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THE EFFECT OF ANCHOVY FLOUR (STOLEPHORUS SP.) AND CASSAVA LEAF FLOUR (MANIHOT ESCULENTA) SUPPLEMENTATION ON HEMOGLOBIN LEVELS IN ANEMIC MICE (MUS MUSCULUS)

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ABSTRACT

Anemia is a condition characterized by reduced hemoglobin levels in the blood and may be associated with inadequate intake of nutrients required for hemoglobin synthesis. Anchovy flour and cassava leaf flour contain nutrients, particularly protein and iron, that contribute to hemoglobin formation. This study aimed to determine the effect of a combination of anchovy flour (Stolephorus sp.) and cassava leaf flour (Manihot esculenta) on hemoglobin levels in anemic mice (Mus musculus). An experimental study with a pretest–posttest design was conducted using 30 anemic mice divided into a positive control group, a negative control group, and three treatment groups, with six mice in each group. P1 received 25% anchovy flour and 75% cassava leaf flour, P2 received 50% of each flour, and P3 received 75% anchovy flour and 25% cassava leaf flour. The intervention was provided for 14 days. Hemoglobin levels were measured before and after the intervention and analyzed using the paired t-test. Mean hemoglobin levels in P1, P2, and P3 increased from 6.3, 6.4, and 6.4 g/dL to 8.4, 9.6, and 11.3 g/dL, respectively. The mean increases were 2.1, 3.2, and 4.9 g/dL, respectively. The paired t-test showed a significant increase in hemoglobin levels in all treatment groups ($p < 0.05$). The highest post-intervention mean was observed in P3. In conclusion, the provision of anchovy flour and cassava leaf flour combinations significantly increased hemoglobin levels in anemic mice.

Keywords: *Anchovy flour, anemia, cassava leaf flour, hemoglobin, mice.*

INTRODUCTION

Anemia is the presence of fewer red blood cells in the blood stream or a decrease in the ability of the blood cells to carry oxygen to body tissues. Anemia is still a major health problem in the world (Gallagher, 2022). According to the WHO, anemia was prevalent among 39.8% of children aged between 6-59 months and 30.7% of women aged 15-49 years in 2019 and 2023 respectively, where 35.5% were pregnant women while 30.5% were non-pregnant women (World Health Organization, 2024). Among the population of Indonesia, the 2023 SKI revealed anemia prevalence rates of 27.7% in pregnant women, 23.8% in children aged 0-4 years and 16.3% in children aged 5-14 years, 15.5% among adolescents aged 15–24 years, 18.0% among non-pregnant women of reproductive age, and 14.4% among adult men (Kemenkes RI, 2023). In Southeast Sulawesi, the 2024 Health Profile reported anemia prevalence rates of 9.1% among pregnant women, 8.79% among prospective brides, and 47.53% among adolescent girls. In Kendari City, anemia prevalence was 13.7% among pregnant women and 5.39% among prospective brides (Dinkes Sultra, 2024).

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Protein and iron are key nutrients involved in hemoglobin synthesis and red blood cell production. Iron acts as an essential component of heme, which forms part of the hemoglobin molecule, while protein contributes to the formation of globin through its amino acid components. Proteins have an important role in the transport of iron throughout the body because transferrin is responsible for carrying it to the bone marrow where red blood cells are formed. Therefore, sufficient protein intake is important not only for globin formation but also for the proper utilization and transport of iron during erythropoiesis. Adequate iron availability is likewise necessary for hemoglobin formation, as iron deficiency can interfere with heme formation and subsequently reduce hemoglobin levels. When protein and iron intake is insufficient, iron transport to the bone marrow and hemoglobin formation may be impaired, which can disrupt red blood cell production and contribute to anemia. Thus, adequate availability of protein and iron supports the formation of hemoglobin and the production of red blood cells (Luhulima et al., 2024).

Anchovy (Stolephorus sp.) is an animal-based protein source with a complete amino acid profile that can be readily utilized by the body, thereby contributing to adequate protein intake and supporting physiological functions (Rexi et al., 2025). Protein provides amino acids required for globin synthesis, while iron is an essential component of the heme group in hemoglobin. In addition, cassava leaves, as a plant-based food source, contain non-heme iron and vitamin C (Alfaruqi et al., 2021). Vitamin C enhances the absorption of non-heme iron, thereby increasing iron availability for hemoglobin formation. The combination of anchovy flour and cassava leaf flour provides protein, heme and non-heme iron, and vitamin C, which are nutrients involved in hemoglobin synthesis and iron utilization. The contribution of these nutrients may support hemoglobin formation through the availability of iron for heme synthesis and amino acids for globin synthesis. Anchovy flour increased the hemoglobin concentration in anemic adolescent females as found by Thalib et al. (2021). Similarly, Arif et al. (2023) observed that cassava leaf extract was effective in increasing the hemoglobin concentration in addition to hematocrit levels in mice. In the same vein, Putriwati et al. (2024) found out that consumption of cassava leaves and iron tablets increased the hemoglobin concentration in pregnant women suffering from anemia. Therefore, anchovies and cassava leaves can be considered as food sources of such nutrients.

