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The Effectiveness of Spirulina in Restoring Body Weight in Anemia-Induced Female Wistar Rats

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Abstract: One of the most prevalent health problems in Indonesia is anemia, particularly among adolescent girls, where concerns about excessive weight gain may reduce adherence to iron supplementation. Spirulina is a potential alternative supplement rich in iron and nutrients that support hematopoiesis and may address these concerns. This study aimed to analyze the effect of spirulina supplementation on body weight in anemic adolescent female Wistar rats. This experimental study used 25 female Wistar rats divided into five groups: normal control (NC) received a standard BR diet, positive control (PC) received a low-iron diet to induce anemia, and three treatment groups received spirulina at doses of 25, 50, and 100 mg/kg body weight (T1–T3) for 21 days. Body weight was measured before anemia induction, after anemia induction, and following supplementation. The results showed a significant overall difference in body weight gain among the groups ($p < 0.001$), which was mainly driven by differences between the normal control and positive control groups. However, post hoc analysis indicated no significant differences in body weight gain between the positive control group and treatment groups 1, 2, and 3 ($p > 0.05$). Body weight in the treatment groups increased by 22.8–27 g, indicating recovery toward baseline values. Although these differences were not statistically significant, spirulina supplementation may contribute to anemia recovery, as reflected by improvements in body weight. In conclusion, spirulina shows potential as a nutritional supplementation strategy that does not induce excessive weight gain while supporting iron-related nutritional needs.

Keywords: anemia, body weight, female adolescents, spirulina, wistar rats

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INTRODUCTION

Iron-deficiency anemia, or a condition where the body experiences decreased red blood cells due to iron deficiency, is highly prevalent among female adolescents in Indonesia. This condition occurs because, during adolescence, females experience menstruation, so the body requires increased iron intake, but an unregulated and haphazard diet promotes severe iron deficiency (Rika Ariana, 2024). This will have an effect on the health of female adolescents, one of which is weight loss and impaired growth.

The prevalence of anemia in female adolescents, based on the data from Indonesian Riskesdas (Indonesian Basic Health Research) and WHO (Indonesia, 2018; WHO, 2021), is reported to reach 30–40%. This percentage is considered high, so special care and treatment are required. One of the factors contributing to iron deficiency is an unbalanced diet. According to the study by Damayanti (2025), female adolescents tend not to pay attention to the food they consume, consuming food with low iron and animal protein, while the intake of green vegetables and other micronutrient sources is insufficient, thereby causing iron levels in the body to be reduced and making them susceptible to anemia. (Krisnasary & Yulianti, 2023) found a significant correlation between iron and protein intake and the prevalence of anemia in female adolescents in rural areas in Indonesia. Meanwhile, a literature study by (Helmyati et al., 2024) states that participation in the Iron Supplementation Program for female adolescents remains low, at approximately 0-25%, with distribution coverage under 50%. Low participation is influenced by a lack of interest in consumption and limited support from family and school.

Solutions provided by the government include implementing the Iron Supplementation Program and encouraging female adolescents to consume one iron tablet during menstruation and once a week when not menstruating (Yanti & Anwar, 2022). However, uncomfortable sensations after swallowing the tablet, unpleasant odor, and side effects, such as nausea, are major reasons for low adherence among female adolescents in taking iron supplements. Thus, a spirulina supplement from freshwater, modified into powder, can be a solution for replacing iron supplements.

Spirulina is proven to be rich in nutrients, such as Fe, protein, vitamins, and antioxidants that have the potential to accelerate anemia recovery. Spirulina has several characteristics and nutritional content that are suitable to be a functional food, as it contains 55-70% protein, 1.3-15% essential amino acids, vitamins, 3-7% minerals, 0.8% chlorophyll, and 6.7-11.7% phycocyanin (Christwardana & Hadiyanto, 2017). Chemical compositions of spirulina include protein (55-70%), carbohydrate (15-25%), essential amino acids (18%), vitamins, minerals, and pigments, such as carotenoids, chlorophyll A, and phycocyanin. Therefore, spirulina has the potential to be a good alternative for many synthetic antimicrobial and antioxidant compounds that have risks for consumers and can improve health (Betaria Sonata Sianturi et al., 2024).

