it was not significantly different from the group administered omega 3 at a dose of 3.24 mg/day. This is because the extract of mackerel oil contains 16.65-20.09% protein and 3-12% fat [15]. Mackerel oil contains 32.8 and 4.5% omega 3 and 6, respectively [16]. Apart from omega 3, omega 6 also has an important role for fetal growth. Omega 6 helps build muscle mass while omega 3 is useful in the mechanism of bone formation to support the formation of fetal extremities. Omega 3 plays a role in calcium absorption, release of calcium through urine, prostaglandin synthesis, lipid oxidation, osteoblast formation and inhibits osteoclastogenesis. High omega-3 plays a role in the formation of trabecular structures and superior cortical, which plays a role in bone density and quality [17].

The administration of mackerel oil extract and omega 3 extract at these doses did not significantly affect fetal brain weight. The dose of mackerel oil extract and omega 3 is 3.24 mg/day whereas according to the RDA, the ideal consumption of fish oil to meet the needs of omega 3 at pregnancy is 2.7 to 5 grams per day in humans or the equivalent of 48.6 - 90 mg/day in rat. The doses of mackerel oil extract and omega 3 administered were still far from the prescribed adequacy rate so that they did not provide significant results in increasing fetal brain weight.

Omega 3 cannot be synthesized by the body itself but must be supplemented by food. Omega 3 is an important component needed in pregnancy because it plays a role in the process of forming the brain and retina and affects the length of pregnancy. Most of the formation of brain structures occurs in the womb. The brain has the ability to transform linoleic and alpha linoleic acids into long-chain fatty acids such as AA and DHA. Fats and fatty acids are important structures that make up white matter [18]. Fats, especially phosphoglycerols and cholesterol, are rich in AA and DHA [19]. Therefore, the consumption of AA and DHA-rich foods as needed will affect the formation and weight of the brain. DHA accumulates in the uterus, which will then be passed on to the fetus through the placenta [9]. DHA and AA accumulate rapidly in the brain during the later stages of pregnancy. The administration of a dose of omega 3 and mackerel oil in accordance with the daily nutritional needs is important for the formation of the fetal brain during pregnancy. The nutritional content of mackerel includes 89 mg/100 g protein, 18.33 mg/100g fat, 78.56 mg/100g amino acids, and 73.34mg/100g carbohydrates [20].

CONCLUSION

Mackerel (*Rastrelliger* sp.) oil extract at dose of 3.24 mg/day and omega 3 at a dose of 3.24 mg/day caused an increase in fetal body weight and length, but it did not influence maternal weight gain and fetal brain weight.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONTRIBUTOR OF AUTHORS

Research concept and design: W, Collection and/or assembly of data: W, SM, AM, Data analysis and interpretation: W, AM, ML, NC, Writing the article: W, HJT, SNK, Critical revision of the article: HJT, SM, Final approval of the article: W, EML.

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