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Effect of Soybean Flour Fortification (Glycine Max) and the Administration of Vitamin C on the Increase in Hemoglobin (Hb) Levels among Adolescents with Anemia at Gresik District

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A B S T R A C T

Adolescents are included in a group that is vulnerable to anemia, as they are in a rapid growth period with high nutritional needs, especially for iron. Unbalanced eating patterns, lack of iron intake, extreme diet habits, and menstruation among adolescent girls may increase the risk of anemia. In Gresik Regency, from January 2024 to January 2025, data on female adolescents in grades 7 and 10 who were identified as having anemia included 3,324 female adolescents (50.2%). Anemia in adolescents not only affects physical health, such as fatigue, dizziness, and decreased endurance, but can also impact concentration, learning achievement, as well as cognitive and emotional development. This research aims to determine whether there is an Effect of Soybean Flour Fortification (Glycine Max) and the Administration of Vitamin C on the Increase in Hemoglobin (Hb). This was a quantitative quasi-experimental study with a one-group pretest-posttest design. The study population involved 78 students with anemia. The purposive sampling technique was applied to obtain 65 respondents as the study sample, based on the Slovin formula. The measuring device used here was a digital hemoglobinometer (portable). A Statistical test was applied using an independent t-test. The study results obtained a P-value of <0.05, which indicated a significant effect of interventions on hemoglobin levels. It can be concluded that there was an effect of soybean flour fortification (Glycine max) and the administration of vitamin C on the increase in hemoglobin (Hb) levels.

Introduction

Anemia is one of the common health problems experienced by adolescents, particularly in developing countries. Such a condition occurs when the body lacks red blood cells or sufficient hemoglobin levels to transport oxygen throughout the body (Samson, Fischer, & Roche, 2022). Adolescents are part of a group that is vulnerable to anemia, as they are in a rapid growth period with high nutritional needs, especially for iron (Bansal *et al.*, 2020).

Factors such as an unbalanced diet, lack of iron intake, extreme diet habits, and menstruation among adolescent girls may increase the risk of anemia. According to data derived from the World Health Organization (WHO), around 25% of adolescents in the world experienced anemia, and this number can even be higher in some regions with high levels of malnutrition. Anemia in adolescents not only affects physical health, such as fatigue, dizziness, and decreased endurance, but can also impact concentration, learning achievement, as well as cognitive and emotional development (Samson, Fischer, & Roche, 2022).

Iron deficiency anemia is a significant public health issue worldwide. Globally, the prevalence of anemia in 2019 was 29.9% (95% uncertainty interval (UI) 27.0%, 32.8%) among women of reproductive age, equivalent to more than half a billion women aged 15-49 years. The prevalence was 29.6% (95% UI 26.6%, 32.5%) among non-pregnant women, and 36.5% (95% UI 34.0%, 39.1%) among pregnant women. Asia was ranked second among continents with the highest percentage of anemia cases, at 39.4%, after Africa (WHO, 2021).

In 2019, the prevalence of anemia among adolescents of productive age in Indonesia was 31.2%. Furthermore, in 2023 and 2024, the prevalence of anemia among women aged 15-24 years remained constant at 32% (Moh RI, 2024). In Gresik District in 2023, 95% of CHC had many cases of anemia among adolescents. In the work area of Bungah Community Health Center (CHC), there were 55.27% cases of anemia. Such findings should be considered, as they relate to adolescent reproductive health (MoH RI, 2022).

Based on a preliminary study conducted at a high school in the work area of Bungah CHC, all 10 adolescents with anemia (100%) were unaware of the importance of balanced nutrition during their productive years. The main cause of anemia, especially iron deficiency anemia, is an unbalanced diet. Such a condition is experienced by many adolescents who did not fully understand the importance of maintaining a healthy body from an early age. A lack of knowledge regarding the appropriate nutritional intake led to their ignorance of the body's essential iron requirements. Adolescence is a crucial stage in growth and development, so special consideration regarding eating patterns is essential to prevent various health problems, including anemia.

