DEVOTION
Journal of Research and Community Service

Volume 3, Number 10, August 2022
e-ISSN: 2797-6068 and p-ISSN: 2777-0915

CORRELATION OF LEUCINE INTAKE WITH SERUM PROLACTIN AND BREAST MILK PROTEIN LEVELS IN BREASTFEEDING WOMEN

Ni Putu Yunita Sri Lestari[1], Widya Kusuma Wardani[2], Linda Ratnawati[3], Tatit Nurseta[4], and Bambang Rahardjo[5]

[12345]Brawijaya University, Malang, East Java, Indonesia

niputuyunitaa@gmail.com[1], widyawardani02@gmail.com[2], bar_obg.fk@ub.ac.id[3], tns_obg.fk@ub.ac.id[4], linda.ratna@ub.ac.id[5]

KEYWORDS

Amino Acid, Leucine, Prolactin, Milk Protein, Breastmilk

ABSTRACT

Breast milk is the best choice food for a baby's growth and development of infants at some point of the primary 6 months of life. Fulfillment of nutrition during breastfeeding is an important requirement, one of which is the intake of the amino acid leucine which is said to affect prolactin as a regulator of breast milk production and protein synthesis in breast milk. This can support the quality and quantity of breast milk to meet the nutritional needs of infants support infant growth and development and reduce the incidence of stunting at the age of toddlers. This research aims to determine the relationship between leucine intake and levels of prolactin and protein in breast milk in breastfeeding mothers. This research is an analytic observational study with a cross-sectional method. This study carried out normality using the Kolmogorov-Smirnov test and using the Pearson correlation test to determine the relationship between variables. Based on the test results of the relationship between leucine intake and serum prolactin levels, a significance value of 0.005 ($p<0.05$) was obtained with a correlation coefficient value of 0.183 and the relationship between leucine intake and breast milk protein levels obtained a significance value of 0.033 ($p<0.05$) with a correlation coefficient of 0.147. The results of this test showed a significant relationship between these variables. There is a weak correlation between leucine intake and levels of prolactin and protein in breast milk

INTRODUCTION

Lactation is defined as the secretion of milk from the mammary glands and is influenced by a complex hormonal network. Breastfeeding or lactation is one of the effective ways to ensure the health and survival of a child (Lyons, Ryan, Dempsey, Ross, & Stanton, 2020). Breast milk contains all the energy, macronutrients, and micronutrients needed for a baby's growth and development during the first 6 months of life. Breast milk also provides various immunological factors and bioactive components (Thai & Gregory, 2020).

Milk production is regulated by the mother's hypothalamus and hormones produced by the anterior pituitary gland. Prolactin is a polypeptide hormone that is responsible for lactation. Prolactin promotes the growth of the alveoli, which are components of the mammary glands, where milk production occurs. Prolactin stimulates breast alveolar epithelial cells to synthesize components of breast milk, including lactose, casein, and lipids (Rudolph, Russell, Webb, Neville, & Anderson, 2011).

Breast milk protein plays a role in providing nutrition and contributing to infant development. If the consumption of energy and protein is less, it will cause the body to lack

nutrients, so the body will use the available energy and protein stores. If this condition lasts for a long time, energy and protein stores will be depleted, resulting in tissue damage that causes children to experience stunting (Wells et al., 2020).

During lactation, a diet high in red meat, cereals, and eggs were associated with higher protein and energy content in breast milk (Zhang et al., 2021). These findings indicate that the diet of breastfeeding mothers can affect the macronutrient composition of breast milk including breast milk protein and provide a basis for improving child health .

Proteins are composed of amino acids linked to one another. These amino acids play an important role in the development and growth of cells in the body. Certain amino acids, such as leucine, serve as substrates for protein synthesis and are important factors in regulating protein metabolism (Li, Yin, Tan, Kong, & Wu, 2011).

