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The effects of *Jebisa* on nutrient intake and hematology profile in adolescent girls with anemia

MARTHA ARUM NUGRAHENI[1,2], DONO INDARTO[1,3,4,♥], ETI PONCORINI PAMUNGKASARI[1,5], TRI NUGRAHA SUSILAWATI[6], SINU ANDHI JUSUP[4], NINIEK PURWANINGTYAS[7], SETYO SRI RAHARDJO[8], SRI WULANDARI[4], KEZIA ELIAN DEVINA[1]

[1]Postgraduate Program of Nutrition Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia
[2]Department of Food Technology, Faculty of Agriculture, Universitas Tidar. Jl. Barito 1 Magelang 56114, Central Java, Indonesia
[3]Biomedical Laboratory, Faculty of Medicine, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271- 664178/637400 , ♥email: dono@staff.uns.ac.id
[4]Department of Physiology, Faculty of Medicine, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia
[5]Department of Public Health, Faculty of Medicine, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia
[6]Department of Microbiology, Faculty of Medicine, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 5 126, Central Java, Indonesia
[7]Department of Cardiology and Blood Circulation, General Hospital Dr. Moewardi Surakarta/Faculty of Medicine, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia
[8]Department of Pharmacology, Faculty of Medicine, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia

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Abstract. *Nugraheni MA, Indarto D, Pamungkasari EP, Susilawati TN, Jusup SA, Purwaningtyas N, Rahardjo SS, Wulandari S, Devina KE. 2024. The effects of* Jebisa *on nutrient intake and hematology profile in adolescent girls with anemia. Nusantara Bioscience 16: 210-218.* Anemia is a public health problem that often occurs in adolescent girls in developing countries, including Indonesia, mainly due to iron deficiency. Iron supplementation has been implemented for anemia treatment, but there are some side effects for long-term use. The Indonesian Government also fortified wheat flour with iron and folic acid, but the efficacy of fortification remains unclear. Snake fruit seeds contain high iron (Fe) and vitamin C levels but they are usually thrown away. Therefore, this study aimed to analyze the effects of jelly-containing snake fruit seed flour (SSF) and sugar, now called *Jebisa*, on macro and micronutrient intake and hematological profile in adolescent girls with anemia. Thirty tree adolescent girls from five public high schools in Sukoharjo Regency, Central Java, Indonesia, were randomly grouped into three categories: Control group (C) was given plain jelly with Fe tablets, and Treatment groups (T1-T2) were given jelly-containing 18.52 g and 37.04 g SSF, respectively, for 60 days. Data were analyzed using the one-way and repeated measures ANOVA tests followed by the Post Hoc Tukey and the Kruskal-Wallis test for non-parametric data. A significant value was set to $p<0.05$. The results suggested that the average daily energy intake, carbohydrates, and fibers significantly increased in the T1 and T2 groups compared to the C group ($p<0.05$). The average Fe intake in the T2 (19.59±1.70 mg/day) was significantly higher than that of T1 (10.74±2.40 mg/day) and C group (4.74±3.43 mg/day) with $p<0.001$. The T1 group had higher hemoglobin (Hb) levels (11.44±2.01 g/dL) than the C (11.04±1.92 g/dL) and T2 groups (10.89±0.67 g/dL) but were not significantly different. In conclusion, regular consumption of jelly-containing SSF increased the daily intake of energy, carbohydrates, fibers, Fe, and Hb levels in adolescent girls with anemia.

Keywords: Adolescent girl, anemia, hematological profile, nutrient intake, snake fruit seed

Abbreviations: Fe: Iron; SSF: Snake fruit Seeds Flour; *Jebisa*: Plain jelly, SSF, and sugar; C: Control group; T1: Treatment group 1; T2: Treatment group 2; HSD: Honestly Significant Difference; SD: Standard Deviation; BW: Body Weight; Hb: Hemoglobin; BMI: Body Mass Index; MCH: Mean Corpuscular Hemoglobin; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration; Zn: Zinc; RDA: Recommended Dietary Allowances; CYBRD1: Duodenal Cytochrome B

INTRODUCTION

Anemia is a public health problem affecting the world's population in both developed and developing countries (Petry et al. 2016). A study conducted by the Indonesia Basic Health Research (Kemenkes RI, Riskesdas) in 2018 revealed that adolescent girls represent one of the highest-risk age groups with a prevalence of 32% of suffering anemia. In addition, the national coverage of adolescent girls receiving iron supplementation reaches 80.9%, but 98.6% of them take iron supplementation less than 52 tablets per year (Kemenkes RI 2017). Furthermore, Sukoharjo is a Regency in Central Java Province, Indonesia, with a high prevalence of anemia in adolescent girls (22%) (Rahayu et al. 2017).

Generally, anemia in adolescent girls is mainly caused by iron deficiency due to increased iron needs for growth, regular blood loss (menstruation), and an inappropriate diet for weight management (Engidaw et al. 2018). The Indonesian Government has established two national programs to overcome iron deficiency anemia: iron supplementation and fortification. However, iron supplementation in adolescent girls is not evenly distributed in all Indonesian areas, with 26.5% not receiving it. In addition, some adolescent girls reported side effects after taking iron supplementation (Kemenkes RI 2023).

