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The effect of storage on energy, carbohydrate, fat, and protein content of breast milk

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ABSTRACT

Background: Working mothers may need to store their breast milk for later use. However, this method raises concern about the nutrition contents of the milk after storage.

Objective: to compare the levels of carbohydrates, fats, and proteins between fresh breast milk and stored breast milk.

Methods: A prospective cohort study was conducted at the Pediatrics outpatient clinic of Sanglah General Hospital. The energy, carbohydrate, fat, and protein content of breast milk were analyzed using MIRIS Human Milk Analyser (HMA). The difference in the concentration of energy, carbohydrate, fat, and protein between fresh breast milk, refrigerated (for 24-hours), and frozen (for 7 days) breast milk was analyzed using ANOVA (α=0.05).

Results: Eighty-one samples of breast milk from 27 mothers were included in the study. The content of energy in fresh, refrigerated, and frozen breast milk were 65.04±3.51, 64.48±3.33 and 64.03±3.41 ($p<0.001$), respectively. The carbohydrate content were 6.96±0.19, 6.90±0.17, and 6.89±0.18 ($p<0.001$). The fat content were 3.55±0.41, 3.46±0.38, and 3.40±0.38 ($p<0.001$). The protein content were 1.14±0.19, 1.09±0.18, and 1.07±0.18 ($p<0.05$). There was a significant difference between the carbohydrate, fat, protein and energy content between the fresh, refrigerated, and frozen breast milk. The decrease in the content of carbohydrate, fat, protein and energy between the fresh and the refrigerated milk were 0.78%; 2.2%; 3.9%; and 0.83%, respectively. The decrease in the content of carbohydrate, fat, protein and energy between the fresh and the frozen milk were 1.01%; 3.9%; 6.3%; and 1.5%, respectively.

Conclusion: There was a statistically significant difference in the energy and macronutrient content between the fresh and the stored breast milk. However, the difference has no importance clinically, and breastmilk is strongly recommended.

Keywords: Content differences, carbohydrate, fat, protein, energy, breast milk

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INTRODUCTION

Breast milk is widely known as the best food for babies for the first six months of life. It contains a complete, balanced and high-quality nutritional content which ensures optimal infant growth and development.[1] The antibodies in the breast milk protect infants from the invasion of various harmful microorganisms. Breastfed infants will experience lower diarrhea and respiratory infection rate compared with infants who were not receiving breast milk.[1] Moreover, breast milk can also prevent chronic diseases such as obesity, hypertension, diabetes, heart disease, and premature death.[1] Furthermore, breast milk consumption may help reduce family expenses.[1] However, these advantages of breastfeeding do not guarantee mothers' willingness to breastfeed their babies. The data showed that the coverage of exclusive breast milk is still very low. The Indonesian Basic Health Research data (*Riset Kesehatan Dasar*) in 2010 reported that an exclusive breast milk coverage in 6-months infants of 35.3%.[2] In Bali, the coverage of exclusive breast milk in 6-months infants is higher than the national data for 45.5%. The low coverage of breastfeeding practices raises concerns for the government and the community, especially health workers to promote exclusive breastfeeding practice.[2,3]

Mothers have various reasons to stop the exclusive breastfeeding or start weaning before the age of 6 months, or even at the earlier age of 4 months. A study in Denpasar reported that mothers stop exclusive breastfeeding due to their occupation, inadequate breast milk production, and a perception of dissatisfied babies after breastfeeding.[4] To prolong breastfeeding, mothers can be taught to store their breast milk before going to work. It can be kept for 6-8 hours at room temperature (25°C), 2-4 days in the refrigerator, 2 weeks in freezer temperature of -17°C, and 3-6 months in refrigerator temperature of -20°C.[5] However, it is not recommended to store breast milk at a temperature of 38°C because it might reduce the nutritional content.[6] Din et al. (2004) reported a decrease in the levels of macronutrients such as lactose (0.1%), fat (0.4%) and protein (4.1%) in milk stored for 24 hours at the temperature of 4-6°C. Moreover, a decrease in lactose (0.5%), fat (1.2%) and protein (10.9%) is observed in breast milk stored for a single week in the refrigerator (temperature -4 to -8°C).[7]

The primary objective of this study was to compare the level of carbohydrate, fat, and protein in the fresh and the stored breast milk. Our secondary objective was to determine the factors affecting the level of macronutrients in breast milk such as the mode of delivery, maternal age at birth, and the number of parity. The result of this study is expected to be used to provide information mother counseling about stored breast milk and in health counseling on nutrition in infants.

