

ORIGINAL RESEARCH ARTICLE

Food intake of breastfeeding mothers versus protein content of breastmilk in the coastal area of Bengkulu, Indonesia

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Abstract

Maternal intake has a role in the composition of nutritional content, especially breast milk protein, but is rarely studied in fish-producing areas (coastal). This study aimed to assess protein concentrations in human milk and evaluate their changes according to diet. The type of study was analytical observation, cross-sectional research design with a total sample of 50 from 313 breastfeeding mothers of infants 0-6 months domiciled at the Padang Serai Health Center. Dietary intake is collected using the food recall form 2 x 24 hours. Breast milk is collected in the morning as much as 30 ml using an electric breast pump. T-Test Independent analyzed the data. The results showed that as many as 10% of mothers had breast milk protein content above 0.9 g/100mL, and there was a relationship between maternal age, energy, and protein with breast milk protein content. Maternal diet, especially energy and protein, affects the protein content of breast milk. It is recommended that mothers improve their daily intake through nutritionally balanced and varied foods. (*Afr J Reprod Health* 2024; 28 [5]: 22-29).

Keywords: Energy and protein intake; protein content of breastmilk; coast area

Résumé

L'apport maternel a un rôle dans la composition du contenu nutritionnel, notamment en protéines du lait maternel, mais est rarement étudié dans les zones piscicoles (littorales). Cette étude visait à évaluer les concentrations de protéines dans le lait maternel et à évaluer leurs évolutions en fonction du régime alimentaire. Le type d'étude était une observation analytique, une conception de recherche transversale avec un échantillon total de 50 personnes provenant de 313 mères allaitantes de nourrissons de 0 à 6 mois domiciliées au centre de santé Padang Serai. L'apport alimentaire est collecté à l'aide du formulaire de rappel d'aliments 2 x 24 heures. Le lait maternel est collecté le matin jusqu'à 30 ml à l'aide d'un tire-lait électrique. T-Test Independent a analysé les données. Les résultats ont montré que jusqu'à 10 % des mères avaient une teneur en protéines du lait maternel supérieure à 0,9 g/100 ml, et qu'il existait une relation entre l'âge de la mère, son énergie et ses protéines et la teneur en protéines du lait maternel. L'alimentation maternelle, notamment énergétique et protéique, affecte la teneur en protéines du lait maternel. Il est recommandé aux mamans d'améliorer leurs apports quotidiens grâce à une alimentation nutritionnellement équilibrée et variée. (*Afr J Reprod Health* 2024; 28 [5]: 22-29).

Mots-clés: Apport énergétique et protéique ; teneur en protéines du lait maternel ; zone côtière

Introduction

Infancy is the first fastest period in the life cycle, characterized by significant physical growth and development compared to the period that follows. Macronutrient and micronutrient needs per kilogram of infant body weight per day are more significant than at other ages. It is needed to accelerate cell division and DNA synthesis during growth, especially energy and protein. Babies aged 0-6 months can meet their nutritional needs only with breast milk alone, the best and most complete nutritional food supporting babies' growth and development. The benefits of breast milk content for infants are undoubtedly able to reduce the

incidence and risk of death from infectious diseases and reduce the risk of obesity, diabetes, and other metabolic diseases as adults^{1,2}.

Breast milk is a liquid milk secreted from the breast after childbirth obtained through the breastfeeding process. Breast milk is the primary source of nutrition for babies, especially in infants aged 0-6 months, where babies have not been able to digest solid food³. Breast milk contains good nutrients and is complete according to the nutritional needs of babies⁴. The excellent content of breast milk is that it has optimal nutritional content consisting of macronutrients in the form of carbohydrates, fats, and proteins. Good macronutrient content in breast milk is fat as much

as 3.5-4.5 g / 100 mL, carbohydrates (lactose 7 g / 100 mL, glucose 1.4 g / 100 mL, galactose 1g / 100 mL), and protein 0.9-1.2 g / dL^{5,6}.