Prolactin as a regulator of breast milk production consists of 199 amino acids that form a single polypeptide chain with three intramolecular disulfide bonds (Bai et al., 2012). Previous studies have shown that leucine regulates prolactin secretion through a mechanism by which tyrosine levels decrease, which regulates dopamine synthesis. The low dopamine hormone will trigger the production of prolactin. It was also mentioned that leucine directly regulates the release of prolactin from pituitary mammotrophs. Increased intake of leucine is also said to increase plasma prolactin concentrations.

A maternal diet with good nutritional content is very important in determining the quality and quantity of breast milk, one of which is leucine. Leucine is one of the essential amino acids that make up protein and plays an important role in protein synthesis in breast milk by activating the rapamycin pathway and regulating prolactin secretion (Rezaei, Wu, Hou, Bazer, & Wu, 2016). Leucine is one of the nutrients needed by mothers during breastfeeding which will support the protein content of breast milk and milk production so that breastfeeding mothers can provide exclusive breastfeeding with good breast milk protein content. Meeting the protein needs of children during breastfeeding is expected to reduce the incidence of stunting in children (Dewey, 2016).

METHOD RESEARCH

This research is quantitative research with an analytical observational research design. This research was carried out from December 2021 to February 2022 in the city of Surabaya, namely in the Benowo Health Center and Kenjeran District working area The research method used purposive sampling. The number of samples in this study was 110 people who were determined based on the inclusion criteria (mothers who have babies aged 1 to 6 months, babies are given exclusive breastfeeding, mothers and babies are in good health, mothers have low-stress levels, and are willing to be respondents) and criteria exclusion (not present at the time of data collection, refusal during follow-up, mothers taking drugs that affect prolactin production such as domperidone). The researcher studied the intake of leucine with direct interviews multiple 24h food recall methods for 3 days, then the researchers converted the food ingredients data according to the 2014 Individual Food Consumption Survey Book. Intake of the leucine using the USDA National Nutrient Database for Standard Reference Legacy (2018). The researchers collaborated with the Surabaya Prodia Clinical Laboratory to take blood samples and check serum prolactin levels (ECLIA test), and the Faculty of Mathematics and Natural Sciences, University of Brawijaya

laboratory to examine breast milk protein levels (Lowry test). The data is processed using SPSS Type 22.0 Software. This study uses the Kolmogorov-Smirnov test to test for normality, and numerical data obtained were then tested using the Pearson correlation statistical test to determine the relationship between variables.

RESULT AND DISCUSSION

A. RESULT

During the study, 110 breastfeeding mothers who had babies aged 1 to 6 months with several characteristics of the respondents were assessed

Table 1.1. Respondents Characteristics

Sample Characteristics	Amount (people)	Percentage (%)
Age		
<20th	2	1.8%
21-30th	75	68.2%
31-40th	31	28.2%
41-50th	2	1.8%
Educational level		
Elementary School	9	8.2%
Middle School	13	11.8%
High School	56	50.9%
Diploma	9	8.2%
Bachelor	23	20.9%
Profession		
Working mom	31	28.2%
Housewife	79	71.8%
Parity		
Primipara	36	32.7%
Multipara	74	67.3%
Total	**110**	**100%**

Table 1 shows that the majority of respondents are aged 21-30 years (68.2%), followed by ages 31-40 years (28.2%) and the last age <20 years and ages 41-50 years having the same number (1.8%) . based on education level, the majority of respondents have high school education (50.9%) and bachelor's (20.9%), there are 8.2% of respondents with elementary school and diploma educational background. Based on occupation, most of the respondents studied were housewives as many as 79 respondents (71.8%). Respondents who work are dominated by private employees and health workers (doctors, nurses, midwives, dentists). Meanwhile, based on parity, the majority of respondents were multiparous (67.3%).

Based on the study of leucine intake for 3 days, it was shown that of 110 respondents in the Benowo and Kenjeran Health Centers, the average daily intake of leucine was 5.57 grams, the lowest daily intake was 1.35 grams and the largest intake was 10.12 grams.

