Research, Society and Development, v. 11, n. 13, e554111335770, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i13.35770

Availability of macronutrients in lyophilized human milk used to supply the nutritional needs of preterm newborns

Disponibilidade de macronutrientes no leite humano liofilizado utilizado para suprir as necessidades nutricionais de recém-nascidos prematuros

Disponibilidad de macronutrientes en la leche humana liofilizada utilizada para suplir las necesidades nutricionales de los recién nacidos prematuros

Received: 09/25/2022 | Revised: 10/09/2022 | Accepted: 10/11/2022 | Published: 10/15/2022

Patrícia Magalhães Souza
ORCID: https://orcid.org/0000-0001-5916-0744
State University of Maringá, Brazil
E-mail: patricia.magalhaes11@hotmail.com
Isadora Boaventura Ponhozi
ORCID: https://orcid.org/0000-0001-7230-161X
State University of Maringá, Brazil
E-mail: isa.ponhozi@gmail.com
Luciana Pelissari Manin
ORCID: https://orcid.org/0000-0002-5429-5743
State University of Maringá, Brazil
E-mail: lucianapmanin@hotmail.com
Adriela Albino Rydlewski
ORCID: https://orcid.org/0000-0002-5791-4159
State University of Maringá, Brazil
E-mail: adrielaar@hotmail.com
Eloize Silva Alves
ORCID: https://orcid.org/0000-0002-3340-8374
State University of Maringá, Brazil
E-mail: eloizeetaus@gmail.com
Victor Hugo Maldonado Cruz
ORCID: https://orcid.org/0000-0003-2050-9149
State University of Maringá, Brazil
E-mail: victor.hugo.maldonado.cruz@gmail.com
Oscar Oliveira Santos
ORCID: https://orcid.org/0000-0002-9631-8480
State University of Maringá, Brazil
E-mail: oliveirasantos.oscardeoliveira@gmail.com
Jesui Vergilio Visentainer
ORCID: https://orcid.org/0000-0003-3412-897X
State University of Maringá, Brazil
E-mail: jesuivv@gmail.com

Abstract
Human Milk (HM) is the optimal source of nutrition for preterm newborns (PTNBs). Still, adapting the appropriate nutrient supply for these babies is a major challenge. Lyophilization is a common procedure employed to conserve HM that enables reconstitution of the milk in different volumes, therefore it provides better control of nutrient content, and consequently facilitates adapting its nutritional content to better fulfill the needs of PTNBs. Thus, this study assessed the proximate composition of HM, lyophilized HM, and hydrated lyophilized HM. For that matter, mature HM of 20 mothers was collected and sampled into 3 sample pools (HM, lyophilized HM, and hydrated lyophilized HM) for further analysis to define their proximate composition. Results demonstrated that lyophilization increased by 8-fold ash, protein, lipid, and carbohydrate content, and energy values compared to HM. Moreover, dilution of 1 g of lyophilized HM with 3.5 g of water was proven ideal for babies weighing up to 1.5 kg, as it provides macronutrient concentrations that approach the recommended nutritional needs. Furthermore, lyophilized HM displayed potential as a possible replacement of infant formulas since, regarding macronutrients, it can supply the nutritional needs of PTNBs.
Keywords: Human milk; Lyophilization; Reconstitution; Preterm newborns; Proximate composition; Infant formulas.

Resumo

O Leite Humano (LH) é a fonte ideal de nutrição para recém-nascidos pré-termo (RNPT). Ainda assim, adaptar o suprimento adequado de nutrientes para esses bebês é um grande desafio. A liofilização é um procedimento comum empregado na conservação do LH que possibilita a reconstituição do leite em diferentes volumes, pois proporciona melhor controle do teor de nutrientes e, consequentemente, facilita a adequação do seu teor nutricional para melhor atender às necessidades dos RNPT. Assim, este estudo avaliou a composição centesimal de LH, LH liofilizado e LH liofilizado hidratado. Para tanto, LH maduro de 20 mães foi coletado e amostrado em 3 pools de amostras (LH, LH liofilizado e LH liofilizado hidratado) para análise posterior para definir sua composição centesimal. Os resultados demonstraram que a liofilização aumentou em 8 vezes o teor de cinzas, proteínas, lipídios e carboidratos e os valores de energia em comparação com o LH. Portanto, a diluição de 1 g de LH liofilizado com 3,5 g de água mostrou-se ideal para bebês de até 1,5 kg, pois fornece concentrações de macronutrientes que se aproximam das necessidades nutricionais recomendadas. Além disso, o LH liofilizado apresentou potencial como possível substituto das fórmulas infantis, uma vez que, em relação aos macronutrientes, pode suprir as necessidades nutricionais dos RNPT.

Palavras-chave: Leite humano; Liofilização; Reconstituição; Recém-nascidos prematuros; Composição próxima; Fórmulas infantis.

