ORIGINAL ARTICLE

Comparative growth outcomes in very low birth weight preterm infants fed an exclusive human milk diet versus bovine milk-based fortification

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ABSTRACT

Background and aim: Human milk feeding in preterm infants reduces the risk of several neonatal morbidities. However, human milk does not provide sufficient macro- and micronutrients to meet the nutritional requirements of preterm infants. Evidence indicates that, compared with the use of bovine milk-based fortifiers (BMFs), human milk-based fortifiers (HMFs) are associated with a reduced incidence of necrotizing enterocolitis, late-onset sepsis, and mortality, as well as improved feeding tolerance and neurodevelopmental outcomes. Nonetheless, few studies have addressed growth in preterm infants fed an exclusive human milk diet. This study aimed to compare growth rates in very low birth weight (VLBW) infants receiving human milk fortified with an HMF or a BMF.

Methods: This retrospective cohort analysis included preterm infants born with a birth weight ≤1,500 g between January 1, 2021, and December 31, 2024, and admitted to the neonatal intensive care unit. Infants received human milk fortified with either an HMF or a BMF according to standard protocols. The primary outcomes were weight, head circumference, and length growth velocities.

Results: No significant differences in weight, head circumference, or length growth velocities were observed between the two groups until discharge. However, the HMF group had a noticeably slower rate of weight gain before fortifiers were discontinued. Since the duration of fortification varied, assessing growth velocity from birth to discharge may be more clinically meaningful.

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Conclusions: Overall growth from birth to discharge is comparable between HMFs and BMFs in VLBW infants, although early weight gain is reduced with HMFs. (www.actabiomedica.it)

Key words: exclusive human milk diet, very low birth weight, human milk-based fortifier, bovine milk-based fortifier, preterm infant growth

Introduction

Breast milk is recommended for all infants (1). However, it is even more important for the optimal growth and development of premature infants (2). Evidence indicates that human milk has many benefits for preterm infants, including improved feeding tolerance, enhanced gut maturation, a healthy gut microbiome, and improved immune function. These benefits collectively contribute to a reduced risk of several neonatal morbidities and improved neurodevelopmental outcomes (3,4). Premature infants receiving milk from their mother have better feeding tolerance and a lower incidence of necrotizing enterocolitis (NEC) than those fed preterm formula (5). Nevertheless, human milk alone does not supply sufficient macro- and micronutrients to meet the specific nutritional needs of preterm infants (6,7). Consequently, fortifying human milk with multicomponent fortifiers that provide additional protein, calories, electrolytes, and vitamins is necessary. The fortification of human milk helps prevent postnatal growth failure, reduces the risk of mineral deficiencies, and supports optimal long-term neurodevelopmental outcomes (8). Traditionally, fortifiers used for breast milk have been sourced from bovine milk (9). However, in recent years, interest in fortifiers derived from human milk has increased. A meta-analysis of four studies involving 681 infants found significantly lower mortality among those fed human milk-based fortifiers (HMFs) than among those fed bovine milk-based fortifiers (BMFs) (10). Furthermore, a multicenter retrospective cohort study of 1,587 preterm infants with a birth weight <1,250 g found a significantly lower incidence of NEC and mortality among extremely premature infants fed an exclusive human milk (EHM) diet. The EHM group also showed a reduction in late-onset sepsis, bronchopulmonary dysplasia (BPD), and retinopathy of prematurity (ROP) (11). An EHM diet might be associated with better feeding tolerance; a shorter time to achieve full enteral feedings, leading to a shorter hospital stay; and lower overall costs compared with BMFs and formula (12). The composition of HMFs differs from that of BMFs, which may impact growth and weight gain in preterm infants. Few studies have examined growth in preterm infants on EHM diets. A retrospective study of infants under 32 weeks of gestation found that starting fortification at less than 26 kcal/oz on an EHM diet resulted in poorer growth. Fortifying at ≥26 kcal/oz improved weight gain and produced similar length and head circumference growth compared with those of the non-EHM group (13). This study aimed to compare growth rates in very low birth weight (VLBW) infants fed human milk fortified with an HMF or a BMF.

