

# Dose relationship between maternal milk proportion and necrotizing enterocolitis development

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## Research article

**Keywords:** Very low birth weight (VLBW)/extremely low birth weight (ELBW), necrotizing enterocolitis (NEC), feeding intolerance (FI), maternal milk (MM) feeding.

**Posted Date:** December 11th, 2019

**DOI:** <https://doi.org/10.21203/rs.2.18537/v1>

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# Abstract

**Background:** To examine the dose effect of maternal milk (MM) feeding on neonatal necrotizing enterocolitis (NEC) development.

**Methods:** This retrospective study included 305 infants [birth weight < 1 500 g, or gestation age (GA) < 32 weeks]. Curve estimation was performed to evaluate the dose relationship between MM proportion and NEC. Then infants were grouped based on curve trend and multivariable regression analysis was conducted to prove the curve estimation.

**Results:** Rate of NEC for the sample was 7.9% and age at onset was 25.6(16.0–26.0) days. The curve showed that when MM proportion was increased from zero to 20%, rate of NEC increased from 25% to 40% at first and when MM from 20% to 100%, rate dropped from 40% to near zero. In the MM proportion from zero to 40%, rate of NEC changed greatly. The multiple regression analysis showed that compared with MM proportion > 0.70,  $0.4 < \text{MM proportion} \leq 0.7$  was associated with a 5.482-fold higher odds of developing NEC [ $P = 0.032$ ];  $0 < \text{MM proportion} \leq 0.4$  was a 24.99-fold increase in the odds of developing NEC [ $P < 0.001$ ]; MM proportion = 0 was a 9.348-fold increase in the odds of developing NEC [ $P = 0.003$ ].

**Conclusion:** This study estimated how rate of NEC changed with MM proportion increase, MM proportion being greater than 40% had a significant protection against NEC. We should encourage mother for breast milk and alert to NEC when the preterm is mixed feeding with MM proportion less than 40%.

## Background

Necrotizing enterocolitis (NEC) is a life-threatening disease that occurs in approximately from 5–12% of very-low-birth-weight (VLBW) infants ( $1,000 \leq \text{birth weight} < 1,500 \text{ g}$ ) and extremely-low-birth-weight infants (ELBW) infants (birth weight < 1,000 g)[1, 2], with a mortality (case fatality rate) from 15–50%[1, 3]. Therefore, it is imperative to take measures to reduce rate of NEC. Feeding with maternal milk (MM) may be one of the best measures to reduce the rate of NEC, because many studies have found that, compared with pure formula feeding (PF), MM feeding reduces the rate of NEC[4–7] and feeding intolerance (FI)[8]. This effect may be due to several nutritional and immune components that promote a healthy intestinal mucosa barrier function and dampen the hyper inflammatory responses to pathologic bacteria. Apart from its benefit in terms of intestine protection, MM has been reported to decrease the rates of multiple complications[9–12]. Earlier studies established that MM was associated with a decrease in the risk of late onset sepsis (LOS)[13], retinopathy of prematurity (ROP)[14–16], bronchopulmonary dysplasia (BPD)[13]. MM feeding is highly beneficial to infants and should always be practiced during hospitalization. However, not all mothers can provide sufficient MM to meet the specific infant requirements, and their preterm infants have to be fed with mixtures of both MM and PF, or only with PF.

All aforementioned studies are qualitative, and most of them emphasized that the higher concentration of MM reduced the rate of NEC in infants. However, few quantitative studies have been published on the

dose-response relationship between MM and NEC. However, the rates of NEC/FI change with MM proportion increase and the precise proportion of MM that should be applied in clinical practice have not been determined and published. Therefore, as part of a comprehensive study of the MM effects and correlations in VLBW/ELBW infants, we aimed to examine the dose effect of MM from birth to six-week stay in the Neonatal Intensive Care Unit (NICU) on neonatal NEC/FI development by curve estimation charts.

## Methods

### Population

This was an observational retrospective study and had been approved by the ethics committee of Affiliated Shenzhen Maternity & Child Healthcare Hospital, Southern Medical University. All infants born gestational age < 32 weeks or birth weight < 1,500 g admitted to the Affiliated Shenzhen Maternity & Child Healthcare Hospital, Southern Medical University (Shenzhen, China), between 1 June 2017 and 30 June 2018 were eligible for the study, except for any of following reasons: (1) Admission > 24 h; (2) Death due to healing failure or giving up before discharge. Our study was not designed to evaluate the influence of nutrition on death, and thus we excluded infants who died before discharge; (3) A major congenital anomaly or genetic metabolic disease; (4) Spontaneous intestinal perforations or congenital intestinal problems.

### Data collection

Clinical information was collected that included the basic information (admission dates, BW, lengths, gestational age, day age, etc.), pulmonary surfactant (PS), days of total parenteral nutrition, and volume of PF or MM in each meal of each day, NEC (Bell Stage 1B or greater), FI (feeding intolerance), BPD (moderate-heavy), ROP (grade 3–4), IVH (intraventricular hemorrhage, grade 2–4), LOS and final disposition (death, transfer, or discharge home) were extracted from the charts of eligible VLBW/ELBW neonates.

### Outcomes

The primary outcome of this study was the development of NEC (Bell's stage 1B or greater), which was diagnosed by methods of neonatology[17]. In the following cases, the infants will be fasted: (1) suffered from vomiting, blotting, or bloody stools; (2) had poor treatment response; and (3) had unstable vital life signs. They would be diagnosed with NEC once their state got worse, and radiographs, C-reaction protein, blood routine indicated abnormal. The criteria for NEC stage was according to Bell stage criteria.

