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ORIGINAL ARTICLE

Effect of human milk fortification at different volume of feeds in pre-term newborns (< 32 weeks of gestation).

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ABSTRACT… Objective: To assess how feeding volume of `human milk fortification (HMF) influences growth and bone mineral status in preterm infants. **Study Design:** Parallel-arm, Randomized Controlled Trial. **Setting:** Department of Neonatology, RTEH Indus Hospital, Muzaffargarh, Pakistan. **Period:** September 2024 to June 2025. **Methods:** A total of 132 preterm newborns born before 32 weeks of gestation, with Apgar scores below 7 at five minutes, admitted to the NICU within 24 hours and exclusively fed maternal human milk were enrolled. Infants were randomized into early (70–100 ml/kg/day), middle (101–130 ml/kg/day), or late (130–160 ml/kg/day) HMF initiation groups. Growth parameters, bone mineral status (BMS), and complications were recorded at 36 weeks post-menstrual age (PMA). Data were analyzed using ANOVA and chi-square tests, with statistical significance set at p<0.05. **Results:** Among a total of 132 preterm infants, 66 (50.0%) were male, and the mean gestational age was 30.1±1.1 weeks. At 36 weeks PMA, the early HMF group showed significantly greater weight gain (1021.8±156.3 g) and linear growth (3.7±0.8 cm), with higher head circumference increase (3.3±0.7 cm), compared to the late HMF group (p<0.001). Early HMF was associated with lower alkaline phosphatase (309.2±61.6 IU/L) and higher calcium and phosphorus levels. **Conclusion:** The early initiation of HMF at lower enteral feeding volumes in preterm infants is associated with improved growth and BMS at 36 weeks PMA.

Key words: Bone Mineral Status, Human Milk, Preterm, Postmenstrual Age.

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INTRODUCTION

Complications arising from pre-term birth are the primary cause of mortality among children below five years of age.[1] In 2019, preterm birth occurred in approximately 10.9% of live births globally, affecting around 15.2 million infants.[2] Preterm birth holds the highest cause-specific fatality rate, resulting in nearly 1 million deaths, which accounts for approximately one-third (36.1%) of deaths during the neonatal period.[3] Low birthweight (LBW) significantly overlaps with preterm birth, and premature birth with LBW is a significant cause of morbidity and morbidity worldwide.[5-7]

Human milk (HM) is known to be the best source of nutrition for both term and preterm infants.[8,9] The utilization of HM fortification (HMF) is 90–100% in the NICUs of the developed world.[10] Multicentric data from Australia exhibited that HMF was administered to infants ranging from 1250-2500 grams in weight, with 100% usage.[11] Another multicenter study from China analyzing very preterm infants found the mean birth weight as 1,204±261 grams, 1,255±257 grams, and 1293±251 grams in early, middle, and late HMF groups.[12] At the time of discharge, the mean body weight were 2,484±456a grams, 2,397±394 grams, and 2,287±402 grams respectively. The highest increase in body weight were noted in early and middle fortification groups. Another study reported no major differences in terms of weight gain with early or delayed fortification of HM.[13]

Despite advancements in neonatal care, achieving optimal nutrition for preterm infants remains challenging. Human milk fortification is commonly used to meet the increased nutritional needs of preterm babies. However, there is limited research on the effects of fortification at different volumes of feed. Understanding how varying feeding volumes impact the efficacy of human milk fortification is crucial for improving growth and developmental outcomes in preterm infants.

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This study aims to assess how feeding volume of HMF influences growth and bone mineral status (BMS) in preterm infants.

