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## EFFECT OF DADIH AND ZINC SUPPLEMENTATION DURING PREGNANCY ON IMMUNE RESPONSE AND NUTRITIONAL STATUS OF BABY BORN : A PROSPECTIVE COHORT STUDY



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Dadih is an Indonesian traditional fermented buffalo milk produced and consumed by Minangkabau people in West Sumatra, Indonesia. The objective of this study is to follow up the effect of dadih and zinc for 6 months of supplementation during pregnancy. There are 138 samples of pregnant mothers which are divided into 3 groups (group dadih, group dadih zink and group control). 100 g of dadih, 20 g zinc sulfate tablet, and combination of dadih and zinc was given to pregnant mothers six times a week for 6 months of intervention. The result of this study shows that there are 15.2 % stunting infants, 6.5 % underweight, and no wasting infants; 10 % stunting infants, 5.0 % underweight, and 5.0 % wasting infants; and 22.5 % stunting infants, 20.0 % underweight, and 5.0 % wasting infants were found in group dadih, group dadih zinc and group control. Fecal sIgA of infants after follow up are 1964 ng/ml, 1653 ng/ml, and 1419 ng/ml, whose mothers were supplemented with dadih, dadih and zinc, and normal diet, respectively, during pregnancy.

Variable	Intervention			Total (n=126) n (%)	p-value
	Dadih (n=48) n (%)	Dadih Zink (n=40) n (%)	Control (n=38) n (%)		
<b>Age :</b>					
<8months	30 (65,2)	23 (57,5)	19 (47,5)	72 (57,1)	0,25
>8months	16 (34,8)	17 (42,5)	21 (52,5)	54 (42,9)	
<b>Gender :</b>					
Male	24 (52,2)	17 (42,5)	22 (55,0)	63 (50,0)	0,50
Female	22 (47,8)	23 (57,5)	18 (45,0)	63 (50,0)	
<b>Nutritional Status :</b>					
<b>WHZ Z-Score WHO:</b>					
Wasting	0 (0,0)	2 (5,0)	2 (5,3)	4 (3,2)	0,280
Normal	48 (100,0)	38 (95,0)	36 (94,7)	122 (96,8)	
<b>HAZ Z-Score WHO:</b>					
Stunting	7 (14,6)	4 (10,0)	9 (23,7)	20 (15,9)	0,243
Normal	41 (85,4)	36 (90,0)	29 (76,3)	106 (84,1)	
<b>WAZ Z-Score WHO:</b>					
Undernutrition	3 (6,3)	2 (5,0)	8 (21,1)	13 (10,3)	0,033
Normal	45 (93,8)	38 (95,0)	30 (78,9)	113 (89,7)	

Table 1. Frequency Distribution of Characteristics and Nutritional Status of Infants