The secondary outcomes included FI, defined as the presence of any of the following signs: (1) The residues extracted from the stomach by a gastric tube were more than 50% of the last feeding volume (or 30% of the feeding volume last two times or 10% of daily feeding volume); (2) Bloating; (3) Vomiting; (4) Bloody stools (not caused by blood culture-positive sepsis and confirmed NEC defined by X-ray). Weights were measured using scales produced by Hengxing Electronics Company Ltd. (China). The length was

measured with a tape and recorded by NICU nurses. Weight growth velocities were calculated using an exponential model validated in VLBW/ELBW infants by Patel[18] from birth until discharge. This method allows a simple and accurate calculation of an infant's growth rate in g/kg/day over an extended hospitalization. The rate is given by the formula

### **See Formula 1 in supporting information**

Where  $Wt_2$  is the weight at the end of hospitalization,  $Wt_1$  is birth weight and  $T$  denotes the number of elapsed days between the two. Lengths (cm/week) were calculated by subtracting the admission measurements from the discharge measurements and dividing by the hospital length of stay measured in weeks. Other outcomes were defined. For example, LOS was defined as a positive blood culture at > 72 hours of life; ROP was defined as stage Ⅲ or greater; BPD was defined as oxygen dependency at 36 weeks PMA (post-menstrual age); and IVH was defined as stage II or greater. The diagnosis was made by every 2 neonatal consultants on duty and data was collected mainly by 2 persons separately.

## **Feeding practices**

Parenteral nutrition was initiated within two hours after birth and was restarted if enteral feedings were disrupted for six hours. All mothers were encouraged to express MM. Here, there was no donor human milk (DM) for infants to be fed; PF was given if MM volumes were insufficient for the infant needs before six weeks or discharge home. Enteral feedings were started by 5–15 mL/kg/d when the infant was regarded as thermodynamically stable by a neonatologist, with the goal of achieving from 130 to 150 mL/kg/day. When approximately 100 mL/kg/day of enteral feedings were achieved, Human Milk Fortifier powder (HMF) was added, 1 packet to 25 mL of MM to achieve an estimated caloric density of 98.4 J/30 mL (24 kcal/ounce).

## **Statistical analysis**

The sample size calculation was based on the morbidity of NEC in VLBW/ELBW infants. At 90% power and  $\alpha = 0.05$ , 300 infants would be sufficient to detect a significant difference. All analyses were performed using Empower Stats (<http://www.empowerstats.com>) and the statistical package R (3.2.3 version). The procedures performed can be divided into the following main steps.

Step 1. Cohort population description if data were continuous and normal distribution, they are expressed as mean  $\pm$  SD, if not, they were presented as median (lower quartile, upper quartile) [M ( $P_{25}$ ,  $P_{75}$ )]; if data were frequent, they were presented as number/proportions (n/%).

Step 2. In the primary outcome description, the infants were divided into a NEC and non-NEC groups. Comparisons between groups were performed using chi-square tests for categorical variables and t-test for continuous variables which was normal distribution. Spearman rank test was employed for continuous data skewed distribution. FI was also simply distributed.

Step 3. Dose relationship estimation curve estimation was developed to evaluate the dose relationship between the MM proportion and NEC occurrence. Then infants were grouped based on the sensible MM proportion interval and estimation figure trend. Complete PF individuals were defined as PF group, lower group as  $0 < \text{MM} \leq 40\%$ , higher group as  $40\% < \text{MM} \leq 70\%$ , highest group as  $> 70\%$ . Curve estimation was proved by next analyses where the relationship between MM groups and NEC was being explored.

Step 4. Univariate analysis NEC as binary outcome variable, MM groups clarified last step as independent variable, odds ratios (ORs), and 95% confidence intervals (CIs) of each group were assessed by logistic regression analyses, and the highest MM group was used as a reference. Univariate conducted analysis was conducted of the factors related to NEC, including gender, age, gestational age, birth weight (BW), cesarean section, amniotic fluid, antenatal steroids, intrauterine distress, five-min Apgar score  $< 5$ , and PS.

Step 5. Multivariate analysis. The procedure was identical to that described for the univariate analysis, except for the NEC-related factors, which were defined as adjusted variables. A two-sided P-value  $< 0.05$  was considered to indicate a statistically significant difference.

## Results

### *Characteristics of participants*

A total number of 405 VLBW infants (birth weight  $< 1\,500$  g, gestational age  $< 32$  weeks) were admitted to NICU between May 1, 2017 and June 1, 2018. From this sample, 79 infants were excluded because of any of the following reasons: (1) admission  $> 24$ h ( $n = 30$ ) (2) death due to healing failure ( $n = 7$ ) or giving up ( $n = 15$ ); (3) a major congenital anomaly or genetic metabolic disease ( $n = 26$ ); (4) spontaneous intestinal perforations or congenital intestinal problems ( $n = 23$ ). Finally, a total number of 305 infants were included in the study with average gestational age  $29.3 \pm 2.8$ . The average BW was  $1,171.6 \pm 234.2$  (g). There were 174 (54%) males, the average enteral nutrition starting time was  $21.4 \pm 50.7$  (h); there were 192 (63.0%) delivered by cesarean section; there were NEC 24 (7.9%), LOS 7 (2.3%), FI 94 (30.8%), ROP 16 (5.3%), BPD 35 (11.5%), IVH 18 (5.9%). length of hospitalization (follow-up time)  $M(P_{25}, P_{75})$  was 53 (42–76) days, hospital costs 103 222 (72 034–155 069 Yuan). The demographic data, clinical characteristics, and health outcomes of the sample are presented in Table 1.

### *Outcomes description*

#### NEC

Rate of NEC for the sample was 7.9%, and the age at onset  $M(P_{25}, P_{75})$  was 25.6 (16.0–26.0) days. Demographic data, clinical characteristics, and predominant feeding type of NEC cases and non-NEC cases were compared. No statistical differences were found between the infants who developed NEC and non-NEC in BW [NEC:  $1,216.54 \pm 200.95$  g; non-NEC:  $1,167.75 \pm 236.75$  g;  $P = 0.33$ ] and GA [(NEC:  $28.9 \pm 2.0$  weeks; non-NEC:  $29.3 \pm 2.8$  weeks;  $P = 0.49$ )].

