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The importance of mineral supply in preterm infant nutrition

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Abstract

Although human breast milk contains significant biomolecules with major impact on growth and development of preterm babies, a diet based exclusively on this milk over a longer period of time can be associated with the development of micronutrient deficiency in this sensitive group of infants. The aim of this study was to determine the content of micronutrients in human breast milk from the mothers of preterm infants and in infant formula, and the influence of mineral content on the osmolality and potential renal solute load. Human breast milk taken during the lactation period was examined, before and after frozen storage, pasteurization and supplementation with a fortifier, with mineral content being determined by inductively coupled plasma-optical emission spectrometry. Osmolality of preterm milk and infant formula was measured using an osmometer. During the lactation period of mothers of preterm born babies, the mineral content of the breast milk changed. The concentrations of all examined minerals differed in colostrum and in mature milk. However, there were no significant differences in mineral content in mature milk before and after pasteurization and storage. Supplementation of mature milk with a fortifier increased the concentration of minerals, the final osmolality and the potential renal solute load. The mineral content of mature milk was lower than necessary for the optimal growth of preterm infants, so adequate supplementation with a fortifier is needed to provide biologically important minerals. However, the osmolarity of supplemented milk should be monitored due to the potentially increased pressure on the kidneys of preterm infants.

Keywords*: human milk, preterm infant, nutrition, mineral, osmolarity, fortifier.*

INTRODUCTION

Every year about 4,000 babies are born prematurely in Serbia. Adequate nutrition of premature infants is very important for their health and appropriate development. Mothers give birth prematurely for various reasons, and their mammary glands are stimulated to secrete milk [1]. While the milk of mothers of term infants is specially adapted to their needs from the first day of life, with the required content of macronutrients, vitamins, minerals and antioxidants, in the case of mothers of preterm infants, the content and quality of milk differs and depends on the mother's health and nutrition, mammary gland physiology, environmental factors, the cause of prematurity, and whether it is colostrum, transitional or mature milk [2,3].

Although human breast milk contains significant biomolecules with major impacts on preterm babies, a diet based exclusively on such milk over a longer period of time, with contents of protein, sodium, calcium and phosphorus below the level required for bone mineralization, can be associated with the development of nutritional deficiency in this sensitive group of infants [3,4]. Since preterm milk is not adapted to

the energy needs of premature babies, there are different modes of feeding to ensure the growth and development of babies of this sensitive group, who are often cared for in neonatal intensive care units. In milk banks in the units, milk is available for feeding preterm infants, based on the mother's own milk or on donor term milk, which differs in composition from preterm milk. The usual procedures for preparing infants' milk meals after the milk is released from frozen storage in milk banks involve pasteurization and fortification [5].

Fortification, i.e., supplementation with fortifier (FF), after thermal treatments, increases the energy value and content of macronutrients, but the composition of oligo and micronutrients in this type of food also changes in relation to the original milk [6]. Premature infants are very sensitive and have low tolerance to variations in milk nutrition, so adequate supplementation of human milk should ensure appropriate nutrition, gastrointestinal function, and decreased intolerance and necrotizing enterocolitis [7, 8]. Many of them begin life with immature nephrons, and are exposed to a variety stress processes that cause infections and suboptimal nutrition [9]. Trace and mineral elements in the body have different functions in the framework of enzymes and proteins that maintain homeostasis, so their content should be controlled in infant nutrition, in addition to the fact that these elements can affect the osmolality of the ingested food. Trace element requirements are critical during infancy and early childhood due to the very high growth rate of children, and insufficient mineral intake can lead to deficiencies that can impair body function [10, 11].

Macronutrients and minerals increase total osmolality, osmolarity and potential renal solute load (PRSL) of infant food. Osmolality and osmolarity of the solution depend on the number of solute molecules in the human milk. Osmometry by freezing point depression is a reliable method to measure the osmolality in solution per kg, while osmolarity is a measure of milliosmoles of solute per liter, the latter is suitable for clinical settings [12]. Osmolality and osmolarity are important criteria for the feeding process, as high values could indicate several risks, including diarrhea, vomiting, nausea, nitrogen and solute overload, insufficient water to support growth, hypertonic dehydration by exceeding the potential renal solute load and excessive mineral intake predisposing the infant to nephrocalcinosis [13-16].

When human breast milk is not available, infant formula (IF) is an adequate form of nutrition for preterm infants. However, IF has some differences from human breast milk in terms of bioavailability and concentrations of nutrients, and some components can have potential negative interactions with the matrix. The mineral content in human milk is lower than in infant formula, e.g. iron in human milk (0.2 - 0.4 mg/L) is lower than in infant formulas (IF 4 - 12 mg/L), but its bioavailability is more effective than in infant formula, and therefore, the competition between iron and other divalent (Zn and Cu) cations is less [17].

