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The Effectiveness of Moringa Leaf and Tuna Fish Oil Biscuits in Increasing Hemoglobin Levels and Breast Milk Production

[1]Kurnia Dewiani*, [2]Riana Versita, [1]Fitri Ramadhaniati, [1]Endah Purda Listya
Corresponding Author: *kdewiani@unib.ac.id
[1]Midwifery Study Program, Faculty of Mathematics and Natural Sciences, Bengkulu University, Indonesia
[2]Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Bengkulu University, Indonesia

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ABSTRACT

Anemia among breastfeeding mothers during the postpartum period may reduce breast milk production and compromise its nutritional quality. An innovative food-based supplementation strategy in the form of moringa (Moringa oleifera) leaf biscuits combined with tuna fish oil offers a practical intervention with a rich macro- and micronutrient profile that may enhance hemoglobin levels and breast milk production. This study aimed to evaluate the effectiveness of moringa leaf biscuits supplemented with tuna fish oil on hemoglobin levels and breast milk volume among postpartum women. A quasi-experimental study with a pretest–posttest control group design was conducted involving 50 postpartum women recruited from four community health centers. Participants were randomly allocated to an intervention group (n = 30) or a control group (n = 20). The intervention group received moringa leaf biscuits supplemented with tuna fish oil twice daily for 14 consecutive days, while the control group received standard care. Hemoglobin levels and breast milk volume were measured before and after the intervention. The results demonstrated a statistically significant increase in hemoglobin levels in the intervention group following supplementation, whereas no significant change was observed in the control group. The intervention group exhibited a mean hemoglobin increase of 14.03%, compared with 1.21% in the control group. Similarly, breast milk volume increased significantly in the intervention group (64.8%), while only a minimal increase (4.5%) was observed in the control group. These findings suggest that moringa leaf

biscuits combined with tuna fish oil are effective in improving hemoglobin levels and increasing breast milk production among postpartum mothers.

Keywords
Biscuits
Moringa leaves
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Introduction

The postpartum period is a critical phase in a woman's life, marked by significant physiological and psychological changes [1]. One of the most pressing health concerns during this time is postpartum anemia, which is characterized by hemoglobin (Hb) levels below 11 g/dL [2], [3]. Postpartum anemia is often an extension of antenatal anemia, exacerbated by blood loss during childbirth and the physiological demands of lactation. If left unaddressed, postpartum anemia can lead to severe complications, including uterine subinvolution, an increased risk of infection, reduced breast milk production, and impaired infant development [4]. Since exclusive breastfeeding is essential for infant survival and health, especially in low-resource settings like Indonesia, addressing postpartum anemia is not just a public health priority, but a crucial and urgent one [5].

While conventional strategies to combat postpartum anemia, such as iron supplementation, fortified foods, and dietary modifications, are in place, they are not without limitations. Poor compliance, often due to gastrointestinal side effects leading to discontinuation of iron supplements, is a significant issue [6]. Accessibility is another concern, as iron-rich foods like meat and liver may be unaffordable or culturally unacceptable in rural and low-income communities [7]. Moreover, these interventions often provide incomplete nutrition, lacking essential nutrients like protein and fatty acids crucial for optimal postpartum recovery and lactation [8]. Therefore, an innovative, accessible, and culturally acceptable solution that addresses anemia and supports lactation and maternal well-being is urgent.

Indonesia, particularly Bengkulu Province, has underutilized natural resources with exceptional nutritional profiles, such as tuna fish oil (*Thunnus* sp.) and moringa leaves (*Moringa oleifera*). Based on nutrition composition of 100 g dry weight of tuna fish is high in iron (14 mg/kg), protein (50 g), and omega-3 fatty acids [9]. In preliminary studies, it has demonstrated efficacy in improving hemoglobin levels and reducing anemia. It is traditionally used in soft gels and jelly candies for malnourished children, but has never been formulated

for postpartum mothers [10]. Further, moringa leaves are exceptionally rich in bioavailable iron (28 g/100 g dried leaves) and protein (27.1 g/100 g) [11]. They contain phytosterols, which enhance breast milk production (galactagogue effect) [12]. They are proven to boost immunity and accelerate postpartum recovery due to their vitamin A, C, and zinc content [13]. Despite their benefits, no study has explored the combined use of tuna oil and moringa leaves, let alone in a palatable, shelf-stable, easy-to-consume format, such as biscuits. This situation represents a critical research gap with immense potential for improving maternal and infant health outcomes.

