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Low Maternal Seafood Intake During Exclusive Lactation Does Not Significantly Affect Milk Protein Content

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Abstract. *Human milk, which contains complex and highly variably biofluid that nourishes and protects the newborn, is the gold standard for infant nutrition. The biological activity of human milk is significantly influenced by proteins. However, the relationship between crude protein in human milk and the amount of milk consumed by mothers during the exclusive breastfeeding period has not been thoroughly investigated. In the current study, 194 healthy women who were exclusively breastfeeding participated in a cross-sectional study to collect human milk samples and complete a quantitative frequent food questionnaire (FFQ). The consumption of cereals, potatoes, sweet potatoes, leafy vegetables, fruits, other vegetables, legumes, nuts, eggs, meats, dairy products, and seafood was grouped based on the consumption of the mother the day before milk collection. The mid-infrared milk analyzer was used to analyze the samples and determine protein concentration. Using the t-test to analyze the impact of partial factors, and the F-test was employed to evaluate the influence of variables concurrently, at a 5% significance level. The statistical relationship between maternal diet and protein content was evaluated. Seafood consumption was categorized as low compared to other groups. Self-imposed maternal food restrictions may be the cause of the reduced seafood consumption. Human milk has an average protein level of 1.02 g/100 ml. According to the statistics, there was no significant correlation between the crude protein content of human milk and seafood consumption. However, a strong correlation was found between the consumption of eggs, legumes, and nuts, suggesting that these foods may impact on the protein content of human milk (p value <0.05). This finding would suggest that to improve the protein content composition of human milk, nursing mothers should consume more local, high-protein foods.*

Keywords: *food frequency questionnaire; maternal consumption; seafood; human milk; protein content.*

Type of the Paper: Regular Article.

1. Introduction

World per capita consumption of seafood has been increasing steadily over the past five decades [1]. Seafood is a common source of protein in terrestrial areas. Exclusive breastfeeding during six months, followed by continued up to 2 years, is recommended by the World Health Organization to meet infant nutritional need. Human milk is the primary source of nutrition during the first six months of life. Protein is one of the important macronutrients in human milk, which plays a crucial role in infant growth, serves as a carrier for vitamins, hormones, and provides

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protective factors [2]. The proportion of protein in human milk varies across different geographical locations and lactation periods, which may be influenced by the different dietary habits in different cities [3]. Previous studies have reported that variations in human milk macronutrients, including protein, are influenced by maternal dietary intake [4,5].

Maternal consumption during the lactation period has been shown to have long-term effects on children's health and influence various compositions of human milk. Previous research has found that significant changes in human milk composition during lactation are linked to variations in maternal consumption. Recently, a maternal diet rich in fat and oligosaccharides has been found to influence the fatty acid and oligosaccharide profiles in human milk [6,7]. Further research is needed to confirm the validity of the association between maternal consumption and protein content in human milk.

The Food Frequency Questionnaire (FFQ) is an affordable method for assessing group or community food consumption. The advantage of this method is its ability to measure the specific target daily intake over both long- or short-term periods [8]. The validity of the questionnaire has been assessed in the previous research regarding its effectiveness in recording the dietary intake of lactating women [9]. Previous studies on the association between lactating women's intake and human milk composition have been conducted in several countries, such as Latvia and New Zealand [10,11]. However, the relationship between dietary protein source and protein content in human milk has not been thoroughly investigated in recent studies. This present study aimed to examine the correlation between maternal consumption and protein content in human milk by using semi quantitative FFQ.

2. Materials and Methods

2.1 Experimental Design

The recruited lactating mothers provided informed consent before participating in the research. Human milk samples were collected from healthy breastfeeding mothers aged 23-35 years from various cities during the exclusive breastfeeding period. Inclusion criteria, such as being physically healthy without degenerative and infectious diseases, were applied through a pre-screening question. The 194 participants who passed the screening process subsequently proceeded to the FFQ and breast milk expression step.

Human milk samples were expressed in the morning between 8:00 - 11:00 am and collected from both the right and left breasts after full expression (including both foremilk and hindmilk). All infants were healthy and delivered at term (37 - 42 weeks). The samples were then stored at -80°C prior to analysis. Human milk samples were analyzed using a mid-infrared spectroscopy analyzer to obtain protein content data. This method has been validated as an effective tool for

measuring the macronutrient content in human milk [12].

2.2 Semi-quantitative food frequency questionnaire (FFQ) procedure

The semi-quantitative analysis was conducted according to the previous method with the minor modification in the recorded food groups [13]. The foods were clustered into 12 types based on Table 1.

