

## Boiled egg supplementation as an adjunct to iron–folic acid therapy for hemoglobin improvement in pregnant women with mild anemia: a randomized controlled study

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### ABSTRACT

**Background.** Anemia in pregnancy is a common public-health problem, and iron deficiency is its leading cause. Standard care in Indonesia is daily iron–folic acid (IFA) supplementation, but hemoglobin recovery is often incomplete, and the added value of iron-rich animal-source foods over IFA alone has rarely been quantified with a control group. **Objective.** To measure the additional effect of two boiled chicken eggs per day, given together with standard IFA supplementation, on hemoglobin levels in pregnant women with mild anemia, compared with IFA supplementation alone. **Methods.** A randomized controlled quasi-experimental study and weekly repeated measurements was conducted over 21 days in Sewaka Village, Pemalang District, Central Java, Indonesia. From a source population of 65 pregnant women with mild anemia at 29–36 weeks of gestation, 34 were enrolled by purposive sampling and randomly allocated to two groups of 17. The intervention group received two boiled eggs (~100 g) daily plus IFA (60 mg elemental iron and 400 µg folic acid); the control group received the same IFA regimen without eggs. Capillary hemoglobin was measured with an Easy Touch GCHb analyzer at days 0, 7, 14, and 21. The primary analysis was the between-group difference in hemoglobin change from baseline to day 21, tested with an independent-samples t-test. **Results.** Baseline hemoglobin was comparable between groups (intervention  $9.70 \pm 0.40$  g/dL; control  $9.69 \pm 0.41$  g/dL). By day 21, hemoglobin reached  $12.00 \pm 0.45$  g/dL in the intervention group and  $11.50 \pm 0.46$  g/dL in the control group. Both groups improved significantly from baseline (both  $p < 0.001$ ):  $+2.30$  g/dL (95% CI 2.13 to 2.47) in the intervention group and  $+1.81$  g/dL (95% CI 1.52 to 2.10) in the control group. The between-group difference in hemoglobin change was 0.49 g/dL (95% CI 0.17 to 0.81;  $t(32) = 3.10$ ;  $p = 0.004$ ; Cohen's  $d = 1.06$ ), favoring egg supplementation. The gap between groups widened progressively (0.10, 0.30, and 0.50 g/dL at days 7, 14, and 21). **Conclusion.** Adding two boiled eggs daily to standard iron–folic acid supplementation produced a significantly larger hemoglobin increase than supplementation alone over 21 days, with a large effect size. Boiled eggs are an inexpensive, locally available food that can support anemia management in pregnancy alongside routine supplementation.

**Keywords:** boiled egg; hemoglobin; iron–folic acid; pregnancy; anemia; dietary supplementation

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## 1. INTRODUCTION

Anemia in pregnancy is defined as a hemoglobin concentration below 11 g/dL and is one of the most common nutritional disorders worldwide [1]. Iron deficiency is the largest single cause, because pregnancy raises iron requirements while plasma volume expands faster than red-cell mass, lowering hemoglobin concentration through hemodilution [1,8]. The World Health Organization estimates that about 37% of pregnant women aged 15–49 years are anemic, with the highest burden in the African and South-East Asia regions [1,2,3].

In Indonesia, the 2023 Indonesian Health Survey (SKI) reported a prevalence of 27.7% among pregnant women, a decline from 48.9% in the 2018 Basic Health Research (Riskesdas) but still a moderate public-health concern [4,5]. Maternal anemia is associated with fatigue and reduced work capacity, and in more severe cases with prolonged labor and postpartum hemorrhage; for the fetus it is associated with low birth weight and impaired growth [7,8,14].

The national strategy against maternal anemia is daily iron–folic acid (IFA) supplementation, with tablet distribution reaching about 92% of pregnant women [4,6]. Coverage does not guarantee response: adherence is often low, and even adherent women may not fully recover hemoglobin within a single trimester [4,6]. This gap has renewed interest in pairing supplementation with iron-rich, culturally accepted foods.

