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ACUTE RESPONSE OF LEUCOCYTE TOTAL OF WHITE RAT (*RATTUS NORVEGICUS*) WITH VARIATIONS VEGETARIAN DIETARY IN STRENUOUS EXERCISE

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ABSTRACT

A healthy lifestyle is an important thing that needs to be considered as a principle to maintain health level. However, the busyness to fulfill needs makes people pay less attention to apply the right of dietary and physical activity daily, so that have potential to cause disease and can be assessed through the total of leucocyte. This study aims to analyses acute response of leucocyte total of white rats (*Rattus norvegicus*) with variation vegetarian dietary on strenuous exercise. The research was an in vitro experimental through analytical exploratory approach using a previous and posttest design with nonequivalent groups. This study was used a white rat (*Rattus norvegicus*) as a sample with three types of vegetarian diet and a standard dietary. Measurement of total leukocytes were conducted before and after strenuous exercise, which exhaustive swim. The data from each group were analyzed statistically using the pair t-test. The results showed that there was significant change in total leucocyte to intervention and control groups after strenuous exercise, that is quasi vegetarian dietary p=0,028 (p<0,05), lacto-ovo p=0,007 (p<0,05) and standard dietary p=0,045 (p<0,05), while there was no significant change in total leucocyte on vegan vegetarian dietary group after strenuous exercise p=0,752 (p<0,05). The conclusion proved that heavy exercise can cause significant acute response of total leucocyte on rats with vegetarian dietary.

Keywords: vegetarian dietary, total leucocyte, white rats, strenuous exercise

1. INTRODUCTION

Non-communicable disease can be experienced by age groups ranging from childhood to the elderly. The prevalence of non-communicable diseases, for example in Indonesia, is still increasing from year to year. The disease includes cancer from 1.4% increased to 1.8%, stroke 7.0% increased to 10.9%, chronic kidney from 2.0% increased to 3.8%, joint disease 7.3% increased to 11.9%, diabetes from 1.7% increased to 2.0%, hypertension 25.8% increased to 34.1% and obesity from 14.8% increased to 21.8%. The increase in the prevalence of non-communicable diseases occurred within a period of 5 years, that is from 2013 to 2018.[1]

This non-communicable disease can be suffered by the age group of childhood to the elderly. These diseases can be caused primarily by lifestyle factors. An unhealthy lifestyle causes a person to easily suffer from non-communicable diseases which then results in a decrease in the quality of life.[2] Thus, a healthy lifestyle is an important thing that needs to be considered and used as a principle in maintaining health.[3] In addition, a healthy lifestyle is a special concern of the government and becomes one of the benchmarks in achieving health improvement in accordance with the Sustainable Development Goals (SDGs) 2015-2030 program.[4] However, a lifestyle that is lived on a daily basis unconsciously,

such as the wrong diet and improper exercise can cause a person to fall ill.[5] A good diet is one that follows the needs of the body and can be done with a certain diet. Diet is a pattern of consumption of foods that contain balanced calories, multiply fruits and vegetables, are low in saturated fat and cholesterol, and low in sugar and salt.[6] Diets are divided into several forms, one of which is a vegetarian diet.

Currently, vegetarian diets are increasingly being implemented and are believed to be able to prevent diseases, especially non-communicable diseases.[7] In addition, a vegetarian diet is also able to reduce the risk of contemporary diseases such as central obesity and diabetes insipidus.[8] A vegetarian diet is a lifestyle that consumes food not from meat sources, but consumes more animal products such as eggs and milk, vegetables, fruit, grains and nuts.[9] These diets are grouped into three types, that is quasi-vegetarian, lacto-ovo-vegetarian and vegan. Quasi-vegetarians are a diet that does not eat red meat, lacto-ovo-vegetarians predominantly consume animal products (milk and eggs), while vegans do not consume foods of animal origin at all.[10] Besides being able to prevent disease, a vegetarian diet can also provide energy to maintain functional homeostasis and body health and slow down aging.[11] The group of vegetarian dieters has a certain hematological profile. Under normal circumstances, the vegetarian group has low levels of cholesterol, triacylglycerol and LDL.[9] In addition, in the vegetarian variation group (quasi, lacto-ovo, and vegan) have different hematological profile levels, including the count of erythrocytes, leukocytes, glucose levels, LDL, HDL, total cholesterol, triacylglycerols, hemoglobin and mineral substances such as calcium, iron and vitamin B12.[12]

