

Background:

Necrotizing Enterocolitis (NEC) is a serious condition that occurs in approximately 3 to 10% of very low birth weight infants¹. Devastatingly, there is a 50% case-fatality rate among those diagnosed with NEC². The additional cost of hospital charges directly related to medical and surgical treatment of NEC in the very low birth weight infant is \$6.5 million a year, or \$216,666 per survivor³. Human milk (HM), both maternal and donor, provides protective benefits against many morbidities, particularly NEC. Mother's breastmilk (MBM) is the gold standard for enteral feedings because it is most protective. In the absence of MBM, the AAP supports the use of pasteurized, fortified donor human milk (DBM) as a second choice. The US Surgeon General's Call to Action to Support Breastfeeding declares that HM, including MBM and DBM, should be utilized more frequently⁴. Formula is considered the third best choice –the last option. The aim of this study is to determine if pasteurized DBM is protective against necrotizing enterocolitis in very low birth weight (VLBW) infants even when fortified with *bovine-derived* human milk fortifier (HMF).

Feeding unfortified DBM versus preterm infant formula (PTF) in VLBW infants is associated with a lower risk of NEC^{5,6}. However, risk reduction of morbidities, such as NEC, only account for one of the major goals in the neonatal intensive care unit (NICU). Supporting growth and development during hospitalization and after discharge are two other important goals. Optimal growth and development requires adequate nutrition. Pasteurized DBM is not nutritionally adequate for optimal growth and development of premature infants⁵. Protein, sodium and energy density are only a few of the major

components that are too low. To meet the needs of a premature VLBW infant, HMF as an additive is required. HMF increases nutritional content of the enteral feedings and thus supports improved long-term growth and physiological development in premature infants⁷.

There is conflicting data on the benefits of fortified DBM compared to PTF⁸. This is partially due to the ingredient differences that exist between available HMFs. DBM fortified with *human-milk-derived* HMF, but not *bovine-derived* HMF, has been shown to be protective against NEC when compared to PTF⁹. Additionally, there is limited evidence from both observational studies and randomized controlled clinical trials, that an exclusive HM and *human-milk-derived* diet reduces both incidence and severity of NEC^{10,11}.

Due to the limited amount of the evidence and high purchasing costs, *human-milk-derived* HMF (\$6.25/mL) has yet to be identified as the standard of care. Fortifying 50ml of HM (20kcal/ounce) to standard preterm energy requirements (24kcal/ounce) requires either 10ml of human milk-derived HMF (\$62.50) or two packets of bovine-derived HMF (\$2.60)¹². The costs of DBM (\$0.10/mL) and PTF (\$0.03/mL) also influence the budgeting decisions of many hospitals¹³. Moreover, there is a research gap on whether there are benefits to feeding diets that are only partially HM in preterm infants. In an era when preterm births are increasingly common and *bovine-derived* HMF remains the standard fortification choice, it is paramount to investigate the NEC risk compared to PTF.

Objective:

To determine differences in NEC rates between preterm infants fed predominantly MBM, DBM, or PTF.

Methods:*Sample and Setting*

The total sample size is 665 infants; 72 infants were not included due to transfer to another facility or death prior to receiving enteral feeds for greater than 3 days. Of the study population that included the remaining 593 infants, 445 infants were discharged to home, 109 infants were transferred to another hospital for subspecialty care or to be closer to home prior to discharge home. Sixty-eight (68%) of the 593 infants had a length of stay greater than 28 days. The sample included 57 sets of twins, 5 sets of triplets, and 2 sets of quadruplets. It is known that risk of NEC is greater in higher order pregnancies only for the reason that they are at greater risk of lower gestational age (GA) and smaller size of birth¹⁴. Nonetheless, a sensitivity analysis was completed which demonstrated that the addition of higher order births did not change the relationship between the exposure and prevalence of NEC. Therefore, each infant in each set was considered to be an individual subject during analysis.

The study was conducted in Winston-Salem, North Carolina, at Novant Health Forsyth Medical Center (FMC), a referral center for women at high risk for obstetric complications. Information on enteral feeding, infant characteristics and health outcomes were collected on all VLBW (<1500g) infants admitted to the neonatal intensive care unit

one year prior (2008) and two years after (2009-2010) a practice change to offer DM as an alternative to PTF. The practice change occurred in January 2009. Recruitment of infants for this study was not required because data were retrospectively analyzed through patient charts. Data were collected and utilized as part of a unit policy change evaluation that extended over three consecutive years.