Eggs are an inexpensive animal-source food that is widely available in rural Indonesia. One whole chicken egg supplies about 6–7 g of protein and roughly 0.9–1.0 mg of iron, together with zinc and selenium, micronutrients involved in erythropoiesis [12]. Egg iron is mainly non-heme and modestly absorbed, partly because the yolk phosphoprotein phosvitin binds iron, but absorption improves when eggs are eaten with vitamin-C-rich foods, and boiling preserves the protein and mineral content without added fat [12]. Food safety during preparation is also relevant; natural antimicrobial agents have been studied at the same institution for related hygiene applications [16].

Non-pharmacological approaches to support maternal health during the third trimester have received increasing attention, including complementary therapies for pain management [15] and dietary supplementation with iron-rich foods. Several Indonesian studies report that boiled egg consumption raises hemoglobin in anemic pregnant women [9]. Internationally, a randomized trial of daily egg supplementation in young Malawian children found no effect on iron status, but the population, dosage, and concurrent supplementation differed from those in pregnant women receiving iron–folic acid [10]. An education-based intervention that promoted iron-rich foods, including eggs, improved hemoglobin in anemic pregnant women in Nepal [13], and a health-education package improved supplementation compliance and hemoglobin in Saudi Arabia [11]. Most egg-specific studies in Indonesia, however, used a single-group before-and-after design without a control group, so the measured increase could not be separated from the effect of the iron tablets taken at the same time. The specific contribution of eggs over and above standard supplementation has therefore remained unclear.

This study addressed that gap using a two-group controlled design in which both groups received identical IFA supplementation and only the intervention group received boiled eggs. The objective was to measure the additional effect of two boiled eggs per day on hemoglobin levels in pregnant women with mild anemia, compared with IFA supplementation alone.

## 2. METHODS

### 2.1 Study design and setting

This was a randomized controlled study with repeated weekly hemoglobin measurements, carried out over a 21-day period in Sewaka Village, Pemalang District, Central Java, Indonesia in December 2024. Participants were recruited through the village primary health center (Puskesmas) and integrated health posts (Posyandu). The study is reported in line with the applicable items of the CONSORT statement for randomized trials.

## 2.2 Participants

The source population comprised 65 pregnant women with mild anemia receiving antenatal care at the study facilities. Using purposive sampling, 34 women who met the eligibility criteria were enrolled and assigned to an intervention group ( $n = 17$ ) and a control group ( $n = 17$ ). Inclusion criteria were a gestational age of 29–36 weeks, a diagnosis of mild anemia (hemoglobin 9.0–10.9 g/dL), willingness to participate, and no known egg allergy. Women with moderate or severe anemia, or with other conditions known to affect hemoglobin, were excluded. Participant flow is shown in Figure 1.

## 2.3 Intervention

Both groups continued standard antenatal care, including one iron–folic acid tablet daily providing 60 mg of elemental iron and 400  $\mu$ g offolic acid. In addition, the intervention group consumed two whole boiled chicken eggs (approximately 100 g in total) once daily for 21 consecutive days. The control group received the iron–folic acid tablet only, with no egg supplementation. Participants in both groups continued their usual diet with no restriction on other foods. Egg administration followed a written standard operating procedure to keep preparation and timing consistent. Table 1 summarizes the daily and cumulative composition of each regimen.

**Table 1.** Daily and cumulative composition of the two study regimens over 21 days

| Component                                   | Intervention group | Control group |
|---------------------------------------------|--------------------|---------------|
| Boiled eggs per day                         | 2 eggs             | 0 eggs        |
| Total eggs over 21 days                     | 42 eggs            | 0 eggs        |
| Elemental iron from tablet per day          | 60 mg              | 60 mg         |
| Folic acid per day                          | 400 $\mu$ g        | 400 $\mu$ g   |
| Additional dietary iron from eggs per day   | ~2 mg              | 0 mg          |
| Total iron intake from tablets (21 days)    | 1,260 mg           | 1,260 mg      |
| Additional dietary iron from eggs (21 days) | 42 mg              | 0 mg          |

|                                                  |          |          |
|--------------------------------------------------|----------|----------|
| Total intervention-related iron intake (21 days) | 1,302 mg | 1,260 mg |
|--------------------------------------------------|----------|----------|

