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# Effects of *Lactobacillus rhamnnosus* GG vs *Lactobacillus* + *Bifidobacterium* supplementation on feeding progression and gastrointestinal outcomes in preterm neonates: A randomized controlled trial

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**Running title:** Probiotics for preterm neonates

Madiha MSc[1,2], Imran Masood PhD[1], Muhammad Atif PhD[1], Imran Qaisar MD[3]

[1]Department of Pharmacy practice, Faculty of Pharmacy and Alternative medicine, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[2]Department of Pharmacy, The Women University Multan, Multan, Pakistan
[3]Quaid-e-Azam Medical College, Bahawal Victoria Hospital, Bahawalpur, Pakistan

**Authors' email addresses and contributions:**
Madiha: madiha.6375@wum.edu.pk
https://orcid.org/0000-0001-8426-4609
Contribution: methodology, data collection, data analysis, visualization and paper writing.
Imran Masood: drimranmasood@iub.edu.pk
https://orcid.org/0000-0002-9260-9272
Contribution: Conceptualization, project management and supervision
Muhammad Atif: pharmacist_atif@yahoo.com
https://orcid.org/0000-0001-6866-8152
Contribution: data analysis and paper writing.
Imran Qaisar: imran.qaisar78@gmail.com
https://orcid.org/0009-0003-3647-4611
Contribution: data collection and data analysis.

**Corresponding Author:** Dr Imran Masood, Department of Pharmacy Practice, Faculty of Pharmacy and Alternative medicine, The Islamia University of Bahawalpur, Khawajah Fareed Campus, Railway Road, Bahawalpur, Pakistan 63100. Tel: (+00 92) 3336397270. Email: drimranmasood@iub.edu.pk

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## ABSTRACT

**Background and Objectives:** Gut immaturity predisposes several serious morbidities in preterm infants. Though most proposed intervention, the clinical effectiveness of probiotics may vary based on strains administered, dosage regimen, duration of supplementation and timing of initiation. We aimed to evaluate effect of early enteral probiotic supplementation on clinical, feeding and gastrointestinal outcomes in preterm neonates. **Methods and Study Design:** Babies born at <36 + 6 weeks' gestation with a birth weight <2500 grams were randomly allocated into three groups. The control group received 10% dextrose, while the intervention groups received single-strain (SS)-*Lactobacillus rhamnosus GG* or multi-strain-(MS) *Bifidobacterium BB-12*, *Lactobacillus paracasei subsp. paracasei 431*, *Streptococcus thermophilus TH-4*, within first 72 hours after birth. Once daily dose of $1.5 \times 10^9$ colony forming units (CFU) was increased to $3 \times 10^9$ once feeds volume exceeded 50mL/kg/day. Neonates were followed up from birth until 28 days of life. Clinical outcomes, feeding intolerance and time to achieve full feeds (TTF) were determined. Appropriate regression models were used to adjust the effects of covariates. **Results:** There was no significant difference in incidence of mortality, NEC, sepsis or levels of inflammatory markers (TLC and CRP). After adjusting for covariates, MS probiotic group was associated with increased likelihood of achieving TTF ([AHR] = 1.74, 95% CI 1.23–2.48, $p$ = 0.002 and lesser gastric intolerance ([AOR]=0.32, 95% CI:0.14-0.69, $p$ <0.01) compared to control group. Change in weight-for-age z-scores from birth-to-discharge across groups showed border-line significance ($p$ = 0.049). **Conclusions:** Probiotic supplementation was associated with reduced feeding intolerance, but no significant effects were observed on mortality or morbidity outcomes.


**Key Words: probiotics, premature, gastrointestinal, feeding, necrotizing enterocolitis**

## INTRODUCTION

The extent to which countries succeed in preventing the disease burden and adverse outcomes associated with premature birth, serves as a critical measure of their progress in promoting neonatal health worldwide.[1] In past few years, various strategies from intervention to prevention has helped in reducing the overall burden of morbidity and mortality in neonates, and probiotics are one of the safest and most studied choice. Probiotics have shown a pivotal effect on the regulation of immune-modulatory pathways in gut, and are now used routinely for care of pre-term neonates in United Kingdom.[2, 3] Some documented mechanism of actions