Methods

This retrospective cohort study included preterm infants with a birth weight of ≤1,500 g, born between January 1, 2021, and December 31, 2024, and admitted to the neonatal intensive care unit (NICU) at a hospital for obstetrics and gynecology. The study excluded preterm infants with multiple major congenital anomalies, congenital heart disease, inborn errors of metabolism, or conditions requiring surgery within the first week, as well as those who died or received palliative care within the same period. The EHM diet group received mother's own breast milk supplemented with an HMF (Humavant+6), while the control group received mother's own breast milk supplemented with a BMF (PreNAN). Neither group received donor breast milk. The groups were matched for birthweight (±100 g) and gestational age (±7 days). Maternal and neonatal characteristics were collected, including mode of delivery, birth weight, gestational age, sex, Apgar scores at 1 and 5 min, singleton status, premature rupture of membranes, maternal group B streptococcus status, receipt of antenatal steroids, presence of preeclampsia, gestational diabetes, small for gestational age, and evidence of absent or reversed end-diastolic flow velocities on antenatal Doppler studies. The NICU feeding guideline for VLBW infants recommends initiating minimal enteral feeding at approximately 20 ml/kg/day immediately after birth when expressed breast milk is available or within 48 h if donor breast milk or formula is utilized, unless contraindicated by clinical status. Minimal enteral feedings may be maintained for up to 7 days and are not intended for nutritional purposes. The guideline recommends advancing feeds as early as clinically feasible when the preterm infant is stable and not acutely ill. Additionally, initiation of total parenteral nutrition is recommended within 24 h of birth. A standardized feeding protocol is followed for VLBW infants, starting with a cautious advancement rate of approximately 20 ml/kg/day during the first 3 days, which is subsequently increased to approximately 30 ml/kg/day. The HMF was introduced when the infant achieved approximately 60 ml/kg/day of feeds at 0.88 kcal/ml (26 kcal/oz), following parental consent. BMF was initiated once feeding reached approximately 100 ml/kg/day, beginning with one pack per 50 ml of breast milk (22 kcal/oz) for 2–3 days and then increasing to one pack per 25 ml (24 kcal/oz). In accordance with the guideline, human milk-based fortifiers (HMF) are to be gradually substituted with bovine-based fortifiers (BMF) over a three-day period when infants reach 32 weeks gestational age. The fortification protocol was maintained without modification from 2021 through 2024. The initiation times of feeding, the timing of fortifier introduction, and the timing of fortifier discontinuation were documented for all eligible infants. The time to achieve full enteral feeding was defined as the number of days required to reach a minimum enteral intake of 150 mL/kg/day. The primary outcomes were growth velocities of weight, head circumference, and length. These were calculated from birth to fortifier cessation and discharge and compared between the BMF and HMF groups. Weight velocity (g/kg/day) was calculated using the exponential method (14). The length and head circumference velocities (cm/week) were also measured. Growth velocity z scores were based on Fenton curves for infants discharged before 50 weeks of postmenstrual age (PMA) or World Health Organization curves for those discharged after 50 weeks of PMA (15,16). The secondary outcomes included NEC, defined as Bell's stage IIA or greater; BPD grade 2, defined as requiring nasal cannula or non-invasive positive airway pressure at 36 weeks of PMA; severe BPD, defined as requiring invasive mechanical ventilation at 36 weeks of PMA; late-onset sepsis (LOS), defined as a positive blood culture obtained after 72 h of life; and severe intraventricular hemorrhage (IVH), defined as grade 3 or greater and diagnosed by head ultrasound interpreted by a certified radiologist.

Statistical considerations and data analysis

The distribution of variables is summarized using numbers and percentages, means and standard deviations, or medians and interquartile ranges (IQRs), as appropriate. Differences in outcomes and other covariates between the two groups were examined using the chi-square test, Fisher's exact test, t-test, or the Mann–Whitney U test, as appropriate. Statistical analyses were performed using IBM SPSS, version 26 (IBM Corp., Armonk, NY, USA).

Results

The final cohort comprised 26 infants, with a median (IQR) gestational age of 27 weeks (25–30) and a median (IQR) birth weight of 730 g (700–1100). No significant differences in maternal or neonatal characteristics were observed between the HMF and BMF groups (Table 1).

