Title: Effect of community-initiated kangaroo mother care on fecal biomarkers of gut function in low birthweight infants in North India: a randomized clinical trial

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ABSTRACT (Unstructured)

This individually randomized trial was conducted to estimate the effect of promoting community-initiated kangaroo mother care (ciKMC) in low birthweight (LBW) infants on gut inflammation and permeability. Participants included 200 stable LBW infants (weighing 1500-2250 g) in North India enrolled between May to October 2017. The ciKMC intervention included promotion and support of continuous skin-to-skin contact and exclusive breastfeeding through home visits. The mothers in the intervention arm were supported to practice ciKMC until 28 days after birth, i.e. the neonatal period, or till the baby wriggled out of KMC position, if earlier. Infant stool specimens were collected during the first week of birth, and within one week after end of the neonatal period. Concentrations of fecal neopterin (nmol/L), myeloperoxidase (ng/mL), and alpha-1-antitrypsin (μg/mL) were determined using ELISA, and composite enteric enteropathy (EE) score at end of the neonatal period was calculated by principal component analysis. We did not find any substantial difference in means between the ciKMC and control arm infants in the log-transformed values of neopterin (0.03; 95% CI -0.15 to 0.21), myeloperoxidase (0.28; 95% CI -0.05 to 0.61) and alpha-1-antitrypsin (0.02; 95% CI -0.30 to 0.34). The mean (SD) composite EE score was 13.6 (7.5) in the ciKMC and 12.4 (8.3) in the control arm infants, and the adjusted difference in means was negligible, 0.4 (95% CI -1.8 to 2.7). Our findings suggest that the promotion of ciKMC did not affect gut inflammation and permeability in our target population of low birthweight infants in North India.

Key words: Kangaroo mother care, Enteropathy, Gut inflammation, Gut permeability, low birth weight, infant

INTRODUCTION

Gut function among young children in low-income communities is postulated to be one of the important drivers of poor growth. Environmental enteric dysfunction (EED) is described as a broad syndrome with various alterations in gut function including increased gut inflammation, altered gut permeability, villous blunting, and crypt hyperplasia as a consequence of chronic exposure to enteropathogens.[1] EED leads to a vicious cycle of reduced intestinal absorptive capacity, which in turn causes protein-energy and micronutrient malnutrition, and thereby poor growth.[1, 2, 3] A multicentric cohort study in 8 countries showed that children with the highest enteric enteropathy (EE) score (calculated using the three fecal biomarkers neopterin, myeloperoxidase and alpha-1-antitrypsin) grew 1.08 cm less than those with the lowest EE score during the following 6-month period.[4] EED is of greater concern in infants born preterm or low birth weight, who are at higher risk of enteric infections and growth faltering.[5, 6, 7]

It is unclear whether EED, gut inflammation, or permeability can be prevented early in life. It is speculated that antibiotics or probiotics, improved infant feeding, zinc supplementation, or water, sanitation, and hygiene (WASH) interventions might improve gut function by reducing the enteric pathogen load, gut inflammation and/or permeability.[1] However, studies have not been able to demonstrate clear reproducible changes in fecal markers of gut function following these interventions.[8, 9, 10, 11] The World Health Organization (WHO) and the Government of India recommend Kangaroo Mother Care (KMC), an intervention encompassing skin-to-skin contact (SSC) and exclusive breastfeeding, to improve survival in low birth weight (LBW) babies.[12, 13, 14] A large randomized controlled trial in India among 8402 LBW infants demonstrated that promotion of community-initiated KMC (ciKMC) improved post-enrolment neonatal survival by 30%.[15] In addition, it reduced the risk of possible serious bacterial infection, diarrhea, and severe underweight during the neonatal period, i.e. the first 28 days of life. It is plausible that KMC reduces gut inflammation and permeability in LBW infants as the baby is placed in a

protective environment in SSC and exclusively breastfed, reducing the chance of enteropathogen exposure and clinical infection.

Our primary objective was to estimate the effect of ciKMC promotion in LBW infants on gut inflammation and permeability at the end of the neonatal period. In addition, we estimated the effect of ciKMC promotion on enteric enteropathy. We hypothesized that the promotion of ciKMC reduces gut inflammation and permeability as reflected in a lower concentration of fecal neopterin, myeloperoxidase, and alpha-1-antitrypsin, and enteric enteropathy as reflected in a lower composite EE score and EE index.

