Journal of Health and Nutrition Research

Vol. 4, No. 3, 2025, pg. 1418-1426, https://doi.org/10.56303/jhnresearch.v4i3.1008

Journal homepage: https://journalmpci.com/index.php/jhnr/index

e-ISSN: **2829-9760**

Iron, Enhancer, and Inhibitor Intake in Relation to Ferritin and Hemoglobin Levels Among Adolescent Girls in Pekalongan, Central Java

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ORIGINAL ARTICLES

Submitted: 27 October 2025

Accepted: 26 November 2025

Keywords:

Iron intake, Inhibitor, Ferritin levels, Hemoglobin levels, Adolescent girls

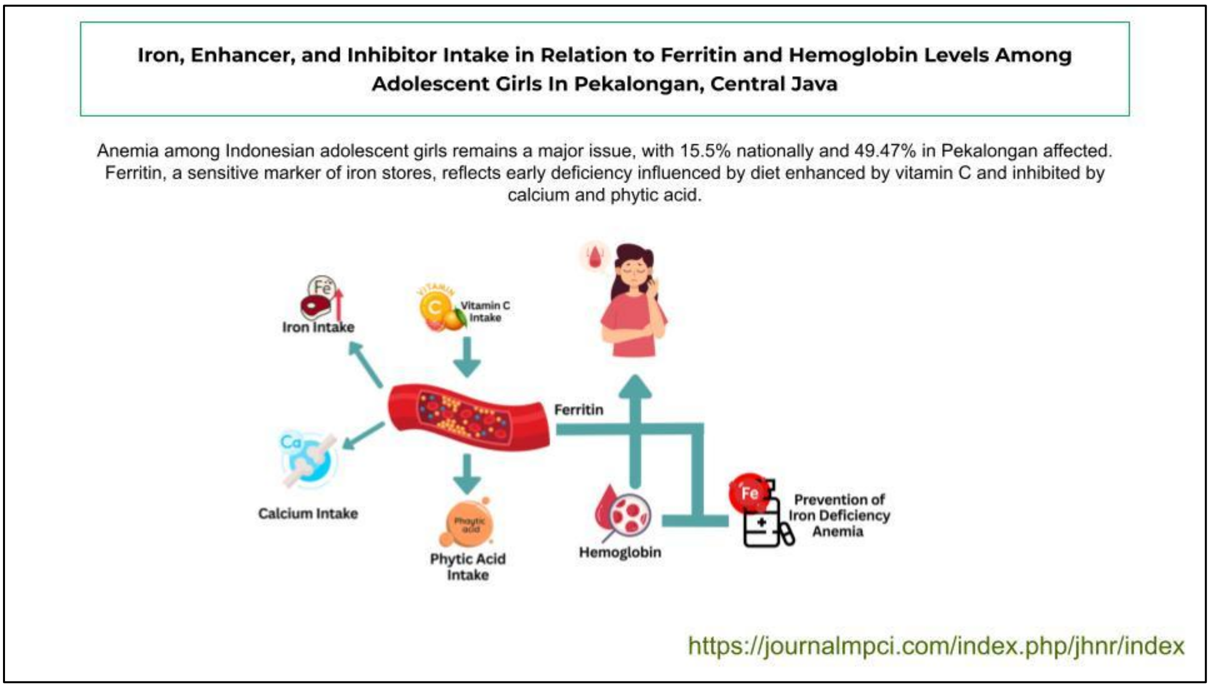
ABSTRACT

Anemia among Indonesian adolescent girls remains a major issue, with 15.5% nationally and 49.47% in Pekalongan affected. Ferritin, a sensitive marker of iron stores, reflects early iron deficiency influenced by diet, enhanced by vitamin C, and inhibited by calcium and phytic acid. This study aimed to investigate the relationship between iron, enhancer, and inhibitor intake and ferritin and hemoglobin levels in adolescent girls. An observational study with a cross-sectional design was conducted among 80 female students in Pekalongan, Central Java. Intake of iron, protein, vitamin A, vitamin C, fiber, calcium, and phytic acid was assessed using a Semi-Quantitative Food Frequency Questionnaire (SQ-FFQ). Hemoglobin levels were measured using a Hematology Analyzer, and ferritin was analyzed using the ELISA method. Data were analyzed using Pearson or Spearman correlation and multiple linear regression tests. The results show that ferritin levels were significantly correlated with iron ($r = 0.293$; $p = 0.008$) and vitamin C intake ($r = 0.298$; $p = 0.007$), and negatively correlated with calcium and phytic acid ($r = -0.673$; $p < 0.001$). Hemoglobin levels were correlated with protein ($r=0.232$; $p=0.035$), calcium ($r=-0.240$; $p=0.032$), and phytic acid ($r=-0.241$; $p=0.032$). Multivariate analysis revealed that ferritin levels were influenced simultaneously by iron, vitamin C, and calcium intake (CI: 95%, $p < 0.001$), while hemoglobin levels were influenced simultaneously by protein, fiber, and phytic acid intake (CI: 95%, $p = 0.019$). Iron, vitamin C, calcium, protein, fiber, and phytic acid intake influence ferritin and hemoglobin levels. Ferritin should be used as an early marker of iron deficiency, and interventions promoting higher iron and vitamin C intake while limiting inhibitors are recommended for adolescent girls.

Key Messages:

- The findings support the need for nutrition education and intervention programs focusing on increasing iron- and vitamin C–rich food intake while moderating calcium- and phytate-rich foods during iron meals to prevent anemia progression.

GRAPHICAL ABSTRACT



INTRODUCTION

Hemoglobin levels below the normal range, which cause anemia in adolescents, represent a serious nutritional problem, with Indonesia showing one of the highest prevalence rates. Data from the 2023 Indonesia Health Survey (SKI) indicate that 15.5% of Indonesian adolescents have low hemoglobin levels(1). In Pekalongan District, 49.47% of adolescent girls aged 15–18 years were recorded as having hemoglobin levels below normal. This figure indicates that iron deficiency remains common among adolescents, particularly females, who require higher nutrient intake due to growth and menstruation(2).