The objective of this study was to examine the quality of milk in the largest milk bank for preterm infant nutrition in Serbia. For that purpose, the mineral components (Na, K, Ca, P, Cl, Mg, Fe, Zn, Cu), osmolality, osmolarity, and PRSL were determined in order to monitor the quality of milk in the nutrition of this sensitive group of infants.

EXPERIMENTAL

Study samples

The study was conducted at the Institute of Neonatology in Belgrade, Serbia. The study included ten healthy women without any medicament therapy who delivered prematurely before 37 weeks of gestation and who intended to breastfeed their infants. An exclusion factor for the study was smoking. The study protocol was approved by the Ethical Committee (No. N82401/4) on April 18, 2014. All participants were informed of the purpose of the study and assured of its confidentiality and each signed an informed consent. Data on the average basic, non-personal characteristics of mothers and infants are given in **Table 1**.

Table 1. Clinical and baseline characteristics of study participants.

Age (years)	
Median	27 ± 3
Range	27 – 36
Body mass index (BMI)	
Median	22.92 ± 3.57
Range	17 – 29
Weight mother (kg)	
Median	64.80 ± 9.18
Range	50 – 92
Gestation (weeks)	
Preterm (<37 weeks)	
Median	31.27 ± 2.21
Range	27 – 36
Weight infant (g)	
Median	1560.00 ± 418.61
Range	950.0 – 2470.0

Milk samples were collected during the lactation period: colostrum up to the 5th day postpartum, and mature milk 21 days after delivery. Mature preterm milk samples: 1. Mature milk; 2. Pasteurized milk (Holder pasteurization, 62.5 ℃ for 30 min); 3. Milk after 7

days' frozen storage (-20 ℃); 4. Milk after frozen storage -20 ℃ for 7 days followed by pasteurization, and; 5. Milk supplemented with fortifier [18].

Multicomponent fortifier (FF) as a 5% solution (5 g FF/100 mL of mature milk) is a standard form of fortification of human milk for the nutrition of preterm infants, and it is based on the average values of nutrients in human milk [6]. Multicomponent FF (produced in Serbia) had the following ingredients: maltodextrin, whey protein concentrate, mineral premix, micronutrient premix, lecithin and soybean oil. Total macronutrients in 5 g of FF were 1.04 g of protein, 3.22 g of carbohydrates and 0.22 g fat (**Table 2**).

Infant formula (IF PRE) for preterm and low-birth weight infants (produced in Serbia), and which had total 2.4% protein, 9.0% carbohydrates and 3.73% fat, was prepared by dissolving 16 g in 100 mL of demineralised water. Ingredients of the IF PRE are shown in **Table 2**. All liquid samples were homogenized by an Ultrasonic homogenizer Sonopuls 2000.2 (Berlin, Germany) prior to measurement. Macronutrient content (protein, fat, lactose and total carbohydrates) of mature milk before and after supplementation with FF, as well as of IF PRE was determined as described in Lugonja et al, 2022 [18].

Table 2. Nutritional composition of fortifier powder and infant formula for preterm and low-birth weight infants.

Nutritional composition in 100 g of powder	FF	IF PRE
Protein	20.8 g	15.0 g
Carbohydrate	64.3 g	56.3 g
Fat	4.4 g	23.3 g
Sodium	530 mg	252 mg
Potassium	640 mg	682 mg
Calcium	1430 mg	751 mg
Phosphorus	848 mg	395 mg
Chloride	382 mg	387 mg
Magnesium	120 mg	42 mg
Iron	33.4 mg	5.1 mg
Zinc	17.4 mg	4.4 mg
Copper	1.3 mg	0.5 mg
Manganese	120 µg	31 µg
Iodine	134 µg	78 µg
Selenium	56 µg	10 µg
Vitamin A	4600 µg	1937 µg
Vitamin D	85 µg	340 µg
Vitamin E	28 mg	8 µg
Vitamin K	240 µg	26 µg
Vitamin C	514 mg	70 mg
Thiamine	2.24 mg	565 µg
Riboflavin	2.88 mg	800 µg
Niacin	8.6 mg	5.6 mg
Vitamin B6	2.96 mg	385 µg
Folic acid	734 µg	350 µg
Pantothenic acid	3.0 mg	2.5 mg
Vitamin B12	8.4 µg	1.5 µg
Biotin	186.2 µg	10 µg
Choline	146 mg	75 mg
Inositol	160 mg	30 mg
Taurine	28 mg	38.7 mg
L-Carnitine	30 mg	12 mg

Mineral determination

Mineral components in preterm human milk before and after supplementation, and in infant formula, were quantified by inductively coupled plasma-optical emission spectrometry (ICP-OES) with axial configuration on a Spectro Arcos model that was purchased from Spectro Analytical Instruments GmbH (Kleve, Germany). It was controlled using Smart Analyzer Vision Software (version 5.01.0928) and connected to an ASX-520 auto sampler (CETAC). Spectro ICAL solution (10x concentrate, Berd Kraft Der Standard) was used for self-checking and self-adjustment of the instrument (over the entire polychromator). Human milk samples were diluted 10 times in ultrapure water before analysis. Amounts (1 g) of IF samples were made up to 100 mL with ultrapure water, due to the higher concentrations of microelements in this matrix. Zn, Cu and Fe were measured directly without digestion and possible interference from organic matter during the detection of the microelements was accounted for in the calculation. Minerals K, Ca, Na, P, Mg Cl were expressed as mg/100 mL, and microelements Zn, Fe, Cu as µg/100 mL [19, 20].

