

Oral presentation

***Dadih* and Zinc Supplementation during Pregnancy Benefits Pregnancy Outcomes and Humoral Immune Response in West Sumatera, Indonesia**

Helmizar^a

^aDepartment of Nutrition Faculty of Public Health, Andalas University, Padang, Indonesia

Keywords

Dadih · Zinc supplementation · Pregnancy outcome · sIgA

Background/Aims: *Dadih* is a dairy product obtained from fermented buffalo milk in West Sumatra Indonesia. It contains various nutrients for human body, and source of probiotics that are beneficial for prenatal supplementation and pregnancy outcomes. **Methods:** We conducted randomized control trial in which 138 pregnant mothers at second trimester were randomly assigned to three groups 1). Control groups, 2). *Dadih* groups, and 3), *Dadih* and zinc groups. The mothers in *dadih* and zinc group received *dadih* tube (100 gram) six times a week and one packed of 20 mg zinc tablet weekly for six months of intervention. Mother's weight gain was measured every month. After six month of intervention, fecal secretary immunoglobulin A (sIgA) and anthropometric of newborn infants were measured in all groups. **Results:** The average maternal weight gain was 8.9 + 3.7 kg, the lowest was in the control group and the highest was in *dadih* and zinc groups (9.2+3.8 kg). We found significant difference on infant's birth weight compared to control, i.e. 163.9 (95% CI:-43.8 - 371.8) gram in *dadih* group and 236.5 (95%CI:-13.4 - 459.7) in *dadih* and zinc group after adjusted for ideal maternal weight gain ($p < 0.05$). There were no significant differences reduction of sIgA in mothers and in infants at the beginning and after six month of intervention among three groups ($p > 0.05$). **Conclusion:** *Dadih* and zinc supplementation were beneficial for improving maternal weight gain and pregnancy outcomes, but have no effect on immune response both for mothers and infants.

INTRODUCTION

Poor health and malnutrition before and during pregnancy are related to mothers mortality, neonatal death, and pregnancy outcomes. The fact is that the mothers mortality rate increases progressively even though the system has been implemented such as the labor coverage policy (Jampersal). Although the policy was launched by Jampersal with a very clear purpose to reduce mothers mortality (MMR) and infant mortality (IMR), the mothers mortality rate in 2007 was 228 / 100,000 live births, from the 2012 IDHS data, the mothers mortality rate increase progressively to 359 / 100,000 live births in Indonesia.

In Indonesia, most of babies suffer from anemia with zinc deficiency. A research in Indonesia found that 25% of breastfeeding mothers, and 17% of their babies had low plasma zinc concentrations. As a result, zinc deficiency causes various manifestations including poor growth, immunity disruption and developmental delay (Dijkhuizen et al, 2001).

Zinc deficiency increases the risk and severity of various infections, obstruct physical growth, and effects pregnancy. Global recognition of the importance of zinc nutrition in public health has been issued in recent years and more experiments are gathered in the design and implications of zinc intervention program (Brown KH et al 2009).

Several studies have shown that there are effects of food supplements containing zinc and probiotics on the body immune response. Studies in healthy children and premature infants have shown an increasing in sIgA excretion in response to probiotic interventions (Fukushima Y et al, 1998). Probiotic combination *L.plantarum* IS-10506 at doses of 10¹⁰ CFU / day and 8 mg of elemental zinc supplementation in a double-blind, placebo-Controlled randomized, pre-post trial showed a potential ability to improve zinc status in pre-school age children. Supplementation with probiotics *L.plantarum* IS-10506 and zinc for 90 days resulted in a humoral immune response that increased significantly, as well as an increase in zinc status (Surono IS et al, 2014).

Dadih is a fermented buffalo milk produced and consumed by ethnic groups in West Sumatra Province and is considered beneficial to human health. Dadih is a typical Minangkabau food by the indigenous people calling it dadiah, which is very popular in several districts in West Sumatra Province especially in Bukittinggi, Padang Panjang, Solok, Lima Puluh Kota and Tanah Datar. Understanding the transformation of buffalo milk into Dadih is necessary to understand its benefits on nutritional status and human health. The main changes in Dadih fermentation caused by lactic acid bacteria (LAB) produce specific health benefits as indicated by two strains of one probiotic Dadih as *E.faecium* IS-27526 isolated from Dadih (2.31 x 10⁸ CFU / day) in 125 ml of low-fat milk at very high temperatures (Surono IS, 2015).