Although previous studies have demonstrated the potential of anchovy and cassava leaf-based interventions to improve hemoglobin levels, the available evidence remains limited to separate applications of these food sources or their use with other interventions. These studies have not evaluated anchovy flour and cassava leaf flour together as a single intervention in anemic mice. Furthermore, previous research has not compared different proportions of the two ingredients to examine whether variations in formulation are associated with differences in hemoglobin response. This gap indicates the need for further investigation of the combined formulation using different proportions in anemic mice.

The novelty of this study lies in evaluating the combined use of anchovy fish flour (Stolephorus sp.) and cassava leaf flour (Manihot esculenta) at three different proportions

(25:75, 50:50, and 75:25) in anemic mice. Unlike previous studies that primarily investigated these ingredients separately, the present study evaluates their combined use as a single intervention and compares different formulation proportions. This approach provides additional evidence regarding the role of formulation proportion in the hemoglobin response and identifies the proportion associated with the greatest increase in hemoglobin levels. In the present study, the P3 formulation, containing 75% anchovy fish flour and 25% cassava leaf flour, produced the greatest increase in hemoglobin levels among the treatment groups. These findings provide additional evidence on the potential of combining locally available animal- and plant-based food sources at different proportions to support hemoglobin improvement in anemic mice.

RESEARCH METHOD

This research employed an experiment using laboratory setup, specifically the pretest post-test design to investigate the effects of a mixture of anchovy flour (Stolephorus sp.) and cassava leaf flour (Manihot esculenta) on the levels of hemoglobin of anemic mice (Mus musculus). The pretest-posttest design was selected so that the levels of hemoglobin can be measured from the same experimental subjects before and after the treatment. This design also allowed the researchers to assess changes in hemoglobin levels following the induction of anemia and subsequent administration of the intervention. The comparative analysis of the levels of hemoglobin pre-intervention and post-intervention helped assess the effects of the combination of anchovy flour and cassava leaf flour on hemoglobin. The study was conducted at the Laboratory of the Faculty of Medicine, Halu Oleo University, from May 14 to June 7. The study involved thirty female BALB/c mice which were then separated into five groups, each with six members: KP and KN being positive and negative controls, respectively, and P1, P2, and P3 being the treatment groups. The KP and KN groups were fed with normal food alone while P1, P2, and P3 were fed with anchovy flour and cassava leaf flour at different ratios.

Ethical approval for this study was obtained from the Research Ethics Commission of the Institute for Research and Community Service (LPPM), Universitas Halu Oleo, under approval number 642/UN29.20.1.2/PG/2026. The research procedures began with acclimatization of the mice, followed by measurement of their initial hemoglobin levels (pretest). The mice were first induced with anemia using sodium nitrite at a dose of 0.1 mL/mice/day for three consecutive days, after which hemoglobin levels were measured to confirm the anemic condition. Following anemia induction, the positive control group (KP) received standard pellet feed at 3–5 g/mice/day and drinking water at 4–8 mL/mice/day throughout the 14-day intervention period, without receiving any additional treatment. The negative control group (KN) received the same sodium nitrite induction at a dose of 0.1 mL/mice/day for three consecutive days and subsequently received only standard pellet feed at 3–5 g/mice/day and drinking water at 4–8 mL/mice/day for the remainder of the 14-day intervention period, without receiving the anchovy flour and

cassava leaf flour intervention. The treatment groups received the respective combinations of anchovy flour and cassava leaf flour orally once daily for 14 consecutive days. Group P1 received a dose of 24.4 mg/20 g body weight, comprising 25% anchovy flour and 75% cassava leaf flour; Group P2 received 32.7 mg/20 g body weight, comprising 50% anchovy flour and 50% cassava leaf flour; and Group P3 received 40.9 mg/20 g body weight, comprising 75% anchovy flour and 25% cassava leaf flour. The intervention volume was determined based on the body weight range of the experimental mice and the maximum administration volume considered acceptable for mice. The minimum and maximum body weights of the mice were used in the calculation to establish an appropriate administration volume, resulting in a volume of 0.7 mL/mice/day. Each formula was administered orally in this volume daily for 14 days. After completion of the intervention, the hemoglobin level was taken again using an EasyTouch GCHb instrument. Before measurement, the instrument and test strips were prepared according to the manufacturer's instructions to ensure proper measurement procedures.

