

## Effect of spinach–sorghum cookie supplementation on hemoglobin concentration and maternal weight gain among pregnant women: a quasi-experimental study

*Efek Suplementasi Kue Bayam-Sorghum pada Konsentrasi Hemoglobin dan Penambahan Berat Badan Ibu di Kalangan Wanita Hamil: Studi Kuasi-Eksperimental*

Witri Priawantiputri<sup>1\*</sup>, Umi Mahmudah<sup>1</sup>, Mulus Gumilar<sup>1</sup>

<sup>1</sup>Jurusan Gizi Poltekkes Kemenkes Bandung, Bandung, Indonesia

\*Email: witri.priawantiputri@staff.poltekkesbandung.ac.id

### ABSTRACT

**Background:** Anemia during pregnancy remains a significant public health problem and is associated with adverse maternal and neonatal outcomes, including hemorrhage, preterm birth, miscarriage, and low birth weight. The development of locally sourced iron-rich supplementary foods, such as spinach and sorghum products, may help improve maternal nutritional status.

**Objective:** This study aimed to assess the association of spinach-sorghum cookie supplementation with changes in hemoglobin levels and body weight among pregnant women.

**Methods:** A quasi-experimental study using a one-group pretest–posttest design without a control group was conducted among 19 second- and third-trimester pregnant women selected through purposive sampling. Participants received 40 g/day of spinach-sorghum cookies as a supplementary snack for 14 consecutive days. Hemoglobin levels and body weight were measured before and after the intervention. Data were analyzed using the Paired Sample t-test with a significance level of 0.05.

**Results:** Mean hemoglobin levels increased from 11.41 g/dL before the intervention to 12.95 g/dL after the intervention ( $p=0.004$ ). Mean body weight increased from 67.54 kg to 69.03 kg following the intervention ( $p<0.001$ ). Spinach-sorghum cookie supplementation was associated with increased hemoglobin levels and body weight over the 14-day study period.

**Conclusion:** Fourteen-day supplementation with spinach–sorghum cookies was associated with increased hemoglobin levels and body weight among pregnant women, with mean increases of 1.54 g/dL and 1.49 kg, respectively. These findings suggest that spinach-sorghum cookies have potential as a locally sourced supplementary food to support maternal nutrition; however, controlled studies are needed to confirm their effectiveness.

**Keywords:** anemia, body weight, hemoglobin, pregnant women, spinach-sorghum cookies

### ABSTRAK

**Latar Belakang:** Anemia pada kehamilan masih menjadi masalah kesehatan masyarakat yang penting dan berhubungan dengan berbagai luaran buruk pada ibu dan bayi, termasuk perdarahan, persalinan prematur, keguguran, dan berat badan lahir rendah. Pengembangan pangan tambahan kaya zat besi berbahan pangan lokal, seperti bayam dan sorgum, berpotensi mendukung status gizi ibu selama kehamilan.

**Tujuan:** Penelitian ini bertujuan untuk menilai hubungan pemberian cookies bayam-sorghum dengan perubahan kadar hemoglobin dan berat badan pada ibu hamil.

**Metode:** Penelitian quasi-eksperimental dengan rancangan one-group pretest–posttest tanpa kelompok kontrol dilakukan pada 19 ibu hamil trimester II dan III yang dipilih menggunakan teknik purposive sampling. Partisipan menerima kukis bayam-sorgum sebanyak 40 g/hari sebagai makanan tambahan selama 14 hari berturut-turut. Kadar hemoglobin dan berat badan diukur sebelum dan sesudah pemberian intervensi. Data dianalisis menggunakan uji Paired Sample t-test dengan tingkat kemaknaan 0,05.

**Hasil:** Rata-rata kadar hemoglobin meningkat dari 11,41 g/dL sebelum intervensi menjadi 12,95 g/dL setelah intervensi ( $p=0,004$ ). Rata-rata berat badan meningkat dari 67,54 kg menjadi 69,03 kg setelah intervensi ( $p<0,001$ ). Pemberian cookies bayam-sorgum berhubungan dengan peningkatan kadar hemoglobin dan berat badan selama periode penelitian 14 hari.

**Kesimpulan:** Pemberian cookies bayam–sorgum selama 14 hari berhubungan dengan peningkatan kadar hemoglobin dan berat badan pada ibu hamil, dengan rerata peningkatan masing-masing sebesar 1,54 g/dL dan 1,49 kg. Temuan ini menunjukkan bahwa cookies bayam-sorgum berpotensi menjadi makanan tambahan berbahan pangan lokal untuk mendukung status gizi ibu selama kehamilan, namun penelitian dengan kelompok kontrol masih diperlukan untuk mengonfirmasi efektivitasnya.

**Kata kunci:** anemia, berat badan, cookies bayam-sorgum, ibu hamil, hemoglobin

## INTRODUCTION

The first 1.000 days of life, spanning from conception to a child's second birthday, represent a critical period that profoundly influences growth, development, and long-term health outcomes. Maternal nutritional status during pregnancy plays a fundamental role in supporting optimal fetal growth and development during this period.<sup>1</sup> Consequently, inadequate maternal nutrition may adversely affect both maternal and fetal health.

