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Effects of Low Protein Diet on Body Weight and Hematological Parameters in Rats

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Abstract: Malnutrition is a condition in which the body is deficient or excessive in macronutrients and micronutrients such as carbohydrates, fats, proteins and vitamins. Malnutrition causes changes in body weight and blood tissue that inhibit the development of hematopoietic cells, resulting in anemia, leukopenia and bone marrow hypoplasia. This study aimed to examine the effect of feeding a diet with a protein level of 12% and 20 % for 6-week to Wistar rats. The parameters seen are the impact on body weight and selected hematological parameters included white blood cell, monocyte, lymphocyte, granulocyte, hemoglobin and mean corpuscular volume. This study found that rats fed with 12% protein showed significant differences in body weight and several hematological parameters such as monocytes, lymphocytes, hemoglobin and mean corpuscular volume compared to normal.

Keywords: body weight, hematological, low protein diet, Malnutrition.

Introduction

Malnutrition is a condition in which the body is deficient or excessive in macronutrients and micronutrients such as carbohydrates, fats, proteins and vitamins. Until now, protein malnutrition is still the most serious problem in the world (FAO, 2015). Malnutrition takes several forms, including undernutrition, stunting, wasting, underweight, overweight and micronutrient deficiencies. Protein energy malnutrition is the most common form of malnutrition. Globally in 2022, it is estimated that 149 million children under 5 years old will experience stunted growth, 45 million will be underweight and 37 million will be obese (WHO, 2024). Malnutrition can affect the hematopoietic system, causing disruption in the production of all blood cell lines. Blood has a high rate of renewal, the hematopoietic system requires a large and organized supply of nutrients for the proliferation and maturation of mesenchymal and hematopoietic stem cells. (Santos *et al.,* 2017). Current research shows that changes caused by malnutrition are closely related to the predominant presence of anemia, leukocytosis and thrombocytosis in children under five who suffer from malnutrition (Getawa *et al.,* 2020).

Malnutrition also affects the immune system. Decreased lymphocytes have been reported to occur in individuals suffering from protein malnutrition. Decreased leukopoiesis interferes with the formation of a rapid and tailored immune response and causes leukopenia. Leukopenia is characterized not only by a decrease in the number of leukocytes but also by disturbances in the processes carried out by leukocytes. This condition alter granulocyte-macrophage colony- stimulating factors, macrophage colony-stimulating factors and the production of colony-stimulating factors by macrophages, which causes reduced immune responses and the formation and maturation of blood cells (Oliveira *et al*., 2016). Many studies have been conducted regarding malnutrition and its relationship to hematological parameters, such as the study by Hastreitet *et al.* (2020) which analyzed the impact of PEM on the hematopoietic system, there is arrest of the hematopoietic cell cycle and impaired differentiation. Getawa *et al.* (2020) studied the relationship between PEM and diseases such as anemia, leukocytosis and thrombocytosis. Amir (2023) also stated that PEM makes consistent changes in immune competence such as weakening the antibody response. Previous

research shows that Protein Energy Malnutrition (PEM) has a significant impact on the hematopoietic system in various aspects. However, it is not yet known whether nutritional interventions such as consuming a low-protein diet produces similar effects to PEM conditions. Therefore, the purpose of this research is to examine the effect of feeding a diet with a protein level of 12% and 20 % for 6-week to Wistar rats. The parameters seen are the impact on body weight and selected hematological parameters.

Materials and Methods

Animal and Research Design

Five-week-old male Wistar rats *(Rattus norvegicus*) were acquired from the animal housing in Muaropalam, West Sumatra, Indonesia. One week prior to the experiment, animals were housed in the laboratory with a temperature of 25°C, 60% humidity, and a 12-hour light-dark cycle. Standard pellet feed and water were provided on an unlimited basis. During a six-week period, five male Wistar rats were fed pellet feed containing 12% protein. Rats were given food and water ad libitum.

Body Weight and Hematological Analysis

Body weight measurements were conducted on a weekly basis using a laboratory animal scale (Islam *et al*., 2021). Following the completion of a six-week treatment period, all animals were subjected to sacrifice. Blood sampling through the cardiac in anesthetized rats. Blood was taken on the left side of the chest between the 3rd and 4th costae next to the sinister sternum. Then the sample is transferred into an EDTA tube which is analyzed using a hematology analyzer

Statistical Analysis

Paired sample t-test was used for analysis of the differences between Normal and Low Protein Diet groups. p-value is inferior or equal to 0.05.

