



Relationship between nutrient intake and iron levels of foremilk and hindmilk in breastfeeding mothers

Hubungan asupan zat gizi dengan kadar zat besi foremilk dan hindmilk pada ibu menyusui

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Abstract

Infants who are not exclusively breastfed are at a risk of iron deficiency. Iron deficiency affects motor development, intelligence, and endurance. Adequate nutrient intake can increase breast milk iron levels, because maternal food intake greatly affects the amount of iron in breast milk. This study aimed to determine the relationship between maternal nutrient intake and iron levels in the breast milk. This study used a cross-sectional approach from October to December 2024. The subjects were 90 mothers who breastfed babies aged 2-24 weeks, with a probability proportional to size. Nutrient intake and breast milk iron stage were the variables that were studied. Data were analyzed using the Pearson's correlation test and multiple linear regression analysis. Results showed that foremilk iron levels ranged from 60,62 µg/dL to 632,94 µg/dL, while hindmilk iron levels varied from 127,44 µg/dL to 680,38 µg/dL. Iron intake was measured between 13,17 g and 115,6 g, showing a significant correlation between nutrient intake and breast milk (p < 0,05). In addition, it was found that breastfeeding mothers' iron intake was the most influential factor on the breast milk iron stage. In conclusion, maternal iron intake significantly affects breast milk iron levels. Ensuring adequate maternal iron intake, supported by vitamin C consumption, can help increase the availability of iron in breast milk, thereby benefiting infant growth and development.

Keywords: Iron sufficiency, iron, breastmilk iron, foremilk, and hindmilk

Abstrak

Bayi yang tidak mendapatkan ASI eksklusif berisiko mengalami kekurangan zat besi. Kekurangan zat besi memengaruhi peningkatan motorik, kecerdasan, dan daya tahan tubuh. Asupan zat gizi yang cukup dapat meningkatkan kadar zat besi ASI, karena asupan makanan ibu sangat mempengaruhi jumlah zat besi dalam ASI. Penelitian bertujuan untuk mengetahui hubungan antara asupan zat gizi ibu dengan kadar zat besi dalam ASI. Penelitian menggunakan pendekatan cross-sectional pada bulan Oktober-Desember 2024. Subjek adalah ibu yang menyusui bayi usia 2-24 minggu sebanyak 90 orang, secara probability proportional to size. Asupan zat gizi dan tahapan zat besi ASI merupakan variabel yang diteliti. Data dianalisis dengan menggunakan uji hubungan Pearson dan analisis regresi linier berganda. Hasil menunjukkan bahwa kadar zat besi foremilk berkisar antara 60,62 µg/dL hingga 632,94 µg/dL, sedangkan kadar zat besi hindmilk bervariasi antara 127,44 µg/dL hingga 680,38 µg/dL. Asupan zat besi diukur antara 13,17g dan 115,6g, menunjukkan adanya korelasi yang signifikan antara asupan nutrisi dan ASI (p-value < 0,05). Selain itu, ditemukan bahwa asupan zat besi ibu menyusui merupakan faktor yang paling berpengaruh terhadap tahapan zat besi ASI. Kesimpulannya, asupan zat besi ibu secara signifikan mempengaruhi kadar zat besi ASI. Memastikan asupan zat besi ibu yang cukup, didukung dengan konsumsi vitamin C, dapat membantu meningkatkan ketersediaan zat besi dalam ASI, sehingga bermanfaat bagi

pertumbuhan dan perkembangan bayi.

Kata Kunci: Kecukupan zat besi; zat besi; zat besi ASI; Foremilk; dan Hindmilk

Introduction

In developing countries, such as Indonesia, nutrition issues remain a significant public health challenge. Inadequate nutritional intake can lead to various diseases and potentially death. Family consumption patterns play an important role in determining nutritional intake, with external factors including the natural, social, cultural, economic, and religious environment, while internal factors include values, emotional management, psychiatric conditions, and nutritional knowledge (Daniels et al., 2019; Hanim, 2020; Sánchez et al., 2020).

