Growth of Very Low Birth Weight Infants Who Received a Liquid Human Milk Fortifier: A Randomized, Controlled Multicenter Trial

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ABSTRACT

Objectives: To evaluate growth (weight, length, head circumference, and knee–heel length [KHL]) in very low birth weight (VLBW) infants (500–1500 g) who received human milk with a liquid fortifier (LHMF) with high protein and fatty acid content versus a traditional powder fortifier (PHMF) for 45 days or until discharge.

Methods: This was a multicenter, randomized, controlled trial. An intention-to-treat analysis was performed to determine adverse events and withdrawal causes. We also performed an efficacy analysis involving the infants who completed at least 2 weeks of study.

Results: Of the 158 infants enrolled in the study, 146 completed at least 2 weeks, and 125 completed the entire study. The biodemographic characteristics were similar between groups, with no differences in increments of weight (22.9 vs 22.7 $g\,kg^{-1}\,day^{-1}$), length (1.03 vs 1.09 cm/week), head circumference (0.91 vs 0.90 cm/week), or KHL (3.6 vs 3.3 mm/week). The KHL increment was greater in infants weighing >1 kg receiving LHMF (3.7 vs 3.2 mm/week, $P = 0.027$). Although there were no significant differences in serious adverse events, the incidence difference of the composite outcome death/necrotizing enterocolitis between groups warrants attention (1.3% with LHMF and 8.1% with PHMF).

Conclusion: There were no differences in the overall growth between VLBW infants receiving either fortifier.

Key Words: human milk fortifiers, neonatal nutrition, randomized controlled trial, very low birthweight infants

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What Is Known

- Human milk (HM) has many advantages for very low birth weight (VLBW) infants, reducing the risk of several comorbidities and improving neurodevelopment.
- HM does not provide sufficient nutrients for rapid growth in these infants.
- HM fortifiers adding protein and other nutrients, improve their postnatal growth.

What Is New

- In this middle-income region, multicenter, controlled trial, we found no differences in growth between VLBW infants receiving a HM fortifier with higher protein content versus a traditional one, except for a greater increase in knee-to-heel length in those >1000 g.
- Serious adverse events and comorbidities were similar between groups.

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''Nutrition of Premature Infants With Human Breastmilk Fortifier (EFORT-LM)''

This study was partially supported by a funding from Mead Johnson Nutrition. All participating institutions were provided with both types of study fortifiers, as well as funds for patient enrollment, data registration, milk/fortifier preparation and for buying supplies for the participating mothers to transport their milk. Drs Masoli and Mena received honoraria for conducting the study during a 2-year period. The other authors report no conflicts of interest.

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The survival of premature very low birth weight (VLBW) infants (<1500 g) has improved worldwide over the last decades. The most critical period for growth and development of several organs, particularly the brain, is the last trimester of pregnancy. Thus, for premature VLBW infants, these developmental processes occur after birth. Inadequate nutrition and/or poor postnatal growth during hospital stay have been associated with neurocognitive impairments (1–3). Accordingly, optimization of nutritional care of preterm infants is crucial for improving neurodevelopment and other outcomes (4–5).

Feeding VLBW infants human milk (HM) has significant advantages in terms of reducing morbidity risks such as necrotizing enterocolitis, retinopathy of prematurity, and bronchopulmonary dysplasia; furthermore, it has proved to be beneficial for cognitive and behavioral prognosis in the long term (6–10). These beneficial effects are far more evident when these infants receive their own mother's milk primarily (8–10). Notwithstanding these benefits, HM by itself does not have sufficient amounts of specific nutrients to suffice for the crucial period when extremely preterm newborns should be developing rapid growth (11–13). The use of HM fortifiers (HMF) which provide additional protein, calcium, and phosphorus among other nutrient contents, has been reported to improve their postnatal growth (14–16).

There is no current consensus regarding the optimum amount of protein content in fortifiers as well as whether using higher amounts of proteins truly represents a better alternative than traditional fortifiers in terms of supporting growth. The existing evidence is insufficient as most studies have small samples and involve single centers in high-income regions (17).

The aim of our study was to compare the growth rate (weight, length, head circumference, and linear growth) in VLBW infants in a South American multicenter setting, receiving either liquid HMF (LHMF), with a high protein content and fatty acid content (long-chain polyunsaturated fatty acids [LC-PUFAs]) or a traditional powder HMF (PHMF) for 45 days or until hospital discharge.