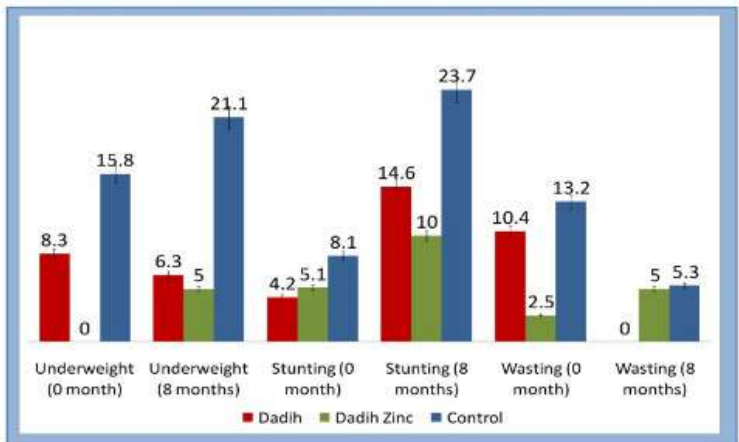


Figure 1. Nutritional Status of Infants during 8 months of follow up

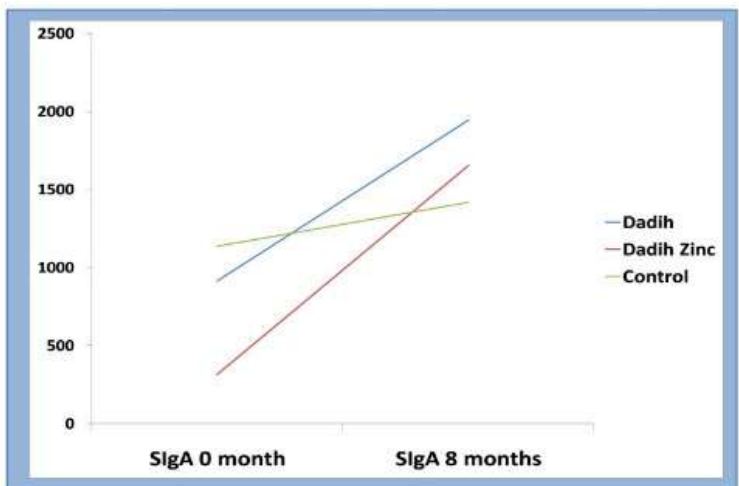


Figure 2. sIgA of infants during 8 months of follow up



Dadih supplementation to mother



Measurement of infant weight after intervention



Measurement of infant height after intervention



Examination of mother and infant sIgA

### Research Documentation

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**Effects Of *dadih* And Zinc Supplementation During Pregnancy On Immune Response And Nutritional Status of Baby Born : A Prospective Cohort Study**

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## Abstract

**Background and objectives:** *dadih* is an Indonesian traditional fermented buffalo milk produced and consumed by the West Sumatran Minangkabau ethnic group in Indonesia with its nutritional composition and indigenous lactic acid bacteria beneficial for human health. The objective of this study is to follow up the effect of *dadih* and zinc given to pregnant mothers for 6 months on fecal sIgA level and nutritional status of infants aged 4 - 10 months (mean = 8 months) infants.

**Materials and Methods:** A randomized controlled trial was conducted in two districts on 138 pregnant mothers for 6 months, randomly assigned into three groups, namely control, *dadih*, combination of *dadih* and zinc groups. 100 g of *dadih*, 20 g zinc sulfate tablet. Then, the combination of *dadih* and zinc groups was supplemented to expecting mothers, respectively, six times a week during 6 months intervention study. Anthropometric measurement, Fecal secretory immunoglobulin A (sIgA) of infants were assessed.

**Results:** No significant difference of fecal sIgA between *dadih* group and *dadih* zinc group as compared to control group ( $p > 0.05$ ). Fecal sIgA of infants aged 4 - 10 months were  $2076.7 \pm 1224.3$  ng/ml,  $1583.11 \pm 1064.13$ , and  $1426.78 \pm 1213.56$  ng/ml. The mothers of those infants were supplemented with *dadih*, *dadih* and zinc, and normal diet, respectively, during pregnancy. There were 15.2 % stunting, 6.5 % underweight, and no wasting infants; 10 % stunting, 5.0 % underweight, and 5.0 % wasting infants; and 22.5 % stunted, 20.0 % underweight, and 5.0 % wasting infants were found in expectant mothers supplemented with *dadih*, *dadih* and zinc and without any supplementation (control group), respectively. The percentage of stunting in 8 months and older infants tended to reduce: 28.57 %, 31.25 % and 11.76 % in expecting mothers supplemented with control, *dadih* and *dadih*-zinc, respectively.

**Conclusion:** *dadih* and zinc are potential for improving nutritional status of the baby born and pregnant mothers are recommended to supplement them during pregnancy, in order to reduce the prevalence of stunting.