The quantity and quality of breast milk are significantly influenced by the nutritional intake of the mother during breastfeeding. Breast milk is the optimal source of nutrition for infants in the first six months to two years of life. Breast milk is rich in essential nutrients, such as protein, non-protein, fat, oligosaccharides, vitamins, and minerals. In addition, breast milk contains enzymes, hormones, growth factors, and various bioactive substances that play important roles in protecting the health of babies (Daniels et al., 2019; Hanim, 2020; Sánchez et al., 2020).

Iron deficiency is the most common nutritional deficiency in various populations, especially in children. Iron is essential for the human body, especially during the growth and development of babies. Approximately two-thirds of the world's children are iron-deficient, with 20-25% of preschoolers suffering from iron deficiency anemia. Infant iron adequacy is crucial for physical and cognitive development during the first 1,000 days of life, including the gestation period to the first two years of life (Atkins et al., 2016; Basrowi & Dilantika, 2021; Hernell et al., 2015).

Although breast milk contains iron, the infant's iron needs after six months of age tend to increase. Babies that receive exclusive breastfeeding have a lower risk of infection and show better growth. Breast milk is considered the best source of nutrition because it provides a variety of additional benefits that support the baby's development and maintain maternal health (Atkins et al., 2016; Hernell et al., 2015).

During the breastfeeding period, the mother's nutritional needs increase because of the high-energy expenditure for breast milk production. A balanced and varied diet is essential to support maternal metabolism. Chronic deficiencies in certain nutrients can harm maternal health, deplete nutrient reserves, and degrade breast milk quality (Bzikowska-Jura et al., 2021). Adequate intake of protein, iron, and vitamin C by breastfeeding mothers contributes to the production of quality breast milk, which supports the optimal physical, mental, and intellectual development of the baby (Dror & Allen, 2018).

Iron plays an important role as a structural component of various enzymes that are involved in metabolic processes. Infants are particularly susceptible to the effects of iron deficiency because of the rapid growth and development of the brain during this period (Dror & Allen, 2018). Iron is an essential mineral responsible for cognitive function and hemoglobin formation. The diet of breastfeeding mothers significantly affects iron levels in their breast milk (Daniels et al., 2019; Sánchez et al., 2020).

Research has shown that adequate nutritional intake by mothers can increase iron levels in breast milk, both in foremilk (initial breast milk) and hindmilk (final breast milk). Physiological differences between foremilk and hindmilk are important for analysis because of their different nutritional compositions. Foremilk has a higher lactose content, which helps with iron absorption, whereas hindmilk is richer in fat. Iron level measurements in both types of breast milk provide a comprehensive picture of the availability of iron in infants (Daniels et al., 2019; Sánchez et al., 2020).

Previous studies indicate that infants who are born full-term, formula-fed, and breastfed for less than four months—in contrast to those who are breastfed for at least four months—have a higher risk of iron deficiency (Pluymen et al., 2018). In the West Tulang Bawang Regency, the prevalence of anemia in breastfeeding mothers reached 45,7%, which is higher than the national average of 37,1%. The geographical and socioeconomic conditions of this region, with the majority of the population working as

rubber and oil palm farmers, contribute to less diverse and low-iron consumption patterns (Data from the Tulang Bawang Barat Regency Health Office, 2023).

Although several studies have examined the relationship between maternal nutritional intake and breast milk composition, there is still a gap in the literature regarding the specific correlation between maternal nutritional intake and iron levels in foremilk and hindmilk, particularly in Indonesia. Based on this description, this study aimed to analyze the relationship between nutritional intake and iron levels of foremilk and hindmilk in breastfeeding mothers in West Tulang Bawang Regency to provide a scientific basis for interventions to improve the nutritional status of breastfeeding mothers and infants in the region (Atkins et al., 2016; Bzikowska-Jura et al., 2021; Dror & Allen, 2018).

Methods

This is a descriptive analytical study with a cross-sectional approach that was carried out from October to December 2024 in the Tulang Bawang Barat Regency. The study population included all breastfeeding mothers who had babies aged 0–6 months and were domiciled in the region. A total of 90 respondents were selected using the Probability Proportional to Size (PPS) technique, where three health centers were designated as cluster units based on the proportion of the number of breastfeeding mothers in each region, namely, Kibang Budi Jaya Health Center, Candramukti Health Center, and Sukajaya Health Center. The selection of respondents in each health center was based on the size of the target population, so that the sample distribution reflected a proportional comparison.

