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Longitudinal changes in haemoglobin levels following supervised weekly iron-folic acid supplementation among adolescent girls: A school-based study in Indonesia

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ABSTRACT

Introduction: Anaemia remains a significant public health concern among adolescent girls in Indonesia. Weekly iron-folic acid supplementation is widely implemented; however, evidence from supervised school-based settings is limited. This study aimed to examine changes in haemoglobin levels following supervised weekly supplementation. **Methods:** A quasi-experimental study with repeated measurements was conducted among adolescent girls in one junior high school. Participants received weekly ferrous fumarate (60 mg iron and 400 μg folic acid) for 12 weeks under school supervision. Haemoglobin levels were measured at baseline and follow-up. Data were analysed using General Linear Model Repeated Measures (GLM-RM), with additional analysis of covariance (ANCOVA) to adjust for baseline differences. **Results:** A total of 47 participants completed the study. Haemoglobin levels increased in the intervention group (10.8 to 13.2 g/dL), while minimal change was observed in the comparison group (12.4 to 12.9 g/dL). GLM-RM showed a significant effect of time ($p<0.001$) and time–group interaction ($p=0.001$). However, after baseline adjustment using ANCOVA, the difference between groups was not statistically significant ($p=0.296$). **Conclusion:** Supervised weekly iron-folic acid supplementation was associated with improved haemoglobin levels over time. However, baseline differences may have influenced the observed effects. Strengthening school-based programmes through structured supervision and adherence monitoring may improve implementation. Further research using baseline-adjusted designs is needed to better estimate the true effect of supplementation.

Keywords: adolescent girls, anaemia, haemoglobin, iron-folic acid supplementation, school-based intervention

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INTRODUCTION

Anaemia remains a critical global public health issue due to its far-reaching impacts on overall health status and human capital quality (WHO, 2024). Among adolescents, this condition is particularly significant, as anaemia during this developmental period can lead to both immediate and long-term consequences. These include fatigue, dizziness, decreased concentration, and reduced academic performance, as well as impaired cognitive development, reduced productivity, and an increased risk of reproductive health problems in adulthood (Ministry of Health Republic of Indonesia, 2023a; Figueiredo *et al.*, 2018; Nadhiroh, Micheala & Tung, 2023). The World Health Organization (WHO) has identified anaemia reduction as one of the Global Nutrition Targets, aiming for a 50% decrease in prevalence among women of reproductive age by 2030 (WHO & UNICEF, 2025). Nevertheless, recent global estimates indicate substantial challenges in achieving this target, with anaemia prevalence reaching 30.7% among women aged 15–49 years (WHO, 2025).

Anaemia is a multifactorial condition caused by various factors, including micronutrient deficiencies, infections, inflammation, chronic diseases, and inherited disorders (World Health Organization, 2025). Among these, iron deficiency is recognised as the most common cause of anaemia worldwide, although its contribution varies across populations, accounting for approximately 10% to over 60% of anaemia cases, depending on the context (WHO, 2025). This variation highlights that not all anaemia is attributable to iron deficiency, as multiple underlying causes may coexist. In Indonesia, although specific national estimates of iron deficiency among adolescents are limited, iron deficiency is widely acknowledged as a major contributor to anaemia in this population, particularly among adolescent girls due to increased physiological demands.

The 2023 Indonesia Health Survey reported an anaemia prevalence of 16.3% among children aged 5–14 years and 15.5% among adolescents aged 15–24 years, with consistently higher rates observed among females (Ministry of Health Republic of Indonesia, 2023b). Adolescence is characterised by rapid physical growth and the onset of menstruation, both of which substantially increase iron requirements for haemoglobin synthesis (Deivita *et al.*, 2021; Anwar *et al.*, 2025; Gattermann *et al.,* 2021). Inadequate dietary intake, increased physiological requirements, and poor iron absorption may reduce iron availability and haemoglobin concentration, leading to impaired oxygen transport and disruption of normal physiological function (Vogt *et al.,* 2021).