Anemia remains one of the most prevalent nutritional disorders affecting pregnant women worldwide and continues to be a major public health concern due to its association with adverse maternal and neonatal outcomes. According to the World Health Organization (WHO), anemia during pregnancy is defined as a hemoglobin concentration below 11 g/dL.<sup>2,3</sup> In Indonesia, maternal anemia continues to pose a substantial public health challenge despite ongoing prevention and supplementation programs.<sup>4</sup> National data from the 2013 Basic Health Research (Riskesdas) reported an anemia prevalence of 37.1% among pregnant women, which increased markedly to 48.9% in 2018.<sup>5,6</sup> More recent findings from the 2023 Indonesian Health Survey (Survei Kesehatan Indonesia/SKI) indicated a prevalence of 27.7%.<sup>7</sup> Despite this decline, maternal anemia continues to represent a substantial public health challenge due to its adverse consequences for maternal and neonatal health.

Maternal anemia has been associated with numerous unfavorable pregnancy outcomes, including low birth weight, preterm delivery, miscarriage, antepartum hemorrhage, premature rupture of membranes, and increased maternal morbidity.<sup>8</sup> These complications may further contribute to impaired child growth and development, including an elevated risk of stunting later in life.<sup>9</sup> The etiology of anemia during pregnancy is multifactorial and involves nutritional, physiological, and pathological factors. Major contributors include inadequate dietary intake, iron deficiency, impaired intestinal absorption, blood loss, and chronic medical conditions. Iron deficiency anemia, the most common type of anemia during pregnancy, primarily results from increased maternal iron requirements combined with insufficient intake or bioavailability of iron and other nutrients involved in erythropoiesis, including folate, vitamin B<sub>6</sub>, vitamin B<sub>12</sub>, and vitamin C.<sup>10,11</sup>

Iron requirements increase substantially throughout pregnancy. Daily iron needs rise from approximately 0.8 mg in the first trimester to around 7.5 mg in the third trimester.<sup>12</sup> Iron requirements increase substantially during pregnancy to support fetal and placental growth, expansion of maternal red blood cell mass, and blood losses associated with childbirth. Because many women enter pregnancy with inadequate iron stores, dietary intake alone is frequently insufficient to meet these increased physiological demands, making iron supplementation an important component of routine antenatal care.<sup>10,13</sup>

In addition to iron supplementation, the consumption of nutrient-dense foods rich in iron may help improve maternal nutritional status. Green leafy vegetables and cereals are among the food sources that contribute dietary iron. Spinach contains approximately 3.5 mg of iron per 100 g, while sorghum provides approximately 4.4 mg of iron per 100 g.<sup>14</sup> Furthermore, sorghum is recognized as a nutrient-dense cereal that contains substantial amounts of dietary fiber and essential minerals, including iron. Compared with commonly refined cereal flours, sorghum generally provides higher dietary fiber content and a richer micronutrient profile, making it a promising ingredient for functional food development.<sup>15,16</sup> These nutritional characteristics, combined with the iron content of spinach, support the potential use of spinach–sorghum products as supplementary foods for pregnant women.<sup>15–17</sup>

Cookies are considered a practical vehicle for supplementary feeding programs because they are convenient, shelf-stable, and generally well accepted due to their sweet taste and crisp texture.<sup>18</sup> A spinach–sorghum cookie formulation was previously developed by Rahmat et al., who evaluated its sensory acceptability and nutritional composition. Their laboratory-based study identified the selected formulation and reported that each 100 g of cookies contained 459.6 kcal of energy, 70.53 g of carbohydrates, 10.3 g of protein, 17.07 g of fat, 10.33 g of dietary fiber, and 2.51 mg of iron. However, the study was limited to laboratory-based product development and nutrient analysis and did not examine maternal nutritional outcomes following cookie consumption among pregnant women.<sup>19</sup>

Previous studies have also reported favorable changes in maternal nutritional indicators following supplementary feeding during pregnancy. Chandra Dewi et al. reported a mean weight gain of  $5.80 \pm 2.01$  kg among pregnant women with chronic energy deficiency who received supplementary foods.<sup>20</sup> Similarly, Amareta et al. found that pregnant women receiving supplementary feeding had a mean hemoglobin level of  $11.11 \pm 0.92$  g/dL.<sup>21</sup> However, unlike the supplementary foods evaluated in those studies, the spinach–sorghum cookie formulation developed by Rahmat et al. has not yet been investigated among pregnant women. Therefore, evidence regarding changes in hemoglobin levels and body weight following supplementation with this formulation is not yet available.

Therefore, this study aimed to assess the association of spinach–sorghum cookie supplementation with changes in hemoglobin levels and body weight among pregnant women using a one-group pretest–posttest design. It was hypothesized that mean hemoglobin levels and body weight would be higher after 14 days of spinach–sorghum cookie supplementation than before supplementation. The findings are expected to provide preliminary evidence supporting the utilization of locally available food resources as supplementary nutrition during pregnancy.

## METHODS

### Study design

This study employed a quasi-experimental study using a one-group pretest–posttest design, in which hemoglobin levels and body weight of pregnant women were measured

before and after the intervention. The study did not include a control group. The research was conducted in October 2025 in the working area of Pasirkaliki Public Health Center, Cimahi City, Indonesia.