Result and Discussion

Body Weight Analysis

Low-protein diets have a very significant impact on body weight. The results of the study in Figure 1 show that mice fed low-protein diets experienced continuous weight loss, reaching an average of 14.15% of their initial body weight in the sixth week. In contrast, mice fed normal diets with 20% protein content experienced a very significant increase in body weight, an average of 99.76% in the same period.

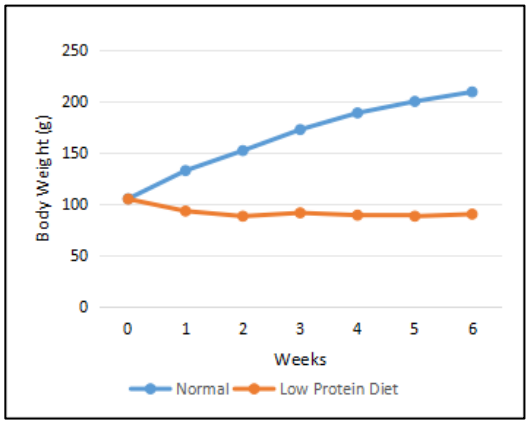

Figure 1. Effect of low protein diet for 6 weeks on rat body weight.

Protein malnutrition reduces body mass in humans and animals by impairing metabolic processes (Schaible & Kaufmann, 2007). Studies conducted by Bray et al. proved that providing adequate protein intake can increase body weight because protein affects energy use and muscle mass accumulation (Bray *et al*. 2012).

Hematological Analysis

The results of this study were presented in Figure 2. Lower mean values in malnourished rats were observed in the values of white blood cells, lymphocytes, granulocytes, hemoglobin and Mean Corpuscular Volume. Meanwhile, the average values that are higher than normal are monocytes.

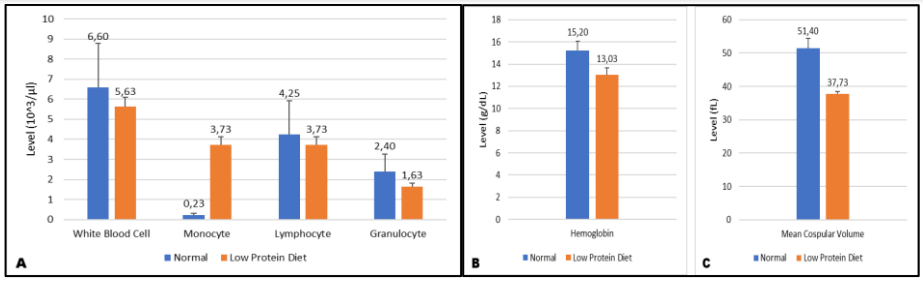

Figure 2. Results of hematological profile analysis.

Figure 2 (A) shows the low levels of white blood cells in the malnourished group compared to normal, especially lymphocytes and granulocytes. This is because malnutrition can affect physiological processes, such as hematopoiesis and immune responses. Modification of the organism's specific and nonspecific immune responses can cause structural changes in the lymphoid organs and areas of the thymus, resulting in decreased production of hematopoietic cells (Nova et al., 2002). Bone marrow is a complex tissue consisting of several hematopoietic cell lineages at various stages of development. Under normal conditions, the ratio of the number of lymphocytes, monocytes, granulocytes, and red blood cells produced in the bone marrow will vary from person to person. However, this difference is still within the range considered normal. This shows that although the process of blood cell formation is the same, there is natural variation in this process in each person. Malnutrition disrupts the bone marrow's ability to produce white blood cells, especially lymphocytes. Lack of nutrients needed for the process of blood cell formation can inhibit the growth and division of lymphocyte progenitor cells in the bone marrow (Touw & Geijn, 2007; Nova *et al*., 2002).

