ORIGINAL RESEARCH ARTICLE

Food-to-food fortification: Effectiveness of red spinach-enriched snakehead fish floss for maternal anemia

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Abstract

Iron deficiency anemia remains a major public health challenge among pregnant women, while adherence to iron supplementation is frequently limited by gastrointestinal side effects. This study evaluated the effectiveness of a ready-to-eat red spinach-fortified snakehead fish (*Channa striata*) floss as a complementary food-based intervention for improving hemoglobin levels among pregnant women with anemia. A quasi-experimental pre-post study involved 60 pregnant women with mild-to-moderate anemia in Malang Regency, Indonesia. Participants were allocated to an intervention group receiving fortified fish floss alongside routine iron-folic acid supplementation or a control group receiving standard supplementation alone for 21 days. Hemoglobin concentrations were measured before and after the intervention and analyzed using paired and independent t-tests. The intervention group demonstrated a greater increase in hemoglobin levels than the control group ($p<0.001$). These findings suggest that integrating a locally produced ready-to-eat fortified food into routine antenatal care may enhance hemoglobin recovery as a complementary nutritional strategy. *(Afr J Reprod Health 2026; 30 [20]:111-120).*

Keywords: anemia, food-based intervention, functional food, pregnancy, snakehead fish

Résumé

L'anémie ferriprive demeure un défi majeur de santé publique chez les femmes enceintes, alors que l'observance de la supplémentation en fer est souvent limitée par des effets secondaires gastro-intestinaux. Cette étude a évalué l'efficacité d'un effiloché de poisson-tête de serpent (Channa striata) enrichi en épinard rouge-un produit prêt à consommer-en tant qu'intervention nutritionnelle complémentaire visant à améliorer le taux d'hémoglobine chez les femmes enceintes anémiques. Une étude quasi-expérimentale de type pré- et post-intervention a été menée auprès de 60 femmes enceintes souffrant d'une anémie légère à modérée dans le kabupaten de Malang, en Indonésie. Les participantes ont été réparties soit dans un groupe d'intervention recevant l'effiloché de poisson enrichi en plus de la supplémentation habituelle en fer et acide folique, soit dans un groupe témoin recevant uniquement la supplémentation standard, et ce, pendant 21 jours. Les concentrations d'hémoglobine ont été mesurées avant et après l'intervention et analysées à l'aide de tests t pour échantillons appariés et indépendants. Le groupe d'intervention a présenté une augmentation du taux d'hémoglobine plus importante que le groupe témoin ($p<0,001$). Ces résultats suggèrent que l'intégration d'un aliment enrichi prêt à consommer, produit localement, aux soins prénatals habituels pourrait favoriser le rétablissement du taux d'hémoglobine en tant que stratégie nutritionnelle complémentaire. *(Afr J Reprod Health 2026; 30 [20]: 111-120).*

Mots-clés: anémie, intervention alimentaire, aliment fonctionnel, grossesse, poisson-tête-de-serpent

Introduction

Iron deficiency anemia (IDA) remains one of the most prevalent nutritional disorders affecting pregnant women worldwide and continues to contribute substantially to maternal and neonatal morbidity, particularly in low- and middle-income countries.[1,2,3] The increased physiological demand for iron during pregnancy frequently exceeds dietary intake, placing women at elevated risk of anemia and its associated adverse outcomes, including preterm birth, low birth weight, impaired fetal development, postpartum complications, and increased maternal morbidity.[4,5,6] Although routine iron-folic acid (IFA) supplementation remains the cornerstone of maternal anemia prevention, its effectiveness in many settings is limited by poor adherence related to gastrointestinal side effects, inadequate dietary diversity, and insufficient consumption of iron-rich foods.[7,8] Consequently, complementary nutritional

strategies that are both biologically effective and feasible for long-term integration into routine antenatal care remain urgently needed.

To address these challenges, several complementary approaches have been implemented alongside iron supplementation, including food fortification, dietary diversification, nutrition education, and promotion of locally available nutrient-rich foods.[9,10] Among these, food-based interventions have gained increasing attention because they simultaneously improve dietary quality, provide multiple essential nutrients, and are more compatible with habitual eating practices than pharmaceutical supplementation alone. Food-to-food fortification, which combines locally available animal- and plant-source foods to enhance nutritional value, represents a particularly promising strategy in resource-limited settings.[11,12] Animal-source foods not only provide highly bioavailable protein and micronutrients but also contain the *meat factor*, which enhances intestinal absorption of non-heme iron derived from plant foods.[13,14] Conversely, iron-rich vegetables contribute iron, folate, vitamin C, and antioxidant compounds that support erythropoiesis and improve iron metabolism.[9,15,16] Therefore, combining complementary local food resources into practical dietary products may provide a sustainable strategy for improving maternal hemoglobin status.