Adolescents are among the groups most vulnerable to nutritional problems, particularly anemia, as they are in a period of rapid growth and development that requires higher intakes of both macro- and micronutrients (3). This risk is higher among adolescent girls due to increased nutritional needs related to growth and menstruation, which are often unmet because of poor eating habits or restrictive diets aimed at preventing weight gain, potentially leading to malnutrition(4). Adolescence, defined by the WHO as the age group of 10–19 years, is a critical period for reproductive maturation and physical development, making this stage more vulnerable to anemia and malnutrition. The risk is even higher in developing countries, where low socioeconomic conditions and inadequate dietary habits further exacerbate the problem(5).

Hemoglobin and ferritin status are influenced by iron intake and its interactions with other nutrients(6). Enhancing factors such as vitamin C, vitamin A, and protein are known to improve iron absorption, while inhibiting factors such as calcium, phytic acid, and fiber can reduce it(7). Serum ferritin reflects high sensitivity and predictive value in cases of mild anemia as well as in non-anemic conditions(8). An imbalance between iron intake and its enhancers and inhibitors may affect iron stores and hemoglobin levels in adolescent girls(9). Although anemia in adolescents can result from various complex factors, iron deficiency remains the most common primary cause. This condition depends not only on the amount of iron consumed but also on the efficiency of its absorption process(10).

A study conducted among adolescent girls in 2014 found a relationship between the consumption of iron absorption inhibitors such as tannins (from tea) and phytates (found in grains, legumes, and cereals) and hemoglobin levels. The intake of these inhibitors can reduce the efficiency of iron absorption and contribute to low hemoglobin levels. Therefore, nutrition education is essential to increase iron intake while reducing the consumption of inhibitors among adolescent girls(11). The European Prospective

https://doi.org/10.56303/jhnresearch.v4i3.1008

Investigation into Cancer and Nutrition (EPIC) study reported that each 1 mg increase in daily heme iron intake was significantly associated with higher serum ferritin concentrations.(12). Several studies have examined the relationship between nutrient intake and hemoglobin levels; however, most have only assessed hemoglobin without measuring ferritin as an indicator of iron stores. In fact, ferritin levels often decrease earlier than hemoglobin levels. Moreover, studies that simultaneously analyze the effects of iron, enhancers, and inhibitors on both hemoglobin and ferritin levels among adolescent girls remain limited in Indonesia(13-15).

