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Original Article

Comparison Of Hematocrit, MCV, MCH And MCHC Amount Between Rats On Vegan Dan Standard Diet After Routine Physical Exercise

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ABSTRACT

Background: The prevalence of non-communicable diseases in Indonesia, such as diabetes mellitus and obesity, continues to increase. The main factors are diet errors and mistakes in sport.

Methods: This study used an in vivo experimental method with a pretest-posttest design with a non-equivalent group. The research sample consisted of 16 white rats, which were divided into two groups based on diet. The treatment given was in the form of dietary and routine physical exercise. Blood sampling was conducted to analyze hematocrit, MCH, MCV and MCHC levels.

Results: The results showed that the change in body weight of the white rats on vegan diet was higher than the standard diet group. Routine physical exercise had significant effect on hematocrit levels in the standard white rats (p=0.034) compare to the vegan diet. In addition, routine physical exercise had a significant effect on MCV (p=0.026), MCH (p=0.027) and MCHC (p=0.026) levels in the vegan compared to the standard diet white rats.

Conclusion: Changes in the number of hematocrits in the standard diet white rats is significant compared to the vegan diet group. Meanwhile, the levels of MCH, MCV and MCHC changed significantly in the white rats that were given a vegan diet compared to the white rats on a standard diet after routine physical exercise.

INTRODUCTION

The prevalence of disease in the world continues to increase from year to year. The same thing happened in Indonesia, where the prevalence of communicable (infectious) and non-communicable diseases continued to increase. Based on reports from the Indonesian Directorate General of Health, infectious diseases such as tuberculosis have the second-highest number of sufferers in the world. In addition, non-communicable diseases have also increased, for example,

diabetes mellitus from 1.74% to 2.03%, and the prevalence of obesity in 2013 was recorded at 14.82%, increasing to 21.84% in 2018.[1] An increase in diseases, especially non-communicable diseases, can be caused by several factors, including diet and lifestyle. Failure to apply a good diet and lifestyle in everyday life can result in a decrease in quality of life.[2]

Today, there is one type of diet that is trending in society, namely the vegetarian diet. Practicing this diet can be a way to maintain a healthy body through daily food nutrition.[3] There are several types of vegetarian diets, one of which is the vegan type. This type of vegetarian diet is one that obtains a source of protein in the form of vegetable protein. Implementation of this diet can prevent the body from suffering from diabetes and obesity, as well as impaired cardiovascular function (hypertension and stroke).[4] In addition to diet, lifestyle factors such as being active in sports or physical exercise have also been reported to be able to prevent disease, especially non-communicable diseases. Regular physical exercise can improve the function of body systems, such as increasing energy formation through breaking down body fat, increasing physiological immunity, and being able to reduce stress and prevent depression.[5] In addition, regular physical exercise can produce responses and changes in the body's hematological components, which then become indicators of body health, such as hematocrit and other red blood cell counts.[6]

Aspects of the hematological profile that can experience changes due to diet or physical exercise factors include the levels, structure, and function of these hematological components. The hematological profile in the human body includes the number of blood cells (erythrocytes, leukocytes, leukocyte count, platelets), hemoglobin, hematocrit, MCV, MCH, and MCHC, as well as blood proteins.[7] Food consumed is a source of molecules that make up hematological components, including carbohydrates, lipids, proteins, nucleic acids, vitamins, and mineral substances. A diet that takes into account the adequacy of iron intake in food can affect the concentration of erythrocytes, hemoglobin, and hematocrit levels in the blood.[8] A vegetarian diet is known to be able to affect the concentration of blood components such as cholesterol, which is reported to have increased after undergoing the diet.[9] In addition, on the vegetarian diet, it was also found that intake of folate and vitamin B12 was lower than in groups of people who adopted a non-vegetarian diet.[10]

In addition to dietary factors, the levels of hematological components such as hematocrit, MCV, MCH and MCHC in the blood can be influenced by the physiology of body movements through routine physical exercise. Exercise causes the mechanisms of several body systems, such as the muscular, cardiovascular and respiratory systems to take place at a higher rate than at rest. This is characterized by an increase in oxygen consumption and the body's metabolic processes.[11] Besides causing an acute response and adaptation to the body's organ systems, changes in the concentration of components and proteins in the blood can be affected by the intensity and type of physical exercise performed.[12] Until now, there has been little research which revealed the blood hematological component profile of the vegan group after routine physical exercise. Based on this, researchers conducted research to analyze the comparison of hematocrit, MCV, MCH and MCHC levels between white rats on a vegan diet and a standard diet after routine physical exercise.

