TOTAL BACTERIA AND ACIDITY LEVEL OF EXPRESSED BREAST MILK IN COVID-19 SURVIVING MOTHERS

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ABSTRACT

Background: Breast milk is the best food for babies. Breast milk contains all the nutrients needed for optimal growth and development. However, breast milk is prone to bacterial growth because the high content of carbohydrates, vitamins, minerals, proteins, and fats which is suitable for bacterial growth. This study aimed to examine total number of bacteria and acidity level of expressed breast milk in COVID-19 surviving mothers.
Subjects and Method: This was a descriptive study conducted at Universitas Muhammadiyah Kudus, Central Java in June 2021. A total of 5 breastfeeding COVID-19 surviving mothers was selected for this study. The study variables were the total number of bacteria and acidity level. The data were analyzed descriptively.
Results: Bacteria were present in the expressed breast milk after 2x24 hour incubation. In the first dilution (10:1), the number of bacterial colonies = 124-367. In the second dilution (10:2), the number of bacterial colonies = 4-150. In the third dilution (10:3), the number of bacterial colonies= 0-103. The higher dilution of expressed breast milk, the smaller number of bacteria. The number of bacteria colonies in the expressed breast milk of COVID-19 surviving mothers was acceptable according to the standard. Likewise, the acidity of expressed breast milk varied according to storage time. On day 0, the average pH ranged from pH 6.8 to 7.3. On day 2, the average pH ranged from pH 6.6 to 7.0. The acidity level of the expressed breast milk among COVID-19 surviving mothers was acceptable according to the standard.
Conclusion: The number of bacteria colonies in the expressed breast milk of COVID-19 surviving mothers is acceptable according to the standard. The acidity level of the expressed breast milk among COVID-19 surviving mothers is acceptable according to the standard.

Keywords: expressed breast milk, bacteria, acidity, COVID-19 surviving mothers

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BACKGROUND

Breast milk (ASI) is the best food for babies at the beginning of their life because breast milk contains all the nutriaents that can be used for optimal growth and development of babies. Therefore, the government has socialized the exclusive breastfeeding program for infants 0-4 months or up to 6 months if possible. However, the phenomenon of the COVID-19 pandemic is a challenge for breastfeeding mothers to continue to give breast milk to their babies. This is also influenced by the health status of the mother, the number of mothers who died, the limited support and the decrease in the number of visits by breastfeeding mothers to health facilities such as public health center and integrated

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healthcare center. Meilinda (2021), the success of breastfeeding mothers especially during the pandemic lies in the experience of breastfeeding and good support for mothers to continue breastfeeding.

The United Nations Children's Fund (UNICEF) and the World Health Organization (WHO) recommend that children should only be given breast milk (ASI) for at least six months, with the aim of reducing child morbidity and mortality. Solid food should be given after the child is 6 months old, and breastfeeding should be continued until the child is two years old (WHO, 2020).

Coronavirus infection in pregnancy has demonstrated that it is capable of causing adverse clinical outcomes including life-threatening maternal illness which in some cases requires hospitalizetion, intensive care and ventilatory support (Schwartz and Graham, 2020). According to indications from the Italian Society of Neonatology, if a mother previously identified as positive for COVID-19 is asymptomatic or paucisymptomatic, hospitalization is reasonable management and direct breastfeeding is preferable, ensuring strict infection control measures including proper personal hygiene. good. wash hands before touching the baby and wear a mask (Salvatori et al., 2020).

The Indonesian Doctors Association (IDAI) ASI Task Force Wiyarni Pambudi said breastfeeding mothers who were confirmed positive for COVID-19 could still provide exclusive breastfeeding for their babies. Precisely based on research results, breast milk in positive COVID-19 mothers has a high antibody content. Mothers who are confirmed positive, it turns out that in their breast milk, Immunoglobulin A and G antibodies flow, Lactalbumin, Lactoferrin etc. which are specifically a fortress of resistance against SARS-CoV-2, called natural passive immunization, are given to mothers who have survived COVID-19 the baby. Antibodies to SARS-CoV-2 specific IgA and specific IgG antibodies in the breast milk of COVID-19 survivors were able to persist for 7-10 months after infection.