Keywords: *dadih*, sIgA, nutritional status, Infants, follow-up study

## **Introduction**

Malnutrition experienced by children is a manifestation of stunting, wasting, intra uterine growth retardation (IUGR), low body mass index (BMI) and various micronutrient deficiencies.(1) The results of research conducted on children under 2 years old in West Sumatra Province in 2013, showed a prevalence of stunting by 21% and children with zinc deficiency by 63%.(2)

The proportion of stunting children 2 years of age increases due to the low intake of primary nutrients based on the recommended Nutrition Adequacy Rate (NAR) where the average percentage of children's energy intake is 42.8% NAR, and the average protein intake is 53.38% NAR. The combination intervention of nutritional supplementation and psychosocial stimulation of *manjajais* able to increase the nutrient intake of children, the average energy intake increased by 155.30 calories (+256.74 SD) and the average protein intake increased by 8.96 (+12, 15 SD). Psychosocial stimulation itself can improve children's nutritional intake at the end of the intervention due to close interaction between the child and mother or caregiver the child's appetite is also better.(2)

Insufficient nutrition and infection are direct causes of malnutrition in infants and children (2). The lack of macro nutrient intake is always followed by a lack of micronutrients with a very large influence on the growth and development of children. According to the World Bank, nutritional intervention efforts need to be done since early childhood based on that malnutrition under 2 years old and it will have an impact on the decline in physical growth, brain development, intelligence and productivity in the future, where this effect is largely irreversible (3).

Providing food of supplementation for children is very important especially to optimize the physiological functions of the body including the immune system. Food supplementation

can improve the body's immune function of vulnerable types, such as infants, toddlers, and people with immune disorders. Safeguarding the impact on this group can improve the quality of health in the future.(3)

*Dadih* is a typical Minangkabau dairy product that is produced through the application of natural fermentation methods with buffalo milk in bamboo tubes with conditions that tend to be facultative anaerobic and use banana leaves as a cover. *Dadih* is traditionally produced in several regions of West Sumatra Province. In general, *dadih* is served as side dish, snack, complementary traditional ceremonies, and traditional medicine.(4)

Meanwhile, since *dadih* is a type of milk product fermented by lactic acid bacteria, the benefit of *dadih* is more than just a food. Lactic acid bacteria which have been successfully isolated amounted to 36 strains, some of which are probiotic. Lactic Acid Bacteria found in *dadih* is 108 CFU with various therapeutic functions including improving microbial balance in the digestive system, and the immune system.(5)

The results of the previous research on 160 pregnant women in Bukittinggi City and Agam Regency in West Sumatra Province stated that 30 people or 18.7% pregnant women with zinc deficiency (serum zinc <8.6 mg / L) where the condition is malnourished. If this condition is not followed up, there will be a major impact on increasing maternal morbidity and child development.(6) One of the right solutions and the sustainability of interventions provided with the use of local food ingredients is giving *dadih* and zinc to pregnant women in order to have a sustainable effect on the child's immunity and the child's growth and development by using the potential of local wisdom in the Minangkabau, West Sumatra. The next research is conducted to determine the sustainability of the effects of *dadih* and zinc supplementation during pregnancy on immune response and nutritional status of infant's birth in West Sumatra Province.

## **Methods**

### **Design of Study**

This study is an observational design with a prospective cohort study which a continuation and prior intervention study with experimental quasy design to see the follow-up effect of intervention *dadih* and zinc supplementation during pregnancy on immunity and nutritional status of infant's.

### **Time and Place**

This study was conducted in Agam District and Bukittinggi City, West Sumatra Province held from January to June 2018.

### **Population, Sample, and Sampling Technique**

The population of this study were mothers and infants born during the study period, while the samples were all infants from the results of the previous study, as many as 138 infants were selected purposively with inclusion criteria were mothers and infants living in the study area, and the willingness of parents to participate in the study by signing informers who have been prepared. The exclusion criteria are sick children or in care at the time of initial data collection, or with other congenital defects.

### **Variable**

The study variables consisted of: independent variables *dadih* supplementation intervention group, the combination of *dadih* and zinc supplementation intervention groups and the control group; variables between nutrition intake and parenting practices; dependent variables are immunity status, growth and development of children and confounding variables (Covariat) are characteristics of children and parents.

