The Effect of Giving Pork Oil and Egg Yolk as Components of a High Fat Diet on The Hematological and Cholesterol Profiles of Mice (*Mus musculus*)

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ABSTRACT

Background: This study examined the role of a high-fat diet, especially one containing animal fat, in its influence on the hematology of the organism, with mice as research subjects. Hematology, as the study of blood components, is the focus for analyzing the impact of a high-fat diet, which includes animal fats such as lard and egg yolks. Although fats are essential for cell function and nutrient absorption, excessive intake can increase the risk of serious health problems. **Purpose:** To fill the gap in knowledge by evaluating the effect of lard and egg yolk on the hematological profile of mice. **Methods:** True Experimental with a Pretest-Posttest Control Group design. The sampling technique used is Simple Random Sampling. The parameters yolk). The sampling technique used is Simple Random Sampling. The parameters observed include blood hematological profiles (Erythrocyte Count, Hemoglobin, and Erythrocyte Index) and cholesterol levels. **Results:** Expected to provide in-depth insight into the impact of high-fat diets on blood health, provide important contributions to understanding the human health implications, and support the development of more effective nutritional guidelines for managing diet and preventing metabolism-related diseases. **Conclusion:** It can be concluded that giving pork oil with added egg yolk can increase erythrocyte levels, hemoglobin profile, erythrocyte index (MCV and MCH), and cholesterol levels.
Keywords: egg yolk, lard, mice, high fat diet, hematology

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INTRODUCTION

Diet and nutritional intake play an important role in regulating body health, including its influence on the hematological system. Hematology is the study of blood components, which involves the analysis of various blood parameters such as the number of red blood cells, white blood cells, platelets, and other components. Diet is one of the external factors that can influence blood hematology and cholesterol profiles (Busia *et al.*, 2016).

A high-fat diet is a diet trend that has become a major topic of conversation in the world of nutrition and health. A high-fat diet is a type of diet that has a feed composition with a high fat content. High-fat diets can consist of various types of fat, including animal fats such as lard and egg fat, which contain large amounts of saturated fat and cholesterol. Fat is an important component in the diet and has a vital role in providing energy, maintaining cell function, and absorbing certain nutrients. However, excessive fat intake has been known to increase the risk of serious health problems. This kind of diet can affect the composition and function of blood in the organism (Erni *et al.*, 2014).

One of the important aspects of assessing the impact of a high-fat diet on the organism is the monitoring of

hematological parameters. Although many studies have been conducted on high-fat diets, research on its effects on mice with a focus on hematological parameters is still limited. Therefore, the aim of this study is to fill this gap in knowledge by investigating the effect of administering lard and egg yolk as components of a high-fat diet on the blood hematology and cholesterol profile in mice (*Mus musculus*).

It is hoped that the results of this research will provide a deeper understanding of how a high-fat diet, especially one containing animal fat, can affect blood health and its possible impact on humans. as well as making significant contributions to understanding the impact of high-fat diets on organismal health (Singh *et al.*, 2022). Additionally, this research may also help in the development of better nutritional guidelines for managing diet and preventing metabolism-related diseases related to fat intake.

METHODS

Study design

The design of this research is True Experimental with a Pretest-Posttest Control Group design. The samples were divided into two groups: negative control group (only given pellets) and positive control group (given pellets, lard, and egg yolk). The sampling technique used is Simple Random Sampling. The parameters observed include blood hematological profiles (Erythrocyte Count, Hemoglobin, and Erythrocyte Index) and cholesterol levels.

Time and Place of Research

This research was carried out at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University as a place for raising and treating experimental animals. This research was conducted on November 9 - November 16 2023.

Samples

The research sample consisted of 10 male *Mus musculus* mice aged approximately 2 months with a body weight ranging from 20-30 grams. The choice of male mice is influenced by hormonal factors compared to female mice. 1 ml of each blood sample was collected, transferred to an EDTA tube, then to an Eppendorf tube for further testing using a hematology analyzer.

Animal treatment

This research used male mice (*Mus musculus*), with a body weight of between 20-30 grams. The number of mice used was 10 mice. Experimental animals were given lard and egg yolk a total of twice a day for one week. Giving lard and egg yolk to mice is done orally, which will be mixed into the mice's feed pellets. The dose of lard and egg yolk used in this study was 1:1 where egg yolk and lard were 0.2 ml each, where the administration was divided into two stages, namely morning and evening (total lard + egg yolk per day /mouse = 0.4 ml). The research was carried out with two treatments, in one treatment there were a minimum of five animals in one treatment, including: Treatment (K-): Group of mice (*Mus musculus*) which were not given lard and egg yolk. Treatment (K+): A group of mice (*Mus musculus*) were given pork oil and egg yolk orally in the morning and evening.