Resumen

La leche humana (LH) es la fuente óptima de nutrición para los recién nacidos prematuros (RNPT). Aún así, adaptar el suministro de nutrientes apropiado para estos bebés es un gran desafío. La liofilización es un procedimiento común empleado para conservar LH que permite la reconstitución de la leche en diferentes volúmenes, por lo tanto, proporciona un mejor control del contenido de nutrientes y, en consecuencia, facilita la adaptación de su contenido nutricional para satisfacer mejor las necesidades de los RNPT. Por lo tanto, este estudio evaluó la composición proximal de LH, LH liofilizado y LH liofilizado hidratado. Para el caso, se recolectó LH maduro de 20 madres y se tomaron muestras en 3 grupos de muestras (LH, LH liofilizado y LH liofilizado hidratado) para su posterior análisis para definir su composición próxima. Los resultados demostraron que la liofilización aumentó 8 veces el contenido de cenizas, proteínas, lípidos y carbohidratos, y los valores de energía en comparación con LH. Además, la dilución de 1 g de LH liofilizado con 3,5 g de agua demostró ser ideal para bebés de hasta 1,5 kg, ya que proporciona concentraciones de macronutrientes que se acercan a las necesidades nutricionales recomendadas. Además, la LH liofilizada mostró potencial como posible reemplazo de las fórmulas infantiles ya que, en cuanto a macronutrientes, puede suplir las necesidades nutricionales de los RNPT.

Palabras clave: Leche humana; Liofilización; Reconstitución; Recién nacidos prematuros; Composición proximal; Fórmulas infantiles.

1. Introduction

Human milk is the greatest source of nutrition for newborns, being considered the ideal foodstuff due to its ability to adapt its composition throughout lactation to meet the newborn requirements for growing and development (Andreas et al., 2015; Pons et al., 2000). HM is classified into three groups with distinct properties based on the lactation period of the mother, which are: colostrum, transitional, and mature (Ballard & Morrow, 2013; Lawrence & Lawrence, 2016; Živković et al., 2015).

Breastfeeding has a positive effect on the cognitive and brain development of the child, therefore, the practice should be encouraged and strongly advised as the only source of nutrition for children up to six months old, and later supplemented for two or more years (González & Visentin, 2016). Still, it is imperative to meet the specific nutritional demands of preterm newborns (PTNBs), which makes adapting the supply of nutrients required for adequate nutrition, aimed at decreasing the growth deficit of newborns, a major issue (O'Connor et al., 2008).

PTNBs exhibit physiological and enzymatic immaturity, hence, their nutritional needs are different from those of term newborns. Even for PTNBs, where breastfeeding is not contraindicated, problems may arise due to tolerance. Therefore, feeding HM to the PTNB is strongly recommended since it has been demonstrated that the practice favors faster weight gaining whilst lessening the risk of medical complications and future development of food allergies (Ahammad et al., 2018).

The key recommendation is that PTNBs are breast-fed HM collected from their birth mother or HM that has been donated by a Human Milk Bank (HMB), which shall be fed to the newborn orally through a small cup or enteral nutrition if necessary, based on the degree of prematurity and difficulty in sucking. The HMB is an organization founded with the intent of

collecting, trialing, processing, pasteurizing, storing, and distributing HM donated by lactating women who produced HM in excess, thus offering adequate nutrition to several newborns (Updegrove et al., 2020, Fang et al., 2021). HM of preterm mothers physiologically adapts to meet the nutritional demands of the PTNBs, modifying itself accordingly throughout the lactation period from gestational age to birth (World Health Organization, 2003; Nessel et al., 2019).

HM is highly perishable, as such, conservation techniques are crucial to increase its shelf life. Lyophilization is a simple and efficient procedure that could be employed to preserve HM in HMBs since it can preserve HM without undermining its nutritional properties (Cortez & Soria, 2016). Lyophilization is based on sublimation, which brings forth advantages compared to regular freeze-drying methods since it preserves the nutrients' structure. This method utilizes low temperatures to remove humidity, increasing stability during storage and transporting, and lessening the likelihood of degradation (Picaud & Buffin, 2017). HM submitted to this procedure can be reconstituted in different volumes to better meet the nutritional needs of PTNBs, by enabling better control of nutrient content (Oliveira et al., 2019).

Prescription of lyophilized HM reconstituted in different volumes to PTNBs that cannot be breastfed directly could be an alternative to supply the nutritional needs of these neonates, which are higher than those of term newborns. Hence, this study aimed to assess the proximate composition of liquid, lyophilized, and reconstituted lyophilized HM that could be used as an individualized form of nutrition for non-extreme PTNBs that weigh approximately 1.5 kg, can be fed HM, and do not require parenteral nutrition.