Snakehead fish (*Channa striata*) is an indigenous freshwater fish widely recognized for its high-quality protein, albumin, essential amino acids, and minerals that support erythropoiesis and tissue repair.[17,18,19] Meanwhile, red spinach (*Amaranthus tricolor*) contains considerable amounts of iron, folate, vitamin C, calcium, and bioactive antioxidant compounds involved in red blood cell formation.[20,21] Importantly, the nutritional interaction between these ingredients extends beyond their individual nutrient composition. Animal protein from snakehead fish may enhance the bioavailability of non-heme iron supplied by red spinach through the *meat factor*, while vitamin C naturally present in red spinach facilitates the reduction of ferric iron into the more absorbable ferrous form. This complementary nutritional mechanism provides a strong biological rationale for combining both ingredients as a food-based intervention to support hemoglobin synthesis during pregnancy. Previous studies have demonstrated that food products fortified with indigenous ingredients possess favorable nutritional composition and high sensory acceptability, indicating their potential as functional foods. Likewise, Ummah et al. reported that supplementation using snakehead fish combined with *Moringa oleifera* significantly improved hemoglobin levels among pregnant women with anemia, providing evidence that locally available nutrient-rich foods can effectively complement routine iron supplementation.[22] Nevertheless, these studies primarily focused either on product development and nutritional characterization or on freshly prepared food combinations requiring continuous preparation before consumption. Such approaches may reduce convenience, consistency of intake, and scalability when implemented within routine maternal nutrition programs. Consequently, evidence remains limited regarding whether a ready-to-eat fortified functional food, developed from locally available ingredients and intended for routine daily consumption, can provide clinically meaningful improvements in maternal hemoglobin levels while offering greater practicality for implementation in antenatal care.

To address this implementation gap, the present study evaluated the effectiveness of red spinach-fortified snakehead fish floss as a ready-to-eat complementary food-based intervention for pregnant women with anemia. Unlike previous investigations that emphasized formulation development, nutritional characterization, or the clinical benefits of freshly prepared food combinations, this study focused on evaluating the clinical effectiveness of a shelf-stable fortified food specifically designed for routine dietary use during pregnancy. Therefore, the novelty of this research lies not merely in replacing *Moringa oleifera* with red spinach, but in translating locally available nutrient-rich ingredients into a clinically evaluated ready-to-eat functional food with greater feasibility for sustainable implementation within maternal nutrition programs. This implementation-oriented approach extends previous evidence by moving beyond product innovation toward evaluating how locally developed functional foods can realistically support routine antenatal nutritional management.

The findings of this study are expected to strengthen the evidence supporting food-based interventions as complementary strategies alongside conventional iron supplementation, particularly in

settings where maternal anemia remains prevalent despite established antenatal nutrition programs. By demonstrating the effectiveness of a culturally acceptable, shelf-stable, and locally produced fortified food, this research may contribute to the integration of indigenous food resources into maternal nutrition policies and primary health care services. Ultimately, the study provides translational evidence supporting scalable, community-based approaches for improving maternal hemoglobin status and reducing the burden of anemia during pregnancy.

Method

Study design

This study employed a two-stage experimental approach consisting of a preliminary product selection phase followed by a clinical effectiveness evaluation. The preliminary phase involved formulation optimization, sensory acceptability assessment, and laboratory nutritional analysis to identify the most appropriate fortified fish floss formulation. Since these activities served only to determine the intervention product, detailed product development procedures are not presented in this article. Instead, the present study focuses on evaluating the clinical effectiveness of the selected ready-to-eat fortified food as a complementary nutritional intervention for pregnant women with anemia. The clinical phase used a quasi-experimental pretest-posttest controlled design. This design was selected because the intervention was implemented under routine antenatal care conditions at public primary health care facilities, where individual randomization was not operationally feasible and could increase the risk of contamination between participants. Therefore, the study was designed to evaluate the effectiveness of the intervention under real-world service conditions while maintaining comparable clinical management between study groups. The study was conducted at selected primary health care centers in Malang Regency, East Java, Indonesia.

Participants and sample size

The target population comprised pregnant women diagnosed with iron deficiency anemia who attended routine antenatal care services at participating primary health care facilities.