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are: competitive reduction of potentially pathogenic bacteria and fungi, improving the epithelial barrier to inhibit translocation of bacteria into the intestinal wall, alleviating the inflammatory reactions to feeding and colonizing bacteria in preterm infants.[4] A study published recently, demonstrated Bifidobacterium strains are ecosystem engineers that lead to a hastened microbiome maturation in extremely premature infants.[5] In neonates, including preterm, additional beneficial effects of probiotics are inferred by the development of the intestinal microbiota, determining early colonization pattern and improving long term health. As the use and diversity of probiotic products grows, so does the confusion on how to choose the best probiotic for your patient with a particular condition.[6, 7] Therapeutic efficacy of probiotics is highly contingent upon the specific species or strains used, the dose administered and disease being targeted, with each factor modifying the variability of host response. A dosage of 5 billion colony-forming units (CFU) or greater per day was significantly more effective than a lower dosage for gastrointestinal (GIT) conditions but physicians and patients are encouraged to use third-party analysis results when selecting a product. The duration of probiotic use is generally based upon the indication to be treated.[8, 9] Studies have established efficacy of probiotics to reduce complications in preterm babies such as necrotizing enterocolitis (NEC), late onset sepsis (LOS), feeding intolerance and to improve overall growth and neurodevelopment.[10-12] As per American Gastroenterological Association guidelines, of various options the combination of Lactobacillus and Bifidobacterium species have been supported by moderate- or high-quality evidence for the prevention of mortality and morbidity in preterm infants.[13] Earlier in 2018, The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN)-Working Group for Probiotics and Prebiotics, published a document using a network meta-analysis (NMA) approach to identify strains with greatest potential efficacy for preventing major neonatal morbidities. It inferred that supplementation of single strain – *L. rhamnosus GG* or a mix of three probiotic strains (*Bifidobacterium infantis*, *Bifidobacterium. lactis* and *Streptococcus thermophilus*) has shown superior effects to various other options,[14] Conversely, some studies showed no significant difference in outcomes with supplementation of single strain versus multi-strain probiotic in preterm neonates.[15-17] Moreover, emerging evidence also suggests that effectiveness of a probiotic may differ with ethnicity. A meta-analysis on the use of probiotics in acute rotavirus diarrhea demonstrated attenuated therapeutic benefits among Asian children compared to Caucasian children.[18] Similarly, the temporal window of probiotic initiation is critical, supplementation commenced within a week after birth has been reported to provide more beneficial outcomes among preterm than late introduction.[19] Although studies from

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neighboring countries are available but these findings have been inconsistent with considerable variations.[20-23] No trial has been conducted in Pakistan, to establish species-specific role of probiotics in preterm supplementation. Furthermore, the lack of clarity about the type of probiotic or dosing regimen used is missing, yielding inadequate evidence about efficacy and safety of probiotics in neonatal survival. Thereby, limiting their routine use in neonatal intensive care units.

We aimed to study the effect of early supplementation with single-strain versus a multi-strain probiotic on clinical, feeding and gastrointestinal outcomes in the premature neonates of local population.

## MATERIALS AND METHODS

A double-blinded randomized controlled trial (RCT) was conducted at a tertiary level neonatal care unit in Bahawal Victoria Hospital, Pakistan between May 2023 and November 2023. The Institutional Ethics Committee approved the study (168-2023-/PHEC). The trial was prospectively registered with the Chinese clinical trial registry (ChiCTR2300071089). We included preterm babies of gestational age (GA) between <35 + 6days, birth weight (BW) <2500 grams (g), received and tolerated at-least one feed within 72 hours (h) of birth. Neonates with major congenital malformations, gastrointestinal anomalies, early onset sepsis (C-reactive protein (CRP) >10 mg/L in the first 72 h of life) and renal insufficiency were excluded. Based on the initial weight (more or less than 1500 g), neonates are fed 2 or 3 hourly. Mother's own milk used as first preference, preterm formula or mixed feeds provided if mother did not produce sufficient milk. Preterm formula (Isolac Pre) was used either in addition to human milk (HM) or alone. As per guidelines, feeding volumes are gradually increased until a target volume of 150 mL/kg/day is reached.[24]

### *Randomization*

A computer-generated block randomization procedure was used with variable block size of 3 and 6, to randomly allocate participants to three study groups at a 1:1:1 allocation ratio. The allocation concealment was performed using a serially numbered opaque sealed envelope. The randomization sequence was prepared and delivered to hospital pharmacy by a research statistician not associated with the study. The pharmacist prepared intervention medication or placebo in identical syringes labelled with allocation numbers and dispatched it to the neonatal intensive care unit. Thus, participants, care providers and those accessing patient outcomes were blinded to the intervention.

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***Procedure***

An informed written consent was obtained from the parents of eligible neonates, and recruited infants were supplemented as per randomized list within first 72 h of the birth. The control group (C) received 1 mL of 10% dextrose water, which was prepared in the pharmacy by adding 55.5 mmol of analytical-grade dextrose to 100 mL of sterile water. Of intervention groups, SS received single strain probiotic - PREPRO GG (Matrix Pharma, Pakistan) containing *Lactobacillus rhamnosus GG* - 5 billion CFU and MS received multi-strain probiotic- AMYBACT (Lucky Core Industries, Pakistan) containing *Bifidobacterium BB-12*, *Lactobacillus paracasei subsp. paracasei 431* (*Lactobacillus casei-431*), *Streptococcus thermophilus TH-4*) 2 billion CFU of each strain The products were commercially manufactured and purchased directly from their respective suppliers registered with the Drug Regulatory Authority of Pakistan (DRAP).