METHODS

We conducted a prospective cohort study at the Pediatric outpatient clinic of Sanglah Hospital in August 2016. Mothers who gave birth to single a term living children a month to 6 months prior to the study and exclusively breastfed at the time of the study were included in this study. Mothers who suffered from chronic diseases, malnutrition, and use drugs affecting the breast milk production were excluded. We used consecutive sampling where subjects were enrolled until the minimum sample size was reached. A written informed consent was obtained from each mother. The study obtained approval from the Research Ethics Committee of Medicine Faculty of Udayana University, Sanglah General Hospital, Denpasar, Indonesia.

Breast milk was collected from mothers by manual milking or self-pumping or an assisted-pumping by a trained nurse. Before breast milking, the mothers were asked to wash their hands with soap on running water. The breast milk was placed in three different sterile pouches, 10 ml each. The milk the three pouches were treated differently. The first pouch was analyzed for its energy, carbohydrate, fat and protein content within the first 6 hours. The second pouch was refrigerated for 24 hours and then analyzed. The third pouch was frozen in -4 to -8°C freezer for seven days and then analyzed. All of the samples were analyzed with MIRIS Human Milk Analyser (HMA) for the energy and macronutrient content (carbohydrate, fat, protein).

The demographic data such as the infant's age and sex, mode of delivery and the number of parity were collected. Data were analyzed by a computer program. The data distribution was tested for normality with the Shapiro-Wilk test. The mean difference of each macronutrient level in all three groups was analyzed using ANOVA. When significant results were obtained, a post-hoc analysis was used to assess the different groups. A p-value of less than 0.05 was considered statistically significant.

Table 1 The subject characteristics

	n=27	
Characteristics	**f**	**%**
Maternal age		
Infant age		
1-3 month	13	11.1
4-6 month	14	22.2
Sex		
Female	16	59.3
Male	11	40.7
Delivery mode		
Spontaneous	14	51.9
Cesarean Section	13	48.1
Parity		
1	13	48.1
2	9	33.3
3	5	18.5

RESULTS

We obtained 81 breast milk samples from 27 mothers. The maternal age mean was 30.74 years (range: 25-37 years old). The infant age mean were and 3.56 months. As many as 16 (59.3%) infants were females, and 11 (40.7%) were males. The parity status ranged from 1 to 3 children. The detailed characteristics can be seen in Table 1.

There was a statistically significant difference in the energy, carbohydrate and fat content between the fresh breast milk, the refrigerated breast milk, and the frozen (p-value <0.05). There was also a significant difference in the protein content of the three samples. (Table 2)

A *post hoc* analysis presented in Table 3 showed a significant mean difference in the energy, carbohydrate, fat and protein contents between the fresh and the refrigerated breast milk, the fresh and the frozen breast milk, and the refrigerated and the frozen breast milk. Between the fresh and frozen breast milk, there were the highest differences in the energy content as many as 1.0 Kcal/100ml (95% CI 0.78-1.22), carbohydrate 0.08 g/100 mL (95% CI 0.39-0.12), fat 0.14 g/100 mL (95% CI 0.11-0.18) and protein 0.07 g/100 mL (95% CI 0.05-0.98). When we compared the fresh to the refrigerated breast milk, the percentage of the decrease in the energy was 0.83%, carbohydrate 0.78%, fat 2.2 %, and protein 3.9%. When the fresh was compared to the frozen breast milk, the percentage of the decrease in the energy content was bigger: 1.53%, carbohydrate 1.1%, fat 3.99%, and protein 6.36%.