METHODS

The primary study objective was to compare the growth rates: weight gain ($g\,kg^{-1}\,day^{-1}$), length (cm/week), head circumference (cm/week) and knee-heel length (KHL) (mm/week) of premature infants fed predominantly HM fortified with either a LHMF (experimental) or a PHMF (control) (both from Mead Johnson Nutrition) over a 45-day period or until discharge. The energy (kcal) per 100 mL between both fortifiers is similar; the specific nutrient content is shown in Table 1, Supplemental Digital Content, *http://links.lww.com/MPG/C539*.

Secondary Outcomes

Serious adverse events (death, necrotizing enterocolitis, late onset sepsis and combined outcomes), bronchopulmonary dysplasia and days of oxygen use, retinopathy of prematurity, extra uterine growth restriction, serum chemistries, and length of stay.

Inclusion Criteria

Premature infants with birth weight ≤1250 g (amended to ≤1500 g in November 2018); gestational age ≤31 6/7 weeks; appropriate for gestational age, by Fenton curves (18); predominantly fed with HM (own mothers milk or donors milk) with an enteral intake of $80\,mL\,kg^{-1}\,day^{-1}$ of unfortified HM at study entry (study day 0).

Exclusion Criteria

Underlying congenital malformation, 5-minute Apgar score ≤4, major surgery before study day 0, grade 3 or 4 intraventricular hemorrhage (19), use of glucocorticoids for 3 consecutive days on or before study day 0, consumption of >3 feedings of fortified HM before study day 0, fluid restriction to $<120\,mL\,kg^{-1}\,day^{-1}$, creatinine level >2 mg/dL on or during the previous 7 days before study day 0, and oxygen requirement of >40% on study day 0 if the infant was mechanically ventilated (higher than 40% of oxygen concentrations were accepted if the infant was on non-invasive ventilation).

Reasons for Withdrawal from the Study

Consumption of <50% of the total enteral intake as HM for >96 consecutive hours, more than 96 consecutive hours of fasting, transfer to another center, and parental decision to withdraw.

All recruited infants were included in an intention-to-treat analysis to evaluate the incidence of major adverse events. Infants who completed at least 2 weeks of study were considered for the primary outcome analysis (effectiveness analysis). Those who completed the study (45 days or hospital discharge) were analyzed for secondary outcomes.

Study Design

This was a multicenter, triple blinded, randomized, controlled trial with two parallel groups, conducted in nine centers in Chile, all of which belonged to the NEOCOSUR Neonatal Network.

Study Population

NEOCOSUR is a South American cooperative, voluntary, nonprofit association that prospectively monitors VLBW infants' outcomes in the region (*www.neocosur.org*). It uses standardized diagnostic criteria and an online registry system. The study sites were all tertiary centers (six public and three private). Only one center had a human milk bank.

Randomization and Blinding

The participants were randomly assigned to either the control or the experimental group in a 1:1 ratio. The allocation sequence was centralized and computer-generated. Randomization was stratified by birth weight (≤1000 g or >1000 g). Sealed, opaque envelopes were sent to participating centers. Whenever an eligible patient was recruited, the personnel in charge of preparing enteral feedings opened the next numbered envelope in sequence, according to the infant's birth weight strata. Allocation concealment was maintained throughout the whole process of randomization; the investigators, treating physicians, nurses, patients (their guardians), and the statistician were blinded to group status.

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After informed consent was signed by the infant's guardian, they were randomized on study day 0 (enteral intake of $>80\,mL\,kg^{-1}\,day^{-1}$) to receive either fortifier for 45 days or hospital discharge, which ever occurred first. Both fortifiers were added to HM at half concentration on study days 1 and 2; if well tolerated, full concentration was used on study day 3 and maintained throughout the remaining study period; however, caloric increment was allowed at the discretion of the attending physician, either by increasing fortifier concentration or adding supplementary nutrients. Availability of breast milk was maximized using evidence-based strategies.

Outcomes Measures

Anthropometric Measurements

Daily weights were obtained with the newborns undressed, without diapers, on the same electronically calibrated scale. Body length and head circumference were measured to the nearest 0.5 cm once a week using a preterm infant length board and a flexible, non-stretchable cloth or vinyl tape. The highest value between three measurements was recorded for length and head circumference. KHL was measured weekly with a standardized caliper (20) (knemometer) to the nearest mm; the average of five measurements was recorded. Extrauterine growth restriction was defined as weight below the 10th percentile at corrected gestational age on day 45 of the study or at discharge using Fenton curves (18).