Table 1. Food groups in semi-quantitative FFQ

Food Group	Food Item
Cereals and cereals product	Rice, rice porridge, millet, black rice, wheat noodle, wheat bun/pancake, clay over rolls, wheat dough stick, corn, corn flour
Potatoes	Potato, potato snack, potato noodles
Sweet potatoes	Sweet potato, sweet potato snack, sweet potato noodle, sweet potato product
Leaf vegetables	Bok choy, Chinese cabbage, spinach, lettuce, coriander leaf, water spinach, kelp, celery
Fruits	Pineapple, jackfruit flesh, coconut, watermelon, durian mango, papaya, banana, bayberry, coconut, loquat, cantaloupe, watermelon apple, pear, peach, plum, apricot, date, cherry, grape/raisin, dragon fruit, persimmon, mulberry, kiwi, strawberry, kumquat, pomelo, longan/ dried longan, jujube
Other vegetables	Radish, carrot, wax gourd, calabash, cucumber, lettuce stem, bamboo shoot, asparagus stem, lotus root, water bamboo, straw/button mushroom, gold needle mushroom, oyster mushroom, wood ear fungus, shitake mushroom, silver ear fungus
Legumes	cowpea, snow peas, kidney beans, soybeans, chickpeas, lentils, lupins, mesquite, carob, tamarind, alfalfa clover, mung beans, tofu, soymilk, soybean curd sheet, soymilk powder, mung bean product, rea bean, adzuki bean porridge, other beans.
Nuts	Peanut, hazelnuts, chestnut, acorns, almond, pecan, pistachios, walnuts, Brazilian nuts
Eggs	Quail's, Goose's, Duck's, Hen's
Poultry, meat dan meat products	Pork and the organs, chicken and the organs, duck and the organs, goose, pigeon, lamb, beef, meat/pork/lamb/poultry product (such as floss, cornet)
Milk dan dairy product	Milk, whole milk powder, cream/butter/canned, full fat, cheese
Seafood	Carp, eel, abalone, clam, oyster, scallop, sea cucumber, squid, octopus, fish

The FFQ, based on the previous method [14], was conducted 24 hours before the day of human milk donation by the trained investigators. The mother's consumption was recorded using questionnaires, including portions to determine the weight of the foods. The results were then inputted and tabulated in the Golden Key Maternal Nutrition Software (Wincome, Shanghai China).

2.3 Statistical analysis

With a 5% significance threshold, the relationship between maternal diet and protein content was statistically assessed using the F-test to explore the effect of factors simultaneously, and the t-test to examine the effect of partial variables. The Pearson correlation test was used to assess the correlation between maternal consumption and crude protein content. Analysis was performed using R (R development core team, Vienna). The data were presented and visualized using both R and Excel.

3. Results and Discussion

3.1 Maternal characteristics and consumption

Maternal characteristics could influence the macronutrient composition of human milk [15]. The characteristics of the respondents are shown in Table 2. Based on the characteristics of the lactating women recruited in the present study, the average maternal age was 34.38 years. At this age, the lactating women were still producing enough human milk for their infants, as well as donating it for this study. Body mass index (BMI) is often used as an indicator of overall health and body function in lactating women. A higher BMI, compared to the normal standard, could affect the initiation of breastfeeding [16]. The present study showed a normal average BMI, which may indicate that the lactating women were able to initiate the exclusive breastfeeding successfully.

A previous study found that primiparous women had higher protein and lower carbohydrate content than multiparous ones [15]. The maternal characteristics in this study ranged from first to second parity, which may give an advantage to the protein result. No preterm infants were included in this research, as their inclusion could affect the differences in the macronutrient content of human milk.

Table 2. Respondent characteristics of the research

Characteristics	Median ± In quartile range
Maternal age (y)	34.38±4.18
Maternal BMI (kg/m^2)	21.38±11.85
Parity	1.25±0.55
Preterm infant	0

