

**FINAL REPORT**

**PRODUCTION AND CLINICAL IMPACT STUDY OF  
MICRONUTRIENTS FORTIFIED RICE FOR TEEN GIRLS IN  
ISLAMIC BOARDING SCHOOL IN MEDAN, INDONESIA**

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In Collaboration with GIZ**



## I. INTRODUCTION

### 1.1 Background

Indonesia as a middle-income country by wide regional disparities: cities with modern shopping malls contrast sharply with the country's poor rural areas. Indonesia is a member of G20 – the Group of Twenty of the world's major industrial and emerging economies – and is playing an increasingly important role in the region. Reforming social protection system is anchored firmly as one of the government's national priorities in the five-year medium-term development plan and will be continued to play a key role in the 2015-2019 development plans. Social protection is seen as one of the foundations for economic growth and social cohesion.

Indonesia has become one of the 17 countries with concerning nutrition problems, placing the country's nutrition status at the same level as African countries. It is conveyed by the Deputy of Human Resources & Culture of Ministry of National Development Planning (BAPPENAS). Both under and over nutrition problems are facing by Indonesians, such as underweight, stunting, obesity, anemia, vitamin A and Iodine deficiencies. According to presidential decree (PERPRES) No. 43/2013 on Acceleration Nutrition Improvement, the main focus of the target groups of the nutrition programs are teenagers, woman at reproductive age, pregnant woman, lactating woman, and young children, particullary among poor, isolated communities and disaster victims such as refugees.

Rice plays an important roles in Indonesians diet. About 97,7% of Indonesian consume rice every day (MOH 2015) with the mean intake was 232,3 grams per capita per day (BPS 2015). The Indonesian population still consumes less than 50% of the amounts of vitamins and minerals required especially iron, calcium, vitamin B1, B2, B3, and B9 (Hardinsyah et al. 2012). Iron Deficiency Anaemia (IDA) is a serious health issue that compromises the cognitive development of young children and increases the risk for maternal death at birth. According to Riskesdas 2013 (Indonesian Basic Health Research) of Ministry of Health, 37,1 % pregnant woman, 28,1% children under-five years and 26,4% of school children are anemic. There is no much change in the prevalance of anemia among children under-five and school children.

Vitamin A is an essential micronutrient especially relevant for the eyesight, immune functions and growth. Urban poor families, rely on cheap staple foods which are lack of vitamin A content. According to the Micronutrient Initiative (MI) subclinical forms of vitamin A deficiency affect 14.6 % of children under five in Indonesia which is considered a significant public health problem.

Other nutrients also tend to be lower. Deficiencies in vitamin B1 and B3 often occur in populations which consume milled rice as their main staple food. Rice is also a poor source of folic acid, which is important for fetus development. Zinc which plays important roles in bone development and the immune system, is usually in adequate in Indonesian diet. Children with inadequate zinc intake are likely to be stunting and suffer more often from diarrhoea, further compromising

their growth. Stunting is prevalent among Indonesian children. Update from data of Riskesdas 2013 of Ministry of Health, the prevalence of stunting among children under-five years was 37,2% and among school children (5-12 years) was 30,7 %.

Evidence from developed countries showed that the health and academic performance of primary school children are improved through the introduction of school food and health programmes. Although some have recognised the value of school food and health programmes, there is still a lack of realistic and affordable concepts, as well as the required expertise. Very few sustainable programmes run on a regional basis. Many are only carried out in a few individual schools and are thus far from reaching their full potential.

The Better Rice Initiative Asia (BRIA) is a larger Public Private Partnership (PPP). The program aims at fostering the cooperation between the German private sector and public sector institutions such as GIZ; and the objective is to promote sustainable growth in agricultural production and improved access to nutrition in emerging and developing countries include Indonesia. There are two BRIA components in Indonesia namely Agriculture and Nutrition. The nutrition component consists of oil fortification and rice fortification.

Deutsche Gesellschaft fur Internationale Zusammenarbeit (GIZ) has been working in Indonesia since 1975 and opened its office in Jakarta the same year. Currently, GIZ believe that food fortification is one of the strategies for improving nutrition and health of school children and refugees in the disasters area. However, a clinical study in Indonesia on this field is required as a basis for future sustainable programs. In addition, fortified food such as wheat flour, noodle, salt has been proven to effectively reduce deficiencies of various micronutrients in different countries.

Based on the above considerations, the Food and Nutrition Society (PERGIZI PANGAN) Indonesia in collaboration with BRIA Indonesia and GIZ conducted a study on fortified rice production and clinical impact study to gain evidence on the effectiveness of fortified rice for school children, to reduce micronutrient malnutrition in Indonesia.

## **1.2 Objectives**

The general objective of this study is to gain evidence on the efficacy of fortified rice for boarding school teenagers girl's students on improving nutritional status of the teenagers girl's students.

The specific objectives of this study are:

1. To produce and supply of quality fortified rice for boarding school teenagers girls .
2. To analyze the clinical efficacy of rice fortification for boarding school teenagers girls on improving their nutritional status.

## II. METHOD

### 2.1 Time and Location

The study is being conducted from January to September 2016, in Medan of North Sumatra Province, Indonesia. Considering the adequate time for study preparation (administration, approaching the school and the local government, workshop, recruiting and training of enumerators and field technicians, baseline data collection, and logistic preparation), therefore the intervention was started on the 1<sup>st</sup> of March 2016. The duration of intervention was about four months from March to June 2016. Since the fasting month of ramadhan started at the second weeks of June, the end of this study was at 11<sup>th</sup> June 2016. Four month is the minimum requirement to assess the changes of the nutrition biomarker, such as haemoglobin, ferritin, zinc serum, folic acid, vitamin A.

### 2.2 Research Design

The study design that was applied for the clinical efficacy of fortified rice was randomized control trial among teenage girl students. This study obtained an ethical approval from Faculty of Medicine, University of Indonesia (No: 97/UN2.F1/ETIK/2016).

### 2.3 Samples

The minimum sample size for this study was calculated based on the formula of comparing two means of haemoglobin levels of the two groups (intervention group and control group) as follows:

$$n = (Z_{\alpha/2} + Z_{\beta})^2 * 2 * \sigma^2 / d^2$$

where  $Z_{\alpha/2}$  is the critical value of the normal distribution at  $\alpha/2$  (1.96),  $Z_{\beta}$  is the critical value of the normal distribution at  $\beta$  (0.8),  $\sigma^2$  is the population variance of haemoglobin (576); and  $d$  is the different of haemoglobin would like to detect (10 g/L). This figures based on the study by Husein SZ, et al, (2014) on micronutrient fortified rice for children in India.

The minimum sample size based on the above formula was 91 subjects for each group. Considering loss to follow up about 10%, than the total subject for this study is 100 subjects for each group (100 subjects for intervention group and 100 subjects to control group). Then, after collecting baseline data, subjects were allocated equally and randomly into intervention and control groups.