Wheat flour fortified with iron, zinc, thiamine, riboflavin, and folic acid has been used to treat anemia in Indonesia since 2002 (Kendrick et al. 2015)However, the efficacy of iron fortification remains debatable. It is possible to make alternative iron fortifications using local ingredients from Indonesian natural products such as *Moringa* leaves flour and fermented buffalo milk (*dadih*). A previous study reported that consuming 4 g *Moringa* leaves flour in *cilok* (street food, ball-shaped dumping made from starch) for 15 days could increase Hb levels by 1 g/dL in female adolescents with anemia (Ariendha et al. 2022). In contrast, consumption of 100 g/day *dadih* for 14 days slightly decreased Hb levels by 0.6 g/dL and serum ferritin levels by 3.9 ng/mL in normal adolescent girls (Budiyatri et al. 2024). Administration of 60 mg iron supplements, combined with 200 mL of cava smoothie from ripe Cavendish bananas, buttery avocados, pure honey, and Sukkari dates, consumed every 2 days for 28 days could increase Hb levels by 2.54±1.24 g/dL, MCH by 1.61±0.99 pg, MCHC by 1.60±0.99 g/dL, and MCV by 1.97±1.19 fL in adolescent girls with moderate anemia (Aryani et al. 2023). Another study reported that consumption of jelly candy containing jelly, snake fruit, banana, and ferrous fumarate for 13 weeks can increase 0.65±0.39 mg/dL Hb levels in female adolescents with mild anemia (Megawati et al. 2023). However, the study does not state the daily dose of jelly candy given to female adolescents and which part of the snake fruits were used in the study.

Snake fruit (*Salacca edulis* Reinw) is a native Indonesian plant whose annual production has increased from 0.89 million in 2018 to 1.14 million in 2022 (Badan Pusat Statistik 2023). This fruit plant can be found in several regions in Indonesia, such as Central Java, North Sumatra, Bali, East Java, and North Sulawesi Provinces. However, the most popular snake fruit comes from the Sleman Regency of Yogyakarta Province, known as "*salak pondoh*," the center of snake fruit plantation and production in Indonesia. A previous study revealed that snake fruit seeds and peels have been utilized for food production with high nutritional values and health benefits (Mazumdar et al. 2019; Kumoro et al. 2020). In addition, snake fruit seeds contain secondary metabolites such as tannins, quinones, monoterpenes, sesquiterpenes, alkaloids, and polyphenolics (Febyawati et al. 2023). Based on our previous studies, 100 g of snake fruit seed flour (SSF) contains 7.5 g of protein, 19.9 mg of iron, 5.79 mf of Zn, and 152.21 mg of vitamin C. Administration of 3.72 g/100 g Body Weight (BW) SSF increases BW, Hb levels, erythrocyte and hematocrit account in female rats with anemia (Indarto et al. 2023). However, a higher amount of SSF is needed when given to adolescent girls with anemia. In addition, the improvement of SSF processing in our study could increase by 1.10 g of protein, 2.67 mg of iron, 0.04 mg of vitamin C, and 0.36 mg of zinc compared to the previous SSF.

The Indonesian Government also has a national program to overcome undernutrition in toddlers, school children, and pregnant women. For example, biscuits fortified with 18 micronutrients have been used as supplementary food for pregnant women (Kemenkes RI 2017). However, there is no supplementary food for anemia in children, adolescent girls, and pregnant women. Several studies have formulated supplementary foods by fortification with iron into cookies (Landim et al. 2016), candy (El-Tahan and Alfky 2016), and chocolate (Mostafa 2023). However, supplementary food for anemia in the form of jelly has not been established. Based on Sani's study (2014), we formulated jelly containing 6 g plain jelly, 29±5 g SSF, and 22.5±5 g sugar for anemia supplementary food (Indarto et al. 2022), hereinafter called *Jebisa* (Indonesian: *jeli biji salak*). This original formulation is granted a patent with IDS 000005116 by the General Directorate of Intellectual Properties, the Indonesian Ministry of Law and Human Rights. Therefore, this study aimed to analyze the effects of jelly-containing SSF (*Jebisa*) on macro and micronutrient intake and hematological profile in adolescent girls with anemia.

MATERIALS AND METHODS

Materials

Fresh snake fruit seeds were purchased from a farmer in Donokerto Village, Turi Sub-district, Sleman Regency, Yogyakarta Province, Indonesia, from which were processed into SSF using the existing method with IDS 000005116 patent number (Indarto et al. 2022). Plain jelly powder was purchased from PT. Satelit Sriti Pandaan, Surabaya, East Java, Indonesia (MD 818513068111). The main ingredients used to make *Jebisa* in this research study were SSF, plain jelly powder, sugar, and water, which were also reported in our previous study (Indarto et al. 2022).

Methods

Research design

This randomized control trial (RCT) study with a pre-posttest control group design was conducted in five public senior high schools (SMAN) 1 Weru, Nguter, Kartasura, Mojolaban, and SMAN 3 Sukoharjo, Central Java, Indonesia, which were determined using the stratified random sampling. The sample size was calculated using the formula of Charan et al. (2021). The final sample size for each group was at least 11 adolescent girls. The study included adolescent girls in senior high schools who were aged 16-18 years old and had <11 g/dL Hb levels, except for those with infectious diseases such as tuberculosis, dengue fever, malaria, typhoid, and helminthiasis, who were menstruation during the screening, taking Fe, and vitamin C supplements, and who were allergies to flour-based foods. Before the research intervention, ethical clearance was acquired from the Medical Research Ethics Committee, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia Number 165/UN27.6/KEPK/2018.

