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Original article

Effect of Holder pasteurization on macronutrients and energy content of pooled donor human milk

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Abstract

Background: Donor human milk (DHM) is the best option for preterm nutrition when mother's milk is unavailable. For its proven benefits on the life and health of premature babies, DHM should be part of the essential newborn care. The fortification of human milk is necessary to ensure adequate growth and consequent good neurodevelopment. Holder pasteurization is routinely practiced in human milk banks (HMBs) to ensure safety of DHM but can impact the macronutrient content. The aim of this study was to explore the effect of Holder pasteurization on fat, protein, lactose and energy content of DHM and compare our data with the literature.

Methods: Protein, lactose, fats and energy of 100 DHM pools from 87 women were analyzed before and after Holder pasteurization using Miris HMA™ (Human Milk Analyzer, Miris AB, Uppsala, Sweden), with the infrared spectroscopic method. The mean macronutrient contents before and after Holder pasteurization were compared using paired t-tests, and the variations in the concentration of the components were calculated as Delta%. The data obtained were compared to other studies with the same purpose.

Results: We observed a reduction in fat (3.12 ± 1.64 vs 2.55 ± 0.85, with Delta% -14.9 ± 13.0 and p-value < 0.0001), T protein (1.05 ± 0.26 vs 0.89 ± 0.20, with Delta% -8.9 ± 63.0 and p-value < 0.0001), energy content (61.38 ± 18.66 vs 55.00 ± 8.27, with Delta% -8.1 ± 9.4 and p-value 0.0001), while no significant changes were observed for lactose content (6.35 ± 0.80 vs 6.43 ± 0.58, with Delta% 6.5 ± 56.7 and p-value 0.3735). Data in the literature on the effect of Holder pasteurization on DHM macronutrients are variable, and the only constant element is the non-variation of the carbohydrate content.

Conclusion: Holder pasteurization decreased protein, fat and energy content of DHM. The lactose content has not been affected after the Holder pasteurization. After having assessed a remarkable variation in the macronutrient content in comparison with other studies, the adjustable fortification, especially if based on the composition data, might be more accurate. In addition, despite the fact that Holder pasteurization is actually the method recommended by the international HMB guidelines, as it provides a compromise between microbiological safety and nutritional/biological quality of DHM, studies on alternative methods capable of treating DHM preserving the milk's components are desirable.

Keywords

Donor human milk, human milk pasteurization, macronutrient content.

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Introduction

The technological advances in the biological field of the last decades have made it possible to totally revolutionize the concept of breast milk, which is currently considered not only a simple nourishment for the newborn and infant, but a complex living and dynamic biological system capable of adapting to their characteristics and need [1].

If the benefits of breast milk known about health in the short and long term are numerous [2-6], these are even more valuable for premature babies, for which the extensive use of human milk is considered a life-saving element [7-14]. The milk of one's mother is to be preferred in the diet of the premature baby [15] and, when mother's milk is not available, the alternative recommended by all the scientific societies and the institutions that deal with health [16-21] is the milk donated by generous donors collected by the human milk banks (HMBs). The reality of HMBs has grown particularly in the last 10 years all over the world, and the total number of HMBs has tripled [22]. Due to the need for microbiological integrity and safety for the recipient infant, donor human milk (DHM) needs a specific pasteurization treatment. The Holder method is currently recommended by all national and international guidelines; even if it represents the best compromise between quality and microbiological safety of DHM, it seems however to impact its content in macronutrients and bioactive factors [23-27]. The present discussion articulates in this context and studies the comparison of the composition data on HMB milk before and after pasteurization. The results could represent a useful element also for the optimization of the fortification of human milk [28-32]. This is a necessary procedure to allow an adequate growth, indispensable for the correct development of the premature newborn, especially in its neurological aspect, which seems to be most affected by proper nutrition [33-36].

Description of the study

Aims

- Assessing the impact of the Holder pasteurization method on the macronutrients of DHM by statistically comparing the individual values of the nutrient components before and after pasteurization;
- comparing our data with the results of previous studies conducted for the same purpose;
- identifying operational elements useful for optimizing the enteral feeding of premature babies.