METHODOLOGY

Ethics Statement

Ethics approval was obtained from the Society for Applied Studies' ethics committee (SAS/ERC/KMCS/2017) and the Regional Committee for Medical and Health Research Ethics (REK) in Western Norway. The study was registered with Clinical trials registry-India (CTRI/2017/04/008430). Written informed consent including permission for storage of specimens for future research was obtained from the mothers of the eligible infants before enrollment.

Study design and participants

This individually randomized clinical trial was developed as a sub-study within a larger primary trial (ClinicalTrials.gov NCT02653534).[15, 16] The trial was conducted in rural and semi-urban low-income populations of Faridabad and Palwal districts in Haryana, India. As part of the primary trial[15, 17], pregnant women were followed up by a surveillance team periodically till delivery. Newborns weighing 1500 to 2250 g and their mothers were eligible for inclusion if they were

screened within 72 hours of birth. Infants were excluded if KMC had already been initiated in a birth facility, or infants were unable to feed, had breathing problems, gross congenital malformations, less than normal movements, or mothers were not living with their babies or intending to move away over the next six months. Additionally, in this sub-study, we excluded twins and triplets. In the primary trial enrolments were done between July 2015 to October 2018. For evaluation of fecal biomarkers, we enrolled consecutive 200 infants from May 2017 onwards who provided consent; only one eligible child was enrolled from each household (Figure 1).

Intervention and Usual Care

The ciKMC intervention comprised of promotion and support of continuous and prolonged SSC and exclusive breastfeeding. The intervention delivery team visited homes of the infant-mother dyads allocated to the ciKMC trial arm to initiate and support KMC. The team visited on days 1, 2, 3, 5, 7, 10, 14, 21, and 28 after birth to observe and solve any problems related to KMC Mothers and family members were taught to daily record the duration of SSC. Mothers were counselled to practice SSC for as long as possible during day and night, with the assistance of other family members. Visits continued till 28 days of age or if the baby wriggled out of KMC position and no longer accepted SSC, whichever was earlier. Referral of ill infants in both trial arms was facilitated through government Accredited Social Health Activists (ASHAs).[18] All infants in the intervention and control arms received usual care, i.e. home-based postnatal care visits by ASHAs as implemented through the health system.[19]

Study Outcomes

The primary outcomes were the concentration of the individual fecal biomarkers viz. neopterin (nmol/L), myeloperoxidase (ng/mL), and alpha-1-antitrypsin (µg/mL) at the end of the neonatal period (day-28 of birth up to plus 7 days). Other outcomes were EE score and EE index which is

a composite score calculated using three fecal biomarkers neopterin, myeloperoxidase and, alpha-1-antitrypsin. Neopterin is an indicator of T-helper cell 1 activity. Myeloperoxidase reflects neutrophil activity in the intestinal mucosa while alpha-1-antitrypsin indicates intestinal permeability and protein loss. These fecal biomarkers have been previously used in multiple studies as proxy measures of gut inflammation and permeability.[3, 20, 21, 22]

Assessment of Outcomes

Stool specimens were collected at baseline within the first week of birth and within 1 week after the end of the neonatal period. A stool kit consisting of a cold box with 4 ice-packs, a labelled sterile (gamma-irradiated) stool container with a spatula, a plastic nappy, and tissue paper was provided to the participant's mother. The process of specimen collection was demonstrated to the mother and family members by the field worker. The mother was instructed to store ~5g stool in the cold box only between 6 AM and 3 PM to enable prompt transportation to the laboratory. The mother called the study team upon specimen collection, after which a fieldworker transported the cold box to reach the Clinical and Research Laboratories, Society for Applied Studies (CRL SAS), New Delhi within 6 to 8 hours. The specimens were stored without fixatives[4] in a -80ºC freezer until analysis.

Laboratory analysis was initiated in CRL SAS, New Delhi, in October 2018. Fecal biomarkers of gut function were analyzed by the ELISA method using the automated EVOLIS™ Twin Plus system (BioRad, California, USA). We used IBL International Kit (Hamburg, Germany) for neopterin assessment; K6630 IDK MPO ELISA kit (Immundiagnostik AG, Bensheim, Germany) for myeloperoxidase; and the Human A1AT kit (Immuchrom Gmbh, Heppenheim, Germany) for alpha-1-antitrypsin. All three kits were verified to identify the acceptable range of values for accuracy using the manufacturer standards, inter and intra-assay precision, and linearity before conducting the experiments with the study stool specimens. Standard kit instructions were

followed; specimens with values out of range were diluted as required and the dilution factor was accounted for when calculating the final values.