#### Data source and sampling procedure

Primary data were collected directly from pregnant women who participated in the study. The study population consisted of second- and third-trimester pregnant women attending antenatal care services in the working area of Pasirkaliki Pasirkaliki Public Health Center. Participants were recruited using purposive sampling because not all pregnant women attending the health center met the eligibility criteria required for the intervention. This sampling approach enabled the recruitment of participants with relatively homogeneous characteristics, including second- or third-trimester pregnancy, age 19–40 years, routine consumption of iron supplementation tablets, parity of fewer than four children, absence of chronic diseases, and willingness to participate throughout the intervention period. The use of purposive sampling was intended to obtain a homogeneous sample that would facilitate the assessment of changes in hemoglobin levels and body weight following spinach–sorghum cookie supplementation.

The minimum required sample size was calculated using the formula for comparing two population means proposed by Lemeshow et al.22:

$$n = \frac{2\sigma^2 (Z\alpha + Z\beta)^2}{(\mu_1 - \mu_2)^2}$$

Where  $n$  is the minimum required sample size,  $\sigma$  is the assumed standard deviation,  $Z\alpha$  is the standard normal deviate corresponding to a two-sided significance level of 5% (1.96),  $Z\beta$  corresponds to a statistical power of 90% (1.28), and  $(\mu_1 - \mu_2)$  represents the expected mean difference. The calculation assumed a significance level of 5% ( $\alpha = 0.05$ ), a statistical power of 90% ( $\beta = 0.10$ ), a standard deviation of 3.2 kg based on Amareta et al.,23 and an expected mean difference of 3.441 kg based on Chandradewi et al.20 The corresponding standardized effect size (Cohen's  $d$ ) was **1.08**, indicating a large effect size. Based on these assumptions, the minimum required sample size was 19 participants. Therefore, a total of 19 eligible pregnant women were enrolled in the study.

#### Variables of the study

The independent variable in this study was spinach–sorghum cookie supplementation administered daily for 14 consecutive days. The dependent variables were maternal hemoglobin level (g/dL) and body weight (kg), which were measured before the intervention (pretest) and after the intervention period (posttest). Information regarding iron supplementation consumption and dietary intake was also collected to describe participant characteristics and to assist in the interpretation of the study findings.

#### Measurement and instruments

**Table 1. Nutrient composition of spinach–sorghum cookies per serving (50 g)**

| Nutrient          | Amount per serving<br>(50 g) | Recommended dietary intake (women<br>19–49 years) | Contribution<br>(%) |
|-------------------|------------------------------|---------------------------------------------------|---------------------|
| Energy (kcal)     | 229.3                        | 2200                                              | 10.4                |
| Carbohydrate (g)  | 33.5                         | 310                                               | 10.6                |
| Protein (g)       | 3.8                          | 60                                                | 6.3                 |
| Fat (g)           | 8.9                          | 60                                                | 14.8                |
| Dietary fiber (g) | 4.78                         | 27.5                                              | 17.0                |
| Iron (mg)         | 2.2                          | 18                                                | 12.2                |

The intervention product consisted of spinach–sorghum cookies prepared from spinach, sorghum flour, powdered sugar, palm sugar, egg yolk, margarine, butter, cornstarch, salt, vanilla, and baking powder. Participants received one package (50 g; four cookies) daily as a morning or afternoon snack for 14 consecutive days. The nutrient composition of the spinach–sorghum cookies is presented in Table 1. The serving size of 50 g was selected because it provides approximately 10–15% of the recommended daily nutrient intake from snacks for women aged 19–49 years. According to the Indonesian Food and Drug Authority (BPOM), the product qualifies as a source of iron because it contains 4.39 mg of iron per 100 g, exceeding the minimum requirement of 3.3 mg per 100 g.

Participants' compliance with cookie consumption was monitored by nutrition graduate enumerators through weekly home visits. Daily reminders were provided via WhatsApp to encourage participants to consume one package of cookies (four cookies) each day. Compliance was recorded using a daily cookie consumption form, accompanied by photographs of the remaining package when applicable. Participants were considered compliant if they consumed 3–4 cookies per day, equivalent to 21–28 cookies per week.

Maternal body weight was measured using a calibrated Camry digital weighing scale with a precision of 0.1 kg before the intervention (pretest) and after the 14-day intervention period (posttest). Before each measurement, the scale was placed on a flat, hard, and stable surface and was checked to ensure it displayed 0.0 kg. Participants were measured barefoot, wearing light clothing, and without carrying personal belongings. Each measurement was performed twice. If the difference between the two measurements exceeded 0.2 kg, a third measurement was performed, and the average of the two closest values was recorded. The weighing scale was checked daily before use and periodically verified using a standard 5-kg calibration weight. If a deviation greater than  $\pm 0.1$  kg was identified, the scale was recalibrated or replaced before data collection.

Hemoglobin concentration was measured using the EasyTouch GCHb Multi-Function Monitoring System with hemoglobin strips based on capillary blood sampling. Measurements were performed by trained medical laboratory personnel before the intervention (pretest) and after the 14-day intervention period (posttest), with results recorded in g/dL. Before blood collection, participants' fingers were cleaned with 70% alcohol and allowed to dry. A sterile disposable lancet was used to obtain capillary blood from the fingertip. The first drop of blood was discarded, while the second drop was applied to the hemoglobin strip according to the manufacturer's instructions. Hemoglobin concentration was displayed automatically on the device and recorded on the observation form. Used lancets and strips were discarded in a safety box following standard laboratory procedures. Before daily use, the instrument was checked for its physical condition, display function, battery capacity, and compatibility between the code chip and the hemoglobin strip lot number according to the manufacturer's recommendations. Hemoglobin strips were stored according to the manufacturer's instructions and were not used beyond their expiration date or if the packaging was damaged.

