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# Snake Fruit Seeds Flour-Fortified Jelly for Supplementary Feeding in Adolescent Girls with Anemia

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**Abstract.** Anemia still becomes a public health problem over the world. Adolescent girls are one of the most susceptible groups who suffer anemia and have significant impacts on their health and study achievement. Fortification with high Fe natural ingredients is commonly used to overcome anemia in public setting. Therefore, the aim of this study was to evaluate the effect of snake fruit seed flour (SSF) on body weight (BW) and BMI/age of adolescent girls with anemia. A total of 20 adolescent girls participated in this study and randomly divided in control group and treatment group. They received 3 x 100 g jelly/week for 8 weeks. BW and BMI/age was measured in day 0, 30, and 60. Collected data were analyzed using paired t-test and ANOVA and p value < 0.05. Average of BW in control group was higher than in treatment group but it was not statistically different (p = 0,374). Average of BMI/age in control group was higher than in treatment group but it was not statistically different (p = 0,348). Administration of SSF does not influence BW and BMI/age in adolescent girls with anemia. Further investigation is required to verify whether or not SSF contains high carbohydrate and fibers.

## 1. Introduction

The global prevalence of anemia is 29.0% (496.3 million peoples) [1] so that anemia becomes a serious public health problem in developed and developing countries [2]. In Indonesia, adolescent girls are one of the highest risk age group with anemia and the prevalence is 26.5% [3]. Anemia is a condition that is characterized by reduction of erythrocytes amount and hemoglobin (Hb) concentration as an oxygen transporter throughout the human body below normal [4].

Adolescence is the second fastest stage of growth and development after childhood period. For their physical and physiological changes, they need higher amount of nutrients increase nutritional needs [5, 6]. It is not surprising that adolescent girls have 10 times higher risk of anemia than adolescent boys



because they undergo regular blood loss during menstruation [7]. However, other factors also contribute to anemia such as low nutrient intake, excessive physical activity, and anemia-limited knowledge [7,8].

Beside of increase of anemia incidence, low nutrition intake also affects nutritional status of adolescent girls. Body mass index (BMI) is the most important indicator for evaluation of nutritional status of adolescent girls [9]. Based on data of Riskesdas (2013), the prevalence of very thin (0.1%), thin (0.4%) and obese (5.9%) nutritional status has increased from 2010 to 2013 [4].

Anemia in adolescent girls can affect not only their human body but also school achievement [10]. In general, anemia can cause symptoms of lethargy, weakness, fatigue, tired and forgetful [11]. Anemia also decreases physical health and ability [5]. Even the incidence of anemia can also reduce economic productivity [12].

Oral iron supplementation becomes the standard therapy for anemia including in Indonesia. However, oral iron supplementation in adolescent girls does not distribute evenly in secondary schools in all areas in Indonesia. Iron-fortified foods are another alternative for anemia treatment for which snacks are often chosen like cookies, candies, chocolate and jelly [5, 13, 14, 15]. Adolescents are more likely to consume snacks than high densed foods. Jelly, for example, is more suitable for iron fortification in adolescent girls because of low energy and anytime consumption.

In Indonesia, snake fruits are cultivated in some regions but snake fruit pondoh is the most popular that is found in Pakem and Turi districts, Sleman Regency. Snake fruit seeds become waste and have not been used for medical purposes. From our research group, we have made SSF that has the content of Fe (26.8 mg / 100 g), Zn (4.65 mg / 100 g), v it C (0.82 mg / 100 g), and protein (3.7%). Therefore, the purpose of this study was to evaluate the effect of SSF jelly on BW and BMI/age in adolescent girls with anemia.

## 2. Methods

### 2.1. Experimental design

This study was a randomized control trial with pre-posttests group control design. It was performed in adolescent girls who came from senior high schools (SMA) in the Sukoharjo Regency from January to May 2019. A purposive sampling was used to select districts where represented the prevalence of anemia (high, moderate, and low) in adolescent girls and then selection of SMA used a simple random sampling. We got five SMAs: SMA N 1 Mojolaban, SMA N 1 Kartasura, SMA N 1 Nguter, SMA N 1 Weru, and SMA N 3 Sukoharjo. The protocol of this study was approved by the Ethical Committe of Medical Research, Faculty of Medicine, Unersitas Sebelas Maret, Surakarta (165/UN27.6/KEPK/2018). Selected adolescent girls agreed to follow this study if they signed the informed consent.

