Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2020 Apr 15; 8(B):558-562.
https://doi.org/10.3889/oamjms.2020.4173
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Pediatrics*

The Influence of Probiotic on *Dadiah* as Fermented Buffalo Milk Product for Breastfeeding Women with Normal Bacterial Flora of the Gastrointestinal Tract

Netti Suharti[1]*, Linosefa Linosefa[1], Asih Kumala[2], Eva Chundrayetti[3], Andani Eka Putra[1]

[1]Department of Microbiology, Faculty of Medicine, Universitas Andalas, Padang City, Indonesia; [2]Biomedicals Program, Faculty of Medicine, Universitas Andalas, Padang City; Indonesia; [3]Department of Pediatrics, Faculty of Medicine, Universitas Andalas, Padang City, Indonesia

Edited by: Mirko Spiroski
Citation: Suharti N, Linosefa L, Kumala A, Chundrayetti E, Putra AE. The Influence of Probiotic on *Dadiah* as Fermented Buffalo Milk Product for Breastfeeding Women with Normal Bacterial Flora of the Gastrointestinal Tract. Open Access Maced J Med Sci. 2020 Apr 15; 8(B):558-562. https://doi.org/10.3889/oamjms.2020.4173.
Keywords: Dadiah; Breastfeeding mothers; Feces; Normal flora; *Lactobacillus plantarum*
***Correspondence:** Netti Suharti, Department of Microbiology, Faculty of Medicine, Universitas Andalas, Padang City, West Sumatera Province, Indonesia. E-mail: nettisuharti@med.unand.ac.id
Received: 09-Dec-2019
Revised: 15-Feb-2020
Accepted: 20-Mar-2020

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

INTRODUCTION: *Dadiah* is a fermented buffalo milk product containing many potential lactic acid bacteria as probiotics. Lactic acid bacteria and their derivative products can prevent the onset of various diseases such as spur health and heart work, good food to increase stamina and endurance, prevent colorectal cancer, and improve intestinal microflora.

AIM: This study aims to determine the influence of probiotic on *dadiah* as fermented buffalo milk products for breastfeeding women with normal bacterial flora of the gastrointestinal tract.

METHODS: The study was conducted on 28 breastfeeding mothers who were divided into two groups with the same proportion, i.e., mothers who received *dadiah* ice cream and not. Interventions were performed for 1 month and analysis was performed on an infant's feces. DNA isolated from feces and *Lactobacillus plantarum* concentrations were analyzed using real-time polymerase chain reaction. The standard curve designed to get the concentration of bacteria appropriately. Data analysis used SPSS version 20.0. Numerical data were analyzed by used unpaired t-test and paired t-test. A $p < 0.05$ was considered statistically significant.

RESULTS: The results showed that the mean intestinal microflora from the infant before the *dadiah* ice cream given was 4.2×10^7 CFU/g and after the *dadiah* ice cream was 8.6×10^8 CFU/g ($p < 0.05$) while in the control group, the mean *L. plantarum* intestinal infants before administered whey ice cream is 2.6×10^7 CFU/g and thereafter 1.3×10^7 CFU/g ($p > 0.05$).

CONCLUSION: Based on the results of this study, it can be concluded that there is an increase in *L. plantarum* concentration after the provision of *dadiah* ice cream in breastfeeding mothers.

Introduction

Dadiah is a fermented buffalo milk product originating from West Sumatera Province, Indonesia, which is a traditional foodstuff [1]. The curd is a buffalo milk product fermented naturally in bamboo containers at room temperature for 24–48 h. Dadiah is usually served by mixing with a glutinous rice stick that has been flaked and added some liquid of sugar. The curd is also delicious to eat with hot rice and sauce [2].

In the previous study, it is known that the *dadiah* contains potent lactic acid bacteria as probiotics. The probiotic bacteria contained in the curd are *Lactobacillus* and *Bifidobacterium* [3]. Lactic acid bacteria and their derivative products are able to prevent the onset of various diseases such as spurring health and the workings of the heart, good food to increase stamina and endurance, prevent colorectal cancer, improve intestinal microflora, repair the intestinal condition affected by antibiotic treatment, treat diarrhea caused by antibiotics, viruses, and bacteria, and produce digestive enzymes, and antimutagen and anticarcinogenic [4].

Probiotics will be effective if able to survive well in environmental conditions such as in the stomach that is low pH environment conditions and not damaged by enzymes produced by the stomach. *Lactobacillus* is one of the most common general of lactic acid bacteria in the gastrointestinal tract [5].