#### **Data collection**

Baseline data collection included measurements of hemoglobin level and body weight, assessment of dietary intake using a Semi-Quantitative Food Frequency Questionnaire (SQFFQ), and collection of information regarding iron supplementation consumption. Before the intervention, all participants received education on avoiding

foods that may inhibit iron absorption and were encouraged to continue consuming iron supplementation tablets or multiple micronutrient supplements (MMS) throughout the study period.

Participants then received one package of spinach–sorghum cookies daily for 14 consecutive days. Following the intervention period, hemoglobin level and body weight were reassessed using the same procedures and instruments as those used at baseline. Information regarding iron supplementation consumption and dietary intake was collected for all participants. Iron supplementation consumption was obtained based on participants' self-reported intake of iron supplements during pregnancy, while dietary intake was assessed using the SQFFQ. These data were used to describe participant characteristics and were considered in interpreting the study findings. However, iron supplementation consumption and dietary intake were not statistically controlled in the analysis. Gestational age was partially controlled through the inclusion criteria by including only pregnant women in the second and third trimesters, thereby minimizing variation due to gestational age. Other factors that may influence hemoglobin levels and body weight, including comorbidities, bleeding, infection, pre-pregnancy nutritional status, and adherence to iron supplementation, could not be fully controlled and are acknowledged as study limitations.

#### Ethical considerations

This study obtained ethical approval from the Health Research Ethics Committee of Poltekkes Kemenkes Bandung under approval number No. 4/KEPK/EC/IX/2025. The ethical clearance was valid from 13 October 2025 to 13 October 2026. Prior to participation, all respondents were informed about the objectives, procedures, benefits, and potential risks of the study and provided written informed consent. Participant confidentiality and anonymity were maintained throughout the research process.

#### Data analysis

Data were analyzed using statistical software. Descriptive statistics were used to summarize participant characteristics. Data normality was assessed using the Shapiro–Wilk test. Differences in hemoglobin levels and body weight before and after the intervention were analyzed using the paired *t*-test. Statistical significance was determined at a *p*-value of <0.05.

## RESULTS

**Table 2. Characteristics of Participants**

| Characteristics         | n(%)/ Mean ± SD |
|-------------------------|-----------------|
| Age (years)             |                 |
| <35                     | 16(84.2)        |
| ≥35                     | 3 (15.8)        |
| Gestational age (weeks) |                 |
| <28                     | 16(84.2)        |
| ≥28                     | 3(15.8)         |
| Parity                  |                 |
| <3 children             | 17(89.5)        |
| ≥3 children             | 2(10.5)         |
| Educational attainment  |                 |
| Elementary school       | 5(26.3)         |
| Junior high school      | 10(52.6)        |

| Characteristics                    | n(%) / Mean $\pm$ SD |
|------------------------------------|----------------------|
| Senior high school                 | 4(21.1)              |
| Occupation                         |                      |
| Housewife                          | 19(100.0)            |
| Baseline hemoglobin ( g/dL)        | 11.41 $\pm$ 1.72     |
| Baseline body weight (kg)          | 67.54 $\pm$ 16.15    |
| Dietary iron intake                |                      |
| Mean iron intake ( mg/day)         | 47.38                |
| <18 mg/day                         | 2 (10.5)             |
| $\geq$ 18 mg/day                   | 17 (89.5)            |
| Baseline anemia status             |                      |
| Anemia                             | 8 (42.1)             |
| Non-anemia                         | 11(57.9)             |
| Compliance with cookie consumption |                      |
| Non-compliant (14/week)            | 4 (21.1)             |
| Compliant (21–28/week)             | 15 (78.9)            |

Table 2 presents the baseline characteristics of the participants. Most participants were younger than 35 years (84.2%), were in the second trimester of pregnancy (84.2%), had fewer than three children (89.5%), and had completed junior high school (52.6%). All participants were housewives. The mean baseline hemoglobin level was 11.41  $\pm$  1.72 g/dL, and the mean baseline body weight was 67.54  $\pm$  16.15 kg. At baseline, 42.1% of participants were classified as anemic. Based on the SQFFQ assessment, the mean dietary iron intake was 47.38 mg/day, with 89.5% of participants consuming at least 18 mg/day of iron. Furthermore, 78.9% of participants were compliant with the prescribed cookie consumption during the intervention period.

**Table 3. Hemoglobin Levels of Pregnant Women Before and After Sorghum–Spinach Cookie Supplementation**

| Variable          | Mean $\pm$ SD    | Min | Max  | Median | p-value |
|-------------------|------------------|-----|------|--------|---------|
| Pre-intervention  | 11.41 $\pm$ 1.72 | 8.9 | 14.3 | 11.5   | 0.004   |
| Post-intervention | 12.95 $\pm$ 1.84 | 8.3 | 15.9 | 12.9   |         |

Table 3 presents hemoglobin levels before and after the 14-day sorghum–spinach cookie supplementation. The mean hemoglobin level increased from 11.41  $\pm$  1.72 g/dL before the intervention to 12.95  $\pm$  1.84 g/dL after the intervention. The paired-samples t-test showed a statistically significant difference between the pre- and post-intervention measurements ( $p = 0.004$ ).