*IFA, iron–folic acid. Iron content of eggs is estimated at approximately 1 mg per egg.*

## 2.4 Outcome and measurements

The outcome was capillary hemoglobin concentration, measured from a peripheral blood sample using an Easy Touch GCHb point-of-care analyzer with a lancet, auto-click device, and alcohol swabs. Measurements were taken at four time points: baseline (day 0) and days 7, 14, and 21. Values were recorded on a structured observation sheet. The primary outcome was the change in hemoglobin from baseline to day 21, compared between groups.

Report the manufacturer and measurement precision of the Easy Touch GCHb analyzer (for example, coefficient of variation) and state whether it was calibrated before use.

## 2.5 Statistical analysis

Continuous data are summarized as mean  $\pm$  standard deviation (SD). Normality of hemoglobin values and change scores was assessed with the Shapiro–Wilk test, and all variables were consistent with a normal distribution, so parametric tests were used. Baseline comparability between groups was examined with an independent-samples t-test. Within-group change from baseline to day 21 was tested with a paired-samples t-test, and the four-time-point trajectory with repeated-measures analysis of variance.

The primary analysis was the between-group difference in hemoglobin change from baseline to day 21, tested with an independent-samples t-test on change scores. Effect sizes were reported as Cohen's  $d_z$  for within-group change and Cohen's  $d$  for the between-group difference. A two-sided  $p$ -value below 0.05 was considered statistically significant. Analyses were performed with SPSS Statistics version 30.0 (IBM Corp., Armonk, NY, USA).

## 2.6 Ethical considerations

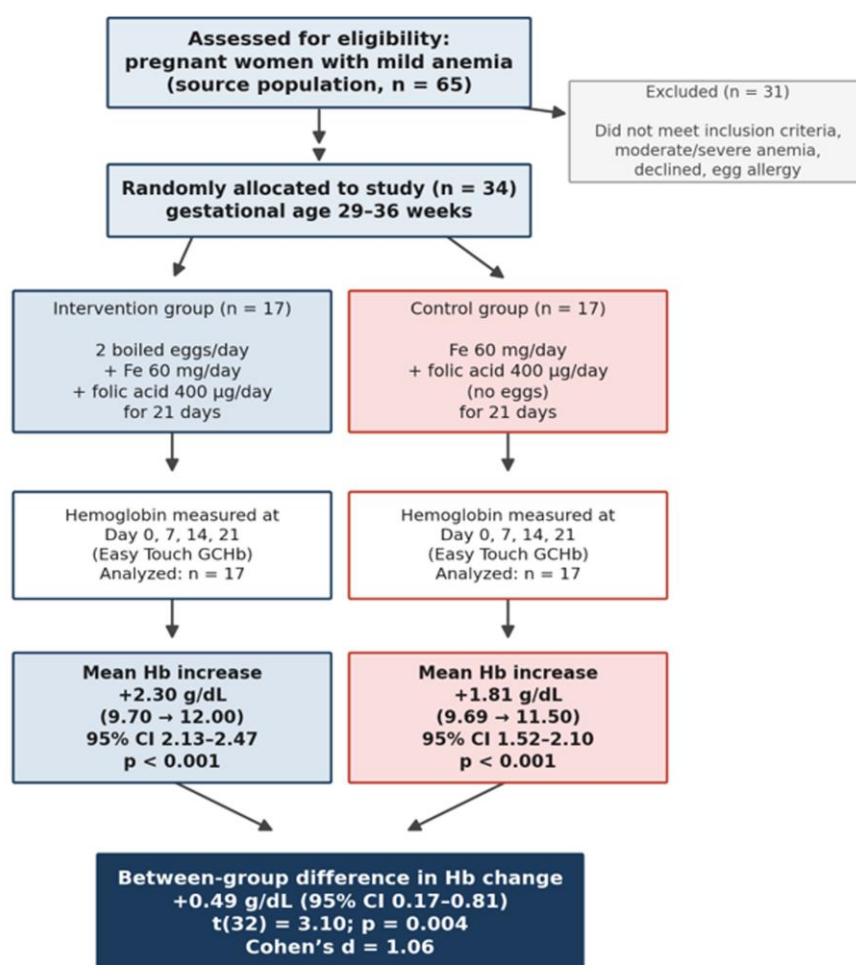
The study was approved by the Health Research Ethics Committee of Universitas Karya Husada Semarang, and an ethical clearance certificate was issued before data collection (No.