Freshly reconstituted contents of the allocated sachets were prepared and dispensed in 5cc syringes concealed with allocation label for bed side administration by nursing staff. All the preparations looked similar and made by registered qualified pharmacist under sterile conditions of pharmacy. The single dose ($1.5\times10^9$ CFU/day as 1 mL of the reconstituted solution) was given every day via the feeding tube or orally as applicable until reaching feeds of 50 mL/kg/day. It was increased thereafter to $3\times10^9$ CFU/day once feeds exceed 50mL/kg/day. Neonates were followed up from birth to a maximum of 28 days, death, discharge to peripheral hospitals or home, whichever time point came first. During periods when feeds were withheld, supplementation was not given, and it was resumed once feeds were restarted.

***Outcomes***

NEC and mortality were prespecified as primary clinical outcomes, while sepsis and other neonatal morbidities, feeding intolerance, and growth-related outcomes were considered secondary outcomes. Morbidity and mortality data was collected on daily basis for four weeks (planned duration of probiotic administration) unless neonate is discharged/transferred/died. Bell's classification was used for grading Necrotizing Enterocolitis NEC.[25] Clinical sepsis with proof of blood culture positive for causative agent was defined as confirmed sepsis. Blood samples were collected at day 7, 14, 21 and 28 of the study to measure total leukocyte count (TLC) and C-reactive protein (CRP) levels using Sysmex XN-1000TM Hematology Analyzer and Cobas-501 Chemistry Analyzer, respectively. An upper normal level of CRP is accepted as 10mg/L.[26] Achievement of time to full feed (TTF, 150mL/kg/d) and occurrence

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of feeding intolerance in intervention and control groups were compared. Under the feeding regime, the day feed started is described as the day of first feed received, and the first day that the neonate received 150 mL/kg or more is described as full feeds reached.[27] Sign and symptoms for gastric tolerance were recorded through a standard questionnaire. A gastrointestinal tolerance was assessed daily using an adapted gastrointestinal/digestive tolerance scoring approach based on the method described by Jacquot et al.[28] and subsequently used in preterm infants.[15] The assessment incorporated gastric residual volume, emesis/regurgitation, abdominal distension, stool pattern, and nil per os (NPO) status. NPO status was included as an additional clinical indicator of feeding interruption. Each component was assigned a predefined score, and a total score >2 was considered indicative of feeding intolerance. The complete scoring criteria is provided in Supplementary Table 1.

***Sample size***

Sample size was calculated based on estimates for probiotics in preventing NEC, a proven marker of gastrointestinal health in preterm neonates.[12] Assuming a placebo NEC rate of 14%, 29 and pooled odds ratios of 0.35 for SS and 0.44 for MS probiotics, the expected NEC rates were 5.3% and 6.7%, respectively. A total of 210 infants (70 per group) provided 80% power ($\alpha = 0.05$) to detect these differences. Accounting for 15% attrition, 249 infants were enrolled (83 per group).

***Statistical analysis***

Categorical variables were expressed as numbers and percentages. Continuous variables were assessed for distribution and represented as mean ± SD or median (IQR) accordingly. Variables were compared across groups with the suitable parametric and non-parametric tests based on normality. Crude and adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated for TTF by using Cox proportional hazards regression (Cox analysis). BW, GA, and feed type were included as covariates based on univariable associations or clinical relevance to feeding progression. Achievement of full feed was defined as event, while infants who did not achieve full feeds during follow-up period were treated as censored at their last observed time point. The proportional-hazards assumption was assessed graphically using log-minus-log survival plots. Model fit was assessed using Cox–Snell residuals, and multicollinearity was ruled out using variance inflation factors. Binary logistic regression was used to estimate the odds ratios (ORs) for gastrointestinal feeding intolerance and its individual binary components. The corresponding continuous measures, including

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days with emesis, abdominal distension, abnormal stools, increased gastric residual volume, and NPO, were analyzed using generalized linear models (GLMs) with appropriate distributions and link functions. A sub-group analysis of all outcomes was performed for extremely preterm neonates with GA <28 weeks. Statistical analyses were performed using SPSS v23, and $p$ <0.05 was considered statistically significant. Intention-to-treat analysis was done.