## Feeding intolerance

The FI rate of the sample was 30.6%. We compared the demographic data, clinical characteristics, and predominant feeding type of the FI and non-FI cases. There were no statistical differences between infants who developed FI and non-FI in BW [FI:  $1,176.05 \pm 229.36$  (g); non-FI:  $1,161.53 \pm 245.79$  (g);  $P = 0.62$ ] and GA [FI:  $29.3 \pm 2.9$  (weeks); non-FI:  $29.1 \pm 2.5$  (weeks);  $P = 0.54$ ].

### *Four groups classified by curve estimation chart*

The curve estimation (Figure 1) showed that the rate of NEC was around 25% when MM proportion was 0 (PF), with the increase in the MM proportion, the rate of NEC initially rose, which continued until MM reached around 20%, the highest rate reached was 40%. At MM greater than 20%, the rate of NEC declined correspondingly to the increase in the MM proportion. At a MM value of 40%, the rate of NEC was approximately 25%, which was similar to that of PF feeding. The curve increased to 40% (MM = 20%) at first and then decreased to 25% (MM = 40%); it was reduced when MM was within the range from 0% to 40%. When MM proportion was increased from 40% to 100%, rate of NEC dropped from 25% to near zero. When MM proportion was increased from 0 to 100%, rate of IVH and BPD (Figure 2) was only slightly changed (5%), while rate of ROP and LOS (Figure 1) incidence decreased from approximately 25% to zero with no inflection points. Considering the slope changes in rate and groups interval, especially for NEC, we classified the cases into four groups: PF group, lower group ( $0 < MM \leq 40\%$ ), higher group ( $40\% < MM \leq 70\%$ ), and the highest group ( $>70\%$ ).

### *Comparisons among the four groups*

Comparison between four groups was conducted. The results showed that there were no statistical differences between four groups in length of hospitalization, change in length, BPD, ROP and IVH ( $P > 0.05$ ). But there was statistical significance in weight gain, NEC, LOS, and FI ( $P < 0.05$ ). Of the 24 infants who developed NEC, 8.3% (2/24) cases were in PF group; 62.5% (15/24) cases were in  $0 < MM \leq 40\%$  group; 20.8% (5/24) cases were in  $40 < MM \leq 70\%$  group; 8.3% (2/24) cases were in  $>70\%$  group. The results clearly showed that 91.6% (22/24) NEC cases happened within  $0 \leq MM \leq 70$ ), 68.1% (64/90) cases who suffered FI also happened within these two groups. The trend of rate of NEC and FI was similar with curve estimation (Table 2).

### *Univariate analysis*

Univariate analysis was conducted to find the relationship between MM and outcomes primarily. The results showed that comparing to MM  $> 0.7$  group,  $0.4 < MM \leq 0.7$  [OR(CI): 6.822 (1.288, 36.121),  $P = 0.024$ ],  $0 < MM \leq 0.4$  [OR(CI): 27.443 (6.047, 124.553),  $P < 0.001$ ], MM = 0 [OR(CI): 9.471 (1.253, 71.585),  $P = 0.029$ ] remained significantly associated with risk of developing NEC. Comparing to MM  $> 0.7$  group,  $0.4 < MM \leq 0.7$  [OR(CI): 4.677 (2.395, 9.133),  $P < 0.001$ ],  $0 < MM \leq 0.4$  [OR(CI): 10.779 (5.390, 21.555),  $P < 0.001$ ], MM = 0 [OR(CI): 4.661 (1.688, 12.868),  $P = 0.029$ ] remained significantly associated with risk of

developing FI. While the higher MM, the weight gain in hospital would be fewer [ $\beta$ (CI):0.506(0.126, 0.885),  $P=0.009$ ]. There was no relationship between LOS and MM ( $P>0.05$ ) (Table 3).

### *Multiple regression analysis*

Multiple regression analysis showed that GA[OR(CI):0.688(0.490, 0.968),  $P=0.032$ ], MM>0.7 served as the reference-feeding group, 0.4<MM≤0.7[OR(CI): 6.482(1.180, 35.608),  $P=0.032$ ], 0<MM≤0.4[OR(CI): 25.99(5.312, 127.146),  $P<0.001$ ], MM=0[10.348(1.287, 83.239),  $P=0.003$ ] remained significantly associated with risk of developing NEC. Only MM was entered into the model of PI, 0.4<MM≤0.7[OR(CI): 5.247(2.590, 10.631),  $P<0.001$ ], 0<MM≤0.4[OR(CI): 15.125(6.915, 33.084),  $P<0.001$ ], MM=0[OR(CI): 4.449(1.547, 12.794),  $P=0.006$ ], other considered variables were excluded ( $P>0.05$ ). MM had no relationship with LOS (CIs of ORs included 1, all  $P>0.05$ ) after excluding bias variables except for birth weight [OR(CI): 0.993(0.987, 0.999),  $P=0.017$ ]. Higher MM, the weight gain in hospital would be fewer [ $b$ (CI): -1.478(-2.604, -0.352),  $P=0.010$ ] (Table 4).

## **Discussion**

The results (Fig. 1) in this study revealed that the highest rate of NEC occurred in infants who are on mixed feeding with a low MM proportion. From the curve estimation, it becomes clear that PF feeding is even better than mixed feeding when a mother can provide only little MM for her infant. When the proportion of MM is equal to 40%, the likelihood of developing NEC is the same as that of PF. As the proportion of MM increases, the risk of NEC gradually decreases. Four groups classified based on the curve trend also prove to be convincing by multiple regression analysis. The multiple regression analysis showed that compared with MM > 0.70, 0.4 < MM ≤ 0.7 is associated with a 5.482-fold higher odds of developing NEC[OR(CI): 6.482(1.180, 35.608),  $P = 0.032$ ]; 0 < MM ≤ 0.4 is a 24.99-fold increase in the odds of developing NEC:[OR(CI): 25.990(5.312, 127.146),  $P < 0.001$ ]; MM = 0 is a 9.348-fold increase in the odds of developing NEC[OR(CI): 9.348(1.287, 83.239),  $P = 0.003$ ]. In addition, gestational age is also independent risk factor for NEC. The smaller and less mature ELBW/VLBW is easier to develop NEC [ $\beta$ (CI):0.688(0.490, 0.968),  $P = 0.032$ ].