**Table 4. Maternal Body Weight Before and After Sorghum–Spinach Cookie Supplementation**

| Variable          | Mean $\pm$ SD     | Min  | Max   | Median | p-value |
|-------------------|-------------------|------|-------|--------|---------|
| Pre-intervention  | 67.54 $\pm$ 16.15 | 43.9 | 102.0 | 64.9   | 0.000   |
| Post-intervention | 69.03 $\pm$ 15.67 | 46.5 | 103.0 | 66.1   |         |

Table 4 presents maternal body weight before and after the 14-day sorghum–spinach cookie supplementation. The mean body weight increased from 67.54  $\pm$  16.15 kg before the intervention to 69.03  $\pm$  15.67 kg after the intervention. The paired-samples t-test

indicated a statistically significant difference between the pre- and post-intervention measurements ( $p < 0.001$ ).

## DISCUSSION

### Characteristics of Respondents

Most participants were younger than 35 years (84.2%). Maternal age between 20 and 35 years is generally considered the optimal reproductive age and is associated with a lower risk of anemia and other pregnancy-related complications compared with pregnancies at advanced maternal age. According to the World Health Organization (WHO) and the Indonesian Ministry of Health, maternal nutritional status and physiological adaptation during pregnancy are influenced by age, with younger reproductive-age women generally demonstrating better pregnancy outcomes than women aged over 35 years.<sup>12,22</sup>

Most respondents were in the second trimester of pregnancy (<28 weeks of gestation), a critical period characterized by rapid fetal growth and substantial expansion of maternal blood volume. During this stage, iron requirements increase considerably to support maternal erythropoiesis and fetal development.<sup>23</sup> Therefore, nutritional interventions containing iron-rich ingredients, such as spinach and sorghum, may contribute to improving maternal hemoglobin levels during this period.

Approximately 89.5% of respondents had fewer than three children. High parity has been recognized as a risk factor for maternal anemia because repeated pregnancies increase cumulative nutritional demands and may progressively deplete maternal iron reserves, particularly when recovery time between pregnancies is insufficient.<sup>13</sup> Consequently, most respondents in this study may have had relatively better iron reserves, potentially enhancing their response to the nutritional intervention.

Educational attainment may also influence maternal knowledge regarding nutrition during pregnancy and adherence to dietary interventions. Higher educational attainment has been associated with improved nutrition knowledge and greater adherence to recommended antenatal interventions, including iron-folic acid supplementation, which may contribute to a lower risk of anemia during pregnancy.<sup>24</sup> This finding is consistent with the high compliance observed in the present study, where 78.9% of participants consumed the recommended number of sorghum-spinach cookies during the intervention period.

### Hemoglobin Levels of Pregnant Women

The findings demonstrated a significant increase in maternal hemoglobin levels following the consumption of sorghum-spinach cookies. The mean hemoglobin concentration increased by 1.54 g/dL, from 11.41 g/dL before the intervention to 12.95 g/dL after the intervention. This increase may be attributed to the nutritional composition of the cookies. Spinach provides non-heme iron, folate, and vitamin C, nutrients that are involved in red blood cell production and iron metabolism.<sup>25,26</sup> In addition, sorghum supplies protein, dietary fiber, magnesium, iron, and other essential micronutrients that support overall nutritional status and hematopoietic processes.<sup>15</sup>

Although spinach and sorghum naturally contain anti-nutritional factors, including oxalates, tannins, and phytates, thermal processing during cookie production may reduce the activity or concentration of some of these compounds, particularly tannins and phytates, while also modifying oxalate levels depending on processing conditions. Consequently, the inhibitory effects of these anti-nutrients on mineral availability may be partially reduced.<sup>27,28</sup> In addition, spinach is a natural source of vitamin C, an established enhancer of non-heme iron absorption. Ascorbic acid promotes iron absorption by reducing ferric iron ( $\text{Fe}^{3+}$ ) to the more absorbable ferrous form ( $\text{Fe}^{2+}$ ) and by forming

soluble iron–ascorbate complexes that help counteract the inhibitory effects of phytates and polyphenols on iron absorption.<sup>29</sup> These mechanisms may partly explain the significant improvement in hemoglobin levels observed in the present study, with mean hemoglobin increasing from 11.41 g/dL at baseline to 12.95 g/dL after 14 days of supplementation.

The increase in hemoglobin of 1.54 g/dL within 14 days is biologically plausible among pregnant women with mild anemia or borderline iron stores, particularly when routine iron supplementation is accompanied by improved dietary intake. The intervention cookies contributed approximately 12.2% of the daily iron requirement, 6.3% of the protein requirement, and additional energy intake, which may have supported erythropoiesis when combined with routine iron supplementation. Oral iron supplementation increases circulating iron availability and supports erythropoiesis, leading to a relatively rapid improvement in hemoglobin concentration when iron deficiency is not severe.<sup>30,31</sup> In addition to the routine iron (Fe) tablets provided through antenatal care, the intervention cookies supplied additional nutrients that support erythropoiesis, particularly iron and protein, which may have contributed to hemoglobin synthesis.<sup>32</sup> Consequently, the observed improvement in hemoglobin levels was likely the result of the combined effects of routine iron supplementation and the nutritional contribution of the cookies, rather than the cookies alone. Therefore, the increase in hemoglobin cannot be attributed solely to the intervention cookies. Furthermore, the relatively large standard deviation of hemoglobin change (SD = 2.05 g/dL) indicates substantial inter-individual variability, suggesting that the response to the intervention differed among participants and was likely influenced by differences in baseline nutritional status, iron stores, dietary intake, and individual physiological conditions.