The minimum sample size was calculated using the formula for comparing two independent means with a significance level (α) of 0.05, statistical power of 80%, and an anticipated moderate effect size derived from previous food-based intervention studies among pregnant women with anemia. The calculation indicated that at least 27 participants per group were required. To compensate for potential attrition and incomplete follow-up, the sample size was increased by approximately 10%, resulting in a final target of 30 participants per group (60 participants in total). Participants were recruited consecutively according to predefined eligibility criteria until the required sample size was achieved. Eligible participants were women in the second or third trimester of pregnancy with mild-to-moderate iron deficiency anemia (hemoglobin 8.0-10.9 g/dL), singleton pregnancy, routine attendance at antenatal care services, receipt of standard iron-folic acid supplementation, willingness to participate, and provision of written informed consent. Women with severe anemia (Hb <8.0 g/dL), multiple pregnancy, chronic diseases, pregnancy complications requiring referral, known allergy to fish or spinach, or inability to complete the intervention were excluded.

Group allocation

Because this study adopted a quasi-experimental design, participants were not individually randomized. Allocation was performed at the health service level to minimize contamination between intervention and control participants who attended the same antenatal sessions. Eligible pregnant women were recruited consecutively from participating primary health care facilities and assigned according to the intervention schedule implemented within each facility. Facilities providing the fortified food intervention constituted the intervention group, whereas facilities continuing routine antenatal nutritional management without fortified food constituted the control group. Both groups received identical antenatal care services delivered under the same district maternal health program. To evaluate baseline comparability, demographic characteristics, gestational age, and baseline hemoglobin concentrations were assessed before the intervention. Because baseline

hemoglobin differed between groups, the primary effectiveness analysis was based on changes in hemoglobin concentration (ΔHb) rather than post-intervention values alone.

Intervention product

The intervention consisted of a ready-to-eat snakehead fish (*Channa striata*) floss fortified with red spinach (*Amaranthus tricolor*), prepared using the optimal formulation identified during the preliminary product selection phase. The selected formulation contained 25 g of red spinach leaves per production batch and demonstrated the highest sensory acceptability together with the highest protein, calcium, and iron contents among all evaluated formulations. Unlike freshly prepared food combinations, the fortified fish floss was developed as a shelf-stable complementary food requiring no additional preparation before consumption, thereby improving practicality, storage stability, and potential integration into routine maternal nutrition programs.

Intervention procedures

Participants in the intervention group received the fortified fish floss in addition to routine antenatal nutritional care, whereas participants in the control group received routine antenatal nutritional care only. Each participant in the intervention group consumed 150 g/day of fortified fish floss for 21 consecutive days. The intervention duration was determined based on previous food-based nutrition intervention studies demonstrating measurable short-term changes in hemoglobin concentration within approximately three weeks while maintaining participant adherence during community-based implementation. Products were distributed weekly by the research team. Participants were instructed to consume the product as part of their daily meals without altering their habitual dietary patterns. Compliance was monitored using daily consumption records, weekly follow-up visits, and remaining product inspection. Participants whose compliance was below 80% were excluded from the final analysis. Both groups simultaneously received identical iron-folic acid tablets containing 60 mg elemental iron and 400μg folic acid according to the Indonesian national antenatal guideline. Compliance with iron tablet consumption was assessed using participant checklists and pill counts during follow-up visits.

Outcome measurements

The primary outcome was the change in hemoglobin concentration (ΔHb), calculated as: ΔHb = Post-intervention Hb - Baseline Hb. Hemoglobin concentration was measured immediately before the intervention and after completion of the 21-day intervention using a calibrated portable digital hemoglobin analyzer operated by trained health professionals following the manufacturer's standard operating procedures. The instrument was calibrated before each measurement session, and standardized blood collection procedures were implemented to minimize measurement variability. Secondary outcomes included participant adherence to the intervention protocol.

Control of potential confounding factors

Several procedures were implemented to reduce potential confounding. Both study groups: received identical iron-folic acid supplementation, attended routine antenatal care according to the same district guideline, received identical nutrition counseling, were followed over the same intervention period. Participants were advised to maintain their usual dietary habits and physical activity throughout the study. Because this pragmatic community-based study aimed to evaluate intervention effectiveness under routine service conditions, dietary intake outside the intervention was not quantitatively assessed using a Food Frequency Questionnaire or 24-hour dietary recall. Accordingly, residual dietary confounding cannot be completely excluded and has been acknowledged as a limitation of the present study.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics. Continuous variables are presented as means ± standard deviations, whereas categorical variables are summarized as frequencies and percentages. Data normality was assessed using the Shapiro-Wilk test. Baseline demographic and clinical characteristics between groups were compared using the independent-samples *t*-test or chi-square test, as appropriate. Within-group

changes in hemoglobin concentration were analyzed using the paired-samples *t*-test. Considering the observed difference in baseline hemoglobin concentrations between groups, the primary effectiveness outcome was defined as the mean change in hemoglobin concentration (ΔHb) rather than the post-intervention value alone. Between-group differences in ΔHb were evaluated using the independent-samples *t*-test. This analytical approach was selected to minimize the influence of baseline imbalance and provide a more appropriate estimate of intervention effectiveness. Statistical significance was established at $p < 0.05$ using two-sided tests.