Low lymphocyte values may also be associated with changes in the thymus that are greatly reduced in children during severe protein restriction. The degree of thymus atrophy is closely correlated with lymphocyte depletion and a decrease in thymus-dependent lymphocytes is also associated with impaired immunity (Smith & Cipriano, 1987). Granulocyte formation is greatly influenced by a cytokine called granulocyte-colony stimulating factor (G-CSF). G-CSF, as a powerful hematopoietic growth factor, stimulates progenitor cells to develop into granulocytes. having important regulatory activity in granulopoiesis (Touw & Geijn, 2007). Granulocytes produced in the BM and after maturity are released into the blood and tissues. Acts as an innate immune response and the body's first line of immune defense. Lower granulocytes are caused by compromised GMP, affecting granulopoiesis (Hastreiter *et al*., 2020). Previous studies have shown that malnutrition not caused by other diseases can cause a decrease in the number of granulocyte cells and making them susceptible to infection (Hastreiter *et al*., 2020).

Figure 2 (A) shows a high increase in monocyte levels in the malnourished group. It has been reported that protein malnutrition is one of the major factors that weakens the immune system, which impairs both natural and acquired immunity (Schaible & Kaufmann, 2007). Monocytes and macrophages, which are vital in fighting infections, are significantly affected by this condition., increasing levels are a sign that malnutrition conditions allow inflammation or infection to occur. Several studies have shown that abnormalities in the immunoregulatory system and their impact on susceptibility to infection have been widely reported in malnourished patients (Borelli *et al*., 2004; Nova *et al*., 2002; Corware *et al*., 2014). The study from Corware *et al.* (2014) showed an increase in the number of monocyte cells in the bone marrow of malnourished mice, indicating a disturbance in the formation of blood cells (hematopoiesis). Previous research by Borelli *et al.*(2004) also supports these findings, by reporting an increase in the number of monocytes in the blood of up to 2.5 times in mice with similar conditions."

Low mean values were also observed in hemoglobin and mean corpuscular volume values. These findings were similar to previous studies, erythropoiesis evaluation was carried out on 50 malnourished and 50 healthy infants and showed significant differences in several blood

parameters. Malnourished infants had significantly lower red blood cell counts, hemoglobin levels, and young red blood cells.(El Nawawy *et al*., 2002). Another findings, Kalicki *et al.* (2019) stated that short- and long-term protein deficiency disrupts several blood parameters (e.g. decreased hemoglobin levels, MCV, MCH), impairs immune function and increases sensitivity to infections. The main factors causing the observed changes may be due to adaptation to decreased basal metabolism and lean body mass, as well as changes in body fluid volume, especially plasma. Increased plasma volume significantly contributes to changes in hematological parameters such as hematocrit and hemoglobin. (Fondu *et al*., 1978; Saka *et al*., 2012)

Conclusion

Rats fed with 12% protein showed significant differences in body weight and shows similar effects to protein energy malnutrition (PEM) parameter such as lower, lymphocytes, hemoglobin and mean corpuscular volume compared to normal.The monocytes lever are higher which may indicate an infection occur.

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Reference

Amir, Mellova Amir (2003) "Effects Of Protein-Energy Malnutrition On The Immune System," *Makara Journal of Science*: Vol. 7: Iss. 2, Article 4.

Bray, G. A., Smith, S. R., De Jonge, L., Xie, H., Rood, J., Martin, C. K., Most, M., Brock, C., Mancuso, S., & Redman, L. M. (2012). Effect of dietary protein content on weight gain, energy expenditure, and body composition during overeating: a randomized controlled trial. *Jama*, *307*(1), 47-55. Corware. DOI: 10.1001/jama.2011.1918

Corware, K., Yardley, V., Mack, C., Schuster, S., Al-Hassi, H., Herath, S., Bergin, P., Modolell, M., Munder, M., Müller, I., & Kropf, P. (2014). Protein energy malnutrition increases arginase activity in monocytes and macrophages. *Nutrition & metabolism*, *11*, 1-10. DOI: https://doi.org/10.1186/1743-7075-11-51

El Nawawy, A., Barakat, S., El Walily, T., Abdel Moneim, D. A., & Hussein, M. (2002). Evaluation of erythropoiesis in protein energy malnutrition. *EMHJ-Eastern Mediterranean Health Journal, 8 (2-3), 281-289, 2002*. PMID: 15339115

Fondu, P., Hariga-Muller, C., Mozes, N., Neve, J., Van Steirteghem, A., & Mandelbaum, I. M. (1978). Protein-energy malnutrition and anemia in Kivu. *The American journal of clinical nutrition*, *31*(1), 46-56. DOI: https://doi.org/10.1093/ajcn/31.1.46

Food and Agriculture Organization. (2015). The State of Food Insecurity in the World 2015. Meeting the 2015 international hunger targets: taking stock of uneven progress. https://www.fao.org/policy-support/tools-and-publications/resources-details/en/c/469455/

Getawa S, Getaneh Z, Melku M. Hematological Abnormalities and Associated Factors Among Undernourished Under-Five Children Attending University of Gondar Specialized Referral Hospital, Northwest Ethiopia. J Blood Med. 2020 Dec 18;11:465-478. doi: 10.2147/JBM.S284572.