The minimum sample size for this study, considered also other related biomarkers measured in this study (ferritin, folate, vit A/retinol, zinc, and CRP). An analyzes from the previous studies using the standard deviations of several biomarkers that were measured in this study (hemoglobin, ferritin, folate, vit A/retinol, zinc, and CRP), showed that the minimum sample sizes range from 22 to 211 subjects. (Table 2.1)

Table 2.1 Minimum sample size based on several biomarkers of the previous studies

Outcome	Reference	Target	Duration	control		intervensi		n minimal
				n	SD	n	SD	
Hemoglobin	Thankachan 2012	Children 6-12 yrs	6 month	76	7	76	7.4	152
Hemoglobin	Moretti 2006	Children 6-13 yrs	7 month	92	12	92	10.5	185
Hemoglobin	Haas 2005	Women 18-45 yrs	9 month	100	10	92	10	192
Ferritin	Moretti 2006	Children 6-13 yrs	7 month	92	15	92	18	186
Ferritin	Haas 2005	Females 18-45 yrs	9 month	100	1.95	92	1.1	211
Retinol	Haas 2005	Females 18-45 yrs	9 month	100	45.2	92	49.05	192
Retinol	Thankachan 2012	Children 6-12 yrs	6 month	55	1.25	50	1.4	105
CRP	Moretti 2006	Children 6-13 yrs	7 month	92	6.3	92	6.2	184
Folate	Haas 2005	Females 18-45 yrs	9 month	100	1.8	92	1.8	192
Zinc	Haas 2005	Females 18-45 yrs	9 month	100	0.175	92	0.16	193
Hemoglobin	Nadimin 2011	Females 18-22 yrs	2 month	10	1.07	10	0.56	22

Having the study being located in an Islamic boarding school, where the students have limited access to fruits and vegetables, and also red meat, as commonly occurred among Indonesia teenagers, the researcher's assume that the prevalence of anemia similar to the national prevalence. The majority of the anemia was assumed to be caused by iron deficiency anemia, rather than genetic disorders such as Thalasemia. Studies in Indonesia has shown that the prevalence of thalasemia is quite low, which is 2%.

The inclusion criteria of the subjects were teenage girls who have already experienced menstruation in boarding school, aged 14-18 (grade 4, grade 3 Intensive and grade 5), have symptoms of anemia (that was assessed through self administered questionnaire on experience of lethargic and weak), and willing to involve in this study. Subjects were excluded if they have a history of chronic diseases, obese, blood donation during the last 3 months, and regular fasting (intermittent fasting, and fasting on Monday and Thursday).

#### 2.4. School Meal and Food Vendor

In the boarding school, each student is provided meals. The meals were given six-days for every week (Tuesday to Sunday), three meals a day (breakfast, lunch and dinner). Every meal given to student always contain rice, side dish and vegetable soup. The side dishes are rotated in the schedule consists of one the following dishes: egg, fish, chicken, tempe, or tofu. Vegetables soup are usually served once in a day, at lunch or dinner time. The menu cycle was changed every 2 weeks. The school meal was cooked and prepared centrally by the school kitchen.

Although the school provides 3 meals every day, the school also has food vendor where the students allowed to buy food, which is usually as snack and drink from the food vendor. Most of snack and drink sold in the food vendor were packed food from food industries. The food vendor manage by the school.

## 2.5. Nutrients Premix

Considering main purpose of this study is to improve micronutrient deficiencies in order to reduce prevalence anemia, the micronutrients provide in the premix are iron, zinc, thiamin, folic acid, vitamin B12, niacin, and vitamin A. The level of these micronutrients in the premix was determined in order to fulfill more than 75% of the RDA (Recommended Dietary Allowance) of the selected micronutrients from fortified rice, that was consumed 225 gram per day (450 gram cooked rice per day). Content of vitamins B were higher than their RDA because their are prone to heat when cooking, however they were far lower than their upper level. The premix was designed and produced by DSM of Singapore (Table 2.2).

Table 2.1 Composition of premix\* and estimated content of micronutrient in fortified rice

Nutrient	In the premix	Unit	Fortified rice with 2% kernel (100 g)	Unit	Contain in 225 g of rice per day	Unit	Upper level	RDA	% RDA
Iron	5400	mg/kg	10,8	mg	24,3	mg	45	26	93,5
Zinc	2570	mg/kg	5,1	mg	11,6	mg	34	16	72,3
Thiamin	500	mg/kg	1,0	mg	2,3	mg	ND	1,1	204,5
Folic acid	72560	ug/kg	145,1	ug	326,5	ug	800	400	81,6
Vit B12	1630	ug/kg	3,3	ug	7,3	ug	ND	2,4	305,6
Niacin	6000	mg/kg	12,0	mg	27,0	mg	30	12	225,0
Vit A	141,77	mg/kg	283,5	mcg	638,0	mcg	2800	600	106,3

\* Based on premix lable from the producer (DSM)

## 2.6. Rice Fortification

Fortified rice was produced by PT FITS Mandiri, and experienced factory in producing. The dried rice kernel was subsequently packaged into alluminium foil pack, each pack for 2 kg rice kernel then was stored in a cold room at 19-22<sup>o</sup> C before sending to boarding school in Medan. The scematic process on the production of rice kernel shown in the following Figure.

The mixing was done by research assistants together with trained kitchen personnel, every week on Monday (day of fasting). Each Monday the fortified rice was produced for the amount of 225kg. This amount was packed in 18 sacks @12.5 kg (a sack for breakfast, a sack lunch and a sack for dinner too). A total of 18 sacks of rice fortification was coded to show the intervention week and the serial number of sacks, as shown in Table 2.3. For example the sack code is M1-H3-W2, it means the fortified rice sack is for the first week intervention (M1), on the third day of the week that is Thursday (H3) and for lunch time (W2).

Table 2.3 Coding system of the rice fortification in the ware house

M (Week of Intervention)	H (Day of Intervention in a Week)	W (Meal time in a day)
Because of the intervention for 16 weeks then the code was labelled as M1 to M16	Because there are six days of the week of intervention then the code was labelled as H1 through H6 H1 = Tuesday H2 = Wednesday H3 = Thursday H4 = Friday H5 = Saturday H6 = Sunday	Because there are three meals a day, then the code was labelled as W1 to W3 W1 = Breakfast W2 = Lunch W3 = Dinner

The sacks in the warehouse was arranged on three rows and six levels in a dry place above the floor boards or pallets (sacks was not come into direct contact with the floor and was avoid interference from rodents, insects and moisture). Checking the sacks of fortification rice was done every week (at the middle of the week) i.e. On Thursday to check the existence of the sacks according to the code number, condition and status of storage sacks by field coordinator.

