

The Effect of Exclusive Breastfeeding on The Acidity Level of Infant's Feces

Asri Noviyanti

Midwifery Departement, Poltekkes Kemenkes Palembang

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ABSTRACT

Breast Milk is important to support the growth of the composition of an infant's gut after birth. Breast milk contains bacteria that are beneficial to the infant's gut. These bacteria play the role to inhibit the growth of harmful bacteria in an infant's body that can cause infections with keeping the acidity of the infant's gut. This study aims to determine the relationship of the total colony of LAB in breast milk and pH feces or infant based on the stage of lactation. This study was an observational study with a cross-sectional design conducted on 25 postpartum mothers who breastfed exclusively in the working territory of Andalas, Ambacang, and Ikur Koto Community Health Center, Padang. Samples were selected by consecutive sampling. Data normality was tested by Shapiro-Wilk, Friedman test for assessing difference, Spearman and Pearson correlation test to assess the correlation between total colony lactic acid bacteria in breast milk and pH feces of infants. The result shows a significant difference in total colony LAB in lactation stage with $p=0,021$ ($p<0,05$) and median the highest LAB in colostrum is 2600000 CFU/ml. And significant difference pH infant's feces with $p=0,001$ ($p<0,05$) and median on transitional stage is 5,42. The result show was a significant correlation between total colony lactic acid bacteria in breast milk and pH feces of infants on transitional stage with $p=0,001$ ($r=-0,729$)

E-mail:

asri@poltekkespalembang.ac.id

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1. Introduction

Infant mortality in developing countries is still high. In 2014 the Infant Mortality Rate (IMR) in Indonesia was 23 deaths per 1000 live births. It is generally caused by infection, particularly gastroenterocolitis and respiratory infections. Gastroenterocolitis infections are often found due to bacteria such as diarrhea. Until now, diarrhea is still the third largest contributor to morbidity and mortality in children in Indonesia. (Kesehatan & Indonesia, n.d.; Lara-Villoslada et al., 2007; Widoyono, 2011)

The West Sumatra Health Office reported the incidence of diarrhea in infants (0-1 years) in 2015 as many as 9,592 people and diarrhea in children under five as many as 109,458 people. Meanwhile, the incidence of infant diarrhea in the city of Padang in 2015 was 741 people and toddler diarrhea was 9,616 people. Diarrhea is currently included in the order of the 10 most common diseases in the city of Padang. (Padang, 2016; RI, 2014)

Mother's Milk has bioactive components that have two functions, namely protecting infants from pathogenic microorganisms from the environment and providing stimulation and maturation of the digestive, immune and neuroendocrine systems. (Smith & Charter, 2010)

Lactic acid bacteria are one of the bioactive components of breast milk. These bacteria are a group of bacteria that play a role in the growth of normal flora in the intestine and are beneficial for the ecosystem of the human digestive tract. These bacteria can be used as potential probiotics so that they can prevent several types of diseases in infants. Types of lactic acid bacteria found in

breast milk such as the genus *Staphylococcus*, *Lactococcus*, *Enterococcus*, and *Lactobacillus*.(J.C et al., 2016; Lara-Villoslada et al., 2007)

The composition of the microbiota contained in breast milk shows a relationship with maternal perinatal factors such as delivery model, lactation stage, gestational age, nutritional status, and use of drugs and antibiotics.(Boix-Amorós et al., 2016; Gomez-Gallego et al., 2016) Previous studies conducted on breast milk from 3 different times (colostrum, breast milk) 1 month, breast milk 6 months). The results showed that there were differences in the microbiota of the three breast milks. The highest lactic acid bacteria were found in colostrum which was dominated by *Staphylococcus*, *Streptococcus*, *Veillonella*, *Leptotrichia* and *Prevotella* bacteria.(Cabrera-Rubio et al., 2012)

These lactic acid bacteria will synthesize lactic acid which is beneficial for the baby's health. This lactic acid synthesis plays a role in lowering the intraluminal pH in the intestine, thereby inhibiting the proliferation of pathogenic microorganisms and the implantation of pathogenic bacteria in the baby's intestines. Therefore, the pH of the feces of babies who consume breast milk tends to be more acidic.(Suraatmaja, 2007)