Biscuits offer a revolutionary delivery mechanism for these nutrients due to cultural acceptability and widely consumed across socioeconomic groups in Indonesia [14]. They are easy to distribute, non-perishable, portable, and scalable for public health programs. Unlike pills or powders, biscuits are palatable and convenient, increasing adherence. Nutrient synergy: combining tuna oil (iron and omega-3s) with moringa (iron and galactagogues) creates a dual-action formula that combats anemia and boosts lactation.

Postpartum anemia remains a significant public health concern in resource-limited settings, contributing to impaired maternal health and suboptimal lactation outcomes. This study aims to develop and evaluate a novel functional food intervention in the form of a tuna oil–moringa (Moringa oleifera) biscuit for postpartum mothers in Bengkulu, Indonesia. The specific objectives are to: (1) formulate a biscuit containing optimal concentrations of tuna oil and moringa leaf extract to enhance iron bioavailability and support lactation; (2) assess its effectiveness in improving hemoglobin levels and breast milk production compared with standard iron supplementation; and (3) evaluate its acceptability among postpartum mothers to determine feasibility for sustained use.

The potential impact of this research is substantial. The intervention offers a scalable and culturally appropriate strategy for addressing postpartum anemia by utilizing locally available, sustainable food resources. Furthermore, it has the potential to strengthen national breastfeeding promotion efforts, thereby contributing to reductions in infant morbidity, mortality, and malnutrition. By integrating principles of nutrition science, food technology, and local knowledge, this study advances a comprehensive, women-centered approach to postpartum care that extends beyond anemia treatment to promote overall maternal and child health.

Material and Methods

This study was carried out in five stages: (1) preparation of tuna fish oil extract, (2) preparation of biscuits, (3) product characterization and preference testing, (4) health ethics review, and (5) preclinical testing 1. This study received ethical approval from the Health Research Ethics Committee of Poltekkes Kemenkes Bengkulu (Approval No. KEPK.BKL/624/07/2024). 1st-3rd stages were conducted at the Pharmacy Laboratory of Faculty of Mathematics and Natural Sciences, Bengkulu University and Indonesian National Agency of Drug and Food Control (BPOM), while the 4th was carried out by the Indonesian Health Ethics Commission (KEPK), and the 5th stage was preclinical testing 1.

Tuna fish biscuits and moringa leaf flour were prepared in collaboration with the Bengkulu City Community Health Center. This research was conducted in the working area of the Bengkulu City Community Health Center. The clinical component of Stage 5 employed a quasi-experimental study using a pretest–posttest control group design. Participants were randomly recruited from four community health centers: Pasar Ikan, Kualo Lempuing, Bawah, and Beringin Raya Padang Serai, yielding a total sample of 50 postpartum mothers. Of these, 30 participants were assigned to the intervention group and received the biscuit supplementation, while 20 participants were allocated to the control group and did not receive the biscuits.

The inclusion criteria comprised postpartum mothers who were actively breastfeeding infants aged 0–6 months and who provided informed consent to participate in the study. Exclusion criteria included mothers experiencing breastfeeding-related complications, such as breast engorgement, mastitis, blocked milk ducts, or other lactation disorders, as well as postpartum mothers who had experienced intrauterine fetal death (IUFD) or infant mortality. The dropout criteria included respondents who did not consume the biscuits as recommended twice a day, those who moved to an address outside the Bengkulu City area, which was unknown to the researcher, and who withdrew from the study. The biscuit intervention was given for 14 days at a total of 100 g/day, consumed in two 50 g portions in the morning and afternoon. Respondents were closely monitored for compliance with biscuit consumption, accompanied by monitoring of protein intake, which must amount to 70–77 g/day, through a food recall observation sheet.

Results and Discussions

The results of Stage 1, which focused on biscuit production, began with the extraction of tuna fish oil. Fresh tunas were selected from a local fish auction facility, then cleaned and cut

into small pieces prior to oil extraction. The extraction was performed using a dry-rendering oven-baking technique to obtain clear, high-quality oil. The oil yield was obtained after heating at 40–80 °C for two hours, resulting in a reddish-colored oil.

Physicochemical analysis indicated that the highest iodine value (7.76) was achieved at an extraction temperature of 80 °C. The lowest peroxide value (5.67 meq/kg) was observed at 40 °C, while the lowest moisture content (0.76%) was recorded at 80 °C. Free fatty acid levels increased proportionally with higher extraction temperatures. In addition, the combined moringa leaf flour and tuna fish oil contained 24.25% albumin.