Osmolality, osmolarity and PRSL

Osmolality was measured using freezing point depression (cryoscopy) by an OsmoTECH™ Single-Sample Micro-Osmometer (Advanced Instruments) [21], while osmolarity was calculated according to Equation 1 (E1). For calibration, we used Clinitrol™ 290 Reference Solution with 290 mOsmol/kg H_2O. Osmolality was expressed mOsmol/kg H_2O, and osmolarity as mOsmol/L.

Osmolarity (mOsmol / L) = Osmolality (mOsmol / kg H_2O) x kg H_2O / L (E1)

The Fomon method for determination of the potential renal solute load (PRSL) in preterm infant food was based on the sum of the intake of nitrogen (expressed as mmol urea, i.e., mg nitrogen divided by 28) and minerals, sodium, potassium, phosphorus and chloride [15, 22], as expressed in Equation 2 (E2). Results for PRSL were given as mOsmol/L.

PRSL (mOsmol / L) = N / 28 + Na + K + P + Cl (E2)

Determination of total antioxidant capacity

Ferric reducing antioxidant potential (FRAP), as a direct method for measuring the total antioxidant capacity of biological fluids, was assessed according to Benzie and Strain. At low pH, reduction of a ferric 2,4,6-tripyridyl-s-triazine [Fe (III)TPTZ] complex to the ferrous 2,4,6-tripyridyl-s-triazine [Fe (II)-TPTZ] complex, which has an intense blue color, is monitored by measuring the change in absorption at 593 nm. Aqueous solutions of $FeSO_4 \cdot 7H_2O$ (100–1000 μM) were used for the calibration, and the results were expressed as FRAP values (μmol Fe^{2+} / L) [18, 22].

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Science (SPSS software package, version 25.0; SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± standard deviation (SD). The Pearson correlation coefficient was used to examine the relationship between two observed parameters. All $p < 0.05$ were considered significant.

RESULTS

A summary of the mineral contents of the studied milk types (per 100 mL) is shown in **Table 3**. Results are expressed as mean ± SD for each mineral in different phases of lactation and after thermal/storage treatments. The most concentrated elements in colostrum and mature milk were K and Cl, followed by, in order of decreasing concentration, Ca, Na, P, Mg, Zn, Fe and Cu. The concentrations of all examined minerals differed in colostrum and mature milk. Colostrum contained higher concentrations of micronutrients (Na, Ca, P, Cl, Mg, Fe, Zn, Cu) compared to mature milk, and to milk after storage and pasteurization.

K and Cu concentrations increased during lactation, as the expressed liquid changed from colostrum to mature milk. The concentrations of Ca, Na, P, Cl, Mg and the microelements Fe and Zn were highest in the first week and then decreased over lactation. Concentrations of K, Mg, Na, P, Fe and Cu remained stable dur-

Table 3. Mineral content in preterm human breast milk during lactation, after frozen storage and pasteurization.

Milk type	Na	K	Ca	P	Cl	Mg	Fe	Zn	Cu
Colostrum	15.2 (8.1)	42.4 (9.3)	27.8 (6.3)	15.9 (7.0)	60.4 (12.5)	3.0 (0.5)	35 (5.1)	564 (27.9)	26 (1.3)
Mature milk	14.5[a*] (7.2)	56.2[a*] (5.4)[b]	26.5[a*] (7.9)	13.6[a*] (4.1)	44.4[a*] (14.1)	2.5[a*] (5.6)	32[a*] (2.3)	298[a*] (13.6)	31[a*] (2.3)
Pasteurized milk	14.8[b] (7.3)	52.6[b] (11.8)	24.9[b] (4.4)	13.9[b] (3.8)	40.8[b] (14.2)	2.6[b] (0.3)	30[b] (2.7)	286[b] (11.7)	33[b] (1.3)
Frozen-stored milk	14.7[b] (5.6)	51.4[b] (1.4)	25.0[b] (3.3)	13.7[b] (2.3)	43.2[b] (9.2)	2.6[b] (2.9)	34[b] (2.9)	279[b] (19.6)	27[b] (6.1)
Frozen-stored and pasteurized milk	14.0[b] (3.8)	53.4[b] (2.4)	25.5[b] (3.8)	13.3[b] (6.1)	43.6[b] (19.5)	2.6[b] (0.4)	26[b] (1.3)	278[b] (10.3)	27[b] (7.4)