2. Methodology

2.1 Sampling

This study has been forwarded to, and approved by the Research Ethics Committee (CEP), under process number 2.797.476, of the Universidade Estadual de Maringá (UEM, Maringá, Paraná, Brazil). Samples of mature HM were collected from 20 different mothers, under a cooling temperature of 4 °C at the Human Milk Bank (HMB) located at the Hospital Universitário de Maringá (Maringá, Paraná, Brazil). Then, the volume collected of HM was homogenized into a sampling pool, with approximately 2 L, and stored at -40 °C in a vertical MFV/UFV ultra-freezer (Terroni Equipamentos Científicos, Paulicéia, São Paulo, Brazil). From the previously formed sample pool, 1 L was separated for lyophilization and further analyses.

2.2 Lyophilization

Aliquots of 80 mL from the mature HM sample pool (evenly allocated into 12 flasks of 80 mL, totaling 960 mL of the sample) were lyophilized in an SLH-50 lyophilizer (Terroni Equipamentos Científicos, Paulicéia, São Paulo, Brazil), under the following instrumental conditions: the temperature of the tray and condenser was set at 40 °C and -55 °C, respectively, and the procedure was carried out under constant pressure of 100 mbar. Lyophilization was over under 48 h. It is important to note that the equipment used was developed and adapted exclusively for samples of LH, and operates under strict hygiene standards, inert atmosphere, and aseptic conditions.

2.3 Reconstitution of lyophilized Human Milk

Approximately 1.0 g of lyophilized HM was weighed and mixed with 7.94 g of ultrapure water in order to reconstruct the sample. The procedure was carried out based on the water loss throughout lyophilization.

2.4 Proximate Composition

Moisture, ash, and crude protein content analyses were carried out as described by AOAC/2005 (Association of Official Analytical Chemists, 2005). Moisture content was determined gravimetrically on a micro-processed oven with forced air renewal (Sterilifer) operating at 105 °C for 4 h. Ash content was acquired by dry-ashing samples in a furnace (Quimis) at 600 °C for 6 h. Crude protein content was calculated employing the semi-micro Kjedahl method, by converting the total nitrogen content using a multiplication factor of 6.38.

Lipid content was determined as described by the Folch, Lees, & Sloane Stanley (1957) method, which utilizes a sample:solvent ratio of 1:20 and chloroform:methanol ratio of 2:1 v v^{-1}. Carbohydrate content was acquired by simple subtraction [100 - (moisture content – ash content – crude protein – total lipids)] and expressed as Nifext fraction (nitrogen-free extract). Energy values were calculated by multiplying values for crude protein, carbohydrate, and total lipids content by 4.00, 4.00, and 9.00 kcal g^{-1}, respectively, and results were expressed as kcal 100 g^{-1} (Anvisa, 1998).

2.5 Statistical Analysis

Analyses required to define the proximate composition of samples of liquid, lyophilized and reconstituted HM were performed in triplicate and results were expressed as mean ± standard deviation. Values for proximate composition of samples were submitted to variance analysis (ANOVA) and means were compared by Tukey's test with a 95% significance level through Assistat 7.7. software (Silva, 1996).

3. Results and Discussion

3.1 Proximate Composition

Table 1 provides the experimental data regarding the analyses required for obtaining the proximate composition and (g 100 g^{-1}) and energy values (Kcal 100 mL^{-1} or Kcal 100 g^{-1}) of mature liquid, lyophilized, and reconstituted lyophilized HM.

Table 1. Proximate composition (g 100 g^{-1}) and energy values (Kcal 100 mL^{-1} or Kcal 100 g^{-1}) of mature liquid, lyophilized, and reconstituted lyophilized HM.

Samples	Moisture	Ash	Crude Protein	Total Lipids	Carbohydrate	Energy value
HM	$86.84^{b} \pm 0.58$	$0.21^{b} \pm 0.03$	$1.25^{b} \pm 0.20$	$3.60^{b} \pm 0.55$	$7.78^{b} \pm 0.22$	$70.88^{b} \pm 1.69$
HM lyophilized	$1.00^{c} \pm 0.07$	$1.71^{a} \pm 0.03$	$9.21^{a} \pm 0.51$	$30.09^{a} \pm 0.53$	$58.00^{a} \pm 0.95$	$527.98^{a} \pm 2.95$
HM reconstituted	$88.41^{a} \pm 0.34$	$0.21^{b} \pm 0.01$	$1.27^{b} \pm 0.26$	$3.97^{b} \pm 0.07$	$6.42^{b} \pm 0.50$	$64.87^{c} \pm 1.36$

Results were expressed as mean ± standard deviation (SD) of triplicates. Values with different lower-case letters in the same column are significantly different ($p < 0.05$) according to Tukey's test. Source: Authors (2022).