Nutritional characteristics and intake data were collected through direct interviews using standardized questionnaires and the Semi-Quantitative Food Frequency Questionnaire (SQ-FFQ), which have previously been tested for validity and reliability in the context of similar populations. Breast milk sampling was carried out at 08.00–10.00 WIB with a manual milking method by the mother using her hands assisted by a sterilized breast pump. Foremilk is obtained

from milking in the first five minutes, while hindmilk is collected afterwards. The minimum volume of breast milk collected for each type was 10 mL. The sample was immediately stored in a special breast milk storage bag, placed in a cooler box at a stable temperature of -18°C using ice gel, and frozen before being sent to the DKI Jakarta Regional Health Laboratory for iron content analysis. During the storage and shipping process, strict procedures are implemented to prevent contamination or degradation of sample quality.

This study was approved by the Health Research Ethics Commission of the Faculty of Medicine, Diponegoro University (number: 445/EC/KEPK/FK UNDIP/VIII/2024). Data were analyzed using Pearson's correlation test and multiple linear regression, with a significance level of $p < 0,05$.

Result and Discussion

Characteristics of Breastfeeding Mothers

This study gathered a sample of 90 breastfeeding mothers of infants aged between 2 and 24 weeks living in Tulang Bawang Barat District. The table below presents the characteristics of the respondents, including their age, protein intake, iron intake, vitamin C intake, and iron content in both foremilk and hindmilk.

Table 1 shows that of the 90 respondents, the average age of breastfeeding mothers was 27,13 years ($\text{SD} = 3,96$), with an age range of 19–36 years. The average protein intake was recorded at 107,45 grams per day ($\text{SD} = 24,19$), with a range of 65,63–189,64 grams. The iron intake had an average value of 35,39 grams ($\text{SD} = 21,71$ g) and ranged from 13,17 to 115,6 grams. For vitamin C intake, the average was recorded at 133,56 mg ($\text{SD} = 65,85$), with a minimum value of 15,81 mg and a maximum of 301,72 mg.

The iron levels in foremilk ranged from 60,62 to 632,94 $\mu\text{g/dL}$, with an average of 238,42 $\mu\text{g/dL}$ ($\text{SD} = 96,63$). Meanwhile, hindmilk showed higher iron levels, averaging 332,47 $\mu\text{g/dL}$ ($\text{SD} = 118,94$), with a range of 127,44 to 680,38 $\mu\text{g/dL}$. These data show considerable variation in iron levels in breastfeeding mothers' breast milk, which may be influenced by individual factors including daily consumption patterns and lifestyle habits.

Table 1. Frequency distribution of respondent characteristics

Variables	Mean±SD	Min-Max
Mother's age	27,13 ± 3,96	19-36
Protein Intake	107,45 ± 24,19	65,63-189,64
Iron intake	35,39 ± 21,71	13,17-115,6
Vit C intake	133,56 ± 65,85	15,81-301,72
Foremilk Iron Stage	238,42 ± 96,63	60,62-632,94
Iron Content of Breast Milk Hindmilk	332,47 ± 118,94	127,44-680,38

Table 2. Frequency distribution of breastfeeding habits of mothers

Breastfeeding Mom's Habits	Yes		No	
	n	%	n	%
Drinking coffee/tea ≥ 3 cups/day	32	35,6	58	64,4
Sleep Late at Night	13	14,4	77	85,5

Table 2 shows that as many as 35,6% of respondents reported consuming ≥ 3 cups of tea or coffee per day. This habit has the potential to lower the bioavailability of non-haem iron in the diet, especially when consumed in conjunction with iron sources. These findings support the report of Zare et al. (2019), who showed that regular consumption of tea/coffee can significantly inhibit iron absorption and, in the long term, can reduce the mother's iron reserves, which ultimately impacts iron levels in breast milk. (W. Basrowi & Dilantika, 2021).

Relationship between Nutritional Intake and Breast Milk Iron Levels

The factors associated with breast milk iron levels based on the results of this study are shown in Table 3.