METHODS

This was a single-center, parallel-arm, randomized controlled trial conducted in the Department of Neonatology at RTEH Indus Hospital, Muzaffargarh, Pakistan. The study was carried during September 2024 to June 2025 following ethical approval from the institutional review board (letter: IHHN_IRB_2024_05_002, dated: 27-Aug-2024). Inclusion criteria were preterm newborns, born before 32 weeks of gestation, with an Apgar score below 7 at five minutes, admitted to the NICU within 24 hours after birth, and who were exclusively fed human milk of their respective mother. Exclusion criteria were the receipt of formula feed prior to 32 weeks post-menstrual age (PMA), major congenital anomalies, or human milk with a caloric content exceeding 26 kcal/oz before 32 weeks PMA. Sample size was calculated using G*Power (version 3.1.9.7), taking an effect size (f) of 0.40, alpha error probability of 5%, power of 95%, degrees of freedom of 5, and three groups, resulting in a total required sample of 130 infants, 132 participants (44 per group) were enrolled.

Following informed, written consent from the mother, eligible infants were randomized by the lottery method into one of three groups, early HMF, middle HMF, or late HMF, according to the volume of enteral feed at which fortification was initiated. The early HMF group began fortification at 70–100 ml/kg/day, the middle HMF group at 101–130 ml/kg/day, and the late HMF group at 130–160 ml/kg/day. Group allocation was concealed and performed by a staff nurse blinded to the intervention protocols. Enteral feeds were commenced per unit protocol under the supervision of the attending neonatologist, with human milk provided by the mother and commercial powdered HMF (Nan, Nestle) used for fortification. Initial HMF dosing was started with a quarter or half dose, increased as tolerated to the full dose as per product instructions. Feeding volumes was advanced by 10–20 ml/kg/day to a maximum of 170–200 ml/kg/day, with full enteral feeding defined as at least 150 ml/kg/day administered for more than 24 hours. At enrollment, demographic and perinatal data were recorded. BMS was evaluated via laboratory measurement of alkaline phosphatase, calcium, and phosphorus. Primary outcome measures as growth parameters, assessed at 36 weeks PMA using calibrated scales. Secondary outcomes included BMS, as well as complications. A dedicated proforma was be used for all study data entry.

Data were analyzed using IBM-SPSS Statistics version 26. Quantitative variables were expressed as mean and standard deviation, and categorical variables as frequency and percentage. Analysis of variance (ANOVA) was used for comparison of quantitative outcomes across groups, while the chi-square test or fisher's exact test were applied for categorical variables. A p-value below 0.05 was considered statistically significant.

RESULTS

Among a total of 132 preterm infants, 66 (50.0%) were male, and the mean gestational age was 30.1±1.1 weeks. The mean gestational age at birth was 30.2±1.1 weeks in the early HMF, 30.0±1.2 weeks in the middle group, and 30.1±1.0 weeks in the late group (p=0.697). The mean birth weight was 1242.9±165.2 grams, 1238.8±152.0 grams, and 1239.4±161.6 grams in the early, middle, and late HMF, respectively (p=0.992). The majority of infants in each group were delivered by cesarean section, comprising 79.5% in early and late HMF, and 81.8% in the middle HMF group (p=0.953), as shown in Table-I.

Infants in the early HMF group demonstrated the greatest weight gain (1021.8±156.3 grams), compared to 998.4±162.5 grams in the middle HMF group and 846.1±178.0 grams in the late HMF (p<0.001). In early HMF group the mean increase in length was 3.7±0.8 cm, compared to 3.6±0.7 cm, and 2.7±0.9 cm in the middle and late groups, respectively (p<0.001). Head circumference gain was significantly higher (p<0.001) in the early HMF (3.3±0.7 cm), and middle HMF group (3.2±0.7 cm), compared to the late HMF group (2.6±0.8 cm). Table-II is showing details about the comparison of 36 weeks PMA among study participants of study groups.

Mean alkaline phosphatase levels were lowest in the early HMF group (309.2±61.6 IU/L), followed by the middle (324.0±59.4 IU/L) and late HMF groups (381.2±66.7 IU/L), with the difference reaching statistical significance (p<0.001). The mean serum calcium was significantly higher in the early HMF group (9.3±0.6 mg/dl) and the middle group (9.1±0.7 mg/dl), compared to the late group (8.7±0.7 mg/dl), and the difference turned out to be statistically significant (p<0.001). The Mean serum phosphorus was highest in the early HMF group (5.7±0.9 mg/dl), followed by the middle (5.6±0.8 mg/dl), and late HMF groups (5.0±0.8 mg/dl), with p<0.001. Comparison of BMS at 36 weeks PMA among study groups is shown in Table-III.