It can be seen from Table 1 that mature HM demonstrated moisture, ash, crude protein, and total lipid contents, and energy values of 86.84 g 100 g^{-1}, 0.21 g 100 g^{-1}, 3.60 g 100 g^{-1}, 7.78 g 100 g^{-1}, and 70.88 g 100 g^{-1}, respectively. However, data from the table demonstrates that lyophilizing HM revealed a statistically significant increase in ash (1.71 g 100 g^{-1}), crude protein (9.21 g 100 g^{-1}), total lipids (30.09 g 100 g^{-1}), and carbohydrate content (58.00 g 100 g^{-1}), and energy value (527.97 g 100 g^{-1}) whereas, as expected, a substantial decrease in moisture content (1.00 g 100 g^{-1}) was verified.

The findings of this study broadly support the work of other studies investigating the proximate composition of unprocessed HM in the same lactation phase. Furthermore, in accordance with the current results, previous studies have identified similar ranges for moisture (87.73-89.85%), ash (0.21-0.24%), total lipids (1.30-3.48%), crude protein (1.12-1.27%),

and carbohydrate content (6.97-7.50%), and energy values (61.81 g 100 g^{-1}) (Santos et al., 2021; Silva et al., 2007; Brasil, 2015; Leite et al., 2019; Shi et al., 2011; Léké et al., 2019; Rydlewski et al., 2019).

Regarding lyophilized HM, previous studies on mature HM conducted by Martysiak-Żurowska et al., (2022) and Castro-Albarrán et al. (2016), reported a moisture content of 3.92% and 1.64%, respectively, which concurs with the results obtained by this study. Maintaining the moisture content of lyophilized HM under 5% is particularly crucial since higher values directly affect the perishability of the product due to an increase in bacterial, enzymatic, and oxidative activity (Martysiak-Żurowska et al., 2022).

The findings above strongly imply that investigating the proximate composition of mature HM before and after lyophilization would greatly contribute to a deeper understanding of how the procedure affects the product.

As previously explained, the purpose of submitting HM to lyophilization is to remove water from the product through sublimation and decrease its perishability (Pisano et al., 2019). Several advantages strengthen the application of the lyophilization procedure, for instance, inhibition of microbial growth, enhancement of shelf life whilst preserving flavor and nutritional characteristics, volume reduction, ease of transport, and lipid oxidation hindering (Morais et al., 2016). Furthermore, the procedure does not impact the food composition, but instead, concentrates the endogenous macronutrients and micronutrients (Morais et al., 2016; Vishali et al., 2019), which is corroborated by the results acquired for liquid and lyophilized HM (Table 1). Such points are indeed advantageous, directly and indirectly, to the HMBs and PTNBs that may eventually need HM from an HMB for its complete development.

3.2 Reconstitution of Human Milk and value of macronutrients for preterm newborns (PTNBs)

Following lyophilization, HM was reconstituted through the addition of ultrapure water. For that matter, ordinarily, the volume of water required to reconstruct the sample is defined by gauging the volume lost during lyophilization and mixing the same amount with the powdered sample. The results for moisture (88.41 g 100 g^{-1}), ash (0.21 g 100 g^{-1}), crude protein (1.27 g 100 g^{-1}), total lipids (3.97 g 100 g^{-1}), and carbohydrate (6.42 g 100 g^{-1}) content, and energy values (64.87 g 100 g^{-1}) of reconstituted lyophilized HM summarized in Table 1 demonstrate that lyophilizing the sample did not modify its macronutrients composition.

Interestingly there is little to no published data regarding reconstitution of HM with different volumes of water, focusing primarily on adapting the nutrient content to better suit the nutritional need of the PTNBs. Thus, a table (Table 2) was built with the macronutrient content of distinct dilutions of reconstituted HMs, calculated based on the mass of lyophilized HM and water used to reconstruct it, consequently generating the mass of reconstituted lyophilized HM obtained and its specific macronutrient composition.

Table 2. Reconstitution of lyophilized HM with distinct water volumes and the macronutrient content for such products expressed as g 100 g^{-1} (crude protein, total lipids, and carbohydrate content).

Dilution	Lyophilized HM (g)	Water (g)	Reconstituted HM (g)	Crude Protein	Total lipids	Carbohydrates
1	1	7	8	1.27	3.97	6.42
2	1	3.5	4.5	2.54	7.94	12.84
3	1	1.75	2.75	5.08	15.88	25.68
4	1	0.875	1.875	10.16	31.78	51.36
5	1	0	1	9.21	30.09	58.00

Source: Authors (2022).

It can be seen from Table 2 that decreasing the volume of water used to reconstruct the sample increases the crude protein, total lipid, and carbohydrate content of the dilution. This is exceptionally advantageous since PTNBs require special care due to their degree of biochemical immaturity, accelerated growth, and higher incidence of clinical complications (Silva & Mura, 2011). Thus, nutritional care for this neonate profile aims to mimic the nutritional conditions of the mother`s womb by providing the nutrients required for growth and development (Accioly, Saunders, & Lacerda, 2009). In addition, from adequate nutritional support, there is a reduction in risk factors that can increase the possibility of complications from prematurity (McNelis, Fu, & Poindexter, 2017).