Research Design

The study identified infant feeding type and health outcomes by utilizing a retrospective chart review. Inclusion criteria were infants with a birth weight <1500 grams and receipt of enteral feedings for at least 4 days during hospitalization. For all infants, parenteral nutrition was begun within two hours of life and initiated at any time during the hospitalization if enteral feedings were not tolerated for more than 24 hours. Enteral feedings were begun when the infant was regarded as stable by the attending neonatologist and advanced according to an established protocol for feeding for this neonatal intensive care unit (NICU). When approximately 100 to 120 ml/kg/day of enteral feeding were achieved and subsequently tolerated, parenteral nutrition was discontinued and HMF was added, 1 packet to 25 ml of either MBM or DM. Powder HMF was used and the brand did not change throughout this study period. HMF was continued until the infant's weight reached 2500 grams or until hospital discharge. Bovine milk exposure was defined as receiving either PTF or HMF.

All mothers of the VLBW infants, regardless of pre-delivery feeding plans, were encouraged and counseled by an International Board Certified Lactation Consultant (IBCLC) to express maternal milk, unless a medical contraindication was present. The

North American Human Milk Banking Association procedures for collection, storage, and handling of MBM and DBM were followed when counseling the mother and in handling milk in the NICU¹⁵. Beginning in January 2009, mothers were also educated about and subsequently offered donor milk, as an alternative to preterm formula, if quantity of maternal milk was insufficient to meet infant needs until post-menstrual age of 34 weeks. Written informed consent was obtained and placed on the infant's chart when the mother agreed. PTF was given if refused to provide consent for DBM and there were insufficient volumes of MBM for infant feedings or if the mother chose to formula feed and refused to consent to donor milk.

Group classification was based on predominant feeding type, which was informed by proportion of total feeding volume received during first 28 days of life. Nutritional intake was recorded daily. Predominant feeding type was defined as the type that comprised greater than a 50% proportion of enteral feeding volume. Each infant was categorized into one of the following groups: Group A (>50% proportion of MBM), Group B (>50% proportion of DBM), or Group C (>50% proportion of PTF) during the first 28 days of life. Eight of the infants in the original study sample could not be categorized into a predominant feeding type as neither MBM, DBM, or PTF comprised >50% proportion.

When subspecialty care was required that was not provided at FMC, such as surgery, infants were transferred to Brenner Children's Hospital at the Wake Forest University Baptist Medical Center in Winston-Salem, North Carolina. Nutritional intake were not collected after transfer except in the case of required NEC surgery.

The institutional review boards of Wake Forest Health Sciences and Forsyth Medical Center (FMC) approved this study.

Outcome Measures

The primary outcome of interest was necrotizing enterocolitis (NEC). NEC was defined using Modified Bell's staging criteria of two or greater, indicating the presence of pneumatosis intestinalis or pneumoperitoneum.¹⁶ Two pediatric radiologists, who were blinded to the type of feeding that was being received by the infant, confirmed diagnosis of NEC. All cases of stage 2 or greater NEC were treated with ten consecutive days of antibiotics and bowel rest.

Secondary outcome measures include differentiation between NEC that requires medical and pharmaceutical intervention, and the more severe form of NEC that requires more invasive surgical therapies. Feeding type as a continuous variable, feeding advancement rates and weight gain velocity, were also documented and observed in this sample population across the predominant feeding groups.

Data Analysis

Data were analyzed with the use of SPSS Statistics 21 software (Armonk, New York, USA). Chi-square was used to assess group differences. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals. To adjust for potentially confounding variables, analysis of covariance was used for continuous variables and logistic regression [odds ratios (OR) with 95% confidence intervals (CI)] was used for dichotomous variables. Infant characteristics, which have been previously identified

through research¹⁷ as risk factors for NEC and those significantly different in bivariate analyses, were entered into the model.. These included gestational age (GA) or birth weight, 5-minute Apgar score<6, small for gestational age classification, Medicaid participation, first feeding type, predominant feeding group, and time to full enteral feedings. GA and birth weight were highly correlated and therefore all statistical analyses were conducted adjusting for gestational age and then again for birth weight. The results were similar and therefore only the results with GA are included. Group A, the MBM predominant feeding group, was identified as the reference group since it is considered the optimal method for feeding infants.