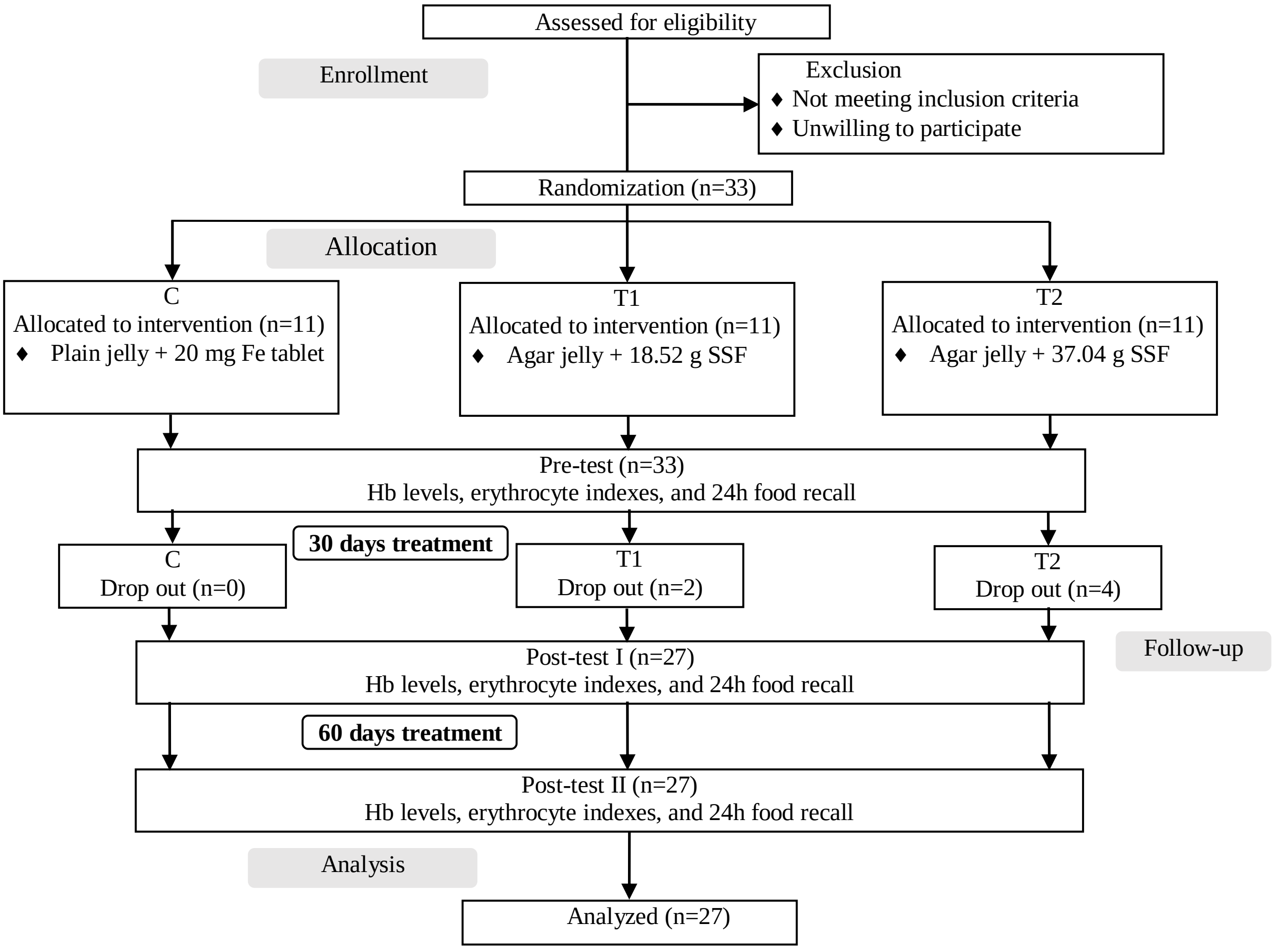

Figure 1. Consort flow diagram for selecting adolescent girls in five senior high schools in Sukoharjo Regency, Central Java, Indonesia.

Jebisa *treatment*

Before the study began, all adolescent girls in five senior high schools were educated about daily nutrition intake and asked to complete personal biodata. Following this, the selected adolescent girls and their parents were carefully guided through the informed consent process, ensuring full understanding and agreement. A venous blood sample was then taken from the upper left arm to determine the Hb level. Suppose the Hb level was less than 11 g/dL (WHO 2011). The research subjects should take 400 mg oral Albendazole in a single dose to treat worm infection, which results in chronic intestinal bleeding. Figure 1 summarizes the selection process of adolescent girls to get the final 27 research subjects. Initially, as mentioned above, we screened adolescent girls in five senior high schools in Sukoharjo Regency and got 33 research subjects that met the inclusion criteria. The selected adolescent girls were randomly allocated into three groups: Control (C), given plain jelly with 20 mg Fe tablets, and Treatment groups (T1 and T2), given jelly-containing 18.52 g and 37.04 g SSF, respectively. All research subjects in the three groups consumed their daily foods and drinks as usual while taking *Jebisa* thrice weekly for 60 days. At the day 30 intervention, however, two and four research subjects in the T1 and T2 groups dropped out of this study for several reasons. Finally, 27 research subjects completed this study and were used for further analysis.