Concentration of mineral substances in human milk expressed in mg/100 mL (Na, K, Ca, P, Cl, Mg) and μg/100 mL (Fe, Zn, Cu). The results are expressed as mean (SD). Different superscript letters indicate a statistical significance between milk types: a = mature milk and colostrum (*P < 0.05), b = milk after thermal treatment and mature milk (*P < 0.05).

ing and after thermal treatments. The greatest differences were seen in Cl and Zn concentrations, which decreased during lactation, so were higher in colostrum than in mature milk. There were no significant differences in the mineral content in mature milk before and after pasteurization and storage. Zn concentration in the milk decreased during lactation. The Zn to Cu molar ratio ranged between 21.6 for colostrum to 9.61 for mature milk, with no significant difference between mature milk samples after thermal treatments. The Ca to P mass ratio was 1.75 in colostrum, and increased to between 1.79 and 1.91 in mature milk.

Figure 1 shows the macronutrient (protein, fat, lactose and total carbohydrate) concentrations in human breast milk, milk after supplementation with fortifier and IF for nutrition of preterm infants.

The protein content in human breast milk is low in relation to the needs of preterm infants, which is in the range of 2.4 g/100 kcal [4]. By supplementing milk with FF, the concentration of proteins was significantly increased, the protein content of milk supplemented with fortifier was similar to that of IF specialized for preterm nutrition. The concentration of total carbohydrates was significantly higher and fat content slightly higher in the supplemented milk, which also contributed to higher energy of those infant meals (87.6 kcal (367 kJ)/100mL) compared to IF PRE (79.2 kcal (332 kJ)/100mL).

Table 4 shows the comparative energy and mineral characteristics of mature milk, milk supplemented with FF and IF PRE, together with the range of values reported in the literature for preterm human milk. Human milk supplemented with fortifier achieved significantly greater energy content than unfortified milk. Supplementation of mature milk with the fortifier increased the concentration of minerals. The concentration of total investigated mineral (measured as ash) increased ($p<0.05$) in mature breast milk after supplementation with fortifier. The total mineral substances in the mature breast milk (10 milk samples) increased (measured increases ranged from 0.29 to 0.50 g/100 mL) after the milk was fortified. Contents of Na, K, Mg, Cl, Ca and P were significantly changed after supplementation (**Table 4**).

Looking at supplementation, there were statistically significant differences between the mean osmolality (osmolarity) in non-supplemented milk, and milk after supplementation with fortifier, which achieved higher osmolality (osmolarity). Fortifier (5 g/ 100mL) increased the osmolality and osmolarity of milk solution to 324 ± 14 and to 276 ± 15 mOsmol/L, respectively. The potential renal solute load of mature milk