The duration of lactation affects the macronutrient content of breast milk more than the mother's age or other variables. The first breast milk to come out, or colostrum, has high protein and oligosaccharides, while mature milk has a higher proportion of lipid and casein levels. The nutritional composition of breast milk changes with the duration of breastfeeding⁷⁻⁹. Recent studies have concluded that the causes of non-optimal milk production and content are poor nutritional intake of breastfeeding mothers, unbalanced food menus, irregular food consumption, and breast milk storage that affects breast milk content¹. The nutritional intake of breastfeeding mothers is very influential in helping the process of breast milk formation, including the quality and amount of^{10,11}. The better the food consumed by nursing mothers, the higher the achievement of maternal nutrient intake, and vice versa if the intake of nutrients in nursing mothers is low, it will affect the adequacy of nutrient intake and cause low quality of breast milk production¹².

The consumption of breastfeeding mothers must be diverse and balanced in the amount and type of food consumed. The nutritional needs of nursing mothers should be more than those of mothers who do not breastfeed¹³. RDA (2019) recommends that the first six months require additional energy and macronutrient intake: energy 330 kcal/day, carbohydrates 45 grams/day, fat 2.2 grams/day, and protein 20 grams/day. Additional nutritional needs for breastfeeding can be estimated from the amount and composition of breast milk released. In addition to the amount of milk that can be excreted differs from mother to mother, and depending on the stage of lactation, the composition of the milk itself differs depending on the time of breastfeeding (morning, afternoon, evening, night) and the stage of breastfeeding: the amount of fat present in breast milk increases meaning that the fat in breast milk that comes out in the final stage of breastfeeding is higher than at the time of initial breastfeeding.

Breast milk protein is very closely related to the protein intake of nursing mothers. The mother's protein intake will be broken down into several amino acids, primarily essential amino

acids obtained only from food because the body cannot produce them, and they play an essential role in synthesizing breast milk protein in the mammary glands. If these amino acids in breast milk are insufficient, it eventually causes disrupted metabolism, which impacts the baby's growth of the baby³. The type of protein consumed by one-fifth should come from animal protein such as meat, fish, eggs, milk, and yoghurt, and the rest comes from vegetable protein such as tofu, tempeh, nuts, and others. Mothers who often consume tempeh and tofu will increase the essential amino acid phenylalanine^{14,15}.

Contradicting the findings of Bzikowska-Jura *et al.*¹⁶ assert that there is no significant relationship between maternal food intake and the protein content in breast milk. According to their study, providing protein intake to nursing mothers has a minimal or insignificant effect on the protein content in breast milk. However, this contradicts the results of research conducted by Prastiyani and Nuryanto³, which demonstrated a correlation between protein intake and the protein content in the breast milk of breastfeeding mothers during the first six months. This study suggests that higher protein intake among breastfeeding mothers leads to increased protein levels in breast milk.

In Indonesia, research is still rare regarding the fulfillment of adequate intake for breastfeeding mothers, until now it is still more focused on targeting pregnant women, even though if the adequacy of breastfeeding mothers is met, it plays a big role in the quality of breast milk, including protein content. The study was conducted in coastal areas in Bengkulu City, considering that this area produces fish, and no study has analyzed the protein content of breast milk in mothers who breastfeed infants aged 0-6 months. This study aimed to assess protein concentrations in milk and evaluate their changes according to dietary intake and demographic parameters (such as age, parity, and length of breastfeeding).

Methods

This study was a cross sectional design conducted and approved by the Padang Serai Public Health Center, Bengkulu Province. The research obtained approval from the Poltekkes Kemenkes Bengkulu Ethics Commission with the reference number KEPK.BKL/220/05/2023.

The study population was 313 all breastfeeding mothers of infants aged 0-6 months in the Padang Serai working area. The sample size in this study was calculated using the Lemeshow formula, as many as 50 respondents. The inclusion criteria were willing to be samples, breastfeeding mothers who had babies aged 0-6 months and not sick. The exclusion criteria were that mothers did not live permanently in Padang Serai.