Materials and methods

The population consists of 87 women enrolled during the period from January 2015 to June 2016, according to the national ministerial guidelines followed by "AllattiamoLaVita", HMB of the Neonatal Intensive Care Unit (NICU) of "Casa Sollievo della Sofferenza" Hospital of San Giovanni Rotondo, Italy. All the donors involved in the study, as per the protocol, signed an informed consent form to donate their milk at the time of enrollment also for clinical or research use.

DHM was obtained at home using a manual or electric breast pump (prevailing method). The bottles used were disposable in sterile polypropylene and, once filled within 24 hours, were frozen in the freezer at -20°C. When transporting milk samples from home to the HMB, thanks to a home collection service, the recommended conditions related to the cold chain are met. According to the national guidelines, the time between milk collection and pasteurization cannot exceed 3 months [37].

After having created the pool with DHM of 3 different mothers, in order to increase their nutritional and caloric power, the samples to be sent to the laboratory for microbiological analysis and the samples to be submitted for macronutrient analysis before pasteurization were taken.

The pasteurization system used is the Holder method with exposure to 65.2°C for 30 minutes.

Other samples were taken at the end of the pasteurization, also this time both for microbiological investigations and for the analysis of post-pasteurization macronutrients.

The 100 samples of the study were brought to a temperature of about 37-40°C and then centrifuged for 5/8 seconds, according to the settings of the Sonicator® centrifuge (Uppsala, Sweden), so as to guarantee the best solubilization of lipids and breakage of casein micelles. Subsequently, the 100 samples were examined via Miris HMA™ (Human Milk Analyzer, Miris AB, Uppsala, Sweden), an instrument that uses an infrared spectroscopic method for the analysis of the constituents of human milk, in particular the protein content, lactose and fats expressed in g/100 ml and the energy expressed in kcal/100 ml.

The data obtained were collected in a dedicated database, created primarily for this analysis and realized with:

- sample number;
- pool lot number;
- fats (before and after pasteurization);
- crude protein (C protein: total amount of protein) (before and after pasteurization);
- lactose (before and after pasteurization);
- dry residue (DR) (before and after pasteurization);
- calories (before and after pasteurization);
- true protein (T protein: content of proteins without non-protein nitrogen) (before and after pasteurization).

The descriptive data on proteins, fats, lactose and energy were expressed as mean and standard deviation (mean ± SD), being data with normal distribution. The macronutrient content before and after pasteurization was compared using a paired t-test.

The variations in proteins, fats, lactose and energy were also calculated as a percentage of reduction (Delta%), which represents the ratio between the difference of macronutrients before and after pasteurization and the value of macronutrients before pasteurization. The t-test was performed in order to assess any difference between the decrease in proteins, fats and lactose. A p-value of 0.05 was considered significant. All statistical analyses were performed using the SPSS® software (SPSS® version 20, SPSS, Chicago, IL) from the Medical Statistics Service of "Casa Sollievo della Sofferenza".

A population study was also carried out considering some variables related to the sample of women recruited in the study, such as maternal age, gestational age at delivery, type of birth, birth weight, parity, beginning of donation and volume of DHM.

Study population

Our general population has an average age of 33.57 years, with a minimum of 17 years and a maximum of 46 years, and 36.8% of women underwent cesarean section (**Tab. 1**).

The average gestational age at birth is 37.59 weeks, with a minimum of 23 weeks and a maximum of 42 weeks, and the average birth weight was 2,963 g, with a minimum of 550 g and a maximum of 4,530 g.

In detail, the donors who gave birth prematurely are 18 (20.7%) and contributed to the donation with a total of 237,750 ml (average of 13,208 ml), which corresponds to 35% of the entire donation of this population, which amounts to 680,050 ml.

Fifty-eight women started the donation period within the first month after birth (66.7%); 24 started the donation between 1 and 3 months (27.6%); 5 women started donating after the third month (5.7%).

The women donated an average of 7,816 ml of milk, with a maximum peak of 80 liters. The minimum was 50 ml. The main share of enlisted donors (65.11%) was multiparous and the remaining part (34.89%) was primiparous.

Table 1. Population data.