Assessment of serum prolactin levels was carried out by examining blood samples in breastfeeding mothers. Data on serum prolactin levels showed an average value of 129.2 ng/mL with the lowest level of 13.10 ng/mL and the highest level of 345.9 ng/mL. This

shows that on average breastfeeding mothers in the Benowo and Kenjeran health centers have normal prolactin levels. While the assessment of breast milk protein levels was carried out by examining breast milk samples in the laboratory, the results showed that the average value of breast milk protein levels in respondents was 1.12 g/dL with the lowest level being 0.93 g/dL and the highest level being 1.47 g/dL. This data shows that breastfeeding mothers in the Benowo and Kenjeran health centers have normal milk protein levels on average.

Table 1.2. Results of Kolmogorov-Smirnov test

	Leucine intake	Serum prolactin	Breastmilk protein
N	110	110	110
Asymp. Sig	.079	.200	.200

Note: The data is normally distributed if the results of the one-sample Kolmogorov-Smirnov test show a significance value of > 0.05.

The results of the data normality test showed that the value of leucine intake, serum prolactin levels, and breast milk protein levels were normally distributed with a significance value >0.05.

Table 1.3. Relationship of leucine intake with serum prolactin and breastmilk protein levels

Independent variable	Dependent variable	p-value	R2
Leucine intake	Serum prolactin	0.005	0.183
	Breastmilk protein	0.033	0.147

A bivariate test was performed using the Pearson correlation test. The correlation value or relationship between the intake of leucine and serum prolactin levels was obtained at a significance value of 0.005 which indicates that the p-value <0.05 means that the intake of leucine has a relationship with serum prolactin levels. Based on the results of the regression test between the intake of leucine and serum prolactin levels, it is known that there is a correlation of 0.183 (18.3%). Assessment of the correlation between intake of leucine and protein content of breast milk showed a significant value of 0.033 which indicated a relationship between intake of leucine and protein content of breast milk. The results of the regression test showed that there was a correlation of 0.147 (14.7%) between the intake of leucine and the protein content of breast milk.

B. DISCUSSION

Research conducted on 110 respondents showed a significant relationship between leucine intake and serum prolactin levels. Based on the study by de Ridder et al. (2014) stated that a mild increase in dietary leucine can also affect the synthesis and release of prolactin, and the normal development of the estrous cycle. Leucine is also said to regulate the release of prolactin from the pituitary either directly or by lowering dopamine levels in the brain. Leucine is one of the amino acids that make up protein, consumption of foods with a high

protein diet can increase prolactin concentrations through the mechanism of increasing the expression of SNAT2 simultaneously not only in the mammary glands during lactation but also in the liver and adipose tissue.

The relationship between amino acid supply and milk production is complex. Previous studies in experimental animals have shown that amino acid intake has been shown to affects circulating hormone concentrations. One possible mechanism of action of amino acids is through stimulation of endocrine responses, in particular through increased endogenous production of insulin and prolactin, two hormones are known to be important in the control and maintenance of lactation (De Ridder, Farmer, De Lange, Shoveller, & Luimes, 2014). The nutritional needs of breastfeeding mothers increase during breastfeeding to support the growth and development of the baby. The amount of breast milk consumed by infants and the nutritional content of breast milk is often used to assess nutritional adequacy during the lactation process. However, the volume of breast milk produced can be significantly reduced if the mother is malnourished.

Besides nutrition, many other factors such as milk production are controlled through a neuroendocrine feedback system. The baby's sucking stimulates mechanoreceptors in the nipple and areola, which send nerve signals to the brain. The anterior pituitary then releases prolactin, which stimulates milk production. Stimulus during breastfeeding can inhibit the release of dopamine by the hypothalamus thus allowing for higher prolactin production (Romano et al., 2013).

Previous research has consistently shown a decrease in prolactin levels in response to physical and psychosocial stress. Prolonged exposure to stress is said to affect milk ejection, leading to suppression of lactation secondary to inhibition of milk synthesis as a result of incomplete emptying of the breast. The breast emptying rate is a factor that regulates milk synthesis.