### **Data Processing and Statistical Analysis**

The data collected is done by cleaning, editing, coding and entry and analyzed using computerization. Correlation and Chi-square analysis were used to see the trend of child growth and development relationship to each group and Multilevel Analysis will be used to analyze the effects of interventions with various impact indicators such as the results of anthropometric

measurements, the child's immunity status based on the characteristics of the sample and region. Data analysis was performed using SPSS Version 20.00 for windows with a significant level  $p < 0.05$ .

## Result

### Mother and Infant's Characteristics

Maternal characteristics are illustrated by maternal age, Body Mass Index (BMI), and number of children family income. 126 children in the sample who have been successful in the follow-up of 138 mothers had received the intervention during pregnancy as shown in Table 2.

**Table 1.** Characteristics of Pregnant Mothers and Infants

Variable	Intervention			Total (n=126)	p-value
	<i>dadih</i> (n=46)	<i>dadih Zinc</i> (n=40)	Control (n=40)		
<b>Age of pregnant mother:</b>					0,39
<20years	1 (2,2%)	0 (0,0)	0 (0,0)	1 (0,8)	
20-35years	36 (78,3)	32 (80)	27 (67,5)	95 (75,4)	
>35years	9 (19,6)	8 (20)	13 (32,5)	30 (23,8)	
<b>BMI of pregnant mother:</b>					0,18
<23,5	20 (43,5)	13 (34,2)	22 (57,9)	55 (45,1)	
23,5-25	8 (17,4)	4 (10,5)	3 (7,9)	15 (12,3)	
>25	18 (39,1)	21 (55,3)	13 (34,2)	52 (42,6)	
<b>Total of children:</b>					0,89
Do not have children	13 (28,3)	9 (22,5)	9 (22,5)	31 (24,6)	
1-2children	25 (54,3)	21 (52,5)	23 (57,5)	69 (54,8)	
>3children	8 (17,4)	10 (25)	8 (20)	26 (20,6)	
<b>Age :</b>					
<8months	30 (65,2)	23 (57,5)	19 (47,5)	72 (57,1)	0,25
≥8months	16 (34,8)	17 (42,5)	21 (52,5)	54 (42,9)	
<b>Gender :</b>					
Male	24 (52,2)	17 (42,5)	22 (55,0)	63 (50,0)	0,50
Female	22 (47,8)	23 (57,5)	18 (45,0)	63 (50,0)	
<b>Nutritional Status :</b>					
WHZ Z-Score WHO:					
<b>Wasting</b>	0 (0,0)	2 (5,0)	2 (5,0)	4 (3,2)	0,30
Normal	46 (100,0)	38 (95,0)	38 (95,0)	122 (96,8)	
HAZ Z-Score WHO:					



<b>Stunting</b>	7 (15,2)	4 (10,0)	9 (22,5)	20 (15,9)	0,30
Normal	39 (84,8)	36 (90,0)	31 (77,5)	106 (84,1)	
WAZ Z-Score WHO:					
<b>Undernutrition</b>	3 (6,5)	2 (5,0)	8 (20,0)	13 (10,3)	0,05
Normal	43 (93,5)	38 (95,0)	32 (80,0)	113 (89,7)	