Based on this, the present study was conducted to analyze the relationship between iron, enhancer, and inhibitor intake with ferritin and hemoglobin levels among adolescent girls in the working area of Doro Public Health Center, Pekalongan District. The results are expected to provide a scientific basis for healthcare professionals and policymakers in formulating strategies for the prevention and management of anemia, thereby improving the health status of adolescent girls in the Doro Public Health Center area.

METHODS

This study employed an observational approach with a cross-sectional design to analyze the relationship between iron intake, iron enhancers, and inhibitors with ferritin and hemoglobin levels among adolescent girls. The study was conducted in the working area of Doro Public Health Center, Pekalongan Regency, Central Java, from April to May 2025. The study population consisted of all adolescent female students within the health center's working area. A total of 80 adolescent girls were included as samples. The inclusion criteria were adolescent girls aged 15–18 years, able to communicate well, not following a special diet, and willing to participate in the entire research process. The exclusion criteria included respondents who did not complete the SQ-FFQ form or were menstruating at the time of sample collection.

Data on iron intake, enhancers, and inhibitors were collected using a Semi-Quantitative Food Frequency Questionnaire (SQ-FFQ) that was previously developed and validated for Indonesian populations(16). Ferritin levels were analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA) method with General Biological Corp Human Ferritin reagents, and the results were read at a wavelength of 450 nm using a microplate reader at the GAKY Laboratory, Faculty of Medicine, Diponegoro University, after serum collection and separation were conducted at the Pekalongan Regional Health Laboratory. Ferritin levels below 15 µg/L were classified as low according to WHO criteria(17). Hemoglobin levels were measured using a Hematology Analyzer at the Pekalongan Regional Health Laboratory, with a cut-off point of <12 g/dL indicating low levels for adolescent girls(18).

The data obtained were analyzed in ratio form using SPSS software. Normality testing was first conducted using the Kolmogorov–Smirnov method to determine the data distribution. If the data were normally distributed (p-value > 0.05), the relationships between variables were tested using the Pearson Product Moment correlation test, whereas non-normally distributed data (p-value < 0.05) were analyzed using the Spearman Rank test. Variables showing a p-value < 0.25 in the correlation test were further analyzed using Multiple Linear Regression to assess the strength of multiple variable relationships with ferritin and hemoglobin levels among adolescent girls. Before performing multiple linear regression, the assumptions of normality, linearity, and multicollinearity were tested. Multicollinearity was assessed using the Variance Inflation Factor (VIF) and tolerance values, with VIF < 10 and tolerance > 0.10 considered acceptable indicators of no multicollinearity. Nutrient intake adequacy was determined based on the Indonesian Recommended Dietary Allowances (RDA) issued by the Ministry of Health of Indonesia (2019) for adolescent girls aged 15–18 years. Each nutrient intake was expressed as a percentage of the corresponding RDA and classified into three categories: low intake (<80% of the RDA), adequate intake (80–120% of the RDA), and excess intake (>120% of the RDA) (19).

CODE OF HEALTH ETHICS

Ethical approval for this study was granted by the Health Research Ethics Committee of the Faculty of Medicine, Diponegoro University, under approval number 077/EC/KEPK/FK-UNDIP/IV/2025.

https://doi.org/10.56303/jhnresearch.v4i3.1008

RESULTS

Table 1 presents the characteristics of the respondents. The mean age was 15.74 years (range: 15–17 years). The mean hemoglobin and ferritin levels were 13.89 g/dL (range: 11.20–16.10 g/dL) and 33.11 μg/L (range: 4.00–170.00 μg/L), respectively. The average intakes of protein, vitamin A, and vitamin C were 84.41 g/day, 280.38 μg/day, and 78.28 mg/day, respectively. The mean intakes of iron, fiber, calcium, and phytate were 24.00 mg/day, 11.28 g/day, 1148.29 mg/day, and 1890.94 mg/day, respectively.