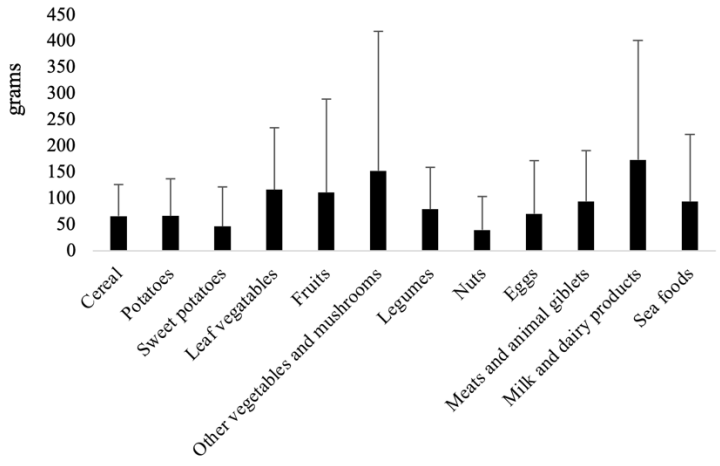

Fig. 1. Maternal food consumption

Maternal consumption can have a positive or negative effect on human milk composition [17]. Based on the semi-quantitative FFQ, milk and dairy products had the highest consumption among lactating women (Fig 1). Dairy products are important for lactating women due to the perception that dairy and its derivatives are rich in nutrients, which are needed for daily

consumption to enrich human milk production and quality [18]. Vegetables were the second most consumed group. In the Asian diet, non-leafy vegetables such as mushrooms, carrots, and radishes were commonly used as main dishes, condiments, and side dishes in the daily menu. Leafy vegetables such as spinach, Brussels sprouts, and water spinach dominated the diet over the staple foods. The World Health Organization (WHO) recommends a minimum intake of 400 grams of vegetables for the prevention of chronic diseases [19]. Based on the WHO recommendation, the lactating women recruited in this study met the required intake from the combined categories of vegetables and fruits. The consumption pattern was comparable to that observed in a previous study analyzing the dietary habits of lactating women [20]. Seafood was categorized as the least consumed animal protein compared to eggs, meat and dairy products. This could be due to the difficulty in finding fresh seafood and self-restriction, resulting in lower consumption.

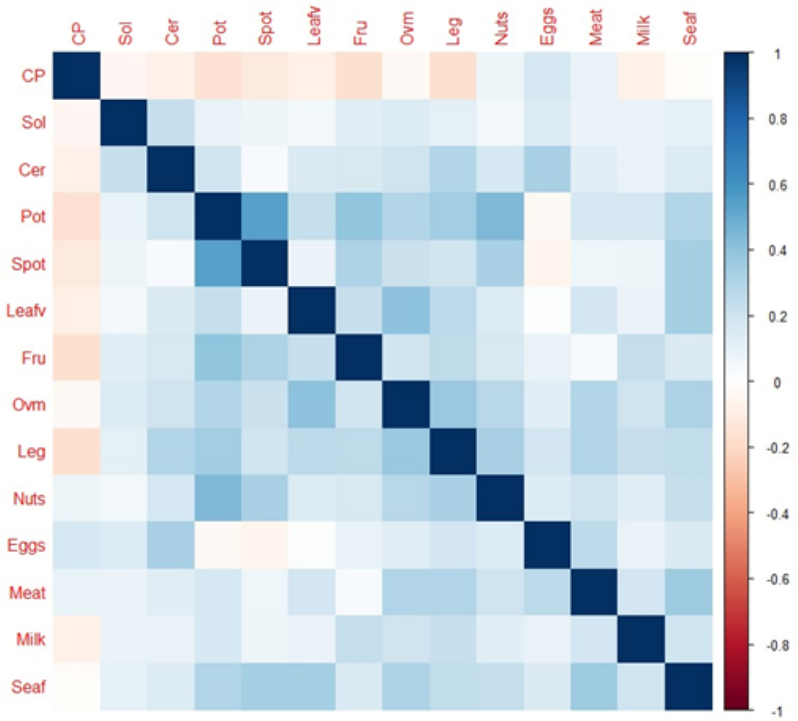

Fig. 2. Correlation plot of food consumption and crude protein in human milk

3.2 Association between human milk and maternal consumption

Based on Fig. 2, the correlation plot showed the values between crude protein and all the categories of food. The blue color indicated a strong positive correlation, while the red color represented a strong negative correlation. The result showed the low correlation between maternal consumption and crude protein content in human milk. Thus, crude protein in human milk may be influenced by many factors, not just maternal consumption. Since protein concentration varies across the lactation period, gender, and economic status [21–29].