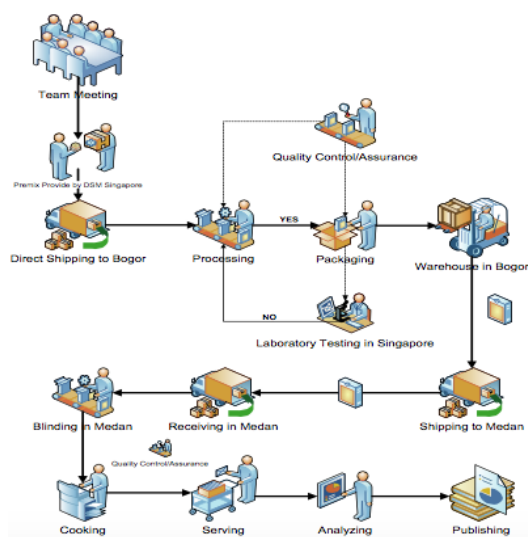
## 2.7. Cooking and Distribution of Fortified Rice

The research team was provide the cookware to cook for fortified rice in accordance with the boarding school kitchen manager order (boiler, stove, furnace and baskets of rice-3 pieces with different colors). The research team trained the kitchen staff about: saving the premix kernel, mixing the premix kernel, packing the fortification rice, coding and sewing the sacks of fortified rice, how to wash and cook the fortification rice, how to move the rice from the boiler to a particular basket, how to use the remaining cooked fortified rice on the basket (was carried by enumerators), how to manage the fortified rice to the enumerators in the dining room.

Every kitchen staff, who would cook the rice, took one sack of fortified rice (@ 12.5 kg) in accordance with a predetermined code. At the same time, the enumerators checked and match the sacs code by week, day and meal times in the kitchen. The kitchen staff poured one sack of fortified rice into a plastic bucket to wash the rice. Rice washed as usual in the kitchen but only for once. The fortified rice which had been washed, put into a steamer of hot water. The kitchen staff stirred the rice cooked in a steamer as usually done in the kitchen until the rice is cooked well, about one hour. The fortified rice that has been cooked poured into 3 (three) pieces of the special colored basket as a sign of the fortification rice from non-fortified rice. Both fortified and non-fortified rice with the dishes and vegetables in a sealed container was taken by car to the dining room (canteen) as usual. The meaning of colors of the basket only known by kitchen staff and servants.

About 15 minutes before the meal time a cup of cooked rice (150 gram) was placed to the plate that was already coded to the differentiate between control and intervention group. Then, each plate was given to the subjects according to their sit. At the meal time after praying, subjects consumed the rice together with

side dish and vegetable soup provided in her dining table (Figure 2.1).



## 2.8. Data Collection

The variables to be collected for the efficacy studies are: identity of subject, socio-economy conditions of subjects, body weight, body height and food intake of subjects. The main outcome variables to be measured are Hemoglobin level, Ferritin level, CRP (C-Reactive Protein) level, Zinc serum level, vitamin A, B9 (folic acid) and B12 level of the subjects through blood collection and laboratory analysis. In addition, other possible related outcome are also to be collected such as absenteeism, morbidity and cognitive score (Table 2.4).

Table 2.4. Summary of variable and outcome collected and measured

Variables and Outcomes	Baseline	Endline	Method
1. Variables be collected for production and supply of fortified rice	Quantity of rice kernel		Weighted and Recorded
	Quantity of fortified rice		
	Level of iron in fortified rice and fortified cooked rice		Accredited food laboratory analysis (Saraswati)
	Level of zinc in fortified rice and fortified cooked rice		
	Level of folic acid in fortified rice and fortified cooked rice		
	Level of Vitamin A in fortified rice and fortified cooked rice		
	Identity of subject		
2. Socio-economic variables be	Socio demography conditions of subjects	Body weight	Interview and observation



Variables and Outcomes	Baseline	Endline	Method
collected for the efficacy studies	Economy conditions of subjects and family		Direct measure
	Body weight		
3. Anthropometric status and diet be collected for the efficacy studies	Body height	Body height	SQ-FFQ
	Food intake of subjects	Food intake of subjects	
4. The main outcome variables to be measured for the efficacy studies	Hemoglobin level	Hemoglobin level	Accredited laboratory analysis (SEAMEO, UI)
	Ferritin level	Ferritin level	
	CRP (C-Reactive Protein) level	CRP (C-Reactive Protein) level	
	Zinc serum level	Zinc serum level	
	Folic Acid level	Folic Acid level	
	Vitamin A	Vitamin A	

## 2.9. Data Management and Analyzes

Data management were started by the developing a Code Book containing variable codes and the description of each code according to the information on the screening questionnaire. Research assistant performed data entry in pairs. On the template provided in accordance to Code Book. They checked the accuracy of data entry by re-entry 10% the number of subjects and the examination results of descriptive statistics (minimum value, maximum value and average each variable).

Data cleaning was done by considering the logic of data obtained from the subject and verify the correctness of the data. The things to consider in this process are as follows: a) Completeness of data, b) The normal value and the extreme value (minimum-maximum) of data on age, weight, height, weight of groceries from the food intake data, information socio-economic, and the others. Any of abnormal data were verified through the examination of primary data questionnaire or original form. If the fault lies in the entry process, the data were corrected as stated in the primary data. However, if irregularities or incompleteness of data derived from the subject, the enumerators are asked to interview directly concerned the subject back and asked about the incomplete data. The information obtained was repaired at the primary data and data base. If the database is complete and the extreme value has been verified, the data ready for analysis.

Data analysis by using the SPSS Statistics 20 software. The descriptive analysis was applied to obtain a description of the data in general, whereas the comparative test was applied compare the data between the control and intervention groups.

The cut-off point applied to determine of biomarker status as shown in Table 2.5. The analysis of the effectiveness of the fortified rice was done by applying a linear regressions for continuous dependent variables namely hemoglobin level, ferritin level, folic acid level, vitamin A level, zinc level, that were analyzed with the independent variables such as delta CRP level, initial folic acid level, delta vitamin A level, delta zinc level, initial ferritin level and potential confounders. Since the subject was consists only female teenagers with a narrow age group (15-17 years old), the age and sex variables did not include as in the independent variables in the regression analysis.

Table 2.5. Cut off point applied to determine of biomarker status

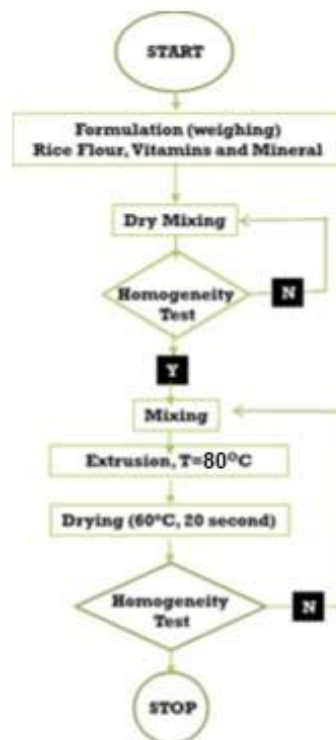
Category	Cut Off	Source
<b>Folic Acid</b>	<b>(ng/mL)</b>	WHO (2012)
Deficient	<5,9	
Normal	6-20	
Elevated	>20	
<b>Hb</b>	<b>(mg/L)</b>	WHO (2011)
Severe Anemia	<8	
Moderate Anemia	8-10,9	
Mild anemia	11-11,9	
Non Anemia	>= 12	
<b>Ferritin</b>	<b>(ng/mL)</b>	Gibson (2005)
Defisien	<15	
<b>Zinc</b>	<b>µg/dL</b>	WHO/FAO (2004)
Deficient	<10.7	
Low	10.7-20	
Acceptable of High	>20	
<b>Retinol</b>	<b>µg/dL</b>	WHO/FAO (2004)
Deficient	<0.7	
Low	<1.05	
Acceptable of High	≥ 1.05	
<b>CRP</b>	<b>(mg/L)</b>	http://mayomedical laboratories.com /test-catalog
Normal	≤ 8	
Abnormal	> 8	