249/KEP/UNKAHA/SLE/VIII/2024, August 2024). Written informed consent was obtained from all participants before enrolment.

### 3. RESULTS

#### 3.1 Participant characteristics

Thirty-four pregnant women at 29–36 weeks of gestation with mild anemia completed the study, 17 in each group; there were no dropouts. Participant flow is shown in Figure 1.



**Figure 1.** Participant flow through the study, from the source population of 65 pregnant women to two analyzed groups of 17, with the primary between-group result at day 21.

The two groups were similar in maternal age, body mass index, parity distribution, and baseline hemoglobin (Table 2), supporting comparability at the start of the study.

**Table 2.** Baseline characteristics of participants by group (n = 34)

| Characteristic                 | Intervention (n=17) | Control (n=17) |
|--------------------------------|---------------------|----------------|
| Maternal age, years, mean ± SD | 28.82 ± 2.83        | 28.82 ± 2.83   |

|                                                |              |              |
|------------------------------------------------|--------------|--------------|
| Body mass index, kg/m <sup>2</sup> , mean ± SD | 24.71 ± 1.30 | 24.65 ± 1.19 |
| Gestational age                                | 29–36 weeks  | 29–36 weeks  |
| Primigravida (parity 0), n (%)                 | 4 (23.5)     | 4 (23.5)     |
| Parity 1, n (%)                                | 8 (47.1)     | 8 (47.1)     |
| Parity 2, n (%)                                | 4 (23.5)     | 4 (23.5)     |
| Parity ≥3, n (%)                               | 1 (5.9)      | 1 (5.9)      |

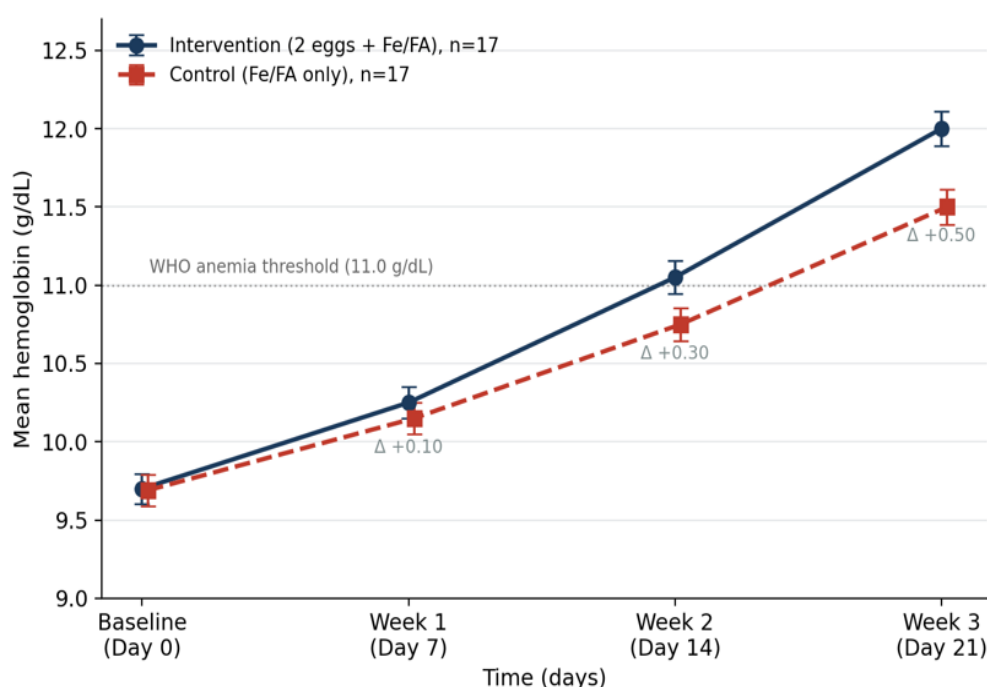
Baseline hemoglobin, 9.70 ± 0.40 9.69 ± 0.41  
g/dL, mean ± SD