The conclusion of this study that a higher MM decreases the rate of NEC for ELBW/VLBW is consistent with the findings of many other investigations. A meta-analysis published in 2018 showed a considerable reduction in the incidence of NEC caused by the increase in the dose of HM (human milk)[19]. When it comes to HM, one point needs to be stated: HM in this previous meta-analysis included MM and DM (donor milk), whereas in the present analysis, we included only MM. In fact, both DM and MM contain woman's milk, having no discrimination, therefore the results of this study and this meta-analysis could be taken to compare. Observational studies[20–26] and interrupted time series[27, 28] included in this meta-analysis for this comparison which shows a significant reduction in NEC rate [RR(CI): 0.53(0.42, 0.67),  $n = 8778$ ). A comparison between any HM and PF in some observational cohort studies[29, 30] included in this meta-analysis presents that there is a clear effect of any HM included on the reduction in NEC occurrence [RR(CI): 0.51(0.35, 0.76),  $n = 3783$ ). Overall, the meta-analysis gives a conclusion that any

volume of HM is better than PF, this is not completely consistent with this study. Our present findings revealed that a low MM is not better than PF, it has protection against NEC only when MM > 40%. Some comparisons in this study also present that higher MM (MM > 0.5, 6/214) protects against NEC,  $\chi^2 = 25.382$ ,  $P < 0.001$ , but there is no differences between any MM (MM > 0, 22/286) and PF (MM = 0, 2/19) for decreasing NEC, Fisher's  $P = 0.452$ . The grouping and comparison approach, which is similar to meta-analysis, can serve as an objective tool to measure the effect of MM on NEC. Inconsistent with the results of previous studies, the research concluded that only when MM reaches a certain proportion (40%), the higher the proportion of MM, the lower the incidence of NEC instead of any proportion of MM can protect infants from NEC.

The research of postnatal transmission of HIV(Human immunodeficiency virus) has showed that significant differences in intestinal permeability between breast-fed and formula-fed infants were observed through 6 weeks of age. Mixed feeding might not maintenance of the intestinal mucosal barrier. Early exposure to antigens is more likely to cause sensitization or to stimulate local or systemic immune responses[31]. This could explain our result that the highest rate of NEC occurred in infants who are on mixed feeding with a low MM proportion. The differences between the findings of this study and those of other meta-analyses /studies might have appeared because most of the studies on MM and NEC have been conducted in European and American populations, whereas the population in this study was Asian. It is well known that the influence of the genetic factors vary in different populations, and the same exposure may result in different race effects. Therefore, the results of this study might not be completely in agreement with those of other investigations.

The curve estimation trend for FI was similar to that of NEC (Fig. 1). When MM was around 25%, the risk for FI had its highest value. Nevertheless, the risk declined after MM was elevated above a value of 0.4. The results of multiple aggression analysis showed that, except for MM > 0.70, any other MM proportion increased the risk for developing FI:  $0.4 < MM \leq 0.7$  [OR(CI): 5.247(2.590, 10.631),  $P < 0.001$ ],  $0 < MM \leq 0.4$  [OR(CI): 15.125(6.915, 33.084),  $P < 0.001$ ], MM = 0 [OR(CI): 4.449(1.547, 12.794),  $P = 0.006$ ]. No other variables are independent risk factors for FI in the model. In the present analysis, the other variables were not independent risk factors for FI and were thus removed from the regression model. Therefore, we presume that FI was probably largely influenced by the feeding practices. MM protected ELBW/VLBW against FI and should be advocated as also highlighted earlier[28].

Similarly to Clowning's<sup>[20]</sup> statement, the weight growth rate was significant low in the infants with an HM intake. We also consider that MM does not support the weight gain either in the univariate analysis or multivariate analysis control-related factors. Compared to a lower MM proportion group, the weight gain of a higher group will decrease - 0.571 [ $\beta$ (CI): -0.571(-0.992, -0.150),  $P = 0.008$ ] (Table 4). MM cannot provide sufficient calorie for infants to grow[32], so HMF is necessary when feedings arrive to 100 mL/kg/day. However, the results show that HMF has no relationship with weight growth in this study, which may be influenced by other factors, for example BW and GA (both P-values < 0.050). BW [ $\beta$ (CI): -0.004(-0.006, -0.002),  $P = 0.001$ ] and GA [ $\beta$ (CI): 0.238(0.010, 0.466),  $P = 0.041$ ] are independent related factors.



From the curve estimation, we can see that the rate of BPD development declines slightly with the MM increase (Fig. 2). It is easily understood that there is no difference between groups for rate of BPD development (Table 2). Which is consistent with previous studies[33]. In addition, MM feeding practices neither have effect on IVH nor ROP ( $P > 0.005$ , Table 2), there are also consistent with some study[33]. The rate of LOS declined when MM proportion increased (Fig. 1); there were differences among four groups in LOS rate (Table 2). However, the results of the univariate and multivariate regression did not indicate a significant effect of MM in LOS rate (Table 3 and Table 4). It is probably because the effect was so little that it could not be detected based on the current statistical effect. Overall, the impact of MM on BPD, ROP, IVH and LOS has not yet been determined[19].

The limitations of this study are associated with its retrospective design. Hence, it is not possible to conclusively confirm the existence of a causal relationship between MM and NEC development in this population. We attempted to control the confounding factors by adjustments for group differences and known risk factors for NEC in the multivariate logistic regression analysis. However, this is not a randomized study, and the groups differed in ways that could not possibly be corrected in the multivariate analyses.