Characterization of moringa leaf flour revealed that, per 100 g, the nutritional composition consisted of 13.61% moisture, 5.96% ash, 76.90% protein, 0.55% fat, and 3.53% carbohydrates, along with micronutrient contents of 3.09 mg zinc and 4.43 mg iron. These values comply with the quality standards for moringa leaf flour and fish oil as specified by the Indonesian National Standard (SNI) 01-2715-1996/Rev. 92.

Stage 2 involved biscuit formulation using four treatment groups: F0, F1, F2, and F3. F0 served as positive control and did not contain moringa leaf flour or tuna fish oil. Formulations F1, F2, and F3 contained moringa leaf flour combined with tuna fish oil at concentrations of 10%, 15%, and 20%, respectively. All other ingredients were maintained at constant quantities across formulations, including wheat flour (250 g), moringa leaf flour (30 g), cornstarch (30 g), egg yolks (2), refined sugar (100 g), margarine (150 g), powdered milk (50 g), vanilla essence (5 g), chocolate chips (to taste), and salt (5 g).

Biscuit preparation was conducted using standard kitchen equipment, including mixing bowls, spoons, molds, dough rollers, and an oven. The preparation process began by creaming refined sugar, egg yolks, margarine, tuna fish oil, salt, and vanilla essence until a homogeneous mixture was obtained. Subsequently, wheat flour, moringa leaf flour, powdered milk, and cornstarch were gradually incorporated and mixed until a smooth dough was formed. The dough was portioned into 10 g unit, shaped using molds, and topped with an appropriate amount of chocolate chips. The biscuits were then baked in an oven for 25 minutes until fully cooked.



Fig. 1. Moringa and tuna oil biscuits

Table 1. Macronutrient, iron, and sulfite levels in biscuits based on formulas (F0–F3) compared to SNI (Indonesian National Standard)

Component	Unit	Formula F0* (Control)	Formula F1*	Formula F2*	Formula F3*	Standard Limits SNI / Codex†
Protein	% weight	1,01 ± 0,20	5,20 ± 1,20	5,50 ± 0,21	9,30 ± 4,20	≥ 9% (SNI Nutritious Biscuits)
Fat	% weight	20,01 ± 0,30	25,74 ± 0,20	30,00 ± 3,01	31,00 ± 0,10	≥ 9,5% (SNI Nutritious Biscuits)
Carbohydrates	% weight	53,01 ± 0,20	59,16 ± 3,01	70,00 ± 0,11	70,00 ± 2,30	≥ 70% (SNI Nutritious Biscuits)
Iron (Fe)**	mg/ 100 g	1,10 ± 0,05	2,40 ± 0,12	3,90 ± 0,08	6,20 ± 0,15	≥ 4,5 mg/100 g (SNI Fortification)
Sulfite	ppm	5,2 ± 0,3	8,7 ± 0,4	11,0 ± 0,2	13,5 ± 0,5	≤ 300 ppm (Codex/SNI 7388:2009)

* F0–F3 denote biscuit formulations ranging from the control (F0) to the formulation with the highest level of functional ingredient addition (F3).
** Iron (Fe) represents the content of an essential mineral involved in prevention of anemia.
† The maximum permitted sulfite level for biscuit products is in accordance with the Codex Alimentarius and the Indonesian National Standard (SNI 7388:2009).

Table 1 presents an evaluation of the nutritional composition of biscuits across four formulations, with F0 serving as the control and F1–F3 representing the developed formulations. The results indicate that modifications in raw material composition substantially enhanced the nutritional quality of the biscuits. The analysis encompasses three principal components: macronutrient content (protein, fat, and carbohydrates), micronutrient content (iron), and food safety parameters as reflected by sulfite levels. All values are expressed as mean ± standard deviation (SD), providing insight into measurement variability and reproducibility across sample replicates. Lower standard deviation values indicate greater consistency and reliability of the measurements, whereas higher values suggest increased variability that may warrant further evaluation of the formulation or production process.