Hastreiter, A. A., Guilherme Galvão dos Santos, Ed Wilson Cavalcante Santos, Edson Naoto Makiyama, Primavera Borelli, Ricardo Ambrósio Fock. Protein malnutrition impairs bone marrow endothelial cells affecting hematopoiesis. *Clinical Nutrition*. Volume 39, Issue 5, 2020,Pages 1551-1559.

Hastreiter, A. A., Makiyama, E. N., Borelli, P., & Fock, R. A. (2020). Impairment of G-CSF receptor on granulocytic progenitor cells causes neutropenia in protein malnutrition. *Nutrition*, *69*, 110540. DOI:https://doi.org/10.1016/j.nut.2019.06.021

Islam, M. M., Farag, E., Mahmoudi, A., Hassan, M. M., Atta, M., Mostafavi, E., Alnager, I. A., Farrag, H. A., Eljack, G. E. A. Bansal,

D., Haroun, M., Abdeen, R., Al-Romaihi, H., Al-Zeyara, A. A., Almaliki, S. A. & Mkhize-Kwitshana, Z. (2021). Morphometric study of Mus musculus, Rattus norvegicus, and Rattus rattus in Qatar. *Animals*, *11*(8), 2162. DOI: https://doi.org/10.3390/ani11082162

Kalicki, B., Lewicka, A., Jęderka, K., Leśniak, M., Marszałkowska-Jakubik, J., & Lewicki, S. (2019). Vitamin B6 improves blood parameters in rats fed a protein-deficient diet and subjected to moderate, long-term exercise. *Central European Journal of Immunology*, *44*(1), 23-32. DOI: https://doi.org/10.5114/ceji.2019.83266

Nova, E., Samartin, S., Gomez, S., Morande, G., & Marcos, A. (2002). The adaptive response of the immune system to the particular malnutrition of eating disorders. *European journal of clinical nutrition*, *56*(3), S34-S37. DOI: https://doi.org/10.1038/sj.ejcn.1601482

Oliveira, D. C. D., Hastreiter, A. A., Borelli, P., & Fock, R. A. (2016). The influence of protein malnutrition on the production of GM-CSF and M-CSF by macrophages. *Brazilian Journal of Pharmaceutical Sciences*, *52*, 375-382. DOI: http://dx.doi.org/10.1590/S1984-82502016000300003

Saka, A. O., Saka, M. J., Ojuawo, A., Abdulkarim, A., Bilamin, S., Latubosun, L., & Adeboye, M. (2012). Haematological profile in children with protein energy malnutrition in North Central Nigeria. *Glob J Med Res*, *12*(4), 1-7. Online ISSN: 2249-4618

Santos Ed W, Dalila C Oliveira, Graziela B Silva, Maristela Tsujita, Jackeline O Beltran, Araceli Hastreiter, Ricardo A Fock, Primavera Borelli, Hematological alterations in protein malnutrition, *Nutrition Reviews*, Volume 75, Issue 11, November 2017, Pages 909–919, https://doi.org/10.1093/nutrit/nux041

Schaible, U. E., & Kaufmann, S. H. E. (2007). Malnutrition and infection: complex mechanisms and global impacts. *PLoS medicine*, *4*(5), e115. DOI: https://doi.org/10.1371/journal.pmed.0040115

Smith, C. (1987). Serum ferritin levels in Shetland Ponies with experimentally-induced acute inflammation (commencing day zero) compared to normal control animals. *Vet Pathol*, *24*, 354-356.

Touw, I. P., & van de Geijn, G. J. (2007). Granulocyte colony-stimulating factor and its receptor in normal myeloid cell development, leukemia and related blood cell disorders. *Front Biosci*, *12*(1), 800-15. DOI: https://doi.org/10.2741/2103

World Health Organization. (2024). Malnutrition (Fact Sheet). https://www.who.int/news-room/fact-sheets/detail/malnutrition