The strengths of this study are as follows:

- (1) The precise volumes of MM and PF were determined, and the proportion of MM feeding was defined as the exposure variable rather than volume of feeding to control the bias caused by feeding healthier infants with greater volumes;
- (2) This meta-analysis and many of the aforementioned studies did not conduct a quantitative estimation. Only the continuous rate changes were established with the increase in the MM dose. Using a quantitative approach, this study clarifies the relationship between MM dose and NEC rate, making it more accurate and reliable.
- (3) Based on the curve trend and interval between groups, the current study did a more accurate classification than those previous studies. Besides, incomplete data are minimized using multiple datasets including the electronic medical record.

In summary, this retrospective study revealed a quantitative relationship between the ratio of NEC to FI as a function. A MM proportion higher than 40% exerted significant protective effects against NEC. When MM proportion is higher than 70%, it might protect preterm infants from NEC. We should encourage mother for breast milk and alert to NEC when MM proportion is less than 40%.

## Declarations

### Ethics approval and consent to participate

The Shenzhen Maternity and Child Health Care Hospital Institutional Ethical Committee approved the collection and usage of the clinical information for research purposes before the investigation was

initiated. The informed consent for participation in the study was obtained from their parents.(IEC No. [2019]-034).

### **Consent for publication**

Not applicable.

### **Availability of data and materials**

The raw dataset analyzed in the current study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that they have no competing interests.

### **Funding**

This study was supported by Shenzhen Science and Technology Innovation Committee (JCYJ20160429102107498 to C.Y. and JCYJ20180306173125699 to X.C.); Shenzhen Medical Sanming Project (SZSM201612045); Shenzhen Health and Family Planning Commission (201606028 to Y.L.) and institutional project (FYA2018018 to C.Y.). The funders were not involved in the study design, data collection, analysis, interpretation, or manuscript preparation.

### **Authors' contributions**

BL collected data and wrote the manuscript. XX and YZ collected data. XX, PS and CY constructed article design. FY implemented quality control on the selection of participants. XC and FY revised the manuscript. All authors read and approved the final manuscript.

### **Acknowledgements**

We thank all neonates and parents enrolled in the study.

## **Abbreviations**

MM: Maternal milk; NEC: Necrotizing enterocolitis; GA: Gestation age; VLBW: Very low birth weight; ELBW: Extremely low birth weight; FI: Feeding intolerance; PF: pure formula feeding; LOS: Late onset sepsis; ROP: Retinopathy of prematurity; BPD: Bronchopulmonary dysplasia; NICU: Neonatal intensive care unit; PS: Pulmonary surfactant; IVH: Intraventricular hemorrhage; PMA: post-menstrual age; DM: Donor human milk; HMF: Human Milk Fortifier powder; BW: birth weight; HM: human milk; HIV: Human immunodeficiency virus; CI: Confidence interval; OR: Odds ratios.

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## Tables

Table 1. Characteristics of the participants

| Character                 | (Mean + SD)/(n,%)  |
|---------------------------|--------------------|
| Age, d                    | 16.9 ± 8.3         |
| Gestational age, wk       | 29.3 ± 2.8         |
| Birth weight, g           | 1171.6 ± 234.2     |
| Milking time, h           | 21.4 ± 50.7        |
| Male                      | 174 (57.2)         |
| Cesarean section          | 191 (62.8)         |
| Amniotic fluid            | 290 (95.4)         |
| Antenatal steroids,       | 167 (55.3)         |
| Test tube baby            | 49 (16.1)          |
| Intrauterine distress     | 25 ( 8.2)          |
| Cure                      | 279 (91.8)         |
| Automatic discharge       | 19( 6.2)           |
| 5-min Apgar score < 5     | 2(0.7)             |
| Hospital costs            | 121422.3 ± 69340.5 |
| Length of hospitalization | 61.4 ± 27.1        |
| PS <sup>1</sup>           | 159 (52.6)         |
| HMF <sup>2</sup>          | 175 (57.9)         |
| NEC <sup>3</sup>          | 24 ( 7.9)          |
| FI <sup>4</sup>           | 93 (30.6)          |
| LOS <sup>5</sup>          | 7 ( 2.3)           |
| ROP <sup>6</sup>          | 16 ( 5.3)          |
| IVH <sup>7</sup>          | 18 ( 6.0)          |
| BPD <sup>8</sup>          | 35 (11.6)          |

<sup>1</sup>PS, pulmonary surfactant; <sup>2</sup>HMF, Human milk fortifier; <sup>3</sup>NEC, Necrotizing enterocolitis; <sup>4</sup>FI, feeding intolerance; <sup>5</sup>LOS, Late onset sepsis; <sup>6</sup>ROP, retinopathy of prematurity; <sup>7</sup>IVH, Intraventricular hemorrhage; <sup>8</sup>BPD, Bronchopulmonary dysplasia. Note: Continuous data

expressed as mean (standard deviation) or median (interquartile range), and categorical data as n (%). a Deaths (22) were excluded in this analysis.

Table 2 Comparison of the outcomes of the different groups

| Outcomes                            | MM=0<br>(19)     | 0<MM≤0.40<br>(59) | 0.4<MM≤0.70<br>(64) | MM>0.70<br>(163) | $\chi^2/t$ | P      |
|-------------------------------------|------------------|-------------------|---------------------|------------------|------------|--------|
| Hospitalization, d                  | 50.0(35.0, 67.0) | 46.0(41.0, 75.0)  | 51.0(40.0, 77.0)    | 58.0(42.0, 78.0) | 5.657      | 0.13   |
| Length growth, cm/wk                | 0.8(0.5, 1.2)    | 0.8(0.5, 1.0)     | 0.9(0.7, 1.1)       | 0.8(0.7, 1.1)    | 4.906      | 0.179  |
| Weight growth, g/kg/d               | 14.0(12.2, 14.7) | 11.3(10.5, 12.5)  | 11.1(9.9, 12.2)     | 10.7(9.8, 11.6)  | 29.827     | <0.001 |
| NEC <sup>1</sup> , n (%)            | 2(10.5)          | 15(25.4)          | 5(7.8)              | 2(1.2)           | 31.364     | <0.001 |
| FI <sup>2</sup> , n (%)             | 8(42.1)          | 37(62.7)          | 27(42.2)            | 22(13.5)         | 56.1       | <0.001 |
| LOS <sup>3</sup> , n (%)            | 1(5.3)           | 4(6.8)            | 2(3.1)              | 0(0)             | 11.057     | 0.004  |
| IVH <sup>4</sup> , grade 2-4, n(%)  | 0(0)             | 2(3.4)            | 1(1.6)              | 2(1.2)           | 1.647      | 0.641  |
| ROP <sup>5</sup> , grade 3-4, n(%)  | 3(15.8)          | 13(22.4)          | 6(9.4)              | 36(22.1)         | 5.409      | 0.145  |
| BPD <sup>6</sup> , grade 2-4, n (%) | 1(5.3)           | 6(10.2)           | 7(10.9)             | 21(13.0)         | 1.188      | 0.757  |