In addition to running a diet, it is also known that exercise can improve the health and fitness of the body by utilizing energy from the food consumed. Exercise benefits the health of the body including improving cardiovascular function, respiration and body immunity and being able to reduce psychological disorders such as stress and depression.[13] The busyness in meeting the needs of the day in this modern era makes people less do sports, lazy to participate in sports activities and embarrassed to exercise regularly, so that when there is an opportunity, the exercise done often reaches fatigue.[14]

The lack of knowledge related to how to exercise correctly and appropriately, makes people tend to do sports until they are tired.[15] Exhausting physical activity can cause health problems, among them acute dehydration due to excessive expenditure of body heat, muscle pain caused by the accumulation of lactic acid and oxidative stress that arises due to low antioxidants.[1] In addition, exhausting exercise can also cause changes in hematological indicators and the metabolic profile of plasma.[16] In the vegetarian group, the exercise performed can cause some acute changes in the physiology of the body's defense system through blood component factors that play an important role in maintaining the body's immunity, such as the number of white blood cells or total leukocytes.[17]

Although there have been several studies looking at the effect of strenuous exercise on total leukocytes in both experimental animals and humans,[18] there is still very little research on the acute response of leukocytes total after heavy intensity exercise, specific to the vegetarian diet group.[19] Thus, this study was conducted to determine the acute response of leukocyte total of white rat with variations in the vegetarian diet in strenuous exercise.

2. METHOD

The tools used in the study were rat cage, rat eating and drinking place, disposable syringe (1 ml) and (3 ml) (Terumo Brand, Japan), gastric sonde and

half-circumference sonde, hemostat, blade and scalpel, cloth and rubber gloves, masks, scales, aquarium / water container, aluminum plate (ballast), ice box, ice replacement and EDTA tube. The ingredients used in the study were sterile aquadest, alcohol, female mice (strain of Sprague Dawley), standard rat food and drinks and blended vegetarian feed.

Obtained white rats are adapted for two weeks. White rats were kept in groups (7 white rats/cage). A quadrangular-shaped cage measuring 40 cm long x 20 cm wide x 10 cm high covered with wire. Bedding on the cage in the form of wooden shavings that are replaced twice a week. During maintenance, feed is given as much as 10% of body weight, which is about 15-20 grams/ head/day in the form of standard feed which is distributed into two feedings, that is morning and evening. Drinking water is given ad libitum through a drinking bottle (source). White rats are kept in a room with a temperature of about 22-25 degrees Celsius with a slowness ranging from 55-65% and a lighting cycle of 12 hours of light/12 hours of darkness.[20]

Vegetarian feed is formulated using the composition of foodstuffs according to the type of vegetarian diet given to white rats. The result of the concoction is in the form of a mixed ration with standard feed according to the type of vegetarian diet in stages. The vegetarian diet foodstuffs of each type are mashed while the standard feed is re-mashed until it becomes flour.[21] Then 75% (15 grams / head / day) standard feed that has been fine is taken and mixed with 25% (5 grams / head / day) of vegetarian food ingredients according to the type of vegetarian diet that will be given to white rats using a mixer until homogeneous. Next, the mixed materials are put into the pelleting machine. The resulting pellets are immediately dried using an oven with a temperature of 60^0C until dry, then wrapped in a plastic bag that has been labeled with a vegetarian feed type number.[22] Furthermore, the proportion of vegetarian diets is increased until it reaches 100% vegetarian diets. The weight of the feed given is increased by 20% every 3 days in anticipation of the growth of white rats.[23]

As soon as vegetarian feed is produced, the omnivorous diet of white rats will gradually be changed to a vegetarian diet. A total of 4 treatments were carried out in this study, that is a quasi-vegetarian type vegetarian diet, lacto-ovo-vegetarian, vegan and omnivorous. The change in the form of the diet in each group was carried out for 4 weeks and adapted until the 8th week.[24]