Table 3. Relationship between nutrient intake and breast milk iron levels

Variables	R	p-value
Foremilk		
Protein Intake	0,297	0,005
Iron Intake	0,256	0,015
Vitamin C Intake	0,254	0,016
Hindmilk		
Protein Intake	0,221	0,036
Iron Intake	0,269	0,010
Vitamin C Intake	0,208	0,049

Table 3 shows a significant positive correlation between protein, iron, and vitamin C intake and iron levels in foremilk and hindmilk samples. The correlation coefficients for protein intake were $r = 0,297$ ($p = 0,005$) and $r = 0,221$ ($p = 0,036$) for foremilk and hindmilk,

respectively. Iron intake showed a correlation of $r = 0,256$ ($p = 0,015$) with foremilk and $r = 0,269$ ($p = 0,010$) with hindmilk. Vitamin C had a correlation of $r = 0,254$ ($p = 0,016$) and $r = 0,208$ ($p = 0,049$) for foremilk and hindmilk, respectively. This positive relationship indicates that increased nutrient consumption, especially iron, is associated with increased iron levels in breast milk. Research by El-Farrash et al. (2012) and Daniels et al. (2019) supports these findings, suggesting that maternal micronutrient intake during breastfeeding plays a role in determining mineral levels in breast milk, including iron. Vitamin C enhances non-heme iron absorption by reducing Fe^{3+} to its more easily absorbed form (Fe^{2+}), as reported by Henjum et al. (2014). In contrast, inhibitory compounds such as tannins and phytates, which are often found in tea, coffee, and cereals, can decrease iron absorption.

Proteins, vitamins, and minerals play important roles in the metabolism of cells. Apart from zinc and copper, most proteins, vitamins, and minerals do not change in breast milk, but the concentration of these minerals can change according to the mother's diet and characteristics. The ionic properties of iron electron transfer allow it to function as a cofactor for enzymes involved in various oxidation-reduction reactions, including the synthesis of amino acids, neurotransmitters, collagen, and hormones (Pasricha et al., 2021). Iron that enters through food is not sufficient to cause anemia. Henjum's study in Bhaktapur, Nepal, showed that 70% of breastfeeding mothers had insufficient iron intake, and there was a weak relationship between body iron intake (P -Value = 0,03, 95% CI 0,014-0,045) (Henjum et al., 2014).

The availability of iron minerals varies significantly among different food sources, depending on whether they contain haem or non-haem iron, as well as the presence of substances that increase or inhibit iron

absorption. A literature review found that taking one gram of vitamin C increased iron absorption threefold. However, 10 mg of vitamin C can double iron absorption (Holm et al., 2017). Previous research on the Seluma Regency conducted by respondents revealed that the amount of vitamin C consumed by respondents was low. In addition, there was a significant difference between the consumption of vitamin C and hemoglobin, as well as the amount of vitamin C consumed by pregnant women who were anemic and non-anemic, with fairly low p-values (0,000, 0,039, and 0,026, respectively). In addition, vitamin C intake can linearly increase iron bioavailability (Flora, 2024).

Interview findings regarding family eating habits, appetite, and local culture revealed a low vitamin C intake. In West Tulang Bawang Regency, 43,3% of breastfeeding mothers avoided consuming chili peppers while breastfeeding, while only 15,5% avoided consuming petai daily. Breastfeeding mothers who consume caffeine experience a decrease in their milk supply. Additional effects include infant sleep problems and impaired iron metabolism. The results of a study in Tulang Bawang Barat District revealed that a simple and monotonous daily diet has low bioavailability. When a person consumes cereals, tubers, and at least 30 g of fish meat or vitamin C-rich foods daily (25 mg/day), up to 5% of the iron is absorbed by the body. If the daily menu includes cereals, root vegetables, animal-based foods, and 25-75 mg/day of vitamin C, 10% of the iron will be absorbed by the body. If the daily menu consists of a lot of meat, meat, and fish (90 g/day). Foods with high amounts of vitamin C (75 mg/day) will be absorbed in 15% of the results of the study. showed a significant relationship between the frequency of consumption of meat, vegetables, and legumes and the concentration of iron in breast milk (Bzikowska-Jura et al., 2021).