Infants in the HMF group received their first minimal enteral feeding at a median of 2 days, reached full feeds in 10 days, and started receiving the HMF at 7 days. In comparison, the BMF group began minimal enteral feeding after 2 days, achieved full feeds

Table 1. Maternal and neonatal characteristics

Characteristic	HMF (n=13)	BMF (n=13)	p value
Gestational age (week)	26 (24–29)	27 (25–30)	0.479
Birth weight (g)	830 (700–1125)	830 (700–1020)	0.92
Birth length (cm)	31 (31–34)	34 (31–35)	0.545
Birth Head circumference (cm)	23.0 (22.0–26.0)	24 (22–25)	0.801
BW < 3rd centile	1 (7.7)	0 (0.0)	NS
Male	4 (30.8)	5 (38.5)	1
Caesarean delivery	6 (46.2)	7 (53.8)	0.695
Apgar score 1 min	6 (4–7)	6 (4–6)	0.687
Apgar score 5 min	8 (7–8)	7 (7–8)	0.204
Multiple	4 (30.8)	2 (15.4)	0.645
PROM	3 (23.1)	2 (15.4)	1
Chorioamnionitis	1 (7.7)	0 (0.0)	NS
Maternal GBS	1 (7.7)	0 (0.0)	NS
Antenatal steroids	11 (84.6)	11 (84.6)	1
Preeclampsia/eclampsia/HELLP	3 (23.1)	5 (38.5)	0.673
Antepartum hemorrhage	0 (0.0)	4 (30.8)	NS
Gestational diabetes	4 (30.8)	3 (23.1)	0.658
Abnormal doppler	1 (7.7)	3 (23.1)	0.593

Data are presented as median (IQR) or number (percentage). *Abbreviations:* BMF, Bovine milk–derived fortifier; BW, Birth weight; GBS, Group B Streptococcus; HELLP, Hemolysis, Elevated Liver enzymes, and Low Platelets; HMF, Human milk–derived fortifier; IQR, Interquartile range; NS, not significant; PROM, Premature rupture of membranes.

in 8 days, and started receiving the BMF at 12 days. Feeding increments followed protocol for both groups. No significant differences were observed between the HMF and BMF groups in terms of the weight (12.4 [11.4–13.5] versus 12.8 [12.1–14.2] g/kg/day), head circumference (0.79 [0.70–0.85] versus 0.71 [0.65–0.88] cm/week), or length (0.96 [0.85–1.12] versus 0.88 [0.81–1.20] cm/week) growth velocity until discharge. These findings remained consistent even after adjusting for the duration of fortification. The adjusted Beta [95% confidence interval] for weight was 2.8 [-0.542–6.142] with a P-value of 0.096, for length was 0.141 [-0.133–0.41] with a P-value of 0.298, and for head circumference was 0.043 [-0.176–0.261] with a P-value of 0.688. In contrast, the weight growth velocity until fortifier discontinuation was lower in the HMF group than in the BMF group (7.6 [5.90–9.50] versus 12.6 [11.9–14.3] g/kg/day, p < 0.001 (Table 2).

Fortification duration differed notably between groups: the HMF group had a median of 18 (10–29) days, while the BMF group had 45 (22–54) days (p = 0.029). Given this significant difference in the duration of fortification, the growth velocity measured from birth to discharge may be a more clinically meaningful parameter for comparing outcomes between the two groups. No significant differences in NEC, BPD, IVH, hospital stay, or pre-discharge mortality were observed between the two groups. The BMF group showed non-significant increases in ROP and LOS, whereas the HMF group demonstrated a non-significant increase in the duration of mechanical ventilation (Table 3). However, because the sample size was very small, these findings are treated as exploratory observations rather than definitive evidence of equivalence and should be considered as hypothesis-generating.

Table 2. Primary outcomes

	HMF (n=13)	BMF (n=13)	P value	Adjusted Beta (95% CI) & P value
Age at start (days of life)	2 (2–2)	2 (1–2)	0.579	
Age at full feed (days of life)	10 (9–15)	8 (8–10)	0.034	
Age at fortification (days of life)	7 (6–8)	12 (9–14)	0.002	
Volume EBM at fortification (ml/kg/day)	80 (60–100)	160 (150–180)	<0.001	
Age at fortifier discontinuation (days of life)	28 (16–45)	54 (34–65)	0.014	
Weight at fortifier discontinuation (grams)	1150 (950–1310)	1760 (1520–1915)	<0.001	
Weight at fortifier discontinuation Z score	-1.35 (-1.76 – -1.01)	-1.92 (-2.46 – -1.68)	0.029	
Weight velocity until fortifier discontinuation*	7.60 (5.90–9.90)	12.60 (11.90–14.30)	<0.001	
Weight at discharge or death (grams)	2100 (1855–2510)	2080 (1900–2160)	0.92	
Weight at discharge or death z-score	-1.72 (-2.19 – -1.16)	-2.15 (-2.40 – -1.56)	0.448	
Weight velocity until discharge*	12.4 (11.4–13.5)	12.8 (12.1–14.20)	0.448	2.8 (-0.542 - 6.142) [P=0.096]
Length velocity until discharge#	0.96 (0.85–1.12)	0.88 (0.81–1.20)	0.801	0.141 (-0.133 - 0.41) [P=0.298]
HC velocity until discharge#	0.79 (0.70–0.85)	0.71 (0.65–0.88)	0.96	0.043 (-0.176 - 0.261) [P=0.688]