The observed improvement may also be associated with the relatively high compliance rate among participants, with 78.9% consuming the cookies as recommended throughout the intervention period. Adequate adherence is an important determinant of the effectiveness of nutritional interventions because consistent nutrient intake is required to support erythropoiesis and maintain iron homeostasis.<sup>24, 10</sup> In addition, variations in baseline hemoglobin concentration, individual nutritional status, and iron bioavailability may influence the magnitude of hemoglobin response among pregnant women.<sup>13</sup> In addition, the palatable taste and practical form of cookie-based products may have facilitated adherence to the intervention. Acceptability and ease of consumption have been identified as important factors influencing adherence to nutritional interventions during pregnancy, which in turn may affect nutritional outcome.<sup>10,24</sup> Therefore, the increase in hemoglobin levels observed in this study is likely attributable not only to the nutrient content of the sorghum–spinach cookies but also to the high level of participant adherence throughout the intervention period. The improvement in hemoglobin levels is also influenced by consistent adherence to iron supplementation and dietary intake. Iron status response during pregnancy is highly dependent on baseline iron stores, supplementation adherence, and bioavailability of dietary iron.<sup>33</sup>

#### **Iron Metabolism, Hepcidin Regulation, and Mechanism of Anemia in Pregnancy**

Iron metabolism plays a central role in the development of anemia during pregnancy. Dietary iron exists in two forms, namely heme iron and non-heme iron. Non-heme iron, which is predominantly found in plant-based foods such as spinach and sorghum, has lower bioavailability because its absorption is strongly influenced by dietary enhancers and inhibitors. Vitamin C is known to increase non-heme iron absorption by reducing ferric iron (Fe<sup>3+</sup>) to ferrous iron (Fe<sup>2+</sup>), while phytates, polyphenols, and oxalates may

reduce iron absorption in the intestine.<sup>34</sup> Iron absorption mainly occurs in the duodenum via divalent metal transporter 1 (DMT1), followed by export through ferroportin into circulation and binding to transferrin for delivery to the bone marrow. In the bone marrow, iron is utilized for hemoglobin synthesis during erythropoiesis. Disruption in this pathway may contribute to reduced hemoglobin production and iron-deficiency anemia.<sup>34,35</sup>

Hepcidin is the key regulatory hormone of systemic iron homeostasis. It is produced by the liver and regulates iron availability by binding to ferroportin and inducing its degradation, thereby reducing intestinal iron absorption and iron release from macrophages. During pregnancy, hepcidin levels are physiologically suppressed, particularly in the second and third trimesters, to increase iron availability for maternal erythropoiesis and fetal growth.<sup>36,37</sup>

In pregnancy, suppression of hepcidin enhances dietary iron absorption and mobilization of iron stores. However, inflammation or infection may increase hepcidin levels and reduce iron availability, potentially contributing to anemia even when iron intake is adequate.<sup>36,37,13</sup> Spinach–sorghum cookies in this study may contribute to improved iron intake and micronutrient availability. Spinach provides non-heme iron, folate, and vitamin C, while sorghum contributes iron, protein, and energy that support erythropoiesis and overall maternal nutritional status.

#### **Maternal Body Weight**

Maternal body weight also increased significantly after the intervention, with a mean increase of 1.49 kg, from 67.54 kg to 69.03 kg. This finding suggests that sorghum–spinach cookies may have contributed additional energy and nutrients needed during pregnancy. Sorghum provides complex carbohydrates, dietary fiber, and essential micronutrients, whereas spinach contributes vitamins and minerals that support maternal nutritional status.<sup>15,26</sup> Overall, one serving of the intervention cookies contributed approximately 10.4% of daily energy requirements, 10.64% of carbohydrate requirements, 6.3% of protein requirements, 17% of dietary fiber requirements, and 12.2% of daily iron requirements for pregnant women, thereby complementing the habitual diet during pregnancy.

The average weight gain observed in this study is consistent with recommendations from the Institute of Medicine and current obstetric guidelines, which suggest that women during the second and third trimesters generally gain approximately 0.35–0.50 kg per week, depending on pre-pregnancy body mass index.<sup>38</sup> Given the duration of the intervention, the observed increase falls within an acceptable range and does not indicate excessive gestational weight gain.

These findings are in line with previous studies showing that nutrient-dense supplementary foods can support maternal nutritional status and gestational weight gain.<sup>20,21</sup> However, differences in weight gain across studies may be influenced by maternal nutritional status, gestational age, dietary intake, adherence, intervention duration, and the nutrient composition of the foods provided.<sup>22,39</sup>

Maternal weight gain during pregnancy is a physiological process influenced by multiple components, including fetal growth, placental development, amniotic fluid, expansion of maternal blood volume, and tissue accumulation. Maternal weight gain during pregnancy is a physiological process influenced by fetal growth, placental development, amniotic fluid, expansion of maternal blood volume, maternal tissue accumulation, and fluid retention. Therefore, short-term changes in body weight cannot be attributed solely to dietary interventions.<sup>38</sup> Since all participants were in the second and third trimesters of pregnancy, the observed mean weight gain of 1.49 kg likely reflected not only the nutritional contribution of the intervention cookies but also normal

physiological changes associated with pregnancy, including fetal growth, placental development, expansion of maternal blood volume, and fluid retention.