**Table2.**Frequency Distribution of Characteristics and Nutritional Status of Infants

Variable	Intervention				<i>p-value</i>
	<i>dadih</i>	<i>dadih Zink</i>	Control	Total	
	(n=48)	(n=40)	(n=38)	(n=126)	
	n (%)	n (%)	n (%)	n (%)	
<b>Age :</b>					
<8months	30 (65,2)	23 (57,5)	19 (47,5)	72 (57,1)	0,25
≥8months	16 (34,8)	17 (42,5)	21 (52,5)	54 (42,9)	
<b>Gender :</b>					
Male	24 (52,2)	17 (42,5)	22 (55,0)	63 (50,0)	0,50
Female	22 (47,8)	23 (57,5)	18 (45,0)	63 (50,0)	
<b>Nutritional Status :</b>					
WHZ Z-Score WHO:					
<b>Wasting</b>	0 (0,0)	2 (5,0)	2 (5,0)	4 (3,2)	0,30
Normal	46 (100,0)	38 (95,0)	38 (95,0)	122 (96,8)	
HAZ Z-Score WHO:					
<b>Stunting</b>	7 (15,2)	4 (10,0)	9 (22,5)	20 (15,9)	0,30
Normal	39 (84,8)	36 (90,0)	31 (77,5)	106 (84,1)	
WAZ Z-Score WHO:					
<b>Undernutrition</b>	3 (6,5)	2 (5,0)	8 (20,0)	13 (10,3)	0,03
Normal	43 (93,5)	38 (95,0)	32 (80,0)	113 (89,7)	

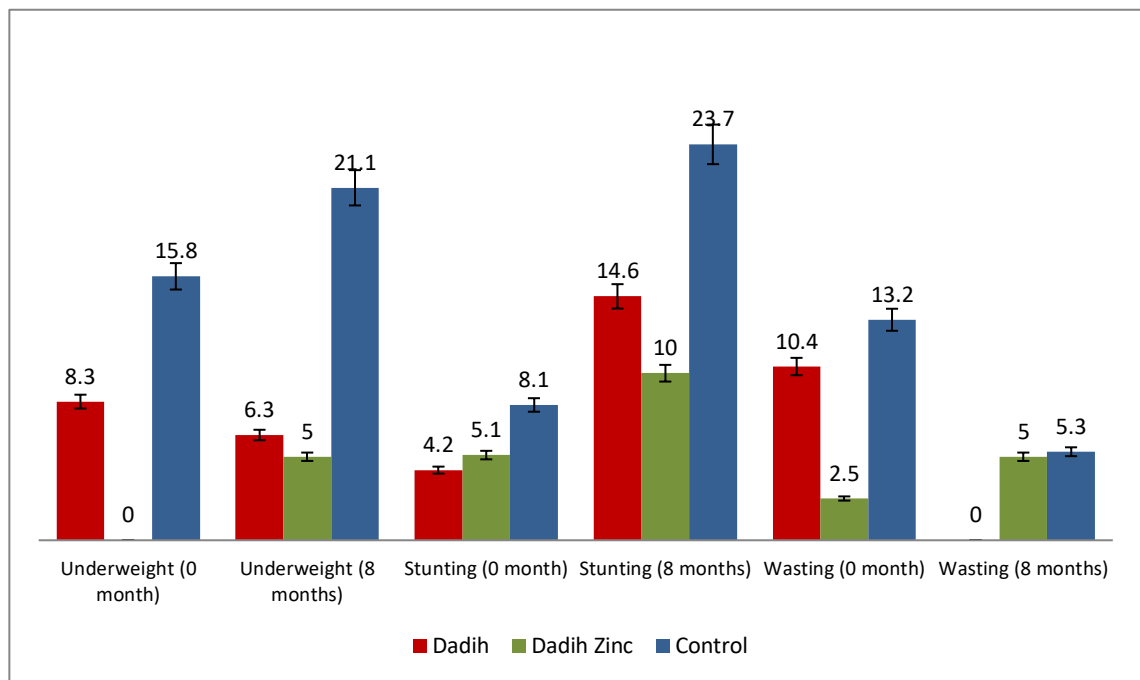
Based on the table above, the overall subject shows that the educational characteristics of mothers are mostly graduated from high school and college which is 65.9% and the remaining is 34.1% graduated from junior high school. The characteristics of the mother's occupation were mostly housewives 66.7% and most were > 20 years old 98.2%. A total of 46.2% of mothers with BMI > 25, this condition shows some mothers with more nutritional status. 54.8% mothers have 1 - 2 children and most mothers 65.1% with low socioeconomic status. Statistically, there were no significant differences in maternal characteristics according to each treatment group ( $p > 0.05$ ).