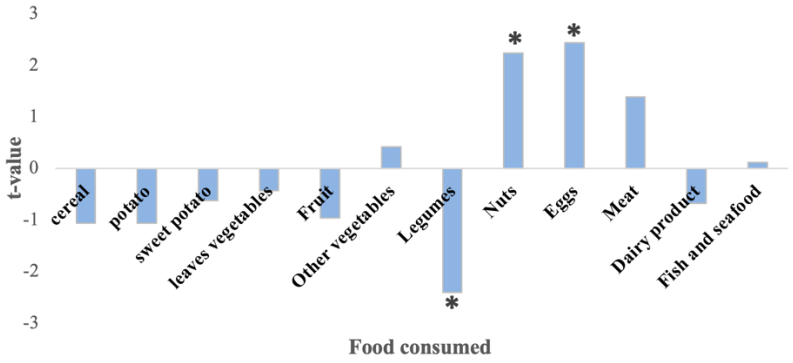

Fig. 3. T-test analysis of association of crude protein with maternal consumption

The statistical analysis began with an assumption test, including normality, multicollinearity, and homogeneity test, to assess the homogeneity of the data used in this research. The result showed that the data met all the assumptions, allowing t-test analysis to be conducted. The results of the t-test are shown in Fig. 3. Significant results were observed in legumes, nuts, and eggs in relation to crude protein content in human milk. Thus, crude protein in human milk was influenced by intake of legumes (p-value = 0.0171), nuts (p-value = 0.0261) and eggs (p-value = 0.0157).

The t-value indicated either a positive or negative association. Nuts and eggs were positively associated with crude protein in human milk, meaning that lactating women who consumed nuts had higher levels of crude protein in human milk. The positive correlation with nuts may be due to the ability of certain types of nuts, such as pecan, to increase the protein ratio in the in vivo study [30]. Nuts contain all three macronutrients including carbohydrate, fat, and protein. In addition, nuts have a high protein nitrogen value and have been recognized as a source of protein [31].

A significant positive association was also found between egg consumption and crude protein content. This suggests that the egg consumption could positively affect the crude protein level. Generally, eggs are known as an affordable source of nutrition, rich in protein. Egg is also used in a variety of dishes, both sweet and savory, ranging from the appetizer to dessert. This association between egg intake and human milk protein has been observed previously [32,33]. The previous results showed that mothers who consumed eggs had higher ovalbumin content in their milk compared to the placebo group [32]. Ovalbumin is a major egg protein that can also be found in human milk. Its presence in human milk can be detected within 8 hours of egg consumption [33].

In contrast, legumes had a negative association with crude protein, meaning that high consumption of legumes could reduce the protein content in human milk. Despite the fact that 38% of legumes are high in protein [34], this category also plays a role as a galactagogue, which boosts human milk production [35]. Traditional galactagogues commonly used in society include legumes such as tofu and soy milk [36]. In the Asian diet, tofu is a popular dish often mixed with either

vegetables or animal-based dishes, such as meat and egg. The consumption of galactagogues may produce more water in human milk and dilute the macronutrient content. Meanwhile, further analysis in a bigger population and biochemical analysis are needed to clearly understand the reason behind the negative association between legumes and human milk protein.

4. Conclusions

In conclusion, this study found a positive association between the consumption of protein-rich local foods, such as eggs and nuts, and the improvement of crude protein content of human milk. Meanwhile, the consumption of local galactagogues, including legumes, which could increase human milk production, may affect milk dilution and decrease protein content in human milk.

Abbreviations

FFQ Food Frequency Questionnaire
WHO World Health Organization
BMI Body Mass Index

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Ratna Nurmalita Sari: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft; **Nuramaliyah**: Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – review and editing.

Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest.

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References

[1] Nurdiani R, Prihanto AA, Firdaus M. Seafood as source of protein-based functional foods. Encycl Mar Biotechnol 2020:2987–97. https://doi.org/10.1002/9781119143802.ch133.
[2] Picciano MF. Nutrient composition of human milk. Pediatr Clin North Am 2001;48:53–67. https://doi.org/10.1016/S0031-3955(05)70285-6.
[3] Sari RN, Pan J, Zhang W, Li Y, Zhu H, Pang X, et al. Comparative Proteomics of Human Milk From Eight Cities in China During Six Months of Lactation in the Chinese Human Milk Project Study. Front Nutr 2021;8:1–10. https://doi.org/10.3389/fnut.2021.682429.
[4] Barrera C, Valenzuela R, Chamorro R, Bascuñán K, Sandoval J, Sabag N, et al. The impact of maternal diet during pregnancy and lactation on the fatty acid composition of erythrocytes and breast milk of chilean women. Nutrients 2018;10:1–14. https://doi.org/10.3390/nu10070839.
[5] Keikha M, Bahreynian M, Saleki M, Kelishadi R. Macro- and Micronutrients of Human

Milk Composition: Are They Related to Maternal Diet? A Comprehensive Systematic Review. Breastfeed Med 2017;12:517–27. https://doi.org/10.1089/bfm.2017.0048.