Data collection of anthropometries, daily nutrition intake, and hematology status

Anthropometric data were collected by measuring BW and height before, during, and after interventions. The BW measurement used a Gea weight scale with 0.1 kg accuracy, while the height measurement utilized a stature meter with 0.1 cm accuracy. Body Mass Index for age (BMI/age) was determined using the z-score: subject's individual BMI – (BMI standard median/BMI standard deviation) and then classified into underweight, normal, overweight, and obese (Kemenkes RI 2020). Data on daily nutrition intake were also assessed three times (day 1, 30, and 60 of interventions) using the 24-hour food recall questionnaires and then converted into macro and micronutrient intake using free Nutrisurvey 2007 software (www.nutrisurvey.de). The venous blood samples collected at 1st, 30th, and 60th-day interventions were utilized to measure Hb levels and erythrocyte indexes: Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), and Mean Corpuscular Hemoglobin Concentration (MCHC) ratio, performed at the Rahanu

Clinic Laboratory, Karanganyar Regency, Central Java, Indonesia.

Statistical analysis

The characteristics of research subjects were presented as mean ± standard deviation for numerical data, while numbers and percentages were used for categorical data. The following variables were analyzed using a one-way ANOVA test: height, BMI, egg allergy, seafood allergy, parental income, carbohydrate intake, and MCV. Post-hoc tests, including the reliable Tukey's Honestly Significant Difference (HSD) and repeated measures ANOVA, were used to ensure the validity of the results. The Kruskal-Wallis test analyzed age, BW, Hb levels, energy, protein, fat, fiber, Fe, Zn, Vitamin C, MCH, and MCHC. Mann-Whitney and Friedman tests were then applied to these variables. A p-value of <0.05 was considered statistically significant, further reinforcing the reliability of the statistical methods.

RESULTS AND DISCUSSION

Characteristics of research subjects

This study involved 33 adolescent girls from five senior high schools in Sukoharjo Regency. Table 1 shows that the characteristics of research subjects among the three groups were similar, except for the mothers' income with p=0.04. The mean BW in the T2 group (53.36±10.27 kg) was higher than that of the C (49.73±7.80 kg) and T1 groups (46.77±6.44 kg). The same pattern was also observed in height and BMI variables. However, the T1 group had slightly higher Hb levels (10.37±0.76 g/dL) than the C and T2 groups, and most research subjects had mild anemia. Egg and seafood allergies were found in all groups (less than 25%). Most parents in all groups had monthly incomes below the regional minimum salary. Regarding mothers' income, there were research subjects in the T2 group (28.5%) whose mothers earn a salary above the regional minimum. In contrast, the other groups had mothers' incomes below (100%) the regional minimum salary.

***Jebisa* consumption increased macronutrient and fiber intake**

Before *Jebisa* treatment, we evaluated macronutrient and fiber intake in all groups (Table 2). All research subjects among the three groups had low energy, fat, protein, and fiber intake and did not differ significantly (p>0.05) except for carbohydrate intake. The mean carbohydrate intake in T2 groups (165.37±40.37 g/day) was significantly higher than that of C (123.77±48.04 g/day; p=0.146) and T1 (109.36±41.33 g/day; p=0.047) groups. The T2 group also exhibited the highest energy and fat intake, though this was not a statistically significant difference (p=0.41). The highest protein and fat intake were observed in the C groups (34.98±11.19 g/day) and T1 groups (38.29±24.68 g/day).

After 30 days of treatment, the daily intake of energy, protein, and fat decreased, while the daily intake of carbohydrates and fiber increased in the T1 and T2 groups. The C group had a lower daily intake of macronutrients and fiber compared to the 0-day treatment. All mean differences were not significant (p>0.05). However, the mean carbohydrate intake in T2 group (205.30±97.64 g/day) was significantly higher than that of T1 (189.63±36.89 g/day; p=0.872) and C (96.73±49.38 g/day; p=0.004) groups. A slightly increased fat intake was found in the T2 group (31.37±22.29 g/day). In contrast to day 30 of treatment, all daily intake of macronutrients and fiber in the T1 and T2 groups increased; at the same time, energy, carbohydrate, and fiber intake increased significantly (p=0.004; p<0.001; p=0.01, respectively) for 60 days of treatment. The energy intake in the T2 group was 62.44% of the Recommended Dietary Allowances (RDA) for Indonesian female adolescents, which was significantly higher than the energy intake in the T1 (50.22%) and C (40.86%) groups. Meanwhile, the carbohydrate intake in T2 was 91.73%, significantly higher than in T1 (65.08%; p=0.034) and C (33.44%; p<0.001) groups. In comparison to the C group (3.98±3.66 g/day), the mean fiber intake in T1 (6.67±3.77 g/day) and T2 (8.88±3.23 g/day) groups also differed significantly (p=0.01). After 60 days of treatment, daily carbohydrate intake showed significant differences in T1 (p=0.005) and T2 (p=0.02) groups.