The low correlation value of 18.3% can be caused by several factors that can affect prolactin production. In this study, only food intake (leucine) was studied, and controlled stress factors were eliminated as confounding factors by selecting respondents who only had a high level of stress. low. Other factors such as breastfeeding time before blood sampling, menstrual phase, especially the luteal and follicular phases, sleep quality, sexual activity, duration of breastfeeding, and breastfeeding frequency could not be controlled by researchers.

The relationship between leucine intake and breast milk protein also showed significant results. The results of this study are supported by the research of (Reitelseder et al., 2011) who showed that leucine significantly affects the regulation of casein (milk protein). Milk protein is an important component of milk and a critical index that affects milk quality. It is reported that amino acids can increase the protein content of milk. Previous research found that leucine itself regulates the synthesis of milk proteins in the epithelial cells of the cow's mammary glands. It was noted that leucine enhances milk protein synthesis via the mammalian target of rapamycin (mTOR) and Janus kinase 2/signal transducer and activator of the transcriptional signaling pathway 5 (JACK2/STAT5). In the mammary gland,

leucine regulates various biological processes, such as cell proliferation and milk synthesis (αs-casein, β-casein, and κ-casein).

Individually, each amino acid has a different effect on protein signaling and the rate of synthesis of the casein fraction. A strategy to increase post-absorption efficiency is to decrease the present percentage of food and supplement it with specific amino acids that have the greatest effect on the regulation of translation and protein synthesis. This will increase or maintain mammary gland requirements and amino acid extraction and reduce the recycling of splanchnic tissue (Apelo, Knapp, & Hanigan, 2014).

The amount of essential amino acids in the diet is important for milk protein synthesis, but the ratio of essential amino acids in the diet is more important (Appuhamy et al., 2014). In a previous study, a significant interaction was observed between methionine and leucine indicating a higher response to β-casein. The use of optimal concentration ratios of 4 essential amino acids or optimal concentrations of single essential amino acids substantially increased β-casein expression. The four amino acids are histidine, lysine, methionine, and leucine with a ratio of 5:6:1:7 which efficiently increase the expression of β-casein.

The balance of the proportion of amino acid intake in the diet is very important in protein nutrition (Hoffer, 2016). Imbalances between chemically or structurally similar amino acids lead to amino acid antagonism, which is manifested as reduced food intake, abnormal behavior, and impaired growth. This was also stated by (Hoffer, 2016) that when increasing milk protein, the level of supply of a single amino acid is important, but it is also necessary to evaluate the supply of balanced amino acids. Concerns with amino acid balance have increased. Several authors have shown that increased milk production may be related to an increased or balanced supply of amino acids that results from protein supplementation. Protein synthesis in milk is a very complex and high-cost process because the conversion efficiency of food protein into milk protein is very low (Carr & Golding, 2016).

CONCLUSION

There is a weak relationship between leucine intake and serum prolactin and breast milk protein levels in breastfeeding mothers in the Benowo and Kenjeran health centers, Surabaya. Suggestions for future researchers, further research is needed on other amino acids/micronutrients that can affect prolactin and protein synthesis in breast milk.

REFERENCES

Apelo, S. I. Arriola, Knapp, J. R., & Hanigan, M. D. (2014). Invited review: Current representation and future trends of predicting amino acid utilization in the lactating dairy cow. *Journal of Dairy Science*, *97*(7), 4000–4017.

Appuhamy, JADRN, Nayananjalie, W. A., England, E. M., Gerrard, D. E., Akers, R. M., & Hanigan, M. D. (2014). Effects of AMP-activated protein kinase (AMPK) signaling and essential amino acids on mammalian target of rapamycin (mTOR) signaling and protein synthesis rates in mammary cells. *Journal of Dairy Science*, *97*(1), 419–429.

Bai, W. L., Yin, R. H., Jiang, W. Q., Luo, G. B., Yin, R. L., Li, C., & Zhao, Z. H. (2012). Molecular characterization of prolactin cDNA and its expression pattern in skin tissue of Liaoning Cashmere goat. *Biochemical Genetics*, *50*(9), 694–701.