Results:

The study sample consisted of 593 VLBW infants. Twenty-six infants were diagnosed with NEC in this study population. Incidence of NEC was significantly negatively associated with proportion of maternal milk exposure in enteral feedings during the first 28 days of life [Odds ratio 0.23 (95% CI 0.07, 0.69), $p = 0.009$]. For every 25% increase in MBM proportion the odds of NEC decreased by 77%. Formula exposure in enteral feedings was significantly associated with incidence of NEC [OR 3.268 (95% CI 1.224 - 8.727), $p = 0.018$]. In other words, the greater the proportion of formula, the greater the risk of NEC. DBM exposure in enteral feedings appears to be protective against NEC; however, this association was not significant [OR 0.495 (95% CI 0.151-1.621), $p > 0.05$]. These data were calculated by conducting a bivariate association between DBM exposure and the incidence of NEC.

Infants were grouped according to the predominate feeding type during the first 28 days of life: Group A (>50% proportion of MBM, n = 347), Group B (>50% proportion of DBM, n = 135), or Group C (>50% proportion of PTF, n = 111). Infant characteristics by predominant feeding type are presented in Table 1. The 28-day feeding composition and rate of NEC in each group is shown in Figure 1. There were nine (2.5%) cases of confirmed NEC in Group A, seven (5.3%) cases in Group B and ten (9.1%) cases in Group C. Infants in Group C had significantly higher birth weights (Group A: 1012±291g MBM, Group B: 1043±276g DBM, Group C: 1162±263g) and GA (Group A: 27.8±2.6 MBM, Group B: 28.3±2.6 DBM, Group C: 29.4±2.7wk).

No differences in gender, race, small for gestational age classification, central line placement indications, or day of life (DOL) of first enteral feed administration were observed between the three groups. Groups B and C had significantly higher Medicaid participation rates than Group A ($p<0.001$). Infants in both Group A took longer to achieve 100ml/kg/day ($p<0.01$). Bovine HMF and PTF exposure occurred approximately 9.5 days earlier in Group C compared to Group A and B ($p<0.001$). Weight gain (g/kg) per day was greater in Group C and Group A when compared to Group B ($p<0.01$). After controlling for gestational age, the group differences between the DOL that 100ml/kg/day was achieved and weight gain at 28 days of life were no longer significant.

Results of the logistic regression analyses are shown in Table 2. After controlling for group differences and previously identified risk factors for NEC, GA and Medicaid participation remained significant independent risk factors for NEC along with feeding group. Compared to Group A, Group B was associated with a three-fold increase (OR 3.00 (CI 1.04 – 8.63), $p = 0.04$), and Group C with a six-fold increase (OR 6.26 (CI 2.31

– 17.00), $p < 0.01$) in the odds of developing NEC. Moreover, of the infants in each predominant feeding type, NEC surgery was required in 1% (4/350) of Group A, in 0% (0/133) of Group B, and 3.6% (4/109) of the Group C.

Discussion:

HMF addition to HM is vital for VLBW infants due to reduced volume intake and increased nutritional needs of these premature infants. Human-derived HMF is inaccessible to many hospitals due to cost. This research study suggests that adding bovine-derived HMF does not significantly increase the risk for NEC, and is considerably lower in risk for NEC than preterm infant formula. To our knowledge, no other study has assessed risk of NEC using enteral feeding type as a continuous variable for the first 28 days of life using MBM, DBM, and PTF. The benefit of a study like ours is that we can start to understand the dose relationship between risk of NEC and type of enteral feeding. Our analysis shows that even a minor increase in MBM can considerably reduce an infant's risk of NEC. This has previously been shown to indirectly reduce healthcare costs and directly increases the infant's and the family of the infant's quality of life¹².

Our data suggests that although the incidence of confirmed NEC cases in the DBM group was not statistically different than PTF group, DBM may serve to protect infants by lessening the severity of NEC and thus reducing surgical interventions. In our study, fortified DBM appears to provide limited benefit over PTF in reducing the risk of NEC. This may be due to small sample size of infants who were received predominately DBM and predominately PTR. On the other hand, although not statistically significant,

those whose predominant feeding type was PTF had an approximately four-fold risk of requiring NEC surgery compared to DBM group.

The finding of this study contributes to the knowledge available for enteral feeding in VLBW preterm infants and the knowledge available for bovine-derived HMF use in the NICU. The strengths and limitations of this study are important to understand when comparing our findings to those of other studies. Strengths include the fact that there is complete data on volume and composition of all enteral feeds for infants in the study population prior to hospital discharge to home or another institution, and that the data were collected consecutively at the same hospital pre and post donor milk policy procedures. Additionally, FMH NICU has a standardized feeding regime informed by an infant's gestational age to which every attending neonatologist abides. This provided consistency among infant enteral feeding prescriptions.