SD, standard deviation.

### 3.2 Hemoglobin over time

Baseline hemoglobin was almost identical in the two groups (9.70 ± 0.40 g/dL in the intervention group and 9.69 ± 0.41 g/dL in the control group; difference 0.01 g/dL). Hemoglobin rose in both groups at every

measurement, consistent with the iron–folic acid supplementation received by all participants. In the intervention group, mean hemoglobin increased to 10.25 ± 0.41 g/dL at day 7, 11.05 ± 0.43 g/dL at day 14, and 12.00 ± 0.45 g/dL at day 21. In the control group, it increased to 10.15 ± 0.42, 10.75 ± 0.44, and 11.50 ± 0.46 g/dL over the same time points. By day 21 the intervention group had passed the 11 g/dL threshold used to define anemia in pregnancy, whereas the control group reached it more slowly. These values are shown in Table 3 and Figure 2.



**Figure 2.** Mean hemoglobin trajectory over 21 days; error bars show the standard error of the mean. The between-group gap ( $\Delta$ ) widened from 0.10 g/dL at day 7 to 0.50 g/dL at day 21. The dotted line marks the WHO anemia threshold of 11.0 g/dL in pregnancy.

**Table 3.** Mean hemoglobin (g/dL) by group at each time point, with between-group difference

| Time point       | Intervention (mean ± SD) | Control (mean ± SD) | Difference (I – C) |
|------------------|--------------------------|---------------------|--------------------|
| Baseline (day 0) | 9.70 ± 0.40              | 9.69 ± 0.41         | +0.01              |
| Week 1 (day 7)   | 10.25 ± 0.41             | 10.15 ± 0.42        | +0.10              |
| Week 2 (day 14)  | 11.05 ± 0.43             | 10.75 ± 0.44        | +0.30              |
| Week 3 (day 21)  | 12.00 ± 0.45             | 11.50 ± 0.46        | +0.50              |

Change (day 0 → 21)

|       |       |       |
|-------|-------|-------|
| +2.30 | +1.81 | +0.49 |
|-------|-------|-------|

Note: I, intervention; C, control; SD, standard deviation.

### 3.3 Within-group change

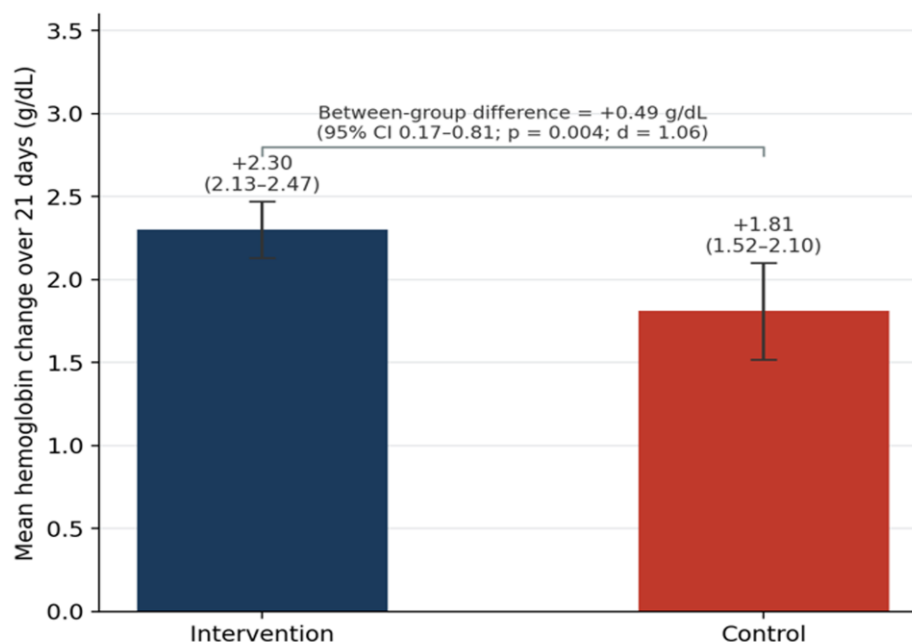
Both groups showed a statistically significant rise in hemoglobin from baseline to day 21. In the intervention group the mean increase was 2.30 g/dL (95% CI 2.13 to 2.47; paired  $t(16) = 28.34$ ;  $p < 0.001$ ; Cohen's  $d_z = 6.87$ ). In the control group the mean increase was 1.81 g/dL (95% CI 1.52 to 2.10; paired  $t(16)$