Sample Size

With 95% confidence and 90% power, a total sample size of 168 infants (84 in each arm) was deemed sufficient to detect at least a 0.5 SD difference in the mean concentration of fecal biomarkers between the trial arms. Assuming a 15% attrition due to loss to follow-up or failed stool specimen collection or processing, we enrolled 200 infants (100 in each of the ciKMC and control arms) into our trial.

Statistical Analysis

Analyses were conducted on an intent-to-treat basis using STATA version 16 (Stata Corporation, College Station, TX). Given the right-skewed distribution of the fecal biomarkers, we reported medians with interquartile ranges (IQRs), presented violin plots for both study arms, and log-transformed (natural logarithm) the data prior to statistical analyses. Pearson correlation coefficient in pairwise comparisons was estimated for the three fecal biomarkers.

Using the approach of MAL-ED investigators[4], a composite score based on the percentile category of the myeloperoxidase, neopterin, alpha-1-antitrypsin concentrations was developed using the weightage factor as per principal component analysis. The principal component analysis indicated a 6-fold higher weight for myeloperoxidase and neopterin compared to alpha-1-antitrypsin. The EE score calculation is shown in the equation below, where myeloperoxidase, neopterin, alpha-1-antitrypsin categories are defined as 0 (≤ 25th percentile), 1, (25–75th percentile), or 2 (≥ 75th percentile). The score ranged from 0 to 26.

EE score = 6(neopterin category) + 6*(myeloperoxidase category) + 1*(alpha-1-antitrypsin category)*

To calculate the EE index we used Stata's "factor" command including the three log-transformed fecal biomarker variables and thereafter generated the index using the "predict" command. The value of this index ranged from -4.3 to 1.73.

We applied the Student's t-test to estimate if there were any substantial differences in the unadjusted mean of the log-transformed concentrations of the fecal biomarkers at 1 month of age between the ciKMC arm and the control arm. We used multivariable generalized linear models (GLM) of the Gaussian family with an identity link to estimate the difference in means of the log-transformed fecal biomarkers between the trial arms adjusted for its baseline concentration and unequally distributed potential confounding factors at baseline. Unequal distribution of a potential confounding factor was *a priori* defined as a relative difference of more than 10% across the study arms.[23] Wealth quintiles, WASH factors (toilet facility and source of drinking water), birth order, baby sex, weight at enrolment, and gestational age were the potential confounders associated with the primary outcome at $P < 0.10$ in univariable analysis.

Subgroup analyses, decided *a priori*, were conducted to estimate whether the effect of ciKMC on gut inflammatory biomarkers at the end of 1 month after birth was different in preterm infants (<37 weeks gestation) compared to term infants (≥37 weeks gestation). Gestational age was estimated from the ultrasonography reports, when available, or based on the last menstrual period as documented in hospital records or as per maternal recall, in the given order of preference.

RESULTS

Stool specimens were collected from all 200 infants enrolled in the trial at baseline and the end of the neonatal period. Fecal biomarker assessments were completed in 99% to 100% of the infants in both study arms for all three biomarkers (Figure 1).

The mean (SD) age when the baseline stool specimens were collected was 4.2 (1.6) days in the ciKMC arm and 5.1 (1.2) days in the control arm. The mean (SD) age of stool specimen collection at the end of the neonatal period was 31.6 (3.4) days in the ciKMC arm and 32.0 (5.5) days in the control arm. Baseline characteristics were similar in the two study arms other than for wealth quintiles (lower 3 quintiles), availability of toilet facility in the household, source of drinking water (public tap), birth order (≥5), and sex of the baby, where the relative differences between study arms exceeded 10% (Table 1).