Data collection started in May until June 2023. The mothers were given an explanation of the purpose of the study and instructed to sign the respondent consent form. Home visits of selected mothers were carried out, interviews using a self report questionnaire conducted in the morning about maternal and infant characteristics, number of children, and mother dietary intake using 2 x 24-hour food recall.

Breastmilk was collected in the morning, with a total of 30 mL of milk extracted from one breast using an electric breast pump. The collected breastmilk was then transferred to dark, sterile plastic tubes without preservatives and promptly stored in a cool box at -80°C. Breastmilk protein content was analyzed in Bengkulu University laboratory using a formol titration test in latest week of June 2023. The data were analyzed with independent T-Test, with statistical results of p-values less than 0.05.

Results

The study results reveal significant associations between energy intake exceeding 80% of the Recommended Dietary Allowance (RDA) and breast milk protein content, as evidenced by the average breast milk protein content of 1.17 g/100mL among the four individuals with such high energy intake levels. Regarding protein intake, 43 individuals consumed below 80% of the RDA, correlating with an average breast milk protein content of 0.45 g/100mL. Similarly, fat intake mirrored energy intake patterns, with four individuals consuming above 80% of the RDA and displaying an average breast milk protein content of 0.57 g/100mL. This research showed a relationship between energy or protein intake and breast milk protein content (p value < 0.05). There was no significant relationship between fat, micronutrients intake and breast milk protein content.

Table 1: Demographics characteristics mothers and infant

| Variables | n | % |
|--------------------------|----|-----|
| Maternal Characteristics | | |
| Maternal age, Years | | |
| 17 – 29 | 23 | 46 |
| 30 – 49 | 27 | 54 |
| Number of Children | | |
| Primipara | 19 | 38 |
| Multipara | 31 | 62 |
| Macronutrients intake | | |
| Energy, Kkal | | |
| < 1720 | 46 | 92 |
| ≥ 1720 | 4 | 8 |
| Protein, g | | |
| < 48 | 43 | 86 |
| ≥ 48 | 7 | 14 |
| Lipid, g | | |
| < 48 | 46 | 92 |
| ≥ 48 | 4 | 8 |
| Carbohydrate, g | | |
| < 272 | 50 | 100 |
| ≥ 272 | 0 | |
| Micronutrient | | |
| Vitamin A, RE | | |
| < 480 | 33 | 66 |
| ≥ 480 | 17 | 34 |
| Vitamin B1, mg | | |
| < 0,88 | 50 | 100 |
| ≥ 0,88 | 0 | |
| Vitamin B2, mg | | |
| < 0,88 | 48 | 96 |
| ≥ 0,88 | 2 | 4 |
| Vitamin B6, mg | | |
| < 1,04 | 45 | 90 |
| ≥ 1,04 | 5 | 10 |
| Vitamin C, mg | | |
| < 60 | 47 | 94 |
| ≥ 60 | 3 | 6 |
| Infant Characteristics | | |
| Infant age, Weeks | | |
| 0-16 | 18 | 36 |
| 17-24 | 32 | 64 |
| Protein content,g/100 mL | | |
| < 0,9 | 45 | 90 |
| ≥ 0,9 | 5 | 10 |

In contrast, vitamin A intake among 33 individuals fell below 80% of the RDA, correlating with an average breast milk protein content of 0.53 g/100mL. Only two individuals had vitamin B2 intake exceeding 80% of the RDA, with an average breast milk protein content of 0.45 g/100mL.