Morbidities

Confirmed necrotizing enterocolitis (modified Bell's staging criteria) (21), late-onset sepsis (positive blood culture), incidence of bronchopulmonary dysplasia (defined as oxygen requirements at 36 weeks postmenstrual age or discharge (22), and retinopathy of prematurity (23) were recorded.

Laboratory Data

Blood urea nitrogen (BUN), calcium, alkaline phosphatase, phosphorus, pH, bicarbonate, and base excess (BE) were recorded every 2 weeks.

Enteral and Parenteral Nutritional Intake

HM, formula, and parenteral nutrition volume were recorded.

Interim Analysis

A third-party security interim analysis was performed at half of the recruitment to evaluate the differences in the incidence of serious adverse events.

Statistical Analysis

The primary response variable was weight gain, expressed in $g\,kg^{-1}\,day^{-1}$. The sample size was determined so that this study would have a power of 80% to detect a clinically relevant difference of $1.6\,g\,kg^{-1}\,day^{-1}$ when testing at an alpha level of 0.05, using a two-tailed test. Assuming a standard deviation of $3.2\,g\,kg^{-1}\,day^{-1}$, 63 participants per group were required.

Infant's weights at evaluation days were used when assessing enteral volumes at different ages. If missing, a linear imputation was performed using the two nearest weight measurements (one before and one after the required measurement). Abnormal BE and bicarbonate levels were defined as at least one measurement of less than −6 or less than 18 units, respectively.

Numerical variables were described as means and standard deviations when they had symmetric distribution or as medians and first and third quantiles when distribution was asymmetric. Categorical variables were described as n and percentages. When comparing between both HMF, the *t*-test or Mann-Whitney test was used for numerical (symmetric or asymmetric) variables, and chi-square tests or Fisher exact test were used to compare categorical variables. Additionally, differences between groups with their 95% confidence intervals (95% CI) were obtained using exact methods when comparing means or proportions and bootstrap methods when comparing medians.

Statistical tests were conducted at an alpha level of 0.05 and were two-sided. Statistical analyses were performed using SPSS 17.0 (24).

RESULTS

Between August 2015 and October 2019, a total of 158 VLBW infants were included in the study; among them, 146 infants completed at least 2 weeks and 125 completed the entire study. Causes for withdrawal from the study were lack of HM (17), serious adverse events (12) and transfer to other centers (4).

The mean age at study entry was 14 days, with no significant difference between the groups. As intended, all participants received HM predominantly, with only 13.8% receiving formula in the LHMF group and 16.9% in the PHMF group. Both groups were also similar in terms of age at initiation of total parenteral nutrition (within the first day of life) as well as in duration, 13.4 versus 14.8 days, in the LHMF group and the PHMF group, respectively (non-significant differences).

Almost all infants received their mothers' milk, only 19 infants—all from the only center that has a milk bank—received bank HM (partially). Table 1 shows the results of our intention-to-treat analysis, including the overall recruited population. The biodemographic characteristics were similar between the study groups. The incidence of serious adverse events (death, late-onset sepsis, and necrotizing enterocolitis) also showed no significant differences between the groups; however, there was a trend toward a lower rate of death/necrotizing enterocolitis, as a composite outcome, in the LHMF group ($P = 0.069$).

Table 2 shows main outcome results: anthropometric measurements of both groups. Overall, there were no differences in weight, length, head circumference or KHL growth between the groups. There was, however, a statistically significant difference in KHL growth between groups within the largest birth weight strata, that is, infants $>1000\,g$ ($P = 0.027$), favoring those receiving LHMF. There were no differences in any anthropometric measurements when categorizing the infants by sex.

As intended, all patients received fortification at full strength (24 cal/oz) from study day 3. Fortification was further increased in 46% of overall patients, with no difference between groups. Figure 1 shows the caloric intake and enteral feeding volumes at different study periods. There were no significant differences among the groups, averaging approximately $130\,kcal\,kg^{-1}\,day^{-1}$ and $155\,mL\,kg^{-1}\,day^{-1}$ throughout the study. Although nutritional supplements (such as casein and/or medium-chain triglycerides) were added to the fortified HM, these were only used intermittently in 10 infants (no difference between groups).

The secondary outcomes showed no significant differences between the groups in any of the clinical variables analyzed.

