Characteristics of Amino Acid, Micronutrient and Probiotic Isolated from Dadih and Their Benefits for Pregnant Mothers and Outcomes in West Sumatra, Indonesia

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Abstract

Dadih is a traditional fermented buffalo milk consumed by the Minangkabau people in West Sumatera Province, Indonesia, especially in Bukittinggi, Padang Panjang, Solok, Lima Puluh Kota, and Tanah Datar districts. The objective of this study was to identify amino acid and strain of acid lactic bacteria isolated from dadih and to evaluate the benefit of *dadih* intervention during pregnancy on the nutritional status of an infant's birth. This study was initiated with DNA extraction from bacterial cultures, Ribosomal DNA amplification, and sequencing, analysis of amino acid using UPLC. The intervention was conducted in two districts on 138 pregnant mothers for 6 months. A prospective cohort study was conducted on a follow up of a previous randomized controlled trial. This study was conducted in two districts in West Sumatera Province from April 2018 to July 2019. Spontaneous probiotic species of dadih strain were identified in this study: Lactobacillus Plantarum, Lactobacillus casei, Lactobacillus durans, Leuconostoc pseudo mesentoroides, and Lactobacillus cactis. Glutamic acid was the highest quantity of non-essential amino acid in dadih (16.28 mg/g), while Lysine and Leucine were the highest essential amino acids (7.22 and 6.42 mg/g). The study revealed that after a 6-month intervention, about 66.7% of infants birth weight > 3000 grams and only 2.1% of infants having birth weight < 2500 grams for Dadih Group and about 60.0% had infant birth weight > 3000 grams and 4.4% infants with low birth in Control Group. The result of this study shows that there are more benefits in pregnant mothers' weight gain and infants' birth weight until a six-month follow up from pregnant mothers in Dadih Group than Control Group. The results of the follow-up study show that infants from mothers supplemented with Dadih Zinc Group have significantly less undernutrition status as compared to the Control Group (p<0,05). The results also show that dadih has a sufficient amount of protein to fulfill the nutritional need for pregnant mothers and

Keywords: Amino acid, probiotic, *dadih*, pregnant mothers, outcomes, Indonesia

1. Introduction

Dadih is a traditional food that is popular in several West Sumatra districts, such as Bukittinggi, Padang Panjang, Solok, Lima Puluh Kota, and Tanah Datar district. *Dadih* is a source of probiotics that can increase human health (Surono 2015a, 2016). It can be referred to as a Minangnese yogurt. Although has been part of the Minangkabau people's diet for along time, its consumption has decreased in recent years because most people, especially the youth, are unaware of its nutritional values. Therefore, it is important to reintroduce *dadih* to the community by using different methods, such as a bamboo cup as its packaging.

For this purpose, many kinds of bamboo can be used, such as Talang bamboo and *Buluah Lapoh*. *Talang* bamboo diameter is about 5-8 cm while *buluah Lapoh* has a smaller diameter ±3-4 cm. First, the bamboo should be cleaned and dried before used. Bamboo can be cleaned either with a duster or with a coconut husk. Bamboo is dried under the sunlight for 10-15 minutes. *Dadih* makers still use traditional ways to produce *dadih*. Sometimes they do not pay much attention to sanitation and hygiene while using a non-pasteurized process and unclean bamboo cups. Because this rather unsafe process has been passed down from generation to generation,

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it is hard to persuade *dadih* makers to adopt new methods.

As technology and science advance, government and certain parties such as researchers have contributed to the development of *dadih* makers in West Sumatra. Sequence training on making *dadih* is provided to the local community to enable them to produce high-quality *dadih*. Its quality improvement is expected to attract consumer interest.