METHOD

This study applied an in vivo experimental method using a pretest-posttest design with non-equivalent groups. The research sample used was a female white rat (Rattus norvegicus) of the Sprague-Dawley strain, 8–9 weeks old. Determination of sample size using the Federer formula with a total sample of 16 individuals. The tools used in the study included analytical balances, pelleting machines, aquariums, stopwatches,

hematocrit pipettes, 5 ml EDTA tubes, and ice boxes. While the ingredients used are 70% alcohol, hyena coloring, standard feed, and vegetarian blended feed, The dependent variables in this study were the hematocrit, MCV, MCH, and MCHC levels of white rats. While the independent variable in this study was the form of diet, namely the vegan-type vegetarian diet and the standard diet.

White rats were housed separately for each group, namely 4 rats per cage. The bedding of the cage uses wood shavings, which are replaced twice a week. The amount of feed given is as much as 10% of body weight per white rat, which is as much as 20–25 grams per head per day. Feeding is done twice, namely in the morning and evening. While giving drinking water is done ad libitum and checking the volume of water in each cage every day, White rats were placed in a room with a temperature of 25 °C, humidity ranging from 60 to 65%, and a room light cycle of 12 hours dark and 12 hours light.[13]

During the feeding stage, each rat was acclimatized for a week using a standard diet. After the acclimatization time was complete, each white rat was weighed using an analytical balance (in units of grams) and blood samples were taken before treatment. Next, the rats were transferred to cages that had been labeled according to the treatment group, namely the vegetarian and standard diet groups. In the vegetarian diet rat group, vegetarian food was given in stages, namely in the first week, in the form of a 25% vegetarian + 75% standard mixture. Furthermore, in the second week, they are given a feed mixture of 50% vegetarian and 50% standard. In the third week, white rats were fed a standard 75% vegetarian plus 25% concoction. Then, the fourth week is given 100% vegetarian food.[14]

Next, at the routine physical exercise intervention stage, each white rat was given treatment in the form of swimming for 15 minutes. The swimming treatment on white rats was carried out three times per week in the afternoon. At the beginning of this stage, each rat was first adapted to swimming for 3 minutes every day for 3 days. During the swimming treatment in white rats, the water temperature was controlled around 32–26 °C. Then, after each swimming treatment, the bodies of the white rats were dried and exposed to light so that their body temperatures could return to their initial levels.[15] The research procedure was continued by taking blood samples after the diet treatment and routine physical exercise for four weeks. The blood sample that has been taken is transferred to the EDTA tube, which already contains an anticoagulant substance. The blood sample was then quickly put into an ice box and analyzed using a hematology analyzer.

The statistical data obtained is tabulated into a file using the MS program. Excel. Data analysis used in this study included univariate analysis, namely the average body weight and levels of the dependent variable, as well as bivariate analysis using t and Wilcoxon tests. The significance value uses the standard alpha value, p 0.05, through the SPSS version 24.0 application.

RESULT AND DISCUSSION

Measuring the body weight of white rats was carried out before feeding using an analytical balance for five times of data collection. Based on Table 1, the average body weight of white rats before treatment in the vegan diet group was 218.8 grams, while the standard diet group was 214.3 grams. Furthermore, the body weight of the white rats on the vegan diet increased every week, with a range of 6.4–12.2 grams per week. Meanwhile, the standard diet white rat group experienced an increase in weight ranging from 4.2-11.3 grams per week. At the end of the study, the mean body weight of the vegan diet group of rats was higher than that of the standard diet group of rats, with an average difference in weight of 10.0 grams. The changes in body weight of white rats every week in both groups can be seen in **Table 1.**

Table 1. Average Weekly Body Weight of White Mice During the Study

Diet Type	Average Weekly Body Weight of White Mice Every Week (gram)				
	Start	1	2	3	4
Vegetarian (Vegan)	218,8	230,3	236,7	246,0	255,5
Standard	214,3	220,3	230,8	241,3	245,5