Zinc deficiency contributes to morbidity and mortality, especially in pregnant mothers, but the relevant data are still very limited. Zinc deficiency during pregnancy is associated with poor pregnancy and birth outcomes, including infertility, fetal death, intrauterine growth retardation, preterm birth, and low birth weight (Ramakrishnan, 2010). Until now, the study on local product intervention - the combination of dadih and zinc supplementation that can be given to pregnant mothers has not been well explained.

METHODS

Design and Location of the Research

The study design was quasi-experimental with a randomized Control design for pre and post intervention trials. The research was conducted in the City of Bukittinggi and the Agam Regency of West Sumatra Province. The research were done for two stages. the first stage was on September 2016 to June 2017 and the follow-up until December 2018.

Subject and Population

The main population of this study included all pregnant mothers in 10-12 weeks of pregnancy or the first and two trimesters. For a total sample from three groups, there were 180 pregnant mothers. The inclusion criteria are pregnant mother in 12-16 weeks of pregnancy.

Intervention

During pregnancy, mothers on zinc supplements and combined probiotic with zinc supplements will receive 20 mg elemental zinc per day. All supplement will prepare in bubble packs of ten tablets each. Health-care workers will instruct pregnant mothers to consume 1 tablet daily between meal. Every mother in this group will get one packed zinc tablet weekly and they have to consume it at 07-11 in the morning 20 mg daily for six month of intervention. Pregnant mothers in the Dadih group and Dadih zinc group receive 100 g of Dadih with various flavors such as fruit or palm sugar six times a week for 144 days or six months of intervention.

sIgA fecal analysis

The sIgA fecal analysis was examined from 100 mg fecal samples and resuspended with BPS. Centrifuging 5000 RPM was done for 10 minutes. Fecal samples from pregnant mothers and infants were collected in the morning and transported immediately to the laboratory and stored at -80°C. The SIgA was measured with ELISA kit (K8870 and K6500, immundiagnostik, Bensheim, Germany).

RESULTS

The Effect of First Trimester BMI on Birth Weight to Mothers Who Receive Dadih Supplementation

Mother who received Dadih supplementation only have 2.1% babies with low birth weight. Among 138 mothers which are samples of the research have 16.7% babies with low birth weight.

Table 1. The Effect of First trimester BMI on Birth Weight to mothers Who Receive Dadih Supplementation

Birth Weight	BMI at First trimester			Total	<i>p-value</i>
	<18.5	18.5-25	>25		
	n=2	n=26	n=20	N=48	
<2500	0.0	2.3	0.0	2.1	0.22
2500-3000	100.0	32.6	0.0	33.3	
>3000	0.0	65.1	100.0	64.6	

Table 1 shows that there are 64.6% of babies with birth weight>3000 g. 100% babies with birth weight>3000 g are from mothers who have BMI>25 at first trimester.

The Effect of First Trimester BMI on Birth Weight to Mothers Who Receive Dadih Zinc Supplementation

Mothers who received Dadih Zinc supplementation have higher number of babies with low birth weight compared to mothers who received Dadih supplementation, which amounts to 11.1 %. mothers with BMI>25 at the first trimester have 66.7% babies with birth weight>3000 g.

Table 2. The Effect of First Trimester BMI on Birth Weight to Mothers Who Received Dadih Zinc Supplementation

Birth Weight	BMI at First trimester			Total	<i>p-value</i>
	<18.5	18.5-25	>25		
	n=2	n=27	n=16	N=45	
<2500	50.0	10	0.0	11.1	0.43
2500-3000	0.0	25	33.3	24.4	
>3000	50.0	65	66.7	64.4	

Effect of Dadih and Dadih Zinc Supplementation on sIgA Fecal Level of Mothers

In this study, it was found that sIgA fecal levels in the Dadih group were 2741.8 ± 1292.9 ng / mg. This is higher than the group that received the Dadih zinc combination and control, each with sIgA 2679.8 ± 1050.9 and 2375.2 ± 909.8 , however this value was not statistically different ($p > 0.05$).