One of dadih's benefits is lactic acid produced from its fermentation at room temperature for 24-48 hours. Soruno (2016) reported that *Lactobacillus Plantarum* strain IS-10506 is dominant in *dadih*. Lactococccus is the dominant microbiota composition founded in all samples of *dadih* from West Sumatra (Venema, K and Surono, IS, 2018). Lactic acid is considered as a probiotic that plays an important role in regulating the digestive system of humans and animals. Some other studies suggest that the result of bacterial isolation in *dadih* has 36 dominant lactic acid strains. Metabolism of lactic acid can inhibit pathogenic bacteria, improve the immune system, prevent constipation, lower cholesterol, antimutagenic, anticarcinogenic, antivaginitis, and produce vitamin B and bacteriocin (Pato, 2003). The possible mechanism of actions includes inhibition of pathogenic bacteria. There was no adverse effect of probiotics on pregnancy outcomes including gestational age, birth weight, malformation and complication of pregnancy (Wibowo et al., 2015). The objective of this research is to identify the characteristics of amino acid, micronutrient and probiotic *dadih* in Agam and Tanah Datar Districts and follow up on the sustainability benefit of the effect of *dadih* and zinc supplementation during pregnancy on the outcomes. This research is of importance because not only does it seek to increase *dadih* quality but it can also be used as a theoretical basis for further intervention research.

2. Research Material and Method

Dadih from Agam and Tanah Datar districts, West Sumatra Province, was used in this study. A community intervention trial was conducted from January to December 2018 and follow up in 2019. Laboratory measurement was conducted in several locations such as the Biotechnology and Microbiology Laboratory of the Faculty of Medicine, Andalas University, Saraswanti Indo Genetech Laboratory Bogor, and Laboratory of 1st base in Singapore.

Identification of Lactic Acid Bacteria (LAB) of Dadih with Polymerase Chain Reaction (PCR)

Firstly, a bacteria culturing of *dadih* in *de Man Ragosa Sharpe Agar* (MRSA) was performed. Secondly, ±2-3 osecolony of bacterial was put into 1 mL *Phosphate Buffered Saline* (PBS). Thirdly, Pellet was isolated based on the kit sample method. DNA isolation step started from digestion, binding, washing, and elution. After the isolation step, the DNA was treated with PCR by using the PCR tool. Before using the PCR tool, provide primer forward (16S63F): 5'-CAG GCC TAA CAC ATG CAA CTC-3' + 273 μL buffer TE, primer reverse (16S63F): 5'-GGG CGG AGT GTA CAA GGC-3' + 218 μL buffer TE. After that, 10 μL of each primer was added with 90 μL molecular water. Mixing and running sample compositions can be seen in the following tables 1 and 2. Furthermore, the sample was sequenced at 1St base Laboratory, Singapore.

Table 1. Sample composition

Composition	Total (µL)
dream Taq Green Master	12.5
Forward primer (16S63F): 5'-CAG GCC TAA CAC ATG CAA CTC-3'	0.5
Reverse primer (16S63F): 5'- GGG CGG AGT GTA CAA GGC-3'	0.5
ddH_2O	10.0
Pellet sample	1.5

Table 2. Running condition of sample

Running condition	Temperature (°C)	Time	Cycle
Pra denaturation	94	3 min	
Denaturation	94	1 min	
Annealing	30	30 sec	35
Extention	30	30 sec	
End extension	72	5 min	

Analysis of Amino Acid, according to Nollet (1996) and Water Acquity UPLC H Class (2012)

Amino acid testing was done at Saraswanti Indo Genetech Laboratory– Bogor. Samples of *dadih* were taken from *dadih* makers in Agam and Tanah Datar districts. Amino acid hydrolysis with HCl, derivatization of amino acid, and amino acid analysis with UPLC equipment with column condition AccQ. Tag Ultra C18 1.7 μL (2.1 x 100 mm), the temperature at 49 °C, motion phase: gradient system composition, motion flow rate phase: 0.5 mL/minute, detector: PDA, wave length 260 nm, and volume injection1 μL.

Ca, Fe, Zn, and I, Mineral Analysis According to AOAC (2011)

1 g of *dadih* was added into the vessel then added 5 mL of HNO₃. After that, it was extruded with *nova wave digestion* (ramp: 10" temperature at 150 °C, hold: 15"). Temperature was gradually lowered to room temperature. Then 25 mL *dadih* solution was dissolved with 5 mL of HCl, 1 mL of HNO₃ and aqua bides tuntiltera mark. The last step was the measurement with ICP-OES

Design of Study Follow-up

This study is an observational design with a prospective cohort study continuation follow up of previous intervention study with experimental quasy design to find out the effect of *Dadih* and Zinc supplementation during pregnancy on immunity and nutritional status of infants'.