Iron undergoes a process of reduction by iron (Fe^{3+}) to iron (Fe^{2+}), so that it is easily absorbed by the digestive tract. The form of iron consumed and the presence of foods that increase (elevate) or inhibit (inhibit) iron absorption affect the absorption stage of iron citrate, tannins, oxalic acid, and antacids inhibit the absorption of iron by intestinal receptors. The greater the mother's habit of consuming animal protein, plant-based proteins, and available vegetables and fruits that are high in

vitamin C in daily consumption, the higher the iron status and hemoglobin levels of breastfeeding mothers. This is supported by a study in Poland that found that total iron intake is positively correlated with breast milk concentration. This means that the intake of mineral-rich foods is a positive factor in the breast milk content. Food intake by breastfeeding mothers is essential for maintaining the energy and nutrition of mothers and their babies. Maternal nutritional intake during pregnancy and breastfeeding, along with iron status during pregnancy, affects the iron content of breast milk. This relationship is explained by lactogenesis, a process initiated by a hormonal or neuroendocrine response that leads to the synthesis of breast milk, which begins in the 16th week of pregnancy. In the last few weeks of pregnancy, iron is transferred by the mother to the baby through a process known as the transplacenta. The body's source of iron is mostly the recycling of old erythrocyte cells by macrophages, and only 10% of iron comes from food that the body can absorb. Low iron intake during pregnancy and lactation can be attributed to insufficient iron intake from daily sources of minerals due to the lack of knowledge of breastfeeding mothers, larger family sizes, and lack of nutrients needed for breastfeeding (Achebe & Gafter-Gvili, 2017).

Multivariate Analysis Results

The result of multivariate analysis (Table 4) after multiple linear regression tests that had the most influence on breast milk iron levels (foremilk and hindmilk) was iron intake.

The results of the multiple linear regression are presented in Table 4. Iron intake was a significant predictor of iron levels in foremilk ($B = 0,350$, $p = 0,024$) and hindmilk ($B = 0,351$, $p = 0,023$). The R^2 values of 0,57 for foremilk and 0,59 for hindmilk indicate that the model explains 57% and 59% of the variation in iron levels in each type of breast milk. The remaining variables are likely to be influenced by other variables that were not analyzed in this study. These findings corroborate the results of the clinical trials by Holm et al. (2017), who showed a significant increase in iron levels in breastfeeding mothers after iron supplementation interventions.

Iron is absorbed by the duodenum into the jejunum of the small intestine through plasma transferrin, which contains approximately 3-4 mg of iron in the blood plasma, to the spinal cord

in the erythropoietic framework, where it creates erythrocyte and hemoglobin molecules that are released into the bloodstream. To transport iron to the surface of the erythroid precursor, transferrin uses specific receptors. In erythroid cells, iron enters the mitochondria and combines with protoporphyrins to form haem.

Furthermore, transferrin transports iron to different tissues throughout the body, as well as to iron reserves in the blood. (Pasricha et al., 2021) undergo metabolic changes that increase nutrient absorption. Hyperphagia is an indicator of lactation owing to the extensive colonization of bacteria on the metabolically active surface of the intestinal lumen. The mother's intestine plays an important role in optimizing the function of the breast glands to meet the nutritional, microbiological, immunological, and neuroendocrine needs of the growing baby. The bacteria and microbiota that provide immunity (enteroimmunity) to the mother are transferred to the baby through various pathways, directly

from the mother to the baby. Food components containing antigens and microbes are repaired or temporarily associated with the intestine-related lymphoid tissues. Food components containing antigenic and microbial substances are permanently or temporarily connected by intestinal-associated lymphoid tissues. The mammary gland compartment is connected to the intestinal mucosa, and the location of the lymphoid tissue system associated with the mucosa determines the tolerability of a substance and identifies harmful substances. Consequently, macrophages and dendritic cells activate the immune response, triggering the activation of supporting T cells, regulatory T cells, or natural killer cells as needed. After exposure to antigens in the intestine, lymphocytes migrate to the exposed milk mucosa where they are prepared to transport the appropriate microbiota. These bacteria move during pregnancy and lactation (Sazawal and Dhingra, 2011).