Table 2: The effect of maternal age, parity and age on mean protein content

| Variable | Protein Content Mean ± SD | p-value |
|--------------------------|---------------------------|---------|
| Maternal Characteristics | | |
| Maternal age, Years | | |
| 17 – 29 | 0,63 ± 0,35 | 0,01 |
| 30 – 49 | 0,43 ± 0,18 | |
| Number of Children | | |
| Primipara | 0,55 ± 0,28 | 0,57 |
| Multipara | 0,50 ± 0,29 | |
| Infant Characteristics | | |
| Infant age, Weeks | | |
| 0-16 | 0,62 ± 0,35 | 0,10 |
| 17-24 | 0,48 ± 0,24 | |

Table 3: The effect of macronutrients and micronutrients on mean protein content

| Variables | Protein Content Mean ± SD | p-value |
|-----------------------|---------------------------|---------|
| Macronutrients intake | | |
| Energy, Kkal | | |
| < 1720 | 0,47 ± 0,21 | 0,00 |
| ≥ 1720 | 1,17 ± 0,33 | |
| Protein, g | | |
| < 48 | 0,45 ± 0,20 | 0,00 |
| ≥ 48 | 0,98 ± 0,39 | |
| Lipid, g | | |
| < 48 | 0,52 ± 0,28 | 0,74 |
| ≥ 48 | 0,57 ± 0,38 | |
| Micronutrient | | |
| Vitamin A, RE | | |
| < 480 | 0,53 ± 0,28 | 0,78 |
| ≥ 480 | 0,51 ± 0,30 | |
| Vitamin B2, mg | | |
| < 0,88 | 0,53 ± 0,30 | 0,70 |
| ≥ 0,88 | 0,45 ± 0,07 | |
| Vitamin B6, mg | | |
| < 1,04 | 0,52 ± 0,30 | 0,92 |
| ≥ 1,04 | 0,54 ± 0,13 | |
| Vitamin C, mg | | |
| < 60 | 0,52 ± 0,28 | 0,97 |
| ≥ 60 | 0,53 ± 0,50 | |

Moreover, 45 individuals had vitamin B6 intake below 80% of the RDA, corresponding to an average breast milk protein content of 0.52 g/100mL. Additionally, 47 individuals consumed vitamin C below 80% of the RDA, with an average breast milk protein content of 0.52 g/100mL.

Discussion

Based on the study's results, the average protein content of breast milk in mothers aged 17-29 years was 0.63 g/100mL, whereas in mothers aged 30-49 years, it was 0.43 g/100mL. This aligns with the results of research conducted by Koutsiafti, Sultani, and Malissiova (2021), which reported average breast milk protein content of 1.96 g/dL for mothers aged < 32 years and 1.83 g/dL for mothers aged ≥ 32 years. The protein content of breast milk can be influenced by factors such as the mother's age and physical growth processes, particularly among younger mothers.

Furthermore, variations in protein composition are affected by factors such as parity, maternal health status, and the mother's physical condition. Interestingly, the mean protein concentration remained relatively stable across groups of women with different parity statuses. This finding contrasts with the research results of Czosnykowska-Łukacka et al., who found that in women with only one child, the levels of total and accurate protein in breast milk were statistically significantly lower compared to women with two children¹⁹.

The mean value of protein concentrations was not stable at a similar level in group breastfeeding of 0-16 weeks and 17-24 weeks (0.62 ± 0.35 and 0.48 ± 0.24). This suggests that as the baby grows older, the protein content of breast milk decreases. In line with the results of research by Gidrewicz and Fenton, (2014) infants aged 7-9 weeks have an average breast milk protein content of 1.3 g / dL, and at the age of 10-12 weeks have an average breast milk protein content of 1.2 g / dL²⁰. Similarly, Nommsen et al. (1991) reported an average protein content of 1.2 g/dL from birth to 2 weeks of breastfeeding, with a relative decrease in protein composition after 4-6 weeks post-delivery²¹. In contrast, the study of Valentine et al., (2010) that the concentration of breast milk protein is not influenced by the mother's diet but is associated with an increase in Body Mass Index (BMI) and more breast milk volume. Additionally, Nommsen et al. proposed that with an increase in breast milk production, there is a corresponding decrease in breast milk protein levels^{22,21}.