## RESULTS

During six months, 249 neonates with mean age of 1.97±0.67 days were randomized into three groups (C: Control, SS: Prepro, MS: Amybact) as shown by the Consolidated Standards of Reporting Trials flow diagram in Figure 1. Subjects in placebo group received a mean of 15.79 ± 7.2 doses and subjects in group SS and MS received 15.6 ± 6.5 and 16.1 ± 6.4 doses respectively. The median (IQR) day of initiation of the study drug or placebo was day 3 (2–3) of life. Baseline characteristics of the three groups were similar (Table 1). Treatment discontinuation occurred in 14.0%, 15.5%, and 16.0% infants in the control, single-strain SS, and multi-strain MS groups, respectively. For the prespecified primary outcomes, mortality occurred in 10 (12.8%), 6 (7.7%), and 6 (7.5%) infants in the control, single-strain (SS, and multi-strain MS groups, respectively, with no statistically significant difference between groups ($p$ = 0.464), as shown in Table 2. The overall incidence of NEC was 10.2%, 5.1%, and 6.2%; $p$ = 0.428 for C, SS and MS respectively. Descriptive analysis of culture positive sepsis cases showed Staphylococcus and Enterobacter species associated infections predominated across three study arms. TLC and CRP levels were comparable across groups. The peak CRP value (mg/L) was observed in control group (229), compared to SS (188) and MS (211) with $p$ = 0.051. Mean TLC across three study arms is presented in Figure 2. Weight-for-age z-scores showed border line significance ($p$ = 0.049), with median changes of −1.24, −1.19, and −1.08 in the control, SS, and MS group, respectively, over the first 4 weeks of life.

The mean age of the infants to achieve full enteral feeding was similar across three groups control, SS and MS 8.32 ± 94.5, 7.4 ± 3.7 and 7.3 ± 3.7 days, respectively, $p$ = 0.241. In the Cox proportional hazards analysis, 219 (92.8%) reached full feeds during the follow-up period, while 17 (7.2%) were censored. Unlike unadjusted analysis (crude model), an adjusted model for GA, BW, and type of feeding, showed intervention groups reaching full feeds earlier compared to the control group (SS: (AHR) 1.77, 95% CI 1.26–2.50, $p$ = 0.001; MS: AHR 1.74, 95% CI 1.23–2.49, $p$ = 0.002) as shown in Table 3. A more rapid decline in the probability of remaining below full feeds among intervention groups compared with the

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control group in adjusted model, indicates an earlier attainment of full feeds as presented in Figure 3. The Cox regression model was statistically significant (likelihood ratio test: $\chi^2$ = 241.08, df = 6, $p < 0.001$), suggesting that covariates improved the prediction of TTF.

Mean gastric intolerance score for control, SS and MS was 1.60 ± 0.61, 1.34 ± 0.55, and 1.31 ± 0.56 ($p$ = 0.003), similar to percentage (%) of neonates with gastric intolerance 30, 18.1 and 13.3 ($p$ = 0.012) across three groups. Among the individual components of the gastrointestinal tolerance score, significant group differences were observed for emesis and NPO status. Emesis occurred in 50.0%, 30.7%, and 22.5% of infants in the placebo, SS, and MS groups, respectively ($p$ = 0.001), while NPO status was observed in 33.3%, 19.2%, and 16.2%, respectively ($p$ = 0.025). Complete regression outputs for the gastrointestinal symptoms that showed significant between-group associations in univariate analysis are provided in Table 5, including crude and adjusted estimates, 95% CI, and statistical significance ($p$-value). Results indicate a consistent directionally similar protective effects in intervention groups relative to control group. For NPO days, only the MS group showed a significant reduction (incidence rate ratio (IRR) = 0.51, $p$ = 0.027). After adjustment, BW retained most statistical significance in these multivariate models. In very preterm (GA <28 weeks) all clinical and feeding progression outcomes were non-significant among the placebo, SS, and MS groups (Supplementary Table 2).

## DISCUSSION

An underdeveloped mucosal barrier and enzyme system, with increased intestinal permeability, predisposes impaired nutrition and inflammatory response in premature neonates leading to serious complications.[14] During the study, overall prevalence of NEC and sepsis was 7.02% and 10.16% in enrolled neonates comparable to other studies in Pakistan.[29-31] Incidence of Nec ≥Stage II and culture proven sepsis in the preterm population of placebo, SS and MS groups was not significantly different. Most of these neonates were very preterm (<28 weeks GA), particularly identifying ineffectiveness of probiotics in prevention for this age group as further established by sub-group analysis.[15, 32, 33] A review has reported that multi-strain probiotic supplementation was associated with lower trend of mortality yet failed to reach statistical significance.[34] Though median CRP levels were similar across groups, the placebo group showed border line significance with higher peak CRP levels ($p$ = 0.05) and number of neonates with CRP levels >10 mg/L across all four weeks of follow up. Biologically plausible as evidence suggested that a four-strain probiotic supplementation enhanced the production of anti-inflammatory cytokines (IL-10 and IL-6) and inhibits the