<sup>1</sup>NEC, Necrotizing enterocolitis; <sup>2</sup>FI, feeding intolerance; <sup>3</sup>LOS, Late onset sepsis; <sup>4</sup>ROP, retinopathy of prematurity; <sup>5</sup>IVH, Intraventricular hemorrhage; <sup>6</sup>BPD, Bronchopulmonary dysplasia. Note: Continuous data expressed as mean (standard deviation) or median (interquartile range), and categorical data as n (%). a Deaths (22) were excluded in this analysis.

Table 3 Univariate analysis of the MM group data

| Outcomes         | Variable        | B       | S.E.     | Wald   | P      | OR(95%CI)              |
|------------------|-----------------|---------|----------|--------|--------|------------------------|
| NEC <sup>2</sup> | Constant        | -4.388  | 0.711    | 38.041 | <0.001 | 0.012(- -)             |
|                  | MM <sup>1</sup> |         |          |        |        |                        |
|                  | MM>0.70         |         |          | 21.334 |        |                        |
|                  | 0.4<MM<=0.70    | 1.92    | 0.85     | 5.098  | 0.024  | 6.822(1.288, 36.121)   |
|                  | 0<MM<=0.40      | 3.312   | 0.772    | 18.418 | <0.001 | 27.443(6.047, 124.553) |
|                  | MM=0            | 2.248   | 1.032    | 4.746  | 0.029  | 9.471(1.253, 71.585)   |
| FI <sup>3</sup>  | Constant        | -1.858  | 0.229    | 65.677 | <0.001 | 0.156(- -)             |
|                  | MM              |         |          |        |        |                        |
|                  | MM>0.70         |         |          | 48.9   | <0.001 |                        |
|                  | 0.4<MM<=0.70    | 1.543   | 0.341    | 20.407 | <0.001 | 4.677(2.395, 9.133)    |
|                  | 0<MM<=0.40      | 2.378   | 0.354    | 45.213 | <0.001 | 10.779(5.390, 21.555)  |
|                  | MM=0            | 1.539   | 0.518    | 8.826  | 0.003  | 4.661(1.688, 12.868)   |
| LOS <sup>4</sup> | Constant        | -21.203 | 3148.157 | <0.001 | 0.995  | <0.001(- -)            |
|                  | MM              |         |          |        |        |                        |
|                  | MM>0.70         |         |          | 0.843  | 0.839  |                        |
|                  | 0.4<MM<=0.70    | 17.769  | 3148.157 | <0.001 | 0.995  | 52112095.480(- -)      |
|                  | 0<MM<=0.40      | 18.582  | 3148.157 | <0.001 | 0.995  | 117489087.992(- -)     |
|                  | MM=0            | 18.313  | 3148.157 | <0.001 | 0.995  | 89748608.883(- -)      |
| weight-growth    | Constant        |         | 0.241    | 45.602 | <0.001 | 10.968(10.494, 11.441) |
|                  | MM              |         | 0.193    | 2.621  | 0.009  | 0.506(0.126, 0.885)    |

<sup>1</sup>MM, maternal milk; <sup>2</sup>NEC, Necrotizing enterocolitis; <sup>3</sup>FI, feeding intolerance; <sup>4</sup>LOS, Late onset sepsis.

Table 4 Multiple regression analysis of the MM group data

| Outcomes         | Variables            | B       | S.E.   | Wald/ <i>t</i> | <i>P</i> | OR/ $\beta$ (95%CI)    |
|------------------|----------------------|---------|--------|----------------|----------|------------------------|
| NEC <sup>3</sup> | Constant             | 4.638   | 4.659  | 0.991          | 0.320    | 103.375(- -)           |
|                  | GA <sup>1</sup> (wk) | -0.373  | 0.174  | 4.606          | 0.032    | 0.688(0.490, 0.968)    |
|                  | MM <sup>2</sup>      |         |        |                |          |                        |
|                  | MM>0.70              |         |        | 17.919         | <0.001   |                        |
|                  | 0.4<MM<=0.70         | 1.869   | 0.869  | 4.623          | 0.032    | 6.482(1.180, 35.608)   |
|                  | 0<MM<=0.40           | 3.258   | 0.81   | 16.174         | <0.001   | 25.99(5.312, 127.146)  |
| FI <sup>4</sup>  | MM=0                 | 2.337   | 1.064  | 4.826          | 0.028    | 10.348(1.287, 83.239)  |
|                  | Constant             | -2.28   | 2.575  | 0.784          | 0.376    | 0.102(- -)             |
|                  | MM                   |         |        |                |          |                        |
|                  | MM>0.70              |         |        | 49.617         | <0.001   |                        |
|                  | 0.4<MM<=0.70         | 1.658   | 0.36   | 21.173         | <0.001   | 5.247(2.590, 10.631)   |
|                  | 0<MM<=0.40           | 2.716   | 0.399  | 46.271         | <0.001   | 15.125(6.915, 33.084)) |
| LOS <sup>5</sup> | MM=0                 | 1.493   | 0.539  | 7.673          | 0.006    | 4.449(1.547, 12.794)   |
|                  | Constant             | -34.813 | 9201.5 | <0.001         | 0.997    | <0.001(- -)            |
|                  | BW(g)                | -0.007  | 0.003  | 5.684          | 0.017    | 0.993(0.987, 0.999)    |
|                  | MM                   |         |        |                |          |                        |
|                  | MM>0.70              |         |        | 1.286          | 0.732    |                        |
|                  | 0.4<MM<=0.70         | 17.773  | 2833.6 | <0.001         | 0.995    | 52328193.662(- -)      |
| Weight growth    | 0<MM<=0.40           | 18.875  | 2833.6 | <0.001         | 0.995    | 157449333.652(- -)     |
|                  | MM=0                 | 18.435  | 2833.6 | <0.001         | 0.995    | 101404844.123(- -)     |
|                  | Constant             |         | 3.407  | 3.451          | 0.001    | 11.756(5.049, 18.463)  |
|                  | BW <sup>6</sup> (g)  |         | 0.001  | -3.296         | 0.001    | -0.004(0.001, -3.296)  |
|                  | MM                   |         | 0.572  | -2.585         | 0.010    | -1.478(-2.604, -0.352) |

