The Effect of Storage Time on Colonization of Lactic Acid Bacteria in Breast Milk

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ABSTRACT
Breast milk is the best food for babies in early life. Ignorance of employed mothers about how to store breast milk is an inhibiting factor for exclusive breastfeeding. Improper storage of breast milk can reduce the number of bacteria in breast milk. The probiotic bacteria of breast milk are important bacteria for infants and affects the gut microbiota as adults. One of the probiotic bacteria of breast milk is Lactic Acid Bacteria (LAB). The purpose of this study was to analyze the effect of storage time on the colonization of LAB. The type of research is a true experiment. The research sample is breast milk from employed mothers, totaling 5 samples. Each sample was given the same treatment, which was stored for 5 days, 7 days and 14 days in the refrigerator (-4°C) and freezer (-4°C). Data were analyzed by one way Anova. The results showed that there is a significant difference in the mean of lactic acid bacteria colonization based on storage time with p value = 0.00, both stored at -4°C and stored at -15°C. The conclusion of the study was that there was the effect of the storage time on the total LAB colonization.

Keywords: *Storage time, Lactic Acid Bacteria, Breast milk.*

1. INTRODUCTION

Breast milk is the best food for babies in early life. Breast milk plays a very important role in achieving optimal growth and development. The World Health Organization (WHO) recommends exclusive breastfeeding until the baby is 6 months old and breastfeeding continues until the baby is 2 years old. [1]

Breast milk is an important source of bacteria for the developing baby, affecting the gut microbiota in adulthood and lifelong health. Breast milk also contains hundreds to thousands of diverse bioactive molecules. These bioactive molecules protect against infection, inflammation, organ development, colonization of healthy microbial breast milk and contribute to immune maturation. Components of breast milk such as oligosaccharides play a role in encouraging the growth of some microbes. In addition, lysozyme, lactoferrin, and antimicrobial peptides can inhibit the growth of pathogenic bacteria [2].

The neonatal period is a critical stage for microbial breast milk colonization in the gastrointestinal tract. Early microbial colonization of breast milk is influenced by one of the factors related to the storage method of breast milk. Globally, the complex and dynamic composition of breast milk promotes the healthy growth and development of infants. Therefore, WHO recommends exclusive breastfeeding for the first 6 months of life. A large number of microbes are known to colonize breast milk in the intestines of infants in the first days and weeks of life. [3-5]

The many benefits of breast milk for the baby's life make giving breast milk a very important and priority thing for babies. However, the reality is that exclusive breastfeeding has not been maximized in the community, especially working mothers. There are many factors that hinder the provision of exclusive breast milk which are felt by working mothers because of their ignorance of how to store and manage breast milk. Storage of breast milk that is not appropriate in terms of temperature, time and container makes the quality of breast milk given decrease [2].

There are changes in the microbiota/bacteria found in breast milk during storage. The types of microbiota found in breast milk are the normal flora on the skin and probiotic bacteria known as lactic acid bacteria (LAB) [2].

The purpose of this study was to analyze the effect of storage time on microbiota colonization of lactic acid bacteria in breast milk. This research is expected to have benefits as an educational effort for the community in proper and optimal storage of breast milk and also as a preventive effort in preventing diseases in infants and efforts to optimize infant growth and development through the provision of breast milk.

2. METHODS

This research is true experimental research with Pretest-Posttest Control Group design. The research population is women who work in the work area of the Air Dingin Health Center, Padang City, Indonesia. The sample is 5 samples taken by consecutive sampling technique. 10 ml of breast milk was expressed from each working mother and put into sterile plastic. Then, the breast milk was brought to the Livestock Technology Laboratory, Faculty of Animal Husbandry, Andalas University using a cooler bag for storage and bacteria count check. Each sample was given the same treatment, which was stored for 5 days, 7 days and 14 days in the refrigerator (-4°C_ and freezer (-15°C). Examination of lactic acid bacteria colonies using the Quebec Colony Counter Unit. Data analysis using One Way Anova test.

3. RESULTS

Table 1. Differences in the Average Number of Lactic Acid Bacterial Colonies in Breast Milk Based on storage time at -4°C

Storage time in Refrigerator (-4°C)	Mean (CFU/ml)	p
Day 0 (control)	$104,2 \times 10^6 \pm 26,8 \times 10^6$	0,00
Day 5	$1,8 \times 10^6 \pm 1,4 \times 10^6$	
Day 7	$1,4 \times 10^6 \pm 0,9 \times 10^6$	
Day 14	$0,4 \times 10^6 \pm 0,6 \times 10^6$	

Table 1 shows the average total colonies of lactic acid bacteria stored at refrigerator temperature (-4°C) decreased from before storage ($104,2 \times 10^6 \pm 26,8 \times 10^{6)}$ until the 5th day of storage ($1,8 \times 10^6 \pm 1,4 \times 10^6$), on the 7th day ($1,4 \times 10^6 \pm 0,9 \times 10^6$) and on 14th day ($0,4 \times 10^6 \pm 0,6 \times 10^6$). Statistical test showed a significant difference in the mean of lactic acid bacteria colonization based on storage time with p value = 0.00.