***Jebisa* consumption increased Fe, Zn, and Vitamin C intake**

We also evaluated micronutrient intake in all groups (Table 3). The T1 group had the highest Fe (9.82±1.45 mg/day) and Zn (5.00±2.55 mg/day) intake, but the C group (32.07±45.71 mg/day) had the highest vitamin C intake. All mean differences were not significant (p>0.05). After 30 days of treatment, the daily intake of Fe, Zn, and Vitamin C decreased in the C and T1 groups, but the daily intake of Fe and Zn increased in the T2 group. The mean Fe intake in the T2 group (10.90±3.60 mg/day) was significantly higher than that of the T1 and C groups (8.78±1.66 mg/day; 3.69±0.87 mg/day; p<0.001). A slightly increased Zn intake was found in the T2 group (4.44±1.37 mg/day), but it was higher than in the C group (3.79±1.45 mg/dL) and lower than the T1 group (4.72±1.80 mg/dL) The mean differences among groups were not statistically different (p>0.05). The mean vitamin C intake in the T2 group (10.90±3.60 mg/day) was significantly higher than in the T1 and C groups 8.78±1.66 mg/day; 3.69±0.87 mg/day; p<0.001).

After 60 days of treatment, all groups increased their daily intake of Fe, Zn, and vitamin C. Daily intake of Fe significantly increased in T1 (p=0.04) and T2 (p=0.03) groups, compared to the same groups before treatment. The mean Fe intake in the T2 group (19.59±1.70 mg/day) was significantly higher than that of the T1 (10.74±2.40 mg/day; p=0.003) and C (4.74±3.43 mg/day; p=0.001) groups. The higher mean of Zn intake was found in the T2 group (5.92±2.46 mg/day) compared to the C (4.55±2.95 mg/day) and T1 (5.65±1.65 mg/day) groups, but it did not differ significantly (p=0.14). In comparison to the C group (25.22±30.55 mg/day), the mean vitamin C intake in T1 (29.56±43.76 mg/day) and T2 (41.47±40.68 mg/day) groups also did not differ significantly (p=0.73).

Table 1. Characteristics of research subjects treated with or without *Jebisa* (n=27)

Variable	C	T1	T2	P
Age (old)[a]	15.91±0.83	15.89±0.60	15.86±0.69	0.99
BW (kg)[a]	49.73±7.80	46.77±6.44	53.36±10.27	0.28
Height (m)	1.53±0.06	1.53±0.05	1.56±0.05	0.51
BMI (kg/m^2)	21.25±3.15	20.00±2.49	22.04±4.62	0.48
Hb (g/dL) [a]	9.90±0.89	10.37±0.76	10.24±0.72	0.28
- mild (10-12 g/dL)	6 (54.55%)	7 (63.64%)	7 (63.64%)	0.37
- moderate (8-10 g/dL)	5 (45.45%)	4 (36.36%)	4 (36.36%)	
Egg allergy	1 (9.09%)	1 (11.11%)	0 (0%)	0.70
Seafood allergy	2 (18.18%)	2 (22.22%)	1 (14.29%)	0.93
Parent's Income (IDR)				
Father				
< regional minimum salary	7 (63.64%)	7 (77.78%)	3 (42.86%)	0.39
≥ regional minimum salary	4 (36.36%)	2 (22.22%)	4 (57.14%)	
Mother				
< regional minimum salary	11 (100%)	9 (100%)	5 (71.43%)	0.04*
≥ regional minimum salary	0 (0%)	0 (0%)	2 (28.57%)	

Note: [a] Statistical test with Kruskal-Wallis and the other variables with One-Way ANOVA. * Significant value of p<0.05. C: Control; T1: Treatment 1; T2: Treatment 2

Table 2. Effects of *Jebisa* on macronutrient and fiber intake in adolescent girls with anemia (n=27)

Macronutrient and fiber intake	Day	Mean ± SD (%RDA)			P
		C (n= 11)	T1 (n= 9)	T2 (n= 7)	
Energy (kcal/day) [a]	0	943.42±303.07 (44.92%)	921.07±383.33 (43.86%)	1066.77±185.95 (50.80%)	0.41
	30	728.55±328.28 (34.69%)	911.68±297.87 (43.41%)	927.50±562.40 (44.17%)	0.27
	60	858.04±480.55 (40.86%)	1054.63±330.12 (50.22%)	1311.25±323.15 (62.44%)	0.04*
	p	0.20	0.53	0.14	
Carbohydrate (g/day)	0	123.77±48.04 (41.26%)	109.36±41.33 (36.45%)	165.37±40.37 (55.12%)	0.05*
	30	96.73±49.38 (32.24%)	189.63±36.89 (63.21%)	205.30±97.64 (68.43%)	0.002*
	60	100.33±54.93 (33.44%)	195.24±70.71 (65.08%)	275.20±48.19 (91.73%)	<0.001**
	p	0.41	0.005*	0.02*	
Protein (g/day) [a]	0	34.98±11.19 (53.82%)	34.87±15.99 (53.65%)	31.63±12. 01 (48.66%)	0.72
	30	23.48±9.92 (36.12%)	28.65±12.89 (44.08%)	30.33±14.23 (46.66%)	0.67
	60	33.88±15.51 (52.12%)	37.68±11.84 (57.97%)	39.92±15.61 (61.42%)	0.54
	p	0.06	0.41	0.49	
Fat (g/day) [a]	0	34.28±11.05 (48.97%)	38.29±24.68 (54.70%)	31.20±11.95 (44.57%)	0.91
	30	27.51±17.56 (39.30%)	29.02±20.61 (41.46%)	31.37±22.29 (44.81%)	0.94
	60	35.85±30.05 (51.21%)	39.16±11.08 (55.94%)	39.78±18.74 (56.83%)	0.60
	p	0.36	0.25	0.53	
Fiber (g/day) [a]	0	5.53±3.19 (18.43%)	4.47±2.24 (14.90%)	7.86±1.76 (26.20%)	0.06
	30	4.57±2.16 (18.23%)	5.34±1.87 (17.80%)	6.13±2.11 (20.43%)	0.23
	60	3.98±3.66 (13.27%)	6.67±3.77 (22.23%)	8.88±3.23 (29.60%)	0.01*
	p	0.35	0.41	0.22	