Female adolescents often have concerns about weight gain. Beauty standard in Indonesia strongly influences the mindset of female adolescents to maintain an ideal body shape and avoid obesity, thereby influencing their choices of food and supplements. Several studies show that Spirulina has been reported to have a role in improving iron-deficiency anemia through increased Hb level and erythrocyte parameters. However, findings regarding the effect of spirulina on body weight

are still inconsistent; some studies report weight gain, while other studies do not find significant changes. To date, no studies have examined the ability of spirulina in recovering anemia and its effect on body weight. This condition emphasizes further study to examine whether spirulina is able to improve anemia without causing weight gain, which is a concern for many female adolescents. Therefore, a study was conducted using experimental animals, using Wistar rats as the animal model, because this strain has been widely used in the induction of iron-deficiency anemia, has a stable hematological response, and is close to the human hematopoiesis mechanism. Moreover, its metabolic characteristics are fairly representative of examining weight changes due to supplementation. According to this consideration, Wistar rats were considered appropriate to examine the ability of spirulina in improving anemia without causing weight gain

METHODS

Design, Time, and Place

This study was an experimental study using a Randomized Block Design (RBD) with five treatment groups and three repetitions. This study aimed to evaluate the effect of spirulina supplementation on body weight in adolescent female Wistar rats induced with anemia. There were five treatment groups, based on the preliminary study:

CN : Negative Control (normal rats, administered a standard BR feed)

CP : Positive Control (anemic rats, administered a low-iron (Fe) diet)

T1 : Anemic rats were administered spirulina 25 mg/kgBW

T2 : Anemic rats were administered spirulina 50 mg/kgBW

T3 : Anemic rats were administered spirulina 100 mg/kgBW

Population and Sample

The number of experimental animals was determined based on the Federer formula, with each group consisting of 5 rats. Thus, a total of 25 female Wistar rats aged 5-8 weeks and weighing 150-200 g were used.

Equipment and Materials

The equipment used was an experimental animal cage, a digital scale, and feeding tubes. Materials included AIM-isolated spirulina (cultivated in freshwater adapted from seawater, obtained from PT. AIM, Klaten), female Wistar rats from Gunung Kidul cultivation, BR feed, low-iron diet, distilled water, aluminum sulfate (used as an anemia-inducing agent) (Adyani et al., 2018), and drinking water.

Intervention Technique

Rats were acclimatized for 7 days prior to the experiment. Anemia was induced by oral administration of aluminum sulfate at a dose of 67.5 mg/kgBW for 10 consecutive days using a feeding tube, combined with a low-iron (Fe) diet in pellet form (30–40 g/day). This procedure successfully established an iron-deficiency anemia model in female Wistar rats.

Following anemia induction, spirulina supplementation was administered orally for 21 days according to the assigned dose for each treatment group. For each administration, spirulina powder

was freshly dissolved in 1 mL of distilled water (aquades) to ensure uniform concentration and ease of oral gavage. The prepared solution was then administered directly to the rats using a feeding tube.

During the treatment period, rats were observed daily for physical condition and signs of toxicity. Body weight was measured at three time points: day 0 (baseline), day 10 (post-anemia induction), and day 37 (after completion of spirulina treatment), to evaluate body weight changes associated with anemia and recovery.