The working principle of probiotics works anaerobically to produce lactic acid resulting in a decrease in the pH of the gastrointestinal tract that blocks the development and growth of pathogenic bacteria. In contrast to pathogenic bacteria inhabiting the area of the gastrointestinal wall to develop the disease, probiotic bacteria inhabit the gastrointestinal mucosa that also results in changes in the composition of bacteria present in the gastrointestinal tract [6].

Infants aged 0–6 months, just need breast milk as food [7]. Breast milk is easily digested and directly absorbed by the baby. Lack of nutrition, allergic, colic, constipation, and obesity is less likely to occur in babies

who consume breast milk [8]. In breast milk, there are probiotics that can help the colonization process to prevent the development of harmful bacteria in the baby's intestines. This is caused by a special probiotic substance in breast milk that selectively feeds only good bacteria. In fact, some studies suggest that good bacteria in the mother's digestion can move the baby's body through breast milk [6].

Adequate intake of probiotics can prevent babies from certain types of respiratory infections and diarrhea and can increase the baby's immune system as a whole. In addition to good for infants, probiotics are also very good for breastfeeding mothers include: Reducing the risk of infection in the breast, such as mastitis, for women who give birth by cesarean section, probiotics help restore the condition of the intestine as before, probiotics can also help restore the bodyweight of the mother after childbirth [9].

The intestinal microflora is defined as the bacterial flora of the feces because the distal intestinal flora (ileum-colon) is almost identical to that found in feces [10]. Gastrointestinal microflora has several functions such as protein synthesis and vitamins, aids digestion, and absorption and serves to inhibit the growth of pathogenic bacteria by producing organic acids, bacteriocin as an anti-bacterial and replacing attached bacterial pathogens on the surface of the gastrointestinal epithelium. Under normal circumstances, the number of beneficial microflora is more than the number of pathogenic bacteria. The number of pathogenic bacteria and beneficial bacteria must be in a balanced state to maintain gastrointestinal health. It is important to maintain the balance of intestinal microflora to achieve optimal growth and development [6].

In addition to probiotics in breast milk, there is also protection, including IgA, IgM, IgD, IgG, IgE, immune, lactoferrin, lysozyme, and oligosaccharide antibodies which at the time of breastfeeding affect the exposure of microorganisms in the baby's gastrointestinal tract. Furthermore, the baby is given breast milk whereby during probiotic breastfeeding, these immunoglobulin cells and components are transferred to the baby so as to help maintain a good intestinal microflora balance of the baby to maintain the health of the baby's digestive tract, keep the immune system or infant immunity until the baby's physical activity becomes better [11].

Materials and Methods

Study design and research sample

This research used an equivalent pre- and post-test with control group design where the measurement of case and control group subject before and after treatment. The ethical document has been reviewed by the Research Ethics Committee of the Faculty of Medicine Universitas Andalas, Padang City, Indonesia. Research subjects were breastfeeding mothers who had infants between 6 and 12 months. Total subjects were 28 people consisting of 14 control groups and 14 treatments received *dadiah* ice cream. Interventions were performed for 1 month and analysis was performed on an infant's feces. DNA isolated from feces and *Lactobacillus plantarum*.

Operational definitions

The variables of this study included that the independent variable is *dadiah* feeding. The dependent variable is the normal bacterial flora of the gastrointestinal tract.

Research procedure

Bacterial identification is based on the species of lactic acid bacteria found in infant stools. Identification of bacterial strains using a 16sRNA primer followed by sequencing. Isolation of feces DNA using QiAamp DNA isolation kit (Qiagen, Inc.). Working procedures adapted to the research protocol. Quantification of bacteria was performed used real-time polymerase chain reaction (PCR) with a hybridization probe technique. In this study, the primers and probes were from the journal according to the species found. Quantification of the bacteria was carried out in three stages, i.e., target DNA amplification, making normal curves of target bacteria from known isolates of concentration, and calculating target bacterial concentrations.

Data analysis

Data are presented in tables and graphs. Bacterial concentration was performed in the form of CFU/ml. Data analysis used SPSS version 20.0. Numerical data were analyzed for the first used Shapiro–Wilk test. Numerical data were analyzed by used unpaired t-test and paired t-test. A $p < 0.05$ was considered statistically significant.

Results

The study was conducted on breastfeeding mothers who have babies aged 6–12 months. The total subjects were 28 subjects from two groups, the first group was 14 subjects who were given *dadiah* ice cream and the second group 14 subjects were given ice cream does not contain *dadiah*.