Pig oil and egg yolk were given orally to the treatment group (K+). In the negative control treatment group (K-), no intervention was given, but blood was drawn for hematological examination. Meanwhile, in the positive control treatment group (K+), after being given treatment, hematology and cholesterol examinations will be carried out.

The data collection method was carried out in several stages, namely carrying out hematological examinations on the negative control (K-

) sample group without any treatment. Blood tests that focused on hematology (total erythrocytes, hemoglobin levels, and erythrocyte index) and cholesterol were carried out on the positive control sample group (K+) after being given treatment.

Data analysis

The existing data has been presented and analyzed descriptively. Data obtained by determining the hematology profile was carried out using a Hematology Analyzer. In this test, parameters are produced in the form of erythrocyte count, hemoglobin level, and erythrocyte index (MCV, MCH, MCHC).

RESULT AND DISCUSSION

Total Erythrocyte Profile

Based on the results of the examination using a hematology analyzer, it was discovered that erythrocytes were lower in the treatment group of mice (K-), namely 3.99 x 106 /mm3. Erythrocytes were higher in the treatment group of mice (K+), namely 5.67 x 106 /mm3. There was an increase in erythrocytes in the group of mice (K+) treated with lard and egg yolk. The total value of erythrocytes/Red Blood Cells (RBC) is presented in the form of a bar diagram as in Figure 1.

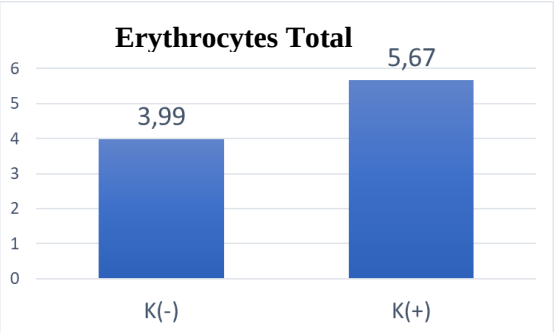

Figure 1. Bar diagram of total erythrocytes of mice in treatment groups (K-) and (K+).

Figure 1. shows that administration of pork oil and egg yolk has an influence on the total number of erythrocytes in mice. The total erythrocytes of the treatment group (K-) showed lower results than the treatment group (K+) with a dose of lard and egg yolk of 0.4 ml/head/day.

The fatty acids in egg yolks are a source of unsaturated fatty acids which play a role in the development of erythrocyte cells. These unsaturated fatty acids act as components of phospholipid membranes which play a role in the stability of erythrocyte cell membranes. The content of vitamin C, vitamin E and lycopene in egg yolk functions as an antioxidant which can inhibit the oxidation of LDL (Low Density Lipoprotein) by free radicals so that the erythrocyte membrane will be protected from lipid peroxidation by LDL-ox. This mechanism allows erythrocyte cells to remain stable even though they have been exposed to free radicals originating from the egg yolk diet. Iron and folic acid are essential for erythrocyte synthesis and are erythrocyte maturation factors. Zinc has a direct effect on membrane protein conformation and/or interactions between proteins in cell membranes. The addition of zinc to feed is thought to extend the life span of erythrocytes so that erythrocytes remain in circulation longer. Meanwhile, erythrocyte production (erythropoiesis) continues.

Hemoglobin Level Profile

Based on the results of examinations using a hematology analyzer, it was discovered that hemoglobin levels were lower in the treatment group of mice (K-), namely 5.0 x g/dL. Hemoglobin levels were higher in the treated group of mice (K+), namely 7.5 x g/dL. There was an increase in hemoglobin levels in the group of mice (K+) treated with pork oil and egg yolk.

The hemoglobin level (HGB) values were presented in the form of a bar chart as in Figure 2.

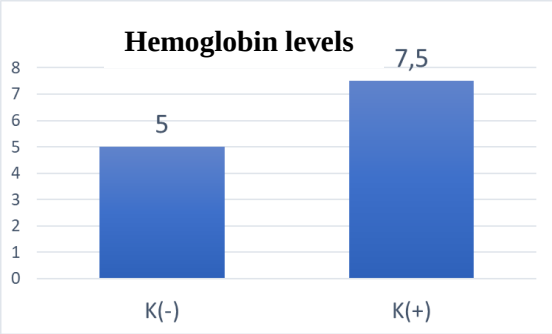

Figure 2. Bar diagram of hemoglobin levels of mice in treatment groups (K-) and (K+).