Figure 1

Oral Spirulina Administration in Wistar Rats



Source: Personal documents

Data Processing and Analysis

Data analysis was conducted to evaluate changes in body weight (ΔBW). Body weight change (ΔBW) was calculated as the difference between body weight after treatment and body weight following anemia induction. Prior to hypothesis testing, data normality was assessed using the Shapiro–Wilk test. Parameters with normally distributed data ($p > 0.05$) were further analyzed using one-way analysis of variance (ANOVA). Homogeneity of variance was evaluated using Levene's test. When the assumption of homogeneity was violated ($p < 0.05$), the Games–Howell post hoc test was applied to identify differences between groups, as it is appropriate for unequal variances. All statistical analyses were conducted using a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

All 25 female adolescent Wistar rats were successfully induced to experience anemia on day 10. After that, treatment with spirulina supplementation was performed, with monitoring of the rats' hematological levels and body weight. Thus, rats' body weight approached pre-anemia levels was obtained in day 37 or 27 days of treatment with spirulina supplement. Based on Table 1, the normal group (CN) showed the greatest increase in body weight, indicating normal growth without anemia occurred, while in the positive group (CP), rats induced with anemia without spirulina supplementation show a significant decrease in body weight, indicating that anemia inhibits the growth of rats and does not recover naturally without treatment.

Table 1

Body Weight Profile of Anemic Female Wistar Rats

Group	Initial BW (g)	Anemia BW (g)	Final BW (g)	ΔBW
CN	131.2 ± 9	145.8 ± 10.8	213.8 ± 23.4	68 ± 29.7
CP	109 ± 17.3	91.6 ± 13.6	73.5 ± 11.9	-14.75 ± 18.2
T1	155 ± 8.2	127.6 ± 10.5	150.4 ± 19.6	22.8 ± 10.3
T2	149.4 ± 19	125 ± 13.9	147.8 ± 11.9	22.8 ± 17
T3	150 ± 11.7	121.6 ± 21	148.6 ± 13.6	27 ± 8.3

Groups administered with spirulina treatment (T1–T3) experienced body weight gain after an initial decrease during anemia induction, with an increase ranging from 22.8 to 27 grams, which successfully approached the initial body weight. This finding indicates that spirulina supplementation, administered at specific doses for each group, supported body weight recovery in anemic rats without causing excessive weight gain. This result is consistent with the study by Leal-Esteban et al. (2021), which reported that young women consuming spirulina did not experience significant increases in body weight or BMI.

In contrast, a study by Jumiyati et al. (2023) reported significant weight gain in obese anemic rats receiving spirulina supplementation. This discrepancy may be attributed to several biological and methodological factors. Obese rats have metabolic profiles that differ from those of adolescent rats, including leptin dysfunction and a greater tendency for fat accumulation. Under these conditions, spirulina may not only improve hematological status but also promote adipose tissue accumulation, resulting in greater weight gain in obese rats compared to non-obese adolescent rats (Diniz et al., 2021). Additionally, the severity of iron deficiency may influence the recovery response. In cases of severe anemia, reduced appetite and metabolic disturbances often occur, and body weight gradually recovers toward normal levels as hematological status improves. Conversely, in obese anemia, energy reserves remain high, allowing improved hematological status to enhance metabolism and accelerate body weight gain beyond normal levels (Eltahan et al., 2023).

Table 2

Statistical analysis of body weight change

Statistical test	Value	p-value
One-way ANOVA	F (4.19) = 11.615	< 0.001*
Shapiro-Wilk (normality)	-	> 0.05
Levene's test (homogeneity)	-	0.014*

*Significant at $p < 0.05$

To determine whether the differences were statistically significant, a One-Way ANOVA test was conducted, and the results showed that weight gain (ΔBB) between treatment groups was F (4.19) = 11.615 ($p < 0.001$). Normality test using Shapiro-Wilk showed that all groups were normally distributed ($p > 0.05$), while the homogeneity test using Levene's Test showed that variance was not homogeneous with a p-value of 0.014, then the analysis was followed by Post Hoc Games-Howell. The results showed that the normal group (CN) was significantly different from the positive group (CP) with a p-value of 0.009, while the results between the normal group and the three treatment groups

(T1-T3) did not show significant differences. The comparison between the positive group (CP) and the three treatment groups (T1-T3) showed that the p-value approached significance (0.056 - 0.09), indicating that there was body weight recovery due to the administration of spirulina supplement.