TABLE 1. Intention to treat analysis and serious adverse events in 158 VLBW infants that entered the study

Variable	Liquid HMF (n = 76)	Powder HMF (n = 82)	Difference (95% CI)	*P* value
Antenatal steroids (%)	95.7	92.7	3.0 (−4.2–10.2)	0.507
BW (g); mean ± SD	1035.2 ± 225.1	1031.9 ± 178.1	3.29 (−59.5–66.1)	0.919
GA (wk); mean ± SD	27.7 ± 1.9	27.9 ± 1.7	−0.12 (−0.68–0.42)	0.656
Females (%)	50.7	52.9	−2.2 (−17.7–13.3)	0.777
Apgar 1 min <3 (%)	18.7	10.6	8.1 (−2.9–19.1)	0.146
NEC (%)	1.3	5.8	−4.5 (−10.1–1.1)	0.217
LOS (post-admission) (%)	9.3	5.8	3.5 (−4.7–11.7)	0.550
Deaths (%)	1.3	3.5	−2.2 (−6.9–2.5)	0.624
NEC & LOS (%)	1.3	0.0	1.3 (−1.2–3.8)	0.466
NEC or death (%)	1.3	8.1	−6.8 (−13.2 to −0.3)	0.069
NEC or LOS or death (%)	9.3	14.0	−4.7 (−14.6–5.2)	0.365

BW = birth weight, GA = gestational age, HMF = human milk fortifier, LOS = late onset sepsis, NEC = necrotizing enterocolitis, SD = standard deviation, VLBW = very low birth weight.

Although there were significantly low mean BE and bicarbonate levels in the LHMF group, there was no significant difference in the percentage of infants presenting with abnormal BE (<−6) or bicarbonate (<18) between the study groups (Table 2, Supplemental Digital Content, *http://links.lww.com/MPG/C540*).

DISCUSSION

Our main finding was that there were no differences in the overall growth between infants receiving either fortifier, except for an increase in KHL in infants >1000 g receiving LHMF. Standardized KHL is a very sensitive index expressing linear growth in a relatively short period of time (20). This finding may represent a potential advantage of LHMF that could be further explored.

In the largest single-center controlled trial including 106 VLBW infants, the authors found that those fed with an acidified LHMF with higher protein content (same as our study) had significantly higher weight, length, head circumference, and linear growth gain rates than those receiving PHMF (25). These results are in line

TABLE 2. Main outcomes according to type of human milk fortifier in 146 VLBW infants, stratified by BW or sex

Variables	Liquid HMF		Powder HMF		Difference	*P* value
	N	Mean ± SD	n	Mean ± SD	(95% CI)	
Weight gain (g/Kg/day)						
Total	67	22.9 ± 5.5	79	22.7 ± 5.1	0.21 (−1.52–1.96)	0.804
BW ≤ 1000 g	27	24.4 ± 5.6	38	23.2 ± 5.8	1.14 (−1.69–3.98)	0.423
BW > 1000 g	40	22.0 ± 5.3	41	22.3 ± 4.5	−0.29 (−2.48–1.90)	0.792
Males	34	22.7 ± 5.9	38	23.9 ± 5.2	−1.20 (−3.80–1.40)	0.360
Females	31	23.0 ± 5.1	39	21.7 ± 4.9	1.31 (−1.09–3.72)	0.279
Length increment (cm/wk)						
Total	66	1.03 ± 0.31	74	1.09 ± 0.37	−0.05 (−0.17–0.06)	0.342
BW ≤ 1000 g	27	1.08 ± 0.26	34	1.09 ± 0.40	0.004 (−0.17–0.18)	0.961
BW > 1000 g	39	1.00 ± 0.34	40	1.09 ± 0.36	−0.09 (−0.25–0.05)	0.217
Males	33	1.01 ± 0.36	35	1.07 ± 0.39	−0.06 (−0.25–0.11)	0.470
Females	31	1.07 ± 0.27	37	1.11 ± 0.37	−0.04 (−0.19–0.12)	0.628
HC increment (cm/wk)						
Total	67	0.91 ± 0.30	76	0.90 ± 0.25	0.01 (−0.08–0.10)	0.854
BW ≤ 1000 g	27	0.91 ± 0.30	36	0.89 ± 0.27	0.05 (−0.10–0.20)	0.498
BW > 1000 g	40	0.90 ± 0.31	40	0.90 ± 0.24	−0.02 (−0.14–0.09)	0.662
Males	34	0.87 ± 0.30	35	0.89 ± 0.28	−0.01 (−0.15–0.13)	0.874
Females	31	0.91 ± 0.30	39	0.90 ± 0.23	0.01 (−0.11–0.14)	0.806
KHL increment (mm/wk)						
Total	62	3.6 ± 0.9	74	3.3 ± 1.1	0.28 (−0.05–0.62)	0.098
BW ≤ 1000 g	24	3.3 ± 0.7	35	3.4 ± 1.2	−0.01 (−0.51–0.48)	0.958
BW > 1000 g	38	3.7 ± 1.0	39	3.2 ± 1.0	**0.48 (0.05–0.91)**	**0.027**
Males	32	3.6 ± 0.8	35	3.5 ± 1.0	0.06 (−0.38–0.52)	0.759
Females	30	3.5 ± 1.0	37	3.1 ± 1.1	0.47 (−0.03–0.98)	0.070