Figure 2 shows that giving pork oil and egg yolk has an influence on the hemoglobin levels of mice. The hemoglobin level of the treatment group (K-) showed lower results than the treatment group (K+) with a dose of lard and egg yolk of 0.4 ml/head/day. Eggs as a livestock product are an animal product that is nutrient dense and rich in high quality protein. The vitamins contained in eggs are vitamins A, D, B complex and B12 as well as other minerals such as iron, calcium, phosphorus, sodium and magnesium. The rich content of eggs can increase hemoglobin if consumed regularly.

Erythrocyte Index Profile

Based on the results of the examination using a hematology analyzer, it was discovered that the Mean Corpuscular Volume (MCV) value was lower in the treatment group of mice (K-), namely 40 µm3. The MCV value was higher in the treated mice group (K+), namely 46 µm3. There was an increase in the value in the group of mice (K+) treated with pork oil and egg yolk. The Mean Corpuscular Volume (MCV) value is presented in the form of a bar diagram as in Figure 3.

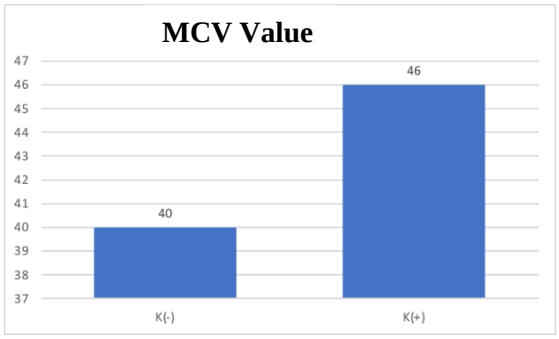

Figure 3. Bar diagram of MCV values for mice in treatment groups (K-) and (K+).

Figure 3 shows that normal MCV ranges from 45 - 55 fL (Connell, 2015). In this study, the MCV results obtained from the positive control treatment with a high fat diet with egg yolk and lard were 46 fL, which means that it was at the normal MCV value compared to the MCV results from the negative control treatment with standard feed.

Based on the results of the examination using a hematology analyzer, it was discovered that the Mean Corpuscular Hemoglobin (MCH) value was lower in the treatment group of mice (K-), namely 12.5 pg. The MCH value was higher in the treated mice group (K+), namely 13.2 pg. There was an increase in the value in the group of mice (K+) treated with pork oil and egg yolk. The Mean Corpuscular Hemoglobin (MCH) value was presented in the form of a bar diagram as in Figure 4.

Figure 4 shows that Mean Corpuscular Hemoglobin (MCH), in the normal value of female mice is 15.80 pg, while in male mice it is 15.40 pg (Wirth-Dzięciołowska *et al*, 2008). In this study, the MCH result of the negative control treatment (standard feed) was 12.5 pg and the positive control result of the high fat diet (egg yolk and lard) was 13.2

pg. It is known that the MCH results obtained from the positive control treatment have increased compared to the negative control (standard feed), but these results still do not show the achievement of normal MCH values.

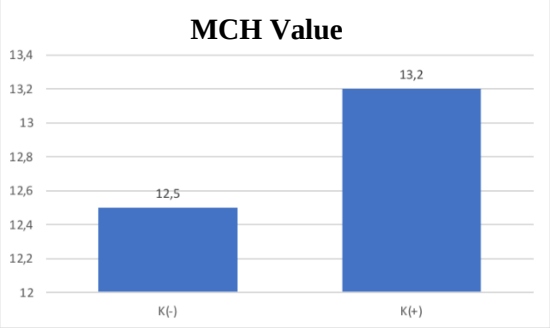

Figure 4. Bar diagram of MCH values for mice in treatment groups (K-) and (K+).

Based on the results of the examination using a hematology analyzer, it was discovered that the Mean Corpuscular Hemoglobin Concentration (MCHC) value was higher in the treatment group of mice (K-), namely 31.2 g/dL. The MCH value was lower in the treated mice group (K+), namely 28.8 g/dL. There was a decrease in the value in the group of mice (K+) treated with lard and egg yolk. The Mean Corpuscular Hemoglobin Concentration (MCHC) value was presented in the form of a bar diagram as in Figure 5.