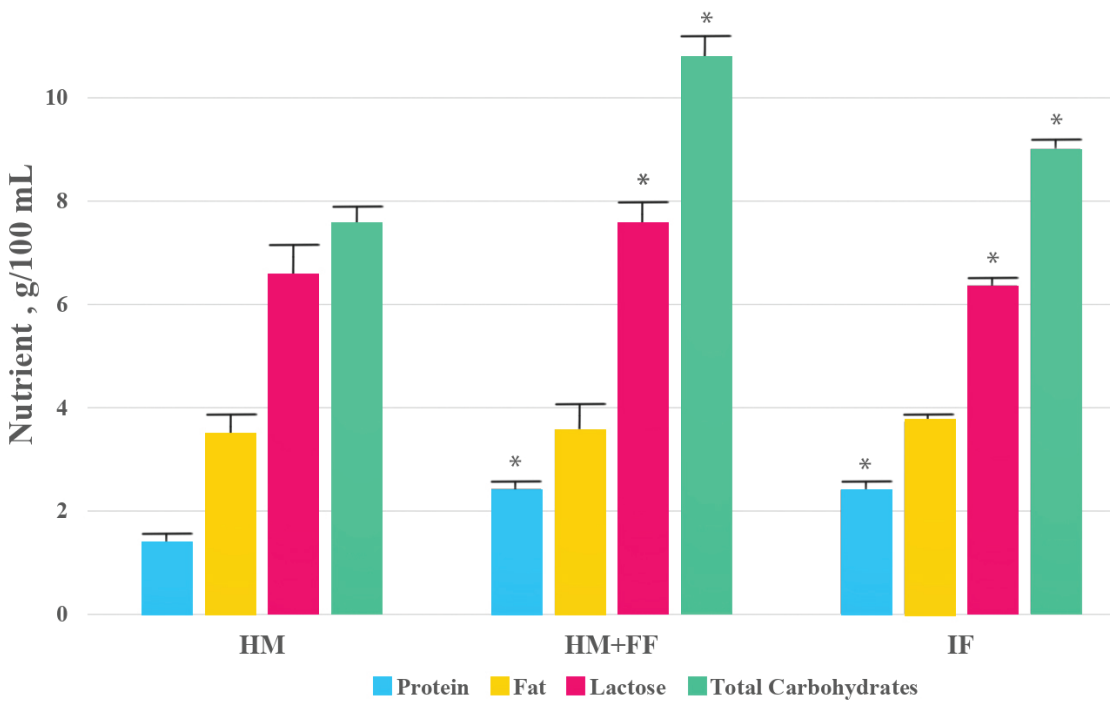

Figure 1. Comparison of macronutrient profiles (protein, fat, lactose and total carbohydrates) of mature milk (HM), mature milk supplemented with fortifier (HM+FF) and infant formula for preterm and low-birth weight infants (IF). The results are expressed as mean (SD). Different superscripts indicate a statistical significance between milk types. * = HM+FF compared with mature milk ($P < 0.05$), # = IF compared with mature milk ($P < 0.05$).

Table 4. Energy and mineral content in preterm infant nutrition: mature breast milk, mature breast milk supplemented with fortifier and infant formula for preterm and low-birth weight infants.

Nutrient concentration	HM Mean (SD)	HM + FF Mean (SD)	IF PRE Mean (SD)	Recommended intake per 100 kcal [4]
Energy, kJ/100 mL	277 (33)	367 (34)	332 (29)	110 – 135 kcal/kg/day
Energy, kcal/100 mL	66.0 (7.8)	87.6 (8.0)a*	79.2 (7.0) b*	
Total mineral substances (ash) g/100 mL	0.23 (0.04)	0.50 (0.05) a*	0.45 (0.04) b*	0.2
Calcium, mg/100 mL	26.5 (7.9)	99.1 (5.4) a*	121.0 (0.7) b*	110 – 130[4]
Phosphorus, mg/100 mL	13.6 (4.1)	56.1 (3.1) a*	63.3 (0.4) b*	55 – 80[4]
Ca:P ratio	1.95 : 1	1.77 : 1	1.91 : 1	1.5 – 2 [27]
Sodium, mg/100 mL	14.5 (7.2)	40.4 (4.4) a*	39.6 (0.6) b*	63 – 105[4]
Potassium, mg/100 mL	56.2 (5.4)	88.3 (3.1) a*	107.7 (1.0) b*	60 – 120[4]
Chloride, mg /100 mL	44.4 (14.1)	64.4 (12.1) a*	61.8 (0.3) b*	95 – 161[4]
Osmolarity, mOsmol/L	134 (27)	276 (15) a*	277 (10) b*	≤ 400 [21]
Osmolality, mOsmol/kgH_2O	152 (31)	324 (17) a*	290 (10) b*	≤ 450 [21]
PRSL, mOsmol/L	118 (23)	215 (14) a*	199 (7) b*	221 [15]

PRSL – potential renal solute load. The results are expressed as mean (SD). Different superscript letters indicate a statistical significance between milk types: a = HM+FF with mature milk (*P < 0.05), b = IF and mature milk (*P < 0.05)

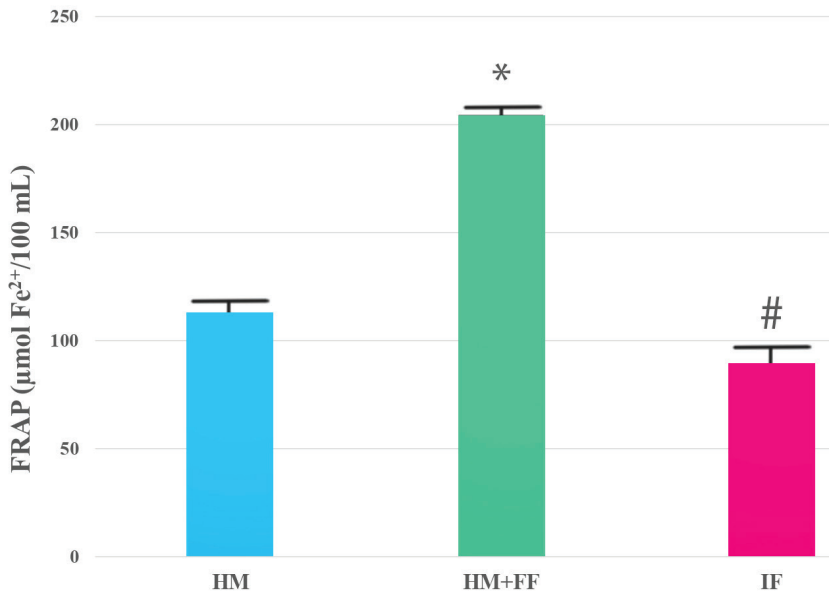

Figure 2. Total antioxidant capacity in preterm infant nutrition: mature milk (HM), mature milk supplemented with a fortifier (HM+FF) and infant formula for preterm and low-birth weight infants (IF) determined by the ferric reducing antioxidant potential (FRAP) method. The results are expressed as mean (SD). Different superscripts indicate a statistical significance between milk types. * = HM+FF compared with mature milk (P < 0.05), # = IF compared with mature milk (P < 0.05).

after fortification increased by $PRSL_{HM+FF}$- $PRSL_{HM}$ = 97 ± 9 mOsmol/L. However, the nutritional properties of IF were not significantly different from those of human milk supplemented with fortifier.