Note: [a] Statistical test with Kruskal-Wallis and the other variables with One-Way ANOVA. * Significant value of p<0.05 and ** significant value of p<0.001. C: Control; T1: Treatment 1; T2: Treatment 2

***Jebisa* consumption increased Hb levels in adolescent girls with anemia.**

Hemoglobin (Hb) levels are a crucial indicator for the assessment of anemia (Figure 2 and Table 4). The mean Hb levels increased in all groups: C (10.59±1.61 g/dL), T1 (10.90±1.42 g/dL), and T2 (10.60±0.61 g/dL) groups after 30 days of treatment. However, the T1 group (11.44±2.01 g/dL) had higher mean Hb levels than that of the C (11.04±1.92 g/dL) and T2 (10.89±0.67 g/dL) groups after 60 days of treatment, which did not differ significantly (p>0.05). Table 4 indicated that some female adolescents in the C (36.36%) and T1 (55.56%) groups had normal Hb levels after 60 days of treatment. Only two female adolescents in the C group (18.18%) and one female adolescent in the T1 group (11.11%) showed increased Hb levels following 30 days of treatment. At the end of treatment, only female adolescents with mild anemia in both C and T1 groups showed improved anemia, resulting in normal Hb levels. One female adolescent in the C group, however, exhibited moderate anemia at the outset and subsequently transitioned to mild anemia. Notably, the T2 group did not demonstrate any improvement in anemia, yet the increased Hb levels among the groups did not differ significantly (p<0.05).

Table 3. Effects of *Jebisa* on micronutrient intake in adolescent girls with anemia (n=27)

Micronutrient intake	Day	Mean ± SD (%RDA)			P
		C (n=11)	T1 (n=9)	T2 (n=7)	
Fe (mg/day) [a]	0	5.11±1.82 (34.07%)	9.82±1.45 (65.47%)	4.94±2.08 (32.93%)	0.75
	30	3.69±0.87 (24.60%)	8.78±1.66 (58.53%)	10.90±3.60 (72.67%)	<0.001**
	60	4.74±3.43 (31.60%)	10.74±2.40 (71.60%)	19.59±1.70 (130.60%)	<0.001**
	p	0.22	0.04*	0.03*	
Zn (mg/day) [a]	0	4.44±1.67 (49.33%)	5.00±2.55 (55.56%)	4.07±1.04 (45.22%)	0.98
	30	3.79±1.45 (42.11%)	4.72±1.80 (52.44%)	4.44±1.37 (49.33%)	0.48
	60	4.55±2.95 (50.56%)	5.65±1.65 (62.78)	5.92±2.46 (65.78%)	0.14
	p	0.63	0.42	0.29	
Vitamin C (mg/day) [a]	0	32.07±45.71 (42.76%)	21.72±24.37 (28.96%)	28.39±20.24 (37.85%)	0.59
	30	3.69±0.87 (4.92%)	8.78±1.66 (11.71%)	10.90±3.60 (14.53%)	<0.001**
	60	25.22±30.55 (33.63%)	29.56±43.76 (39.41%)	41.47±40.68 (55.29%)	0.73
	p	0.09	0.68	0.12	

Note: [a] Statistical test with Kruskal-Wallis and others with One-Way ANOVA. * Significant value of $p<0.05$ and ** significant value of $p<0.001$. C: Control; T1: Treatment 1; T2: Treatment 2

Table 4. Effect of *Jebisa* on Hb levels in adolescent girls with anemia (n=27)

Day	Hb levels	C (n=11)	T1 (n=9)	T2 (n=7)	P
0	Moderate	5 (45.45%)	4 (36.36%)	4 (36.36%)	0.29
	Mild	6 (54.55%)	7 (63.64%)	7 (63.64%)	
30	Moderate	4 (36.36%)	3 (33.33%)	1 (14.29%)	0.52
	Mild	5 (45.45%)	5 (55.56%)	6 (85.74%)	
	Normal	2 (18.18%)	1 (11.11%)	-	
60	Moderate	3 (27.27%)	3 (33.33%)	1 (14.29%)	0.60
	Mild	4 (36.36%)	1 (11.11%)	6 (85.74%)	
	Normal	4 (36.36%)	5 (55.56%)	-	