BW = birth weight, HC = head circumference, HMF = human milk fortifier, KHL = knee to heel length, SD = standard deviation; VLBW = very low birth weight.

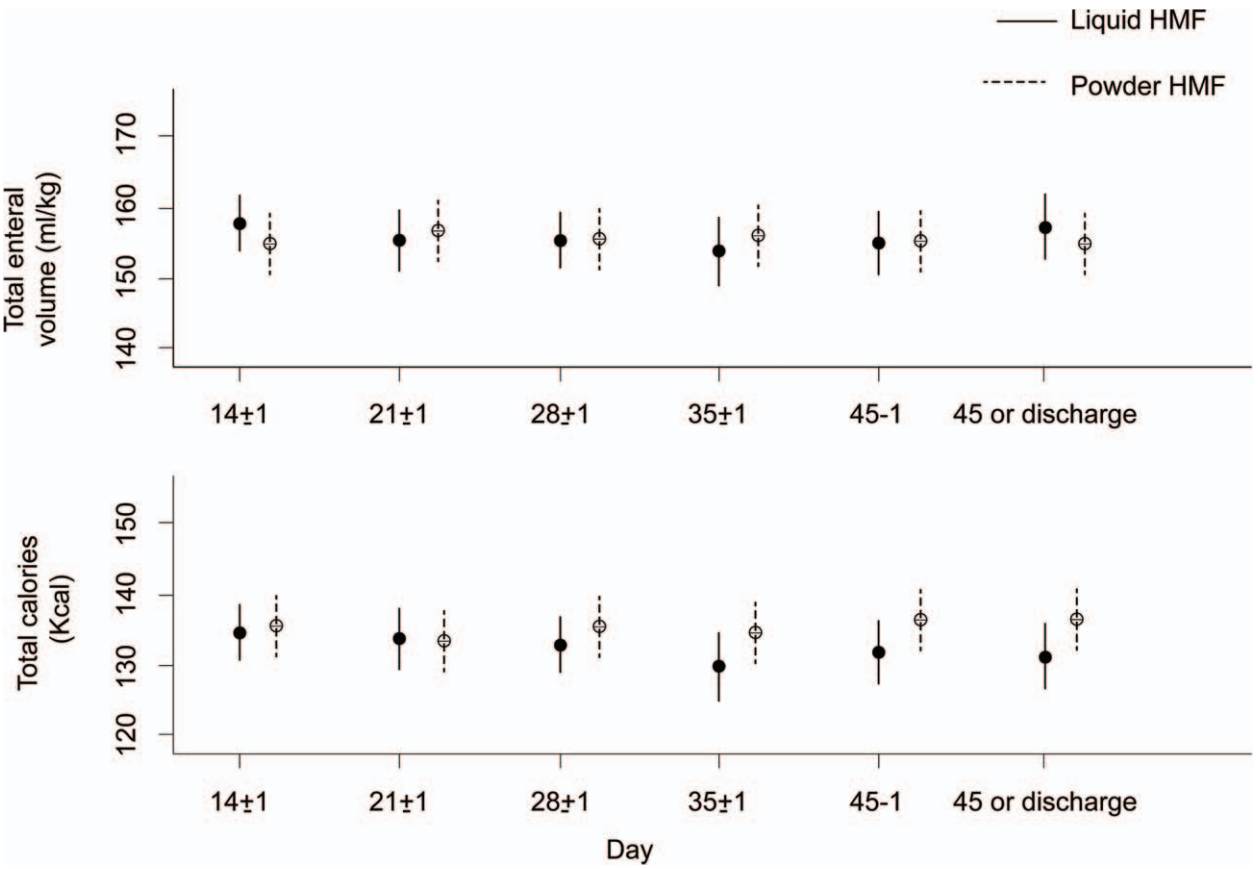

FIGURE 1. Total enteral volumes (ml/kg/day) and Total estimated calories (kcal) at different study days. Upper panel. Mean and 95% confidence intervals for total enteral volumes (ml/kg/day) at different study days, comparing liquid and powder fortifier. No differences between fortifiers or across days were found. Lower panel. Mean and 95% confidence intervals for total estimated calories (kcal) at different study days, comparing liquid and powder fortifier. No differences between fortifiers or across days were found. HMF: human milk fortifier.