### 2.2. Selection of subjects

Sample size of our study was calculated using a formula from Jaykaran and Tamoghna [16] with 95% confidence level and 80% power test. Therefore, we needed at least 9 students for each group to avoid loss of follow up [17]. Adolescent girls were recruited as research subjects if they met inclusion criteria: 15-18 years old and having moderate anemia with Hb levels 80 - 109 g / dL. A total 38 adolescent girls met the inclusion criteria but only 22 adolescent girls participated in this study and were randomly divided into 2 groups. During 8 weeks intervention, there were two adolescent girls did not complete the study because of rejecting eating the jelly.

### 2.3. Intervention

SSF was the main ingredients of jelly and was added to agar-agar flour (PT. Dunia Bintang Walet – Jakarta, Indonesia), sugar, and mineral water, based on the procedure of Sani study [15]. Boiled jelly was put into a 100 ml plastic cup and was allowed to cool at room temperature for 1-2 hours. Every research subject in the C group was received 150 g jelly, which was equal to 129 mg Fe and were given three times/week for 8 weeks. Meanwhile T group received a cup of jelly, which contain 37 g of SSF and were equal to 30 mg Fe. Jelly was distributed to research subjects on Monday, Wednesday and Friday.

#### 2.4. Data collection and statistical analysis

Data of subject characteristics including age, height, history of allergy and illness were collected using open questionnaires. Measurements of BW and height used a Gea weight scale and microtoise respectively before, during, and after intervention. Body Mass Index (BMI) was calculated by dividing  $BW/height^2$  and nutrition status was based on z-score [9]. Characteristics data were presented as number, percentage or mean  $\pm$  standard deviation. The differences of pre-posttest data were statistically analyzed using the paired t-test and one way anova followed by Tukey post hoc test was used to determine differences among four different groups with p value  $< 0.05$ .

### 3. Results and Discussion

In this study, adolescent girls with anemia were given snacks in the form of jelly fortified with Fe supplement in the control group and SSF for the treatment group. Fortification is the addition of a nutrient to food. Fortification can be used to prevent the occurrence of iron deficiency, so that it can increase the value of health. Jelly is made as it is in general with only added ingredients which refers to the manufacture of high Fe pudding with modifications [15]. Jelly with the selected formulation was then tested for safety by the Center for Industrial Pollution Prevention Technology, Semarang. The test results show that observing SFF jelly meets the quality requirements according to SNI 01-35532-1994.

In this RCT Study, 11 adolescent girls were in the C group whereas only 9 adolescent girls were in the T group due to not eating jelly completely (**Table 1**). All research subjects aged between 15 and 17 years old. The mean age of research subject was  $15.91 \pm 0.83$  years old in the C group and  $15.89 \pm 0.60$  years old in the T group. From anthropometric data, the mean height of research subjects in the C group is similar to the mean height of research subjects in the T group. However, higher mean BW was observed in the C group ( $49.73 \pm 7.80$  kg), compared to the T group ( $46.77 \pm 6.44$  kg). In Indonesia the average size of the weight and height of adolescent girls is 50 kg and 154 cm [5]. Average of BW and height subjects in this study showed a smaller than the national average. The average of WB in the C group was around 49 kg while in the T group it was in the range of 46 kg. The average of height in the C group and T group was in the same range around of 153 cm.

**Table 1.** Characteristics of Research Subjects who Participated in The RCT Study.

	Group		P value
	Control	Treatment	
Mean age (years old)	$15.91 \pm 0.83$	$15.89 \pm 0.60$	0.952
Sebaran umur :			
15 years old	4 (36%)	2 (22%)	0.414
16 years old	4 (36%)	6 (67%)	0.527
17 years old	3 (28%)	1 (11%)	0.317
Mean height (cm)	$152.92 \pm 5.66$	$153.01 \pm 5.49$	0.971
Mean BW (kg)	$49.73 \pm 7.80$	$46.77 \pm 6.44$	0.374
Nutritional status:			
Severely wasted	-	-	-
Wasted	-	-	-
Normal	9 (82%)	7 (78%)	0.617
Overweight	1 (9%)	2 (22%)	0.564
Obesity	1 (9%)	-	-
History of allergy			
Eggs	1 (9%)	1 (11%)	1.000
Seafoods	2 (18%)	2 (22%)	1.000
History of illness	1 (9%)	-	-