Ethical considerations

The study protocol was approved by the Health Research Ethics Committee of Noor Huda Mustofa University before participant recruitment. All participants received written and verbal explanations regarding the study objectives, procedures, potential benefits, and possible risks before providing written informed consent. Participation was voluntary, and confidentiality of participant information was maintained throughout the study.

Results

Participant characteristics

A total of 60 pregnant women with mild-to-moderate anemia who met the eligibility criteria were enrolled in the study. Thirty participants were allocated to the intervention group and thirty to the control group. All participants completed the 21-day intervention period and were included in the final analysis, resulting in a 100% completion rate.

The intervention product was selected from a preliminary formulation study before the clinical evaluation. Baseline clinical characteristics were assessed before the intervention to determine comparability between groups. Both groups received identical routine antenatal care services, including iron-folic acid supplementation and nutritional counseling. Although the intervention group had a slightly lower baseline hemoglobin concentration than the control group, all participants met the inclusion criteria for mild-to-moderate anemia.

Table 1: Baseline Hemoglobin Concentration of Study Participants

Variable	Control	Intervention
Hemoglobin before intervention (g/dL), Mean ± SD	**10.77 ± 0.685**	**9.91 ± 0.888**

Table 2: Changes in Hemoglobin Concentration After the 21-Day Intervention

Variable	Control	Intervention
Baseline hemoglobin (g/dL)	**10.77 ± 0.685**	**9.91 ± 0.888**
Post-intervention hemoglobin (g/dL)	**11.33 ± 0.207**	**11.35 ± 1.609**
Mean change (ΔHb) (g/dL)	**0.56**	**1.44**
Within-group ***p***-value	**0.0001**	**0.0001**

Changes in hemoglobin concentration following the intervention

The Shapiro-Wilk test indicated that hemoglobin concentrations in both study groups were normally distributed before and after the intervention (all $p > 0.05$).

Therefore, parametric statistical analyses were considered appropriate for subsequent comparisons.

Following the 21-day intervention, significant improvements in hemoglobin concentration were observed in both study groups. In the control group, the mean hemoglobin concentration increased from 10.77 ± 0.685 g/dL before intervention to 11.33 ± 0.207 g/dL after intervention ($p = 0.0001$). In the intervention group, mean hemoglobin concentration increased from 9.91 ± 0.888 g/dL to 11.35 ± 1.609 g/dL ($p = 0.0001$).

Because baseline hemoglobin concentrations differed slightly between groups, intervention effectiveness was primarily evaluated using the change in hemoglobin concentration (ΔHb). The intervention group demonstrated a substantially greater mean increase in hemoglobin concentration (ΔHb = 1.44 g/dL) compared with the control group (ΔHb = 0.56 g/dL), indicating that supplementation with ready-to-eat red spinach-fortified snakehead fish floss produced greater improvement than routine antenatal nutritional management alone.

Discussion

The present study provides evidence that integrating red spinach-fortified snakehead fish (Channa striata) floss into routine antenatal nutritional care may enhance hemoglobin recovery among pregnant women with mild-to-moderate anemia. Unlike previous studies that primarily emphasized food formulation or nutritional composition, this study evaluated the clinical effectiveness of a ready-to-eat local functional food when implemented alongside standard antenatal care. The findings suggest that improving dietary quality through culturally acceptable local foods can complement existing maternal anemia management strategies. Rather than replacing iron supplementation, the intervention appears to strengthen routine nutritional care by providing additional dietary support that facilitates hematological recovery. These findings also emphasize the importance of translating locally available food resources into clinically applicable nutritional interventions. The present study therefore contributes not only to the evidence supporting food-based approaches for maternal anemia but also to their practical implementation within routine antenatal care services.

The observed improvement in hemoglobin concentration is biologically plausible because erythropoiesis depends on multiple interconnected physiological processes rather than iron availability alone.[23,24]

Hemoglobin synthesis requires adequate iron for heme formation, while dietary protein provides amino acids necessary for globin synthesis. In addition, folate and vitamin B12 support DNA synthesis during erythrocyte maturation, whereas vitamin C enhances non-heme iron absorption by converting ferric iron into its more absorbable ferrous form.[25,26,27] Consequently, successful correction of maternal anemia requires an adequate nutritional environment that supports iron utilization, red blood cell production, and tissue oxygen transport. These mechanisms explain why food-based nutritional interventions may provide additional hematological benefits beyond pharmaceutical supplementation alone. From a physiological perspective, correction of maternal anemia depends on improving the efficiency of iron utilization rather than simply increasing iron intake. Consequently , dietary interventions capable of supporting multiple metabolic pathways involved in erythropoiesis may generate greater clinical benefits than single-nutrient approaches.