Isolates of early cultures and subculture products in single colonies are shown in Figure 1.

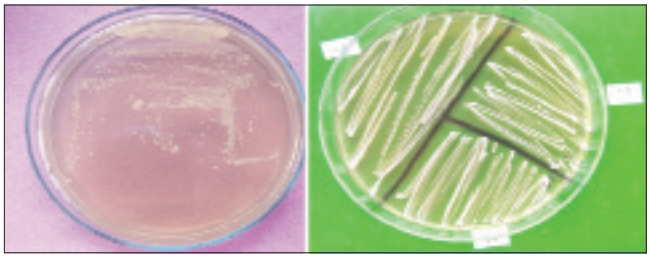

Figure 1: Isolates of early cultures and subculture products in single colonies

Figure 1, known as the identification of infant feces bacteria, begins with microbiological identification in the form of gram staining and culture. Cultures were performed on MRS, a specific medium against lactic acid-producing bacteria. The results of gram staining showed that all the isolates were Gram-positive grams. The isolates mixed in the early cultures were separated by colony morphology so that a single isolate was obtained.

Results of electrophoresis of PCR products 16sRNA and sequencing are shown in Figure 2.

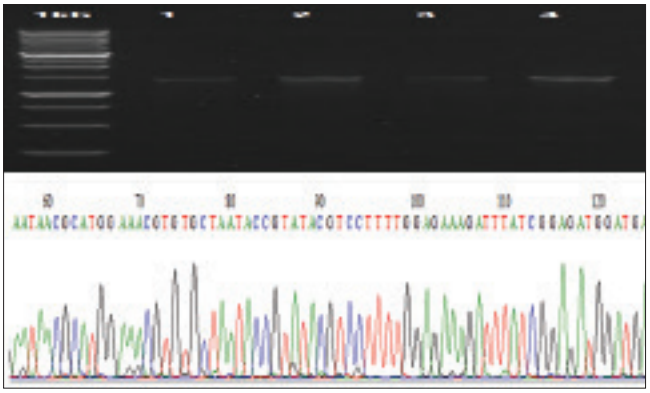

Figure 2: Results of electrophoresis of polymerase chain reaction products 16sRNA and sequencing

Figure 2, known as DNA isolation, was performed on the feces and continued with amplification using a 16sRNA primer with a size of about 1300 bp. The PCR product is further sequenced and aligned using BLAST. Based on the sequencing data obtained, several species of lactic acid bacteria in infants are *L. plantarum*, *Weissellia cibaria*, *Lactobacillus gasseri*, *Lactobacillus salivarius*, and *Lactobacillus ducreyi*.

Sequencing data compared with culture results showed that the largest proportion of bacteria is *L. plantarum*, so in the next stage, we used *L. plantarum* as a guide for analysis.

Mean of intestinal *L. plantarum* of infants aged 6–12 months in breastfeeding mothers given *dadiah* ice cream (Table 1).

Table 1: Mean of intestinal *L. plantarum* of infants aged 6–12 months in breastfeeding mothers given *dadiah* in the treatment group

Variable	Before	After	p-value
	Mean±SD (CFU/g)	Mean±SD (CFU/g)	
Intestinal *L. plantarum* in the treatment group	$4.2\times10^{7}\pm7.2\times10^{7}$	$8.6\times10^{8}\pm1.3\times10^{9}$	0.001

L. plantarum: *Lactobacillus plantarum.*

Table 1, known in the treatment group, it was found that the mean intestinal microflora of the infant before the *dadiah* was given was 4.2×10^{7} CFU/g and after the *dadiah* was 8.6×10^{8} CFU/g. Statistical analysis showed a significant difference in the number of *L. plantarum* in feces before and after has given *dadiah* ($p < 0.05$).

Mean of intestinal *L. plantarum* of infants aged 6–12 months in breastfeeding mothers given *dadiah* (Table 2).

Table 2: Mean of intestinal *L. plantarum* of infants aged 6–12 months in breastfeeding mothers given *dadiah* in the control group

Variable	Before	After	p-value
	Mean±SD (CFU/g)	Mean±SD (CFU/g)	
Intestinal *L. plantarum* in the control group	$2.6\times10^{7}\pm4.2\times10^{7}$	$1.3\times10^{7}\pm2.9\times10^{7}$	0.001

L. plantarum: *Lactobacillus plantarum.*

Table 2, known in the control group, it was found that the mean of *L. plantarum* of the infant intestinal before the *dadiah* was given was 2.6×10^{7} CFU/g and after the given was 1.3×10^{7} CFU/g. Statistical analysis showed no significant difference in the number of *L. plantarum* in feces before and after has given *dadiah* ($p > 0.05$).

