www.jpnim.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2026;15(2):e150208
doi: 10.7363/150208
Received: 2026 May 18; accepted: 2026 Jul 14; published online: 2026 Aug 07

Review

Inside human milk fortifiers: composition and clinical rationale

Giulia Maiocco[1,2*], Elena Maggiora[1,2*], Sonia Deantoni[3], Guido E. Moro[4], Enrico Bertino[4], Massimo Agosti[5,6]

[1]Neonatology, University of Turin, Turin, Italy

[2]Regina Margherita Children Hospital – Sant'Anna Hospital, Turin, Italy

[3]Neonatal Intensive Care Unit and Paediatric Department, Santa Croce Hospital, Moncalieri, Italy

[4]Italian Association of Donor Human Milk Banks (*Associazione Italiana Banche del Latte Umano Donato* – AIBLUD), Milan, Italy

[5]Division of Neonatology, Woman and Child Department, "F. Del Ponte" Hospital, ASST-Settelaghi, Varese, Italy

[6]Italian Society of Neonatology (*Società Italiana di Neonatologia* – SIN), Milan, Italy

*These Authors share co-first authorship

Abstract

Human milk (HM) represents the gold standard for infant's nutrition, including preterm and very low birth weight (VLBW) infants, owing to its well-documented benefits in reducing neonatal morbidity and supporting long-term neurodevelopmental and metabolic outcomes. However, HM alone does not meet the nutritional requirements of VLBW infants as defined by current European Society for Paediatric Gastroenterology, Hepatology and Nutrition guidelines, making fortification with protein, energy, and micronutrients a widely adopted strategy in Neonatal Intensive Care Units.

This article reviews the quantitative and qualitative aspects of HM fortification, with a focus on fortifiers currently available in Italy.

From a quantitative standpoint, multicomponent fortifiers provide additional protein and carbohydrates within recommended osmolality limits, though evidence on optimal protein intake thresholds and fortification strategies remains inconclusive. Lipid supplementation, particularly with docosahexaenoic acid and arachidonic acid, is recommended to increase the caloric intake, to address long-chain polyunsaturated fatty acid deficiency and to support neurodevelopment.

Regarding qualitative aspects, bovine milk-derived fortifiers remain the standard of care despite the fact that exposure to bovine milk-derived products is not optimal with respect to tolerance and the reduction of the risk of necrotizing enterocolitis. HM-derived fortifiers, despite their theoretical advantages, have not demonstrated clear superiority over bovine milk-derived products in terms of morbidity or growth outcomes, and raise significant ethical and economic concerns. Donkey milk-derived fortifiers

represent an emerging and promising alternative, given their compositional similarity to HM, hypoallergenic properties, and preliminary clinical evidence showing improved feeding tolerance with comparable auxological and neurodevelopmental outcomes in VLBW infants.

Future perspectives point toward the industrial development of donkey milk-based fortifiers as a clinically feasible and ethically sustainable alternative to current bovine milk-derived formulations.

Keywords

Human milk fortification, preterm nutrition, very low birth weight infants, bovine milk-derived fortifier, human milk-derived fortifier, donkey milk-derived fortifier.

Corresponding author

Sonia Deantoni, Neonatal Intensive Care Unit and Paediatric Department, Santa Croce Hospital, Moncalieri, Italy; email: deantoni.so09@gmail.com.

How to cite

Maiocco G, Maggiora E, Deantoni S, Moro GE, Bertino E, Agosti M. Inside human milk fortifiers: composition and clinical rationale. J Pediatr Neonat Individual Med. 2026;15(2):e150208. doi: 10.7363/150208.

Introduction

Human milk (HM) represents the gold standard for the nutrition of all neonates. The American Academy of Pediatrics (AAP) and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommend its use also in preterm and very low birth weight (VLBW) infants, prioritizing mother's own milk given its advantages over formula feeding in terms of reduced incidence of necrotizing enterocolitis (NEC), sepsis, bronchopulmonary dysplasia, and retinopathy of prematurity, as well as long-term neurodevelopmental, metabolic, immunological, psychological, social, and economic benefits [1, 2].