Protein is a key determinant of the suitability of biscuits as a functional food. Formula F0 contained only 1.01 ± 0.20% protein, which is substantially below the minimum requirement of 9% stipulated by the Indonesian National Standard (SNI) for nutritious biscuit products. Protein levels increased markedly in F1 (5.20 ± 1.20%) and F2 (5.50 ± 0.21%); however, these formulations still failed to meet the required standard. Only formula F3 achieved and exceeded the minimum threshold, with a protein content of 9.30 ± 4.20%. This improvement is likely attributable to the incorporation of protein-rich ingredients such as moringa leaf flour, milk powder, and fish oil. Nevertheless, the relatively high standard deviation observed in F3 indicates considerable variability between production batches, potentially arising from uneven ingredient distribution, differences in oven temperature, or inconsistencies in mixing duration. To ensure reproducibility and product sustainability,

stricter control of processing parameters and quality management systems is required to stabilize protein content across batches.

Regarding fat content, all formulations exceeded the minimum SNI requirement of 9.5%, beginning with F0 (20.01 ± 0.30%). Fat levels increased progressively in F1 (25.74 ± 0.20%), F2 (30.00 ± 3.01%), and F3 (31.00 ± 0.10%), indicating that fat constituted a major contributor to total energy content. Fat plays an important role in biscuit quality by enhancing texture, improving flavor, and extending shelf life through reduced microbial growth. However, excessively high fat levels should be carefully monitored, particularly in relation to saturated fat content, as this may negatively influence the perceived healthfulness of the product. Among the formulations, F2 exhibited the greatest variability (±3.01%), suggesting inconsistencies in fat measurement or ingredient incorporation. In contrast, the low standard deviation observed in F3 (±0.10%) indicates effective control of fat composition during production.

Carbohydrates in F0 (53.01 ± 0.20%) and F1 (59.16 ± 3.01%) remained below the minimum SNI standard of 70%. In contrast, formulations F2 (70.00 ± 0.11%) and F3 (70.00 ± 2.30%) met the required threshold. Carbohydrates serve as the primary energy source in biscuit products and are typically derived from cereal- or starch-based ingredients such as wheat flour, cassava flour, or rice flour. The achievement of optimal carbohydrate levels in F2 and F3 indicates that reformulation using complex carbohydrate-rich ingredients was successful. The low variability observed in F2 suggests homogeneous formulation and effective raw material control, whereas the higher standard deviation in F3 highlights the need for further refinement of starch ratios and dough mixing techniques.

One of the most significant outcomes of the reformulation process was the enhancement of iron (Fe) content, a micronutrient essential for anemia prevention. Formula F0 contained only 1.10 ± 0.05 mg/100 g of iron. This value increased in F1 (2.40 ± 0.12 mg/100 g) and F2 (3.90 ± 0.08 mg/100 g); however, only F3 (6.20 ± 0.15 mg/100 g) exceeded the minimum fortification threshold of ≥4.5 mg/100 g recommended by World Health Organization (WHO) guidelines and adopted in several SNI regulations. From a nutritional perspective, this increase represents a major achievement, as iron content is a critical indicator in the development of functional foods aimed at addressing anemia. The consistently low standard deviations across all formulations, including F3, indicate successful formulation and uniform distribution of iron-rich ingredients.

Food safety was assessed through analysis of sulfite levels, which are commonly used preservatives in processed foods, including biscuits. Sulfite concentrations increased gradually from F0 (5.2 ± 0.3 ppm) to F1 (8.7 ± 0.4 ppm), F2 (11.0 ± 0.2 ppm), and F3 (13.5 ± 0.5 ppm). Despite this upward trend, all values were far below the maximum permissible limit of 300 ppm established by the Codex Alimentarius and the Indonesian National Standard, indicating

that all formulations are safe for consumption. The observed increase in sulfite levels may be associated with the greater complexity of the formulations, including the use of additional binding agents or emulsifiers containing sulfur-based compounds. Given that sulfites may trigger adverse reactions in sensitive individuals, particularly those with asthma, continued monitoring and strict control remain essential. The low standard deviations observed across formulations suggest consistent and well-managed preservative usage.

Overall, formula F3 emerged as the most optimal formulation, as it met all minimum SNI requirements for protein, fat, carbohydrate, and iron content while remaining within safe limits for sulfite levels. Nevertheless, variability observed in certain parameters, particularly protein and carbohydrate content, indicates that further improvements in production techniques, such as enhanced dough homogenization and standardized raw material specifications are necessary. Additional evaluations, including sensory acceptability, shelf-life stability, and microbiological safety testing, are required to complete a comprehensive assessment of product quality. Provided that these evaluations yield favorable results, the F3 formulation demonstrates strong potential for development as a nutritious functional biscuit to support community-based nutrition interventions in Indonesia.