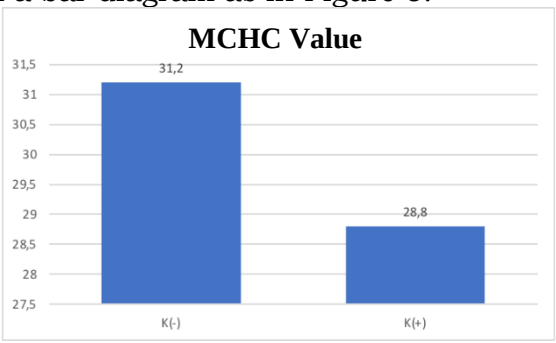

Figure 5. Bar diagram of MCHC values for mice in treatment groups (K-) and (K+).

Figure 5 shows that Mean Corpuscular Hemoglobin Concentration (MCHC), the normal value is 32-36 mg/dl (Mayangsari, 2021). In this study, it was found that the MCHC results of the positive control treatment (diet high in lard and lard) were lower and below the normal MCHC value compared to the MCHC results of the negative control treatment (standard feed). This can be caused by the animal experiencing iron deficiency anemia. Iron deficiency anemia is anemia caused by a lack of iron availability in the body, resulting in insufficient iron needed for erythropoiesis (Intantri, 2020). Pork oil has a relatively low iron content, namely around 0.35 mg (Anon, 1970).

From the erythrocyte index examination that was carried out on mice treated with a diet high in lard and egg yolk, the MCV results were normal, MCH was below normal, and MCHC was below normal. A normal MCV indicates normocytic anemia (Maner, 2022). Causes include nutritional deficiencies, kidney failure, and hemolytic anemia.

Cholesterol Level Profile

The results of examinations using the GCU Meter showed that cholesterol levels in the treatment group of mice (K+) were 139 mg/dl and 184 mg/dl, exceeding the normal threshold (40-130 mg/dl) (Erni *et al*, 2014).

Cholesterol levels in the blood generally come from the food consumed. The more consumption of fatty foods will result in increased cholesterol levels in the blood. Egg yolks and lard are food sources that contain lots of cholesterol. Egg yolks and animal fats are sources of cholesterol and saturated fat intake which can increase plasma cholesterol levels (Busia *et al.*, 2016).

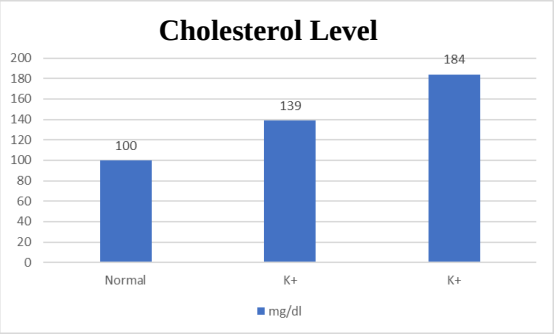

Figure 6. Cholesterol level values for mice in normal, treatment groups (K+) were 139 mg/dl, and (K+) were 184 mg/dl.

Figure 6 shows that the group of mice (K+) that were treated with lard and egg yolk experienced an increase in cholesterol levels compared to normal levels in mice. Pork fat contains high levels of saturated fatty acids. Saturated fatty acids cause an increase in triglyceride levels and a decrease in HDL, resulting in a greater risk of atherosclerosis. Consuming excessive saturated fat causes hyperlipidemia with increased LDL cholesterol levels. Hypercholesterolemia can cause endothelial dysfunction. Disruption of endothelial function causes endothelial permeability to increase so that LDL can enter the intima (Maramis *et al.*, 2014).

CONCLUSION

Based on research that has been carried out, namely "The Effect of Giving Lard and Egg Yolks as Components of a High Fat Diet on the Hematological and Cholesterol Profiles of Mice (*Mus musculus*)", which has an effect on the treatment that has been given is an increase in the number of erythrocytes, hemoglobin profile of mice that have been given treatment, erythrocyte index (MCV and MCH) and cholesterol levels of mice. It can be concluded that giving pork oil with added egg yolk can increase erythrocyte levels, hemoglobin profile, erythrocyte index (MCV and MCH), and cholesterol levels.

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Author Contribution

A.D.L, M.C.R, R.Y.R, and A.U.A Conceptualization, R.J.A contributed to the animal study, D.D.P analyzed data, A.L.G draft preparation, D.D.S writing review and editing.

Competing Interest

The authors declare that there are no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

All trials were examined and approved by the Faculty of Veterinary Medicine at Airlangga University's Ethical Approval Committee assessed this work, and it was given ethical approval under No. 1.KEH.081.06.2023.

Data Availability

The article includes data that was used to support the study's conclusions.

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