### III. PRODUCTION AND SUPPLY OF FORTIFIED RICE

#### 3.1. Production of Fortified Rice

Fortified rice was produced by mixing rice kernel and rice with the ratio 2:100 using mixer machine. The rice kernel was produced by mixing nutrient premix, rice flour and water using extruder machine. The kernel was made from a mixture of 45 kg rice flour (IR 42), 5 kg nutrients premix, and 4,5 L water to produce 40 kg of rice kernel. The mixture was homogenized with a mixer. The rice flour was made by PT FITS Mandiri. The rice kernel from extruder was dried at 80° C for 2 hours until the water content of the premix kernel is about 14%.

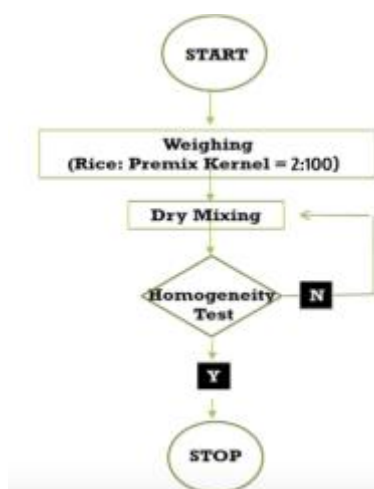
The dried rice kernel was subsequently packaged into aluminium foil pack, each pack for 2 kg rice kernel then was stored in a cold room at 19-22° C before sending to boarding school in Medan. The schematic process on the production of rice kernel shown in the following Figure.



The rice kernel was transported to the boarding school in Medan by aeroplane for about two hours flight. The production of fortified rice was done through a mixing of rice kernel with rice in which the variety is the same as the rice variety commonly consumed at the boarding school. The composition of fortified rice was 2% of rice kernel plus 98% of rice (as shown in the following Figure).

The level of 2% rice kernel used in this study was based on 1) the quantity of the cooked rice commonly consumed by subject in each meal, namely 150 gram cook rice, which is equal to 75 gram rice (uncook rice); 2) the micronutrient adequacy target more than 75% RDA ; and 3) the result of organoleptic/sensoric test from five levels of rice kernel (1%, 1,5%, 2%, 2,5%, and 3%) that was done prior to the intervention.

The mixing was done by using a hexagonal mixer with a capacity of 25 kg powered electrically. The quality of hexagonal mixer machine and homogeneity testing were done in Bogor before the mixer before it was sent to Medan. The mixer was installed in the warehouse of kitchen (mounted firmly on the floor and connected with electrical power).



The mixing was done by research assistants together with trained kitchen personnel, every week on Monday (day of fasting). Each Monday the fortified rice was produced for the amount of 225kg. This amount was packed in 18 sacks @12.5 kg (a sack for breakfast, a sack lunch and a sack for dinner for six days). The sacks were kept safely in the warehouse closed to the kitchen.

Quality checks of the nutrients content of the rice kernel and fortified rice were performed by an accredited laboratory analysis, namely in Laboratory Saraswanti in Bogor. The result of analyses as shown in Table 3.1.

Table 3.1 Composition of fortified rice and cooked fortified rice

Nutrient	Unit	Fortified rice with 2% kernel (100 g)	Cooked fortified rice (100 g)	Cooked fortified rice (450 g/day)	Upper level	%RDA
Iron	mg	10,8	5,5	24,8	45	95,5
Zinc	mg	5,2	2,3	10,3	34	64,7
Thiamin	mg	2,0	0,5	2,3	ND	212,7
Folic Acid	ug	145,2	47,4	213,3	800	53,3
Vit B12	ug	3,2	1,0	4,3	ND	180,0
Niacin	mg	ND	ND	ND	30	ND
Vit A	mcg	283,6	120,6	542,7	2800	90,4

The dried rice kernel was subsequently packaged into alluminium foil pack, each pack for 2 kg rice kernel then was stored in a cold room at 19-22<sup>0</sup> C before sending to boarding school in Medan. The scematic process on the production of rice kernel shown in the following Figure.

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#### IV. CHARACTERISTIC OF SUBJECT AND COMPLIANCE

##### 4.1. Characteristic of Subjects

The characteristic of the subject in both control and intervention groups shown in Table 4.1. In general there are no significant differences on the characteristics of subjects between control and intervention group. During the baseline survey, the mean age of subjects were around 16,1 years old. Both groups have an average of normal nutrition status with mean weight 49,4 kg and height 151,5 cm. Most of the subject's parents are high school graduates and above. The family income ranges from 4.9 million to 5.5 million rupiahs. The majority of subjects ethnicity were Javanese and Bataknese.

Table 4.1. Characteristic of Subjects

Characteristics	Control (n=107) Mean ± SD	Intervention (n=108) Mean ± SD	P Value
Age (years)	16,1±0,7	16,2±0,8	0,13
Weight (kg)	49,4±5,9	49,4±6,1	0,95
Height (cm)	151,2±8,4	151,9±5,7	0,62
BMI/Age Z Score	0,2±1,0	0,1±0,8	0,96
Allowance per month (Rupiah)	265.935±149.838	285.880±171.257	0,52
Father's years of education, n (%)			0,88
Never went to school	0 (0,0%)	2 (1,9%)	
1-6 years	3 (2,8%)	10 (9,3%)	
6-9 years	8 (7,5%)	12 (11,1%)	
9-12 years	55 (51,4%)	45 (41,7%)	
>12 years	41 (38,2%)	39 (36,1%)	
Mother's years of education, n (%)			0,29
Never went to school	0 (0,0%)	2 (1,9%)	
1-6 years	9 (8,4%)	10 (9,3%)	
6-9 years	10 (9,3%)	12 (11,1%)	
9-12 years	43 (40,2%)	45 (41,7%)	
>12 years	45 (42,1%)	39 (36,1%)	
Family income (Rupiah)	4.878.972±3.340.101	5.469.611±5.724.427	0,82
Ethnicity, n (%)			0,61
Javanese	23 (21,5%)	27 (25,0%)	
Batak	44 (41,1%)	34 (31,5%)	
Minangkabau	5 (4,7%)	7 (6,5%)	
Melayu	11 (10,3%)	6 (5,6%)	
Gayo	10 (9,3%)	14 (13,0%)	
Aceh	9 (8,4%)	13 (12,0%)	
Others	7 (6,4%)	7 (6,5%)	

## 4.2. Subject's Compliance

During 6 days in a week, from Tuesday to Sunday and three times a day, intervention subjects receive 150 g of cooked fortified rice for each meal. In this study the compliance of subjects in consuming the rice that is served in the boarding school's canteen is 60%, with 60,8% among control and 59,5% among intervention group. There are no significant differences among groups.