Limitations that reduce generalizability of this data include the fact that this is a single institution study, enteral feeding data were not obtained for those infants who transferred institutions, and that this is a retrospective study design thus examination of complete maternal factors such as maternal chorioamnionitis is not possible due to incomplete chart documentation. This study relied on existing data for maternal characteristics. As is true in all non-randomized studies, the observed association may have arisen because of group differences in variables that were not measured, not accurately measured, or incorrectly specified in multi-variate analyses.

Our findings are consistent with a similar study by Schanler et al.⁶ that compared DBM or PTF with MBM as the reference. This was a randomized controlled trial that compared health outcomes of infants who received fortified DBM or PTF as a

supplement to fortified MBM when MBM was not available. Similar to our study, the Schanler et al. study also used bovine-derived HMF. Enteral feeding data for entire hospital stay was the exposure, rather than the first 28 days as used in our study. In summary, that study concluded that DBM, as a MBM substitute, offers limited benefits such as lower rates of NEC, diarrhea and other infections, when compared to PTF. Earlier studies have found that unfortified DBM is protective against NEC^{5,8,10,11}. However, unfortified DBM will not support growth and physiological development in extremely premature infants¹⁸ and therefore is not a viable option and would be unethical to repeat.

Areas for future research would require a randomized trial of VLBW infants with DBM with bovine HMF or PTF with a larger sample size than what has been previously conducted⁵. In the future, it would also be beneficial to include other secondary factors in order to assess the difference in DBM with human milk-derived HMF and DBM with bovine-derived HMF. For instance, research studies have shown that DBM alone can improve feeding tolerance, reduce risk of lung disease and improve lipoprotein profiles¹⁹. Unfortified DBM is not nutritionally adequate and therefore it would not be ethical to randomly assign infants to DBM alone.

Our study provides additional data on the use of DBM and bovine derived HMF and the risk of NEC. These data should encourage health care providers to educate mothers about benefits of her own milk for her baby and about how to express milk for her baby even if she does not plan to breastfeed. NEC diagnoses are closely associated with longer hospital stays and greater hospital costs³. Moreover, increasing the proportion of mother's human milk in an infant's diet could save lives and reduces financial costs¹³.

Acknowledgements:

I would like to thank Dr. Polly Sisk for granting me the opportunity to collaborate with her research team and take this project under my wing so that I may be able to complete my Master's Thesis on this interesting and timely NICU nutrition topic. I am forever grateful. Additionally, I greatly appreciate the guidance and support provided by my Master's Thesis advisor Dr. Anna Maria Seiga-Riz.

Appendix

Table 1: Infant Characteristics By Predominant Feeding Type Group

	Group A (>50% MBM)	Group B (>50% DBM)	Group C (>50% PTF)
Sample Size,	N=347	N=135	N=111
Gestational Age, mean±SD	27.8±2.6 weeks	28.3±2.6 weeks	29.4±2.7 weeks
Birth Weight, mean±SD	1012±291g	1043±276g	1162±263g
Medicaid Participant	57.1%	80.0%*	78.4%*
Male	49.9%	48.1%	48.6%
5-minute Apgar <6	7%	14.1%***	9.9%***
Small for Gestational Age	25.1%	28.9%	30.6%
Central Line Placement	20.5%	12.6%	19.8%
DOL First Feed, mean±SD	2.8±1.8 days	2.6±1.3 days	2.5±1.4 days
DOL 100ml/kg/day, mean±SD	23.0±15.5 days	18.2±11.5 days	16.1±11.9 days**
DOL Bovine-based HMF Exposure, mean±SD	14.2±12.0 days	13.3±11.1 days	4.6±7.6 days*
DOL Bovine-based Premature Infant Formula Exposure, mean±SD	14.3±21.9 days	23.5±20.4 days*	4.1±6.9 days*
Weight gain/kg/day^a, mean±SD	9.4±4.2g	8.1±3.8g	10.6±3.5g**
Race			
Caucasian	55.6%	41.2%	38.7%
Black	32.9%	45.9%	46.8%
Hispanic	11.0%	10.4%	9.9%
Asian	1.4%	0.7%	2.7%