<sup>1</sup>Gestational age; <sup>2</sup>MM, maternal milk; <sup>3</sup>NEC, Necrotizing enterocolitis; <sup>4</sup>FI, feeding intolerance; <sup>5</sup>LOS, Late onset sepsis; <sup>6</sup>BW, Birth weight

## Figures



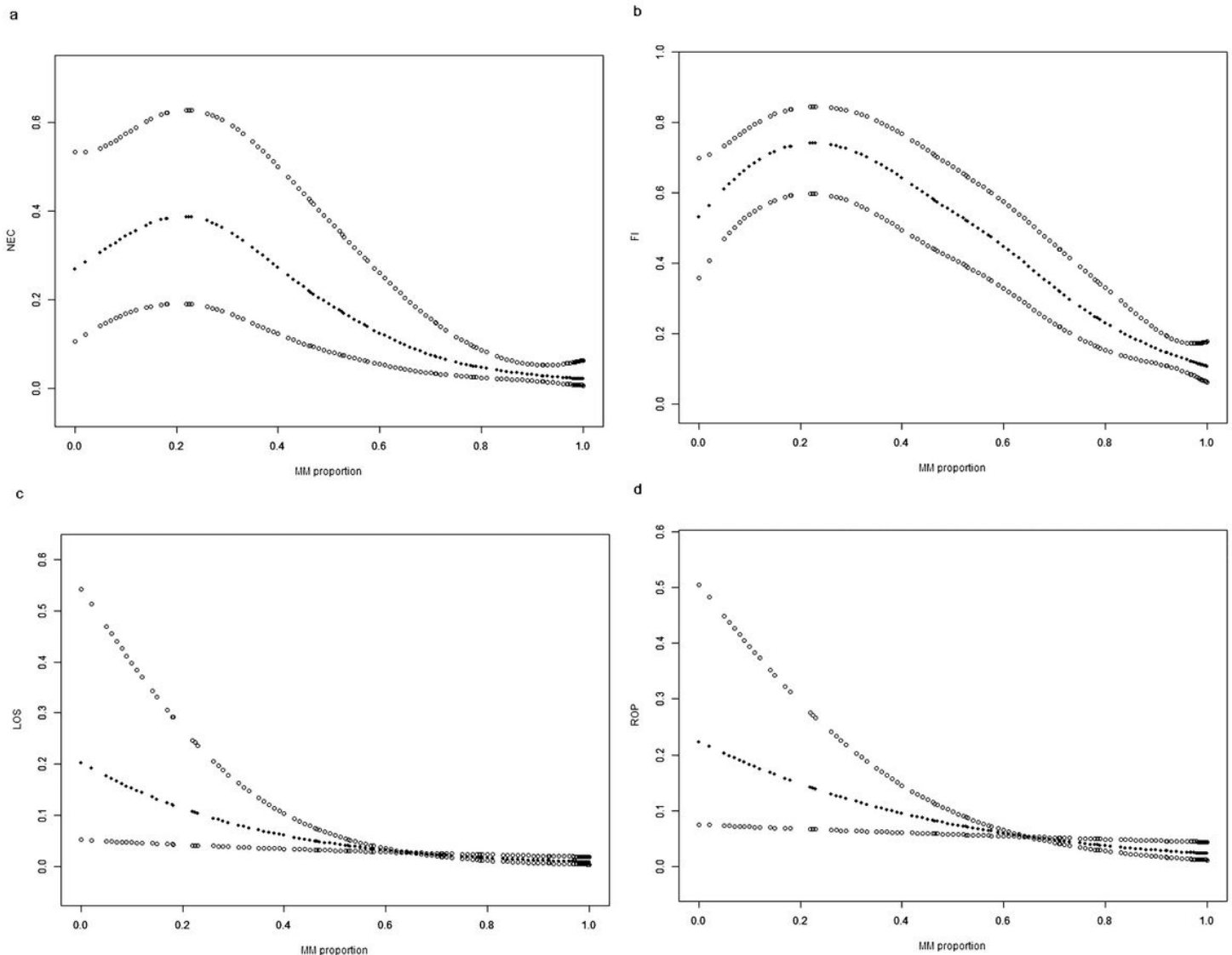


Figure 1 Presentation of the dose relationship between the rates of outcomes and MM proportion. a: The dose relationship between rate of NEC and MM proportion. b: The dose relationship between rate of FI (feeding intolerance) and MM proportion. c: The dose relationship between rate of LOS (late onset sepsis) and MM proportion. d: The dose relationship between rate of ROP (retinopathy of prematurity, grade 3-4) and MM proportion.