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inflammatory chemokines (IL-8, CXCL 10 and MCP-1).[35] Another study has demonstrated that *Lactobacillus casei* supplementation to the tuberculosis patients has significantly modulated the levels of inflammatory cytokines and metabolites.[36] Compared to our results, a prospective cohort study involving 102 preterm infants from Pakistan, showed significant effectiveness of a double-strain probiotic in preventing NEC and LOS, possibly attributable to comparatively longer hospital stay (42 days) then in our study (28 days). [37]

We found that, after adjustment for GA, BW, and feeding type, probiotics remained significantly associated with reduced time to full enteral feeds, suggesting that while these maturational factors strongly influence feeding progression, probiotic may offer an independent benefit, consistent with the physiological evidences. MS probiotic showed the most profound effect in very-low-birth-weight neonates.[38] A study has confirmed that mean blood flow velocity to the intestine after probiotic supplementation was significantly increased ($p$ = 0.035). Therefore, proposing it as an underline mechanism for TTF attainment.[39] Additionally, they promote short-chain fatty acid production, which stimulates enteric neurons and smooth muscle activity. The precise mechanism for different probiotic strains remains unclear and warrant further research.[40] When compared in a meta-analysis with 106 trials, both single or combination probiotics proven as the most effective intervention in reducing the TTF (-1.94 days and -2.03 days respectively) compared with placebo or prebiotics.[41]

The use of probiotics is also found to be efficacious in reducing the occurrence of gastric intolerance (OR = 0.38, 95% CI=0.27, 0.55, $p$ <0.001).[42] Abovementioned results were also supported in our study by efficacy of probiotics in reducing gastric intolerance and associated symptoms. Compared to control, the adjusted odds of developing gastric intolerance were 65% lower in SS and 68% lower in MS. The reduction in NPO days solely in MS, further supports its effectiveness in maintaining gastrointestinal integrity, feed continuity, and functional adaptation of the immature gut.[5] The decrease in GA effect in adjusted multivariate model, suggests that birth weight was predominant risk factor for feeding intolerance in three groups.[43] A recent study, on predictive ability of different factors for feeding disorders in preterm infants, has showed that though birth GA remains significant in multivariate analysis, the area under curve (AUC) of BW for predicting feeding disorders was highest 0.725 ($p$ <0.001, 95% CI: 0.642–0.808), with a sensitivity of 60.0% and a specificity of 80.7%.[44] The non-significant association of feed type with TTF and gastric intolerance suggests that poor gastrointestinal maturity and functionality may override nutritional benefit of feed composition in determining feeding advancement.[45, 46]

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Change in the weight for age z-scores from birth to discharge showed that the decline appeared less pronounced in MS group, indicating that intervention exhibited a trend toward meaningful results ($p$ = 0.049) but must be confirmed by further studies with appropriate sample size for weight gain changes.[38] The parameters to determine weight outcomes are variable across studies including average daily weight gain, final discharge weight, and weight gain velocity, that may cause variability in results or effects.[47] Sub-group analysis of extremely premature neonates showed no difference across three study arms for any study outcomes. However, these findings must be interpreted cautiously, as a smaller number of neonates with GA <28 weeks, makes the analysis underpowered to detect clinical differences. Our findings are comparable to the studies, where the probiotic supplementation was started earlier and showed significant effect on gastric intolerance and selected associated symptoms but showed insignificant impact on mortality or major morbidities of prematurity such as NEC or sepsis.[32, 33, 48] No probiotic-related infection, adverse effect or treatment discontinuation attributable to the study intervention was observed, indicating that the benefits likely outweigh the rare risk of probiotic-associated sepsis.[49]

***Conclusion***

This study evaluated the effects of single- and multi-strain probiotic supplementation on major prematurity related complications, gastric tolerance, feeding progression, and weight changes in preterm neonates. Compared to placebo, probiotic supplementation was associated with lower feeding intolerance, and decreased emesis and NPO outcomes. The MS probiotic showed stronger associations with these outcomes, with reduced odds of gastric intolerance symptoms occurrence and lower incidence rate ratios IRR for days with emesis and NPO status. However, neither probiotic regimen significantly reduced NEC, sepsis, mortality, or improved discharged weight gain z-score.

This is the very first registered clinical trial comparing effects of single strain versus multi-strain probiotics supplementation on clinical and feeding outcomes among preterm neonates in local population. There are few limitations of the study. The probiotic supplementation was limited to 28 days, restraining the ability to determine developmental and metabolic outcomes beyond neonatal phase. Secondly, as this was a single-center study, multi-center studies are warranted to validate the generalizability of our findings across different neonatal populations.