Table 1. Frequency Characteristic of Respondent (n=80)

Characteristic	Result
Age (years)	15.73 (15.00-17.00)
Hemoglobin levels	13.89 (11.20-16.10)
Ferritin levels	33.11 (4.00-170.00)
Iron intake	24.00 (9.00-75.00)
Enhancer intake:	
Protein	84.41 (47.00-173.00)
Vitamin A	280.37 (80.00-551.00)
Vitamin C	78.27 (17.00-334.00)
Inhibitor intake:	
Fiber	11.27 (3.00-39.00)
Calcium	1148.29 (310-2100)
Phytic acid	1890.94 (511-3458)

Note: data are presented as mean (minimum–maximum)

Figure 1 illustrates the distribution of adolescents' characteristics by age, anemia status, ferritin levels, and nutrient intake. Most respondents were 16 years old (43.8%). The majority were non-anemic (93.8%), shown in green, while anemic respondents (6.3%) are represented in red. Most individuals had normal ferritin levels (66.3%), indicated in green, and low ferritin levels (33.8%), indicated in red. For nutrient intake (iron, protein, vitamins, fiber, calcium, and phytic acid), the chart uses yellow for *low intake*, green for *adequate intake*, and red for *excess intake*. Overall, most respondents had adequate hemoglobin and ferritin levels; however, vitamin A and fiber intake were relatively low compared to other nutrients.

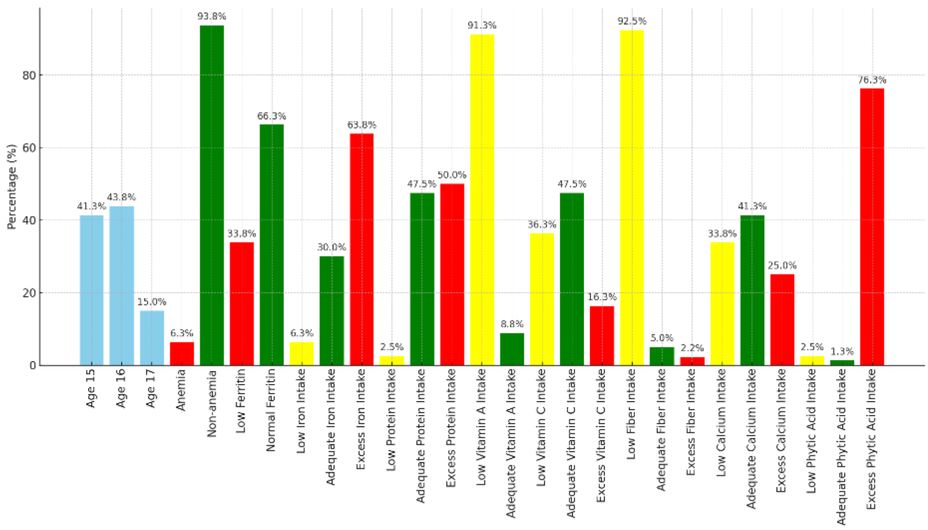


Figure 1. Bar Chart of Respondent Characteristic

Note: Red indicates anemia, low ferritin, or excess nutrient intake; Green indicates non-anemia, normal ferritin, or adequate intake; Yellow indicates low nutrient intake;Blue represents age groups. Nutrient adequacy was classified according to the Indonesian Recommended Dietary Allowances (RDA, 2019): Low (<80% RDA), Adequate (80–120% RDA), and Excess (>120% RDA).

Tables 2 and 3 present the relationship between nutrient intake and ferritin and hemoglobin levels among adolescent girls. Based on Table 2, iron intake was significantly associated with ferritin levels (p = 0.008). Among the enhancer factors, vitamin C intake was significantly associated with ferritin levels (p =

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0.007), whereas protein (p = 0.073) and vitamin A intake (p = 0.583) showed no significant associations. In the inhibitor group, calcium and phytate intake demonstrated significant associations with ferritin levels (p < 0.001), while fiber intake showed no significant relationship (p = 0.686).