To address this issue, the Ministry of Health of the Republic of Indonesia has implemented a nationwide weekly iron-folic acid supplementation (IFAS) programme through the *Aksi Bergizi* initiative in schools, with a target of providing at least 52 tablets per year to adolescent girls (Ministry of Health Republic of Indonesia, 2023a). Evidence from other settings has shown that school-based weekly iron-folic acid supplementation (WIFAS) can improve haemoglobin levels and micronutrient status among adolescent girls (Bolka *et al.,* 2025). However, the effectiveness of this programme remains suboptimal, primarily due to low adherence among adolescents (Apriningsih *et al.*, 2020). National data further indicate that compliance with IFAS among adolescents remains below 20% in several regions, influenced by limited understanding of the benefits of supplementation, concerns regarding perceived side effects, and inadequate nutrition education (Ministry of

Health Republic of Indonesia, 2023b). This is supported by previous evidence indicating that nutrition education is associated with improved knowledge and reduced risk of anaemia among adolescent girls (Rahmiwati *et al.,* 2023).

At the regional level, anaemia among adolescent girls remains a significant public health concern in the West Java Province. The 2024 SIGIZI report documented an anaemia prevalence of 24.9% among seventh-grade students and 25.1% among tenth-grade students, figures that are notably higher than the national average (Ministry of Health Republic of Indonesia, 2024; West Java Provincial Health Office, 2024). In Depok City, where this study was conducted, haemoglobin screening in 2023 revealed that 36.3% of 27,766 female students were anaemic despite iron-folic acid supplementation (IFAS) coverage exceeding 93% (Apriningsih *et al.*, 2020; Depok City Health Office, 2024; Depok City Health Office, 2025). These findings indicate a substantial gap between programme coverage and health outcomes, suggesting that supplementation distribution alone is insufficient without effective adherence, monitoring, and supportive implementation strategies.

Previous studies evaluating the effectiveness of iron-folic acid supplementation in Indonesia have predominantly employed simple pre–post designs or two-point comparisons, which are limited in their ability to capture dynamic longitudinal changes in haemoglobin levels. Such approaches fail to adequately account for within-subject variation across multiple time points and do not distinguish patterns of change between intervention and comparison groups. Methodological literature indicates that commonly used statistical approaches, such as ANOVA, often do not appropriately model longitudinal data due to the assumption of independent observations, despite the inherent correlation among repeated measurements within individuals. This limitation may lead to biased and imprecise estimates and potentially inaccurate conclusions regarding intervention effects (Murphy, Weaver & Hendricks, 2022). Therefore, more robust analytical approaches, such as repeated measures analysis or linear mixed-effects models, are required to comprehensively evaluate intervention effectiveness.

To address this gap, the present study used the General Linear Model Repeated Measures (GLM-RM) as a statistical analysis method to evaluate changes in haemoglobin levels over time following weekly iron-folic acid supplementation among adolescent girls. This study aimed to generate empirical evidence on the longitudinal effects of supervised iron-folic acid supplementation and to inform the refinement of school-based anaemia prevention strategies in Indonesia.

METHODOLOGY

Study design and setting

A quasi-experimental study with a comparison group and repeated measurements was conducted to evaluate changes in haemoglobin levels among adolescent girls receiving weekly iron-folic acid supplementation. The study was carried out at At Taufik Islamic Junior High School, Cimanggis District, Depok City, Indonesia, from August to October 2025. The study population consisted of female students aged 12–15 years, with early adolescence defined as 12–13 years and middle adolescence as 14–15 years. All participants were in good health and willing to participate throughout the study period. Participants were excluded if they had any chronic diseases, were menstruating at the time of haemoglobin measurement, or declined to complete the intervention. Participants were allocated into intervention and comparison groups using a non-random assignment approach based on existing class groupings within the school. A total of 47 participants were included in the study, comprising 24 students in the intervention group and 23 students in the comparison group.

Sample size determination

Minimum sample size was calculated using the formula for comparing two paired means, assuming a standard deviation (σ) of 0.9, a 95% confidence level ($Z_{1-\alpha/2}$ = 1.96), and a statistical power of 95% ($Z_{1-\beta}$ = 1.64). The estimated mean haemoglobin values before and after intervention were 11.1 g/dL and 11.8 g/dL, respectively, based on previous studies (Yuanti *et al.*, 2016; Giyanti & Wahtini, 2016). The minimum required sample size was 22 participants per group. To account for potential dropouts, the final sample included 24 participants in the intervention group and 23 participants in the comparison group.