Every week during the four months, the intervention subjects was given fortified cooked rice from Tuesday to Sunday (six days). Monday is a fasting day for students as suggested by the principal of the school. In Figure 4.1, below shows that the average attendance of subjects decreased on day 4 which coincides with Friday. This is because Friday is a free day for the students of Ar Raudhatul Hasanah, hence parents are allowed to visit their children. They often bring in food from outside of the school, causing the subjects to be absent during meal times in the canteen. However there were no significant differences on the attendance of the subjects for meals between control and intervention group.

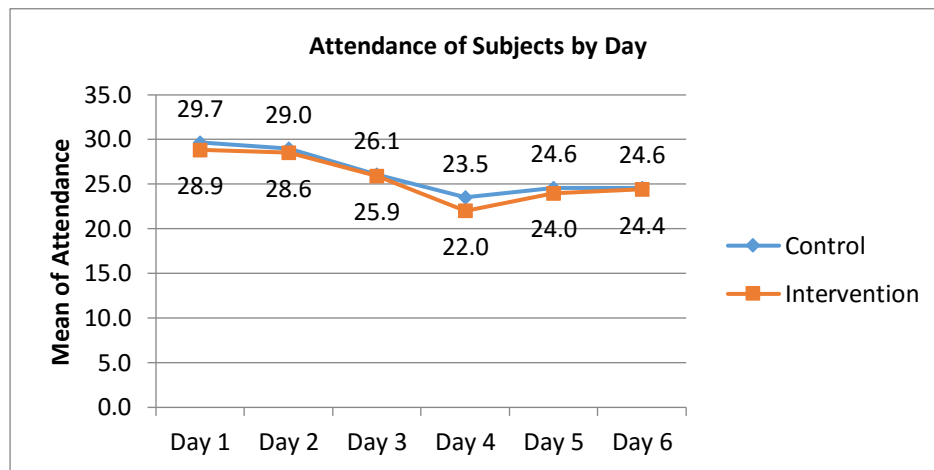


Figure 4.1. The mean attendance of control and intervention subjects by day from day1 to day6 during four months

According to meal time, subjects have the highest attendance during breakfast, meanwhile for lunch and dinner it declines for both group. This is because they felt more hungry in the morning and the canteen is not open in the morning (Figure 4.2).

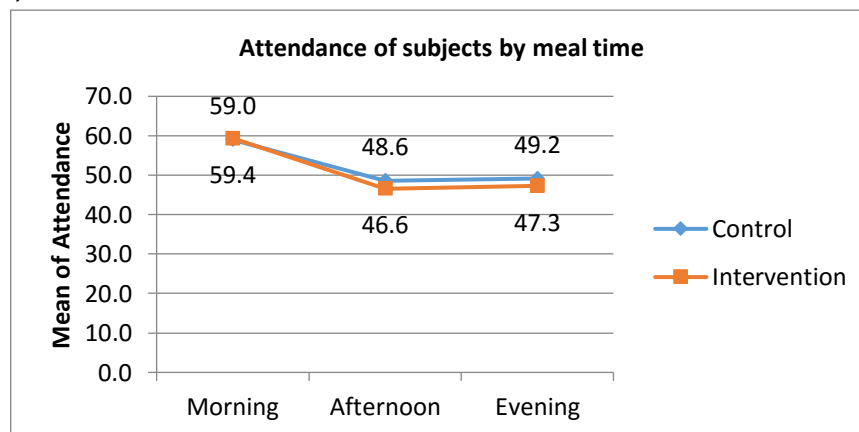


Figure 4.2. Trend of attendance of control and intervention subjects by meal time

Other factors effecting the attendance of subjects were the acceptability of the food provided by the kitchen. Based on the baseline data, most subjects suggested to have chili sauce and soybean sauce to be provided in each meal to increase the acceptability of the meal. Hence, one of the strategies conducted to increase the compliance and appetite is by providing chili sauce and soybean sauce in each dining table during the mealtime. A side from that, we also provided rewards for subjects with the highest attendance. This strategy increased the compliance of subjects in the second week, even though it is not consistent throughout the study (Figure 4.3).

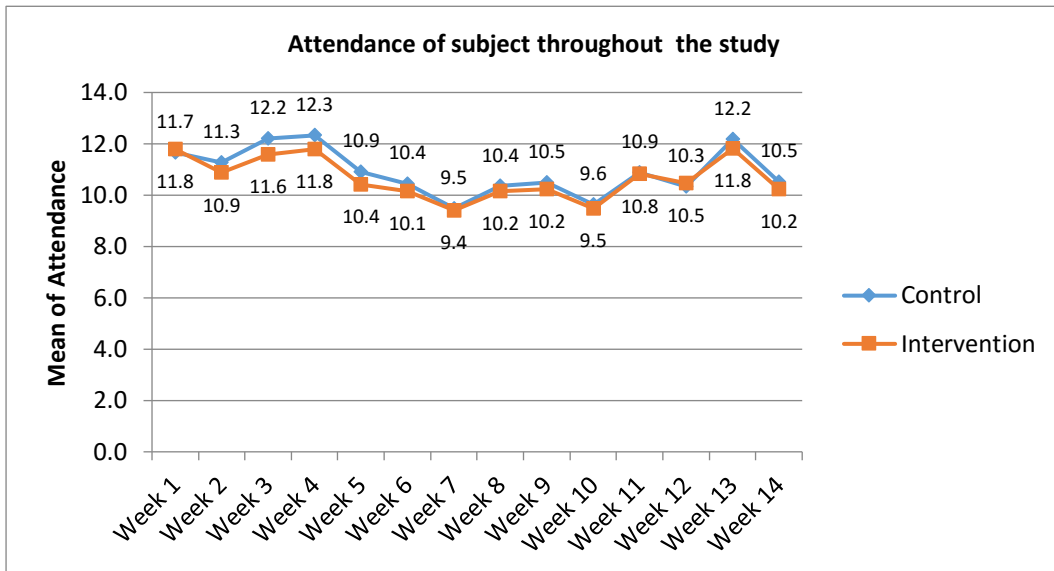


Figure 4.3. Trend of attendance of control and intervention subjects

There is no difference in the mean consumption of rice for both groups, which ranges from 147 grams to 150 grams served (Table 4.2). Although the highest compliance of subjects were during breakfast (Figure 4.2), however the amount of rice consumed is the most. Subjects consume more during lunch and even consume additional rice during the respective meal time.