Note: C: Control; T1: Treatment 1; T2: Treatment 2

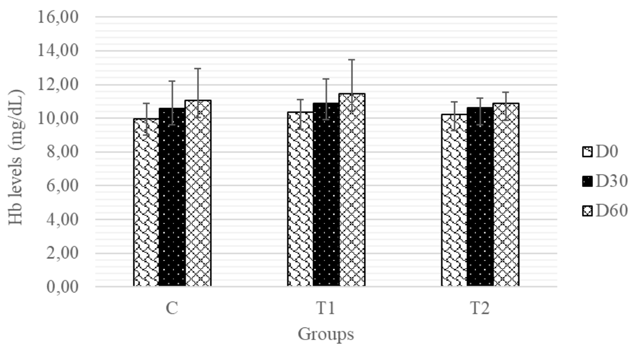

Figure 2. Effect of *Jebisa* on Hb levels in adolescent girls with anemia

***Jebisa* consumption increased the erythrocyte indexes in adolescent girls with anemia.**

Erythrocyte indexes (MCH, MCHC, and MCV ratio) were also evaluated in all groups (Table 5) to confirm anemia etiology. The mean MCV in the T2 group (76.43±5.22 fL) was lower than that of the C (77.45±8.89 fL) and T1 (77.56±8.60 fL) groups, but it did not differ significantly (p= 0.96). The highest MCH and MCHC were observed in the T1 group (24.61±3.82 pg; 31.60±1.65 g/dL) compared to the other groups before treatment, but all mean differences were not significant (p>0.05). The highest erythrocyte indexes were observed in the T1 group compared to the other groups, but it was not significantly different (p=0.66; p=0.76; p=0.48, respectively) following 30 days of treatment. After 60 days of treatment, erythrocyte indexes declined in all groups, returning to levels comparable to those observed before treatment. The decreased MCHC in all groups was significantly lower than 30 days of treatment (p=0.006; p=0.01; p=0.03, respectively).

Discussion

This study evaluated the effects of jelly consumption containing 18.52 g and 37.04 g SSF on dietary intake and hematological profile in adolescent girls with anemia. Consumption of both doses of *Jebisa* three times per week for 60 days significantly increased the daily intake of energy, carbohydrates, fibers, and Fe but did not increase the daily intake of proteins, fats, Zn, and vitamin C. Furthermore, the mean Hb levels increased, but it was not significant. Interestingly, a higher concentration of SSF increased Hb levels from moderate to mild anemia (6: 85.74%), and only one adolescent girl (14.29%) remained in the moderate anemia category. In contrast, 5 adolescent girls had normal Hb levels (55.56%), and 1 (11.11%) adolescent girl had mild anemia, while 3 (33.33%) adolescent girls remained in the moderate anemia category after consuming a lower concentration of SSF in the *Jebisa*. Surprisingly, *Jebisa* consumption had minor effects on MCV, MCH, and MCHC. These findings underscore the encouraging impact of *Jebisa* consumption on Hb levels, providing a strong motivation for further research and application that *Jebisa* consumption may become a supplementary food for anemia in adolescent girls.

Table 5. Effect of *Jebisa* on erythrocyte indexes in adolescent girls with anemia (n=27)

Erythrocyte indexes	Day	Mean ± SD			P
		C (n=11)	T1 (n=9)	T2 (n=7)	
MCV (fL)	0	77.45±8.89	77.56±8.60	76.43±5.22	0.96
Normal: 80-100 fL	30	77.91±9.89	80.33±8.52	76.57±5.16	0.66
	60	74.82±9.64	77.11±6.21	72.57±4.58	0.50
	p	0.71	0.65	0.26	
MCH (pg)[a]	0	23.87±3.63	24.61±3.82	23.27±1.79	0.77
Normal: 27-31 pg	30	26.70±5.19	27.83±4.15	26.73±3.91	0.76
	60	23.45±4.79	24.47±3.48	22.54±1.71	0.52
	p	0.18	0.17	0.01*	
MCHC (g/dL)[a]	0	30.73±1.74	31.60±1.65	30.44±0.54	0.17
Normal: 32-36 g/dL	30	33.98±2.62	34.57±2.08	32.99±2.45	0.48
	60	31.09±2.62	31.63±2.35	31.06±0.97	0.77
	p	0.006*	0.01*	0.03*	

Note: [a] Statistical test with Kruskal-Wallis and the other variables with One-Way ANOVA. * Significant value of $p<0.05$. C: Control; T1: Treatment 1; T2: Treatment 2

Based on the calculation of Fe ingredients in *Jebisa* with a higher concentration of SSF (37.04 g), it is adequate to support Hb synthesis in the human body. Our data showed that the mean Fe intake in adolescent girls with anemia increased approximately four-fold (19.59±1.70 mg/day) compared to the mean Fe intake before *Jebisa* consumption. The low intake of Fe in those adolescent girls is probably caused by the lack of a variety of foods rich in Fe because their monthly parents' income is lower than the regional minimum salary in Sukoharjo Regency (Table 1). Adding SSF into plain jelly will provide affordable supplementary food using natural ingredients from Indonesia. However, SSF dominantly contains ferric (Fe^{3+}) ions, which should be converted into ferrous (Fe^{2+}) ions in the presence of vitamin C and can be absorbed in the small intestine (Florez and Alborzinia 2021). In addition, the absorption rate of SSF is lower than Fe-rich foods derived from animals (35%) (Piskin et al. 2022).