MBM = Mother's Breastmilk

DBM = Donor Breastmilk

PTF = Preterm Infant Formula

DOL = Day of Life

**Significantly different, $p < 0.001$*

***Significantly different, $p < 0.01$*

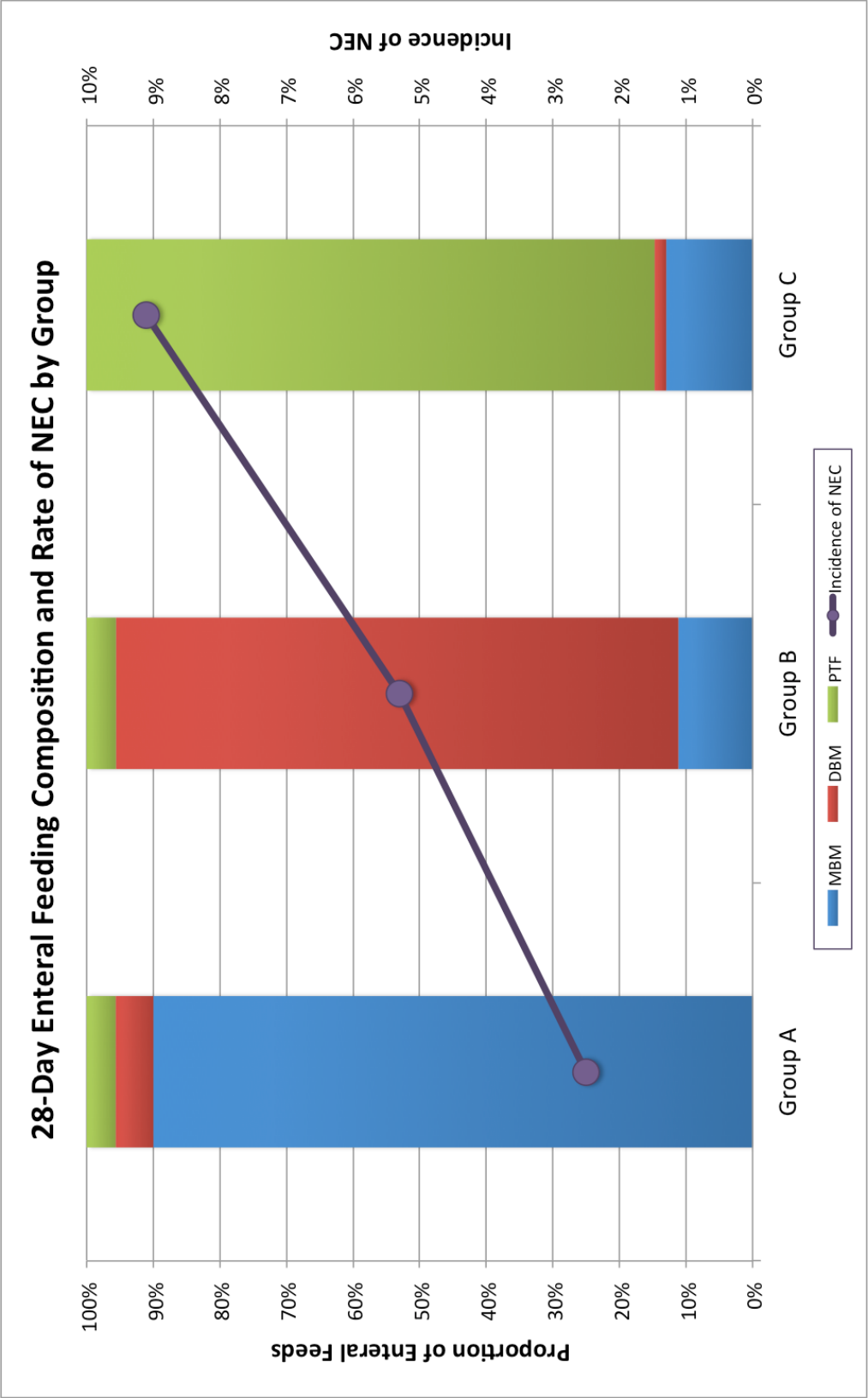
****Significantly different, $p < 0.05$*

^aExcluded infants who were transferred to another facility prior to discharge home.

Table 2: Incidence of NEC

	Odds Ratio	Confidence Interval	P value
GA	1.21	1.02-1.41	0.02
Medicaid	2.94	1.26-7.14	0.01
Predominant Feeding Group			
Group A >50% MBM	1.0 (reference)		
Group B >50% DBM	3.00	1.04-8.63	0.04
Group C >50% PTF	6.26	2.31-17.00	<0.01

Figure 1: Proportion of Total Enteral Feeds Within the First 28 Days of Life and Incidence of NEC by Group



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