Following formulation, an organoleptic evaluation was conducted using a hedonic test involving 10 student panelists. The highest acceptance rates for color (96.67%) and aroma (63.33%) were observed in biscuits containing 20% moringa leaf flour. Texture acceptability was highest at 15% substitution (73.33%), while taste acceptance peaked at 10% substitution (58.33%). Statistical analysis revealed that moringa leaf flour substitution had a significant effect ($p < 0.05$) on aroma, taste, color, and overall appearance. Based on overall panelist acceptance, the biscuit formulation containing 15% moringa leaf flour (F3) was selected as the most acceptable formulation.

The final stage involved a preclinical evaluation of the biscuit's effectiveness in improving anemia status and breast milk volume among postpartum mothers. The demographic and clinical characteristics of the study participants are presented in Table 2.

Table 2. Characteristics of research subjects

Characteristics	Intervention Group (n=30)	Percentage (%)	Control Group (n=20)	Percentage (%)
Age (years)				
20-29	17	56.6	12	60
30-39	12	40	8	40
40-45 Years	1	3.4	0	0
Level of education				
Primary School	0	0	2	10
Middle School	4	13.4	15	75
High School	19	63.3	3	15

Characteristics	Intervention Group (n=30)	Percentage (%)	Control Group (n=20)	Percentage (%)
College	7	23.3	0	0
Employment				
Employee	1	3.4	0	0
Unemployed	29	96.6	20	100
Parity				
1	7	23.4	6	30
≥2	23	76.6	14	70
Body Mass Index (BMI)				
Underweight (<18,4)	0	0	0	0
Normal (18,5-25)	19	63.4	13	65
Overweight (25,1-27)	7	23.3	3	15
Obesity (>27)	4	13.3	4	20
Income (million)				
1-1.5	6	20	9	45
2-2.5	14	46.6	10	50
3-3,5	9	30	1	5
>3.5	1	3.4	0	0
Recent birth history				
Normal	28	93.4	18	90
Cesaria	2	6.6	2	10
Breastfeeding				
Exclusive	19	63.4	12	60
No/mixed	11	36.6	8	40

Table 2 presents the baseline characteristics of study participants in accordance with the predefined inclusion and exclusion criteria. An initial sample of 60 postpartum mothers was recruited; however, 10 participants were excluded during the study period due to noncompliance, six participants did not consume the biscuits daily as prescribed, and four participants did not adhere to the breast milk pumping protocol. Consequently, data from 50 respondents was included in the final analysis. Baseline information collected for all participants included age, education level, occupation, parity, family income, body mass index (BMI), breastfeeding practices, and mode of delivery.

The age distribution of participants was comparable between the intervention and control groups, with the majority aged 20–29 years (56.6% and 60%, respectively), followed by those aged 30–39 years (40% in both groups). This distribution reflects the typical reproductive age range for postpartum women. In terms of occupation, most respondents were not formally employed, accounting for 96.6% of the control group and 100% of the intervention group.

Educational attainment varied across groups, with the intervention group predominantly having completed senior high school (63.3%), while the control group primarily had secondary or senior high school education (75%). These findings indicate that anemia during the postpartum period occurred across different educational levels. Assessment

of nutritional status showed that most participants in both the intervention and control groups had a normal BMI (63.4% and 65%, respectively).

Parity distribution was similar between groups, with multiparous mothers comprising 76.6% of the intervention group and 70% of the control group. Family income among participants in the intervention group was most commonly in the range of IDR 2.0–2.5 million per month (46.6%), whereas in the control group, income was distributed primarily between IDR 1.0–1.5 million (45%) and IDR 2.0–2.5 million (50%). Regarding obstetric history, the majority of participants in both groups had experienced normal vaginal delivery (93.4% in the intervention group and 90% in the control group).

Breastfeeding practices were also comparable between groups. In the intervention group, 63.4% of mothers practiced exclusive breastfeeding, while 36.6% did not. Similarly, in the control group, 60% of mothers exclusively breastfed, whereas 40% did not. Overall, the baseline characteristics between the intervention and control groups were generally well balanced, indicating comparability prior to the intervention.