Discussion

The results of this study showed a significant increase in the number of *L. plantarum* in infant feces after obtaining *dadiah* ($p<0.05$) and this condition was not found in the group that did not receive *dadiah*.

The number of Intestinal microflora of newborns between 10^{9} and 10^{10} CFU/g. The study also showed an increase in the number of intestinal microflora of newborns after being given probiotics [12]. The effect of probiotics on breastfeeding mothers on the number of microbiota *Lactobacillus* and *Bifidobacterium* and the immunity response in infants 0–2 years found that there was a difference in the number of *Lactobacillus* microbiota increased compared to bifidobacterium, i.e., 2.9×10^{9} CFU/g and 1×10^{8}CFU/g [13]. Another study found a significant difference in the number of probiotics of *Lactobacillus* type between breast-fed and formula-fed infants. In breastfed infants, *Lactobacillus* accounts for 70% of total intestinal microflora during infancy, while infants fed only 30% of *Lactobacillus* intake in the intestinal tract [14].

One of the colonization phases of the intestinal tract microbiota is the type of diet or food consumed by the baby. At the time of infant sterile intestinal infections of various microbiota, but when eating habits develop following the adult diet, intestinal channel microbiota will change. In breastfed infants, the baby's intestinal tract contains a large number of lactic acid bacteria such as

Lactobacillus and *Bifidobacterium*, whereas in infants fed intestinal channel milk more dominated by mixed flora and *Lactobacillus* and *Bifidobacterium* less prominent [15].

Babies who get breast milk will have the number of colonies *Lactobacillus* and *Bifidobacterium* in the number of lactic acid bacteria are more than the baby who given formula milk where *Lactobacillus* and *Bifidobacterium* in breast milk are affected by the composition of breast milk rich in BAL factors present in probiotics.

Based on conventional microbiological analysis with fecal culture in a laboratory with good laboratory practices certification during a year period, it was found that intestinal microflora of newborns that activate the immune system is *Lactobacillus* and *Bifidobacterium* which is found in babies consuming breast milk. *Lactobacillus* and *Bifidobacterium* play an important role in activating the baby's immune system, so babies consuming formula are more at risk of allergies and intestinal tract infections. By eating foods rich in probiotics, it improves the intestinal tract. Bacteria in these probiotics play an important role to stimulate the immune system, help the immune maturation, and protect the body from infection. The bacterium *L. plantarum* acts as an immunostimulant agent and *Lactobacillus* Salifaris as an inhibitor of inflammation.

Some of the specific strains of probiotics significantly play an important role in overcoming intestinal problems such as acute diarrhea in children. *In vivo* is also proven by milk supplementation with probiotic content can significantly decrease clinical symptoms of diarrhea. Probiotics are also able to reduce the histopathological changes caused by viruses and the ability of probiotics to prevent the spread of the virus in the intestine as a result of the effects of probiotic immunity. Probiotics have an important role in the management of intestinal tract disorders [16], [17].

Breastfeeding mothers who consume probiotics, then the probiotic content in breastmilk mother can be transferred to the baby through breast milk. Not only beneficial for the baby for the mother alone probiotics but also provide a very large advantage to health. Based on the results of this study, it can be concluded that there is a significant difference related to the amount of *L. plantarum* before and after the provision of *dadiah* in the group receiving *dadiah*, and there was no significant difference in the number of *L. plantarum* before and after in the group that did not get *dadiah*. There was a significant difference in the change in *L. plantarum* between groups receiving and not getting *dadiah*.

Conclusion

Based on the results of this study, it can be concluded that there is an increase in *L. plantarum* concentration after the provision of *dadiah* ice cream in breastfeeding mothers.