Initially, the normality of the data was assessed using the Shapiro-Wilk test. Afterwards, the paired t-test was used to assess the differences in the levels of hemoglobin before and after the intervention in both groups at $p < 0.05$ level.

RESULTS AND DISCUSSION

The mean hemoglobin levels before and after the intervention in the positive control, negative control, and treatment groups are presented below.

Table 1. Mean Hemoglobin Levels Before and After Treatment in Anemic Mice

Group	Pre-Test (g/dL)	Post-Test (g/dL)	Mean Difference (g/dL)	p-value
P1	6.3	8.4	2.1	0,000
P2	6.4	9.6	3.2	0,000
P3	6.4	11.3	4.9	0,000

Table 1 shows that the mean hemoglobin levels increased from 6.3 to 8.4 g/dL in the P1 group, from 6.4 to 9.6 g/dL in the P2 group, and from 6.4 to 11.3 g/dL in the P3 group after 14 days of intervention with a combination of anchovy flour and cassava leaf flour. The paired t-test results showed a significant increase in hemoglobin levels in all treatment groups ($p < 0.05$), with mean differences of 2.1 g/dL in P1, 3.2 g/dL in P2, and 4.9 g/dL in P3. Descriptively, the P3 group showed the greatest mean increase in hemoglobin level among the treatment groups. However, the paired t-test only evaluated the pre–post changes within each group and did not assess statistical differences in hemoglobin increases among P1, P2, and P3. Therefore, the greater mean increase observed in P3 is interpreted descriptively and does not indicate a statistically significant difference in treatment effects among the three proportions.

The research results showed that giving a combination of anchovy fish flour *(Stolephorus sp.)* and cassava leaf flour *(Manihot esculenta)* influenced the increase in

hemoglobin levels in anemic mice. This finding addresses the research goal that the combination of these two local food ingredients can boost hemoglobin levels after a 14-day intervention. The increase in hemoglobin levels across all treatment groups indicates that the combination of anchovy fish flour and cassava leaf flour supports the hemoglobin formation process, although the level of increase varies with each proportion. The P3 group, which received 75% rice anchovy fish flour and 25% cassava leaf flour, showed the greatest increase in hemoglobin levels among the treatment groups. This finding is consistent with the higher proportion of anchovy flour in the P3 formulation, which provides a greater contribution of animal protein and heme iron. Heme iron has high bioavailability, making it more readily absorbed and utilized by the body than non-heme iron. In addition, animal protein contributes to iron transport and utilization during erythropoiesis, supporting the process of hemoglobin synthesis. The protein and iron provided by animal food sources contribute to iron status and hemoglobin formation, which can support an increase in hemoglobin levels (Piskin et al., 2022). These nutritional characteristics provide a biological basis for the greater increase in hemoglobin observed in the P3 group.

The increase in hemoglobin levels observed in the treatment groups may be related to the complementary roles of protein and iron provided by the combination of anchovy flour and cassava leaf flour. Protein supports globin synthesis, while iron is required for heme formation, and both are essential components of hemoglobin. Protein is a macronutrient used in the development of body tissue and red blood cells, particularly hemoglobin. The quality of protein can be described in terms of its essential amino acid composition and digestibility. The biological value of animal protein is higher compared to plant protein since its essential amino acid composition matches human needs more closely (House et al., 2024). In addition, protein provides the amino acids required for globin formation as a component of hemoglobin. Adequate protein availability supports hemoglobin and red blood cell formation, making protein an important nutrient in the process of erythropoiesis (Nurrahman et al., 2026). Furthermore, iron is required for erythropoiesis and hemoglobin formation. Insufficient iron availability can disrupt erythropoiesis and result in inadequate hemoglobin production (Furkaniaty et al., 2023). The vitamin C content of cassava leaves may also support the absorption of non-heme iron, thereby contributing to iron availability for hemoglobin synthesis (Putriwati et al., 2024). The combination of these nutrients may therefore support erythropoiesis and hemoglobin formation, which may explain the increase in hemoglobin levels observed after the intervention.

The protein content in anchovy is 59.32% which consists of eight essential amino acids, one semi-essential amino acid, and six non-essential amino acids. The primary essential amino acids in anchovy include leucine and lysine. Glutamate and aspartate are two abundant non-essential amino acids. These amino acids play a role in protein synthesis, including hemoglobin formation, and support tissue growth and body metabolism. Protein also contributes to iron transport to the bone marrow, facilitates iron utilization, and supports erythropoiesis, thereby contributing to hemoglobin formation.