		Average	Min	Max
Age of donors (y)		33.57	17	46
Gestational age (w)		37.59	23	42
Birth weight (g)		2,963	550	4,530
DHM volume (ml)		7,816	50	80,000
			No.	**%**
Parity	**Primipara**		30	34.89
	Multipara		57	65.11
Delivery	**Vaginal delivery**		55	63.2
	Cesarean section		32	36.8
Start of donation	**Within a month after birth**		58	66.7
	Between 1 and 3 months after birth		24	27.6
	After 3 months after birth		5	5.7

DHM: donor human milk.

Results

In the comparison between the values of macronutrients before and after pasteurization, C protein decreased from 1.58 ± 1.49 to 1.36 ± 1.21, T protein from 1.05 ± 0.26 to 0.89 ± 0.20, lactose from 6.35 ± 0.80 to 6.43 ± 0.58, fats from 3.12 ± 1.64 to 2.55 ± 0.85, calories from 61.38 ± 18.66 to 55.00 ± 8.27, DR from 11.23 ± 2.46 to 10.57 ± 1.28. The difference between the values before and after pasteurization was -0.5711 for fats, -0.2276 for C protein, 0.0763 for lactose, -0.6645 for DR, -6.3816 for calories and -0.1613 for T protein.

The Delta% variations were -14.9 ± 13.0 for fats, 2.7 ± 95.8 for C protein, 6.5 ± 56.7 for lactose, -4.5 ± 8.9 for DR, -8.1 ± 9.4 for calories and -8.9 ± 63.0 for T protein. The p-value was < 0.0001 for fats, 0.2553 for C protein, 0.3735 for lactose, 0.0024 for DR, 0.0001 for calories and < 0.0001 for T protein.

The greatest decrease was in fats and proteins, while lactose remained almost stable. In particular, there was a 14.9% reduction in fats, 8.9% in proteins, 4.5% in DR and 8.1% in caloric content.

These data are summarized in **Tab. 2**.

Discussion

Our study found that the concentration of macronutrients decreased significantly after pasteurization, with the exception of lactose, which has not undergone modifications.

The greatest decrease was in fats and proteins, with a 14.9% reduction in fats, 8.9% in proteins and 8.1% in calories, while lactose remained almost stable.

Since the caloric intake has also decreased and lactose has remained unchanged, we can deduce that the reduction in kcal is due to the decrease in fats.

Other studies [24-26; 37] have previously analyzed macronutrients before and after pasteurization (**Tab. 3**), where the same methods of milk analysis and pasteurization were used (Miris HMA™ [Human

Table 2. Macronutrient content before and after pasteurization (analyzed using Miris HMA™ [Human Milk Analyzer, Miris AB, Uppsala, Sweden]).

Variable	Before pasteurization	After pasteurization	Difference (after - before)	p-value	Delta%
Fats (g/100 ml)	3.12 ± 1.64	2.55 ± 0.85	-0.5711	< 0.0001	-14.9 ± 13.0
C protein (g/100 ml)	1.58 ± 1.49	1.36 ± 1.21	-0.2276	0.2553	2.7 ± 95.8
Lactose (g/100 ml)	6.35 ± 0.80	6.43 ± 0.58	0.0763	0.3735	6.5 ± 56.7
DR	11.23 ± 2.46	10.57 ± 1.28	-0.6645	0.0024	-4.5 ± 8.9
Energy (kcal/100 ml)	61.38 ± 18.66	55.00 ± 8.27	-6.3816	0.0001	-8.1 ± 9.4
T protein (g/100 ml)	1.05 ± 0.26	0.89 ± 0.20	-0.1613	< 0.0001	-8.9 ± 63.0

C protein: crude protein; DR: dry residue; T protein: true protein.

Table 3. Macronutrient content before and after pasteurization (analyzed using Miris HMA™ [Human Milk Analyzer, Miris AB, Uppsala, Sweden]): review.