Table 3. Normality test of Hb levels and breast milk volume

Variable	Data Normality Test (p-value)	
	Intervention	Control
Before		
Hb	0.204	0.578
Breast milk volume	0.468	0.414
After		
Hb	0.069	0.538
Breast milk volume	0.804	0.064

Based on the result of normality test using Shapiro-Wilk, p-value > 0.05 was obtained for all variables. Based on these results, it can be concluded that all variables tested were normally distributed. Thus, further comparative tests will be conducted using a parametric statistical approach, namely paired t-tests on both groups with normally distributed data.

Table 4. Hemoglobin levels after giving moringa leaf biscuits and tuna fish oil

Group	Average Measurement		Percentage (%) Increase	p-value*
	Before	After		
Intervention	10.40	11.86	14.03%	<0.001
Control	11.10	11.23	1.21%	0.506

Table 4 shows a significant difference in the increase in Hb levels before and after biscuit administration, with a p-value < 0.001 and an average increase of 14.03%. Statistical analysis of the data indicates a significant relationship. The control group's results showed no significant difference in Hb levels over 14 days, as indicated by a p-value > 0.05 and an average increase of only 1.21%. These results prove that mothers treated with moringa leaf biscuits and tuna fish oil had higher Hb levels than those without treatment or the control group. In this study, the subjects were conditioned to have relative homogeneity, as seen in Table 2 regarding respondent characteristics. No respondents were in a state of malnutrition; all respondents had a BMI >18.5, and the history of childbirth with cesarean section was only <10%. The homogeneity of respondents is significant in preventing bias in the study.

Anemia is a condition in which the Hb level in the blood is lower than usual [15]. Hemoglobin is a component of red blood cells/erythrocytes that binds to oxygen and delivers it to all body tissue cells. The body tissues require oxygen to perform their functions. Lack of oxygen in the brain and muscle tissue causes symptoms, including a lack of concentration and fitness in performing activities. The diagnosis of anemia based on the WHO is carried out by laboratory examination of hemoglobin/Hb levels in the blood. Adolescent girls and those with childbearing age suffer from anemia if their blood hemoglobin levels are less than 12 g/dL [16], [17].

During pregnancy and the postpartum period, women are at increased risk of iron deficiency due to multiple factors, including inadequate dietary intake and iron supplementation, impaired iron absorption, low socioeconomic status, multiparity, and blood loss during childbirth. These factors contribute significantly to the development of anemia in late pregnancy and the postpartum period. Iron deficiency remains the most prevalent micronutrient deficiency globally and is the leading cause of anemia worldwide. It is estimated that approximately 50% of anemia cases are attributable to iron deficiency [18].

Women at high-risk reproductive ages are more susceptible to postpartum health complications, including anemia. This increased vulnerability is observed among women younger than 20 years, whose organ systems may not yet be fully mature, as well as among women older than 36 years, in whom physiological degeneration may reduce the body's capacity to store sufficient iron to compensate for blood loss during childbirth. Iron plays a critical role as an essential component of hemoglobin, and approximately 73% of total body iron is stored in the form of ferritin, which serves as a reserve to support erythropoiesis and maintain iron homeostasis.

The impact of postpartum anemia includes reduced physical ability, fatigue, and postpartum infections, and this condition can cause postpartum blues and the occurrence of

postpartum depression. Research in 2022 explains that there is a relationship between the history of anemia during pregnancy and the incidence of PP anemia in postpartum mothers [19]. In this case, midwives can provide counseling on preventing anemia in pregnant, postpartum, and postpartum women through good consumption patterns, namely eating with balanced nutrition, so that the prevalence of postpartum anemia can decrease. Sukaisi, in his research, explained that the treatment that can be done for postpartum mothers who experience anemia is by administering blood supplement tablets (Fe) and training in eating menu settings, such as consuming green vegetables or consuming foods that meet iron needs [20]. One option to meet iron needs is to consume green vegetables.

Regular consumption of moringa leaves, whether as vegetables or as juice, is efficacious in treating several types of diseases, one of which is preventing anemia because Moringa leaves are high in iron. Every 100 g of moringa leaves contains 2.3 grams of protein, 3.2 grams of carbohydrates, 3 grams of iron, and 81 grams of calcium. Spinach is also rich in vitamins and minerals, including vitamin A, C, niacin, thiamine, phosphorus, riboflavin, sodium, potassium, and magnesium [21]. The primary function of moringa leaves is to distribute oxygen throughout the body due to their high iron content. Another benefit of iron is that it is a component of cytochromes, proteins that interfere with photosynthesis, which is beneficial for patients with anemia. Spinach also contains substances that can prevent essential oxidation, and phytochemicals that protect the body against various diseases [22].