Besides being high in protein, anchovy is also a good source of iron, with iron content around 1.0–3.0 mg (Tohata et al., 2021). Proteins that contribute to hemoglobin formation are not only obtained from animal food sources but can also be obtained from plant-based foods. Cassava leaves are one such plant protein source with considerable nutritional value and are commonly consumed as food. Every 100 grams of cassava leaves contain about 6.3 g of protein and 2.0 mg of iron (Ayele et al., 2021).

The protein component is present in anchovy at 59.32%, which is made up of eight essential, one semi-essential, and six non-essential amino acids. The dominant essential amino acid is leucine and lysine, while the dominant non-essential amino acids are glutamate and aspartate. These amino acids play a role in protein synthesis, including hemoglobin formation, and support tissue growth and body metabolism. Besides being high in protein, anchovy is also a good source of iron, with iron content around 1.0–3.0 mg (Tohata et al., 2021). Proteins that help in forming hemoglobin don't only come from animal foods; they can also be obtained from plant-based sources. Cassava leaves are one of those plant proteins with pretty high nutritional value and are often used as food. Every 100 grams of cassava leaves contain about 6.3 g of protein and 2.0 mg of iron (Ayele et al., 2021).

The findings herein are consistent with the findings from Thalib et al. (2021) who revealed that consumption of products made using anchovy flour increased hemoglobin in anemic adolescents. Similarly, Arif et al. (2023) established that hemoglobin and hematocrit were improved by the use of cassava leaf extract in anemic mice while Umanilo and Linda (2025) observed increased hemoglobin in anemic pregnant women due to intake of cassava leaves with iron supplements. These findings support the present study by demonstrating that interventions involving anchovy or cassava leaf products are associated with increased hemoglobin levels. However, differences in the type of intervention, study subjects, food form, dose, and intervention duration may contribute to variations in the magnitude of hemoglobin changes among studies. Thalib et al., (2021) used an anchovy flour-based product in adolescent girls, whereas Arif et al., (2023) used cassava leaf extract in mice, and Umanilo and Linda (2025) combined cassava leaves with iron tablets in pregnant women. In contrast, the present study evaluated a combination of anchovy flour and cassava leaf flour as a single intervention in anemic mice using three different proportions. All treatment groups showed increases in hemoglobin levels, with the P3 group showing the highest observed increase. This difference in the magnitude of increase may be related to the varying proportions of the two food ingredients and the resulting nutrient contribution.

The novelty of the present study lies in evaluating anchovy fish flour (Stolephorus sp.) and cassava leaf flour (Manihot esculenta) as a combined intervention at three different proportions in anemic mice. Unlike previous studies that examined anchovy- or cassava leaf-based interventions separately or in combination with other interventions, the present study assessed the two locally available food ingredients together at proportions of 25:75, 50:50, and 75:25 over a 14-day intervention period. This approach provides additional evidence on the use of locally available animal- and plant-based food

ingredients in combination and on the variation in hemoglobin responses across different formulation proportions. The findings therefore extend previous evidence by examining a combined food-based approach to hemoglobin improvement in anemic mice.

These findings have broader implications for the development of food-based nutrition interventions for anemia prevention and management. Anchovy is a locally available food ingredient that has potential as a source of protein and iron and can be utilized as a functional food ingredient in nutrition interventions (Putalan et al., 2021). Cassava leaves are also locally available leafy vegetables that may contribute to improving hemoglobin levels as part of dietary interventions (Handayani & Sugiarsih, 2021).The combination of anchovy rice flour and cassava leaf flour provides an opportunity to utilize these locally available food ingredients as complementary sources of nutrients in food-based nutrition interventions, particularly in settings where access to diverse and nutrient-rich foods may be limited. At the national level, the utilization of locally available food ingredients could support nutrition programs that promote dietary diversification and the use of local food resources as part of efforts to address anemia. At the global level, these findings contribute to the broader evidence supporting locally adapted, food-based approaches to addressing nutritional deficiencies and anemia, particularly in settings where locally available food resources can be incorporated into nutrition interventions. Further research involving human subjects is needed to evaluate the acceptability, nutritional adequacy, safety, and effectiveness of food products developed from this combination before their implementation in broader anemia prevention programs.

CONCLUSION

Based on the research results, it can be concluded that giving a combination of anchovy fish meal (Stolephorus sp.) and cassava leaf meal (Manihot esculenta) affects hemoglobin levels in anemic mice (Mus musculus). All treatment groups showed a significant increase in hemoglobin levels after 14 days of intervention ($p<0.05$), with the highest increase in the P3 group that received a combination of 75% anchovy fish meal and 25% cassava leaf meal. This study shows that the combination of these two local food ingredients has the potential to help increase hemoglobin levels and could become a promising alternative functional food for preventing and addressing anemia.

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