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## SUPPLEMENTARY MATERIALS

All supplementary tables and figures are available upon request from the editorial office, and are also accessible on the journal's webpage (apjcn.qdu.edu.cn).

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## CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

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**Table 1.** Neonatal baseline characteristics

| Characteristics | Control (n=78) | SS (n=78) | MS (n=80) |
|---|---|---|---|
| Weight at birth (g) | 1600 (1250–1810) | 1515 (1200–1900) | 1550 (1200–1810) |
| Gestational age (weeks) | 32.0 (28.4–33.7) | 32.0 (29.2–33.4) | 32.0 (28.8–33.2) |
| Gender | | | |
| Male | 52 (66.6) | 48 (61.5) | 48.0 (60) |
| Reason of pre-mature delivery | | | |
| Spontaneous preterm premature rupture of membranes (SPPROM) | 28.0 (35.9) | 29.0 (37.2) | 32.0 (40) |
| EOPET | 29.0 (37.2) | 28.0 (37.2) | 16.0 (20) |
| SPTL | 5.0 (6.4) | 6.0 (7.6) | 14.0 (17.5) |
| IUGR | 6.0 (7.7) | 5.0 (6.4) | 7.0 (8.8) |
| Delivery method | | | |
| Cesarean section | 55 (70.5) | 56 (71.8) | 71 (88.7) |
| Feed type | | | |
| EBM | 21 (26.9) | 22 (28.2) | 17 (21.2) |
| Formula milk | 30 (38.5) | 32 (41) | 30 (37.5) |
| Both | 27 (34.6) | 24 (30.8) | 33 (41.2) |
| Small for gestational age | 13 (16.6) | 13 (16.6) | 11 (13.7) |
| Apgar Score (5 min) | 8 (8-9) | 8 (8-9) | 8 (8-9) |

EBM, expressed breast milk; EOPET, early-onset pre-eclampsia; IUGR, intrauterine growth restriction; SPPROM, spontaneous preterm premature rupture of membranes; SPTL, spontaneous preterm labour.
[†]Continuous variables are presented as median (interquartile range); categorical variables are presented as number (%).

**Table 2.** Clinical outcomes[†]

| Outcome | Control (n=78) | SS (n=78) | MS (n=80) | *p*-value |
|---|---|---|---|---|
| Mortality | 10 (12.8) | 6 (7.6) | 6 (7.5) | 0.464 |
| Morbidity | 21 (26.9) | 13 (16.6) | 11 (13.7) | 0.085 |
| Nec≥ Stage II | 6 (7.7) | 4 (3.8) | 3 (3.7) | 0.291 |
| Culture proven sepsis | 12 (15.3) | 7 (8.9) | 8 (10) | 0.401 |
| CRP levels (mg/L) | 5 (5.9-9) | 5 (5-7.8) | 5 (5-8) | 0.833 |
| TLC (x $10^9$/L) | 16.4 ± 3.18 | 15.7 ± 2.87 | 15.7 ± 2.91 | 0.728 |
| Δ Weight-for-age z-score | -1.24 [-1.97, -0.90] | -1. 19 [-1.42, -0.90] | -1.08 [-1.31, -0.87] | 0.049 |

[†]Data are mean ± SD or median (IQR) for continuous variables, and n (%) for categorical variables.

**Table 3.** Cox regression analysis for time to full feeding (TTF)

| Group (vs Control) | CHR (95%CI) | p-value | AHR (95%CI) | p-value[†] |
|---|---|---|---|---|
| SS | 1.29 (0.92-1.81) | 0.130 | 1.77 (1.26-2.50) | 0.001 |
| MS | 1.30 (0.94-1.82) | 0.113 | 1.74 (1.23-2.48) | 0.002 |

SS: xxx; MS: xxxx
[†]Adjusted for BW, GA and feeding type

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**Table 4.** Regression analysis of feeding intolerance outcomes

| Outcome | Crude Effect Estimate (95% CI) | p-value | Adjusted Effect Estimate (95% CI) | p-value |
|---|---|---|---|---|
| Development of gastric intolerance† | | | | |
| SS | 0.43 (0.22-0.87) | 0.019 | 0.35 (0.16-0.76) | 0.008 |
| MS | 0.40 (0.19-0.79) | 0.009 | 0.32 (0.14-0.69) | 0.004 |
| Emesis† | | | | |
| SS | 0.44 (0.23-0.85) | 0.015 | 0.38 (0.18-0.78) | 0.009 |
| MS | 0.29 (0.14-0.57) | <0.001 | 0.23 (0.10-0.49) | <0.001 |
| No. of days emesis‡ | | | | |
| SS | 0.54 (0.32-0.90) | 0.017 | 0.51 (0.30-0.88) | 0.015 |
| MS | 0.42 (0.25-0.72) | 0.002 | 0.40 (0.23-0.70) | 0.001 |
| NPO† | | | | |
| SS | 0.47 (0.22-0.99) | 0.48 | 0.38 (0.16-0.89) | 0.26 |
| MS | 0.38 (0.18-0.82) | 0.14 | 0.29 (0.12-0.68) | 0.05 |
| No. of days NPO‡ | | | | |
| SS | 0.66 (0.39-1.12) | 0.125 | 0.58 (0.32-1.04) | 0.067 |
| MS | 0.59 (0.34-1.01) | 0.050 | 0.51 (0.28-0.93) | 0.027 |