Carr, Alistair, & Golding, Matt. (2016). Functional milk proteins production and utilization:

Casein-based ingredients. *Advanced Dairy Chemistry*, 35–66.

De Ridder, K. A. G., Farmer, C., De Lange, C. F. M., Shoveller, A. K., & Luimes, P. H. (2014). Plasma amino acids, prolactin, insulin and glucose concentrations in lactating sows following venous infusion of isoleucine, leucine, lysine, threonine or valine. *Canadian Journal of Animal Science*, *94*(2), 323–330.

Dewey, Kathryn G. (2016). Reducing stunting by improving maternal, infant and young child nutrition in regions such as South Asia: evidence, challenges and opportunities. *Maternal & Child Nutrition*, *12*, 27–38.

Hoffer, L. John. (2016). Human protein and amino acid requirements. *Journal of Parenteral and Enteral Nutrition*, *40*(4), 460–474.

Li, Fengna, Yin, Yulong, Tan, Bie, Kong, Xiangfeng, & Wu, Guoyao. (2011). Leucine nutrition in animals and humans: mTOR signaling and beyond. *Amino Acids*, *41*(5), 1185–1193.

Lyons, Katríona E., Ryan, C. Anthony, Dempsey, Eugene M., Ross, R. Paul, & Stanton, Catherine. (2020). Breast milk, a source of beneficial microbes and associated benefits for infant health. *Nutrients*, *12*(4), 1039.

Reitelseder, Søren, Agergaard, Jakob, Doessing, Simon, Helmark, Ida C., Lund, Peter, Kristensen, Niels B., Frystyk, Jan, Flyvbjerg, Allan, Schjerling, Peter, & van Hall, Gerrit. (2011). Whey and casein labeled with L-[1-13C] leucine and muscle protein synthesis: effect of resistance exercise and protein ingestion. *American Journal of Physiology-Endocrinology and Metabolism*, *300*(1), E231–E242.

Rezaei, Reza, Wu, Zhenlong, Hou, Yongqing, Bazer, Fuller W., & Wu, Guoyao. (2016). Amino acids and mammary gland development: nutritional implications for milk production and neonatal growth. *Journal of Animal Science and Biotechnology*, *7*(1), 1–22.

Romano, Nicola, Yip, Siew H., Hodson, David J., Guillou, Anne, Parnaudeau, Sébastien, Kirk, Siobhan, Tronche, François, Bonnefont, Xavier, Le Tissier, Paul, & Bunn, Stephen J. (2013). Plasticity of hypothalamic dopamine neurons during lactation results in dissociation of electrical activity and release. *Journal of Neuroscience*, *33*(10), 4424–4433.

Rudolph, Michael C., Russell, Tanya D., Webb, Patricia, Neville, Margaret C., & Anderson, Steven M. (2011). Prolactin-mediated regulation of lipid biosynthesis genes in vivo in the lactating mammary epithelial cell. *American Journal of Physiology-Endocrinology and Metabolism*, *300*(6), E1059–E1068.

Thai, Julie D., & Gregory, Katherine E. (2020). Bioactive factors in human breast milk attenuate intestinal inflammation during early life. *Nutrients*, *12*(2), 581.

Wells, Jonathan C., Sawaya, Ana Lydia, Wibaek, Rasmus, Mwangome, Martha, Poullas, Marios S., Yajnik, Chittaranjan S., & Demaio, Alessandro. (2020). The double burden of malnutrition: aetiological pathways and consequences for health. *The Lancet*, *395*(10217), 75–88.

Zhang, Xiaomei, Zhang, Minghui, Lin, Tie, Zhao, Junying, Luo, Zhang, Hou, Juncai, Sun, Baoguo, & Chen, Lijun. (2021). Relationship between traditional maternal diet pattern and breastmilk composition of rural lactating women during the first month postpartum in Shigatse, Tibet. *Food Science & Nutrition*, *9*(8), 4185–4198.