Immunological test results revealed seroconversion of IgM on day 8 after onset and IgG on day 28. IgM and IgG antibodies to SAR-CoV-2 were detected in breast milk, umbilical blood and serum in neonates. (Gao et al, 2020) stated that breast milk is one of the ingredients that is very easy to grow bacteria because the nutritional composition of carbohydrates, vitamins, minerals, protein, and fat is quite high which is very beneficial for growth. microorganisms. The bacteria contained in breast milk include *Lactobacillus spp*, *Staphylococcus spp*, Bacillus spp, *Streptococcus spp*, *Corynebacterium spp*, *Enterococcus spp*. Breast milk contains probiotic bacteria which are widely known to belong to the Lactic Acid Bacteria (LAB) group (Damayanti, 2018).

Breast milk also contains non-pathogenic bacteria originating from the mother's endogenous pathway (Fernandez et al., 2013). The number of bacteria in breast milk causes organoleptic changes, especially in taste, causing breast milk to become sour or change the degree of acidity.

The quality that is considered is the microbial content in milk and is

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closely related to the degree of acidity of milk caused by the number of microbes in milk. The presence of coliform bacteria in food or drink indicates the possibility of enteropathogenic and/or toxigenic microorganisms that are harmful to health (Pelczar and Reid, 1972).

There are several ways that can be used to determine the number of bacteria in milk. There are two basic calculations, namely direct calculations and indirect calculations. Direct calculation is counting the number of bacteria in milk directly using a microscope. While the indirect calculation is to grow bacteria on a growth medium (generally Standard Tryptone-Glucose-Extract Milk Agar), then count the colonies that grow on the media (Hadiwiyoto, 1982).

Some of the damage to milk caused by the emergence of microorganisms include the following: acidification and clumping, slimy, and clumping of milk that occurs without a decrease in pH.

So far, research has only targeted breast milk as an immunity booster from the content of immunoglobulin antibodies for exclusively breastfed infants. There has never been a study that examined the number of bacteria contained in the breast milk of COVID-19 survivors.

Therefore, on this, researchers are interested in knowing the ASIP content of mothers who have survived COVID-19, seen from the results of the identification of the total bacteria and the degree of acidity.

SUBJECTS AND METHOD

1. Study Design

This was a descriptive study conducted at Universitas Muhammadiyah Kudus, Central Java in June 2021.

2. Population and Sample

The population in this study were breast milk used from COVID-19 survivors. A total of 5 breastfeeding COVID-19 surviving mothers was selected for this study.

3. Study Variables

The study variables were the total number of bacteria and acidity level.

4. Operational Definition of Variables

The expressed breast milk of Covid 19 survivors is the volume of dairy products from Covid 19 survivor.

Total bacteria is an estimate of the number of microorganisms present in the sample to represent the number of bacterial colony units (CFU) per gram or milliliter in the sample.

Potential Hydrogen (pH) is the acid or base of a solution from 0 to 14. 7 is neutral. A pH below 7 is acidic and above 7 is a base.

5. Instruments

The instruments used in this research included: breast pumps, breast milk bags, cooler bags, pH meters, thermosmeters, petri dishes, volume pipettes, Erlenmeyer, measuring cups, beakers, test tubes, autoclaves, incubators, cotton swabs, spirit lamps, LAF, refrigerators.

6. Data Analysis

The data were reported descriptively.

RESULTS

Table 1 showed the results of the analysis of the total bacteria from the breast milk of mothers who have

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survived COVID-19 after 2x24 hours of incubation.

Table 2. showed the decrease in pH values due to storage. There is a differrence in the pH value of 5 probands on the day of sampling and after incubation 2x24 hours.