The amount of nutrient content in feed nutrition, especially protein, can affect the increase in body weight of experimental animals. The group of white rats fed a vegan diet obtained a source of protein from plants, namely soybeans, where this food source contains up to 37.7% protein per 100 grams of soy flour.[16] Protein in soybeans is a source of components of amino acids that can act as precursors. body protein building blocks in experimental animals. In addition, regular physical activity is an additional factor in the formation of muscle protein, especially structural protein. This is due to the role of the hormone testosterone, which triggers an increase in structural protein synthesis, so that the body weight of the experimental animals increases.[17]

Analysis of the data on hematocrit levels in the two groups of mice showed that the data were not normally distributed, so the data used was the median value. Based on Table 2, in the vegan diet of white rats, the median value of hematocrit before and after routine physical exercise was the same, namely 46.65%. The results of the significance test showed that routine physical exercise had no significant effect on the hematocrit levels of white rats fed a vegan-type vegetarian diet (p = 0.892). Whereas in the group of rats that were given a standard diet, the amount of hematocrit changed between before and after routine physical exercise. In addition, the significance test showed that there was a significant effect of routine physical exercise on the amount of hematocrit in the standard diet group (p = 0.034). Details of the data analysis can be seen in **Table 2**.

Table 2. Statistical Test Results for Comparison of Hematocrit Levels Between White Rats on a Vegetarian Diet and a Standard Diet After Routine Physical Exercise

Diet Type	Intervention	Median (%)	Min-Max	*p Value*
Vegetarian (Vegan)	Before Exercise	46,65	46,50-49,90	0,892
	After Exercise	46,65	46,60-46,80	
Standard	Before Exercise	47,65	46,60-49,70	0,034
	After Exercise	46,65	46,50-46,80	

Hematocrit, which is defined as the ratio of red blood cells to total blood volume or the percentage of erythrocytes per total blood volume, can be influenced by several factors, including the water content of food, age, place of residence, and degree of dehydration after physical exercise.[18] The intensity of physical exercise can affect the percentage of hematocrit in the blood; after physical exercise, the hematocrit level has decreased significantly. Thus, the higher the intensity of physical exercise performed, the greater the decrease in blood hematocrit levels.[19] In this study, the decrease in hematocrit levels in the white rat group was not significant. This is due to the vegan feed formulation using protein ingredients sourced from soy. Soybeans are also known to contain relatively high levels of folate. Folate has a role in the process of synthesis of red blood cell production, which is one of the components that determines the percentage of blood hematocrit.[20]

Furthermore, in this study, the MCV data obtained were not normally distributed between the two groups. Therefore, the data used to test the effect of physical exercise is the median value in each group. Based on **Table 3**, in the vegan diet group, it can be seen that the MCV value after routine physical exercise was lower than before routine physical exercise. Whereas in the standard diet group, the MCV value decreased by 0.05 fL after routine physical exercise. Next, this table also shows that regular physical exercise has a significant effect on changes in MCV levels in the vegan diet group (p = 0.026). However, routine physical exercise did not have a significant effect on the group of white rats that were given a standard diet (p = 0.458). Complete data regarding the MCV levels of the two groups of white rats after routine physical exercise can be seen in **Table 3.**

Table 3. Statistical Test Results for Comparison of MCV Levels Between White Rats on a Vegetarian Diet and a Standard Diet After Routine Physical Exercise

Diet Type	Intervention	Median (fL)	Min-Max	*p* Value
Vegetarian (Vegan)	Before Exercise	53,75	53,70 - 53,50	0,026
	After Exercise	53,50	53,40 - 53,60	
Standard	Before Exercise	53,50	53,20 - 53,50	0,458
	After Exercise	53,45	53,40 - 53,60	

