Effect of DHA Supplementation on The MDA and SOD Levels in Protein Malnourished Rats

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Abstract. Protein malnutrition interferes with the synthesis of enzymes served as antioxidants while reducing antioxidants concentration in tissues, leading to oxidative stress. One of the most stable markers of oxidative stress is malondialdehyde (MDA). Docosahexaenoic acid (DHA) has an important role in inhibiting oxidative stress and protecting the tissue from peroxidative damage to lipids and proteins, thereby reducing oxidative stress in the tissue. This study aimed to analyze the effect of DHA on the MDA and superoxide dismutase (SOD) levels in protein malnourished rats. This study was carried out using the rat model Rattus norvegicus. After delivery, the rats were classified into two groups, namely, the malnutrition group (MG) and the non-malnutrition group (NMG). The MG was given low-protein diet since birth until 50 days old. The MG was further classified into two subgroups, namely the control malnutrition group with normal feed recovery (CMG), and the treated malnutrition group with normal feed recovery and DHA supplementation for 6 weeks (TMG). At the end of treatment, the blood MDA and SOD levels were assessed. The results showed that the MDA levels in the NMG, CMG, and TMG were 812.75 μM, 647.75 μM, and 624.00 μM respectively. The SOD levels were 19.40 μM, 19.20 μM, and 26.80 μM, respectively. The Kruskal Wallis test showed a significant difference in MDA levels between groups (p = 0.001). The posthoc test using the Mann Whitney test showed that the MDA level in TMG was significantly different from that in NMG, but not significantly different from CMG. The Anova test showed a significant difference in SOD levels between groups (p = 0.000). The Tukey posthoc test showed that the SOD level in TMG was significantly different from that in NMG and CMG. DHA has been shown to play an antioxidant role in malnutrition. DHA can increase the levels of SOD enzymes so that oxidative stress that occurs in malnutrition can be reduced. This is proven by the decrease in MDA levels as a lipid peroxidation product. It can be concluded that DHA supplementation can reduce the MDA levels and increase the SOD levels in protein malnourished rats.

Keywords: Protein malnutrition, DHA, MDA, SOD

1. Introduction
Indonesia is a developing country that still has a number of nutritional problems. Compared to other ASEAN countries, Indonesia is the most complete nutritional problem. The results of the 2018 basic
health research (Riskesdas) reported that the prevalence of malnutrition are 19.6% (Riskesdas 2013) to 17.7% (Riskesdas 2018) [1].

Oxidative stress is the steady state level of oxidative damage in a cell, tissue or organs caused by an imbalance between the production of reactive oxygen species (ROS) and biological systems ability to detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbance in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell [2].

Protein malnutrition interferes with the synthesis of enzymes served as antioxidants while reducing antioxidants concentration in tissues, leading to oxidative stress. ROS degrades polyunsaturated lipids, forming malondialdehyde (MDA). MDA is one of the most stable markers of oxidative stress. Increased levels of lipid peroxidation products in the serum are used as a marker for tissue damage [3].

Docosahexaenoic acid (DHA) has an important role in inhibiting oxidative stress and protecting the tissue from peroxidative damage to lipids and proteins, thereby reducing oxidative stress in the tissue both ex-vivo and in vivo [4].

This study aimed to analyze the effect of DHA on the MDA and superoxide dismutase (SOD) levels in protein malnourished rats.

2. Materials and Methods
2.1. Material
Docosahexaenoic acid (DHA), Rat Standard diet, Diet low protein AIN-76A Purified Rodent Diet contain 6.0% Casein (diets, USA).

2.2. Animal Study
This study was carried out using the rats Rattus norvegicus as the models. This work was approved by the Ethical Committee Faculty of Medicine Lambung Mangkurat University. This research used Posttest Control Group Design. After delivery, the rats were classified into two groups, namely, the malnutrition group (MG) and the non-malnutrition group (NMG). The MG was given low-protein diet since birth until 50 days old [5].

2.3. Administration of DHA
The MG was further classified into two subgroups, namely the control malnutrition group with normal feed recovery (CMG), and the treated malnutrition group with normal feed recovery and DHA supplementation for 6 weeks (TMG).