with those of other small studies, including one single-center trial from India that supplemented small premature infants with high amounts of protein content (26–29); however, other studies have not found this association (30–32). There might be a point at which there is a ceiling effect for enteral protein intake with respect to growth (32).

The use of acidified HMF in VLBW infants is controversial, showing no advantages in growth in most studies (33–34). These include a relatively large multicenter randomized trial that found higher rates of metabolic acidosis in infants using an acidified LHMF (35). Moreover, growth was not significantly different between the study groups. The lack of growth when using an acidified HMF has been attributed to metabolic acidosis (33–35). In our study, we found a statistically lower mean BE and bicarbonate with the LHMF than with PHMF; however, the absolute values were very similar and the difference was of no clinical significance. Additionally, there were no differences in pH or in the incidence of abnormal BE episodes between the study groups.

Our data support the finding of a similar growth comparing both HMF, despite greater amounts of protein content and LC-PUFA in the LHMF. We speculate that this lack of difference could be due to the fact that both study groups presented an optimum weight gain during the study period (averaging 22 g kg^{-1} day^{-1}); in this scenario, it could be difficult to further improve weight gain. This overall good performance has several possible explanations: the sickest infants were excluded; almost all infants received their mother's own milk, which has been associated with greater weight gain than donor's HM (36); additionally, nutritional practices for neonates have improved significantly in Chilean centers and are quite homogenous since the implementation of standardized guidelines (37). Regarding LC-PUFA, their use has been associated with improved child growth (arachidonic acid), including premature infants (38). Furthermore, there is evidence that DHA can protect the retina, brain cortex, lungs, and the intestinal epithelium from oxidative stress (38); however, our study was not designed to assess these effects.

Recommendations from the European Milk Bank Association encourage the use of individualized fortification to optimize nutrient intake, revealing concerns about suboptimal growth achieved in VLBW infants during hospital stay (39). Postnatal growth rate similar to intrauterine growth can be reached only with adequate protein and energy intake (3.5–4.5 g kg^{-1} day^{-1} and 110–130 kcal kg^{-1} day^{-1}, respectively). Both groups in our study population fulfilled these recommendations.

A Cochrane review from 2018 (17) concluded that there is still low-quality evidence regarding the association between HM protein supplementation and growth in VLBW infants. The small sample sizes, low precision, and low-quality evidence precludes any

conclusions about other outcomes such as necrotizing enterocolitis and length of hospital stay. Furthermore, the actual findings may not be generalizable to low-resource countries, as there is a lack of studies in this setting.

Although there were no statistically significant differences in the incidence of serious adverse events, there was a trend toward a lower rate of death/necrotizing enterocolitis in the LHMF group (1.3% vs 8.1%), which represents an important clinical difference; furthermore, this could have reached statistical significance with a larger sample size. A sterile acidified LHMF may be potentially safer in VLBW infants, particularly in this middle-income region setting.

A limitation of this study was the slow recruitment rate. Three reasons contributed to this: we overestimated the proportion of infants that would receive almost exclusive HM feeding during the study period. Second, the exclusion criteria were too stringent. Finally, our initial population considered only infants with birth weight $\leq$1250 g; this subgroup had a relatively higher mortality rate as well as complications that hindered many of these infants from participating in the study. Another limitation is that we did not use a human milk analyzer to measure the exact energy intake of infants; it is a known fact–and this is an issue in most similar studies—that the nutrient content of HM is quite variable. The strengths of the study are that it is a blinded, multicenter, randomized controlled trial, which included a total of 146 infants for the primary outcome. Additionally, this study was performed in a middle-income region, in comparison to other referred studies, mostly from high-income countries.

Large, multicenter, controlled studies comparing different amounts of protein content in HMF are required in the future. They should be designed to determine not only the impact on in-hospital growth, safety, and length of hospital stay but also long-term growth, body composition, and neurodevelopmental outcomes.

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