Time and Place

This study was conducted in Agam District and Bukittinggi City, West Sumatra Province held from April 2018 to December 2018 and held from April to July 2019 for the next follow up.

Population and Sample, Sampling Technique

The populations of this study were mothers and babies born from the results of the previous study in Bukittinggi City and Agam Regency of West Sumatra Province. The samples consist of 126 infants. Stool and blood were withdrawn. The inclusive criteria are mothers who have been supplemented with *Dadih* or *Dadih* Zinc for six months leading to birth. Informed consent was signed by the mothers for willingness to participate in the follow-up study. The exclusion criteria are sickness at the time of initial data collection, or with other congenital defects.

Variables

The study variables consisted of independent and dependent variables, which included the *Dadih* supplementation intervention group, the combination of *Dadih* and Zinc supplementation intervention group and the Control group variables. Dependent variables included immunity status, nutritional status, growth, and development of infants, while the confounding variables are nutrition intake, parenting practices and the characteristics of infants and mothers.

Anthropometry Measurement

The infants' anthropometry measurements which included weight, height, upper arm circumference (MUAC) were measured using a stadiometer/length-board, calibrated digital weight scale and MUAC tape respectively and caliby trained staff following standard procedures (10). At each test moment, anthropometric measurements were repeated and their average was used calculated z-scores for infants' nutritional status based on WHO standards.

Data Processing and Statistical Analysis

The data collection was done by cleaning, editing, coding, and analyzing. Those were computerized. Correlation and Chi-square analysis were used to see the trend of child growth and development relationships with each group. Analysis of variance (ANOVA) was used to analyses difference mean dependent variables such as the mean of anthropometric measurements among three groups. The data analysis was performed using SPSS for Windows Version 20.00 for Windows with p < 0.05 as a significant level.

3. Results and Discussion

Dadih is a food obtained from buffalo milk fermented for \pm 24-48 hours. Dadih has a lumpy shape like tofu, a white color, and smells like milk. Sirait (1993) argues that good quality of dadih has white color and milk-like consistency. Sisriyenni and Zurriyati (2004) observed that white color dadih has a soft texture and unique smell which consumers tend to like. Dadih Consistency is affected by chemical components, such as protein, fat, water, and lactic acid bacteria from fermented milk process. The fermentation process uses Lactobacillus bacteria which can be found in milk. This bacteria produces \(\text{B-galactosidase} \) that can convert lactose into glucose and galactose. Glucose is converted into fructose 6-fosfat through the glycolysis process. The final result of the glycolysis process is lactic acid. (Horton et al., 2012).

3.1 Analysis of Amino Acid

The purpose of the amino acid analysis is to discover amino acid composition in the sample. The data of amino acid composition in *dadih* protein aim to increase its nutritional status, by adding deficient essential amino acid (supplementation) or by mixing its protein with other proteins to get better essential amino acid. *Dadih* has a greater amount of amino acid glutamate than other amino acids (Table 3). Amino acid glutamate exists in D, D, L-, and L- which contribute to form sour taste in food products (Kusnandar 2010). The sour taste in *dadih* is produced by lactic acid during the fermentation process.

Table 3. Result of amino acid analysis

		Composition of Dadih (mg/g)		
No	Parameter	Agam	Tanah Datar	
1	Glutamic acid	16.8	15.6	
2	lysine	7.6	6.8	
3	Proline	6.9	6.3	
4	Leucine	6.6	6.2	
5	Aspartic acid	5.6	5.1	
6	Valine	4.1	3.9	
7	Serine	3.8	3.6	
8	Isoleucine	3.6	3.4	
9	Tyrosine	3.4	3.0	
10	Threonine	3.3	3.2	
11	Phenylalanine	3.0	3.0	
12	Alanine	2.7	2.2	
13	Arginine	2.2	2.0	
14	Methionine	1.8	1.6	
15	Histidine	1.8	1.6	
16	Glisine	1.6	1.4	
17	Tryptophan	1.2	0.8	
18	Sistine	0.3	0.3	

The result of observation on amino acid analysis (Table 3) shows that glutamate acid is the highest non-essential amino acid in both samples and Lysine while leucine as the highest essential amino acid in the same samples.