Even though *Jebisa* has a beneficial effect on daily Fe intake, the daily intake of proteins (39.92±15.61 g/day) and vitamin C (41.47±40.68 mg/day) remained lower than the recommended dietary allowances (46 g/day and 75 mg/day, respectively) (Keats et al. 2018; National Institutes of Health 2021). This is most likely due to the low protein and vitamin C-rich foods such as meat, poultry products, fish, vegetables, and fruits since their parent's income is lower than the regional minimum salary in Sukoharjo Regency. This finding was similar to a recent study, indicating daily vegetable consumption is positively related to nutritional status and Hb levels in adolescent girls (Ilmiyati et al. 2023). This nutrient deficiency also plays a vital role in iron deficiency anemia, which can interfere with the heme formation process and the Hb formation (Adilah et al. 2023). Therefore, the Hb levels of adolescent girls slightly increased, and they continued to still suffer from anemia.

In community-based anemic evaluation, Hb levels represent the most crucial parameter. Our data indicated that jelly consumption containing 18.52 g and 37.04 g SSF could increase Hb levels by 1.1±1.6 g/dL and 0.64±0.78 g/dL in adolescent girls with anemia. The increased Hb levels in our findings were lower than the increased Hb levels in another study after the research subjects consumed 100 g jelly containing 14.9 g SSF and 5.7 g *Moringa* leaves flour (6.17±0.63 g/ dL) (Wijayanti et al. 2021). In addition, the increase of Hb levels in our study was slightly lower than in the previous study with the administration of 7 dates and Fe tablets for 30 days, which could increase Hb levels by 1.28±0.07 mg/dL (Sari et al. 2018). Thus, adding other food ingredients such as chocolate, milk, and eggs into the *Jebisa* is required to increase protein and vitamin C levels, and adolescent girls should take the iron tablet supplementation. The genetic factor may also contribute to the pathogenesis of anemia in adolescent girls with no improvement of Hb levels (moderate anemia). A recent study reported that the AA genotype of a duodenal Cytochrome B (CYBRD1) gene polymorphism, involving the conversion of Fe^{3+} to Fe^{2+} ions, increases the higher risk of anemia, compared to the GG and GA genotypes (Ilmiyati et al. 2023).

The most common anemia in developing countries is caused by iron deficiency, characterized by low erythrocyte indexes such as MCV, MCH, and MCHC (Helmyati et al. 2023). We found that *Jebisa* consumption did not affect the erythrocyte indexes in 30 or 60 days of treatment. In contrast, our results were different from another study that administration of 1.400 mg/day of *Moringa* leaves extract with 200 mg ferrous sulfate for 3 weeks in anemic women aged 16-49 years old increased the MCH by 1.31±4.94 pg and MCHC by 2.46±2.86 g/dL (Suzana et al. 2017). Administration of 100 g cookies containing 16 g sweet basil leaves powder for 4 months could also increase MCV by 1.34 fL, MCH by 1.14 pg, and MCHC by 1.67 g/dL in anemic adolescent girls (Akbar et al. 2024). Administration of fortified chocolate biscuits with iron sulfate for 10 weeks could increase MCV by 2.4±0.2 fL, MCH by 1.7±0.6 pg, and MCHC by 1.1±0.8 g/dL in Mexican schoolchildren with anemia (Quintero-Gutiérrez et al. 2016). Female adolescents with anemia who consumed 2×15 ml/day of date syrup and bee pollen for 14 days could increase MCV by 9.07±10.46 fL, MCH by 3.60±3.88 pg, and MCHC by 1.25±1.66 g/dL (Mony et al. 2022). There were two reasons why *Jebisa* did not affect the erythrocyte indexes. The Fe ion in *Jebisa* from snake fruit seeds has lower Fe absorption than that from animal products (Shubham et al. 2020; Piskin et al. 2022). Secondly, the adolescent girls with moderate anemia were probably unresponsive to the oral Fe since the anemic adolescent girls in the control group who received oral iron tablets also had their erythrocyte indexes remain the same (Table 5).

This study recognizes several limitations because our main findings had limited beneficial effects on nutrient intake and hematology profiles. *Jebisa* in this study consisted of SSF and plain jelly but did not contain Fe^{2+} ions and proteins derived from milk, chocolate, and egg. Theoretically, Fe^{2+} ion absorption is easier than Fe3+ ion absorption, which requires vitamin C to convert into Fe^{2+} ions. The erythrocyte indexes remained unchanged after *Jebisa* consumption in adolescent girls with anemia. We speculated that the genetic factor also contributed to the pathogenesis of anemia in adolescent girls in Sukoharjo Regency because those in the control group also had minor increases in Hb levels and erythrocyte indexes.

In conclusion, regular consumption of *Jebisa* containing SSF increased daily intake of energy, carbohydrates, fibers, Fe, and Hb levels. This does not affect protein and vitamin C intake and the erythrocyte indexes in adolescent girls with anemia. In the future, it is hoped that *Jebisa* can be used as an additional food for anemia with other ingredients of iron sources, such as milk, chocolate, and egg. Therefore, to ascertain the efficacy of the food supplementation, it is essential to assess the hepcidin and matriptase 2 levels and genotype of the CYBRD1 gene. It is also important to know whether *Jebisa* consumption can enhance Hb levels and erythrocyte indexes.

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