Table 4.2. Mean rice consumption among control and intervention groups

Rice Consumption	Control		Intervention		P Value
	Mean	SD	Mean	SD	
Breakfast	143,8	19,4	142,3	17,6	0,272
Lunch	151,6	17,0	155,0	19,2	0,121
Dinner	148,3	18,1	147,2	18,0	0,797
Mean	146,9	17,0	147,6	17,1	0,895



## V. NUTRIENT INTAKE AND NUTRITION STATUS

### 5.1 Nutrient Intake before and after intervention

At the beginning of the intervention, there were no significant differences in nutrient consumption of control and intervention group ( $p > 0.01$ ). After the intervention (end line) there were significant differences found for the intake of iron, zinc, vitamin A, vitamin B12 and vitamin B3, where the intervention group has higher nutrient intake compared to control.

Table 5.1. Macronutrient intake before and after intervention

Nutrients	Control		Intervention		P Value
	Mean (Median)	SD (25 <sup>th</sup> – 75 <sup>th</sup> percentile)	Mean (Media)	SD (25 <sup>th</sup> – 75 <sup>th</sup> percentile)	
<b>Energy (Kkal)<sup>1</sup></b>					
Baseline	1201 (1167)	402 (870-1464)	1273 (1221)	411 (938-1483)	0,339
Endline	1343 (1291)	432 (985-1590)	1292 (1285)	408 (1021-1503)	0,891
Delta	123		64		0,339
P Value	0,012		0,722		
<b>Fat</b>					
Baseline	39,1 (36,4)	17,7 (25,3-41,9)	44,0 (41,3)	19,4 (29,6-56)	0,494
Endline	44,6 (38,1)	23,8 (27,2-58,7)	39,9 (38,5)	18,2 (26,9-49,8)	0,891
Delta	1,7		-2,8		0,494
P Value	0,186		0,185		
<b>Protein</b>					
Baseline	35,3 (33,6)	11,4 (26,4-41,9)	39,8 (36,4)	12,9 (29,8-47,2)	0,076
Endline	40,2 (36,3)	14,5 (29,8-48,6)	38,0 (37,4)	13,1 (29,2-45,5)	0,891
Delta	2,7		1		0,004
P Value	0,028		0,373		
<b>Carbohydrate</b>					
Baseline	178,2 (165,6)	65,2 (131,3-222,9)	180,5 (170,4)	62,9 (133,9-212,9)	0,891
Endline	195,2 (193,7)	54,0 (149,7-229,5)	194,5 (182,1)	65,0 (148,1-233,1)	0,494
Delta	28,1		11,7		0,494
P Value	0,021		0,071		

<sup>1</sup>Data presented as mean value for normally distributed data

This study found that the delta of the nutrient intake (the difference between endline and baseline) were significant for vitamin B3, vitamin B12, vitamin A, zinc, and iron (Table 5.2). This is in line with the number of fortificants added into the fortified rice. Significant differences were also found for protein, however the control group was higher compared to the intervention group. The same pattern was observed for the other macro nutrients, namely carbohydrate, energy and fat, even though it was not significant. This may be due to the fact in this study all subjects were obliged to follow the meal schedules, whereas before such practice have not been done and therefore students are free to eat in the canteen or choose not to.

Table 5.2. Micronutrient intake before and after intervention

Nutrients	Control		Intervensi		P Value
	Mean (Median)	SD (25 <sup>th</sup> – 75 <sup>th</sup> percentile)	Mean (Median)	SD (25 <sup>th</sup> – 75 <sup>th</sup> percentile)	
<b>Iron</b>					
Baseline	5,0 (4,4)	2,8 (3,2-6,2)	5,2 (4,2)	2,7 (3,5-6,6)	0,682
Endline	6,9 (5,4)	4,5 (3,7-8,3)	23,5 (23,8)	7,3 (18,2-28,1)	0,000
Delta	1		19,6		0,000
P Value	0,001		0,000		
<b>Zinc</b>					
Baseline	5,1 (4,8)	1,9 (3,6-6,2)	5,7 (5,4)	2,3 (4,1-6,7)	0,040
Endline	6,3 (5,6)	2,4 (4,5-7,7)	14,0 (14,5)	7,3 (11,1-17,4)	0,000
Delta	0,8		9,1		0,000
P Value	0,000		0,000		
<b>Thiamin<sup>1</sup></b>					
Baseline	0,3 (0,3)	0,1 (0,2-0,4)	0,3 (0,3)	0,1 (0,2-0,4)	0,133
Endline	(0,40) 0,39	0,14 (0,30-0,49)	0,40 (0,40)	0,13 (0,317-0,479)	0,891
Delta	0,09		0,095		0,891
P Value	0,000		0,000		
<b>Folic Acid</b>					
Baseline	5,3 (2,8)	8,9 (0,0-7,8)	6,4 (3,7)	10,0 (1,2-9,5)	0,494
Endline	8,3 (7,5)	7,1 (3,1-10,6)	8,8 (5,4)	16,3 (3,0-10,6)	0,219
Delta	4,7		1,7		0,682
P Value	0,000		0,007		
<b>Vitamin A</b>					
Baseline	298,5 (266,2)	203,4 (162,6-417,2)	317,2 (279,6)	238,6 (185,5-364,8)	0,682
Endline	365,5 (321)	214,2 (205-446,4)	750,4 (747,4)	320,9 (547,3-923,4)	0,000
Delta	54,8		467,8		0,000
P Value	0,015		0,000		
<b>Vitamin B12</b>					
Baseline	1,5 (1,3)	0,8 (0,9-1,8)	1,8 (1,6)	1,0 (1,1-2,4)	0,020
Endline	2,0 (1,7)	1,2 (1,5-4,0)	7,5 (7,7)	2,7 (5,0-8,0)	0,000
Delta	1,5		5		0,000
P Value	0,000		0,000		
<b>Vitamin B3</b>					
Baseline	5,2 (4,9)	2,0 (3,9-6,2)	5,6 (5,3)	2,2 (4,1-7,1)	0,339
Endline	5,7 (5,2)	2,3 (5,0-13,2)	26,1 (26,5)	9,7 (16,8-27,7)	0,000
Delta	3,7		17,4		0,000
P Value	0,048		0,000		

## 5.2 Nutrition status before and after intervention

For nutrition status, significant changes were only seen for weight in the intervention group. This pattern however was not apparent for height and BMI for age z-score. This showed that there is a possible synergetic effect of the various micro nutrients that was consumed leading to higher weight gain compared to control.

Table 5.3. Nutrition status before and after intervention

Variabel	Control (n= 107 )		Intervensi (n= 108 )		P Value
	Mean	SD	Mean	SD	
Weight					
Baseline	49,4	5,9	49,4	6,1	0,95
Endline	49,7	7,6	49,8	6,1	0,57
Delta	0,3		0,4		
P value	0,439		0,006		
Height					
Baseline	151,2	8,4	151,9	5,7	0,37
Endline	150,3	15,4	152,4	4,7	0,37
Delta	-0,9		0,5		
P value	0,589		0,235		
Z-score of BMI/Age					
Baseline	0,20	1,01	0,11	0,84	0,96
Endline	0,26	0,75	0,14	0,77	0,43
Delta	0,06		0,03		
P value	0,395		0,404		

## VI. EFFICACY OF FORTIFIED RICE ON MICRONUTRIENT STATUS

This efficacy study analyzed for six (6) biomarkers of the outcome namely: hemoglobin, ferritin, folic acid, vitamin A serum, zinc serum and CRP. The results of these biomarkers before and after intervention, and the changes in intervention and control group as shown in Table 6.1. The results show that compare to control group, the ferritin level and folic acid level in intervention group were significantly increase after the intervention. While the other four (4) biomarkers (hemoglobin, vitamin A, zinc, and CRP) did not change significantly.