SS: XXX; MS: XXXX; NPO: XXXXXX
†Binary logistic regression (OR) for occurrence
‡Negative binomial regression (IRR) for duration. Adjusted for BW, GA and feeding type
$p < 0.05$ was considered significant

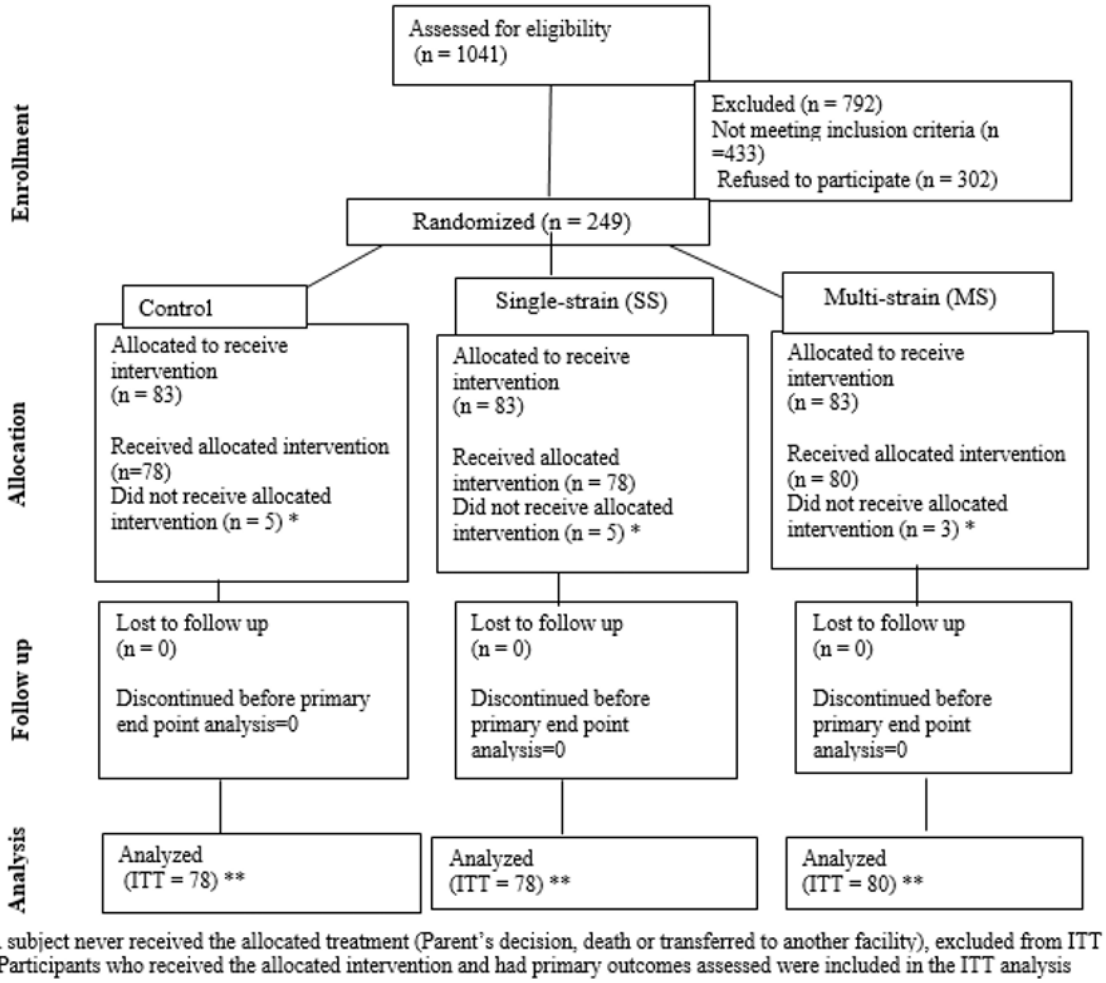


**Figure 1.** CONSORT flow of the study. CONSORT, Consolidated Standards of Reporting Trials.

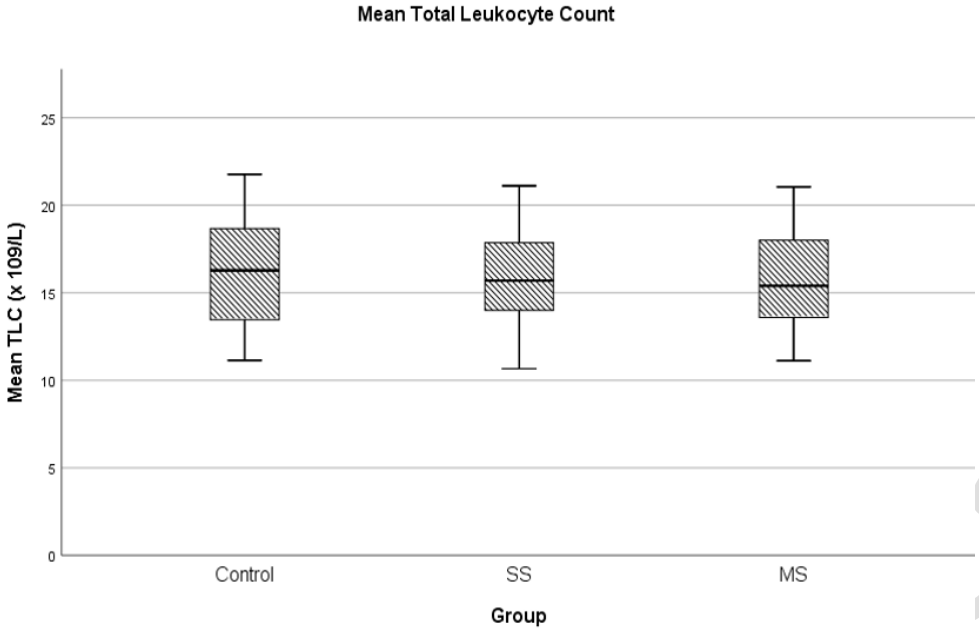


