# The Effect of Vitamin C Administration on Hemoglobin and Hematocrit of Albino Rats (*Rattus norvegicus*)

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**ABSTRACT**

Anemia, a condition characterized by low hemoglobin or red blood cell levels, is a widespread nutritional issue affecting over 2 billion people globally. Iron deficiency, folic acid, and vitamin B12 or C deficiencies are common causes, with vitamin C playing a crucial role in enhancing iron absorption. This study aimed to investigate the impact of various doses of vitamin C supplementation on hematocrit and hemoglobin levels in rats. The experiment employed a True Experimental design with a control group and three treatment groups receiving different doses of vitamin C (1 mg/ml, 3 mg/ml, and 6 mg/ml) over three weeks. Results showed no significant increase in hemoglobin or hematocrit levels across treatment groups compared to the control. Although some variations were observed in the data, particularly with the 6 mg/ml dose showing a slight decrease in hemoglobin levels, the overall effect of vitamin C on these hematological parameters was not substantial. These findings suggest that animal health, feed quality, and vitamin C administration duration influence the outcomes.
**Keywords:** vitamin C, hemoglobin, hematocrit

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## INTRODUCTION

Anemia is when the blood's number of red blood cells or hemoglobin (Hb) levels are below normal. Anemia can be influenced by several factors, namely severe bleeding, iron deficiency, folic acid, vitamins B12 and C, and several diseases such as malaria, worm infections, leukemia, and chronic diseases. Most anemia is related to the digestive system due to inadequate nutritional intake, malabsorption, or chronic bleeding. Anemia in the form of nutritional deficiencies can be anemia iron deficiency, anemia folic acid deficiency in pregnant women, and anemia vitamin B12 deficiency (Muhayati and Ratnawati (2019).

Anemia is one of the world's nutritional problems. According to the World Health Organization (WHO) report, more than 30% of the world's population, or around 2 billion people, have anemia. The prevalence of anemia in Indonesia reaches 21.7%. This figure shows that 26.4% of children aged 5-14 years have anemia, while 57% aged 15-24 experience similar conditions (Ministry of Health of the Republic of Indonesia, 2014).

Iron supplementation or iron tablets (TTD) can treat or prevent anemia. Iron is a vital microelement that plays a role in blood formation through hemoglobin (Hb) synthesis. Hemoglobin binds and delivers oxygen from the blood throughout the body (Indonesian Ministry of Health, 2001). However, TTD consumption often causes unwanted side effects for users, such as nausea, vomiting, stomach cramps, heartburn, and constipation.

Meanwhile, Vitamin C is an essential nutrient that is important for the formation of red blood cells. It can increase the absorption of iron needed to prevent anemia (Kaimudin *et al.*, 2017). Vitamin C inhibits the formation of hemosiderin, a compound that is difficult to mobilize to release iron when needed. Vitamin C deficiency can cause anemia, dry skin, internal eye bleeding, gingivitis, a decreased immune system, delayed wound healing, muscle pain, and easy bruising (Krisnanda, 2020).

The vitamin C content in food creates an acidic environment that facilitates the reduction of ferric iron to ferrous, which is more easily absorbed by the small intestine. Absorption of non-heme iron increases up to fourfold with the help of vitamin C, which converts ferric to ferrous in the small intestine, making it more easily absorbed. Thus, vitamin C increases iron absorption and inhibits the formation of hemosiderin, which is difficult to mobilize to release iron when needed (Yunita and Maigoda, 2023).

## METHODS

This study used the Posttest-only Control Group Design in the form of True Experimental research. Sampling was carried out after the treatment. The control and treatment groups were given feed without additional Vitamin C IPI, and the treatment group was given feed and Vitamin C IPI orally. A conclusion was drawn from the differences that occurred between the groups.

The study was conducted for three weeks, starting with the dissolving of vitamin C supplements, the maintenance and treatment period, and data analysis. The process of making solutions and administering vitamin C supplements using a sonde was carried out at the Experimental Animal Laboratory, Faculty of Veterinary Medicine, Airlangga University, which began in November 2024. Sample testing was conducted at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University, Surabaya.

This study used Simple Random Sampling, where samples were taken randomly. The observed parameters were hemoglobin (Hb) and hematocrit levels in the blood of rats *(Rattus norvegicus)*.

The animal model in this study used eight Wistar strain rats *(Rattus norvegicus)* aged 75-90 days and weighing 200-250 grams. The experimental animals were acclimatized for seven days to adjust to laboratory conditions by providing standard feed and drinking ad libitum. The cage base was given wood husks to maintain the humidity and cleanliness of the cage. The cage was placed away from noise and pollutants with adequate air ventilation. Eight rats were divided into four groups: one control group and three treatment groups. The research design was assigned into four treatment groups i.e., (P1) negative control, (P2) 1 mg/ml vitamin C, (P3) 3 mg/ml vitamin C, and (P4) 6 mg/ml vitamin C.

After one week, the animals adapt to the cage environment, and the experimental animals receive treatment. The experimental animals are given vitamin C supplements dissolved in distilled water every day, with each treatment group receiving different doses, namely graduated doses of 1 mg/ml, 3 mg/ml, and 6 mg/ml. On the seventh day, blood samples will be taken to determine their blood's hemoglobin (Hb) and hematocrit levels.

Blood sampling is carried out using the cervical dislocation method, and then blood is taken through the heart. Cervical dislocation is carried out with the side of the mouse's stomach facing down and the mouse's head away from the tester's body. The base of the tail is

held with the left index finger and thumb. The other left finger is placed on the back to immobilize the mouse. Then, the thumb and index finger of the right hand are placed tightly on the base of the skull. To cause dislocation, this stage can be done by pushing forward and down so that the mouse's body can be stable. This action must be done quickly and firmly to cause separation of the spine from the skull so that immediate death occurs. The skin will still look intact, but there must be a clear visual and palpable separation of the skull from the spine and cervical tissue. The death of the mouse occurs when there is no longer a nervous response from the mouse, but the heart is still beating because blood will be taken.