Soybeans are rich in iron, protein, vitamin B complex (especially B12 and folate), as well as antioxidants. Soybean flour fortification (glycine max) can play a role in increasing hemoglobin (Hb) levels through several physiological mechanisms, mainly due to its nutritional content that supports the hematopoiesis process (red blood cell formation) (Buckner *et al.*, 2016). Non-heme iron is contained in soybeans. Iron is essential for the synthesis of hemoglobin, which is a component of red blood cells that carry oxygen to the body's tissues and organs. Iron is absorbed in the small intestine (duodenum) and transported to the bone marrow to help the formation of erythrocytes (red blood cells) (Alamu, Popoola, and Maziya-Dixon, 2018). Protein in the fortification of soybeans (Glycine max), especially essential amino acids such as lysine, plays a role in the formation of hemoglobin structures and red blood cells. It also supports tissue regeneration and the production of important enzymes in iron metabolism. Folate (vitamin B9) in soybeans is important for erythrocyte maturation in the bone marrow. A lack of folate and B12 may cause megaloblastic anemia, so that sufficient intake will support an increase in Hb levels (Alamu *et al.*, 2017). Isoflavones in soybeans are antioxidants, which protect red blood cells from damage caused by free radicals. Consumption of biscuits made with soybean flour should be balanced with the consumption of vitamin C to facilitate iron absorption (De Freitas Ortega *et al.*, 2025).

Soybean flour, as the study intervention, is considered a local ingredient rich in vegetable iron (non-heme iron), protein, and isoflavones. Additionally, it serves as an alternative supplement that is more economical and easily accessible, particularly in areas with a high prevalence of anemia (Lane &

Richardson, 2014). Vitamin C is scientifically proven to increase the absorption of non-heme iron. Such a combination can be more effective than a single intervention (for example, giving iron without any additional components) (Rathee *et al.*, 2025).

Such a finding is in line with a pseudo-experimental study conducted at State High School 19 of Palembang, which showed that the provision of spritz cookies containing chicken liver and soybean flour could increase Hb levels among adolescent girls with anemia. The mean increase in Hb levels in the treatment group was 3.503 g/dL, while the comparative group showed only a 0.004 g/dL increase ($p < 0.05$).

A study using the One-Group Pre-Test-Post-Test experimental design, conducted in Selorejo Village, Lamongan, revealed that administering soybeans could increase hemoglobin (Hb) levels among adolescent girls. The mean Hb levels before and after the administration of soybeans were 11.27 g/dL and 11.59 g/dl, respectively ($p = 0.00$).

This study is expected to make a scientific contribution in the field of nutrition and public health, particularly regarding alternative anemia control among adolescents using local food-based approaches and supplements.

Methods

This was a quantitative quasi-experimental study with a one-group pre-test and posttest design. The study population consisted of all adolescent girls at "X" high school in the work area of the Bungah Community Health Center of Gresik, comprising 78 individuals, who were selected from September 14 to December 17, 2024. The study samples were selected using a purposive sampling technique, covering adolescents with hemoglobin levels below normal limits or those who had been diagnosed with anemia by medical personnel (Notobroto, 2020). The sample size was determined using the Slovin formula, with a 5% margin of error, for a study population of 78 people, or approximately 65 respondents. Inclusion Criteria: 1) Adolescents aged 12–18 years living in Gresik Regency, 2) Diagnosed with anemia, 3) Willing to be research respondents, 4) Not consuming iron or vitamin C supplements, 5) No allergies. Exclusion Criteria: 1) Having a history of chronic diseases such as thalassemia, kidney failure, or other chronic infections that can affect hemoglobin levels, 2) Having allergies to soy or vitamin C, 3) Undergoing other medical treatments that can affect Hb levels, 4) Not pregnant.

The stages of fortification involved selecting and cleaning soybeans, ensuring they were free from dirt and harmful materials, drying the soybeans to remove their water content, and milling the resulting soybean flour. The composition of fortification biscuits, which applied the F3 Formula, consisted of 30% soybean flour, with a protein content of 10.71 g and an iron content of 9.07 mg (Hamad & Singh, 2025).

Dosage of soybean flour to increase Hb levels.

Several studies in Indonesia and abroad have observed the effective dose of soybean fortification in increasing Hb levels among the school-aged population, specifically 25-30 grams/day for 8 weeks of administration (Uro-chukwu, 2025). In this study, the maximum absorption of iron was

also supported by the administration of Vitamin C. Respondents were also educated not to consume the supplement with tea or coffee, as the tannin content could inhibit iron absorption. Hb levels were assessed using a digital hemoglobinometer (portable). It is essential to adjust the proportion of soybean flour to achieve fortification, aiming to increase both protein and iron content, while also considering the organoleptic acceptance by consumers. Additionally, fortification with iron microcapsules is believed to enhance the iron content in the final product. To implement the intervention in this study, SOP was also used to assess accuracy when providing the intervention.