When mother's own milk is unavailable or insufficient, donor HM (DHM) obtained from a recognized HM bank [1] is the recommended alternative, owing to its well-documented benefits, including a reduced risk of NEC [3], improved enteral tolerance [4], higher rates of breastfeeding at discharge [5], and a possible protective effect against bronchopulmonary dysplasia [6].

However, HM alone is not sufficient to meet the nutritional requirements of preterm infants, as defined by the ESPGHAN 2022 guidelines [2]. In this context, fortification of HM with protein, energy, and micronutrients represents an effective strategy to achieve the nutritional and auxological targets of VLBW infants and is now widely adopted in Neonatal Intensive Care Units (NICUs) [1-30].

Quantitative aspects: which formulations?

The recommended protein intake for a clinically stable infant with a birth weight < 1,800 g is 3.5-4 g/kg/day, and in selected cases an increase up to 4.5 g/kg/day may be considered [2].

The protein content of HM varies with time since delivery and gestational age at birth; in milk from mothers of preterm infants, it averages 1-1.9 g/100 mL [7].

Therefore, even when fed according to recommended milk volumes (150-180 mL/kg/day), preterm infants do not meet their protein requirements with HM alone (these volumes provide approximately 1.8-2.2 g/kg/day of proteins) [7].

Multicomponent HM fortifiers currently available in Italy provide additional proteins, ranging from 1.3 to 1.4 g per 100 mL of HM when used at standard concentrations (4%) [8, 9]. A 2020 *Cochrane* review showed that the use of high-protein fortifiers (≥ 1.4 g/100 mL HM) is associated with faster in-hospital growth compared with moderate-protein (1-1.4 g/100 mL HM) or low-protein (< 1 g/100 mL HM) fortifiers [10]. However, the overall level of evidence is low to moderate, and it remains debated whether a "ceiling effect" exists beyond a certain level of protein intake [11], which in any case should be considered in relation to energy intake (a protein-to-energy ratio between 3 and 3.6 g/100 kcal is considered appropriate) [12].

Given the wide temporal, intra-individual, and inter-individual variability in HM protein content, a standardized fortification approach is often suboptimal in providing adequate nutrient intake. For this reason, different fortification strategies (targeted or adjustable) are currently used [8-13], although they are beyond the scope of this article.

Carbohydrates provide approximately 45-50% of the non-protein energy in HM, with an adequate intake estimated at 11-15 g/kg/day in infants with a birth weight < 1,800 g [2].

Multicomponent HM fortifiers currently available in Italy contain a variable proportion of carbohydrates, ranging from 32% to 37%. In terms

of safety, even fortifiers with higher carbohydrate content do not exceed the osmolality limit recommended by the AAP (< 450 mOsm/kg) within the first 24 hours after reconstitution with fresh HM at standard concentration (4%), although manufacturers recommend their use within a short time frame (maximum 4 hours) [14].

Oligosaccharides, which play an important role in immunity and microbiota modulation, are not included in fortifiers but are naturally present in HM and are preserved despite freezing or pasteurization [15].

Regarding lipids, mature HM is generally able to meet the nutritional requirements of preterm infants (4.8-8.1 g/kg/day in infants with a birth weight < 1,800 g) when administered at recommended volumes (150-180 mL/kg/day of HM provide approximately 5.3-6.3 g/kg/day of lipids) [2, 7].

Since preterm infants are at risk of long-chain polyunsaturated fatty acid (LC-PUFA) deficiency, supplementation with docosahexaenoic acid (DHA) and arachidonic acid (ARA) is recommended to improve growth quality and neurodevelopment, with an ARA/DHA ratio of 0.5-2 [2].

A recent randomized controlled trial evaluated a multicomponent fortifier characterized by higher protein content (1.3 vs 1.1 g/100 mL HM) and increased lipid content (DHA and ARA) at the expense of carbohydrates, showing comparable growth and similar feeding tolerance, with the advantage of reduced osmolality and improved lipid quality intake [16].