Blood is taken from the heart using a 1 ml syringe and needle. The blood sample must be placed in an Eppendorf tube through the tube wall so that it does not lyse. Hematocrit and hemoglobin examinations are carried out using a hematology analyzer, and blood samples collected. Each tube is marked to facilitate recording and analyzing the examination results.

## RESULT AND DISCUSSION

### Hematocrit

The table above shows that the hematocrit level of the control group (P1) was 45.6%. Meanwhile, the treatment groups had hematocrit levels of 39.3%, 45.2%, and 36.65%, respectively (Table 1).

These data indicate that most of the hematocrit levels in the treatment group were still within the normal range. However, hematocrit levels decreased in certain treatment groups, indicating the effect of treatment factors on the rat's physiology.

The results of this study explain that the control group (P1), which was not given vitamin C treatment, had a hematocrit level of 45.6%, reflecting normal and stable conditions without any physiological changes due to treatment. Treatment group P2, given vitamin C at a dose of 1 mg/ml, showed a hematocrit level of 39.3%. Although there was a slight decrease compared to the control group, these results were still within normal limits, indicating that giving low doses of vitamin C did not have a negative effect on the hematocrit levels of albino rats. Furthermore, treatment group P3, which received a dose of vitamin C of 3 mg/ml, recorded a hematocrit level of 45.2%, even slightly higher than the control group. This suggests that moderate doses of vitamin C can play a role in maintaining or slightly increasing hematocrit levels, which may be related to the positive effects of vitamin C in supporting the body's physiological functions. However, different results were found in treatment group P4, which received the highest dose of vitamin C of 6 mg/ml. In this group, hematocrit levels were recorded at 36.65%, indicating the most significant decrease compared to the other groups. This decrease, although still within the normal range, is notable compared to the control group and other treatment groups.

Studies show that stress can affect hematocrit levels, either increasing as an adaptive response of the body to increase oxygen transport capacity or decreasing due to effects such as hemolysis or hemodilution (Pritchard *et al.*, 2020). A decrease in hematocrit levels can also be associated with blood thinning due to increased plasma volume or decreased erythrocytes (Zuhrawati *et al.*, 2010).

Hematocrit levels can be influenced by various physiological factors, including stress and nutritional supplementation, but the response can vary depending on the subject's initial

condition and the research method used.

**Hemoglobin**

The table shows that the hemoglobin level of the control group (P1) was 13.9 g/dL, while the treatment groups had hemoglobin levels of 12.5 g/dL, 14 g/dL, and 11.85 g/dL, respectively (Table 1).

Data on the effect of vitamin C administration on albino rats (*Rattus norvegicus*) shows that hemoglobin levels in each treatment group are generally still within the normal range. The results of this study explain that the control group (P1), which was not given vitamin C treatment, had a hemoglobin level of 13.9 g/dL, which reflects normal and stable conditions without any physiological changes due to treatment. Treatment group P2, given vitamin C at a 1 mg/ml dose, showed a hemoglobin level of 12.5 g/dL. Although there was a slight decrease compared to the control group, this result was still within normal limits, indicating that giving low doses of vitamin C did not have a negative effect on the hemoglobin levels of albino rats. Furthermore, treatment group P3, which received a dose of vitamin C of 3 mg/ml, recorded a hemoglobin level of 14 g/dL, even slightly higher than the control group. This suggests that moderate doses of vitamin C can play a role in maintaining or slightly increasing hemoglobin levels, which may be related to the positive effects of vitamin C in supporting the body's physiological functions. However, different results were found in treatment group P4, which received the highest dose of vitamin C of 6 mg/ml. In this group, hemoglobin levels were recorded at 11.85 g/dL, which showed the most significant decrease compared to the other groups. This decrease, although still within the normal range, was prominent compared to the control group and other treatment groups.

**Table 1.** Results of examination and calculation of hematocrit and hemoglobin levels in albino rats (*Rattus norvegicus*)

| Treatment | Hematocrit | Hemoglobin |
|---|---|---|
| P1 | 45,6 % | 13,9 g/dL |
| P2 | 39,3 % | 12,5 g/dL |
| P3 | 45,2 % | 14 g/dL |
| P4 | 36,65 % | 11,85 g/dL |

A previous study conducted by Innggarsih *et al.* (2022) stated that the environment where rats are kept, including temperature, humidity, and population density, can affect general health and hemoglobin levels. Environmental stress can contribute to decreased hemoglobin levels. Various physiological factors, including stress and nutritional supplementation, can influence hemoglobin levels. However, the response can vary depending on the initial conditions of the animals and the research methods used.

These data show that the control group (P1) is still within the normal range, the treatment group P2 is still within the normal range, the treatment group P3 is still within the normal range, and the treatment group P4 is still

within the normal range. However, hemoglobin levels decreased in certain treatment groups, namely P4. This indicates that treatment factors influence the physiology of rats.

## CONCLUSION

In the treatment group given vitamin C, there was no significant increase in hemoglobin and hematocrit levels compared to the control group. These results indicate that giving vitamin C in specific doses does not significantly affect these hematological parameters. Factors such as the rat's health status, the feed quality, and the duration of vitamin C administration may cause the absence of significant differences.

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## Author Contribution

All authors participated to all aspects of this work, including preparation, research, data collecting and analysis, manuscript drafting, and publication approval.

## Competing Interest

None.

## Ethical Approval

The present study was approved by the Ethics Committee of Faculty of Veterinary Medicine, Airlangga University.

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