Before being given a diet, rat weight weighing was first carried out using digital scales (grams) on all female white rats. The body weight of rats is recorded on the research book. A total of 28 white rats were placed in their respective cages that had been labeled according to the diet treatment group. In accordance with the research design, the mice were divided into 4 groups, that is K, P1, P2, and P3 with the following treatment:

K : Control group, rats who performed strenuous exercise on a regular diet (omnivorous)
P1 : Treatment group, rats who exercised strenuous exercise with a quasi-vegetarian type vegetarian diet
P2 : Treatment group, rats performed strenuous exercise on a lacto-ovo-vegetarian type vegetarian diet
P3 : Treatment group, rats who were subjected to strenuous exercise with a vegan-type vegetarian diet.

Acclimatization of experimental animals was carried out during the week, for the adjustment of the mice to the environmental conditions of the study site, both for the control group and the treatment group. Feeding of tried animals was carried out gradually with tapering techniques on portions of food of experimental animals

during the study.[24] The amount of feed given is 10% of the body weight of the rat. Here are the stages of dietary treatment in experimental animals:
1th Week : Diet Standard (100%)
2nd Week : Standard (75%) + vegetarian (25%)
3rd Week : Standard (50%) + vegetarian (50%)
4th Week : Standard (25%) + vegetarian (75%)
5th Week : Vegetarian diet (100%)

This research uses an experimental method of in vitro laboratory through an analytical exploratory approach with a pre-posttest design with nonequivalent groups. The strenuous exercise treatment given was in the form of swimming to fatigue treatment in each sample of white rats. Before the treatment of swimming until tired, each white rat in each group was given an adaptation of swimming exercises for 10 minutes every day for 3 days with the water temperature controlled in the range of 32-36 degrees Celsius. Further, on the administration of strenuous exercise treatment (swimming to fatigue), the tail of a white rat is tied with heavy metals about 5% of body weight.[25] White rats were put in a container of water and swam alone to the point of exhaustion. Fatigue is achieved when the white rat is unable to maintain its nose outside and is below the surface of the water for 10 seconds.[26]

White rats are positioned in a comfortable and stressed state. Rat blood sampling was carried out through venous vessels in the tail that had been warmed by ± 1 ml of mice after strenuous exercise. Furthermore, the blood taken is transferred into a 1.5 ml eppendorf tube that contains the chemical anti-coagulant compound ethylenediaminetetraacetic acid (EDTA). Then, quickly the EDTA tube containing the blood sample was put into an ice box, then taken to the laboratory to measure the total leukocyte levels using a hematology analyzer.[27] The data obtained were analyzed using a t-test through the SPSS 24.0 application. The research carried out has obtained the approval of the Medical and Health Research Ethics Committee (KEPKK) of the Faculty of Medicine, Sriwijaya University with a certificate worthy of research ethics No. Protocol: 125-2021.

3. RESULT

The body weight of white rats weighed weekly for 5 weeks, since the acclimatization process, had different weight averages between groups of white mice with different types of vegetarian diets as can be seen in the following table 1.

Table 1. Body Weight of a White Rat

Diet Vegetarian	Average (SD) Weight Week to- (gram)				
	1	2	3	4	5
Quasi	203,50 (33,53)	216,33 (38,19)	226,33 (37,12)	232,17 (36,94)	236,67 (36,86)
Lacto-ovo	229,33 (35,57)	242,83 (34,78)	250,33 (33,90)	262,67 (35,73)	268,67 (36,55)
Vegan	218,83 (28,27)	230,33 (32,85)	236,67 (34,63)	246,00 (33,62)	255,50 (38,74)
Standard	214,33 (24,89)	220,33 (23,15)	230,83 (29,69)	241,33 (23,18)	245,50 (21,91)

The body weight of white rats, as seen in Table 1, increased weekly in each group of vegetarian diets. In the quasi-type vegetarian diet, the increase in body weight of white rats occurred every week, that is 12.83 grams (week 1), 10.00 grams (week 2), 5.84 grams (week 3) and 4.5 grams (week 4). Next, in the lacto-ovo type vegetarian diet group, the increase in body weight of white rats every week was 13.50 grams (week 1), 7.5 grams (week 2), 12.34 grams (week 3) and 6.00 grams (week 4). Furthermore, the increase in body weight of white rats with a vegan-type vegetarian diet every week, that is 11.50 grams (week 1), 6.34 grams (week 2), 9.33 grams (week 3) and 9.5 grams (week 4). Whereas in the group of white rats with a diet using standard feed, an increase in the body weight of white rats was recorded, that is 6.00 grams (week 1), 10.5 grams (week 2), 10.5 grams (week 3), and 4.17 grams (week 4).