The macronutrient composition of individual fortifiers is summarized in **Tab. 1**.

All fortifiers currently marketed in Italy are in powder form.

Following reports of possible contamination with *Enterobacter sakazakii*, some fortifiers (not available in Italy) have been developed in liquid form; the main drawback of these formulations is that they partially replace HM, thereby reducing its proportion in the infant's diet [8].

Table 1. Macronutrient intakes provided by different types of multicomponent fortifiers (MCF) and protein concentrates (PC) per 100 mL of fortified preterm human milk (HM).

		Macronutrient intakes per 100 mL of HM			
		Energy (kcal)	Proteins (g)	Carbohydrates (g)	Lipids (g)
Preterm HM [a]		67.0	1.6	7.3	3.5
		Macronutrient intakes after addition of MCF/PC to obtain a total amount of 100 mL of fortified HM			
		Energy (kcal)	Proteins (g)	Carbohydrates (g)	Lipids (g)
Preterm HM + bovine milk-based MCF/PC	Preterm HM + MCF Aptamil BMF (concentration [g per 100 mL of HM]: 4%)	84.2	2.9	8.8	4.2
	Preterm HM + MCF PreNan FM85 (concentration [g per 100 mL of HM]: 4%)	84.4	3.0	8.6	4.2
	Preterm HM + PC Aptamil PS (concentration [g per 100 mL of HM]: 1.2%)	71.1	2.6	7.3	3.5
Preterm HM + donkey milk-based MCF/PC [b]	Preterm HM + MCF Fortilat DMC (concentration [g per 100 mL of HM]: 4%)	82.6	2.5	9.7	3.6
	Preterm HM + PC Fortilat (concentration [g per 100 mL of HM]: 1.2%)	72.0	2.1	7.7	3.6
Preterm HM + HM-based MCF	Preterm HM + MCF Prolact+4 H²MF (concentration [mL per 80 mL of HM]: 25%) [c]	83	2.5	7.7	4.7
	Preterm HM + MCF NeoKare MMF (concentration [g per 100 mL of HM]: 4%) [d]	84.2	2.7	8.9	4.2

HM: human milk; MCF: multicomponent fortifiers; PC: protein concentrates.

Concentrations: the concentrations of bovine milk-derived MCF and PC correspond to the maximum levels generally recommended [9]; the concentrations of donkey milk-derived MCF and PC correspond to the maximum levels recommended according to available evidence [24-27]; the concentrations of HM-derived MCF refer to the manufacturers' recommended formulations: 25% for the 20 mL Prolact+4 H²MF formulation (according to the manufacturer's instructions, each bottle of Prolact+4 H²MF [20 mL] should be mixed with 80 mL of HM [1:4 ratio]; for consistency with the other fortifiers, in this table values were proportionally calculated for 100 mL of fortified HM [corresponding to 20 mL of fortifier]) [29] and 4% for the 1 g NeoKare MMF formulation (according to the manufacturer's instructions, 1 g should be mixed with 25 mL of HM) [30]. In light of the patient's clinical condition and the defined fluid intake allowance, the concentration of HM fortification may be titrated to meet the nutritional targets set by ESPGHAN recommendations [2]. References: [a] see reference [28]; [b] MCF and PC based on donkey milk patented in Italy, not commercially available [24-27]; [c] see reference [29]; [d] see reference [30].

Qualitative aspects: which milk sources?

Traditionally, HM fortifiers are derived from bovine milk. The main protein source consists of whey proteins, selected for their superior digestibility compared with caseins. The latter are sometimes included for their ability to slow gastric emptying, resulting in a more prolonged release of amino acids.

Bovine proteins used in fortifiers are usually subjected to partial or extensive hydrolysis, as this is thought to improve tolerance and reduce inflammatory and allergenic potential [8]. However, hydrolysis alters the bioactive properties of peptides and may also impair product palatability. From an immunological perspective, the degree of hydrolysis is not a reliable predictor of sensitizing potential, as other physicochemical properties – such as peptide size distribution and surface hydrophobicity – also influence immunogenicity [13].