2. Methods

This research is an observational study using a cross sectional design. The research population was all breastfeeding mothers and babies aged 0-4 days in the Andalas, Ambacang and Ikur Koto Padang Health Center Work Areas. Samples were taken using the Consecutive Sampling method, totaling 25 mothers and their babies. (S. Dahlan, 2010)

The collection of breast milk and infant faeces samples was carried out three times, namely at the colostrum stage on day 0-4, the transition stage on day 5-10 and the mature stage on day 11-30. The tools used are Quebec Colony Counter and pH meter.

Data processing is done by editing, coding, entry and tabulating methods. Data analysis was performed by using the Shapiro-Wilk data normality test. Analysis of differences in total LAB colony of breast milk and infant faeces pH by Friedman test followed by post-hoc Wilcoxon analysis. To assess the relationship between the total LAB colony of breast milk and the pH of the baby's feces at the colostrum and transition stages, Spearman was used. While the analysis at the mature stage using Pearson.(M. Dahlan, 2013; S. Dahlan, 2010)

3. Results

Table 1. Characteristics of Maternal Age, Body Mass Index (BMI), Infant Birth Weight

Variable	Mean ± SD	Median (Min-Maks)
Age's Mother	28,80 ± 5,83	-
Body Mass Index Mother	-	22,4 (19,80 – 25,70)
Birth Weight baby	3212 ± 337,04	-

The table above shows that the mean age of breastfeeding mothers is 28.80 ± 5.83 years, the median Body Mass Index (BMI) of breastfeeding mothers is 22.4 (19.80 – 25.70) and the average birth weight of infants is 3212 ± 337. .04 grams

Table 2. Total Colonies of Lactic Acid Bacteria in Mother's Milk Based on Lactation Stage

Lactation Stage	n	Median (Min-Maks) CFU/ml	p
Colostrum	25	2600000 (1300000-6800000)	
Transision	25	2100000 (100000-4300000)	0,021
Mature	25	2300000 (400000-4700000)	

The table above shows that there are differences in the total colonies of lactic acid bacteria in breast milk at different stages of lactation, the value of $p = 0.021$ ($p < 0.05$). Thus, data analysis was continued with Wilcoxon

The results of post hoc analysis showed that there were differences in the total colony of colostrum and transitional lactic acid bacteria with a value of $p=0.002$ ($p < 0.05$), there were differences in the total colonies of colostrum and mature lactic acid bacteria with a value of $p=0.020$ ($p < 0.05$).), but there was no significant difference in the total colony of lactic acid bacteria in transitional breast milk and mature milk with p value = 0.443 ($p > 0.05$).

The characteristics of the BAL gram staining results showed that at the colostrum stage, 20 people (80%), the dominant breast milk contained gram-positive bacteria in the form of Coccus (80%), 15 people (60%) in the transition stage breast milk and 17 people (68%) in the mature breast milk stage.

Table 3. pH of Baby Feces by Lactation Stage

Lactation Stage	N	Median (Min – Maks)	<i>p</i>
Colostrum	25	5,57 (5,45 – 5,59)	0,001
Transision	25	5,42 (5,33 – 5,69)	
Mature	25	5,69 (5,59 – 5,74)	

The table above shows the median pH of the baby's feces based on the lactation stage and the results obtained with a p value = 0.001 ($p < 0.05$), namely there are differences in the pH of the baby's feces based on different lactation stages. Therefore, data analysis was continued with the Wilcoxon Post Hoc test.

The results of the post hoc test showed that there was a difference in the pH of the baby's feces at the colostrum and transitional breast milk stages with p value = 0.001 ($p < 0.05$), there was a difference in the pH of the baby's feces at the colostrum and mature breast milk stages with $p = 0.001$ ($p < 0.05$) and there was a difference in the pH of the baby's feces at the stage of transitional breast milk and mature milk with a value of $p=0.001$ ($p < 0.05$).

The relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces at the colostrum breast milk stage was carried out by Spearman analysis. The results of the analysis showed that there was no significant relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces at the colostrum breast milk stage with a weak relationship with $p = 0.237$ ($r = 0.246$)

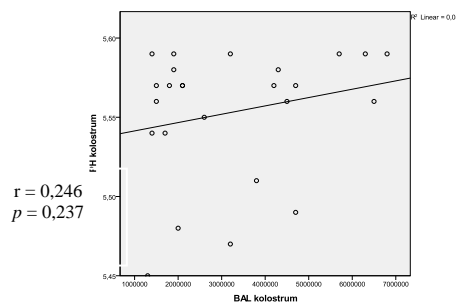


Figure 1. The results of the analysis of the relationship between total lactic acid bacterial colonies in breast milk and the pH of baby feces at the stage of breast milk colostrum

The relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces at the transitional breast milk stage was carried out by Spearman's analysis. The results of the analysis showed that there was a significant relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces at the transitional breastfeeding stage with a strong relationship in the negative direction with $p = 0.001$ ($r = -0.729$) which means the higher the total colony of lactic acid bacteria in breast milk, the higher the total colony of lactic acid bacteria in breast milk. the lower the pH value of the baby's feces.

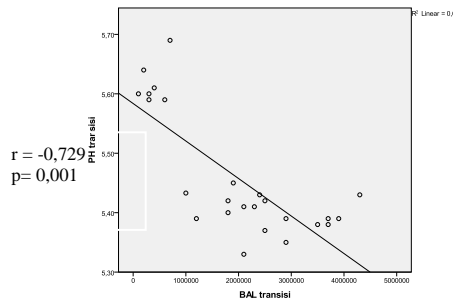


Figure 2. The results of the analysis of the relationship between total lactic acid bacterial colonies in breast milk and the pH of baby feces at the transitional breast milk stage

The relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces at the mature breast milk stage was carried out by Pearson analysis. The results of the analysis showed that there was no significant relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces at the mature breast milk stage with a weak relationship with $p = 0.157$ ($r = 0.292$).

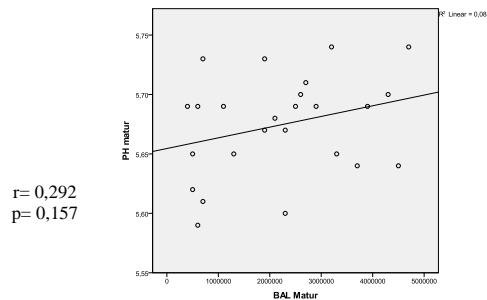


Figure 3. Results of Analysis of the Relationship of Total Lactic Acid Bacterial Colonies in Breast Milk with the pH of Baby Feces at Mature Breast Milk Stage

4. Discussion

The subjects of this study consisted of 25 postpartum mothers who breastfed exclusively, gave birth to babies vaginally, gave birth to term babies without congenital defects and had normal birth weights. And after giving birth, mothers give exclusive breastfeeding to their babies. along with their babies who are in the Andalas Health Center, Ambacang Health Center and Ikur Koto Health Center Padang City.

Postpartum mothers who breastfed exclusively in this study had a mean age of 29 years and a median body mass index (BMI) of 23. Infants of postpartum mothers had an average birth weight of 3200 grams and consisted of 14 infants (56%) male and female. 11 infants (44%) were female.

The results of statistical analysis showed that the median total colony of lactic acid bacteria in breast milk at the colostrum breast milk stage was higher than the transitional and mature breast milk stages. The analysis showed that there were differences in the total colonies of lactic acid bacteria in breast milk based on the lactation stage with a value of $p=0.021$ (<0.05).