Table 1. Total bacteria from breastmilk of COVID 19 survivors

Subjects (Initial)	10^{-1}	10^{-2}	10^{-3}
Subject 1 (P)	367	115	32
Subject 2 (M)	210	133	77
Subject 3 (A)	124	4	0
Subject 4 (A)	382	150	94
Subject 5 (U)	171	149	103

Table 2. pH of expressed breastmilk from COVID 19 Survivors

Derajat Keasaman (pH)	pH (on the day of sampling)	pH (after incubation 2x24 hours)
Subject 1 (P)	7.26	6.84
Subject 2 (M)	6.8	6.71
Subject 3 (A)	7.26	6.90
Subject 4 (A)	6.93	6.63
Subject 5 (U)	7.02	6.96

The calculation of bacterial colonies is mentioned in formula 1.

Formula 1. if the colonies in petri are 25-250 colonies.

$$(N) = \frac{\sum C}{[(1xn1)(0,1xn1)(0.01xn1)]xd}$$

$$= \frac{210 + 124 + 171 + 115 + 133 + 150 + 149 + 32 + 77 + 94 + 103}{[(1x3)(0,1x)(0.01x4)]x0.1}$$

$$= \frac{1358}{[(3) + (0,4) + (0.04)]x0.1}$$

$$= \frac{1358}{[(3.44)]x0.1}$$

$$= \frac{394,767}{x0.1}$$

$$= 3948 \text{ koloni/ml}$$

DISCUSSION

The results of the study are based on table 1. it can be described that the results of the total bacterial analysis showed that there were bacteria in the breast milk of COVID-19 survivors after 2x24 hours of incubation. The higher the dilution of the number of bacteria there was a decrease in the total bacteria.

The first dilution (10-1) of 5 subjects showed that the total number of bacteria was between 124 - 367 bacterial colonies, there was a decrease in the total number of bacteria in the second dilution (10-2) which was between 4-150 bacterial colonies. In the 3rd dilution (10-3) there were no bacteria in one of the subjects, namely subjects 3 (A), and the total number of bacteria in the other subjects was 0-103 bacterial colonies.

ASIP is one of the ingredients that is very easy for bacteria to grow because the nutritional composition between protein and fat is quite high and it is very beneficial for the growth of microorganisms. The total change in bacteria is related to the growth phase of micro-organisms. Several factors that affect the speed of growth of microorganisms are the availability of nutrients, acidity (pH), temperature

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and humidity. In this phase, bacteria require more energy than other phases (Fardiaz, 1992 in Aminah and Isworo, 2012).

Based on table 1, it also explains the results of the calculation of the total bacterial colony, which is 3948 colonies/ml, the number of bacteria is below the threshold so that it can be categorized as good milk. According to Davidson et al. (1993), good milk is milk that does not contain more than 4x106 microbes. The provision of incentives or services is to increase motivation, job satisfaction and employee performance. Incentives are a means of motivation in the form of incentives or incentives that are deliberately given to employees so that a greater enthusiasm arises in them to excel for the organization (Wayan, 2015).

Expressed breastmilk is one of the ingredients that is very easy for bacteria to grow because the nutritional composition between protein and fat is quite high and it is very beneficial for the growth of microorganisms. The total change in the bacteria is related to the growth phase of the micro-organisms.

The results of the analysis of pH or acidity of ASIP during storage showed various changes in the five probands. On day 0, fresh ASIP showed that the average pH range of ASIP ranged from pH 6.8 to 7.26, while on day 2 or after 2x24 hours of storage there was a decrease from 6.63 to 6.96. This is in line with Aminah and Isworo (2012), which shows that the acidity of breast milk that has been stored for five days at a temperature of -5°C has changed. This change in acidity can be caused by bacteria present in breast milk during storage. These bacteria are able to break down lactose into lactic acid, so this condition causes a decrease in the acidity of ASIP on the fifth day.

According to Siahaya (2017) showed that the treatment of long frozen storage has a significant effect on protein levels, pH, the total number of bacteria in breast milk stored for twelve days in the freezer. There is also an effect of storage in the refrigerator (temperature 2-8°C) on the decrease in lactose levels in breast milk (Arifin et al., 2009). According to Estiasih and Ahmadi (2011), at a normal freezing temperature (-18°C), there is a slow decline in quality due to chemical changes or enzyme activity. These changes are accelerated by changes in pH, an increase in solute concentration around the ice, a decrease in water activity, and an oxidation-reduction potential. If the enzyme is not inactivated prior to clotting, damage to the cell membrane causes the enzyme to come into contact with the solute and react.