Table 6.1. Mean and standard deviation of biomarkers before and after intervention in both intervention and control groups of selected subjects (n=215)

Biomarkers	Control			Intervention			Change of Delta
	Before	After	Delta (Change)	Before	After	Delta (Change)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Hemoglobin	12,45	12,08	-0,37	12,03	12,46	0,43	0,80
	± 1,04	± 1,20	± 1,09 <sup>s</sup>	± 1,19	± 0,99	± 0,96 <sup>s</sup>	± 0,14 <sup>s</sup>
Ferritin	23,15	28,53	5,38	26,46	35,15	8,69	3,31
	± 16,39	± 21,88	± 13,22 <sup>s</sup>	± 21,28	± 24,34	± 11,17 <sup>s</sup>	± 1,67 <sup>s</sup>
Folic Acid	9,74	10,50	0,77	10,02	12,40	2,38	1,62
	± 3,54	± 3,97	± 3,52 <sup>s</sup>	± 3,89	± 3,61	± 3,43 <sup>s</sup>	± 0,45 <sup>s</sup>
Vitamin A	1,23	1,20	-0,03	1,23	1,15	-0,07	-0,04
	± 0,37	± 0,42	± 0,30 <sup>ns</sup>	± 0,45	± 0,39	± 0,39 <sup>ns</sup>	± 0,048 <sup>ns</sup>
Zinc	11,32	10,94	-0,38	11,49	11,62	0,13	0,05
	± 2,86	± 2,42	± 2,59 <sup>ns</sup>	± 3,41	± 3,99	± 4,10 <sup>ns</sup>	± 0,469 <sup>ns</sup>
CRP	0,96	1,24	0,28	1,05	0,94	-0,11	-0,39
	± 2,87	± 3,97	± 4,83 <sup>ns</sup>	± 2,07	± 1,55	± 2,11 <sup>ns</sup>	± 0,08 <sup>ns</sup>

s = significant (p < 0,01)

ns = not significant

Further analysis by selecting subject who were consume cooked rice more than 27.000g (a number of 69 subjects who were consume at least 450g of cooked rice per day for at least two months) showed that a consistent result. That mean only serum ferritin level and folic acid level were significantly higher after the intervention.

Table 6.2 Mean and standard deviation of biomarkers before and after intervention in both intervention and control groups of selected subjects (n=69)

Bio markers	Control			Intervention			Change of Delta
	Before	After	Delta (Change)	Before	After	Delta (Change)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Hemo globin	12,66 ± 0,87	12,25 ± 1,12	-0,41 ± 1,02 <sup>s</sup>	11,95 ± 1,08	12,36 ± 0,97	0,40 ± 0,86 <sup>ns</sup>	0,81 ± 0,23 <sup>s</sup>
Ferritin	22,07 ± 14,59	23,45 ± 17,39	1,38 ± 11,49 <sup>s</sup>	26,82 ± 23,24	38,79 ± 29,19	11,98 ± 14,09 <sup>s</sup>	10,59 ± 3,11 <sup>s</sup>
Folic Acid	8,16 ± 3,24	9,36 ± 4,39	1,20 ± 4,13 <sup>s</sup>	8,33 ± 2,80	11,63 ± 3,44	3,30 ± 3,39 <sup>s</sup>	2,10 ± 0,09 <sup>s</sup>
Vitamin A	1,22 ± 0,42	1,12 ± 0,41	-0,10 ± 0,33 <sup>ns</sup>	1,10 ± 0,42	1,06 ± 0,36	-0,03 ± 0,38 <sup>ns</sup>	0,06 ± 0,91 <sup>ns</sup>
Zinc	11,58 ± 2,90	11,52 ± 2,52	-0,07 ± 3,02 <sup>ns</sup>	11,17 ± 2,84	11,88 ± 4,58	0,71 ± 4,36 <sup>s</sup>	0,78 ± 1,09 <sup>s</sup>
CRP	0,43 ± 0,61	1,53 ± 6,11	1,10 ± 5,83 <sup>s</sup>	1,37 ± 2,68	1,37 ± 2,21	0,01 ± 2,78 <sup>ns</sup>	-1,10 ± 0,35 <sup>s</sup>

s = significant (p < 0,01)

ns = not significant

The distribution of subjects by category of CRP and micronutrient status as shown in Table 6.3 In this study the analysis of CRP was included in order to identify whether or not the ferritin level related to inflammation or infection. As shown in Table 6.2 and Table 6.3 the CRP level and status was not different between intervention and control groups. This implies that the ferritin level in this study is not related to the infection.

In this study the effect of intervention (fortified rice) decreased the prevalence of anemia 41.4 %, the prevalence of ferritin deficiency decreased 7.4 %, and folic acid deficiency 3.2 % (Table 6.3). It seems that the lower the prevalence of micronutrient deficiency in the beginning of the study (such as ferritin deficiency and folic acid deficiency) the lower the changes (delta) in the prevalence after intervention.

Based on regression analysis, the changes in (delta) of hemoglobin level of the subjects was affected by the intervention (fortified rice consumption), the delta folic acid as well as the delta of vitamin A (Tabel 6.4).

The delta ferritin level was affected by the intervention of rice fortification but not affected by folic acid level, vitamin A level, zinc level, CRP level and initial ferritin level (Table 6.5). The delta folic acid level was significantly affected by the intervention of rice fortification. It is also affected by initial folic acid level but not by the delta zinc level. We intentionally included zinc level into the regression analysis, since the previous study by Ghishan FK et al (1986) showed that under normal physiological

conditions a mutual inhibition between folate and zinc exists at the site of intestinal transport. Actually in this study the zinc intake in this level did not inhibit folic acid intake (Table 6.6).