Table 2. Total Leukocyte Changes in White Rats

Diet Vegetarian		Average (SD) (10^3/µL)	Difference (SD)	p
Quasi	Pre	4,55 (0,18)	0,02 (0,02)	0,028*
	Post	4,53 (0,17)		
Lacto-ovo	Pre	4,53 (0,17)	0,01 (0,01)	0,007*
	Post	4,52 (0,16)		
Vegan	Pre	4,55 (0,16)	0,01 (0,04)	0,752
	Post	4,54 (0,18)		
Standard	Pre	4,53 (0,17)	0,01 (0,01)	0,045*
	Post	4,51 (0,17)		

Based on the results of statistical analysis, it can be seen in Table 2 that there was a significant total leukocyte acute response after exercise, namely in the group of white rats with a quasi-type vegetarian diet (p=0.028), lacto-ovo (p=0.007) and the standard diet group (p=0.045). Meanwhile, in the group with a vegan-type vegetarian diet, there was no change in the number of leukocytes after strenuous exercise (p = 0.752). In this result, the largest change in the number of leukocytes after strenuous exercise was also found, namely in the quasi-type vegetarian diet group (0.02 x 10^3/µL).

4. DISCUSSION

The difference in the type of diet in this study lies in the type of protein source, so the protein obtained is used for growth, which then has an impact on increasing the body weight of white rats.[28] Furthermore, the substance of essential and non-essential amino acids contained in the vegetarian diet can act as a precursor to the body's structural and functional protein synthesis so that it can increase body weight.[29] In addition, the protein found in each type of vegetarian diet can be a source of energy, apart from the carbohydrate content in the diet. Thus, the process of protein metabolism can also produce free radicals from oxidative cell reactions, which are then able to trigger the immune system response of leukocyte cells.[30]

The group with a vegetarian diet showed some differences in hematological parameters, such as the number of leukocytes, with the group that was on a non-vegetarian diet. The group that was on a vegetarian diet tended to have a lower number of leukocytes compared to non-vegetarians but was still within the normal

range.[31] This is due to the process of propagation (proliferation) and cell fermentation, as in leukocyte cells, influenced by several factors, including vitamin B12 which is not found in vegetarian diets.[32]

Total leukocytes may increase during physical exercise due to the mobilization of leukocyte cells from the lymphatic ducts.[33] However, in this study, on average it showed a decrease in the number of leukocytes. This is because after exercise, leukocyte cells are diagnosed out of the blood vessels to muscle tissue damaged by strenuous exercise in carrying out their functions as agents of the immune system.[34]

In addition, the total acute response of post-exercise leukocytes is also influenced by hormones such as epinephrine and cortisol levels in the blood. The hormone epinephrine can trigger changes in the concentration of leukocytes in the blood during short exercise (less than 1 hour).[35] Drastic changes in the number of leukocytes after strenuous exercise caused by the function of the hormone cortisol, occur as a result of the body's inflammatory response to oxidative stress resulting from such strenuous exercise.[36]

Physical activity in a vegetarian diet group can provide certain benefits in maintaining a healthy body. An acceptable effect for post-exercise vegetarian dieters is a reduced risk of infection and acute disease.[37] Exercise caused a response to the immune system, through an increase in the concentration of antioxidants in the blood due to an increase in the number of leukocytes, as well as an increase in inflammatory biomarkers resulting from the synthesis of leukocyte cells, such as interleukin-6.[38]

5. CONCLUSION

The conclusion of this study was that strenuous exercise caused an acute response of white rat leukocytes with a quasi-type vegetarian diet and lacto-ovo as well as a standard diet. However, white rats with a vegan-type vegetarian diet did not show a significant total acute response to leukocytes.

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