Another important explanation relates to the concept of dietary synergy. Nutrients contained within whole foods interact biologically and collectively influence nutrient utilization.[20,28] Animal-source foods have consistently been shown to enhance the absorption of non-heme iron through the so-called meat factor, thereby increasing iron bioavailability from plant-based foods consumed simultaneously.[12,29] Rather than acting through a single nutrient, the intervention evaluated in this study likely improved erythropoiesis through complementary interactions between animal protein and plant-derived micronutrients. This interpretation supports the growing recognition that whole-food interventions should be evaluated according to their combined physiological effects rather than the contribution of individual nutrients alone. Such an approach is particularly relevant for maternal nutrition, where anemia often results from multiple nutritional deficiencies rather than isolated iron deficiency. This observation reinforces the growing shift from reductionist nutrient-based strategies toward whole-food dietary approaches that better reflect habitual eating patterns and are more feasible for long-term implementation in community settings.

One concern raised during peer review was the potential influence of anti-nutritional compounds, particularly oxalates naturally present in leafy vegetables. Oxalates are known to reduce calcium and non-heme iron absorption under certain dietary conditions.[30,31] Nevertheless, the favorable hematological response observed in this study suggests that the inhibitory effect of oxalates was unlikely to outweigh the overall nutritional benefits of the intervention. Several mechanisms may explain this finding. The enhancing effect of animal protein on iron absorption, concurrent administration of iron-folic acid tablets, and the presence of absorption-promoting nutrients may collectively compensate for the inhibitory activity of oxalates.[32,33] Furthermore, nutrient absorption occurs within complex dietary matrices where inhibitors and enhancers interact simultaneously. Therefore, the effectiveness of food-based interventions should be interpreted according to their overall physiological impact rather than the presence

of individual anti-nutritional compounds. Accordingly, evaluating food interventions within real-world dietary contexts provides more clinically meaningful evidence than assessing isolated nutrient interactions under laboratory conditions.

An important finding is that both study groups demonstrated significant improvement in hemoglobin concentration during the intervention period. This outcome was expected because all participants continued receiving routine iron-folic acid supplementation according to national antenatal care guidelines. Iron supplementation remains the primary intervention for preventing and treating maternal anemia and has consistently been associated with increased hemoglobin levels. However, the greater improvement observed in the intervention group suggests that dietary enhancement provided additional physiological benefits beyond pharmaceutical treatment alone. These findings reinforce current recommendations advocating integrated nutritional approaches that combine iron supplementation with dietary improvement to maximize maternal hematological recovery. Therefore, the intervention evaluated in this study should be considered a complementary nutritional strategy rather than an alternative to established antenatal supplementation programs. This finding supports current international recommendations advocating integrated maternal nutrition strategies in which dietary improvement complements, rather than substitutes for, routine iron-folic acid supplementation.

The difference in baseline hemoglobin concentration between the intervention and control groups also deserves consideration. Participants in the intervention group presented with slightly lower baseline hemoglobin levels than those in the control group, which may raise concerns regarding comparability between groups. Baseline imbalance is relatively common in pragmatic quasi-experimental studies conducted within routine health-service settings because participant allocation is determined by service delivery rather than individual randomization. Therefore, the observed difference should not necessarily be interpreted as evidence of systematic selection bias but rather as an inherent characteristic of implementation research. To minimize this potential bias, intervention effectiveness was interpreted primarily using the magnitude of hemoglobin change rather than post-intervention values alone. Nevertheless, because this study adopted a quasi-experimental design within routine antenatal care settings, residual confounding cannot be completely excluded. Another important limitation is that dietary intake outside the intervention was not quantitatively monitored using validated methods such as Food Frequency Questionnaires or repeated 24-hour dietary recalls. Consequently, differences in habitual dietary intake may have partially contributed to the observed hematological response. Although both groups received identical nutritional counseling and routine antenatal care, future randomized controlled trials incorporating comprehensive dietary assessment are required to better isolate the independent contribution of the intervention. Future investigations employing randomized allocation and statistical adjustment methods such as analysis of covariance (ANCOVA) may further reduce the influence of baseline imbalance and strengthen causal inference.