This is in line with previous research which showed that the number of lactic acid bacteria was higher, especially in colostrum breast milk. At this stage, breast milk is dominated by *Staphylococcus*, *Streptococcus*, *Veillonella*, *Leptotrichia* and *Prevotella*.(Hunt et al., 2011)

The bacteria in breast milk originate from the mother's intestine which will be transferred from the intestine to the mammary gland which is facilitated by hormonal changes during pregnancy. In particular, the hormone progesterone is able to increase intestinal permeability and help transmission into the bloodstream and subsequently to the mammary gland. This is one of the factors that affect the number of lactic acid bacteria in colostrum which is higher than at other stages (Jeurink et al., 2013)

The results of statistical analysis showed that the median pH of infant faeces at the transitional breast milk stage was lower than that of infants at the colostrum and mature breast milk stages. The results of the analysis showed that there was a difference in the pH of the baby's feces based on the lactation stage with p value = 0.001 ($p < 0.05$).

In theory, at the beginning of the baby's life the baby's digestive tract will be colonized by aerobic bacteria such as *E.coli* and *Streptococcus* in the first 24 hours in large numbers. Aerobic germs create a decreased environmental atmosphere and this provides opportunities for the growth of anaerobic bacteria such as *Bacteroides* and *Clostridium*. On the fifth day, digestion will become anaerobic which is occupied by lactic acid bacteria, one of which is *Bifidobacterium*, which appears in large numbers and at the end of the first week there are approximately 108-1011 per gram of feces. (C et al., 2013; Suraatmaja, 2007)

It is also supported by research which states that in the first 24 hours, babies will consume 7-123 ml of breast milk/24 hours with 3-8 times of feeding. Meanwhile, at 6 days of age, babies consume more breast milk, reaching 395-868 ml/day with 5-10 times of feeding. The more often and more babies breastfeed, the more milk microbiota is transferred to the baby. This microbiota will play a role in creating a more acidic feces (Kent et al., 2015)

The results showed that at the transitional breastfeeding stage, there was a significant relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces with a strong relationship with p value = 0.001 ($r = -0.729$) and the direction of the relationship was negative. This means that the higher the total colony of lactic acid bacteria in breast milk, the lower the pH value of the baby's feces.

In contrast to the transitional stage, the colostrum and mature stages showed that there was no very weak relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces with $p = 0.237$; $r = 0.246$ at the colostrum stage and $p = 0.157$; $r = 0.292$ at mature breast milk stage.

The absence of a relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces at the colostrum stage occurred because of the pattern and duration of breastfeeding and the growth pattern of bacterial colonization in the baby's intestines which began with the emergence of aerobic bacteria. Similarly, at the mature stage, at this stage the total colony of lactic acid bacteria in breast milk is not high enough, the levels of HMOs in breast milk which become bifidus growth factors for commensal bacteria in the intestine are low, only around 5-13 g/l. (Yu et al., 2013)

The bacteria in breast milk will be transferred into the baby's digestive tract when breastfeeding as a source of bacteria formation in the baby's intestine. These bacteria have an important role in the growth and development of the human gut which affects the survival of each individual. Intestinal bacteria are also called "organs within organs" or "super organs". Because it has a function in enzymatic reactions and helps the maturation of the intestine after birth (Walker, 2014)

Intestinal bacteria will ferment HMOs which are also contained in colostrum to become Short Chain Fatty Acids (SCFA). SCFA (lactic acid) that is formed is a source of energy for the baby's intestinal epithelial cells which will protect the baby from infection. (IDAI, 2010)

Lactic acid bacteria can produce organic acids such as lactic acid, acetic acid, butyric acid, and propionic acid which can lower the intestinal pH to 3 to 4.5 so that it can kill other bacteria that live in the pH range of 6-8 and protect the colonization of pathogenic bacteria to prevent infection. proliferate. (J.C et al., 2016; Syukur & Purwati, 2013)

5. Conclusion

1. There is a difference in the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces based on the lactation stage
2. There is a difference in the pH of the baby's feces based on the stage of lactation
3. There is no significant relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces at the colostrum breast milk stage
4. There is a significant relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces at the transitional breast milk stage

5. There is no significant relationship between the total colony of lactic acid bacteria in breast milk and the pH of the baby's feces at the mature breast milk stage.

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