Based on the study results, a statistically significant relationship was observed between energy intake and breast milk protein levels ($p < 0.05$). In line with the results of Prastiyani and Nuryanto's research, (2019) it shows a significant relationship between energy intake and breast milk protein levels ($p < 0.05$), which has a medium relationship strength ($r = 0.40$)³. Similarly, the research results by Wardana, Widyastuti and Pramono, showed a relationship between energy intake and breast milk protein content (p -value = 0.031)¹². The research results by Wati *et al.*, also state that energy intake can affect breast milk protein levels²³. In addition to its influence on breast milk protein levels, energy also affects breast milk production. The fulfilment of energy needs in nursing mothers can launch milk production because the energy obtained will be converted into the hormone prolactin. Prolactin release stimulates cells in the alveoli of the breast to produce breast milk. Therefore, maternal nutrition, particularly adequate energy intake, is closely related to milk production. Improved nutritional intake leads to increased production of breast milk³.

It was found that there was a relationship between energy, protein, and fat with breast milk protein content (p -value <0.05). In line with the results of Prastiyani and Nuryanto's research, there is a significant relationship between protein intake (macronutrients) and protein content in breast milk³. Supported by the results of research by Wardana, Widyastuti and Pramono, foods with protein content consumed by mothers will be digested in the body, and one of the stopping places is the breast milk bag¹². The higher or optimal the mother's protein intake, the higher or optimal the protein content in breast milk. Similarly, the results of research by Fujita *et al.*, and Huang and Y. M. Hu, stated that there is a relationship between the protein intake of breastfeeding mothers and the protein content in breast milk^{1,24}. Indeed, the research by Wati *et al.* supports the notion that maternal diet, including protein intake, can influence breast milk protein levels. A mother's diet can affect milk protein through several integrated metabolic pathways²⁵. Furthermore, the mother's food intake of amino acids plays a vital role in synthesizing milk protein in the mammary glands. Protein function depends on its amino acid composition²⁶. There are nine essential amino acids (EAAs) that the human body cannot synthesize and

must obtain from dietary sources. These essential amino acids include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Insufficient EAA intake can lead to metabolic disturbances that can negatively impact the growth of children¹⁵.

The results of the laboratory analysis concluded, the ratio between the protein content of breast milk is less (< 0.9 g / 100 mL) and sufficient (≥ 0.9 g / 100 mL) is 9: 1 or 45 people in the category of less breast milk protein content and five people in the category of sufficient breast milk protein content. Lack of protein intake in nursing mothers is due to a lack of eating diverse and high-protein foods. This is based on the statement of Huang and Y. M. Hu, (2020) who state that the factors that affect breast milk content are food intake, age of breastfeeding mothers, and nutritional status of breastfeeding mother¹. The causes of milk production and content that are not optimal are poor nutritional intake of breastfeeding mothers, imbalanced food menu, irregular food consumption, and breast milk storage that affects breast milk content.

The important information from the 24-hour food recall states that breastfeeding mothers have typically prepare only one meal per day. Food sources commonly consumed by nursing mothers are animal protein (cob fish and chicken meat) and vegetable protein (tempeh and tofu). Many foods containing high protein are readily available in the areas where nursing mothers reside. It is evident from the geographical location of the Padang Serai Health Center, which is adjacent to the Pulai Baai market, a trading center for marine resources at low prices. Based on the description above, researchers argue that maternal food intake impacts the quality of breast milk content. It is observed that the higher the protein intake of nursing mothers, the higher the protein content in breast milk⁴. The results of the metallic study by Xi *et al.*, (2023) showed that the protein content of breast milk ranged (from 1.2 - to 1.59 g/100mL) when compared to our findings, showed a lower number²⁷. This discrepancy may be attributed to variations in the protein intake of mothers residing in different regions, particularly in areas where protein sources are less abundant or diverse.