All mothers in the intervention arm and 4% in the control arm reported practice of SSC during the neonatal period. In the intervention arm, the median (IQR) age of the infant at ciKMC initiation was 27.5 (12.5 to 38.5) hours. The mothers in the intervention arm practiced SSC for a median of 28 days with a mean (SD) of 12.2 (3.1) hours per day. Exclusive breastfeeding prevalence (24-hour recall) at day-28 was 84% in the ciKMC arm and 60% in the control arm (Online supplemental table 1). Diarrhea or dysentery during the neonatal period was reported in 3% in the ciKMC arm and 9% in the control arm infants. The chi-square test showed that there were no significant differences in diarrhea or dysentery between the trial arms (p=0.075)

Pearson's correlation coefficient (*r*) suggested a low correlation between the baseline concentrations of neopterin or myeloperoxidase with alpha-1-antitrypsin (r = <0.1). At baseline, the median (IQR) concentrations of fecal neopterin were 1497 (993 to 2397) nmol/L and 1268 (879 to 1893) nmol/L, myeloperoxidase were 201 (94 to 388) ng/mL and 208 (98 to 340) ng/mL,

and alpha-1-antitrypsin were 282 (148 to 544) µg/mL and 302 (187 to 549) µg/mL in the ciKMC arm and control arm infants, respectively (Figure 2).

At the end of the neonatal period, the median (IQR) concentration of fecal neopterin were 1866 (1022 to 2371) nmol/L and 1689 (1055 to 2355) nmol/L; myeloperoxidase were 324 (137 to 498) ng/mL and 262 (93 to 392) ng/mL; alpha-1-antitrypsin were 310 (164 to 649) µg/mL and 298 (178 to 605) µg/mL in the ciKMC and control arm infants, respectively (Figure 3). The mean (SD) composite EE Score was 13.6 (7.5) in the ciKMC arm and 12.4 (8.3) in the control arm infants.

The adjusted difference in means between the ciKMC arm and control arm in the log-transformed concentration of neopterin was 0.03 (95% CI -0.15 to 0.21), myeloperoxidase was 0.28 (95% CI -0.05 to 0.61), and alpha-1-antitrypsin was 0.02 (95% CI -0.30 to 0.34). The adjusted difference in means in the EE score was 0.44 (95% CI -1.81 to 2.69), that for the EE index 0.17 (95% CI -0.11 to 0.45). Unadjusted analysis showed similar results (Table 2).

In term infants, there was virtually no difference between the study arms in the mean log-transformed concentrations of fecal biomarkers, nor in the EE score or the EE index (Table 3). Among the preterm infants, the adjusted difference in means between study arms in the log-transformed concentration of myeloperoxidase was 0.41 (95% CI 0.02 to 0.82), and that for EE index was 0.38 (95%CI 0.01 to 0.75).

DISCUSSION

We aimed to estimate the effect of ciKMC promotion among LBW infants on fecal biomarkers of gut inflammation and permeability, enteric enteropathy score and index at the end of the neonatal period. In our trial of 200 North Indian LBW infants, we did not find evidence of any

246 substantial effect of ciKMC promotion on the concentrations of fecal neopterin,
247 myeloperoxidase, or alpha-1-antitrypsin, nor on the enteric enteropathy score or index.

248 Our effectively implemented randomization, no loss to follow-up and adjustment for potential
249 confounders makes it unlikely that selection bias compromised the validity of our findings. Errors
250 in the measurement of fecal biomarkers are unlikely, given the use of an automated ELISA
251 system and pre-study kit validation exercises. Similar to the protocol followed in the MAL-ED
252 study sites[4], we did not use any fixatives or protease inhibitors while storing fecal specimens.
253 Therefore, we cannot rule out the possibility of some degree of natural degradation of the
254 biomarker proteins. Nonetheless, given that the fecal specimens were stored within 6-8 hours of
255 stool passage at -80ºC, with no freeze-thaw events, we believe this not to be a major concern.
256 There is a possibility that stool specimens collected during a diarrheal episode may lead to
257 inaccurate measurement of fecal biomarker concentrations. We included all eligible LBW infants
258 in the primary analysis, as history-based ascertainment of diarrhea in neonates is not always
259 reliable. Although we did not find a statistically significant difference in this substudy, the
260 proportion of children with diarrhea or dysentery in the trial arms was comparable to that in the
261 primary trial.[15] A sensitivity analysis excluding the infants with diarrhea during the neonatal
262 period showed similar estimates as described in the results section (data not shown). With the
263 low likelihood of bias, the study findings seem internally valid and suggest that the promotion of
264 ciKMC is unlikely to affect gut inflammation and permeability in the target population of stable
265 LBW infants in our study setting.