Feeding intolerance (p=0.683), necrotizing enterocolitis (NEC) (p=0.592), bronchopulmonary dysplasia (p=0.650), culture-proven sepsis (p=0.760), intraventricular hemorrhage (p=0.760), or mortality (p=0.875) did not exhibited significant differences, and the details are given in Table-IV.

TABLE-I

Comparison of baseline characteristics of participants (N=132)

Characteristics		Early HMF (n=44)	Middle HMF (n=44)	Late HMF (n=44)	P-Value
Gender	Male	22 (50.0%)	21 (47.7%)	23 (52.3%)	0.913
	Female	22 (50.0%)	23 (52.3%)	21 (47.7%)	
Gestational age (weeks)		30.2±1.1	30.0±1.2	30.1±1.0	0.697
Birth weight (grams)		1242.9±165.2	1238.8±152.0	1239.4±161.6	0.992
Apgar score (5-minutes)		6.4±1.0	6.3±1.1	6.5±0.9	0.647
Mode of delivery	Cesarean section	35 (79.5%)	36 (81.8%)	35 (79.5%)	0.953
	Vaginal delivery	9 (20.5%)	8 (18.2%)	9 (20.5%)	

TABLE-II

Comparison of growth outcomes at 36 weeks postmenstrual age (N=132)

Characteristics	Early HMF (n=44)	Middle HMF (n=44)	Late HMF (n=44)	P-Value
Weight gain (grams)	1021.8±156.3	998.4±162.5	846.1±178.0	<0.001
Length incrase (cm)	3.7±0.8	3.6±0.7	2.7±0.9	<0.001
Head circumference	3.3±0.7	3.2±0.7	2.6±0.8	<0.001

TABLE-III

Comparison of bone mineral status at 36 weeks postmenstrual age (N=132)

Characteristics	Early HMF (n=44)	Middle HMF (n=44)	Late HMF (n=44)	P-value
Alkaline phosphatase (IU/L)	309.2±61.6	324.0±59.4	381.2±66.7	<0.001
Calcium (mg/dl)	9.3±0.6	9.1±0.7	8.7±0.7	<0.001
Phosphorus (mg/dl)	5.7±0.9	5.6±0.8	5.0±0.8	<0.001

TABLE-IV

Complications during hospital stay (N=132)

Characteristics	Early HMF (n=44)	Middle HMF (n=44)	Late HMF (n=44)	P-Value
Feeding intolerance	7 (15.9%)	6 (13.6%)	9 (20.5%)	0.683
Necrotizing enterocolitis	1 (2.3%)	2 (4.5%)	3 (6.8%)	0.592
Bronchopulmonary dysplasia	5 (11.4%)	6 (13.6%)	8 (18.2%)	0.650
Culture-proven sepsis	4 (9.1%)	3 (6.8%)	5 (11.4%)	0.760
Intreventricular hemorrhage	3 (6.8%)	4 (9.1%)	5 (11.4%)	0.760
Mortality	2 (4.5%)	3 (6.8%)	3 (6.8%)	0.875

DISCUSSION

This study indicated that early initiation of HMF was associated with significantly greater weight gain, linear growth, and head circumference increment at 36 weeks PMA. The weight gain at 36 weeks PMA was greatest in the early HMF group, with statistically significant differences compared to the late HMF group ((1021.8±156.3 grams vs. 846.1±178.0 grams, p<0.001). Linear growth (p<0.001), and head circumference increment (p<0.001) followed a similar pattern. Thanigainathan et al.[14], comparing early, versus late fortification of HM and found no clear differences variations in growth outcomes. The lack of significant differences in the meta-analysis may relate to methodological differences, smaller sample sizes, and earlier timing of fortification. The present trial, by using an intermediate early threshold and robust sample size, demonstrates a clear benefit to earlier HMF, suggesting that both timing and sufficient feed volumes may be important to maximize the anabolic effects of fortification in preterm infants.[15]