Intervention and measurements

The intervention consisted of administering one iron-folic acid tablet per week for 12 consecutive weeks. Each tablet contained 60 mg of elemental iron and 400 µg of folic acid, in accordance with the Indonesian Ministry of Health guidelines. The supplementation was administered during school hours under the direct supervision of teachers. Participants consumed the tablets in the classroom at a scheduled time each week, typically after meals to minimise gastrointestinal discomfort. Teachers ensured that each participant swallowed the tablet and recorded attendance to monitor compliance. Participants were advised to report any side effects such as nausea or gastrointestinal discomfort. Mild side effects were managed by administering the tablets after meals. No serious adverse events were reported during the study. The comparison group did not receive supervised iron-folic acid supplementation and continued their usual routines. However, both groups received a standard educational session on anaemia prevention prior to the commencement of the study. Haemoglobin levels were measured at four time points: baseline (Month 0), Month 1, Month 2, and Month 3.

Measurements were conducted using an EasyTouch® GCHb 3-in-1 digital haemoglobinometer (Bioptik Technology Inc, Miaoli, Taiwan). Capillary blood samples were obtained via finger prick by trained health personnel; the device was calibrated prior to use to ensure measurement accuracy. Additional data on participant characteristics, including age, nutritional status, and knowledge related to anaemia, were collected using structured questionnaires. Nutritional status was assessed based on measured height and weight, categorised using BMI-for-age criteria. Knowledge was assessed using a structured questionnaire based on validated instruments. Adherence was monitored using structured consumption logs completed at each supplementation session, which recorded the date and participant signature as confirmation of tablet intake.

Data analysis

Data were analysed using IBM SPSS Statistics for Windows version 25.0 (IBM Corp, Armonk, NY, USA). Descriptive statistics were used to summarise participant characteristics. Normality was assessed using the Kolmogorov–Smirnov test. Box's test and Levene's test were used to assess homogeneity of covariance matrices and error variances, respectively. Mauchly's test of sphericity was conducted; when violated, the Greenhouse–Geisser correction was applied. The GLM-RM analysis was used to examine the effects of time and time–group interaction on haemoglobin levels, with particular emphasis on the time-by-group interaction as the primary outcome. Baseline haemoglobin differences between groups were assessed and considered in the interpretation of the results. An additional analysis using analysis of covariance (ANCOVA) was conducted to adjust for baseline haemoglobin differences between groups, with Month 3 haemoglobin level as the dependent variable, group as the fixed factor, and baseline haemoglobin as a covariate. Statistical significance was set at $p<0.05$.

Ethical approval and consent to participate

Ethical approval was obtained from the institutional research ethics committee of Universitas Mohammad Husni Thamrin, Jakarta, Indonesia (Approval No. 0124/S.Ket/KEPK/UMHT/VII/2025). Written informed consent was obtained from both participants and their parents/guardians prior to data collection. Participant confidentiality and anonymity were strictly maintained, and participants were informed of their right to withdraw at any time without penalty.

RESULTS

All participants were female students aged 12–15 years. In the intervention group, 1 participant (4.2%) was classified as an early adolescent (12–13 years) and 23 participants (95.8%) as middle adolescents (14–15 years). In the comparison group, 9 participants (39.1%) were in early adolescence and 14 participants (60.9%) were in middle adolescence. Nutritional status was assessed based on measured height and weight and categorised using BMI-for-age criteria into underweight, normal, and overweight. In the intervention group, 13 participants (54.2%) were underweight, 10 (41.7%) had normal nutritional status, and 1 (4.1%) was overweight. In the comparison group, 8 participants (34.8%) were underweight, 10 (43.5%) had normal nutritional status, and 5 (21.7%) were overweight.

Most participants in both groups had experienced menarche. In the intervention group, 17 participants (70.8%) had already menstruated, while 7 (29.2%) had not yet menstruated. In the comparison group, 22 participants (95.7%) had already menstruated and 1 participant (4.3%) had not yet menstruated. Knowledge regarding anaemia was generally good in both groups. In the intervention group, 17 participants (70.8%) demonstrated good knowledge and 7 (29.2%) had poor knowledge, whereas all participants in the comparison group (100%) demonstrated good knowledge regarding anaemia. These baseline characteristics were described to illustrate the participants' profile in each group. However, baseline haemoglobin differences between groups were assessed and are presented in Table 1.