266 We did not find previous studies that examined the effect of KMC on infant gut function. Some
267 trials evaluated the effect of different interventions like WASH or improved infant feeding
268 practices on gut function but failed to demonstrate clear effects on reducing gut inflammation or
269 permeability.[8, 22] The SHINE l cluster-randomized trial, estimated the effect of improved WASH
270 and infant and young child feeding (IYCF) practices on environmental enteric dysfunction in

children aged 1 to 18 months.[8] The trial found no effect of improved WASH or IYCF interventions on fecal myeloperoxidase, neopterin, alpha-1-antitrypsin levels in the first 6 months of life. Another trial in Bangladesh showed that age-appropriate nutrition counselling plus a lipid-based nutrient supplement substantially reduced neopterin concentration at 3 and 14 months of age, but there was no effect on myeloperoxidase, alpha-1-antitrypsin levels, or lactulose-mannitol ratio, another marker of gut permeability. At 28 months of age, however, myeloperoxidase and the lactulose-mannitol ratio were higher among children in the intervention arm than in the control arm.[22] Our trial did not show evidence of any effect of KMC promotion on fecal biomarker concentration at the end of the neonatal period. Better resource availability could have enabled a longer follow-up period, which would have been useful to study the effect over time.

The median concentrations of myeloperoxidase, neopterin, and alpha-1antitrypsin in our study were lower than that observed in many of the previous studies. This may be because of the specific population of LBW infants included in our trial, the timing of fecal specimen collection and/or differences in the ELISA kits used. We analysed the concentration of fecal specimens which were collected within 7 days of birth and at the end of the neonatal period, whereas in earlier studies fecal specimens were studied mostly in children 3 months and older.[4, 21, 24, 25] A study documenting trends in fecal biomarker concentrations over time suggests that they are probably higher when babies are 3 to 6 months of age than younger infants.[24]

The exploratory subgroup analyses in preterm infants did not suggest any meaningful differences between the study arms in the measured fecal biomarkers at the end of the neonatal period. The somewhat higher myeloperoxidase levels among preterm infants in the ciKMC arm could be an incidental finding.[26] Alternatively, it may be explained by the higher rates of exclusive breastfeeding in the ciKMC arm which are believed to be associated with increased

fecal myeloperoxidase levels.[24] More research on the effect of interventions on gut function in preterm infants and association of breastfeeding with fecal biomarkers of inflammation would be helpful.

The study did not show evidence to support our hypothesis that KMC promotion can reduce fecal biomarkers of gut inflammation and permeability in stable LBW neonates in rural and peri-urban settings in North India. Our study had some limitations. The findings may not be generalizable to different settings, or among unstable or very low birth weight infants. Additional information on maternal nutrition parameters that might influence infant gut function could be useful to document adequate randomization and contextualize our findings. Further research is needed to substantiate our findings and to study if the intervention has an impact on EED, which is a broader entity encompassing several aspects of gut function and systemic inflammation.[27] Biomarkers of EED are seen to be associated with the presence of multiple viral and bacterial pathogens (enteroviruses, adenoviruses, *Campylobacter* spp., and diarrheagenic *Escherichia coli*) in the gut.[25] Future assessment of intervention effects on gut function may consider detection and quantitation of fecal enteropathogens in addition to the biomarkers. The fact that promotion of ciKMC reduced the risk of severe neonatal stunting and wasting[15], yet in the current study we did not find a measurable effect on fecal biomarker concentrations, underscores the need to look for additional mechanisms that can explain growth faltering. Because EED may be influenced by multiple factors it may be worthwhile to explore the role of integrated health interventions on gut function in LBW infants.

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Table 1. Baseline characteristics of the study participants*

Variables	Control arm (n=100) %	ciKMC arm (n=100) %
Household characteristics		
Wealth Quintiles		
Least poor	19	16
Less poor	25	27
Poor	17	26
Very poor	25	13
Most poor	14	18
Religion		
Hindu	86	85
Muslim	10	14
Others	3	1
Type of family		
Nuclear	33	35
Joint	67	65
Toilet not available inside household	17	13
Source of drinking water		
Piped water	20	19
Tube well	31	33
Public tap	28	22
Bottled water	19	25
Other	2	1
Mean number of family members (SD)	7.0 (2.8)	7.6 (3.7)
Maternal and paternal characteristics		
Mean maternal age: years (SD)	24.1 (3.7)	23.1 (3.2)
Mean paternal age: years (SD)	27.2 (4.8)	26.4 (4.2)
Maternal education: years of schooling (SD)	7.1 (5.3)	5.7 (5.2)
Birth related characteristics		
Place of delivery: Home	14	16
Birth order		
1	35	37
2-4	56	56
≥5	9	7
Infant characteristics		
Sex of the baby: Female	52	59
Mean weight at enrolment in gm (SD)	2094.5 (162.1)	2086.2 (139.1)
Mean gestational age in weeks (SD)**	35.9 (1.9)	35.9 (1.6)
Preterm <37 weeks	62	63