It is well established from a myriad of studies that the preterm requires proper nutritional support, and partial or complete lack of said support can lead to risk factors that may increase medical complications throughout prematurity and subsequent growth and development inhibition (McNelis, Fu, & Poindexter, 2017).

Considering that the protein requirements of a PTNB range from 2.7 to 3.5 g / kg a day (Mahan, Escott-Stump, & Raymond, 2013), for a PTNB weighing approximately 1.5 kg, the macronutrient content present in 1 g of lyophilized HM (5.08 g 100 g^{-1}), when diluted into 3.5 g of water (Table 2, dilution 2), come very close meeting the nutritional needs of the neonate. Protein excess offers no benefits, but instead, increases the risk of metabolic problems, such as an increase in ammonia and urea, metabolic acidosis, metabolic and renal overload due to glomerular filtration process immaturity, and may lead to neurological disorders (Waitzberg, 2004). Conversely, the supply of adequate protein content is responsible for providing the amino acids required for the PTNB development, playing a key role in the digestion and absorption of nutrients by the intestinal mucosa and defense against infections (Guo, 2021; Lönnerdal, 2003).

The lipid necessities of a PTNB range approximately between 5.9 and 11.6 g / kg a day (Waitzberg, 2004). The PTNBs are more vulnerable to lack of lipid intake since the intrauterine deposition which happens in the 3° trimester was influenced by the prematurity (Silva e Mura, 2011). Hence, close inspection of Table 2 demonstrates that reconstitution of lyophilized HM with 3.5 g of water (dilution 2) (15.88 100 g^{-1}) may meet the requirement of a PTNB weighing approximately 1.4 kg. This lipid content is essential for neonate growth and development, meeting its greatest energy demands whilst fulfilling primordial metabolic and physiological functions, and acting as thermal insulant (Delplanque, Gibson, Koletzko, Lapillonne, & Strandvik, 2015). According to González & Visentin (2016), HM has lipids essential for infant brain development, making up the central nervous system structure, and playing key roles in development, migration, and nerve cell differentiation. To date, no HM substitutes have been able to mimic its lipid profile, besides these substitutes demonstrate differences in digestion and lipid absorption (López-López et al., 2001).

Regarding carbohydrate requirements, the PTNBs need between 10 and 15 g / kg a day (Mahan, Escott-Stump, & Raymond, 2013). It can be seen from Table 2 that dilution 2 of HM, using 3.5 g of water, contains 25.68 g 100 g^{-1}, consequently matching the requirement of the profile of a 1.7 kg neonate. In HM, the main carbohydrate is lactose, whereas infant formulas (IFs) are evenly comprised of lactose and glucose polymers added aiming to adjust calorie supply, lessen the osmotic load and improve carbohydrate absorption (Vasconcelos, 2011). Since lactose is the main carbohydrate of HM, it has a favorable effect on mineral absorption by increasing the solubility of calcium salts. Besides, lactose and minerals are vital to stabilizing the osmotic pressure of HM, preventing osmotic diarrhea (Guo, 2021).

Therefore, taking into consideration the discussion above, it can be concluded that dilution 2, 1 g of lyophilized HM into 3.5 g of water, is ideal for preterm newborns weighing approximately 1.5 kg since the protein (5.08 g 100 g^{-1}), lipid (15.88 g 100 g^{-1}), and carbohydrate (25.68 g 100 g^{-1}) content supplied by this dilution comes extremely close to meeting the nutritional requirements of this PTNB profile.

3.3 Reconstituted human milk versus Infant formulas

Lyophilized HM can be an excellent alternative to substitute the usage of IFs, since IFs lack in their composition few bioactive compounds, carbohydrates, and proteins that are exclusive to HM (Martin, Ling, & Blackburn, 2016; Brasil, 2014). The superiority of HM over IFs for PTNBs is indisputable and despite previous research in the literature stating the benefits of calcium, phosphorous, and protein supplementation, feeding the neonate with HM is still strongly advised due to its several advantages over IFs, for instance lessening the risk of necrotizing enterocolitis mortality, enhanced immunological response and antibodies that avoid hospital infections, among others (Waitzberg, 2004; Silva & Mura, 2011).

Succinctly, formulas for preterms have higher energy and protein contents, and lower lactose and essential fatty acids content, and must follow the standards defined by the Codex Alimentarius, being capable of supplying the nutritional needs of the infant throughout the first months of life until food introduction, contamination-free, and ensure normal growth and development (Waitzberg, 2004).

The lipid composition of the majority of IFs vary according to the mixture of the different sources of lipid employed, resulting in differences in the structure, composition, and complexity of the fat compared to that of HM since the lipid composition of HM is extremely diverse (Delplanque, Gibson, Koletzko, Lapillonne, & Strandvik, 2015). Thus, producers are advised to offer IFs that mimic as best as possible the nutrient profile of HM whilst, according to the Codex, having an energy value, crude protein, total lipids, and carbohydrate content ranging from 60 to 70 Kcal 100 g^{-1}, 1.8 to 3.0 g 100 mL^{-1}, 4.4 to 6.0 g 100 mL^{-1}, 9.0 to 14.0 g 100 mL^{-1}, respectively (Anvisa, 2011).