= 13.33;  $p < 0.001$ ; Cohen's  $d_z = 3.23$ ). The significant rise in the control group confirms that iron–folic acid supplementation alone was effective, which is why a control group is needed to isolate the contribution of the eggs. These results are summarized in Table 4.

### 3.4 Between-group difference (primary analysis)

The mean hemoglobin change was greater in the intervention group than in the control group, with a between-group difference of 0.49

g/dL (95% CI 0.17 to 0.81; independent-samples  $t(32) = 3.10$ ;  $p = 0.004$ ; Cohen's  $d = 1.06$ ). Because the 95% confidence interval lies entirely above zero, the additional benefit of the eggs is statistically significant, and the effect size of 1.06 is large by conventional criteria. Relative to the control group, the intervention group gained about 27% more hemoglobin (0.49 of 1.81 g/dL). The advantage was progressive rather than sudden: the between-group gap grew from 0.10 g/dL at day 7 to 0.30 g/dL at day 14 and 0.50 g/dL at day 21 (Figure 2). The primary result is shown in Figure 3.



**Figure 3.** Mean hemoglobin change from baseline to day 21 by group; error bars show 95% confidence intervals. The between-group difference of 0.49 g/dL was statistically significant ( $p = 0.004$ ) with a large effect size (Cohen's  $d = 1.06$ ).

**Table 4.** Within-group and between-group analysis of hemoglobin change (baseline to day 21)

| Comparison                  | Mean change (g/dL) | SD    | Statistics                                                |
|-----------------------------|--------------------|-------|-----------------------------------------------------------|
| Intervention (within group) | +2.30              | 0.335 | $t(16)=28.34$ ; $p<0.001$ ; $d_s=6.87$ ; 95% CI 2.13–2.47 |
| Control (within group)      | +1.81              | 0.560 | $t(16)=13.33$ ; $p<0.001$ ; $d_s=3.23$ ; 95% CI 1.52–2.10 |

Between-group difference +0.49 n/a  $t(32)=3.10$ ;  $p=0.004$ ;  $d=1.06$ ; 95% CI 0.17–0.81

*Note: SD, standard deviation of the change score;  $d_z$ , Cohen's  $d$  for paired data;  $d$ , Cohen's  $d$  for the between-group difference; CI, confidence interval.*

## 4. DISCUSSION

In this controlled study, adding two boiled eggs per day to standard iron–folic acid supplementation produced a significantly larger hemoglobin increase than supplementation

alone. The additional effect was 0.49 g/dL over 21 days (95% CI 0.17 to 0.81;  $p = 0.004$ ), with a large effect size (Cohen's  $d = 1.06$ ). Both groups improved significantly, which is expected because both received 60 mg of elemental iron and 400  $\mu\text{g}$  of folic acid daily. The value of the control group is that it separates the effect of the eggs from the effect of the tablets: the 1.81 g/dL rise in the control group reflects the tablets, while the additional 0.49 g/dL in the intervention group reflects the eggs. A single-group design would have attributed the full 2.30 g/dL rise to the eggs and overstated their effect by more than fourfold.