*Data presented are number/ percentages unless indicated otherwise

**67.5% (135/200) had an ultrasound for gestational age assessment

Table 2. Fecal biomarker concentration, enteric enteropathy (EE) score and EE index among study participants at the end of the neonatal period

Fecal biomarkers	**Control arm**	**ciKMC arm**	**Unadjusted** Difference in means (95% CI)	**Adjusted**[†] Difference in means (95% CI)
Log Neopterin nmol/L				
Mean (SD)	7.30 (0.68)	7.37 (0.61)	0.07 (-0.11 to 0.25)	0.03 (-0.15 to 0.21)
Log Myeloperoxidase ng/mL				
Mean (SD)	5.12 (1.29)	5.49 (1.01)	0.38 (0.05 to 0.70)	0.28 (-0.05 to 0.61)
Log Alpha1antitrypsin µg/mL				
Mean (SD)	5.57 (1.09)	5.61 (1.16)	0.04 (-0.27 to 0.35)	0.02 (-0.30 to 0.34)
EE score				
Mean (SD)	12.36 (8.32)	13.57 (7.51)	1.21 (-1.01 to 3.43)	0.44 (-1.81 to 2.69)
EE index				
Mean (SD)	-0.12 (1.12)	0.12 (0.86)	0.25 (-0.03 to 0.53)	0.17 (-0.11 to 0.45)

[†] Adjusted for potentially confounding baseline factors when the relative difference at baseline between trial arms were >10%, i.e., wealth quintiles, toilet facility, source of drinking water, birth order, sex of the baby, and baseline concentration of the respective gut inflammatory markers.

Table 3. Effect of ciKMC on infant fecal biomarkers at the end of the neonatal period in subgroups of preterm and term infants

Gut Biomarkers at 1 month	**Infant subgroup**	**Control arm** Mean (SD)	**ciKMC arm** Mean (SD)	**Unadjusted** Difference in means (95% CI)	**Adjusted** † Difference in means (95% CI)
Log Neopterin nmol/L	Preterm (n=125)	7.27 (0.75)	7.43 (0.61)	0.17 (-0.07 to 0.41)	0.16 (-0.09 to 0.40)
	Term (n=75)	7.37 (0.57)	7.27 (0.60)	-0.10 (-0.37 to 0.17)	-0.26 (-0.55 to 0.04)
Log Myeloperoxidase ng/mL	Preterm (n=125)	5.13 (1.13)	5.51 (0.98)	0.39 (0.01 to 0.76)	0.41 (0.02 to 0.82)
	Term (n=75)	5.11 (1.54)	5.47 (1.09)	0.36 (-0.25 to 0.98)	0.01 (-0.58 to 0.60)
Log Alpha1antitrypsin µg/mL	Preterm (n=125)	5.39 (1.24)	5.55 (1.23)	0.16 (-0.28 to 0.60)	0.24 (-0.21 to 0.69)
	Term (n=75)	5.86 (0.74)	5.70 (1.02)	-0.16 (-0.57 to 0.25)	-0.21 (-0.66 to 0.24)
EE Score at 1 month	Preterm (n=125)	11.7 (7.9)	14.2 (7.6)	2.5 (-0.27 to 5.26)	2.5 (-0.40 to 5.36)
	Term (n=75)	13.5 (8.9)	12.5 (7.2)	-0.99 (-4.77 to 2.80)	-3.84 (-7.76 to 0.08)
EE index at 1 month	Preterm (n=125)	-0.18 (1.1)	0.19 (0.8)	0.37 (0.02 to 0.72)	0.38 (0.01 to 0.75)
	Term (n=75)	-0.04 (1.1)	0.01 (0.8)	0.04 (-0.43 to 0.51)	-0.29 (-0.76 to 0.18)

† Adjusted for potentially confounding baseline factors i.e., wealth quintiles, toilet facility, source of drinking water, birth order, sex of the baby, and baseline concentration of the respective gut inflammatory markers.

Figure 1. Participant flow in the trial

Figure 2. Fecal biomarkers of inflammation and permeability across study arms at baseline

Figure 3. Fecal biomarkers of inflammation and permeability across study arms at the end of the neonatal period