Based on the previous research by Helmizar et al. (2019), protein in *dadih* is estimated to approximately 12.41% and 49.64 g of protein per 100 g of *dadih*. This means that *dadih* does not have a sufficient amount of protein to fulfill the nutritional need for pregnant mothers who need a protein intake of 77 g/day (Indonesian Recommended Daily Intake, 2013). Nevertheless, consuming *dadih* can fulfill half of the protein need of adults in general.

Table 3 shows that the *dadih* sample from West Sumatra has a lower nutritional value of amino acid in comparison with previous research (Usmiati, 2012). This research reveals that *dadih* from West Sumatra has 13.71% protein. Several elements explain this difference: the location where the *dadih* was collected, the processing time and the type of buffalo.

Essential amino acid is the amino acid that can not be produced by the human body. Therefore, human beings should consume foods that have essential amino acids. The type of essential amino acid in *dadih* includes isoleucine, leucine, methionine, phenylalanine, threonine, valine, lysine, histidine (for babies and toddlers), and arginine (for babies only).

Methionine and lysine have limited milk protein synthesis in corn. It shows a low absorption rate in the mammary gland due to the low content of Methionine and Lysin in corn. If the food given has low amino acid, then there will

be absorption limit and degradation in the rumen. Therefore, it is important to balance the specific amino acid in food to be absorbed in the small intestine (Chow et al., 1990). A non-essential amino acid is an amino acid that can be self-produced by the human body, so it has lower consumption priorities compared to the essential amino acid. The non-essential amino acids in *dadih* are glycine, alanine, proline, serine, cysteine, tyrosine, aspirin, glutamine, aspartic acid, and glutamate acid.

Table 4. Result of Micro Nutrient Analysis in Dadih

Composition of Dadih				
No	Micro Nutrient	Agam	Tanah Datar	
1	Calsium / Ca (mg/100 g)	190.54	247.93	
2	Iodine / I (mcg/100 g)	15.93	16.39	
3	Zinc / Zn (ppm)	7.03	9.27	

Analysis of the micronutrient component in *dadih* samples was done to determine the level of micronutrients as the data can be used as a reference for further study related to the intervention of dadih to pregnant mothers. Referring to Indonesian Recommended Dietary Allowance (2013), pregnant mothers aged 19-49 years need 1100 mg of calsium, 150 mcg of iodine, and 13 mg of zinc every day. Consuming *dadih* as much as 100 g/day can fulfill calcium needs as much as 219.24 mg/100 g, 16.16 mcg/100g of iodine, and 0.01 mg/g of zinc. Table 4 shows that the need for calcium, iodine and zinc can not be fulfilled by only consuming 100 g/ day by pregnant mothers. Therefore, nutritional needs can be fulfilled by consuming other foods with calcium, iodine, and zinc, such as meat, fish, vegetable, fruits, etc. Other than consuming *dadih* as much as 100 g/day, zinc supplement estimated to 20 mg should be given to pregnant mothers.

3.2 Bacterial Cultures and Molecular identification Probiotic from Dadih

Every grown bacteria is analyzed with gram coloring to know the positive and negative gram. The result showed that all bacteria is positive gram. Culturing result is identified based on colony morphology, they are the border, surface, size, color, and smell. The colony that has a similar size will be cultured separately. The data showed that 29 colonies were found which consist of 20 aerob and 9 anaerob. The morphology of the colony is diverse, which allows revealing the species. The colony that has different morphology is analyzed further with the sequencing method. Molecular is identified with primer 16sRNA consists of 27F:CGGTTACCTTGTTACGACTT. Both primers will be produced with a size of 1600 bp. The size depends on the type of species, which is about 1300 – 1600bp.