The size of the additional effect is consistent with the nutritional contribution of the eggs. Two eggs add about 2 mg of dietary iron per day, or 42 mg over 21 days, on top of 1,260 mg from the tablets (Table 1). Iron alone is therefore unlikely to explain the whole difference. Eggs also supply high-quality protein and micronutrients such as zinc and selenium that support erythropoiesis, and the additional protein and energy may improve the use of the iron already provided by the tablets [12]. Egg iron is mainly non-heme and modestly absorbed, so the observed benefit is best read as a moderate, food-based addition to supplementation rather than a substitute for it [12].

The progressive widening of the gap between groups is consistent with a cumulative dietary effect. At one week the difference was only 0.10 g/dL, but by three weeks it reached 0.50 g/dL, and the paired within-group effect sizes were very large in both arms because the measurements were tightly clustered. This pattern suggests that studies shorter than about three weeks may underestimate the contribution of food-based measures, and it supports measuring hemoglobin over a period of at least three weeks rather than a single week.

These results extend earlier Indonesian studies of boiled egg consumption in pregnant women, which reported hemoglobin increases but generally used single-group before-and-after designs without a control group [9]. By keeping supplementation identical in both groups, the present study isolates the added value of eggs and reports it as a confidence interval and effect size rather than a raw pre-post change. A randomized trial in Malawian children found that daily egg supplementation did not improve

iron status in a population without concurrent iron–folic acid therapy [10], which is consistent with the view that eggs are best used as an adjunct to, rather than a replacement for, supplementation. Nutrition-education programs promoting iron-rich foods have also improved hemoglobin in anemic pregnant women [13], and structured health-education packages have increased supplementation compliance and hemoglobin levels [11]. The isolated egg effect in the present study is smaller than the raw pre-post increases reported in single-group studies, which is expected once the tablet effect is removed.

#### 4.1 Limitations

This study has several limitations. Although allocation was randomized, the sample was small, so unmeasured differences between groups may have influenced the result; the closely matched baseline characteristics reduce but do not remove this concern. The sample was small (17 per group) and drawn from a single village by purposive sampling, which limits generalizability. Tablet adherence and total dietary intake outside the intervention were not quantified, so residual dietary differences cannot be excluded. Hemoglobin was measured with a point-of-care capillary analyzer rather than a laboratory venous method, which is practical in field settings but less precise. The very large within-group effect sizes reflect unusually small variability in the change scores and should be interpreted with this in mind. Finally, the 21-day period captures short-term change; effects on birth outcomes were not assessed.

Future work should use a larger multi-site sample, laboratory-based hemoglobin and iron-status markers such as ferritin, measurement of tablet adherence and total dietary iron, and follow-up long enough to assess maternal and neonatal outcomes.

## 5. CONCLUSION

Adding two boiled eggs daily to standard iron–folic acid supplementation increased hemoglobin significantly more than supplementation alone in pregnant women with mild anemia over 21 days, with a between-group difference of 0.49 g/dL (95% CI 0.17 to 0.81;  $p = 0.004$ ) and a large effect size. Boiled eggs are inexpensive and locally available, and can be

used alongside routine iron–folic acid supplementation to support the management of mild anemia in pregnancy. Because the sample was small and drawn from a single site, these findings should be confirmed in larger randomized controlled trials.

## DECLARATIONS

### Ethics approval.

Approved by the Health Research Ethics Committee of Universitas Karya Husada Semarang (No. 249/KEP/UNKAHA/SLE/VIII/2024, August 2024); written informed consent was obtained from all participants.

### Consent for publication.

Not applicable; no individually identifiable data are presented.

### Availability of data.

The datasets analyzed are available from the corresponding author on reasonable request.

### Competing interests.

The authors declare no competing interests.

### Funding.

This research received no external funding.

### Authors' contributions.

HW and PF designed the study, collected and analyzed the data, and wrote the manuscript. Both authors approved the final version.

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