Study	Variable	Before pasteurization	After pasteurization	Difference (after - before)	p-value	Delta%
Piemontese et al. [24]	Proteins (g/100 ml)	0.88 ± 0.20	0.86 ± 0.20	-	< 0.0001	-2.51 ± 13.12
	Lactose (g/100 ml)	7.19 ± 0.41	7.11 ± 0.48	-	< 0.0001	-0.92 ± 5.92
	Fats (g/100 ml)	2.91 ± 0.89	2.75 ± 0.48	-	< 0.0001	-4.79 ± 9.47
	Energy (kcal/100 ml)	60.99 ± 8.10	59.38 ± 7.81	-	< 0.0001	-2.48 ± 5.19
Vieira et al. [25]	Proteins (g/100 ml)	1.03 ± 0.39	0.99 ± 0.42	-	< 0.001	-3.9
	Lactose (g/100 ml)	6.33 ± 0.51	6.28 ± 0.54	-	0.427	-
	Fats (g/100 ml)	2.17 ± 1.46	2.05 ± 1.46	-	< 0.001	-5.5
García-Lara et al. [26]	Proteins (g/100 ml)	1.03 (0.96; 1.09)	-	-	0.61	-
	Lactose (g/100 ml)	6.03 (5.92; 6.14)	-	-	0.20	-
	Fats (g/100 ml)	4.88 (4.18; 5.58)	-	-0.17 (-0.29; -0.04)	-	3.5
	Energy (kcal/100 ml)	73.62 (67.42; 79.82)	-	-2.03 (-3.60; -0.46)	-	2.8
Adhisivam et al. [37]	T protein (g/100 ml)	1.6 ± 0.4	1.4 ± 0.3	-	0.01	12.5
	C protein (g/100 ml)	2.0 ± 0.4	1.7 ± 0.2	-	0.02	10
	Lactose (g/100 ml)	6.1 ± 0.5	5.9 ± 0.7	-	0.5	2.2
	Fats (g/100 ml)	3.6 ± 0.5	2.7 ± 0.5	-	< 0.001	25
	Energy (kcal/100 ml)	56.6 ± 6.8	47.5 ± 7.4	-	< 0.001	16

C protein: crude protein; T protein: true protein.

Milk Analyzer, Miris AB, Uppsala, Sweden]; Holder pasteurization: 62.5°C for 30 min). Our results agree with those of Piemontese et al. [24], who analyzed 191 samples of DHM pools, reporting a decrease in lipids, proteins and energy, while in our case lactose did not decrease after pasteurization. On the same trend are the data of Vieira et al., which tested 57 samples of DHM, also finding a reduction in lipids and proteins after pasteurization [25].

In the study by García-Lara et al., instead, the authors observed the reduction of fats and energy on 34 samples of frozen human milk, but not a significant decrease in proteins [26]. In the paper of Adhisivam et al. [37], the pasteurization process reduced protein, fat and energy content of pooled DHM by 12.5%, 25% and 16%, respectively, while carbohydrates were not significantly reduced.

The international and national guidelines on the activity of HMBs recommend pasteurization at 62.5°C for 30 minutes (Holder method) to ensure a safe product for the infants [38]. This method allows a good compromise between the microbiological safety and the nutritional/biological quality of DHM; however, as is known, it can affect some of the nutritional and biological properties of human milk [23].

In this regard, it is worth remembering how modern biological technologies have decreed the irreplaceability of breast milk for the nutrition of all newborns and, in particular, of premature infants, due to the myriad of nutritive and bioactive factors available. This is why the study of the possible effects of the treatment to which DHM is subjected on the many components of milk is of great interest.

In our study, we evaluated the effect on macronutrients which is fundamental to the growth and the development of premature babies [39]. The impact on bioactive factors is observed in other studies [23, 40-45], and the results are difficult to interpret; only a part of the very many factors known so far has been analyzed, and of these the percentages of reduction reported are variable. However, some factors are not affected by the treatment. The most representative example is that of oligosaccharides. They perform many biological functions, and the chromatographic pattern is totally unchanged before and after pasteurization [23]. Or the fatty acids, which are fundamental for the neurodevelopment of the premature baby and are not impacted by the treatment. However, the search for more conservative treatment systems of nutritive and bioactive factors of human milk is active, and the most promising seems to be rapid high-temperature short-time (HTST) pasteurization (just 5-15 seconds at 72°C) [46, 47]. This is a low-impact and safe pasteurization process. The biochemical quality of the milk after HTST pasteurization was evaluated with respect to the standard Holder pasteurization, determining the IgAs secretory content, the protein profile, as well as the lysozyme and the lipase stimulated by bile salts. The content of immunoglobulins and lipase were significantly higher in milk pasteurized with the new method compared to the same milk treated with Holder's standard pasteurization [48-53]. This auspicious system is in an advanced stage of validation (European Milk Bank Association [EMBA] Congress 2019).