In contrast, IFs meant for PTNBs must provide a calorie content between 60 and 70 Kcal per 100 mL (Guo, 2014). The proximate composition of such formulas must contain a crude protein, carbohydrate, and total lipids content ranging from 2-3.5%, 10.4%, and 2.77-4.23%, respectively (Mendonça, Araújo, Borgo, & Alencar, 2017; Fanaro, Ballardini, & Vigi, 2010). As a result, as shown by Table 2, an intermediate between dilution 1 and 2 would certainly meet the parameters recommended by the codex. Diluting 1 g of the powdered sample into 5.25 g of water would possibly generate 6.25 g of a sample with the following proximate composition: 1.91% of crude protein, 5.96% of total lipids, and 9.63% of carbohydrates. Subsequently, satisfying the recommended nutritional requirements.

A study conducted with 24 PTNBs divided into three groups fed with HM provided by HMBs supplemented with 5% of bovine-based protein, vaporized HM, and lyophilized HM, respectively, observed that despite the lack of negative metabolic repercussions while the study was being carried out, the PTNBs fed with HM supplemented with bovine-based protein may

exhibit damaged future cognitive development since the additive increases blood phenylalanine content of the neonates (Thomaz et al., 2014).

Thus far several studies have highlighted the superiority of HM over IFs. Nevertheless, in cases where usage of IFs is mandatory, the nutrient requirements of PTNBs can be met through intervention with lyophilized HM since substitution of HM for IFs provides no immunological protection. Besides, no IF can mimic the impact of HM on the PTNBs' physiological programming and long-lasting beneficial effects on the child's life (Grazziotin & Moreira, 2016).

4. Conclusions

Lyophilizing the samples increased the macronutrient content (crude protein, total lipids, and carbohydrates) of mature HM besides lessening moisture content, subsequently enhancing the perishability of the sample. Reconstitution of powdered HM with several volumes of water can adapt the macronutrient content of the product, either generating a more concentrated or diluted meal. Besides, our results indicated that reconstitution of 1 g of lyophilized HM with 3.5 g of water was the optimal dilution to feed PTNBs weighing approximately 1.5 kg as it provides the amount of nutrients needed by this PTNB profile.

Lastly, the findings reported here shed new light on alternatives for IFs, since the lyophilization and reconstruction procedure of HM enables the adaptation of nutrient concentration to better suit the nutritional needs of neonates according to their weigh, allowing to concentrate or dilute the nutrients to better match the necessities of each neonate profile.

Acknowledgments

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Cargill and Programa Pesquisa para o SUS (PPSUS) for financial assistance, to the research group APLE-A/CNPq; and to the Human Milk Bank of University Hospital of Maringá for its partnership and donation of human milk samples.

References

Accioly, E., Saunders, C., & Lacerda, E. M. A. (2009). *Nutrição em obstetrícia e pediatria*. (2nd ed.); Guanabara Koogan.

Agência Nacional de Vigilância Sanitária – Anvisa (1998). Portaria No. 27, de 13 de Janeiro de 1998. R*egulamento Técnico referente à Informação Nutricional Complementar*. Diário Oficial da União: Brasília, Brazil. https://bvsms.saude.gov.br/bvs/saudelegis/svs1/1998/prt0027_13_01_1998.html.

Agência Nacional de Vigilância Sanitária – Anvisa (2011). Resolução da diretoria colegiada (RDC) No. 43, de 19 de Setembro de 2011. *Regulamento Técnico para formulas infantis para lactentes.* Diário Oficial da União: Brasília, Brazil. https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2011/res0043_19_09_2011.html.

Ahammad, F., Begum, T., & Nasrin, E. (2018). Comparison of feeding intolerance between very preterm and moderate preterm neonates–a prospective cohort study. *Journal of Pediatrics and Neonatal Care*, *8*(4), 6-10.

Andreas, N. J., Kampmann, B., & Le-Doare, K. M. (2015). Human breast milk: A review on its composition and bioactivity. *Early human development*, *91*(11), 629-635.

Association of Official Analytical Chemists – AOAC (2005). *Official Methods of analysis of the Association of Official Analytical Chemists*. (18th ed.); Gaithersburg: AOAC.

Ballard, O., & Morrow, A. L. (2013). Human milk composition: nutrients and bioactive factors. *Pediatric Clinics*, *60*(1), 49-74.

Brasil (2014). A*leitamento materno, distribuição de leites e fórmulas infantis em estabelecimentos de saúde e a legislação*. Ministério da Saúde: Brasília, Brazil. https://bvsms.saude.gov.br/bvs/publicacoes/aleitamento_materno_distribuicao_leite.pdf.