[6] Ueno HM, Higurashi S, Shimomura Y, Wakui R, Matsuura H, Shiota M, et al. Association of DHA Concentration in Human Breast Milk with Maternal Diet and Use of Supplements: A Cross-Sectional Analysis of Data from the Japanese Human Milk Study Cohort. Curr Dev Nutr 2020;4:1–9. https://doi.org/10.1093/CDN/NZAA105.

[7] Biddulph C, Holmes M, Kuballa A, Davies PSW, Koorts P, Carter RJ, et al. Human milk oligosaccharide profiles and associations with maternal nutritional factors: A scoping review. Nutrients 2021;13:1–20. https://doi.org/10.3390/nu13030965.

[8] Sharma S. Nutrition Society Silver Medal Lecture Development and use of FFQ among adults in diverse settings across the globe. Proc. Nutr. Soc., vol. 70, 2011, p. 232–51. https://doi.org/10.1017/S0029665110004775.

[9] Zang J, Luo B, Chang S, Jin S, Shan C, Ma L, et al. Validity and reliability of a food frequency questionnaire for assessing dietary intake among Shanghai residents. Nutr J 2019;18:1–12. https://doi.org/10.1186/s12937-019-0454-2.

[10] Butts CA, Hedderley DI, Herath TD, Paturi G, Glyn-Jones S, Wiens F, et al. Human Milk Composition and Dietary Intakes of Breastfeeding Women of Different Ethnicity from the Manawatu-Wanganui Region of New Zealand n.d. https://doi.org/10.3390/nu10091231.

[11] Aumeistere L, Ciproviča I, Zavadska D, Andersons J, Volkovs V, Ceļmalniece K. Impact of maternal diet on human milk composition among lactating women in Latvia. Med 2019;55:1–12. https://doi.org/10.3390/medicina55050173.

[12] Casadio YS, Williams TM, Lai CT, Olsson SE, Hepworth AR, Hartmann PE. Evaluation of a mid-infrared analyzer for the determination of the macronutrient composition of human milk. J Hum Lact 2010;26:376–83. https://doi.org/10.1177/0890334410376948.

[13] Ding Y, Li F, Hu P, Ye M, Xu F, Jiang W, et al. Reproducibility and relative validity of a semi-quantitative food frequency questionnaire for the Chinese lactating mothers. Nutr J 2021;20:1–12. https://doi.org/10.1186/s12937-021-00678-5.

[14] Liu G, Ding Z, Li X, Chen X, Wu Y, Xie L. Relationship between polyunsaturated fatty acid levels in maternal diets and human milk in the first month post-partum. J Hum Nutr Diet 2016;29:405–10. https://doi.org/10.1111/jhn.12337.

[15] Burianova I, Bronsky J, Pavlikova M, Janota J, Maly J. Maternal body mass index, parity and smoking are associated with human milk macronutrient content after preterm delivery. Early Hum Dev 2019;137:104832. https://doi.org/10.1016/j.earlhumdev.2019.104832.

[16] Wojcicki JM. Maternal prepregnancy body mass index and initiation and duration of breastfeeding: A review of the literature. J Women's Heal 2011;20:341–7. https://doi.org/10.1089/jwh.2010.2248.

[17] Emmett PM, Rogers IS. Properties of human milk and their relationship with maternal nutrition. Early Hum Dev 1997;49. https://doi.org/10.1016/S0378-3782(97)00051-0.

[18] Achon M, Ubeda N, Garcia-Gonzalez A, Partearroyo T, Varela-Moreiras G. Effect of Milk and Dairy Product Consumption on Pregnancy and Lactation Outcomes: A Systematic Review. Am Soc Nutr 2019;10:S74–87. https://doi.org/10.1093/advances/nmz009.

[19] Kanungsukkasem U, Ng N, Van Minh H, Razzaque A, Ashraf A, Juvekar S, et al. Fruit and vegetable consumption in rural adults population in indepth hdss sites in asia. Glob Health Action 2009;2:35–43. https://doi.org/10.3402/gha.v2i0.1988.

[20] Hu R, Eussen SRBM, Sijtsma FPC, Papi B, Stahl B, Jin Y, et al. Maternal dietary patterns are associated with human milk composition in Chinese lactating women. Nutrition 2021;91–92:111392. https://doi.org/10.1016/j.nut.2021.111392.