Table 1 presents the mean haemoglobin levels in the intervention and comparison groups across the four measurement time points. At baseline, the mean haemoglobin level in the intervention group (10.8±1.2 g/dL) was significantly lower than that in the comparison group (12.4±1.0 g/dL) ($p<0.001$), indicating that participants in the intervention group were initially in the anaemic range, while those in the comparison group were within normal limits. No statistically significant differences were observed between the groups at Month 1 ($p=0.343$) and Month 3 ($p=0.362$), while a significant difference was found at Month 2 ($p=0.016$).

Table 1. Mean haemoglobin levels (g/dL) in the intervention and comparison groups

Time point	*Intervention group (Mean±SD)*	*Comparison group (Mean±SD)*	*p-value*
Baseline (Month 0)	10.8±1.2	12.4±1.0	<0.001
Month 1	12.5±1.1	12.8±1.3	0.343
Month 2	12.0±0.9	13.0±1.5	0.016
Month 3	13.2±1.0	12.9±1.1	0.362

Over time, haemoglobin levels in the intervention group increased from 10.8 g/dL at baseline to 13.2 g/dL at Month 3. In contrast, haemoglobin levels in the comparison group remained relatively stable, ranging from 12.4 g/dL to 12.9 g/dL. A temporary decrease in haemoglobin levels was observed in the intervention group between Month 1 (12.5 g/dL) and Month 2 (12.0 g/dL), followed by an increase at Month 3.

Prior to the GLM-RM analysis, assumption testing was conducted. The Kolmogorov–Smirnov test indicated normal distribution (p=0.200). Box's test (p=0.099) and Levene's test (p>0.05) confirmed homogeneity of covariance matrices and error variances. Mauchly's test of sphericity was significant (p=0.009), indicating violation of the assumption; therefore, the Greenhouse–Geisser correction (ε=0.833) was applied.

Results of the GLM-RM analysis (Table 2) showed a significant main effect of time (p=0.001; partial η^2=0.25) and a significant time–group interaction (p=0.001; partial η^2=0.12) in the univariate analysis. Multivariate analysis confirmed a significant effect of time (p=0.001; partial η^2=0.60) and time–group interaction (p=0.001; partial η^2=0.43). The model included time as a within-subject factor and group as a between-subject factor. These findings indicated that haemoglobin levels changed significantly over time and that the pattern of change differed between the intervention and comparison groups. Within-subject contrast analysis (Table 3) was conducted on the combined sample of both groups and showed significant increases in haemoglobin levels between the first and second measurements (p=0.001; η^2=0.371) and between the third and fourth measurements (p=0.006; η^2=0.192), while no significant difference was observed between the second and third measurements (p=0.068).

To account for the significant baseline difference in haemoglobin levels between groups, an additional analysis using ANCOVA was conducted, with Month 3 haemoglobin level as the dependent variable, group as the fixed factor, and baseline haemoglobin as a covariate. Table 4 shows that after adjusting for baseline haemoglobin levels, the difference between the intervention and comparison groups at Month 3 was not statistically significant (p=0.296). Baseline haemoglobin was also not a significant predictor in the model (p=0.594).

Table 2. General linear model repeated measures (GLM-RM) analysis on haemoglobin levels

Effect	*Approach*	*p-value*	*Partial Eta Squared (η^2)*	*Interpretation*
Time	Univariate	0.001	0.25	Significant time effect
Time × Group interaction	Univariate	0.001	0.12	Significant interaction
Time	Multivariate	0.001	0.60	Significant effect across time
Time × Group interaction	Multivariate	0.001	0.43	Strong time–group interaction

Table 3. Within-subject contrast test of haemoglobin levels

Measurement Comparison	*p-value*	*Partial Eta Squared (η^2)*	*Interpretation*
1st vs. 2nd measurement	0.001	0.371	Significant increase
2nd vs. 3rd measurement	0.068	0.082	Not significant
3rd vs. 4th measurement	0.006	0.192	Significant increase

Table 4. Analysis of covariance (ANCOVA) for haemoglobin levels at month 3 adjusted for baseline haemoglobin

Source	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*p-value*	*Partial Eta Squared (η^2)*
Baseline haemoglobin (HB_0)	0.343	1	0.343	0.289	0.594	0.007
Group (intervention vs control)	1.329	1	1.329	1.117	0.296	0.025
Error	52.362	44	1.190			
Total	53.698	46				

Figure 1 illustrates the trend of mean haemoglobin levels across the four measurement time points, showing an increasing trend in the intervention group and relatively stable values in the comparison group.