Table 6.3 Distribution of Subjects by Category of CRP and Micronutrient Status (n=215)

Micronutrient Status	Control n (%)		Intervention n (%)		Delta (%)
	Baseline	Endline	Baseline	Endline	
<b>Hemoglobin</b>					
• Severe Anemia	0 (0,0)	1 (0,9)	0 (0,0)	0 (0,0)	(-0,9)
• Moderate Anemia	10 (9,4)	9 (8,4)	18 (16,7)	5 (4,6)	(-11.2)
• Mild Anemia	10 (9,3)	39 (36,4)	36 (33,3)	15 (13,9)	(-46.5)
• Non Anemia	87 (81,3)	58 (54,3)	54 (50,0)	88 (81,5)	(58.6)
<b>Ferritin</b>					
• Deficient	37 (34.6)	34 (31.8)	38 (35.2)	27 (25.0)	(-7.4)
• Normal	70 (65.4)	73 (68.2)	70 (64.8)	81 (75.0)	(7.4)
<b>Folic Acid</b>					
• Deficient	14 (13.1)	8 (7.5)	11 (10.2) <sup>a</sup>	2 (1.9) <sup>b</sup>	(-3.2)
• Normal	92 (86.0)	97 (90.7)	95 (88.0) <sup>a</sup>	104 (96.3) <sup>b</sup>	(3.6)
• Elevated	1 (0.9)	2 (1.9)	2 (1.9)	2 (1.9)	(-1.0)
<b>Vitamin A (Retinol)</b>					
• Deficient	8 (7.5)	9 (8.4)	11 (10.2)	10 (9.3)	(-1.8)
• Low	24 (22.4)	34 (31.8)	25 (23.1)	39 (36.1)	(3.6)
• Acceptable high	75 (70.1)	64 (59.8)	72 (66.7)	59 (54.6)	(-1.8)
<b>Zinc</b>					
• Deficient	49 (45.8)	50 (46.7)	48 (44.4)	53 (49.1)	(3.8)
• Low	57 (53.3)	57 (53.3)	58 (53.7)	52 (48.1)	(-5.6)
• Acceptable high	1 (0.9)	0 (0)	2 (1.9)	3 (2.8)	(1.8)
<b>CRP</b>					
• Abnormal	2 (1.9)	2 (1.9)	3 (2.8)	0 (0)	(-2.8)
• Normal	105 (98.1)	105 (98.1)	105 (97.2)	108 (100)	(2.8)

Table 6.4 Regression analysis of delta hemoglobin level

Variables	B	Std. Error	t	Sig.
Constant	5,195	0,681	7,634	0,000
Intervention (Control = 0)	0,598	0,127	4,724	0,000
Delta Folic Acid Level	-0,446	0,054	-8,207	0,000
Delta Vitamin A Level	-0,457	0,174	-2,629	0,009
Delta Zinc Level	0,044	0,018	2,489	0,014
Initial Hemoglobin Level	-0,016	0,017	-0,933	0,352

Dependent Variable = Delta hemoglobin Level

Table 6.5 Regression analysis of delta ferritin level

Variables	B	Std. Error	t	Sig.
Constant	4,773	1,565	3,049	0,003
Intervention (Control = 0)	4,118	1,721	2,392	0,018
Initial Folic Acid Level	-0,333	0,241	-1,384	0,168
Delta Vitamin A Level	3,021	2,41	1,253	0,211
Delta Zinc Level	-0,283	0,245	-1,155	0,250
Delta CRP Level	0,266	0,226	1,176	0,241
Initial Ferritin Level	0,033	0,044	0,748	0,455

Dependent Variable = Delta Ferritin Level

Table 6.6 Regression analysis of delta folic acid level

Variables	B	Std. Error	t	Sig.
Constant	4,822	0,638	7,552	0,000
Intervention (Control = 0)	1,74	0,429	4,06	0,000
Initial Folic Acid Level	-0,417	0,058	-7,223	0,000
Delta Zinc Level	-0,015	0,062	-0,236	0,814

Dependent Variable = Delta Folic Acid

The presence study results was similar to the result of the studies done by Angeles-Agdeppa et al (2011), Perignon et al (2015) and Martorell et al (2015). The study among school children and mothers in Philippines showed that the fortified rice decreased anemia prevalence among children (from 17.5% to 12.8%) but not among mothers (from 13.0% to 12.5%) after 9 months of study (Angeles-Agdeppa et al, 2011). Study on the impact of multi-micronutrient fortified rice on hemoglobin, iron and vitamin A status of Cambodian School Children for six months showed the impact on hemoglobin and iron status was limited, partly by sub-clinical inflammation (Perignon et al, 2015).

Another previous study that showed a success story of reducing anemia (19.4 % to 4.0 % among children, 18.4% to 10.2% among women) is the study done by Martorell et al (2015) through mandatory fortification of wheat flour, maize flour, liquid and powdered milk with Iron ferrous fumarate was in Costa Rica which has been placed for the duration for 3 years.

There was one study showed no effect of iron fortified rice on hemoglobin level and anemia status among children of daycare centers for sixteen weeks in Rio de Janeiro (Bagni et al, 2009). Small effect was found in this study done for six months among the school children in Cambodia. The longer this study might result in better efficacy for the other biomarkers. The previous studied showed that the longer the study and the younger the subject of the study, the more likely the studied had an impact on hemoglobin and ferritin level or reducing anemia.

The limitation of this studies were inadequate duration of the study (fifteen weeks) because of fasting month, the lack of side dish effect on the quantity of rice consume by the subjects, as well as a tight schedule for breakfast and lunch for the boarding schools students include the subjects.

In conclusion this efficacy study showed that the rice fortification had a beneficial effect on increasing hemoglobin level, ferritin level and serum level of teenage girl students.



## VII. CONCLUSION AND RECOMMENDATION

### 7.1. Conclusion

This study develop micronutrient fortified rice that was acceptable to be consume by teenage girls. The micronutrient fortified into rice include iron, zinc, folic acid, vitamin B1, vitamin B12 and vitamin A.

This study reveal that consuming micronutrient fortified rice -150g per meal, three meals a day for a period of fifteen weeks- increase hemoglobin level, ferritin level and folic acid level of teenage girls. This study also demonstrated that the efficacy of the micronutrient fortified rice in reducing the prevalence of anemia, ferritin and folic acid deficiencies.

### 7.2. Recommendation

The formula of the fortified rice that already developed could be used for improving hemoglobin, ferritin and folic acid status of teenage girls.

The researchers realized that there are limitations of the studies included inadequate length of the study, lack of side dish (animal protein intake) of the subject and very limited time available for student to eat meal. Further studies are required to identify the efficacy of other micronutrient (other than iron and folic acid) that fortified into the rice that not showed the evidence in this study, such as vitamin A.

**ABSTRACT**  
**MICRONUTRIENT FORTIFIED RICE AND ITS EFFICACY**

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Rice plays important roles in Indonesians diet. Rice could potentially fortified with micronutrient to address nutrition problem such as anemia. About one third of women and teenagers in Indonesia suffering from anemia. The objective of this study was to develop a micronutrient fortified rice and to test its efficacy on reducing micronutrient deficiencies.

The micronutrient fortified into rice include iron, zinc, folic acid, vitamin B1, vitamin B2 and vitamin A. The micronutrient fortified rice to develop base on review on literature, expert review, and sensory test. The efficacy study was done through randomized control trial among 216 school teenage girls in Medan for fifteen weeks. The biomarker analyzed included hemoglobin, serum ferritin, serum vitamin A, serum zinc and folic acid.

The results showed that fortified rice with 2% of rice kernel was accepted organoleptically. This analysis show that consuming micronutrient fortified rice -150g per meal, three meals a day for a period of fifteen weeks- increase hemoglobin level, ferritin level and folic acid level of teenage girls. This imply that the formula of the fortified rice that already developed could be used for decreasing anemic problem among teenage girls.

## APPENDIX

### Preparation



Baseline



## Process of Intervention



Endline