Brasil (2015). *Saúde da Criança: Aleitamento Materno e Alimentação Complementar*. (2nd ed.); Ministério da Saúde: Brasília, Brazil. https://bvsms.saude.gov.br/bvs/publicacoes/saude_crianca_aleitamento_materno_cab23.pdf.

Castro-Albarrán, J., Aguilar-Uscanga, B. R., Calon, F., St-Amour, I., Solís-Pacheco, J., Saucier, L., & Ratti, C. (2016). Spray and freeze drying of human milk on the retention of immunoglobulins (IgA, IgG, IgM). *Drying Technology*, *34*(15), 1801-1809.

Cortez, M. V., & Soria, E. A. (2016). The effect of freeze-drying on the nutrient, polyphenol, and oxidant levels of breast milk. *Breastfeeding Medicine*, *11*(10), 551-554.

Delplanque, B., Gibson, R., Koletzko, B., Lapillonne, A., & Strandvik, B. (2015). Lipid quality in infant nutrition: current knowledge and future opportunities. *Journal of pediatric gastroenterology and nutrition*, *61*(1), 8.

Fanaro, S., Ballardini, E., & Vigi, V. (2010). Different pre-term formulas for different pre-term infants. *Early human development*, *86*(1), 27-31.

Fang, M. T., Grummer-Strawn, L., Maryuningsih, Y., & Biller-Andorno, N. (2021). Human milk banks: a need for further evidence and guidance. *The Lancet Global Health*, *9*(2), e104-e105.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J biol Chem*, *226*(1), 497-509.

González, H. F., & Visentin, S. (2016). Micronutrientes y neurodesarrollo: actualización. *Archivos argentinos de pediatría*, *114*(6), 570-575.

Grazziotin, M. C. B., & Moreira, C. M. D. (2016). In *Amamentação – bases científicas*; Carvalho, M. R.; Gomes, C. F., eds.; Guanabara Koogan: Rio de Janeiro, Brasil, ch. 24.

Guo, M. (2014). *Human milk biochemistry and infant formula. Manufacturing Technology,* 1st ed.; Elsevier: Cambrdige, England.

Guo, M. R. (2021). In *Human Milk Biochemistry and Infant Formula Manufacturing Technology*. Cockle, C., & Ball, M. eds., Woodhead Publishing: Cambrdige, England, ch 2.

Lawrence, R. A., & Lawrence, R. M. (2016). *Breastfeeding: A Guide for the Medical Profession*. (8th ed.), Elsevier: Amsterdã, Netherlands.

Leite, J. A., Migotto, A. M., Landgraf, M., Quintal, V. S., Gut, J. A., & Tadini, C. C. (2019). Pasteurization efficiency of donor human milk processed by microwave heating. *LWT*, *115*, 108466.

Léké, A., Grognet, S., Deforceville, M., Goudjil, S., Chazal, C., Kongolo, G., ... & Biendo, M. (2019). Macronutrient composition in human milk from mothers of preterm and term neonates is highly variable during the lactation period. *Clinical Nutrition Experimental*, *26*, 59-72.

Lönnerdal, B. (2003). Nutritional and physiologic significance of human milk proteins. *The American journal of clinical nutrition*, *77*(6), 1537S-1543S.

López-López, A., Castellote-Bargalló, A. I., Campoy-Folgoso, C., Rivero-Urgel, M., Tormo-Carnicé, R., Infante-Pina, D., & López-Sabater, M. C. (2001). The influence of dietary palmitic acid triacylglyceride position on the fatty acid, calcium and magnesium contents of at term newborn faeces. *Early human development*, *65*, S83-S94.

Mahan, L. K., Escott-Stump, S., & Raymond, J. L. (2013). *Krause Alimentos, Nutrição e Dietoterapia*. (13th *ed.);* Elsevier: Amsterdã, Netherlands.

Martin, C. R., Ling, P. R., & Blackburn, G. L. (2016). Review of infant feeding: key features of breast milk and infant formula. *Nutrients*, *8*(5), 279.

Martysiak-Żurowska, D., Rożek, P., & Puta, M. (2022). The effect of freeze-drying and storage on lysozyme activity, lactoferrin content, superoxide dismutase activity, total antioxidant capacity and fatty acid profile of freeze-dried human milk. *Drying Technology*, *40*(3), 615-625.

McNelis, K., Fu, T. T., & Poindexter, B. (2017). Nutrition for the extremely preterm infant. *Clinics in Perinatology*, *44*(2), 395-406.

Mendonça, M. A., Araújo, W. M. C., Borgo, L. A., & Alencar, E. D. R. (2017). Lipid profile of different infant formulas for infants. *PLoS One*, *12*(6), e0177812.