Exclusive HM feeding has been shown to reduce the risk of adverse events, particularly NEC, compared with feeding based on bovine milk products. Therefore, to ensure an exclusively HM-based diet in the most vulnerable infants, over the past 20 years a US and a UK company have developed HM-derived fortifiers. However, current evidence does not demonstrate their superiority over bovine milk-derived fortifiers in terms of morbidity (including NEC risk) or mortality, nor a clear advantage in growth outcomes [17-19].

The commercialization of DHM and its derivatives also raises ethical concerns. In Italy, the national guidelines for the organization and management of HM banks, published in the Official Gazette (*Gazzetta Ufficiale*) on 8 February 2014 [20], state that DHM cannot be commercialized. Furthermore, the recent *European Parliament and Council Regulation on quality and safety standards for substances of human origin* (SoHO) adopted in June 2024 (*Regulation 2024/1938*) [21], includes DHM, classifying it as a human tissue and consequently excluding it from commercial use.

The high production costs of HM-derived fortifiers must also be considered.

An interesting alternative to bovine milk-derived fortifiers and to HM-derived fortifiers is donkey milk. Among mammalian species, donkey milk has the closest composition to HM in terms of carbohydrates, lipids, and protein profile. Its chemical, biological, and nutritional characteristics have led to increasing interest in its use in clinical nutrition, particularly in children with cow's milk protein allergy [22]. Given its biological properties and demonstrated clinical benefits, donkey milk appears to be a suitable candidate for HM fortifier formulation.

Some bovine milk-derived fortifiers include industrial modulation of the whey-to-casein ratio to better resemble that of HM; this is unnecessary for donkey milk-based fortifiers, as this ratio is already comparable to that of HM. Moreover, individual proteins in donkey milk show much higher sequence homology with HM proteins than those of bovine milk and other ruminants. This homology underlies the hypoallergenic properties of donkey milk and allows avoidance of protein hydrolysis, thereby preserving bioactive properties [22, 23].

An Italian randomized controlled trial demonstrated that supplementation with a donkey milk-derived HM fortifier, isocaloric and isoproteic compared with a bovine counterpart, provides improved feeding tolerance with comparable auxological outcomes in VLBW infants [24]. Subsequent studies in the same population also showed comparable neurodevelopmental outcomes at 18 months and a protective effect against gastroesophageal reflux, with no differences in growth patterns compared with bovine milk-derived fortifiers [24-27].

This donkey milk-based fortifier has been the subject of an Italian patent but is not yet commercially available. Considering its biological characteristics and observed clinical benefits, it appears promising and opens interesting perspectives for future clinical use.

Conclusions

At present, in Italy, the available HM fortifiers are exclusively derived from bovine milk. Looking ahead, the most promising alternatives are formulations based on HM or donkey milk. However, the limitations associated with HM-derived fortifiers are notable, including high costs and ethical concerns related to their commercialization, which is already prohibited in some countries, such as Italy, and by recent European SoHO regulations.

The prospect of industrial production and distribution of donkey milk-based fortifiers may represent a reasonable compromise in terms of cost and feasibility in clinical practice.