**Figure 2.** Mean total leukocyte count was comparable across three groups

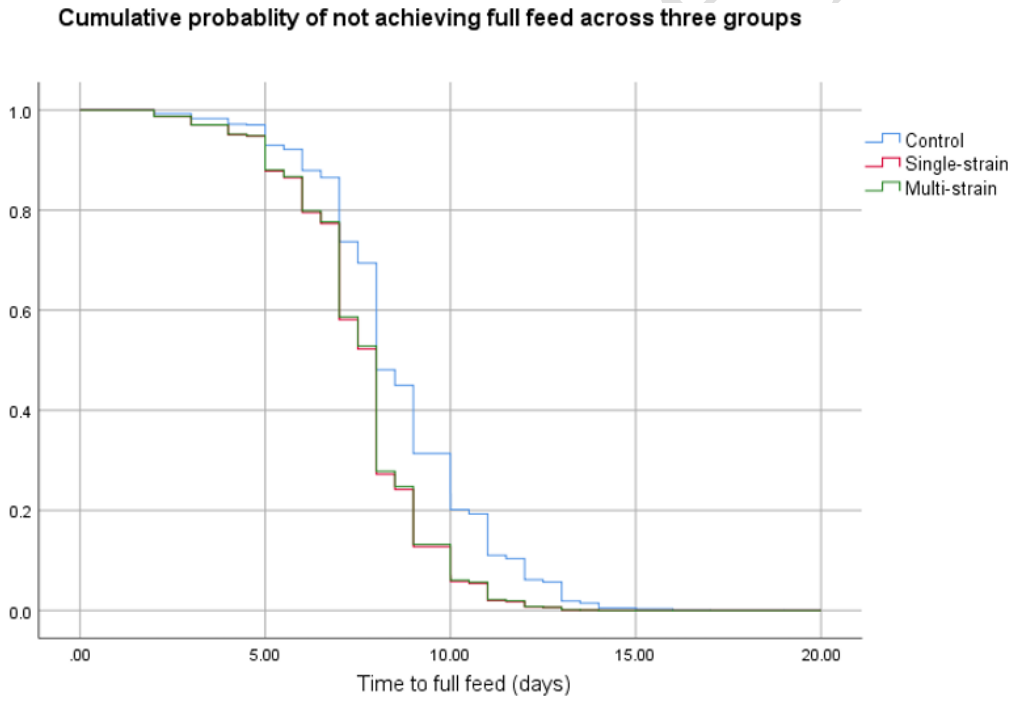


**Figure 3.** Adjusted Cox proportional hazards survival curves for time to full enteral feeds in the control, SS) and MS probiotic groups. Curves are adjusted for BW, GA and feeding type. A steeper decline indicates earlier achievement of full enteral feeds.