Table 3

Post Hoc Games–Howell Results

Comparison	p-value
CN vs CP	0.009*
CP vs T1	0.076
CP vs T2	0.090
CP vs T3	0.056

*Significant at $p < 0.05$

The results demonstrated that iron deficiency can significantly reduce rats' body weight, while the administration of spirulina supplement can prevent further weight loss and support recovery towards normal body weight. The previous studies prove that spirulina can support anemia recovery. (Fatimah & Wulandari, 2024) states that spirulina is more effective in increasing the hemoglobin level in pregnant women with anemia compared to conventional iron supplementation, which is less preferred by adolescents due to its side effects. Iron content in spirulina can be used to improve iron deficiency in vulnerable groups. This finding is in line with the study conducted by (Anggraeni et al., 2024) that supplementation from Spirulina platensis is able to increase body weight in pregnant women, while improving nutritional and hematological status. This is supported by the study conducted by (Risnawati et al., 2025), which reported a significant increase in Hb level in pregnant women who received 300-800 mg/day of spirulina supplement during a short-term intervention.

It is expected that people, especially female adolescents in Indonesia who are vulnerable to anemia, can consume spirulina supplementation to prevent severe anemia that affects body weight. The consumption of spirulina has the potential to improve nutrition without causing concerns about excessive weight gain. This is really relevant, given that many adolescents avoid supplementation due to side effects causing rapid weight gain. The administration of spirulina supplementation can be varied and does not have to be in tablet. This variation is expected to enhance adolescents' interests and motivation to care about their health conditions in preventing anemia. A study conducted by (Nuraini, 2024) reported that spirulina supplementation is processed into cookies for female adolescents with anemia. Therefore, good acceptance and enthusiasm can be obtained, so that spirulina supplementation has strong potential to be a functional food, encouraging people to consume it without hesitation.

Body weight recovery in rats to the normal level after the administration of spirulina occurs along with an increase in hemoglobin level. Improvement in anemia status allows body weight to gradually return toward the initial level before anemia induction. This is in line with the study conducted by (Khateb, 2021), which reported that spirulina supplementation is able to improve hematology status in rats with iron-deficiency anemia. The rat group that received spirulina showed a significant increase in hemoglobin level and the number of red blood cells compared to the anemia control group. This finding indicates that spirulina plays a role in supporting the blood cell formation process by increasing the availability of iron and improving oxygen transportation in the body, thereby

improving the anemia condition gradually. Moderate body weight gain in the anemia rat group after the administration of spirulina supplementation indicates that recovery occurs through the mechanism of improving hematological status, so that spirulina has the potential to be an iron supplement for iron-deficiency anemia without risk of excessive weight gain.

This study has several limitations, namely that it used animal models of anemia, so the results cannot be directly generalized to humans. The parameters used only focus on hematological indicators and weight monitoring; the molecular mechanisms underlying the improvement of anemia after spirulina supplementation were not specifically evaluated. Therefore, further research involving human subjects is needed to strengthen the findings of this study, and molecular research on the improvement of anemia should be conducted.

CONCLUSION

Spirulina supplementation administered to anemic adolescent female Wistar rats did not result in significant differences in body weight gain among the treatment groups receiving doses of 25, 50, and 100 mg/kg body weight ($p > 0.05$). Nevertheless, spirulina supplementation was associated with an improvement in body weight toward baseline values, indicating a potential contribution to anemia recovery. These findings suggest that spirulina may serve as a nutritional supplementation strategy that supports iron-related nutritional needs without inducing excessive weight gain.

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Author Contribution Statement

Zahra Konitah: Conceptualization; Methodology; Investigation; Data Collection; Data Analysis; Writing – Original Draft Preparation. **Sajidan Sajidan**: Supervision; Conceptualization; Project Administration; Writing – Review & Editing. **Artini Pangastuti**: Validation; Writing – Review & Editing; Translation.

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