Table 2. Correlation of Iron, Enhancer, and Inhibitor Intake on Ferritin Levels

Variabel	Median±Min-Max	r-correlation	p-value
Iron intake	21.00±(9.00-75.00)	0.293	0.008
Enhancer intake			
Protein	78.50±47.00-173.00	0.202	0.073
Vitamin A	277.00±80.00-551.00	0.062	0.583
Vitamin C	60.00±17.00-334.00	0.298	0.007
Inhibitors intake			
Fiber	9.00±3.00-39.00	-0.046	0.686
Calcium	1131.50±310-2100	-0.673	<0.001
Phytic acid	1863.50±511-3458	-0.673	<0.001

**Correlation test using rank Spearman*
Note: variables found to be normally distributed were analyzed using Pearson (and reported as Mean±SD), while non-normally distributed variables were analyzed using Spearman (and reported as Median±Min-Max).

According to Table 3, iron intake was not significantly associated with hemoglobin levels (p = 0.886). Among the enhancer factors, vitamin A (p = 0.985) and vitamin C intake (p = 0.543) showed no significant relationships with hemoglobin levels, whereas protein intake showed a significant association (p = 0.038). In the inhibitor group, fiber intake was not significantly associated with hemoglobin levels (p = 0.203), while calcium and phytate intake showed significant associations (p = 0.032).

Table 3. Correlation of Iron, Enhancer, and Inhibitor Intake on Hemoglobin Levels

Variabel	Mean±SD / Median±Min-Max	r-correlation	p-value
Iron intake	21.00±13.18	0.016	0.886[a]
Enhancer intake			
Protein	84.41±23.35	0.232	0.038 [a]
Vitamin A	280.37±139.47	0.002	0.985 [a]
Vitamin C	60.00±17.00-334.00	0.069	0.543 [b]
Inhibitors intake			
Fiber	9.00±3.00-39.00	-0.144	0.203 [b]
Calcium	1148.29±428.65	-0.240	0.032 [a]
Phytic acid	1890.94±705.78	-0.241	0.032 [a]

[a]correlation test using pearson, [b]correlation test using rank Spearman
Note: variables found to be normally distributed were analyzed using Pearson (and reported as Mean±SD), while non-normally distributed variables were analyzed using Spearman (and reported as Median±Min-Max).

Tables 4 and 5 present the results of the multiple linear regression analysis. The multicollinearity test indicated no collinearity issues. Table 4 shows that iron intake, vitamin C intake, and calcium intake were significantly associated with ferritin levels (p < 0.05). Among these variables, calcium intake demonstrated a negative association, whereas iron and vitamin C intake were positively associated with ferritin concentration. The model explained 68.9% of the variation in ferritin levels (Adjusted R^2 = 0.689, p < 0.001).

Table 4. Multiple Linear Regression Analysis of Nutrient Intake and Ferritin Levels

Independent variable	Ferritin levels						
	B	95%CI	Std, Error	Beta	*t*	VIF	*p value*
Iron intake	1,041	0,72-1,26	0,161	0,460	6,468	1,283	0,000
Protein intake	0,059	0,04-0,12	0,094	0,046	0,633	1,360	0,529
Vitamin C intake	0,081	0,02-0,13	0,028	0,188	2,891	1,077	0,005
Calcium intake	-0,040	-0,05 – -0,31	0,005	-0,581	-8,473	1,193	0,000

Adjusted R^2 = 0.689; Model significance (p < 0.001)

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Table 5 indicates that hemoglobin levels were simultaneously influenced by protein, fiber, and phytate intake, with a relatively small contribution of 8.8% to the variation in hemoglobin levels. None of these variables showed a statistically significant effect, although fiber intake tended to have a negative association (β = –0.197; p = 0.077).