This study has several limitations. First, the study used a one-group pretest–posttest design without a control group, making it difficult to completely exclude the influence of external factors on the observed changes in hemoglobin levels and body weight. Second, the relatively small sample size may limit the generalizability of the findings to a wider population of pregnant women. Several confounding factors may have influenced the outcomes of this study. Although data on iron tablet consumption and dietary intake were collected using SQFFQ and participant reports, these variables were not statistically controlled. Therefore, they may affect hemoglobin levels and body weight outcomes. Gestational age was controlled through inclusion criteria; however, other factors such as infection status, comorbidities, baseline nutritional status, and adherence variability were not fully controlled and may contribute to differences in outcomes.

Therefore, future studies with larger sample sizes and randomized controlled designs are recommended to strengthen the evidence regarding the effectiveness of spinach-sorghum cookies during pregnancy.

## CONCLUSION

The provision of sorghum–spinach cookies for 14 consecutive days was associated with improved maternal hemoglobin levels and increased body weight among pregnant women. Mean hemoglobin concentration increased from 11.41 g/dL to 12.95 g/dL ( $p = 0.004$ ), while mean body weight increased from 67.54 kg to 69.03 kg ( $p < 0.001$ ). These findings suggest that sorghum–spinach cookies may contribute to improving maternal nutritional status when provided as a complementary nutritional intervention during pregnancy. However, the findings should be interpreted with caution because of the one-group pretest–posttest design, the absence of a control group, and the relatively small sample size, which limit causal inference and the generalizability of the results.

Further studies with larger sample sizes, longer intervention periods, and randomized controlled trial designs are recommended to confirm the effectiveness of sorghum–spinach cookies and to evaluate their long-term effects on maternal and birth outcomes. If proven effective in future controlled studies, sorghum–spinach cookies may be considered as an alternative supplementary food for pregnant women, particularly in community-based maternal nutrition programs.

## REFERENCES

1. Kesehatan Kementerian Republik Indonesia. Kualitas Manusia Ditentukan Pada 1000 Hari Pertama Kehidupannya. 2017. <https://kemkes.go.id/id/kualitas-manusia-ditentukan-1000-hari-pertama-kehidupannya>
2. World Health Organization. *Guideline on Haemoglobin Cutoffs to Define Anaemia in Individuals and Populations*. WHO; 2024.
3. Lewkowicz AK, Tuuli MG. Identifying and treating iron deficiency anemia in pregnancy. *Hematol Am Soc Hematol Educ Progr*. 2023;2023(1):223-228. doi:10.1182/hematology.2023000474
4. Putri JR, Pujonarti SA, Syafiq A, Endang Laksmningsih Achadi, Perkasa AIKG, Basrowi RW. Factors Associated With the Incidence of Anemia in Pregnant Women. *J Indones Spec Nutr*. 2025;18(1):26-33. doi:10.62817/jkbl.v18i1.408
5. Kemenkes RI. Laporan Riset Kesehatan Dasar (RISKESDAS) tahun 2013 dalam bentuk angka. 2013. Published online 2013.
6. Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan Republik Indonesia. *Riset Kesehatan Dasar (RISKESDAS) 2018*; 2019.

7. BKPK Kemkes RI. Survey Kesehatan Indonesia (SKI) Dalam Angka. *Jakarta, BKPK Kemkes, 2024.*
8. Wang R, Xu S, Hao X, et al. Anemia during pregnancy and adverse pregnancy outcomes: a systematic review and meta-analysis of cohort studies. *Front Glob Women's Heal.* 2025;6(January):1-12. doi:10.3389/fgwh.2025.1502585
9. Nadhiroh SR, Micheala F, Tung SEH, Kustiawan TC. Association between maternal anemia and stunting in infants and children aged 0-60 months: A systematic literature review. *Nutrition.* 2023;115:112094. doi:10.1016/j.nut.2023.112094
10. Cantor AG, Holmes R, Bougatsos C. Screening and Supplementation for Iron Deficiency and Iron Deficiency Anemia During Pregnancy: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force.2024.13546. *JAMA.* 2024;332(11):814-928. doi:10.1001/jama.2024.13546
11. Kirthan JPA, Somannavar MS. Pathophysiology and management of iron deficiency anaemia in pregnancy: a review. *Ann Hematol.* 2024;103:2637–2646. doi:https://doi.org/10.1007/s00277-023-05481-2
12. Kemenkes RI. *Buku Saku Pencegahan Anemia Pada Ibu Hamil Dan Remaja Putri.* Kementerian Kesehatan RI; 2023.
13. Sangkhae V, Fisher AL, Ganz T, Nemeth E. Iron Homeostasis during Pregnancy: Maternal, Placental, and Fetal Regulatory Mechanisms. *Annu Rev Nutr.* 2023;43:279-300. doi:10.1146/annurev-nutr-061021-030404
14. Kemenkes KKR. Tabel Komposisi Pangan Indonesia. Departemen Kesehatan. Jakarta. Published online 2020.
15. Tanwar R, Panghal A, Chaudhary G, Kumari A, Chhikara N. Nutritional, phytochemical and functional potential of sorghum: A review. *Food Chem Adv.* 2023;3(June):100501. doi:10.1016/j.focha.2023.100501
16. Saeed OS, Hong J, Zheng X KR. Sorghum Flour and Sorghum Flour Enriched Bread : *foods.* 2023;12(4221):1-27. doi:https://doi.org/ 10.3390/foods12234221
17. Fernando WMADB, Abdulraheem RA, Kariyawasam KP, et al. Sorghum and Sorghum-Based Products: Nutritional Composition, Prebiotic Potential and Health Benefits in Gut Microbiota Interactions. *Int J Food Sci.* 2025;2025(1):1-16. doi:10.1155/ijfo/7084868
18. Kementerian Kesehatan RI. Petunjuk Teknis Makanan Tambahan Balita dan Ibu Hamil. *Jakarta Direktorat Gizi Masy Kementrian Kesehat Republik Indones.* 2023;6(August):78-81.
19. Rahmat M, Priawantiputri W, Pusparini P. Cookies Bayam Dan TepungSorgum Kaya Akan Zat Besi Sebagai Makanan Tambahan Untuk Ibu Hamil Dengan Anemia. *J Ris Kesehat Poltekkes Depkes Bandung.* 2020;12(2):245-254.
20. Chandradewi A. Pengaruh Pemberian Makanan Tambahan Terhadap Peningkatan Berat Badan Ibu Hamil Kek (Kurang Energi Kronis) Di Wilayah Kerja Puskesmas Labuan Lombok. *J Kesehat Prima.* 2019;9(1):1391-1402.
21. Amareta DI. Hubungan Pemberian Makanan Tambahan-Pemulihan Dengan Kadar Hemoglobin Dan Kenaikan Berat Badan Ibu Hamil Kurang Energi Kronis (Studi Di Wilayah Kerja Puskesmas Jelbuk Kabupaten Jember). *J Ilm Inov.* 2016;15(2). doi:10.25047/jii.v15i2.25
22. World Health Organization (WHO). *WHO Recommendations on Antenatal Care for a Positive Pregnancy Experience.* WHO; 2016.
23. Harper LM, Tita A, Biggio JR. The Institute of Medicine Guidelines for Gestational Weight Gain after a Diagnosis of Gestational Diabetes and Pregnancy. *Am J Perinatol.* 2015;32(3):239-246. doi:10.1055/s-0034-1383846
24. Engidaw MT, Lee P, Fekadu G, Mondal P, Ahmed F. Effect of Nutrition Education during