Bivariate analysis was applied to analyze the correlation between two variables. A comparison between the results of fortification and non-fortification, as well as low Hb levels and normal Hb levels, was applied using the Chi-Square test. A bivariate test was applied to analyze the correlation between two paired t-test variables. This research was conducted in accordance with the ethics agreement No. 148-KEPK, dated September 14, 2024, at the University PGRI Adi Buana Surabaya.

Results

Table 1. Characteristics of the Study Respondents

Characteristics of Respondents	Frequency (n=65)	Percentage (%)
Gender		
Male	25	38.5%
Female	40	61.5%
Age (years)		
15	20	30.8%
16	30	46.2%
17	15	23.0%
Nutritional Status (BMI/Age)		
Thin	18	27.7%
Normal	40	61.5%
Fat	**7**	10.8%
Anemia Category		
Mild (Hb 10-10.9 g/dL)	20	30.7%
Moderate (Hb 8-9.9 g/dL)	25	38.6%
Severe (Hb <8g/dL)	20	30.7 %

Source: Primary Data 2024

Based on the table regarding the characteristics of respondents, it was found that the majority of respondents were female, with 40 respondents (61.5%). Furthermore, regarding age, it was found that most respondents with anemia were aged 16 years (46.2%). Regarding nutritional status, it was found that 40 respondents (61.55%) had anemia. In addition, based on the anemia category, most respondents experienced moderate anemia (Hb 8-9.9 g/dL), with 25 respondents (38.6%) affected.

Table 2. Distribution of Mean Hemoglobin Levels Before and After Intervention (n=65)

Time of Assessment	Mean (SD) Hb (g/dL)	Min - Max Hb	Anemia Category *	Percentage (%)
Before Intervention	10.2 (±0.6)	9.1 – 11.3	Mild Anemia	65 (100%)
After Intervention	11.5 (±0.7)	10.1 – 13.0	Normal	60 (92.3%)

Source: Primary Data 2024

Based on Table 2 regarding the distribution of mean hemoglobin levels before and after intervention, the mean hemoglobin level before intervention was 10.2 (± 0.6) g/dl. After the intervention for two weeks, it was found that the mean Hb level increased to 11.5 (± 0.7) g/dl.

Table 3. Changes in Anemia Status Before and After Intervention (n = 65)

Hemoglobin Level (Hb)	Pre-Test (%)	Posttest (%)	p-value
Normal (Hb 11.0 – 13.5 gram/dL)	0%	55 (84.9%)	**0.0001**
Mild (Hb 10-10,9 g/dL)	20 (30.7%)	5 (7.6%)	
Moderate (Hb 8-9,9 g/dL)	25 (38.6%)	3 (4.5%)	
Severe (Hb<8g/dL)	20 (30.7 %)	2 (3%)	

Source: *Independent t-test*

Based on Table 3, which outlines changes in anemia status before and after the intervention, it was found that before the intervention, most respondents experienced moderate anemia (Hb 8-9.9 g/dL), with 25 respondents (38.6%) affected. After the intervention, most respondents did not experience anemia or had normal Hb levels (Hb 11.0-13.5 grams/dL), with 55 respondents (84.9%) affected. The paired t-test, applied to assess the significant difference in hemoglobin levels before and after intervention within the same group, yielded a p-value of <0.05, indicating a statistically significant change in hemoglobin levels.

Table 4. Effect of Soybean Flour Fortification and Vitamin C on the Increase in Hemoglobin Levels

Time of Assessment	Mean (g/dL)	Deviation Standard (g/dL)	t-value	p-value
Pre-Test	10.4	1.2	10.56	0.0001
Posttest	12.3	1.1		

Based on Table 4, a p-value of <0.05 was obtained, indicating a significant effect of the interventions on hemoglobin levels.

Discussion

Based on data, the majority of respondents were female (61.5%). This is in line with various literature which states that adolescent girls are more susceptible to anemia than adolescent boys. Physiologically, adolescent girls experience regular menstruation every month, which can cause loss of iron and decreased hemoglobin levels if not balanced with adequate iron intake. According to the Ministry of Health of the Republic of Indonesia (2021), around 28% of adolescent girls in Indonesia experienced anemia, mainly due to loss of iron during the menstrual period and poor nutritional eating patterns. A study conducted by Sari et al. (2018) also supported this finding, which found that the prevalence of anemia was higher among adolescent girls due to their higher iron needs during puberty and menstruation (Muchtar et al., 2025).