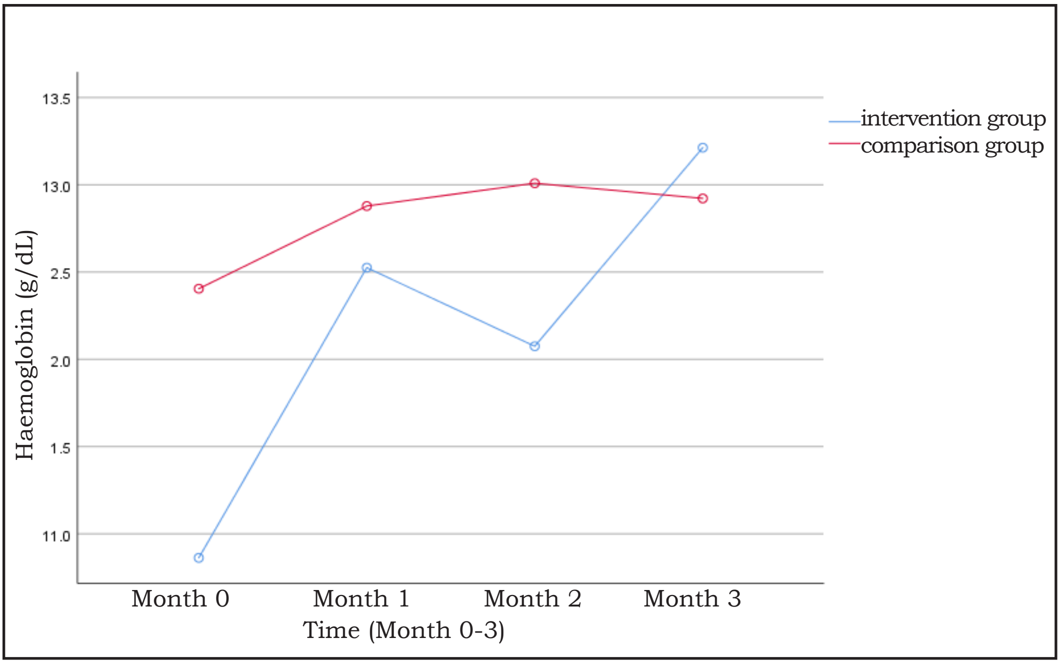

Figure 1. Trend of mean haemoglobin levels (g/dL) across four measurement time points in the intervention and comparison groups

DISCUSSION

This study demonstrated that supervised weekly iron-folic acid supplementation over a 12-week period was associated with an increase in haemoglobin levels among adolescent girls. The GLM-RM analysis showed significant effects of time and time–

group interaction, indicating that haemoglobin levels changed over time and that the pattern of change differed between the intervention and comparison groups. These findings suggest that haemoglobin levels improved over the study period, particularly in the intervention group. The observed increase may reflect improved iron availability through supplementation, which is important during adolescence, a period characterised by rapid growth and increased iron requirements due to menstrual blood loss. These findings are consistent with previous studies showing that school-based weekly iron-folic acid supplementation programmes are associated with improvements in haemoglobin levels and reductions in anaemia among adolescent girls (Bolka *et al.,* 2025).

However, an important methodological consideration in this study is the baseline imbalance in haemoglobin levels between groups, with the intervention group presenting lower initial values within the anaemic range. This condition introduces the possibility of regression to the mean, whereby individuals with lower baseline values may show greater apparent improvement over time independent of the intervention effect. This interpretation is supported by the results of the baseline-adjusted analysis using ANCOVA, which showed that the difference between groups at Month 3 was no longer statistically significant after controlling for baseline haemoglobin levels. These findings indicate that the observed improvement in the intervention group may be partly influenced by initial differences rather than the supplementation effect alone. Therefore, caution is required when interpreting the observed changes as a direct effect of the intervention. This is consistent with evidence suggesting that anaemia is a multifactorial condition, and iron deficiency accounts for only a proportion of anaemia cases globally, indicating that factors beyond iron supplementation may influence haemoglobin levels (WHO, 2025).