Declaration of interest

The Authors declare that they have no conflicts of interest relevant to the content of this article. This work received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Moro GE, Arslanoglu S, Bertino E, Corvaglia L, Montirosso R, Picaud JC, Polberger S, Schanler RJ, Steel C, van Goudoever J, Ziegler EE; American Academy of Pediatrics; European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. XII. Human Milk in Feeding Premature Infants: Consensus Statement. J Pediatr Gastroenterol Nutr. 2015;61(Suppl 1):S16-9.
2. Embleton ND, Jennifer Moltu S, Lapillonne A, van den Akker CHP, Carnielli V, Fusch C, Gerasimidis K, van Goudoever JB, Haiden N, Iacobelli S, Johnson MJ, Meyer S, Mihatsch W, de Pipaon MS, Rigo J, Zachariassen G, Bronsky J, Indrio F, Köglmeier J, de Koning B, Norsa L, Verduci E, Domellöf M. Enteral Nutrition in Preterm Infants (2022): A Position Paper From the ESPGHAN Committee on Nutrition and Invited Experts. J Pediatr Gastroenterol Nutr. 2023;76(2): 248-68.
3. Quigley M, Embleton ND, Meader N, McGuire W. Donor human milk for preventing necrotising enterocolitis in very preterm or very low-birthweight infants. Cochrane Database Syst Rev. 2024;9(9):CD002971.
4. Quigley M, Embleton ND, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev. 2019;7(7):CD002971.
5. Williams T, Nair H, Simpson J, Embleton N. Use of Donor Human Milk and Maternal Breastfeeding Rates: A Systematic Review. J Hum Lact. 2016;32(2):212-20.
6. Villamor-Martínez E, Pierro M, Cavallaro G, Mosca F, Kramer BW, Villamor E. Donor Human Milk Protects against Bronchopulmonary Dysplasia: A Systematic Review and Meta-Analysis. Nutrients. 2018;10(2):238.
7. Boyce C, Watson M, Lazidis G, Reeve S, Dods K, Simmer K, McLeod G. Preterm human milk composition: a systematic literature review. Br J Nutr. 2016;116(6):1033-45.
8. Adamkin DH. Use of human milk and fortification in the neonatal intensive care unit. Semin Fetal Neonatal Med. 2025;30(2):101632.
9. De Rose DU, Maggiora E, Maiocco G, Morniroli D, Vizzari G, Tiraferri V, Coscia A, Cresi F, Dotta A, Salvatori G, Giannì ML. Improving growth in preterm infants through nutrition: a practical overview. Front Nutr. 2024;11:1449022.
10. Gao C, Miller J, Collins CT, Rumbold AR. Comparison of different protein concentrations of human milk fortifier for promoting growth and neurological development in preterm infants. Cochrane Database Syst Rev. 2020;11(11):CD007090.
11. Embleton ND, van den Akker CHP. Protein intakes to optimize outcomes for preterm infants. Semin Perinatol. 2019;43(7):151154.
12. Embleton ND. Optimal protein and energy intakes in preterm infants. Early Hum Dev. 2007;83(12):831-7.
13. Arslanoglu S, Boquien CY, King C, Lamireau D, Tonetto P, Barnett D, Bertino E, Gaya A, Gebauer C, Grovslien A, Moro GE, Weaver G, Wesolowska AM, Picaud JC. 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 22;7:76.
14. Elia S, Ciarcià M, Cini N, Luceri F, Mattei ML, Miselli F, Perugi S, Fanelli A, Dani C. Effect of fortification on the osmolality of human milk. Ital J Pediatr. 2023;49(1):72.
15. Hahn WH, Kim J, Song S, Park S, Kang NM. The human milk oligosaccharides are not affected by pasteurization and freeze-drying. J Matern Fetal Neonatal Med. 2019;32(6):985-91.
16. Picaud JC, Reynolds PR, Clarke P, van den Hooven E, van Weissenbruch MM, van Lingen RA, Goedhart A, Botma A, Boettger R, van Westering-Kroon E, Fusch C, Hascoet JM; RENOIR Study Group. A novel human milk fortifier supports adequate growth in very low birth weight infants: a non-inferiority randomised controlled trial. Arch Dis Child Fetal Neonatal Ed. 2025;110(5):512-9.
17. Reyes SM, Paul TL, Ferry J. Human Milk Fortification and Necrotizing Enterocolitis in Very Low Birthweight Infants: State of Evidence and Systematic Review with Meta-Analysis. Nutrients. 2025;17(21):3384.
18. Huizing MJ, Vizzari G, Bartoš F, Cavallaro G, Gianni ML, Villamor E. Absence of Evidence of Beneficial Effects of Human Milk-Based Fortifier: A Bayesian Model-Averaged Meta-Analysis. Acta Paediatr. 2025;114(12):3309-16.
19. Galis R, Trif P, Mudura D, Mazela J, Daly MC, Kramer BW, Diggikar S. Association of Fortification with Human Milk versus Bovine Milk-Based Fortifiers on Short-Term Outcomes in Preterm Infants – A Meta-Analysis. Nutrients. 2024;16(6):910.
20. Ministero della Salute, Comitato Nazionale Multisettoriale per l'allattamento materno. Linee di indirizzo nazionale per l'organizzazione e la gestione delle banche del latte umano donato nell'ambito della protezione, promozione e sostegno dell'allattamento al seno. Available at: https://www.salute.gov.it/imgs/C_17_pubblicazioni_2127_allegato.pdf, date of publication: 8 February 2014, last access: July 2026.
21. European Parliament and Council. Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for substances of human origin intended for human application and repealing Directives 2002/98/EC and 2004/23/EC. Available at: https://eur-lex.europa.eu/eli/reg/2024/1938/oj/eng, current consolidated version: 17 July 2024, last access: July 2026.
22. Monti G, Bertino E, Muratore Cristina M, Coscia A, Cresi F, Silvestro L, Fabris C, Fortunato D, Giuffrida Gabriella M, Conti A. Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: an in vivo and in vitro study. Pediatr Allergy Immunol. 2008;19(1):90-1.
23. Peila C, Spada E, Deantoni S, Borsani M, Asteggiano M, Chiale F, Moro GE, Giribaldi M, Cavallarin L, Cortinovis I, Coscia A. The Use of a Novel Donkey Milk-Derived Human Milk Fortified in the Neonatal Period Had No Effect on the Frequency of Allergic