Data are presented as median (IQR). *g/kg/day, # cm/week. *Abbreviations:* BMF, Bovine milk–derived fortifier; cm/week, centimeters per week; EBM, Expressed breast milk; g/kg/day, grams per kilogram per day; HC, Head circumference; HMF, Human milk–derived fortifier; IQR, Interquartile range; NS, not significant

Table 3. Secondary outcomes

	HMF (n=13)	BMF (n=13)	p value
Age at discharge	67 (42–104)	69 (43–80)	0.84
NEC ≥ stage 2	2 (15.4)	2 (15.4)	1
ROP ≥ stage 3	0 (0.0)	2 (16.7)	NS
BPD ≥ grade II	2 (16.7)	2 (16.7)	1
LOS	5 (38.5)	7 (53.8)	0.431
IVH ≥ grade III	3 (23.1)	2 (15.4)	1
Days of mechanical ventilation	7.0 (0.6–26.0)	2.0 (0.7–14.0)	0.699
Death	1 (7.7)	1 (7.7)	1

Data are presented as median (IQR) or number (percentage). *Abbreviations:* BMF, Bovine milk–derived fortifier; BPD, Bronchopulmonary dysplasia; HMF, Human milk–derived fortifier; IQR, Interquartile range; IVH, Intraventricular hemorrhage; LOS, Late-onset sepsis; NEC, Necrotizing enterocolitis; NS, not significant; ROP, Retinopathy of prematurity. Missing data: ROP (2), BPD (2), Mechanical ventilation (not required in 4 cases)