Table 5. Result of BAL identification from dadih with primer 16sRNA

No	Species	Quantity	%
1	Lactobacillus plantarum	5	50
2	Lactobacillus casei	2	20
3	Lactobacillus durans	1	10
4	LeuconostocPseudomesentoroides	1	10
5	Lactobacillus lactis	1	10
	Total	10	100.0

3.3 Benefits of Dadih

Pregnant women belong to groups that are prone to nutritional problems. It can be fatal not only for mothers but also for the mothers the child in her wombs. The nutritional condition of a baby is influenced by current nutritional status in the fetus. In other words, the nutritional status of pregnant women is very important for her health and for predicting pregnancy outcomes for mothers and the nutritional status of newborns (Wibowo et al., 2015). This is due to the fetus's dietary intake that can only go through the umbilical cord connected to the mother's body. The nutritional status of pregnant mothers is reflected in its anthropic size. The size of anthropometry pregnant mothers

greatly affects the weight of the baby to be born. If the nutritional status of a mother before pregnancy is good, she, will give birth to a healthy baby, enough months and having normal body weight (Helmizar, 2019).

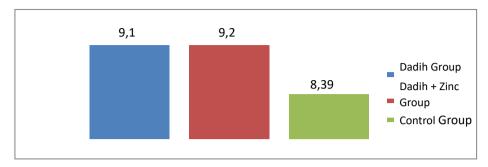


Figure 1. Weight gain of pregnant mothers during six months intervention

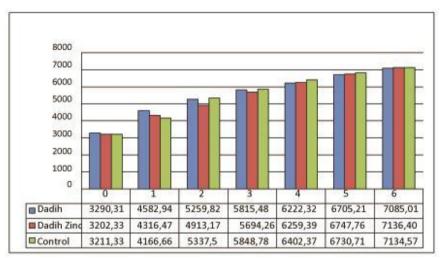


Figure 2. Follow-up infants's birth weight for six months of aged (grams)

Besides intervention to determine weight gain in pregnant women who were divided into 3 groups, namely Dadih, Dadih Zinc, and Control group. The result is the Dadih and Zinc group has the highest weight gain amount 9,26 kilograms and the lowest weight gain is in the Control group with 8,39 kilograms. Based on the diagram above, it can conclude that the highest infant birth weight gain is on Dadih Zinc Group and the lowest is on the Control group. Dadih Zinc group has a constant increase that the other group.

The results of this follow-up study found that there were still infants suffering from stunting, which amounted to 15.9% found in the three intervention groups. This stunting condition was two times higher in the Control group. In stunting, the child's height does not meet normal height standards according to their ages. Short infants 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 (Rokx, C. 2018). Therefore, further subscriptions are needed to improve the stunting condition suffered by infants at that time. There are solutions for these problems, such as feeding complementary with breast milk and Zinc-fortified local foods to infants that might be carried out in the next phase of research.

4. Conclusion

Dadih is a dairy product obtained from fermented buffalo milk in West Sumatra. It contains various species and strains of lactic acid bacteria such as Lactobacillus sp., and Leuconostoc sp., which very beneficial to human health. Lactobacillus sp., L. Plantarum is the dominant species in dadih (50%). This bacteria produces more than 85% lactic acid as its metabolite product. L-lactic acid (Dextrorotatory) is a type of lactic acid metabolite that is useful to human health as it can be absorbed quickly and metabolized in the formation of glycogen in the human body. So, therefore, dadih can be used as a source of functional probiotic food to improve health.

Besides having probiotics, *dadih* contains various nutrients needed for the human body, including aminoacid. The amount of amino acid is greater in *dadih* than in dairy milk because lactic acid bacteria in *dadih* can produce metabolite compounds such as amino acids. Glutamic acid is the highest non-essential amino acid in dadih about 16.28 mg/g, while Lysine and Leucine are about 7.22 and 6.42 mg/g. It is also known that *dadih* of West Sumatra also contains micronutrients such as calcium 219.24 mg/100 g, iodine 16.16 mcg/100 g, and zinc 0.01 mg/g. This amount is not enough to fulfill humans' daily nutritional needs. *Dadih* supplementation and *Dadih* Zinc supplementation has a positive effect on pregnancy outcomes, lowering the proportion of stunting in infants. Acceleration and growth of infants to achieve optimal growth and development should be carried out before they are under two years old

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Competing Interest Statement

The authors declare that there are no competing or potential conflicts of interest.

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