The present findings are consistent with recent international studies reporting that food-based nutritional interventions can improve maternal hemoglobin concentration when combined with routine iron supplementation. During the past five years, evidence from low- and middle-income countries has increasingly emphasized dietary diversification, greater consumption of animal-source foods, and increased intake of micronutrient-rich vegetables as effective complementary strategies for maternal anemia prevention. However, many previous interventions have relied on fresh foods or intensive dietary counseling, both of which require substantial participant commitment and may be difficult to sustain in routine practice.[34] In contrast, the intervention evaluated in this study utilized a ready-to-eat fortified food that requires minimal preparation and can be readily incorporated into habitual eating patterns. Consequently, this study extends existing evidence by demonstrating not only potential clinical effectiveness but also the feasibility of integrating locally produced functional foods into routine antenatal nutritional services. Recent intervention studies have consistently demonstrated that combining dietary improvement with routine iron supplementation results in greater hematological recovery than supplementation alone, particularly in populations with inadequate dietary diversity. Nevertheless , several previous studies

evaluated freshly prepared meals or intensive dietary counseling, approaches that may be difficult to sustain in routine maternal health services because they require substantial participant commitment and continuous food preparation. The present study extends this evidence by demonstrating that a shelf-stable ready-to-eat food can achieve comparable clinical benefits while offering greater practicality for routine implementation. The principal novelty of the present study lies in its implementation perspective. Our previous publications primarily focused on product formulation, sensory acceptability, and nutritional composition of fortified snakehead fish floss, while another study evaluated hematological improvement following consumption of fresh food interventions. In contrast, the present study investigated whether a ready-to-eat fortified food could improve maternal hemoglobin concentration when incorporated into routine antenatal care. Accordingly, the scientific contribution of this study is not the development of a new food product but the generation of preliminary implementation evidence supporting the use of locally available fortified foods as complementary nutritional interventions in maternal health services. This translational perspective distinguishes the present study from our previous work and enhances its relevance for clinical practice and public health nutrition. Accordingly, the novelty of this study should be interpreted from an implementation science perspective rather than from the development of a new food product. The present research bridges the gap between previous formulation studies and practical application within routine maternal health services.

From a policy perspective, these findings have practical implications for antenatal nutrition programs, particularly in countries where maternal anemia remains highly prevalent despite widespread iron tablet distribution. Current maternal nutrition programs continue to emphasize supplementation, whereas comparatively less attention has been directed toward improving dietary quality using locally available foods. Integrating culturally acceptable ready-to-eat fortified foods into routine antenatal care may strengthen existing nutritional interventions by improving dietary diversity and supporting adherence to maternal nutrition recommendations because the intervention utilizes locally available ingredients that are culturally familiar and easy to consume, it may also improve adherence to nutritional recommendations while supporting sustainable community-based maternal nutrition programmes. Nevertheless, these findings should be interpreted as preliminary evidence of complementary benefit. Broader implementation requires confirmation through larger multicenter studies evaluating effectiveness, feasibility, acceptability, and cost-effectiveness before incorporation into national maternal nutrition policies. This implementation approach is also consistent with the WHO recommendation that food-based strategies should complement micronutrient supplementation as part of comprehensive antenatal nutritional care.

This study has several strengths. It evaluated a food-based intervention under routine antenatal care conditions, thereby increasing the practical relevance of the findings for real-world maternal health services. Complete participant follow-up also minimized attrition bias. However, several limitations should be acknowledged. First, the intervention period lasted only 21 days, preventing evaluation of the long-term sustainability of hemoglobin improvement throughout pregnancy. Second, dietary intake outside the intervention was not assessed using validated dietary instruments, limiting the ability to control nutritional confounding. Third, hemoglobin concentration was used as the primary outcome without measuring biomarkers of iron metabolism such as serum ferritin, transferrin saturation, soluble transferrin receptor, or inflammatory markers. Finally, the quasi-experimental design limits causal inference because residual confounding and selection bias cannot be completely eliminated.

Future research should employ adequately powered randomized controlled trials with longer intervention periods extending throughout pregnancy. Comprehensive dietary assessment using Food Frequency Questionnaires or repeated 24-hour dietary recalls should be incorporated to better control dietary confounding. Future studies should also include biomarkers of iron status and inflammation to clarify the biological mechanisms underlying hematological improvement. Finally, implementation research evaluating participant adherence, cost-effectiveness, scalability, and integration within existing antenatal care programs is needed before this complementary food-based strategy can be recommended for wider implementation.