Figure 2 shows the total antioxidant capacity of the preterm infant meals, determined by the FRAP method. Human breast milk had a significantly higher total antioxidant capacity compared to that of IF PRE, and fortification additionally increased the activity of non-enzymatic antioxidants compared to that detected in mature milk.

DISCUSSION

Our study analyzed, in a human milk bank in Serbia and for the first time, the mineral composition of infant meals for prematurely born babies. The design of this study enabled a comparison of mineral concentration in human breast milk during lactation, in IF for preterm infants, as well as the impact of milk fortification on mineral composition, osmolarity, PRSL, TAC, as well as the impact on the quality of food for prematurely born babies.

The composition of milk changes during the lactation period, and after storage and pasteurization [18]. Therefore, it is necessary to monitor the protein concentration of preterm milk, as well as the total energy and mineral content of milk meals. Physiological processes responsible for mineral composition in preterm human milk occur due to differences in hormonal balance and metabolic regulation in women who deliver preterm because of their shorter gestational period [23-25]. The principal mineral constituents of human milk of mothers of preterm infants are K, Ca, Na, P, Mg and Cl. By comparing the concentrations of minerals with the required values, we determined there is a need for some mineral supplementation.

According to these results and to WHO recommendations [26] for the nutrition of preterm infants, human breast milk is insufficient for normal growth, so it is necessary to supplement mature milk with a fortifier in order to increase energy, macronutrients and micronutrients. Multicomponent fortifier is used for standard fortification of human breast milk for feeding preterm infants, by adding it at a level of 5% to the volume of milk. Fortification achieved significantly greater protein and energy content than unfortified milk, which brought it into line with the recommended daily intake for preterm infants [4, 6]. However, this type of supplementation does not take into consideration individual differences in the concentration of macronutrients, especially protein.

Ca absorption decreases the risk of fractures, osteopenia, ensures appropriate mineralisation and depends on Ca and vitamin D intakes, related to absorbed P [27]. The Ca to P mass ratios, in the range of 1.77 and 1.95 for mature milk, fortified milk, and IF, were in line with recommendations of a Ca:P ratio of between 1.5 and 2 [28]. The microelements Fe, Cu and Zn are very important, and the contents of these vary considerably in preterm milk [29]. Zn in milk, unlike in mother's serum [30], did not increase during lactation, but rather, decreased. The Zn:Cu ratio ranged from 20.5 in the first week of lactation to 5.2 for mature preterm milk, which is consistent with recommendations and previous studies that followed the lactation period of preterm women [4, 23].

Mineral content influence osmolarity of human milk. In the nutrition of preterm infants, it should be taken into account that fortification increases osmolarity [31, 32]. Due to the activity of amylase in human milk, which hydrolyzes the dextrin component of fortifier to oligosaccharides, the final osmolality of fortified milk is higher than that of its individual components [33, 34]. The addition of fortifiers concentrates human milk, increases its osmolarity, and can enhance pressure on immature kidneys in some infants. The intensive growth of preterm infants requires a high protein intake, which affects the increased rate of glomerular filtrate and the renal solute load, finally resulting in hypernatremic dehydration. The recommendations from the American Academy of Pediatrics reported that the osmolality of milk supplemented with fortifier should be below 450 mOsm/kg (an osmolarity of 400 mOsm/L) [34, 35]. Trace elements such as Fe, Cu, and Zn, although increased after supplementation with fortifier, do not contribute significantly to the increase in osmolality with their mass, but their contribution to total antioxidant capacity is significant [24]. The total antioxidant capacity of human milk is important in defence against oxidative stress, which is very high in premature babies.

Future guidelines in the nutrition of preterm infants will stipulate individual supplementation, after analysis of human breast milk, with macro- and micronutrient loadings from a high-quality fortifier. Individualized fortification would greatly contribute to the optimization of nutritional properties of breast milk. The application of human milk-based fortifiers requires additional studies due to limited data on efficacy, safety of application and ethical issues.

CONCLUSION

During the lactation period in mothers of babies born preterm, the mineral content of human breast milk changed. Storage and pasteurization did not significantly affect the mineral content. The mineral content of mature milk was lower than necessary for the optimal growth of preterm infants, so adequate supplementation with a fortifier provides biologically important minerals. However, the osmolarity of supplemented

milk should be monitored due to the potentially increased pressure on the kidneys of preterm infants. Finally, it should be emphasized that the obtained results clearly indicate that the use of fortifier is justified and confirm the validity of the applied doctrine in the case of a human milk bank in Serbia.