### Follow-up of Infant's Growth

**Table3.** Differences in Infant's Weight Gain

Variable	<i>dadih</i> (n=46)	<i>dadih</i> +Zink (n=40)	Control (n=40)	Total (n=126)	p-value
<b>Weight gain</b>					
decrease	4 (8,7)	7 (17,5)	7 (17,5)	18 (14,3)	0,49
stable	2 (4,3)	2 (5)	4 (10)	8 (6,3)	
increase	40 (87,0)	31 (77,5)	29 (72,5)	100 (79,4)	

Table 3 above shows the best growth of infants with the highest weight gain is (87.0%) in the *dadih* group and *dadih* + zinc (77.5%) while the lowest proportion was in the control group (72.5% ) Statistically also showed no significant difference in the growth of infant's weight in each intervention group (p <0.05).



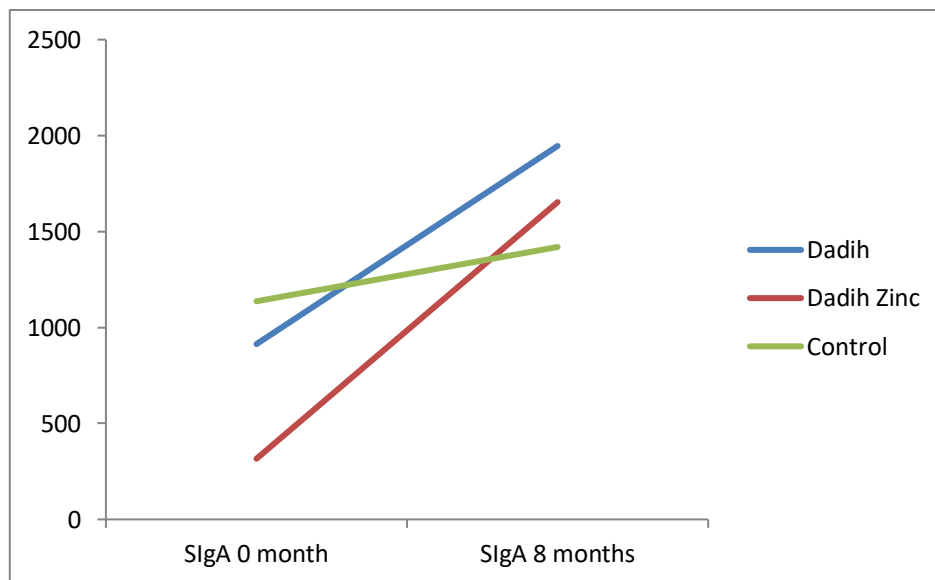
**Tabel 5. Follow up Morbidity on Infants Birth 1-6 months**

Desease	Group				p-value
	<i>dadih</i> n=45	<i>dadih</i> Zinc n=45	Control n=48	Total N=138	
No desease	8.3	28.9	17.8	18.1	0.08
Fever/diarrhea/cough/flu, etc	45.8	33.3	44.4	41.3	
Fever , cold/flu, etc	43.8	28.9	35.6	36.2	
Fever, diarrhea and cold/flu,	0.1	2.1	8.9	4.3	

Based on the table above, children in the *dadih* zinc group is 8.9% were exposed to 3 types of diseases (fever, diarrhea, cough / flu) for 6 months of follow-up and the least is in the *dadih* group 2.1% and control group 2.2%. Whereas the highest proportion 28.9% of infants had no disease during 6 months of follow-up was found in the *dadih* zinc group compared to the *dadih* and control group.

### Effect of *dadih* and Zink on sIgA level

The study showed that sIgA levels at the beginning of the study is  $2951 \pm 960$  ng / ml for the *dadih* group,  $2578 \pm 960$  ng / ml for *dadih* + zinc group and  $2544 \pm 1044$  ng / ml for the control group. Statistical analysis did not show differences in the gastrointestinal sIgA levels of the three groups ( $p > 0.05$ ). After being given the treatment, there was a decrease in sIgA level in the three groups. In the *dadih* group, the sIgA level decrease to  $2773 \pm 1401$  (a decrease of 178 ng / ml, in the *dadih* + zinc group decreased to  $2528 \pm 1015$  (a decrease of 50 ng / ml) and the control group to  $2299 \pm 927$  ng / ml (a decrease of 245 ng / ml) Statistical analysis shows the magnitude of the decline between the three groups did not differ significantly ( $p > 0.05$ ).