Table 4. Multiple linear regression analysis

Foremilk				
Variables	Regression Coefficient (B)	T _{calculated}	Adjusted R ²	p-value
Model 1				
Protein Intake	0,471	1,116	0,040	0,267
Iron Intake	0,368	2,236		0,028
Vitamin c Intake	0,005	0,463		0,644
Model 2				
Protein Intake	0,474	1,144	0,057	0,256
Iron Intake	0,350	2,298		0,24
Model 3				
Constant	167,340		0,054	0,016
Iron Intake	0,372	2,462		
F _{count} = 3,696				
0,029				
R _{square} = 0,57				
Hindmilk				
Variables	Regression Coefficient (B)	T _{calculated}	Adjusted R ²	p-value
Model 1				
Protein Intake	0,481	1,143	0,041	0,256
Iron Intake	0,369	2,244		0,27
Vitamin c Intake	0,048	0,448		0,656
Model 2				
Protein Intake	0,484	1,170	0,59	0,245
Iron Intake	0,351	2,237		0,023
Model 3				
Constant	166,446		0,55	0,015
Iron Intake	0,351	2,312		

F_{count} = 3,768; 0,027; R_{square} = 0,55

Iron is transported by lactoferrin to the gland to regulate iron in the breast glands. This revealed that transferrin and lactoferrin are

functionally and structurally similar iron-binding proteins; therefore, they share the same role in the transport of iron to the breast glands.

Lactoferrin is a 77 kDa glycosylated protein composed of approximately 700 amino acids that show significant similarities to each other. Its polypeptide chains fold into two symmetrical lobes, each capable of binding Fe³⁺ and carbonate ions, showing a strong affinity for iron. The lactoferrin present in breast milk not only makes it difficult for microorganisms to obtain iron but also affects iron absorption. It is possible that there is a positive relationship between iron intake and iron concentration in breast milk because the transfer of iron from plasma to breast milk in the mother's breast gland occurs through passive diffusion (Rodríguez et al., 2021).

Physiological and Clinical Implications

Physiologically, iron in breast milk is affected by lactogenesis, which is controlled by the mother's hormonal system. In this process, iron is transferred from the mother's blood to the breast glands by binding to proteins such as transferrin and lactoferrin (Lönnerdal, 2010). The high lactoferrin content in breast milk also favors increased iron absorption in babies.

Furthermore, the presence of maternal intestinal microbiota and immunological status also affect the transfer of nutrients into breast milk through the entero-mammary axis (Rodríguez et al., 2021). This explains how a mother's diet and intestinal health status can have implications for breast milk quality.

Conclusion

This study showed that there is a significant relationship between the nutritional intake of breastfeeding mothers, especially protein, iron, and vitamin C, and iron levels in foremilk and hindmilk. Among the three nutrients, iron intake has been proven to be the most dominant factor affecting the iron content in breast milk. This emphasizes the importance of iron fulfillment during the lactation period to ensure the quality of breast milk, especially in supporting the iron needs of infants in the early stages of life. The implications of these findings are relevant for efforts to improve the nutritional status of mothers and their babies. Adequate iron content in breast milk plays a crucial role in supporting brain growth, cognitive development, and the immune system of babies, especially during the golden period of the first 1,000 days of life.

Therefore, nutritional interventions for breastfeeding mothers should be a major concern in public health programs.

To maintain iron and vitamin C adequacy during breastfeeding, mothers are encouraged to eat both heme (such as liver, red meat, and fish) and non-heme (such as nuts and greens) combined with natural sources of vitamin C (such as oranges, tomatoes, and guava) to increase iron absorption. However, excessive tea or coffee consumption, especially in conjunction with iron-rich foods, needs to be limited because tannin content can inhibit iron absorption. A nutrition education-based approach and individualized counseling for breastfeeding mothers are also important, especially in areas with a high prevalence of anemia. These findings also support the need to strengthen breastfeeding nutrition policies through increased access to affordable iron and vitamin C supplements, and the integration of nutrition services in the primary healthcare system. Further research is recommended to use biochemical biomarkers and explore other factors such as inflammatory status, oxidative stress, and micronutrient interactions to obtain a more complete picture of the determinants of iron levels in breast milk.

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4286

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