2.4. Measurement of MDA level and SOD level
After six weeks of feeding, the rats were sacrificed using cervical dislocation and blood was collected directly from the heart of the animals through 5 ml syringe into EDTA bottles. The blood collected into the plain bottles was allowed to clot, then centrifuged in 4000 x g and the supernatant was collected into another plain bottle for analysis MDA and SOD levels.

2.5. Statistical analysis.
The data obtained were analyzed using the Kruskall Wallis test and ANOVA with a confidence level of 95%
3. Results
The results showed that the MDA levels in the NMG, CMG, and TMG were 812.75 μM, 647.75 μM, and 624.00 μM respectively. The SOD levels were 19.40 μM, 19.20 μM, and 26.80 μM, respectively. The Kruskal Wallis test showed a significant difference in MDA levels between groups (p = 0.001). The posthoc test using the Mann Whitney test showed that the MDA level in TMG was significantly different from that in NMG, but not significantly different from CMG. The Anova test showed a significant difference in SOD levels between groups (p = 0.000). The Tukey posthoc test showed that the SOD level in TMG was significantly different from that in NMG and CMG.

![Figure 1. Mean of MDA level after supplementation DHA 1 mg/day. NMG= control (well-nourished), CMG = malnourished, TMG = malnourished + supplementation DHA (p=0.001).](image1.png)

![Figure 2. Mean of SOD level after supplementation DHA 1 mg/day. NMG= control (well-nourished), CMG = malnourished, TMG= malnourished + supplementation DHA (p=0.000).](image2.png)

4. Discussion
Experimental protein malnutrition in rats has been shown to reduce antioxidant cellular defence system and to enhance lipid peroxidation in plasma [6].

In the present study, DHA has been shown to play an antioxidant role in malnutrition. DHA can increase the levels of SOD enzymes so that oxidative stress that occurs in malnutrition can be reduced. This is proven by the decrease in MDA levels as a lipid peroxidation product.

Dietary deficiency of protein not only impairs the synthesis of plasma albumin and antioxidant enzymes but also reduces tissue concentrations of antioxidants, thereby resulting in a compromised antioxidant status. Malnourished children were found to have more oxidant damage products and less antioxidant levels. In children with PEM has been suggested to be caused by an imbalance between the
production of these toxic free radicals and antioxidant potential. Experimental study, serum MDA in malnourished children is is a significant increase as compared to control [3].

Protein-deficient diet intake has been shown to influence the activity of drug metabolizing enzymes as well as antioxidant enzymes. Feeding of a protein-deficient diet to rats has been shown to increase lipid peroxidation and induce significant changes in activities of catalase, glutathione peroxidase and superoxide dismutase [7].

Docosahexaenoic acid (DHA) has an important role in inhibiting oxidative stress. DHA are described to have anti-inflammatory effects and to improve endothelial function. In experimental study is that DHA diminished ROS-induced DNA damage in human aortic endothelial cells. H₂O₂-induced γ-H2AX foci formation was decreased by DHA, which indicated that these n-3 PUFAs diminished DSBs, the most severe type of DNA damage [8].

In the present study, was show that intake of DHA causes decreased production of MDA, which may serve a redox signalling role, thereby activating one of the major cellular detoxification pathways. The nuclear factor-E2-related factor-2 (Nrf2) pathway. Nrf2 plays a key role in defense against oxidative stress throughout the body. Nrf2 plays an important role in regulating the expression of genes that encode proteins responsible for decreasing oxidative stress (MDA). It has previously been shown that Nrf2 is important for ischemic lesion repair [9]. Nrf2 including those involved in Phase II detoxification and antioxidant gene expression. Regulation of Nrf2 activity represents a critical step in initiating a cellular antioxidant response to reactive oxygen species [10].

Recent studies reported that daily administration of DHA increased antioxidant enzyme activities and decreased MDA production in the brain [11]. DHA supplementation reduced urinary F2-isoprostane levels, a marker for oxidative stress, as well as enhanced cellular antioxidant defense systems. Lipid peroxidation products, such as malondialdehyde, can be as deleterious as ROS and may even cause DNA damage. Therefore, DHA supplementation given in association with an antioxidant treatment to reduce the deleterious oxidative effects [12].

5. Conclusion
It can be concluded that DHA supplementation can reduce the MDA levels and increase the SOD levels in protein malnourished rats.

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