References

1. Surono IS, Martono PD, Kameo S, Suradji EW, Koyama H. Effect of probiotic L. plantarum IS-10506 and zinc supplementation on humoral immune response and zinc status of Indonesian pre-school children. J Trace Elem Med Biol. 2014;28(4):465-9. https://doi.org/10.1016/j.jtemb.2014.07.009
   PMid:25183688
2. Lutter CK, Chaparro CM. Neonatal period: Linking best nutrition practices at birth to optimize maternal and infant health and survival. Food Nutr Bull. 2009;30(2):S215-24. https://doi.org/10.1177/15648265090302s205
   PMid:20496614
3. Ramakrishnan U. A review of the benefits of nutrient supplements during pregnancy: From iron-folic-acid to long-chain polyunsaturated fatty acids to probiotics. Ann Nestle Eng. 2010;68(1):29-40. https://doi.org/10.1159/000298781
4. Rodrigues MA, Silva DA, Taketomi EA, Hernandez-Blazquez FJ. IgA production, coliforms analysis and intestinal mucosa morphology of piglets that received probiotics with viable or inactivated cells. Pesqui Vet Bras. 2007;27:241-5. https://doi.org/10.1590/s0100-736x2007000600004
5. Ljungh A, Wadström T. Lactic acid bacteria as probiotics. Curr Issues Intest Microbiol. 2006;7(2):73-89.
   PMid:16875422
6. Lara-Villoslada F, Debras E, Nieto A, Concha A, Gálvez J, López-Huertas E, *et al*. Oligosaccharides isolated from goat milk reduce intestinal inflammation in a rat model of dextran sodium sulfate-induced colitis. Clin Nutr. 2006;25(3):477-88. https://doi.org/10.1016/j.clnu.2005.11.004
   PMid:16375993
7. World Health Organization. Guidelines for Evalution of Probiotic in Food. Report of Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotic in Food. London, Ontario, Canada: World Health Organization; 2002.
8. Kos B, Susković J, Vuković S, Simpraga M, Frece J, Matosić S. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. J Appl Microbiol. 2003;94(6):981-7. https://doi.org/10.1046/j.1365-2672.2003.01915.x
   PMid:12752805
9. Yi SH, Jernigan JA, McDonald LC. Prevalence of probiotic use among inpatients: A descriptive study of 145 U.S. Hospitals. Am J Infect Control. 2016;44(5):548-53. https://doi.org/10.1016/j.ajic.2015.12.001
   PMid:26822808
10. Berggren A, Lazou Ahrén I, Larsson N, Önning G. Randomised, double-blind and placebo-controlled study using new probiotic lactobacilli for strengthening the body immune defence against viral infections. Eur J Nutr. 2011;50(3):203-10. https://doi.org/10.1007/s00394-010-0127-6
    PMid:20803023
11. Makino S, Ikegami S, Kume A, Horiuchi H, Sasaki H, Orii N. Reducing the risk of infection in the elderly by dietary intake of yoghurt fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1. Br J Nutr. 2010;104(7):998-1006. https://doi.org/10.1017/s000711451000173x
    PMid:20487575
12. Sanchez M, Darimont C, Drapeau V, Emady-Azar S, Lepage M,

Rezzonico E, *et al*. Effect of *Lactobacillus rhamnosus* CGMCC1. 3724 supplementation on weight loss and maintenance in obese men and women. Br J Nutr. 2014;111(8):1507-19. https://doi.org/10.1017/s0007114513003875

PMid:24299712

13. Senok AC, Ismaeel AY, Botta GA. Probiotics: Facts and myths. Clin Microbiol Infect. 2005;11(12):958-66. https://doi.org/10.1111/j.1469-0691.2005.01228.x

PMid:16307549

14. Hatcher GE, Lambrecht RS. Augmentation of macrophage phagocytic activity by cell-free extracts of selected lactic acid-producing bacteria. J Dairy Sci. 1993;76(9):2485-92. https://doi.org/10.3168/jds.s0022-0302(93)77583-9

PMid:8227651

15. He F, Morita H, Hashimoto H, Hosoda M, Kurisaki J, Ouwehand AC, *et al*. Intestinal *Bifidobacterium* species induce varying cytokine production. J Allergy Clin Immunol. 2002;109(6):1035-6. https://doi.org/10.1067/mai.2002.124894

PMid:12063538

16. Henker J, Laass M, Blokhin BM, Bolbot YK, Maydannik VG, Elze M, *et al*. The probiotic *Escherichia coli* strain Nissle 1917 (EcN) stops acute diarrhoea in infants and toddlers. Eur J Pediatr. 2007;166(4):311-8. https://doi.org/10.1007/s00431-007-0419-x

PMid:17287932

17. Clarke TC, Black LI, Stussman BJ, Barnes PM, Nahin RL. Trends in the use of complementary health approaches among adults: United States, 2002-2012. Natl Health Stat Report. 2015;79:1-6.

PMid:25671660