Conclusion

This study demonstrates that integrating ready-to-eat red spinach-fortified snakehead fish floss into routine antenatal care as a complementary food-based intervention significantly improves hemoglobin recovery among pregnant women with mild-to-moderate anemia beyond standard iron-folic acid supplementation alone. The findings support the potential of locally available, culturally acceptable, and shelf-stable functional foods to strengthen maternal nutrition programmes through practical and sustainable dietary strategies. Although larger randomized studies with longer follow-up and comprehensive dietary assessment are needed to confirm these findings, the present study provides important implementation evidence supporting the integration of locally produced fortified foods into routine antenatal nutritional care for the prevention and management of maternal anemia.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request, subject to appropriate ethical and institutional approvals.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

Wiqodatul Ummah contributed to the study design, data collection, and drafting of the initial manuscript. Santy Irene Putri was responsible for data analysis, interpretation of results, and substantial content editing. Woro Tri Utami participated in the methodology validation and final revision of the manuscript. Tessaldri Sukma Lodu participated in data collection, and interpretation of the findings. All authors have read and approved the final version of the manuscript and take full responsibility for its content.

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References

1. Rosson S, Pavord S. Understanding hepcidin for iron management in pregnancy. Transfus Med. 2025;35(2):109–15.
2. Zhou Y, Lyu Y, Ye W, Shi H, Peng Y, Wen Z, et al. The Prevalence of Anemia among Pregnant Women in China: A Systematic Review and Meta-Analysis. Nutrients. 2024;16(12):1–13.
3. Sun H, Weaver CM. Decreased Iron Intake Parallels Rising Iron Deficiency Anemia and Related Mortality Rates in the US Population. J Nutr [Internet]. 2021;151(7):1947–55. Available from: https://doi.org/10.1093/jn/nxab064
4. Damayanti D, Puspitasari Y, Indasah. Analysis of factors: Prevalence and determinants of anaemia among pregnant women in Mimika, Indonesia. Malahayati Int J Nurs Heal Sci. 2025;8(6):745–50.
5. Stephanie F, Yudhya M E, Purwara D L, Angkasa D, Wahyuni Y. Nutritional Intake and Hemoglobin Levels in Pregnant Women. Media Gizi Pangan. 2021;28(1):27–38.
6. Nivas S, Sahay V. Clinical Evaluation of Anemia Severity in Pregnant Women and Its Association with Maternal Morbidity and Neonatal Outcomes. Int J Pharm Qual Assur. 2025;16(2):393–8.
7. Mamme NY, Roba HS, Fite MB, Asefa G, Abrahim J, Yuya M, et al. Serum folate deficiency and associated factors among pregnant women in Haramaya District, Eastern Ethiopia: A community-based study. BMJ Open. 2023;13(5):1–8.
8. Musammah A. The Relationship Between Total Iron and Folic Acid Intake and Hemoglobin (Hb) Levels. Indones J Midwifery. 2023;3(1):13–20.
9. Malley ZA, Sauli E, Martin HD. Consumption of Mixed Indigenous Vegetables : A Solution to Low Hemoglobin Levels among Pregnant Women in Babati, Tanzania. Curr Res Nutr Food Sci. 2025;13(1):494–507.
10. Jaya N, Sary L, Astriana A, Putri RD. Benefits of Red Spinach (Amaranthus Gangeticus) for Increasing Hemoglobin Levels in Pregnant Women. J Kebidanan Malahayati. 2020;6(1):1–7.
11. Karomah U, Dewi NMPK, Putri LP. Effectiveness of Food Fortification to Anemia in Adolescents : A Systematic