Another method proposed is ultraviolet irradiation, which could have a potential as an alternative to Holder pasteurization in providing safe and high-quality human milk for preterm infants, but has limits, especially with respect to the quantities of milk that can be obtained. The concentrations of lactoferrin, lysozyme and IgA have been described as substantially unchanged [54, 55]. However, other studies are needed to definitively identify a valid alternative method to Holder pasteurization in the treatment of human milk.

The reduction of macronutrients after pasteurization can provide some suggestions with respect to the most suitable method of fortification for the healthy growth of the premature baby [28-32, 56-60]. The present work provides further evidence of the variability of human milk even when subjected to pasteurization, with the emblematic data of García-Lara et al. [26], in which there was even no reduction in the protein content after pasteurization, confirming the usefulness of customizing fortification, monitoring growth and all the parameters indicative of a development. In clinical practice, the most common fortification method used in the world's NICUs is the standard fortification. There is probably not enough awareness yet that the gold standard of human milk fortification for very low birth weight infants is the individualized one, which allows the amount of fortifier to be added each time in a specific way, avoiding the risk of excessive energy intake (overnutrition) or deficit (undernutrition).

In our study, the average age of women in the study population was 33.57 years, going from a minimum of 17 years to a maximum of 46 years: this underlines that there is no right age to donate, and there is no right time to start donating. Our women began to donate 3 days after giving birth or several months after birth.

Furthermore, the average gestational age of these women's pregnancies is 37.59 weeks, with a minimum of 23 weeks and a maximum of 42 weeks, with a birth weight ranging from 550 to 4,530 grams. This highlights that, even in extremely premature deliveries, if there is motivation and maternal will, and if a simple but effective protocol is followed – including breast stimulation within 6 hours of birth and systematically 8 times in 24 hours and skin-to-skin approach, as soon as the clinical conditions allow it –, it is possible to feed the premature babies exclusively with their own milk and also to donate a quota to the HMB [56]. In our population, the percentage of women who gave birth prematurely is particularly high (20.7%), and contributed to the donation of 35% of the total volume.

Moreover, milk coming from women who give birth prematurely is particularly valuable not only because it is more proteic and caloric, but mainly because it has the biological characteristics that best meet the needs of premature babies [30, 57].

The presence of a HMB in the NICU does not represent an unfavorable element for breastfeeding, rather it significantly improves the availability of mother's milk for feeding the premature baby with higher percentages [61-65].

Conclusions

The study shows that:

- Holder pasteurization reduces protein, fats and energy content in a statistically significant manner while not impacting carbohydrates;
- our data are in line with the literature, but with variability in the reduction percentages;
- the results suggest the individualized fortification is a choice for a tailor-made approach to the nutrition of the premature baby;
- the search for more conservative treatment methods of DHM is suitable;
- even mothers of premature babies, if adequately informed and supported, can donate milk.

The results of our study should not represent an element to be interpreted negatively with respect to the quality of human milk donated, but, on the contrary, it should represent a reason for improvement and optimization of the nutrition of this category of babies.

Strength of the study

The study focused on some important aspects of the use of human milk in preterm feeding, drawing two important practical implications: the choice of individualized fortification with respect to the standard fortification, which is still widely used, and the need to activate all effective protocols aimed at promoting milk production by mothers of premature babies of all gestational ages, even the lowest. There are relatively few studies in the literature today that have analyzed the macronutrient content of DHM with our method, and for this reason as well we believe this study can represent a significant contribution.

Limitations of the study

The limitation of this type of analysis is that it allows only the quantitative and non-qualitative evaluation of macronutrients; qualitative evaluation would also add valuable information.

Abbreviations

C protein: crude protein
DHM: donor human milk
DR: dry residue
EMBA: European Milk Bank Association
HMB: human milk bank
HTST pasteurization: high-temperature short-time pasteurization
NICU: Neonatal Intensive Care Unit
T protein: true protein

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Declaration of interest

The Authors declare that there is no conflict of interest.

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