Despite these methodological considerations, it remains important to examine key components of the intervention that may have contributed to the observed changes. Supervision represents a central component of the intervention design and warrants further discussion. In this study, supplementation was implemented in a controlled school setting under direct teacher supervision, ensuring that tablets were consumed as scheduled. In addition, adherence was monitored using structured consumption logs completed at each session, which documented the date and participant signature as confirmation of intake. This approach provides a structured and systematically documented proxy for adherence and reflects a form of direct observation of supplement consumption. Such mechanisms may help reduce common barriers to adherence, including forgetfulness, low motivation, and limited access to supplements. Previous qualitative evidence has shown that adherence to iron supplementation among adolescent girls is strongly influenced by social support and interpersonal factors, including the role of parents, teachers, peers, and health workers in providing information and reminders (Silitonga *et al.,* 2023). This suggests that supervision in school settings may not only ensure tablet intake but also function as a behavioural support mechanism that reinforces adherence. High adherence has been identified as a key factor influencing the effectiveness of supplementation programmes, with previous studies reporting improved outcomes in settings with structured and supervised implementation (Bolka *et al.,* 2025).

Nevertheless, adherence was not quantified using standardised measures such as adherence rates or validated compliance scales. Therefore, although supervised administration and consumption logs suggest consistent intake, the extent to which adherence influenced the observed changes in haemoglobin levels cannot be precisely determined. Given that adherence is a key determinant of supplementation effectiveness, future studies should incorporate quantitative adherence measures to better assess its role in influencing outcomes.

It is also important to consider the difference in intervention exposure between groups. The comparison group did not receive supervised supplementation and continued their usual routines, although both groups received standard anaemia education. This difference in intervention intensity may have contributed to the variation in haemoglobin trajectories observed between groups. The use of repeated measures analysis allowed for the assessment of within-subject changes over time and provided insight into differences in trends between groups.

Overall, the findings of this study suggest that changes in haemoglobin levels were likely influenced by multiple interacting factors, including baseline haemoglobin status, supervision of supplementation, adherence behaviour, and differences in intervention exposure. This indicates that observed improvements over time should not be interpreted as the result of a single factor but rather as a combined effect of these conditions. From a methodological perspective, this study highlights the importance of incorporating baseline-adjusted analyses in intervention research to avoid overestimation of effects and to improve the accuracy of interpretation.

Several limitations should be acknowledged. The study was conducted in a single-school setting, which may limit generalisability. The non-random allocation of participants into intervention and comparison groups may introduce potential selection bias and should be considered when interpreting the findings. Although the calculated sample size met the minimum requirement for detecting mean differences between two time points, it was not specifically powered to detect time-by-group interaction effects in a repeated measures design. Detecting interaction effects generally requires a larger sample size due to the increased complexity of modelling within-subject correlations across multiple time points. Therefore, the present study may have been underpowered to detect smaller or moderate interaction effects, and this limitation should be considered when interpreting the findings. In addition, biochemical indicators of iron status were not assessed, and potential confounding factors such as dietary intake, menstrual patterns, and physical activity were not controlled.

From a practical perspective, these findings suggest that school-based anaemia prevention programmes should not only focus on supplement distribution but also integrate structured supervision, systematic adherence monitoring, and more rigorous evaluation approaches. Strengthening programme implementation with better monitoring systems and appropriate analytical methods may improve both the effectiveness of interventions and the validity of outcome evaluation.

CONCLUSION

This study suggests that supervised weekly iron-folic acid supplementation administered over a 12-week period was associated with improvements in haemoglobin levels among adolescent girls compared with the comparison group. The GLM-RM analysis indicated significant time effects and time–group interactions, reflecting changes in haemoglobin levels over time and differences in the pattern of change between groups. However, these findings should be interpreted with caution, as the observed effects may have been influenced by baseline differences between groups and limitations in adherence measurement. Therefore, causal inferences regarding the effectiveness of the intervention should be made cautiously. Strengthening school-based anaemia prevention programmes through the integration of structured supervision, systematic adherence monitoring, and continuous nutrition education may enhance programme implementation. Further studies using more rigorous designs and larger sample sizes are needed to confirm these findings and provide more robust evidence on the effectiveness of supervised supplementation.

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Authors' contributions

Rusmiati D, conceptualised the study, conducted data analysis, and drafted the manuscript; Wahyuni D and Ama PGB, contributed to study design and data collection; Siwiendrayanti A, contributed to data analysis and manuscript revision; Budiono I, critically reviewed the manuscript. All authors approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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