Morais, A. R. V., Alencar, É. N., Júnior, F. H. X., Oliveira, C. M., Marcelino, H. R., Barratt, G., ... & Elaissari, A. (2016). Freeze-drying of emulsified systems: A review. *International journal of pharmaceutics*, *503*(1-2), 102-114.

Nessel, I., Khashu, M., & Dyall, S. C. (2019). The effects of storage conditions on long-chain polyunsaturated fatty acids, lipid mediators, and antioxidants in donor human milk—A review. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *149*, 8-17.

O'Connor, D. L., Khan, S., Weishuhn, K., Vaughan, J., Jefferies, A., Campbell, D. M., ... & Postdischarge Feeding Study Group. (2008). Growth and nutrient intakes of human milk–fed preterm infants provided with extra energy and nutrients after hospital discharge. *Pediatrics*, *121*(4), 766-776.

Oliveira, M. M., Aragon, D. C., Bomfim, V. S., Trevilato, T. M., Alves, L. G., Heck, A. R., ... & Camelo Jr, J. S. (2019). Development of a human milk concentrate with human milk lyophilizate for feeding very low birth weight preterm infants: A preclinical experimental study. *PloS one*, *14*(2), e0210999.
Picaud, J. C., & Buffin, R. (2017). Human milk—treatment and quality of banked human milk. *Clinics in perinatology*, *44*(1), 95-119.

Pisano, R., Arsiccio, A., Capozzi, L. C., & Trout, B. L. (2019). Achieving continuous manufacturing in lyophilization: Technologies and approaches. *European Journal of Pharmaceutics and Biopharmaceutics*, *142*, 265-279.

Pons, S. M., Bargalló, A. C., Folgoso, C. C., & Sabater, L. (2000). Triacylglycerol composition in colostrum, transitional and mature human milk. *European journal of clinical nutrition*, *54*(12), 878-882.

Rydlewski, A. A., Silva, P. D., Manin, L. P., Tavares, C. B., Paula, M. G., Figueiredo, I. L., ... & Visentainer, J. V. (2019). Lipid Profile Determination by Direct Infusion ESI-MS and Fatty Acid Composition by GC-FID in Human Milk Pools by Folch and Creamatocrit Methods. *Journal of the Brazilian Chemical Society*, *30*, 1063-1073.

Santos, V. J., Baqueta, M. R., Neia, V. J. C., de Souza, P. M., Março, P. H., Valderrama, P., & Visentainer, J. V. (2021). MicroNIR spectroscopy and multivariate calibration in the proximal composition determination of human milk. *LWT*, *147*, 111645.

Shi, Y. D., Sun, G. Q., Zhang, Z. G., Deng, X., Kang, X. H., Liu, Z. D., ... & Sheng, Q. H. (2011). The chemical composition of human milk from Inner Mongolia of China. *Food chemistry*, *127*(3), 1193-1198.

Silva, F. D. A. (1996). The ASSISTAT Software: statistical assistance. In *International conference on computers in agriculture*, *6*, 294-298.

Silva, R. C. D., Escobedo, J. P., Gioielli, L. A., Quintal, V. S., Ibidi, S. M., & Albuquerque, E. M. (2007). Composição centesimal do leite humano e caracterização das propriedades físico-químicas de sua gordura. *Química Nova*, *30*, 1535-1538.

Silva, S. M. C. S. D., & Mura, J. D. (2011). *Tratado de alimentação, nutrição & dietoterapia*. (2nd ed.;) Roca.

Thomaz, D. M., Serafin, P. O., Palhares, D. B., Tavares, L. V., & Grance, T. R. (2014). Serum phenylalanine in preterm newborns fed different diets of human milk. *Jornal de pediatria*, *90*, 518-522.

Updegrove, K., Festival, J., Hackney, R., Jones, F., Kelly, S., Sakamoto, P., & Vickers, A. (2020). *HMBANA Standards for Donor Human Milk Banking: An Overview*. Human Milk Banking Association of North America: Fort Worth, USA. https://www.hmbana.org/file_download/inline/95a0362a-c9f4-4f15-b9ab-cf8cf7b7b866.

Vasconcelos, M. J. O. B. (2011). *Nutrição Clínica - Obstetrícia e Pediatria*. Medbook.

Vishali, D. A., Monisha, J., Sivakamasundari, S. K., Moses, J. A., & Anandharamakrishnan, C. (2019). Spray freeze drying: Emerging applications in drug delivery. *Journal of Controlled Release*, *300*, 93-101.

Waitzberg, D. L. (2004). *Nutrição oral, enteral e parenteral na prática clínica*. (3rd ed.); Atheneu.

World Health Organization (2003). *Global strategy for infant and young child feeding*. https://www.who.int/publications/i/item/9241562218.

Živković, J., Sunarić, S., Trutić, N., Denić, M., Kocić, G., & Jovanović, T. (2015). Antioxidants and antioxidant capacity of human milk. *Acta Facultatis Medicae Naissensis*, *32*(2), 115-125.