Review and Meta-Analysis. Amerta Nutr. 2024;8(3):466–77.
12. Reddy MB, Agbemafle I, Armah S. Iron Bioavailability: Enhancers and Inhibitors. Nutrition and Health (United Kingdom). United States; 2022. 149 p.
13. Ginta S. Effectiveness of the Provision of Snakehead Fish Nuggets and Colored Fruit Extracts to Blood Protein (Total Protein, Albumin, HB) in PLHIV. J Phys Conf Ser. 2020;1665(1).
14. Ganap EP, Amalia RR, Sugmana PA, Hidayati LI, Hakimi M. The effect of snakehead fish (Channa striata) cookies supplementation on fetal growth and birth outcomes: A randomized clinical trial. Med J Nutrition Metab. 2022;15(3).
15. Spórna-Kucab A, Tekieli A, Kisiel A, Grzegorczyk A, Skalicka-Woźniak K, Starzak K, et al. Antioxidant and Antimicrobial Effects of Baby Leaves of Amaranthus tricolor L. Harvested as Vegetable in Correlation with Their Phytochemical Composition. Molecules. 2023;28(1463):1–22.
16. Mardiah SS, Andreyna G, Rismawan W. The Effect Consumption of Red Spinach Juice on Hemoglobin on Pregnant Woman. J Drug Ther. 2021;11(1):104–7.
17. Ummah W, Rahmawati MA, Ningrum NB, Kuswandari E. Increasing Hemoglobin (Hb) Levels on Anemic Postpartum Mothers With Snakehead Fish (Channa Striata) Extract. Proc Int Heal Conf [Internet]. 2024;1(5):159–66. Available from: https://jurnal.unigal.ac.id/IHC/article/view/14926
18. Fajri UN, Hadisaputro S, Soejoenoes A. The Effect of Snake Fish Extract (Channa striata) on Post Cesarean Section Wound Status in Postpartum Anemia Mothers. Indones J Med. 2020;3(2):84–8.
19. Nazir S, Khan N, Azmat H, Saima, Ramzan MM, Davies SJ. Effect of vitamin C (L-ascorbic acid) feed supplementation on the proximate composition, amino acids, and fatty acids profile of bullseye snakehead Channa marulius. Pakistan J Agric Sci. 2022;59(5):869–76.
20. Jahan FI, Bhuiyan MNH, Islam MJ, Ahmed S, Hasan MS, Bashera M, et al. Amaranthus tricolor (red amaranth), an indigenous source of nutrients, minerals, amino acids, phytochemicals, and assessment of its antibacterial activity. J Agric food Res. 2022;10.
21. Rustamsyah A, Noviyanti, Asterina Y, Khairunnisa A. Antioxidant Activity Test of Red Spinach Leaf (Amaranthus Tricolor L.) Extract and Fraction with Methode (2,2-DIPHENYL-1 PICRYLHYDRAZYL). SPECTA J Technol. 2025;9(2):162–70.
22. Ummah W, Putri SI, Utami WT. The Effectiveness of The Combination Snakehead Fish and Moringa Leaves on Increasing Hemoglobin Levels In Pregnant Women With Anemia. In: 6th International Conference of Health, Science and Technology (ICOHETECH). Surakarta: Duta Bangsa University; 2025. p. 164–71.
23. Obeagu GU, Obeagu EI. Complications of anemia in pregnancy An updated overview for healthcare professionals. Medicine (Baltimore). 2025;104(35).
24. Ramadhani J, Hasniah H, Saputri M. Impact of iron supplement tablet compliance on hemoglobin levels in pregnant women. J Innov Res Knowl. 2025;5(2).
25. Bansal A, Suri V, Sikka P, Attri SV, Varma N, Saini SS, et al. B12 Deficiency is the Commonest Cause of Anaemia During Pregnancy in Northern India: Study from a Tertiary Care Institute. Indian J Hematol Blood Transfus. 2024;40(1):78–82.
26. Sobowale OI, Khan MR, Roy AK, Raqib R, Ahmed F. Prevalence and Risk Factors of Vitamin B12 Deficiency among Pregnant Women in Rural Bangladesh. Nutrients. 2022;14(10):1–14.
27. Abrahamowicz W, Darmochwał-Kolarz D, Gonet-Sebastianka J, Hus I, Oleszczuk J. Iron deficiency anaemia in pregnancy. Ginekol i Poloznictwo. 2022;24(2):17–24.
28. Beluska-Turkan K, Korczak R, Hartell B, Moskal K, Maukonen J, Alexander DE, et al. Nutritional gaps and supplementation in the first 1000 days. Nutrients. 2019;11(12):1–50.
29. Simanjuntak R, Siahaan G, U S, Bakara T. Sustainability of freshwater snakehead fish empowerment as animal protein in increasing albumin and hemoglobin levels of breastfeeding mothers Sustainability of freshwater snakehead fish empowerment as animal protein in increasing albumin and hemoglobin l. In: The 6th International Conference on Agriculture, Environment and Food Security. Medan: Institute of Physics; 2023.
30. Fitriani H, Nurlailah N, Rakhmina D. Spinach Contains Oxalic Acid. Med Lab Technol J. 2016;2(2):51.
31. Piskin E, Cianciosi D, Gulec S, Tomas M, Capanoglu E. Iron Absorption: Factors, Limitations, and Improvement Methods. ACS Omega. 2022;7(24):20441–56.
32. Johnson TS, Souza A De, Amit S, Vora A. Safety assessment of red spinach as a potential sports nutrition supplement : An evidence based comprehensive overview. Futur foods. 2025;12(August).
33. Setyawati E, Sumarmi S, Nurasmi N, Irnawati I, Hutagaol IO. Effectiveness of moringa biscuit (Moringa oleifera) and snakehead fish (Channa striata) in improving the nutritional status of pregnant women with chronic energy deficiency. Afr J Reprod Health. 2024;28(10):41–9.
34. Santosh O, Bajwa HK, Indira A, Bano S, Dolma N, Bisht MS, et al. Novel Food Product Development Through Food-to-Food Fortification with Nutrient and Bioactive Compound-Rich Bamboo Shoot. Bamboo Sci Technol. 2023