Moringa leaf contains nutrients, minerals, and essential amino acids. Every 100 g of dried moringa leaves contains twice as much protein as yogurt, seven times more vitamin A than carrots, three times more potassium than bananas, four times more calcium than milk, and seven times more vitamin C than oranges. In addition, moringa leaves also contain high levels of iron and other vitamins. Moringa leaves contain 28.2 mg of iron. Moringa leaves also contain B vitamins, including thiamine, riboflavin, niacin, pantothenic acid, biotin, vitamin B6, vitamin B12, and folic acid. The content of vitamin C, beta carotene, quercetin, and chlorogenic acids makes moringa leaves very rich in antioxidants. Moringa leaf extract, both old and young leaves, showed antioxidant activity against free radicals. The antioxidants in moringa leaves can prevent damage due to oxidation and provide adequate protection against cell damage [9].

As has been shown, moringa leaves contain iron, vitamin B12, folic acid, and vitamin C, which are important in increasing hemoglobin levels in the blood. Wahyunigtyas et al. (2019) added moringa leaf extract to Pukis cakes. The protein content found in moringa leaf extract Pukis was 10%, carbohydrates 12%, vitamin A 4%, vitamin E 10%, vitamin C 294%, calcium 9%, iron 12%, and zinc as much as 2% [23]. Moringa leaves contain several beneficial compounds, which have been observed in previous studies. One of the benefits that can be

obtained is the reduction of anemia. Based on research conducted by Suheti et al. (2020), moringa leaves can increase hemoglobin levels to a greater extent than green beans [24]. In addition, Pratiwi (2020) has also studied moringa leaf tea in anemic teenagers, which has increased hemoglobin levels [25]. Research conducted by Hastuti and Nitia (2022) also states that administering moringa leaf extract can increase hemoglobin levels in young women [26].

Apart from moringa leaves, tuna has the Latin name *Thunnus sp*. This pelagic marine fish belongs to the family *Scombroidae*. Tuna has a cigar-like body with a dorsal fin, the front fin usually separated from the back fin. The vitamin B6 content in tuna supports various important functions in the body. One of the benefits of vitamin B6 in the body is that it helps increase hemoglobin production [22].

Regular supplementation with moringa leaf biscuits combined with tuna oil was associated with a significant increase in Hb levels among postpartum mothers. This effect is likely attributable to the comprehensive macro- and micronutrient composition of the biscuits, particularly their high iron content (24.14 mg/kg), which supports erythropoiesis and hemoglobin synthesis.

Table 5. Breast milk volume after administration of moringa leaf biscuits and tuna fish oil

Variable	Average Measurement		Percentage (%) Increase	p-value
	Before	After		
Intervention	66.0	108.8	64.8%	<0.001
Control	66.5	69.5	4.5%	<0.001

Table 5 demonstrates a statistically significant increase in breast milk volume in the intervention group following biscuit supplementation ($p < 0.001$), with a mean increase of 64.8%. Although the control group, which did not receive the biscuit intervention, also showed a statistically significant increase in breast milk volume ($p < 0.001$), the magnitude of this increase was substantially smaller, with a mean increase of only 4.5%. These findings indicate that postpartum mothers who received moringa leaf–based biscuit supplementation experienced a markedly greater improvement in breast milk volume compared with those who did not receive the intervention.

Breast milk is the primary source of nutrition for infants and provides an optimal balance of nutrients necessary for growth and development. In addition to its nutritional value, breast milk contains immunological components that support the infant's immune system during early life, a period when infants are particularly vulnerable to infections caused by environmental microorganisms [27]. As the most suitable food for infants, breast milk should

be provided exclusively during the first six months of life, as it is uniquely adapted to the infant's digestive capacity. Exclusive breastfeeding during this period has been associated with optimal brain development, as well as enhanced language, motor, and emotional development [28].

The observed increase in breast milk production in both the intervention and control groups can be explained by the normal physiological processes of lactation, which occur in three distinct phases. *Lactogenesis I* takes place during late pregnancy and is characterized by a 10–20-fold increase in prolactin levels, enabling the mammary glands to produce colostrum. This phase is supported by human chorionic somatomammotropin, which exhibits mild lactogenic activity. However, colostrum secretion is partially inhibited by elevated progesterone levels during pregnancy, although production may still occur in some mothers. Lactogenesis II begins at childbirth with placental expulsion, resulting in a decline in progesterone, estrogen, and human placental lactogen levels. This hormonal shift permits the full lactogenic action of prolactin from the pituitary gland, leading to the onset of copious milk secretion [29].