Table 3. Mothers sIgA Fecal

Groups	sIgA level (ng/mg)		p-value
	(Mean + SD)		
Dadih (n=24)	2741.8 ± 1292.9		0.532
Dadih + zinc (n=15)	2679.8 ± 1050.9		
Dadih+Dadih zinc (n=39)	2717.9 ± 1191.5		0.264

Table 4. Means of Mothers sIgA Fecal of dadih and dadih zinc Group

Groups	sIgA level (ng/mg)		Difference (ng/ml)	p-value
	Baseline	Endline		
Dadih (n=21)	2955.6 ± 989.9	2845.1 ± 1356.1	110,5	0.743
Dadih + zinc (n=9)	2935.3 ± 1327.3	2582.6 ± 636.2	352,6	0.449
Dadih+Dadih zinc (n=30)	2949.5 ± 1077.9	2766.3 ± 1181.1	183,1	0.494

Based on the table above, it shows a decrease in mothers fecal levels at the beginning and end of the intervention where the lowest decrease of 110.5 ng / ml was found in Dadih group, while the largest decrease was 352.6 ng / ml in Dadih group but the difference was not significant ($p > 0.05$).

Effect of Dadih and Dadih Zinc Supplementation on sIgA Fecal Level of Infants

The results of the fecal baseline sIgA measurements in all groups shows the Dadih group was $1345.3 (+ 1045.4$ SD) ng / ml, the Dadih zinc group was $731.9 (+575.1$ SD) as shown in the following table.

Table 5. Differences of sIgA Level (ng / ml) Means of Infants from All Groups

Groups	sIgA level of Infants (ng/ml)		Difference	p-value
	Baseline	Endline		
Dadih (n= 21)	$1345.3+1045.4$	$2356.3+1196.5$	$1011.1+1789.8$	0.06
Dadih + zinc (n= 16)	$731.9+575.1$	$1951.9+1157.7$	$1218.5+1135.1$	0.02

From the table above, there were the differences between the levels of sIgA in infants fecal from 0 months (baseline) and after 6 months follow-up (endline), 1011.1 (+1789.8 SD), 1218.5 (+1135.1 SD) and 84.9 (+1599.2 SD). The results of the Paired Sample T-test statistical test showed significant differences in the mean differences in the levels of sIgA in all groups and in Dadih zinc group ($p < 0.05$). From the results of this study it was concluded that dadih supplementation as well as a combination of dadih+ zinc had an effect on the infants birth.

DISCUSSION

Based on the average difference according to the confounding variables, there was a tendency of differences in birth weight according to mother's age (20-35 years), the difference was 162.7 g and the babies whose mothers did not work showed the difference of 163.2 g (95% CI; -71.2 - 397.7) in Dadih group compared to Control group. The birth height showed different as much as 0.8 cm (95% CI; -0.1 - 1.6) in mothers who did not work and mothers with energy consumption > 1800 kcal. From the results of multivariate modeling, it was found that the positive effect of giving Dadih supplementation to the birth weight of infants compared to Control group increased by 163.9 g (95% CI: -43.8 - 371.8) along with the increase of normal mothers weight gain (8-12 kg).

This research is in line with the research of Mora et al (1979) and Lechtig A, et al (2017) that there is a difference in birth weight (105.9 gs) in mothers who received food supplementation for more than 13 weeks and only 102 g found in mothers who received food supplementation less than 13 weeks that were compared to the Control group. Meanwhile, this difference was not significant. The supplementation tends to decrease the prevalence of low birth weight. This study concluded that differences in birth outcomes caused by supplementation program were not caused by confounding variables. The effect of supplementation also has an effect on body weight gain of pregnant mothers, which is very related to the increase in mothers nutritional status which mediates the effect on birth weight.

The results of this study found a greater average birth weight in Dadih zinc group supplementation compared to the Control group where the birth weight mean was 3202.6 (\pm 457.9 SD) but there were no significant differences ($p > 0.05$).

There was 66.7% of pregnant mother in the Dadih zinc group experienced zinc deficiency at the start of data collection and fell to 44.4% after the end of the intervention.

Giving a combination of *Dadih* and zinc supplementation can decrease the prevalence of zinc deficiency in pregnant mother by 22.3%.

Zinc is very necessary for biological functions, such as protein synthesis, cell division, nucleic acid metabolism, immune system, and reproductive system^{15,19}. Without zinc, the body cannot develop and function properly. Zinc deficiency during pregnancy can effect pregnancy outcome and fetal outcome. Some studies show that zinc deficiency can be associated with infertility, congenital anomalies, intrauterine growth retardation, prolonged labor, fetal death, abortion, post partum bleeding, low birth weight, and immune system disfunction.

The effects of zinc deficiency in fertile mothers and pregnant mothers are still rarely known. However, zinc deficiency is considered to have long-term effects on growth, immune system, and body metabolism. 18 of 38 studies reported that there was a relationship between plasma or serum zinc concentrations in mothers on fetal development. Furthermore, 17 of the 41 studies in 1977 and 1994 found that there was a relationship between zinc deficiency during pregnancy and the incidence of low birth weight and delayed fetal growth.