This research can be concluded that the quality of breast milk for mothers who have survived COVID-19 is assessed from the total bacteria and pH is safe and can be consumed by infants because the number of microbial colonies is below the threshold so that it can be consumed. Although the pH has decreased after 2x24 hours of incubation, it is still in the normal pH category so it is safe for babies to consume.

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AUTHOR CONTRIBUTION

Yunita rusidah as the main researcher who collected data. Islami and Shinta Dwi Kurnia writing a publication script.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

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REFERENCES

Buckle KA, Edwards RA, Fleet GH, Wooton MKA, Purnomo, Hari, Semangun H, et al. (1985). Ilmu Pangan. UI Press, Jakarta.

Damayanti S (2018). Pengaruh lama penyimpanan Air Susu Ibu (ASI) terhadap jumlah bakteri Coliform (the effect of the length of storage of breast milk on the number of coliform bacteria). Fakultas Sains dan Teknologi Universitas Islam Negeri Alauddin Makasar.

Dasuki UA, Lengkey AHW, Setiadi E (1981). Pengaruh Perbedaan Metode Pasteurisasi Secara Sederhana dan Pabrik Terhadap Daya Awet, Jenis dan Jumlah Bakteri Susu (Kasus Susu Sapi Rakyat Pengalengan) (The Effect of Differences in Simple Pasteurization Methods and Factory on Durability, Type and Amount of Milk Bacteria (Case of Canned People's Cow Milk). Dari Kumpulan Makalah Kongres Nasional Mikrobiologi ke-3, Perhimpunan Mikrobiologi Indonesia. Jakarta.

Davidson PM, Branen AL (1993). Antimicrobial in Foods 2nd. Marcel Dekker Inc, New York.

Fardiaz S (1992). Mikrobiologi Pangan 1. Jakarta: Gramedia Pustaka Utama

Fernandez L, Langa S, Martin V, Maldonado A, Jimenez E, Martin R, Rodriguest JM (2013). Origin and potential roles in health and disease. Pharmacol. Res. 69, 1-10

Hadiwiyoto S (1982). Teknik Uji Mutu Susu dan Hasil Olahannya. Liberty, Yogyakarta.

Pelczar MJ, Reid RD (1972). Microbiology. McGraw Hill Book Company, New York.

Salvatori G, De Rose DU, Concato C, Alario D, Olivini N, Dotta A, Campana A (2020). Managing COVID-19-Positive Maternal-Infant Dyads: An Italian Experience. Breastfeeding medicine: Breastfeed Med . 15(5), 347–348. DOI: 10.1089/bfm.2020.0095.

Schwartz DA, Graham AL (2020). Potential Maternal and Infant Outcomes from (Wuhan) Coronavirus 2019-nCoV Infecting Pregnant Women: Lessons from SARS, MERS, and Other Human Coronavirus Infections. Viruses, 12(2), 194. DOI: 10.-3390/v12020194

Meilinda V (2021). Keberhasilan Ibu Penyintas COVID dalam Kegiatan Menyusui (The Success of Mothers of COVID Survivors in Breastfeeding Activities). Jurnal Kesehatan Perintis (Perintis's Health Journal), 8(1), 32-38.

Mustika DN, Nurjanah S, Su YN (2020). Shelf life, Total Bactery and Acidity of Pasteurized Milk (ASIP) Based on Storage Timein Cooler Bag. Final Report Research For Beginners.

Gao X, Wang S, Zeng W, Chen S, Wu J, Lin X, Liu Y, et al. (2020). Clinical and immunologic features among COVID-19-affected mother-infant pairs: antibodies to SARS-CoV-2 detected in breast milk. New microbes and new infections, 37, 100752. DOI: 10.-1016/j.nmni.2020.100752.

https://doi.org/10.26911/ICPHmaternal.FP.08.2021.21