Following delivery, milk secretion increases progressively but requires adequate stimulation to be maintained. This process is sustained during Lactogenesis III, the maintenance phase, in which frequent milk removal through breastfeeding or expression stimulates continued milk synthesis. When the breasts are regularly emptied, prolactin secretion is enhanced, thereby promoting increased milk production. Several factors influence this process, including breastfeeding frequency, maternal nutritional status, anemia, and psychological condition [30].

The substantially greater increase in breast milk volume observed in the intervention group (64.8%) compared with the control group (4.5%) may be attributed to the additional nutrient intake provided by biscuit supplementation. Dietary intake in both groups was carefully monitored using food recall methods, with daily energy intake maintained at approximately 2,200–2,300 kcal and protein intake ranging from 70 to 77 g/day. Breastfeeding frequency was also monitored, and all participants in both groups breastfed their infants more than eight times per day. These measures were implemented to ensure homogeneity between groups and to minimize the influence of potential confounding factors on breast milk production.

These findings are consistent with previous studies examining the effects of moringa-based supplementation on lactation outcomes. Research evaluating biscuits made from anchovy flour (*Stolephorus commersonii*) and moringa leaf flour (*Moringa oleifera*) demonstrated significant effects on postpartum maternal weight ($p = 0.006$) and breast milk

volume ($p < 0.001$). Similarly, Zakaria et al. (2016) investigated the effects of moringa leaf extract on breast milk quantity and quality among breastfeeding mothers of infants aged 0–6 months [31]. Participants were divided into two groups: one receiving moringa leaf extract capsules (800 mg per capsule, twice daily; n = 35) and the other receiving moringa leaf powder at an equivalent dose (n = 35). Breast milk quantity and quality were assessed before and three months after the intervention using the Byerley method, with quality evaluated based on iron, vitamin C, and vitamin E content. The increase in breast milk volume differed significantly between the extract and powder groups, with greater increases observed in the extract group; however, no significant changes were observed in breast milk iron, vitamin C, or vitamin E levels ($p > 0.05$). These findings suggest that moringa-based supplementation can enhance breast milk volume without adversely affecting milk quality.

Moringa leaves are recognized for their exceptional nutritional profile, containing substantially higher levels of vitamin A, vitamin E, protein, calcium, and iron compared with commonly consumed foods. In addition, tuna fish contributes valuable macronutrients that support lactation, with 100 g providing approximately 144 kcal of energy, along with substantial fat and protein content. Fish protein concentrate, produced by reducing fat and water content, further increases protein density and contributes to improved maternal energy intake. Adequate maternal nutrition supported by balanced functional foods such as moringa–tuna biscuits may therefore play a critical role in sustaining optimal breast milk production during the postpartum period [32].

Conclusion

This study observed associations between nutritional supplementation and improvements with moringa leaf and tuna fish oil biscuits in increasing hemoglobin levels and breast milk production in postpartum mothers. Our quasi-experimental study observed significant associations between consumption of moringa leaf and tuna fish oil biscuits and improved hematological and lactation outcomes in postpartum mothers. However, these findings demonstrate correlation rather than proven causation, and unmeasured confounders may have influenced results. Future research should investigate long-term outcomes, optimal dosing, and cost-effectiveness for potential public health applications.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Authors



Kurnia Dewiani is a member of the Midwifery Study Program, Faculty of Mathematics and Natural Sciences. She is a lecturer at the author's current institution. (email: kdewiani@unib.ac.id).



Riana Versita is a member of the Pharmacy Study Program, Faculty of Mathematics and Natural Sciences. She was a professional lecturer and loved to be a researcher. (E-mail: riana.versita@unib.ac.id)



Fitri Ramadhaniati is a member of the Midwifery Study Program, Faculty of Mathematics and Natural Sciences. She is a junior lecturer and a maternal-child and reproductive health expert (email: fitriramadhaniati@unib.ac.id).



Endah Purda Listya is a member of the Midwifery Study Program, Faculty of Mathematics and Natural Sciences. She is an expert in maternal-child health and reproductive health (email: eplistya@unib.ac.id)