Table 5. Multiple Linear Regression Analysis of Nutrient Intake and Hemoglobin Levels

Independent variable	Hemoglobin levels						
	B	95%CI	Std. Error	Beta	*t*	VIF	*p value*
Protein intake	0,006	0,01-0,03	0,427	0,145	-1,267	1.132	0,209
Fiber intake	-0,027	-0,057-0,003	0,015	-0,197	-1,796	1.044	0,077
Phytic acid intake	0,000	-0,001-0,000	0,000	-0,165	-1,450	1.122	0,151

Adjusted R^2 = 0.088; Model significance (p = 0.019)

Meanwhile, these findings suggest that the three variables were not the main predictors of anemia, indicating that other factors may play a more dominant role in influencing hemoglobin status among adolescents.

DISCUSSION

The findings of this study show that the prevalence of anemia among adolescent girls was relatively low (6.3%), with a mean hemoglobin concentration of 13.89 g/dL (range 11.20–16.10 g/dL). However, one-third of the respondents (33.8%) had low ferritin levels, with a mean concentration of 33.11 μg/L (range 4.00–170.00 μg/L), indicating early-stage iron deficiency or iron depletion(9). This condition suggests that although hemoglobin levels remain within the normal range, iron stores in the body have begun to decline. Such findings emphasize the importance of assessing ferritin levels as an early indicator of iron deficiency, as relying solely on hemoglobin measurement may overlook latent iron depletion(14).

The correlation analysis demonstrated a positive relationship between iron intake and ferritin levels (r = 0.293), suggesting that higher dietary iron intake contributes to higher ferritin concentrations. This aligns with the biological role of ferritin as the main iron storage protein in the body, which reflects the adequacy of iron reserves. A similar association was observed by Alghamdi (2017) and Reddy et al. (2022), who found that ferritin levels increase proportionally with dietary iron intake(20, 21). Furthermore, vitamin C intake showed a significant positive association with ferritin, confirming its role as an iron absorption enhancer. Vitamin C improves iron bioavailability by reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) form, which is more soluble and readily absorbed in the duodenum(22). This finding is consistent with Herawati (2018) and Fiorentino et al. (2016), who reported that adequate vitamin C intake enhances iron absorption and improves ferritin status among adolescents(20, 23).

In contrast, calcium and phytate intake demonstrated significant negative associations with ferritin levels, reflecting their inhibitory effect on iron absorption. Physiologically, calcium competes with iron for absorption pathways in the enterocytes and may alter ferroportin expression, thereby reducing the transport of iron into circulation. Phytate, on the other hand, forms insoluble complexes with iron, decreasing its solubility and intestinal availability(24). These findings are in agreement with Abioye et al. (2021) and Reddy et al. (2022), who reported that diets high in calcium and phytate significantly decrease iron absorption efficiency. Meanwhile, protein and vitamin A intake showed no significant correlation with ferritin levels, suggesting that ferritin is more strongly influenced by iron balance and absorption-related nutrients than by overall macronutrient intake(21, 24). This result supports findings from NHANES (2017–2020) and Zhang et al. (2023), which also reported weak associations between protein, vitamin A, and ferritin status among adolescents(25, 26).

Protein intake showed a significant association with hemoglobin, indicating that adequate protein plays a vital role in hemoglobin synthesis through its contribution to globin production(27). This finding is consistent with Putriwati (2024), who found that low protein intake increased the risk of anemia among adolescent girls(28). Meanwhile, calcium and phytate intake were negatively associated with hemoglobin,

https://doi.org/10.56303/jhnresearch.v4i3.1008

which supports their known inhibitory effects on iron absorption, as also reported by Permatasari (2024). Conversely, iron, vitamin A, vitamin C, and fiber intakes showed no significant association with hemoglobin levels. This lack of association could be due to low bioavailability of non-heme iron sources and the presence of dietary inhibitors, as well as the influence of non-dietary factors such as menstruation, infection, inflammation, and chronic disease, which can interfere with erythropoiesis(29). Similar results were reported by Marliyana (2025) and Suliburska et al. (2023), emphasizing that hemoglobin concentration is influenced by complex interactions beyond dietary iron intake alone(30, 31).