Discussion

This retrospective study found no significant difference in growth rates (weight, head circumference, or length) between infants given human milk fortifiers and those given bovine milk fortifiers, even after accounting for fortification duration. However, a notable distinction emerged when assessing weight growth velocity up to the point of fortifier discontinuation. Infants in the HMF group exhibited a lower weight growth velocity compared to those in the BMF group. The study also highlighted considerable variation in the duration of fortification between the two groups. This difference in fortification length underscores the importance of selecting appropriate intervals for evaluating growth outcomes. Given this substantial divergence, assessing growth velocity from birth to discharge is likely to provide a more clinically meaningful comparison of growth between infants receiving HMF and those receiving BMF. Our findings are consistent with those of Eibensteiner et al., who examined the impact of HMFs versus BMFs on the growth of extremely low birth weight (<1,000 g) infants (17). They found that the growth velocity from the initiation of fortification until 32+0 weeks was significantly lower in the HMF group than in the BMF group, whereas all growth parameters were comparable between the groups from birth to 37+0 weeks. Both groups had an equal fortification period. A retrospective study by Chou et al. found that starting fortification at less than 26 kcal/oz on an EHM diet led to poorer growth, whereas fortification at 26 kcal/oz or higher was linked to better weight gain (13). A randomized controlled trial by Sullivan et al. (2010) found no significant differences in growth between infant groups receiving HMF at an enteral intake of either 100 mL/kg/day or 40 mL/kg/day and those receiving a BMF (18). Assad et al. (2016) found no difference in growth velocity (g/kg/d) from birth to discharge between infants receiving HMFs or BMFs (12). Although human milk is widely regarded as the optimal choice for preterm infants, there remains a question as to why bovine milk fortifiers (BMF) are sometimes used when human milk fortifiers (HMF) are available. A key consideration is whether the faster growth curve observed with BMF is sufficient justification for its use. The decision to use BMF in place of HMF centers on the growth outcomes associated with each fortifier. While studies have shown that infants receiving BMF may experience a higher weight growth velocity until the point of fortifier discontinuation, overall growth from birth to discharge does not significantly differ between infants fed human milk fortified with HMF and those with BMF. Therefore, the apparent advantage in early growth rate provided by BMF must be weighed against the broader clinical context and long-term growth measures, which remain similar between the two fortifier types. Ultimately, the choice of fortifiers should not rely solely on faster initial growth curves but must also consider the clinical significance of growth outcomes measured throughout the hospital stay, as well as the established benefits of human milk for preterm infants. This underscores the importance of evaluating both short-term and long-term growth effects when selecting the most appropriate fortifier. Lucas et al. reanalyzed a 12-center randomized trial and performed a subgroup analysis (n=114), including only infants receiving a 100% milk-from-the-mother-based diet with fortification and no donor milk or preterm milk formula. The study found an increase in adverse outcomes with bovine milk fortification, including NEC and severe morbidity, defined as NEC requiring surgery or death (19). Although the ideal growth rate for preterm infants is not fully determined, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) recommends that VLBW babies gain 17–20 g/kg/day after initial weight loss (20). It is important to recognize that growth outside the womb differs significantly from fetal growth in utero. The unique challenges and environmental factors encountered after birth may influence the appropriateness of directly replicating intrauterine growth velocities for preterm infants. This raises the question of whether it is necessary or even beneficial to strive for the exact same growth trajectory observed during gestation, given the different physiological and clinical circumstances present in the neonatal setting. In human infants, brain development is prioritized, but in calves, physical growth comes first. A protein-to-energy ratio of 3.4 g/100 kcal supports adequate growth at 26–30 weeks of gestation (21). Human milk has a protein-to-energy ratio of 2.59 g/100 kcal, while fortification with HMF (Humavant 26) and BMF (PreNAN) raises this to 3.2 and 3.45 g/100 kcal, respectively, both suitable for supporting growth. Humavant 26 provides more lipids (5.3 g/100 mL) and fewer carbohydrates (7.8 g/100 mL) than PreNAN (4.6 g/100 mL lipids, 8.5 g/100 mL carbohydrates) does. In very preterm infants, fat energy provision is reduced due to limited digestion and absorption related to low enzyme secretion (21,22). Carbohydrates are easily digested by preterm infants and provide a rapid energy source. The ESPGHAN recommends at least 10.5 g/100 kcal for enteral intake, whereas the human milk+HMF mix offers only 8.4 g/100 kcal, which is below this recommendation. The human milk+BMF mix provides 11.4 g/100 kcal, which meets the guidelines. Optimizing nutrition for preterm infants is complicated by macronutrient variations between HMFs and BMFs. While protein levels were sufficient in both, carbohydrate and fat content varied. A major limitation was inferring nutrient intake from product composition instead of direct measurement. Focusing on appropriate macronutrient ratios and personalized fortification can improve growth outcomes while preserving the benefits of human milk diets. Major outcomes were alike in both groups, but the study's small sample size limits detection of differences. Thus, these results are considered exploratory and hypothesis-generating rather than conclusive. Sample size calculation was feasible only for growth velocity from birth to discharge. A 10% minimally relevant effect, with a weighted mean of 13.35 and SD of 2.3 from previous study (23), requires 96 patients per group to achieve 90% power at a 1% significance level. The main strength of this study lies in being among the few studies to examine EHM diets and growth, with both groups well-matched for birth weight, gestational age, and the use of combinations of milk from the mother and fortifier. This study has some limitations. The retrospective design may have introduced bias, and the small sample size may have reduced statistical power and limited multivariate analysis. Late fortification in both groups may also have affected the observed growth patterns. Another consideration is the difference in the timing of fortifier introduction: the BMF group generally started fortification after reaching full enteral feeds, while the HMF group began before full feeds were achieved. This variation may influence growth outcomes and should be addressed in future studies. Future research should use prospective designs, larger cohorts, and standardized fortifier protocols to better assess the effects of the fortifiers on growth in very preterm infants.

Conclusion

In this retrospective study, overall growth measures from birth to discharge were similar in both groups. However, infants fed mother own milk fortified with an HMF showed a lower weight growth velocity until fortifier discontinuation than did those receiving mother own milk fortified with a BMF. Since the duration of fortification differed among participants, assessing growth velocity from birth to discharge may provide more clinically meaningful insights.

Ethic approval: The study was approved by the Ministry of Health and Prevention ethics board, UAE (MOHAP/REC/2024/21-2024-F-M). The study was conducted in accordance with the ethical standards of all applicable national and institutional committees and the World Medical Association's Helsinki Declaration.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors contribution: MRA and SAA developed theoretical formalism, led collecting and analyzing data, and contributed to the final version of the manuscript.

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