The mean corpuscular volume (MCV) is the value of the average volume or size of erythrocytes and is also commonly referred to as the erythrocyte index value. Changes in MCV in the blood can be used as an indicator of the condition of iron in the blood, so that it can provide an overview regarding the number of red blood cells and hemoglobin levels in the blood.[21] Changes in MCV levels can be influenced by several factors, such as diet and physical exercise. The form of MCV changes that occur is caused by reduced blood plasma volume as a result of the body's dehydration response during routine physical exercise.[22] In addition, a post-exercise decrease in MVC can also be caused by increased energy use by the muscles, which chemically increases blood viscosity and decreases deformability, resulting in an increase in body temperature.[23]

Based on the results of the distribution analysis, data on MCH levels in the two groups obtained were not normally distributed. So that the data taken is in the form of mean data and its significance value is determined using the Wilcoxon test. In Table 4, it is shown that the increase in MCH levels in the group of white rats fed a vegan-tip vegetarian diet was higher than that in the white rats on a standard diet. Analysis of the effects of routine physical exercise showed that routine physical exercise given to the vegan diet group had a significant effect on the MCH levels of white rats (p = 0.027). Whereas in the standard diet white rat group, the routine physical exercise given did not have a significant effect on the MCH levels of the experimental animals (p = 0.102). Other information regarding the effect of regular physical exercise on MCH levels in white rats can be seen in **Table 4.**

Mean Cell Hemoglobin (MCH) is part of the erythrocyte index which describes the ratio of hemoglobin levels to the number of erythrocytes in the blood.[24] This MCH value can also give an idea related to the weight of hemoglobin per erythrocyte cell. Routine physical exercise can be an important factor in changes in blood MCH. After physical exercise, MCH levels increase as a consequence of the increased demand for oxygen by the body through the function of hemoglobin in the erythrocyte cells. In addition, changes in MCH due to physical exercise can affect the distribution of red blood cells, both during physical exercise and during post-exercise rest.[2]

Table 4. Statistical Test Results for Comparison of MCH Levels Between White Rats on a Vegetarian Diet and a Standard Diet After Routine Physical Exercise

Diet Type	Intervention	Median (pg)	Min-Max	*p* Value
Vegetarian (Vegan)	Before Exercise	18,15	18,10-18,20	0,027
	After Exercise	18,45	18,30-18,50	
Standard	Before Exercise	18,50	18,40-18,50	0,102
	After Exercise	18,55	18,40-18,60	

The data from this study also showed that the MCHC levels of white rats in the two groups were not normally distributed. So that the significance test used is an alternative test, namely the Wilcoxon test. Based on Table 5, it can be seen that the MCHC levels in the vegan diet white rat group experienced an increase compared to before routine physical exercise was given. On the other hand, in the white rat group, the standard diet showed a decrease in MCHC levels. In addition, the significance test showed that routine physical exercise given to the vegan diet white rat group caused a significant change in MCHC levels (p = 0.026). Whereas in the standard diet white rat group, the routine physical exercise given did not show a significant change in MCHC levels (p = 0.059). Details of MCHC-level data analysis can be seen in **Table 5**.

Table 5. Statistical Test Results for Comparison of MCHC Levels Between White Rats on a Vegetarian Diet and a Standard Diet After Routine Physical Exercise

Diet Type	Intervention	Median (g/dL)	Min-Max	*p* Value
Vegetarian (Vegan)	Before Exercise	34,10	34,10-34,20	0,026
	After Exercise	34,40	34,30-34,50	
Standard	Before Exercise	34,50	34,40-34,60	0,059
	After Exercise	34,40	34,30-34,60	

The mean corpuscular hemoglobin concentration (MCHC) value was obtained from the calculation of MCHC (g/dL) = Hb (g/dL)/HCT (5%), which is also commonly referred to as the average hemoglobin per erythrocyte level.[24] In this study, there was an increase in MCHC after physical exercise in both the vegan and standard white rat diet groups. This is in line with previous research, which reported that routine physical exercise carried out in the afternoon can increase blood MCHC levels.[25] This can occur due to the intensity of physical exercise performed, which then affects the formation of new red blood cells from the spinal cord to meet the body's need for oxygen during muscle contraction.[26]

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CONCLUSION

Based on the results of the research and analysis, it can be concluded that routine physical exercise has a significant effect on the levels of MCV, MCH and MCHC in the group of white rats fed a vegan-type vegetarian diet. While the amount of hematocrit experienced a significant change in the group of white rats with a standard diet after routine physical exercise

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