CONCLUSIONS

Dadih supplementation for pregnant mothers has a positive effect on infant birth weight and birth height. *Dadih* and zinc supplementation group can decrease the prevalence of zinc deficiency in pregnant mothers amount to 22.3% and it gives better outcome. *Dadih* and zinc supplementation tend to have an effect on immune response, birth weight and birth height compared to *Dadih* and Control group. There was a tendency to decrease the morbidity experienced by infants which can be seen from the type and duration of disease after being followed up. It also has better growth status especially in infants of mothers who were given *dadih* and *dadih* zinc supplementation.

ACKNOWLEDGMENTS

This study is partly funded by Indonesian Danone Institute Foundation. The views expressed herein are those of the individual authors, and do not necessarily reflect those of Indonesian Danone Institute Foundation. We also thank to the Ministry Higher Education of Indonesia, our respondent, *Dadih* farmers and the community health workers for assistance and support.

REFERENCES

1. Dijkhuizen et, al., 2001. Concurrent micronutrient deficiencies in lactating mothers and their infants in Indonesia Am J Clin Nutr 2001; 73:786–91.

2. Gibson, RS. 2005. Principle of Nutritional Assessment, Second Ed. Oxford: Oxford Univ Press.
3. Helmizar (2015). Local Food Supplementation and Psychosocial Stimulation Improve Linear Growth and Cognitive Development among Indonesian Infants Aged 6 to 9 months. *Asia Pacific Journal of Clinical Nutrition*. Doi:10.6133/apjcn.102015.10 Published online: October 2015
4. King JC, Chaffee BW (2012). Effect of zinc supplementation on pregnancy and infant outcomes: a systematic review. *Paediatric and perinatal epidemiology*. 2012; 26 Suppl 1: 118-37.
5. Lönnerdal B (1989). Intestinal Absorption of Zinc. In: Mills CF, editor. *Zinc in Human Biology*. London: Springer-Verlag Berlin Heidelberg; 1989. p. 33-55.
6. Lutter CK and Chaparro CM. Neonatal period: Linking Best Nutrition Practices at Birth to Optimize Maternal and Infant Health Survival. *Food and Nutrition Bulletin*; Vol. 30 Number 2, June 2009; P S215-S224
7. Luoto R, Laitinen K, Nermes M and Isouri E. Impact of maternal probiotic-supplemented dietary counseling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. *British Journal of Nutrition* (2010), 103, 1792-1799
8. Ministry of Health Indonesia (2013). Reporting Basic Health Research (Riskesdas) Indonesia 2013.
9. Ministry of Health Republic of Indonesia (2013). Angka Kecukupan Gizi (AKG) 2013
10. Putra AA, Marlida Y, Khasrad, Azhike and Wulandari (2011). Perkembangan dan Usaha Pengembangan Dadih. *Jurnal Peternakan Indonesia*, Oktober 2011, Vol.13 (3)
11. Ramakrishnan U (2010). A Review of the Benefits of Nutrient Supplements during Pregnancy: From Iron-Folic-Acid to Long-Chain Polyunsaturated Fatty Acids to Probiotics. *Annales Nestlé (English ed)*. 2010; 68(1): 29-40
12. Saskia OS (2001). Zinc Supplementation in Bangladeshi women and infants; Effect on Pregnancy Outcome, Infants Growth, Morbidity and Immune Response. Dissertation. Wageningen Universiteit. The Netherlands
13. Sirait dan Setyanto (1995). Evaluasi mutu dadih di daerah produsen. *Prosiding Seminar Nasional Sains dan Teknologi Peternakan*. Buku I:284-280
14. Syah NP (2006). Health Benefits of Yogurt and Fermented Milks; Manufacturing Yogurt and Fermented Milks. Blackwell Publishing. NewYork.USA
15. Suroso IS, Martono PD, Kameo S, Suradji EW, Koyama H, (2014). Effect of probiotic L. Plantarum IS-10506 and zinc supplementation on humoral immune response and zinc status of Indonesia pre-school children. *J Trace Elem Med Biol*.28:465-9. Doi:10.1016/j.jtemb.2014.07.009
16. Suroso IS (2015). Traditional Indonesian dairy foods. *Asia Pac J Clin Nutr* 2015;24 (suppl 1):S26-S30. Doi:10.6133/apjcn