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REFERENCES

1. Infant and Young Child Feeding: Model Chapter for Textbooks for Medical Students and Allied Health Professionals. Geneva: World Health Organization; 2009. SESSION 2, The physiological basis of breastfeeding. https://www.ncbi.nlm.nih.gov/books/NBK148970/
2. Garcia C, Duan RD, Brevaut-Malaty V, Gire C, Millet V, Simeoni U, et al. Bioactive compounds in human milk and intestinal health and maturity in preterm newborn: an overview. Cell Mol Biol. 2013; 59:108–31.
3. Lönnerdal B. Breast milk: a truly functional food. Nutrition. 2000; 16(7-8):509-11.
4. Agostini C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2010; 50:85-91.
5. De Curtis M, Candusso M, Pieltain C, Rigo J. Effect of fortification on the osmolality of human milk. Arch Dis Child Feta Neonatal Ed. 1999; 81: F141.
6. Arslanoglu S, Boquien CY, King C, Lamireau D, Tonetto P, Barnett D, et al. Fortification of Human Milk for Preterm Infants: Update and Recommendations of the European Milk Bank Association (EMBA) Working Group on Human Milk Fortification. Front Pediatr. 2019; 7:76.
7. Goedicke-Fritz S, Härtel C, Krasteva-Christ G, Kopp M, Meyer S, Zemlin M. Preterm Birth Affects the Risk of Developing Immune-Mediated Diseases. Front Immunol. 2017; 8:1266.
8. Lapidaire W, Lucas A, Clayden JD, Clark C, Fewtrell MS. Human milk feeding and cognitive outcome in preterm infants: the role of infection and NEC reduction. Pediatr Res. 2022; 91(5):1207-14.
9. Carmody JB, Charlton JR. Short-Term Gestation, Long-Term Risk: Prematurity and Chronic Kidney Disease. Pediatrics. 2013; 131(6):1168–79.
10. 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.
11. Almeida CC, Baião DDS, Rodrigues PA, Saint'Pierre TD, Hauser-Davis RA, Leandro KC, et al. Macrominerals and Trace Minerals in Commercial Infant Formulas Marketed in Brazil: Compliance with Established Minimum and Maximum Requirements, Label Statements, and Estimated Daily Intake. Front Nutr. 2022; 9:857698.
12. Pereira-da-Silva L, Virella D, Henriques G, Rebelo M, Serelha M, Videira-Amaral JM. A simple equation to estimate the osmolarity of neonatal parenteral nutrition solutions. J Parenter Enteral Nutr. 2004; 28(1):34-7.
13. Salvia G, De Vizia B, Manguso F, Iula VD, Terrin G, Spadaro R, et al. Effect of intragastric volume and osmolality on mechanisms of gastroesophageal reflux in children with gastroesophageal reflux disease. Am J Gastroenterol. 2001; 96, 1725–32.
14. Pereira GR, Baumgart S, Bennett MJ, Stallings VA, Georgieff MK, Hamosh M, et al. Use of high-fat formula for premature infants with bronchopulmonary dysplasia: metabolic, pulmonary, and nutritional studies. J Pediatr. 1994; 124: 605–11.
15. Ziegler EE, Fomon SJ. Potential renal solute load of infant formulas. J Nutr. 1989; 119(12 Suppl):1785-8.
16. Schell-Feith EA, Kist-van Holthe JE, van der Heijden AJ. Nephrocalcinosis in preterm neonates. Pediatr Nephrol. 2010; 25(2):221-30.
17. Institute of Medicine (US) Committee on the Evaluation of the Addition of Ingredients New to Infant Formula. Infant Formula: Evaluating the Safety of New Ingredients. Washington (DC): National Academies Press (US); 2004. 3, Comparing Infant Formulas with Human Milk. https://www.ncbi.nlm.nih.gov/books/NBK215837/
18. Lugonja N, Marinković V, Miličić B, Avdalović J, Vrvić, M, Spasić S. Effect of storage process on nutritive properties of preterm human milk. Chem Ind Chem Eng Q. 2022; In press. https://doi.org/10.2298/CICEQ220117021L
19. Yaman M, Çokol N. Determination of Trace Elements in Human Milk, Cow's Milk, and Baby Foods by Flame AAS Using Wet Ashing and Microwave Oven Sample Digestion Procedures. At. Spectrosc. 2004; 25, 185–90.
20. Đurović D, Milisavljević B, Nedović-Vuković M, Potkonjak B, Spasić S, Vrvić M. Determination of Microelements in Human Milk and Infant Formula Without Digestion by ICP-OES. Acta Chim Slov. 2017; 64(2):276-82.
21. Kreissl A, Zwiauer V, Repa A, Binder C, Haninger N, Jilma B, Berger A, Haiden N. Effect of Fortifiers and Additional Protein on the Osmolarity of Human Milk: Is It Still Safe for the Premature Infant? J Pediatr Gastroenterol Nutr. 2013; 57:432–7.
22. Fomon SJ. Potential Renal Solute Load: Considerations Relating to Complementary Feedings of Breastfeed Infants. Pediatrics. 2000; 106(5):1284.
23. Sabatier M, Garcia-Rodenas CL, Castro CA, Kastenmayer P, Vigo M, Dubascoux S, et al. Longitudinal Changes of Mineral Concentrations in Preterm and Term Human Milk from Lactating Swiss Women. Nutrients. 2019; 9:11(8):1855.
24. Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. Clin Nutr. 2011; 30:215–20.
25. Hill PD, Aldag JC, Demirtas H, Naeem V, Parker NP, Zinaman MJ, et al. Association of serum prolactin and oxytocin with milk production in mothers of preterm and term infants. Biol Res Nurs. 2009; 10:340–9.
26. World Health Organization. Guidelines on optimal feeding of low birth-weight infants in low-and middle-income countries. Retrieved from https://www.who.int/maternal_child_adolescent/documents/infant_feeding_low_bw/en/ (accessed 21 April 2021)