- Pregnancy on Iron-Folic Acid Supplementation Compliance and Anemia in Low- and Middle-Income Countries: A Systematic Review and Meta-analysis. *Nutr Rev.* 2025;83(7):e1472-e1487. doi:10.1093/nutrit/nuae170
25. Hardinsyah; Supariasa IDN. *Ilmu Gizi Teori Dan Aplikasi*. EGC Medical Publisher; 2019.
  26. Sarma U, TR B. Dietary phytonutrients in common green leafy vegetables and the significant role of processing techniques on spinach: a review. *Food Prod Process Nutr.* 2024;6(1):1-11. doi:10.1186/s43014-023-00192-7
  27. Nageessa WB, Chambal B, Macuamule C. Effects of processing methods on phytate and tannin content of black small common beans (*Phaseolus vulgaris* L.) cultivated in Mozambique. *Cogent Food Agric.* 2023;9(2):1-15. doi:10.1080/23311932.2023.2289713
  28. Huynh NK, Nguyen DHM, Nguyen HVH. Effects of processing on oxalate contents in plant foods: A review. *J Food Compos Anal.* 2022;112:104685. doi:https://doi.org/10.1016/j.jfca.2022.104685
  29. Ems T, Lucia KS, Huecker MR. *Biochemistry, Iron Absorption*. StatPearls; 2023.
  30. World Health Organization (WHO). Daily iron and folic acid supplementation during pregnancy. 2024. Accessed June 28, 2026. <https://www.who.int/tools/elena/interventions/daily-iron-pregnancy>
  31. Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M, Fawzi WW. Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: Systematic review and meta-analysis. *BMJ.* 2013;347(7916):1-19. doi:10.1136/bmj.f3443
  32. World Health Organization. *Nutritional Anaemias: Tools for Effective Prevention and Control*. WHO; 2017.
  33. Mahayasa PD, Winata IGS, Setiawan WA. Iron Deficiency Anemia Treatment in Pregnancy. *Eur J Med Heal Sci.* 2022;4(4):36-37. doi:10.24018/ejmed.2022.4.4.1289
  34. Abbaspour N, Hurrell R, Kelishadi R. Review on Iron and its Importance for Human Health. *J Res Med Sci.* 2014;19(2):164-174.
  35. Camaschella C. Iron-Deficiency Anemia. *N Engl J Med.* 2015;372(19):1832-1843. doi:10.1056/NEJMra1401038
  36. Pasricha SR, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. *Lancet.* 2021;397(10270):233-248. doi:10.1016/S0140-6736(20)32594-0
  37. Koenig MD, Tussing-Humphreys L, Day J, Cadwell B, Nemeth E. Hepcidin and iron homeostasis during pregnancy. *Nutrients.* 2014;6(8):3062-3083. doi:10.3390/nu6083062
  38. American College of Obstetricians and Gynecologists. *Committee Opinion No. 548: Weight Gain during Pregnancy.*; 2013.
  39. Marshall NE, Abrams B, Barbour LA, et al. The importance of nutrition in pregnancy and lactation: lifelong consequences. *Am J Obstet Gynecol.* 2022;226(5):607-632. doi:10.1016/j.ajog.2021.12.035