The multiple linear regression analysis revealed that, simultaneously, iron, vitamin C, and calcium intake significantly influenced ferritin levels (Adjusted $R^2 = 0.689$, $p < 0.001$), explaining 68.9% of the variation in ferritin concentrations. This finding underscores the combined effects of enhancers and inhibitors in determining iron storage. Meanwhile, protein, fiber, and phytate intake had a simultaneous but relatively small effect (8.8%) on hemoglobin levels, suggesting that these nutrients contribute to hemoglobin status, but to a lesser extent. These results are in line with Pan et al. (2024), who highlighted the interactive effects of dietary enhancers and inhibitors on iron metabolism, and with Suliburska et al. (2023), who confirmed the strong inhibitory role of phytate(31, 32).

This study has several limitations that should be acknowledged. First, its cross-sectional design limits the ability to establish causality between dietary intake and ferritin or hemoglobin levels; the associations observed should therefore be interpreted as correlational rather than causal (33). Second, the use of a Semi-Quantitative Food Frequency Questionnaire (SQ-FFQ) to assess nutrient intake may have introduced recall bias, as participants were required to recall their food consumption over an extended period, which could lead to under- or over-reporting (34). Third, the study did not measure inflammatory biomarkers such as C-reactive protein (CRP) or α1-acid glycoprotein (AGP). Because inflammation can elevate ferritin levels independently of iron status, the absence of inflammation adjustment represents a limitation that may influence the interpretation of ferritin as a biomarker of iron stores (35). Finally, other non-dietary factors that may affect hemoglobin concentration, such as menstrual blood loss, parasitic infections, and socioeconomic status, were not analyzed in this study and should be considered in future research.

Overall, this study reinforces that maintaining a balanced dietary intake between enhancers (such as vitamin C and protein) and inhibitors (such as calcium and phytate) is essential for optimizing iron absorption, storage, and hemoglobin synthesis among adolescents. The finding that one-third of participants had low ferritin levels despite normal hemoglobin highlights the need for preventive strategies focusing on dietary improvement and early detection of iron depletion. These results underscore the importance of incorporating ferritin screening into routine adolescent health assessments to identify early-stage iron deficiency that might not be detected through hemoglobin measurement alone (14).

From a public health perspective, the findings provide valuable insights for strengthening nutrition strategies addressing adolescent anemia in Indonesia. Nutrition education programs should emphasize increasing the consumption of iron- and vitamin C–rich foods while limiting inhibitors such as calcium- and phytate-rich foods during iron-containing meals (24). These recommendations align with national initiatives such as the Program Nasional Tablet Tambah Darah (TTD) for adolescent girls, suggesting that dietary counseling should accompany supplementation programs to enhance absorption and adherence. Future interventions could integrate school-based nutrition education, parental involvement, and collaboration with local health centers to promote sustainable dietary behavior and routine ferritin monitoring, thereby preventing progression from iron depletion to iron deficiency anemia and improving overall adolescent health outcomes.

CONCLUSION

This study showed that although the prevalence of anemia among adolescent girls was low, one-third of participants had low ferritin levels, indicating early iron deficiency. Iron, vitamin C, and calcium intake were significantly associated with ferritin levels, while protein, fiber, and phytate intake had limited effects on hemoglobin. Maintaining a balanced intake between enhancers and inhibitors is therefore essential for optimal iron status. Regular ferritin screening, together with increased consumption of iron-

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and vitamin C–rich foods and reduced intake of calcium- and phytate-rich foods during iron meals, is recommended to prevent iron deficiency anemia among adolescents. Further research exploring the temporal and behavioral aspects of dietary patterns and iron metabolism would provide a deeper understanding of these relationships and support the development of more effective nutrition programs for adolescents.

FUNDING

This research received no external funding

ACKNOWLEDGMENTS

Appreciation is also extended to the Pekalongan District Health Office and Doro Public Health Center for their cooperation and assistance in data collection. The authors sincerely thank all participating students for their valuable time and contribution.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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https://doi.org/10.56303/jhnresearch.v4i3.1008