27. Rigo J, Pieltain C, Salle B, Senterre J. Enteral calcium, phosphate and vitamin D requirements and bone mineralization in preterm infants. Acta Paediatr. 2007; 96:969-74.
28. FAO/WHO. Standard for infant formula and formulas for special medical purposes intended for infants. CODEX STAN 72.
29. Sánchez C, Fente C, Barreiro R, López-Racamonde O, Cepeda A, Regal P. Association between Breast Milk Mineral Content and Maternal Adherence to Healthy Dietary Patterns in Spain: A Transversal Study. Foods. 2020; 9(5):659.
30. Đurović D, Milisavljević B, Mugoša B, Lugonja N, Miletić S, Spasić S, et al. Zinc concentrations in human milk and infant serum during the first six months of lactation, J Trace Elem Med Biol. 2017; 41:75-8.
31. Koletzko B, Cheah F-C, Domellöf M, Poindexter BB, Vain N, van Goudoever JB (eds): Nutritional Care of Preterm Infants. Scientific Basis and Practical Guidelines. World Rev Nutr Diet, Basel, Karger, 2021; (122):191–7.
32. Seliga-Siwecka J, Chmielewska A, Jasińska K. Effect of targeted vs standard fortification of breast milk on growth and development of preterm infants (≤ 32 weeks): study protocol for a randomized controlled trial. Trials. 2020; 21(1):946.
33. Escribano J, Luque V, Ferre N, Zaragoza-Jordana M, Grote V, Koletzko B, et al. Increased protein intake augments kidney volume and function in healthy infants. Kidney Int. 2011; 79(7):783-90.
34. Pereira-da-Silva L, Pitta-Grós Dias M, Virella D, Moreira AC, Serelha M. Osmolality of preterm formulas supplemented with nonprotein energy supplements. Eur J Clin Nutr. 2008; 62(2):274-8.
35. Commentary on breast-feeding and infant formulas, including proposed standards for formulas. Pediatrics. 1976; 57:278–85.

Značaj minerala u ishrani prevremeno rođene odojčadi

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Kratak sadržaj

lako humano mleko sadrži brojne biomolekule značajne za rast i razvoj prevremeno rođenih beba, ishrana bazirana isključivo na majčinom mleku tokom dužeg vremenskog perioda može biti povezana sa nedostatkom mikronutrijenata kod ove osetljive grupe odojčadi. Cilj ovog istraživanja je da se utvrdi sadržaj minerala u mleku majki prevremeno rođene dece i infant formuli, kao i njihov uticaj na osmolalitet, osmolaritet i potencijalno opterećenje bubrega rastvornim supstancama. Mineralne komponente infant formule i majčinog mleka u toku perioda laktacije, nakon sladištenja pasterizacije i suplementacije obogaćivačem, određene su primenom tehnike indukovano spregnute plazme – optičke emisione spektrometrije. Osmolalnost uzoraka mleka i infant formule je određena osmometrom. Tokom perioda laktacije majki prevremeno rođenih beba menja se sadržaj minerala u mleku. Koncentracije ispitivanih minerala su se razlikovale u zrelom mleku odnosu na kolostrum. Nije bilo značajne razlike u sadržaju minerala u zrelom mleku pre i nakon pasterizacije i zamrzavanja. Suplementacija zrelog mleka obogaćivačem povećava koncentraciju minerala, ukupnu osmolalnost i potencijalno opterećenje bubrega. Mineralni sadržaj zrelog mleka je manji od potrebnog za optimalan rast prevremeno rođene dece, stoga je neophodna adekvatna suplementacija obogaćivačem kojim se obezbeđuju biološki važni minerali. Međutim, potrebno je pratiti osmolarnost suplementiranog mleka zbog potencijalno povećanog pritiska na bubrege prevremeno rođene dece.

***Ključne reči**: mleko; prevremeno rođena deca; ishrana; minerali; osmolarnost; obogaćivač.*