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Table 2 Macronutrient and energy content analysis of the fresh, the refrigerated, and the frozen breast milk

Contents	Fresh breast milk (mean ± SD)	Refrigerated breast milk (mean ± SD)	Frozen breast milk (mean ± SD)	p-value
Energy (Kcal/100ml)	65.04±3.51	64.48±3.33	64.03±3.41	<0.001
Carbohydrate (g/100ml)	6.96±0.19	6.90±0.17	6.89±0.18	<0.001
Fat (g/100ml)	3.55±0.41	3.46±0.38	3.40±0.38	<0.001
Protein (g/100ml)	1.14±0.19	1.09±0.18	1.07±0.18	<0.05

SD: standard deviation

Table 3 Post Hoc analysis of the fresh breast milk and the stored breast milk

	Mean difference (95 % CI)			
Content	Fresh vs. Refrigerated	Fresh vs. Frozen	Refrigerated vs. Frozen	p
Energy	0.56 (0.36-0.76)	1.0 (0.78-1.22)	0.44 (0.21-0.67)	<0.001
Carbohydrate	0.06 (0.02-0.91)	0.08 (0.39-0.12)	0.02 (0.02-0.42)	<0.001
Fat	0.08 (0.05-0.17)	0.14 (0.11-0.18)	0.063 (0.03-0.09)	<0.001
Protein	0.05 (0.02-0.71)	0.07 (0.05-0.98)	0.03 (0.08-0.44)	<0.001

DISCUSSION

Our study observed a difference in the energy and macronutrient contents between the fresh and the stored breast milk. A study reported a significant difference in the carbohydrate content of fresh breast milk and breast milk stored in a freezer.[8] The result was similar to our findings. We found a significant difference in carbohydrate contents between the fresh, the 24-hours refrigerated, and 7-days frozen breast milk. The difference in carbohydrate content between the fresh ant the refrigerated breast milk was 0.78% and increased to 1.1% when compared to the frozen one. However, the differences were not clinically significant.

A similar result was observed in the fat content between fresh, the refrigerated, and the frozen ones. The differences in fat content between fresh and the refrigerated breast milk was 2.2%; and increased to 3.99% when compared to frozen breast milk. Although the result was not clinically relevant, they were consistent with another study conducted in 2011.[9] A different study in 2016 found similar results.[10] The study showed a significant difference in the content was present between the fresh and the 2-3 days stored breast milk. However, another study in 2004 found no significant differences in content between the breast milk stored for 24 hours in 4-6°C and the breast milk stored for a week at -20°C. Our study found an increase in the fat content in the refrigerated breast milk as much as 0.4% when compared to the fresh one, and as much as 1.2% in the frozen one when compared to the fresh one.[7] The differences may be because different tools were used to assess the breast milk contents. We utilized the MIRIS HMA method, similar to the study showing similar results to ours, while the different result was from the study using an acid hydrolysis technique. Moreover, breast milk contains the enzyme lipase which is used to digest fat.[11] Lipase assists the breakdown of triglycerides to diglycerides and monoglycerides. The storage duration significantly affects the changes in breast milk fat content. It is thought to be due to the activity of lipolytic bacteria that produce lipase enzymes in breast milk. Lipase breaks down fats into fatty acids and glycerol.[11] Another study explained that lipolysis occurred during the first hour of storage and reached 8% at 24 hours of storage.[6]

We observed there was a significant difference in the protein content between the fresh and the refrigerated breast milk at 3.6%. The difference was greater (6:36%) when the fresh was compared to the frozen breast milk. A study by Garza reported a significant protein difference in stored breast milk, especially when the storage time was extended for 48 hours.[13] These results were also consistent with a study conducted by Lev.[8] Din also found a significant decrease in protein content, with 4.1% on 24-hours storage and 10.9% in 7-days storage.[7] Our study obtained a lower percentage compared to Din's study. This can be attributed to the homogeneous data in our study.

The energy content is associated with the decreased levels of fat and carbohydrates. We found significant differences in carbohydrate, fat, protein and energy content. The differences in energy content were 0.83% for the fresh vs. the refrigerated breast milk and 1.53% for the fresh vs. the frozen breast milk. However, the differences were not clinically significant. Studies by Lev and by Garcia-Lara also found similar results.[8,9]

CONCLUSION

Our study found statistically significant differences in energy and macronutrient content were present when the fresh breast milk was compared to the stored ones. The differences had no impact clinically. Direct breastfeeding is strongly recommended. However, stored breast milk facilitates a working mother to continue exclusive breastfeeding for the first six months of her baby's life with no clinically relevant differences in the content of the milk following a proper storage process. Further research is needed to determine the effect of storage on the micronutrient content of breastmilk.

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