[21] Lönnerdal B, Erdmann P, Thakkar SK, Sauser J, Destaillats F. Longitudinal evolution of true protein, amino acids and bioactive proteins in breast milk: a developmental perspective. J Nutr Biochem 2017;41:1–11. https://doi.org/10.1016/j.jnutbio.2016.06.001.

[22] Elwakiel M, Boeren S, Hageman JA, Szeto IM, Schols HA, Hettinga KA. Variability of serum proteins in Chinese and dutch human milk during lactation. Nutrients 2019;11:1–14.

https://doi.org/10.3390/nu11030499.
[23] Shi YD, Sun GQ, Zhang ZG, Deng X, Kang XH, Liu ZD, et al. The chemical composition of human milk from Inner Mongolia of China. Food Chem 2011;127:1193–8. https://doi.org/10.1016/j.foodchem.2011.01.123.
[24] Lönnerdal B. Bioactive Proteins in Human Milk - Potential Benefits for Preterm Infants. Clin Perinatol 2016;11:1–13. https://doi.org/10.1016/j.clp.2016.11.013.
[25] Fujita M, Roth E, Lo YJ, Hurst C, Vollner J, Kendell A. In poor families, mothers' milk is richer for daughters than sons: A test of Trivers-Willard hypothesis in agropastoral settlements in Northern Kenya. Am J Phys Anthropol 2012;149:52–9. https://doi.org/10.1002/ajpa.22092.
[26] Quinn EA. No Evidence for Sex Biases in Milk Macronutrients , Energy , or Breastfeeding Frequency in a Sample of Filipino Mothers. Am J Phys Anthropol 2013;216:209–16. https://doi.org/10.1002/ajpa.22346.
[27] Hahn WH, Song JH, Song S, Kang N mi. Do gender and birth height of infant affect calorie of human milk? An association study between human milk macronutrient and various birth factors. J Matern Neonatal Med 2017;30:1608–12. https://doi.org/10.1080/14767058.2016.1219989.
[28] Lönnerdal B. Human milk proteins: Key components for the biological activity of human milk. Adv Exp Med Biol 2004;554:11–25. https://doi.org/10.1007/978-1-4757-4242-8_4.
[29] Nommsen LA, Lovelady, Cheryl A; Heinig, M Jane; Lonnerdal, Bo; Dewey KG. Determinants of energy , protein , lipid , and lactose concentrations in human milk during the first 12 mo of lactation : the DARLING. Am Soc Clin Nutr 1990;53:457–65. https://doi.org/doi: 10.1093/ajcn/53.2.457.
[30] Tanwar B, Modgil R, Goyal A. Protein quality assessment of pecan [Carya illinoinensis (wangenh.) K. Koch] and pine (Pinus gerardiana wall.) nuts for dietary supplementation. Nutr Food Sci 2022;52:641–56. https://doi.org/10.1108/NFS-08-2021-0260.
[31] Brufau G, Boatella J, Rafecas M. Nuts: Source of energy and macronutrients. Br J Nutr 2006;96:24–8. https://doi.org/10.1017/BJN20061860.
[32] Palmer DJ, Gold MS, Makrides M. Effect of maternal egg consumption on breast milk ovalbumin concentration. Clin Exp Allergy 2008;38:1186–91. https://doi.org/10.1111/j.1365-2222.2008.03014.x.
[33] Metcalfe JR, Marsh JA, D'Vaz N, Geddes DT, Lai CT, Prescott SL, et al. Effects of maternal dietary egg intake during early lactation on human milk ovalbumin concentration: a randomized controlled trial. Clin Exp Allergy 2016;46:1605–13. https://doi.org/10.1111/cea.12806.
[34] Hughes J, Pearson E, Grafenauer S. Legumes-A comprehensive Exploration of Global Food-Based Dietary Guidlines and Consumption. Nutrients 2022;14:1–19. https://doi.org/10.1201/b13349-9.
[35] Goksugur SB, Karatas Z. Breastfeeding and Galactogogues Agents. Acta Medica Anatolia 2014;2:113. https://doi.org/10.15824/actamedica.56572.
[36] Buntuchai G, Pavadhgul P, Kittipichai W, Satheannoppakao W. Traditional Galactagogue Foods and Their Connection to Human Milk Volume in Thai Breastfeeding Mothers. J Hum Lact 2017;33:552–9. https://doi.org/10.1177/0890334417709432.