The results showed that there was no significant relationship between fat intake and breast milk protein content (p -value > 0.05). In line

with the research results by Wardana, Widyastuti and Pramono, (2018) there is no relationship between fat intake and breast milk protein content (p-value = 0.323)¹². The results of the study of Wati *et al.*, also stated that fat intake does not correlate with total breast milk protein²³. It was found that there was no relationship between vitamin A intake and breast milk protein content (p-value = 0.76). This is because vitamin A plays a role in stimulating the secretion of the hormone prolactin in the epithelium of the brain and activating epithelial cells in the alveoli to produce breast milk²⁸. The findings of a study by Chahyanto and Roosita support this notion, indicating a significant relationship between vitamin A intake and breast milk production (p < 0.05). Essentially, higher intake of vitamin A among nursing mothers leads to increased breast milk production, ensuring adequate nutrition for babies²⁹.

Several factors influence variations in breast milk composition, namely maternal age, parity, nutrition, behavior, maternal hormones, environment, baby's gender, duration of breastfeeding, and other factors. Zielinska, shows a positive correlation between the mother's daily food intake and the carotenoid content in breast milk at the 3rd and 6th months³⁰. Several intervention studies show that carotenoids in breast milk increase after consuming foods high in carotenoids. Absolutely, the body lacks the capability to synthesize carotenoids internally, which means that the carotenoids present in breast milk exclusively originate from dietary sources^{31,32}.

It was found that there was no relationship between vitamin B2 intake and breast milk protein content (0.92). Besides carbohydrates, fats, and proteins, breast milk also contains minerals, vitamin K, vitamin A, vitamin D, vitamin E, and water-soluble vitamins. Most water-soluble vitamins, including B vitamins, folic acid, and vitamin C, are present in breast milk. However, it is notable that breast milk lacks vitamin B2 (Riboflavin) and pantothenic acid.

It was finding that there was no relationship between vitamin B6 intake and breast milk protein content (p-value = 0.71). Vitamin B6 converts carbohydrates and proteins into energy³³. Although there was no significant association, vitamin B6 is needed in the early stages of nervous system development³⁴. In addition, the content of vitamin

B6 in breast milk is proven to accelerate infant development, as seen in a significant increase in baby length, head circumference, and body weight³⁵. Vitamin B-6 concentrations in breast milk increase 3- to 4-fold in the first few weeks postpartum, followed by a gradual decline at the end of lactation³⁶. Our finding, it was found that there was no relationship between vitamin C intake and breast milk protein content (p-value = 0.17). In addition to the absence of its relationship with vitamin C intake, based on the results of research by Permatasari and Ule, state that vitamin C also does not affect breast milk production³⁷. The highest vitamin C content of breast milk is found in colostrum and decreases during breastfeeding^{38,39}. Vitamin C is helpful in the body's defense system so that the body can respond to the immune system appropriately. When an individual is deficient in vitamin C, it can increase the risk of experiencing diarrhea and the duration of healing in wounds⁴⁰.

Conclusion and recommendations

The higher the energy and protein intake indicates the higher the protein content of breast milk. Despite living in fish-producing areas, respondents did not show significant improvements in maternal protein consumption. This could be attributed to the low level of education and knowledge among mothers. Hence, it is recommended that health centers educate mothers from pregnancy onwards, encouraging them to enhance their energy intake as well as macro and micronutrient consumption, which play a crucial role in increasing the protein content of breast milk

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Contribution of authors

Betty Yosephin Simanjuntak: developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations, authors contributed to the final version of the manuscript.

Yunita Yunita: designed the model and the computational framework and analysed the data, wrote the manuscript with input from all authors

Arie Krisnasary: authors contributed to the final version of the manuscript.

Anisa: carried out the research and performed the measurements,

Novrizia Sukmawati: carried out the research and performed the measurements.

All authors provided critical feedback and helped shape the research, analysis and manuscript.

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