**Figure 1.** Changing of fecal sIgA infant's level

### Discussion

Analysis of the association between mother sIgA level and zinc showed a relationship pattern that was directly proportional, where the higher the zinc level, the higher the sIgA level. Statistically there is no relationship between the two variables ( $p = 0.197$ ,  $r = 0.227$ ).

Total sIgA (secretory IgA) examination shows the defense system in the gastrointestinal mucosa. IgA is found in many parts of the body's secretions (saliva, mucus, tears, colostrum and milk). The contribution of a constant fragment of sIgA with the mucus component bond allows microbial binding. Secretory IgA is the body's most widely produced antibody through the mucosal system, especially in Mucosal-Associated Lymphoid Tissues (MALT), estimated to be around 3 to 5 g secreted into the intestinal lumen every day.

This study tried to compare the sIgA level in the group that received *dadih* and *dadih* combination with zinc. The results showed a decrease in sIgA level in the three groups, where the largest decrease was found in the control group and the smallest is in the combination of *dadih*-zinc. These results show that the *dadih* with the components of lactic acid bacteria in it are not considered as strange (non-self) components by the body, so that no immunological response occurs. However, this data does not show the development of sIgA at the initial stage after administration of *dadih*. Greater decrease in sIgA level in the control group showed that *dadih* and zinc has a strong influence on maintaining total digestion in the gastrointestinal system for a longer period of time.

Zinc is a very important trace element for the body's immune system that functions as a non-specific immune system such as neutrophils and natural killer cells (5). Zinc does not only affect natural killer cells but also affects the cytolytic activity of T cells (6). Zinc is related to the immune system, where zinc can accelerate the translocation of Nuclear Factor kappa Beta (NF- $\kappa$ B). This protein is a transcription regulator that regulates the production of cytokines, inflammation and so on. Th1 cell activity plays a role in the length of secretory IgA levels remaining high in the digestive tract. This cell activity is affected by the presence of zinc in the circulation.

From the results of this study *dadih* supplementation along with zinc has an effect on increasing the immunity response of infants by one-third without pain during the 6 months of follow-up found in the zinc *dadih* group compared to the *dadih* and control group. Infants who experience pain for 6 months after follow-up have fever, diarrhea, cough or flu. Nearly one-third of babies have suffered from one type of disease or more than one type of disease. The effect of giving the *dadih* and zinc to the mother during this pregnancy can decrease the level of pain experienced by the baby after birth also has an impact on the child's growth and development which is also better than the control group although it is not statistically significant ( $p > 0.05$ ).

The results of this follow-up study found that there were still babies suffering from stunting, which amounted to 15.9% found in the three treatment groups. This stunting condition was twice larger in the control group infants as in the *dadih* and zinc groups. In stunting, the child's height does not meet normal height according to his age(9). Short children are closely related to conditions that occur in a long time such as poverty, poor hygiene and healthy behavior, poor environmental health, poor parenting and low levels of education (10). Therefore, further subscriptions are needed to improve the stunting condition suffered by the baby at this time. The solution of complementary feeding with breastmilk and zinc-fortified local foods is an alternative that might be carried out in the next phase of research.

## **Conclusions**

*dadih* supplementation and *dadih* zinc combination since pregnancy had a positive effect to lower sIgA levels and the proportion of stunting in children which was also lower compared to control group. Acceleration and growth of children to achieve optimal growth and development needs to be given before the child is under two years old. The level of technology preparedness in the form of development of *dadih* products as a supplementary food of local

products in West Sumatra that have high nutritional value needs to be developed as a form of further intervention to improve children's development.

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