Improvement in BMS observed with earlier HMF initiation in this study is supported by evidence that timely provision of protein, calcium, and phosphorus is critical for bone mineralization in preterm infants. The early HMF group demonstrated significantly lower alkaline phosphatase levels compared to the late HMF group (309.2±61.6 IU/L vs. 381.2±66.7 IU/L, p<0.001), along with higher mean serum calcium and phosphorus. Lower alkaline phosphatase values in the early HMF group indicated reduced risk of metabolic bone disease. Tillman et al.[13], retrospectively compared early HMF versus late HMF, and found a lower incidence of elevated alkaline phosphatase levels in the early HMF, although no differences were detected in weight gain at 34 weeks PMA. The similarity in improved BMS between these findings and the current study may reflect the benefit of increased mineral intake when fortification is introduced earlier in the course of enteral feeding.[16]

Clinical safety is a central concern in the timing of HMF fortification. In the present study, the incidence of feeding intolerance did not differ significantly among groups, affecting 7 (15.9%) infants in the early HMF group, 6 (13.6%) in the middle group, and 9 (20.5%) in the late group (p=0.683). The occurrence of NEC was also not statistically different (p=0.592). Thanigainathan et al.[14], found little or no effect of early fortification on NEC risk, and with a systematic review by Kumar et al.[17], which also demonstrated comparable risks. The absence of increased risk with earlier HMF initiation in this setting suggests that concerns about adverse gastrointestinal outcomes should not preclude timely fortification, especially when careful monitoring and incremental dosing strategies are implemented. These findings provide reassurance for clinicians, particularly in low-resource settings, that early fortification can be safely implemented without elevating rates of serious morbidity. The lack of significant differences in rates of bronchopulmonary dysplasia, sepsis, intraventricular hemorrhage, and mortality among the three groups further supports the safety profile of early HMF introduction.

The present findings hold important clinical implications for the nutritional management of preterm infants. Early introduction of HMF at lower enteral volumes enables more rapid achievement of optimal protein and mineral intake, which is critical for catch-up growth and bone mineralization in this population.[18,-21] Researchers have also recommend individualized or targeted fortification strategies, and this study further supports the concept that the timing of standard fortification remains an underrecognized determinant of short-term growth and bone health.[9] Given the strong evidence that preterm infants are vulnerable to nutritional deficits and the proven association between adequate growth and neurocognitive outcomes.[22,23] This may be particularly relevant in resource-constrained environments as the timing of standard fortification is a modifiable factor.[24,25]

Some limitations of the present study warrant discussion. The trial was conducted at a single center, which may limit generalizability, especially given potential differences in clinical protocols, maternal milk composition, and local epidemiology of neonatal complications. Measurement of actual protein and energy intake from breast milk was not feasible, as the study relied on standard calculations and did not utilize point-of-care human milk analysis, which may introduce variability and

bias. Future multicenter randomized trials are recommended, including diverse populations, assessment of actual nutrient intake through human milk analysis, and longitudinal follow-up to evaluate neurodevelopmental and metabolic outcomes.

CONCLUSION

The early initiation of HMF at lower enteral feeding volumes in preterm infants resulted in improved growth and BMS at 36 weeks PMA without raising the risk of common neonatal complications. This study provides support for reconsidering the timing of HMF introduction in standard clinical protocols. Early nutritional optimization is likely to yield meaningful short-term and potentially long-term benefits for the most vulnerable infants.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Muhammad Imran:** Data collection, data analysis.
2	**Muhammad Usman:** Conception, design, proof reading.
3	**Muhammad Sarfraz Ahmad:** Literature review, proof reading.