Table 2. Differences in the Average Number of Lactic Acid Bacterial Colonies in Breast Milk Based on storage time at -15°C

Storage time in Freezer (-15°C)	Mean (CFU/ml)	p
Day 0 (control)	$104,2 \times 10^6 \pm 26,8 \times 10^6$	0,00
Day 5	$3,4 \times 10^6 \pm 1,4 \times 10^6$	
Day 7	$2,1 \times 10^6 \pm 0,6 \times 10^6$	
Day 14	$0,9 \times 10^6 \pm 0,5 \times 10^6$	

Table 2 shows that the mean total colonies of lactic acid bacteria stored at freezer temperature (-15°C) decreased from before storage ($104.2 \times 10^6 \pm 26.8 \times 10^6$) until the 5th day of storage ($3.4 \times 10^6 \pm 1.4 \times 10^6$), day 7 ($2.1 \times 10^6 \pm 0.6 \times 10^6$) and day 14 ($0.9 \times 10^6 \pm 0.5 \times 10^6$). A Statistical test showed a significant difference in the mean of colonization of breast milk with lactic acid bacteria based on storage time with p value = 0.00..

4. DISCUSSIONS

Table 1 shows that the total LAB colonies stored at refrigerator temperature (-4°C) decreased from before storage to day 5, day 7 and day 14. Before storage, the mean colony of lactic acid bacteria was $104.2 \times 10^6 \pm 26.8 \times 10^6$ CFU/ml. After storage, the highest mean colony of lactic acid bacteria was on the 5th day of storage $1.8 \times 10^6 \pm 1.4 \times 10^6$ CFU/ml. On the 7th day of storage, the mean colony of lactic acid bacteria began to decrease slightly to $1.4 \times 10^6 \pm 0.9 \times 10^6$ CFU/ml. Then on day 14, there was a significant decrease, namely $0.4 \times 10^6 \pm 0.6 \times 10^6$.

Likewise, breast milk is stored in the freezer (-15°C). The highest mean colony of lactic acid bacteria was on the 5th day of storage, namely $3.4 \times 10^6 \pm 1.4 \times 10^6$ CFU/ml. On the 7th day of storage, the mean colony of lactic acid bacteria began to decrease slightly to $2.1 \times 10^6 \pm 0.6 \times 10^6$ CFU/ml. Then on day 14, there was a significant decrease, namely $0.9 \times 10^6 \pm 0.5 \times 10^6$. CFU/ml. This study also showed that the average BAL decreased below the normal limit on day 14. This means that breast milk stored for 14 days, both in the refrigerator and in the freezer, does not meet the probiotic standards set by the Indonesian National Standard. According to the Indonesian National Standard, the number of probiotics that have a positive effect on health is $1x10^6$ - $1x10^8$ CFU/ml. [1]

This study is in line with the research of Suharti et al (2016) who also found that there was a significant difference between the mean colonies of lactic acid

bacteria stored on day 1, day 5 and day 14, both stored at -4°C. as well as at temperature (-15°C. At -4°C, the mean colony of lactic acid bacteria decreased from day 1, day 5 and day 14). (12,5 x 10^6 ± 4,63 x 10^6 vs 1,00 x 10^6 ± 0,00 x 10^6 vs 0,52 x 10^6 ± 0,47 x 10^6). It is also on -15^{0}C in colony of lactic acid bacteria decreased drom day 1, 5 and 14 (36,25 x 10^6 ± 11,88 x 10^6 vs 2,37 x 10^6 ± 1,19 x 10^6 vs 0,62 x10^6 ± 0,64 x 10^6). Suharti also found that the average colony of lactic acid bacteria on day 14 was below the standard of probiotics which had a positive effect on health. [1]

The reduction in the number of colonies of lactic acid bacteria due to long storage occurs because the nutrients contained in breast milk required by bacteria for metabolism are also reduced. Lactic acid bacteria are one of the aerobic bacteria that requires nutrition as a growth medium. Some complex nutrients that are most needed by bacteria to survive such as amino acids, proteins, purines and pyrimidines are decreasing in quantity from day to day. This is what causes long storage can reduce the total colony of lactic acid bacteria in breast milk. [1]

AUTHORS' CONTRIBUTIONS

The author contributes in every stage of the research, namely in the initial survey, data collection, data analysis, and making proceedings.

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