Based on the 24-hour food recall (**Table 2**), showed the T group higher than the C group in all macronutrient parameters (energy, carbohydrate, protein, fat, and fiber) but did not show a significant difference ( $p > 0.05$ ). Macronutrient intake on day 60 showed an increase compared to day 0. One of the factors influencing the increase in intake on days 30 and 60 due to the administration of SSF jelly in the study subjects. The average energy intake in both groups showed lower results than the nutritional adequacy rate of Indonesia (AKG). Similarly, energy, carbohydrate, protein, fat, and fiber intake also shows results below the AKG for women in the age group of 15-18 years.

**Table 2.** Mean of Macronutrient Intake of Research Subjects.

Macronutrient	Day	Mean		P value
		C group	T group	
Energi (kcal)	0	1418.90 $\pm$ 679.27	1419.49 $\pm$ 530.30	0.998
	30	1328.26 $\pm$ 416.21	1639.07 $\pm$ 493.06	0.143
	60	1623.05 $\pm$ 729.93	1884.24 $\pm$ 542.48	0.385
Carbohydrate (g)	0	209.96 $\pm$ 105.82	181.50 $\pm$ 75.91	0.508
	30	208.77 $\pm$ 68.10	308.49 $\pm$ 69.58	0.005
	60	246.81 $\pm$ 117.40	327.14 $\pm$ 139.68	0.179
Protein (g)	0	46.78 $\pm$ 20.50	47.74 $\pm$ 14.44	0.907
	30	39.68 $\pm$ 13.62	42.80 $\pm$ 17.44	0.658
	60	49.44 $\pm$ 22.88	55.27 $\pm$ 18.71	0.547
Fat (g)	0	42.64 $\pm$ 22.80	55.01 $\pm$ 36.21	0.363
	30	39.76 $\pm$ 20.07	39.93 $\pm$ 26.03	0.987
	60	50.96 $\pm$ 39.55	55.48 $\pm$ 13.92	0.748
Fiber (g)	0	6.03 $\pm$ 3.23	6.10 $\pm$ 2.74	0.958
	30	10.58 $\pm$ 4.19	8.62 $\pm$ 3.86	0.296
	60	9.96 $\pm$ 5.22	11.16 $\pm$ 5.81	0.632

Nutritional status of research subjects showed different distribution. Distribution of normal, overweight and obesity nutritional status in the C group was 82%, 9% and 9% respectively. Whereas in T group distribution only in normal (78%) and overweight (22%) nutritional status. But the distribution of each nutritional status in the two groups did not show a significant difference with a P value of 0.617 (normal) and 0.564 (overweight). In this study there were no research subjects with nutritional status wasted and several wasted. Around 10% research subjects in both groups had egg allergy and 20% among research subjects had seafood allergy. From all research subjects, there was only 9% having history of illness.

**Table 3.** The Effect of SSF Jelly on BW and BMI/age in Research Subjects with Anemia.

	Day	Mean		P value
		C group	T group	
BW (kg)	0	49.73 $\pm$ 7.80	46.77 $\pm$ 6.44	0.374
	30	49.61 $\pm$ 8.36	46.99 $\pm$ 6.74	0.458
	60	49.15 $\pm$ 9.03	46.16 $\pm$ 6.38	0.421
	P value	0.985	0.963	
BMI (kg/m <sup>2</sup> )	0	21.25 $\pm$ 3.15	20.00 $\pm$ 2.49	0.348
	30	21.08 $\pm$ 3.46	20.03 $\pm$ 2.61	0.407
	60	20.59 $\pm$ 3.63	19.53 $\pm$ 2.61	0.534
	P value	0.897	0.964	

**Table 2** showed the BW average in the C group showed a decrease on the day 30 (49.61  $\pm$  8.36) and returned to the day 60 (49.15  $\pm$  9.03) but did not show a significant difference ( $p = 0.985$ ), while the T group showed fluctuating results, increased in day 30 (46.99  $\pm$  6.74) and decreased in day 60 (46.16  $\pm$

6.38). The increase in BW average in the treatment group was  $0.12 \pm 1.58$  kg but on day 60 it decreased by  $0.46 \pm 1.19$  kg from the day 30, but the difference in values did not show a significant difference ( $p = 0.963$ ). The difference in mean BW in both groups on days 0, 30 and 60 did not show a significant difference, with P values 0.348, 0.407 and 0.534 respectively.