Most respondents were aged 16 years (46.2%), which corresponds to a period of rapid growth, during which there is an increase in iron needs to support physical and cognitive development. At this time, inadequate nutritional intake may increase the risk of anemia. MOH RI (2018) explains that adolescent boys and girls aged 15-18 years have an iron need of about 15 mg/day and 26 mg/day, respectively. An unmet requirement may have a direct impact on blood hemoglobin levels (M. He *et al.*, 2024).

Most respondents with anemia had a normal nutritional status, with as many as 40 respondents (61.55%) reporting this. Such findings indicated that anemia did not only occur among adolescents with malnutrition, but could also be experienced by adolescents with normal nutritional status. Furthermore, anemia is not only influenced by body weight or height, but also by the quality of nutrient consumption, including micronutrients such as iron and vitamin C. A study conducted by Rahmawati et al. (2020)

found a significant relationship between nutritional status and Hb levels; however, anemia can also occur among adolescents with a normal BMI and low iron intake (Kirana & Laili, 2025).

The anemia status of respondents revealed that most respondents had moderate anemia (Hb 8-9.9 g/dL), with 25 respondents (38.6%) affected. This condition indicated that most respondents experienced anemia, which necessitated nutritional intervention. According to the WHO (2015), anemia should be treated immediately, as it may reduce concentration, endurance, and learning achievement in adolescents. The administration of food fortification, such as protein-rich soybean flour and non-heme iron, as well as vitamin C, which enhances iron absorption, is an effective approach to increase Hb levels (De Freitas Ortega *et al.*, 2025).

The distribution of mean hemoglobin levels showed an increase in mean hemoglobin levels among adolescents with anemia after a two-week intervention involving soybean flour fortification and vitamin C administration. The mean Hb content increased from 10.2 ± 0.6 g/dL to 11.5 ± 0.7 g/dL. Such an increase indicated a positive effect of the intervention on the hemoglobin status (Lane & Richardson, 2014).

The increase in Hb levels, both biologically and clinically, can be explained through several scientific mechanisms. Soybean flour is a good source of vegetable iron (non-heme) and contains high-quality protein that supports the formation of red blood cells (Belhaj et al., 2025). However, iron from vegetable sources has a low bioavailability compared to heme iron. Therefore, fortification with vitamin C becomes an important strategy because vitamin C functions as an enhancer of non-heme iron absorption in the body (T. He *et al.*, 2024).

Vitamin C acts by reducing ferrions (Fe^{3+}) into ferro ions (Fe^{2+}), which are more easily absorbed by the intestinal mucosa. Additionally, vitamin C can form a water-soluble complex with iron, which increases its solubility and absorption in the digestive tract (Hurrell & Egli, 2010). Thus, the combination of soybean flour, a source of iron, and vitamin C, an absorption enhancer, provides a synergistic effect in increasing hemoglobin levels (Maggiore & Sciveres, 2024).

The study's findings align with those from several previous studies. A study conducted by Sulistyowati et al. (2018) revealed that supplementing iron with vitamin C among adolescents with anemia resulted in a significant increase in hemoglobin levels within two weeks. Likewise, a study conducted by Fair (2024) found that local food interventions enriched with iron and combined with vitamin C could be an effective solution for managing anemia in adolescents (Pecher *et al.*, 2024).

It is essential to recognize that the adolescent age group is a population vulnerable to anemia due to high iron needs during the rapid growth phase, especially among adolescent girls who experience menstrual blood loss. Therefore, an intervention approach that utilizes local food sources, such as soybean flour, which is easily accessible and combined with vitamin C, is a very effective step in sustainable anemia control programs.

Overall, the results of this study support the hypothesis that soybean flour fortification and vitamin C supplementation can significantly increase hemoglobin levels in the short term. The

application of local food-based interventions that integrate the principles of scientific nutrition is highly recommended as part of adolescent health programs in various regions.

Conclusions

Soybean flour fortification (Glycine max) combined with vitamin C administration was shown to have a positive effect on increasing hemoglobin (Hb) levels in adolescents with anemia. The content of vegetable iron in soybeans, which can be increased in bioavailability by vitamin C, may help significantly improve hemoglobin status. These findings suggest that a local food-based nutritional intervention, combined with essential micronutrients, could be an effective and affordable strategy for managing anemia in adolescents.

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