## Figure 1

. Presentation of the dose relationship between the rates of outcomes and MM proportion. "a": The curve estimation showed that the rate of NEC (Bell Stage 1B or greater) was around 25% when MM (maternal milk) proportion was 0 (pure forum, PF), with the increase in the MM proportion, the rate of NEC initially rose, which continued until MM reached around 20%, the highest rate reached was 40%. At MM greater than 20%, the rate of NEC declined correspondingly to the increase in the MM proportion. At a MM value of 40%, the rate of NEC was approximately 25%, which was similar to that of PF feeding. The curve increased to 40% (MM = 20%) at first and then decreased to 25% (MM = 40%); it was reduced when MM was within the range from 0% to 40%. When MM proportion was increased from 40% to 100%, rate of NEC dropped from 25% to near zero. "b": Rate of FI (feeding intolerance) changed in the same way with NEC. "c": LOS (Rate of late onset sepsis) decreased from approximately 25% to zero with no inflection

points. “d”: ROP (retinopathy of prematurity, grade 3–4) decreased from approximately 25% to zero with no inflection points.

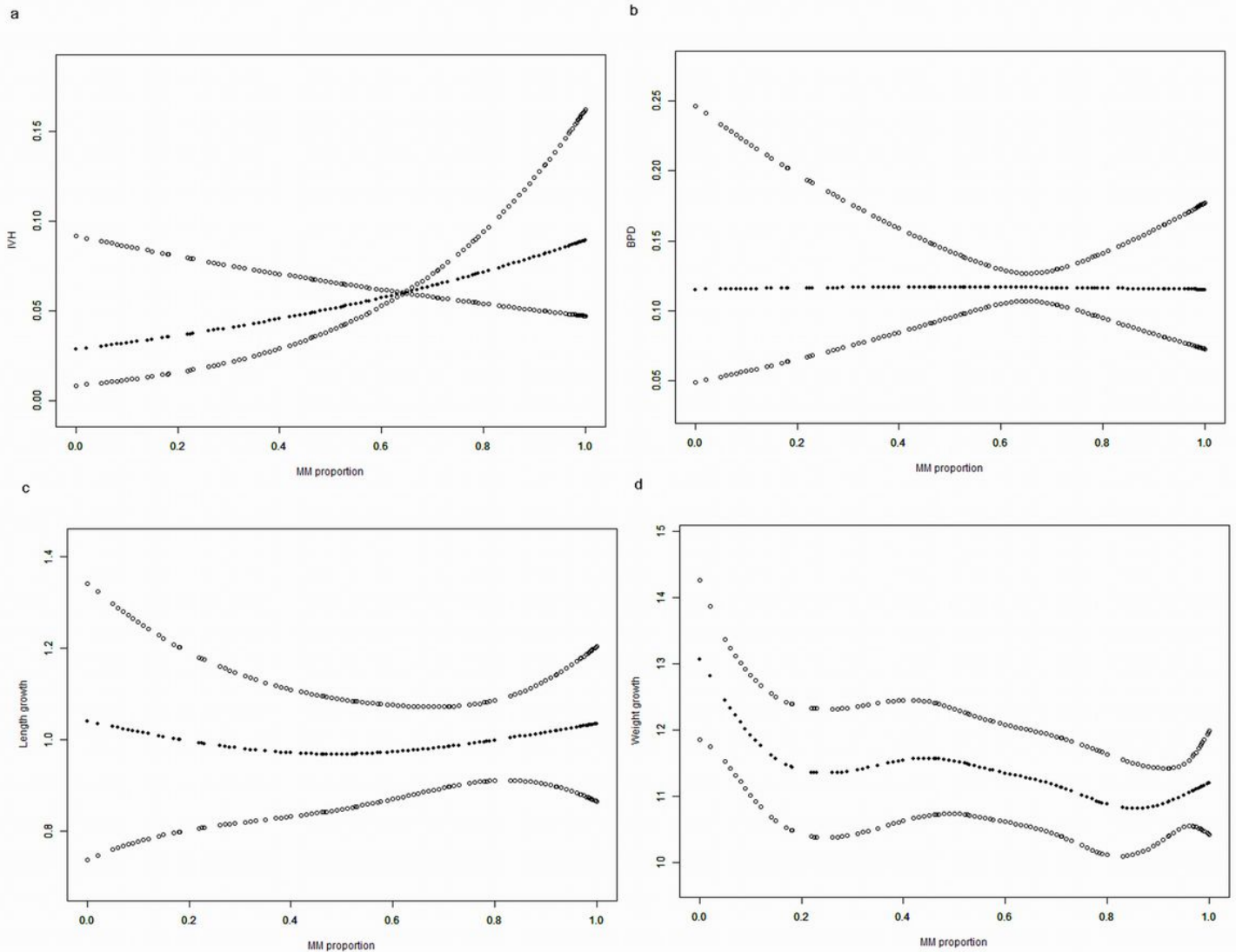


Figure 2 Presentation of the dose relationship between the rates of complications and MM proportion. a: The dose relationship between IVH (intraventricular hemorrhage, grade 2–4) and MM proportion. b: The dose relationship between rate of BPD (bronchopulmonary dysplasia, moderate-heavy) and MM proportion. c: The dose relationship between rate of length growth and MM proportion. d: The dose relationship between rate of weight growth and MM proportion.

## Figure 2

Presentation of the dose relationship between the rates of complications and MM proportion. “a”: When MM (maternal milk) increased from 0 to 100%, rate of IVH(intraventricular hemorrhage, grade 2–4) increased from 3% to 8%. “b”: When MM increased from 0 to 100%, The rate of BPD (bronchopulmonary dysplasia, moderate-heavy) was stable at 12%. “c”: When MM increased from 0 to 100%, The rate of length growth was stable at 1.05%. “part d”: When MM increased from 0 to 100%, the rate of weight growth changed in a complex manner, which might have been caused by the influence of mixed factors, such as birth weight and gestational age. However, the curve trend clearly showed that the higher MM proportion led to a lower weight growth. When infants were fed with PF (pure forum), their highest weight

growth was 13, whereas the lowest weight growth was 11 when their MM proportion was greater than 0.8.

## Supplementary Files

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- [formula1.JPG](#)