The nutritional status indicators used BMI/age which is based on anthropometric measurements of BW and height. The classification indicator of BMI/age according to RI Ministry of Health is divided into five: severely wasted (Z score  $< -3.0$ ), wasted (Z score  $\geq -3.0$  until  $< -2.0$ ), normal (Z score  $\geq -2.0$  until  $\leq 1.0$ ), overweight (Z score  $> 1.0$  until  $\leq 2.0$ ) and obesity (Z score  $> 2.0$ ) [9]. BMI/age showed the same pattern as BW, the average in the C group showed a decrease on the day 30 ( $21.08 \pm 3.46$ ) and day 60 ( $20.59 \pm 3.63$ ), while in the T group the BW increased on the day 30 ( $20.03 \pm 2.61$ ) but fell on the day 60 ( $19.53 \pm 2.61$ ). The difference in mean BMI/age on day 0, 30 and 60 days in the both groups did not showed significant differences, 0.897 and 0.964 respectively. Average of BMI/age on a daily basis did not show a significant difference in the C group and T group which was indicated by the P value on the day 0 (0.348), 30 (0.407) and 60 (0.534).

Basically, nutritional status (BMI/age) does not affect the incidence of anemia [18]. In this study around 80% of research subjects with anemia have a normal nutritional status and just around 20% who have poor nutritional status (overweight and obesity). This result not linear with Yunarsih and Sunny statement that poor nutritional status risks causing anemia [18]. Another statement also said adolescents with normal nutritional status have a small risk to anemia than adolescents with more nutritional status sufficient [19]. Adolescents with normal nutritional status or more who anemia are due to lack of consumption of macro nutrients (energy and protein) and micronutrients (such as nutrients), so that the ingredients used to form red blood cells are not sufficient [19].

SSF fortification in jelly is done to meet the needs of macronutrients and micronutrient adolescent girls with anemia. Macronutrients are obtained like carbohydrates and proteins, while the micronutrients obtained are Fe, Zn and vitamin C. In addition, jelly also contains fiber which is a macronutrient. Fortification is generally carried out by adding a nutrient to a food that is widely consumed [11]. In this study jelly was chosen as fortified food, considering the subjects in this study were adolescents who preferred consumption a low density food (snacks).

This study shows that SSF jelly in anemic adolescent girls does not affect both BW and BMI/age on C group or T group because there was no significant increase or decrease. SSF is a snack that is a food with high energy density [20], because containing macronutrients like carbohidrat, protein, and fiber. This study is in line with the statement of Adhitya et al [21] which stated that giving snacks did not affect nutritional status but had a tendency towards increasing nutrient intake. In the study of Chaterine et al [22], the administration of snacks did not affect the nutritional status of girls and boys, and did not show significant differences in BMI/age. In a study conducted by Leon et al [23], was shown that iron supplementation in children aged 8-12 years with iron deficiency anemia did not show a significant difference in weight and height.

One of the factors that influence nutritional status is physical activity. Basically the nutritional status, physical activity and incidence of anemia are related. There is no significant difference in the administration of jelly, both in the C group and in the T group from days 0 to 60 can be caused by physical activity. According to Silvia et al [24], children with normal nutritional status have a tendency to be more active so that they engage in more physical activity. In this study the majority of research subjects were in normal nutritional status, so it is possible for them to have high physical activity. High physical activity helps the process of energy expenditure, so it is not possible to accumulate energy which causes an increase in BW [25].

In addition, the presence of fiber content in jelly can also be a factor that affects BW or BMI/age. The presence of fiber can reduce BW and reduce macronutrient absorption [26]. High-fiber foods require a longer digestive time, so that young women do not easily feel hungry and reduce excessive food consumption [27]. Therefore young women who were the subjects of this study did not experience an increase in BW, so that it did not affect nutritional status. The disadvantage of this study is that there has been no measurement of physical activity and daily food intake.

#### 4. Conclusions

Jelly SSF as a snack does not show a significant difference of WB and BMI/age in day 0, day 30 and day 60, so observing SSF jelly can be used as a snack alternative for adolescent girl without worrying about increasing WB. However, further research is needed to determine the content in SSF jelly, especially the carbohydrate and fiber content.

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