Manifestations During the First Years of Life: The "Fortilat Trial" Follow-Up. Breastfeed Med. 2024;19(3):223-7.

24. Bertino E, Cavallarin L, Cresi F, Tonetto P, Peila C, Ansaldi G, Raia M, Varalda A, Giribaldi M, Conti A, Antoniazzi S, Moro GE, Spada E, Milani S, Coscia A. A Novel Donkey Milk-derived Human Milk Fortifier in Feeding Preterm Infants: A Randomized Controlled Trial. J Pediatr Gastroenterol Nutr. 2019;68(1):116-23.
25. Cresi F, Maggiora E, Pirra A, Tonetto P, Rubino C, Cavallarin L, Giribaldi M, Moro GE, Peila C, Coscia A. Effects on Gastroesophageal Reflux of Donkey Milk-Derived Human Milk Fortifier Versus Standard Fortifier in Preterm Newborns: Additional Data from the FortiLat Study. Nutrients. 2020;12(7):2142.
26. Peila C, Spada E, Deantoni S, Iuliano E, Moro GE, Giribaldi M, Cavallarin L, Cresi F, Coscia A. The "Fortilat" Randomized Clinical Trial Follow-Up: Neurodevelopmental Outcome at 18 Months of Age. Nutrients. 2020;12(12):3807.
27. Peila C, Spada E, Bertino E, Deantoni S, Percivati F, Moro GE, Giribaldi M, Cavallarin L, Cresi F, Coscia A. The "Fortilat" Randomized Clinical Trial Follow-Up: Auxological Outcome at 18 Months of Age. Nutrients. 2020;12(12):3730.
28. Koletzko B, Wieczorek S, Cheah FC, Domellöf M, van Goudoever JB, Poindexter BB, Vain N. Recommended Nutrient Intake Levels for Preterm Infants. World Rev Nutr Diet. 2021;122:191-7.
29. Prolacta Bioscience Inc. Supplemental product information: Prolact+4 H2MF (20ml). Available at: https://www.prolacta.com/en/resource-library/supplemental-product-information-prolact4-h2mf-20ml/, last access: July 2026.
30. NeoKare Nutrition Ltd. Human milk-derived fortifier from NeoKare Nutrition. Available at: https://neokare.co.uk/pdf/latest/Neokare-mmf.pdf, last access: July 2026.