PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effect of probiotic administration to breastfeeding mothers with very low birth weight neonates on some neonatal and maternal outcomes: study protocol for a randomized, double-blind, placebo-controlled trial
AUTHORS	Alikamali, Maryam; Mohammad-Alizadeh-Charandabi, Sakineh; Mirghafourvand, Mojgan; Gharehbaghi, Manizheh Mostafa; Homayouni-Rad, Aziz; Fardiazar, Zahra; Shahnazi, Mahnaz

VERSION 1 - REVIEW

REVIEWER NAME	***sinha, anju***
REVIEWER AFFILIATION	***Indian Council of Medical Research, Reproductive and Child Health***
REVIEWER CONFLICT OF INTEREST	Na
DATE REVIEW RETURNED	01-Feb-2024

GENERAL COMMENTS	1. The strengths and limitations of the study are not well defined; they should be revised and reworded. 2. Page no. 6, Line no 40: “Probiotics contained only”...? Looks like an incomplete sentence. 3. What is the regulatory requirement for the two strains of probiotics (Lactobacillus paracasei 431 and Bifidobacterium lactis BB-12) in Iran? Are there formulations being marketed with these strains? 4. Page No: 7: Elaborate on the Study hypothesis: Decrease of TSB and PPD by what proportion...? 5. Page no-7, incorrect English. Line no 46: "if they are eligible" or “if eligible” instead of “if are eligible” 6. Pg No 8, Line No 43: “The bottles will be given to the participants in order of entering the study.” Rephrase the sentence for better clarity. 7. The term 'side events' is frequently used throughout the manuscript. It would be relevant to reclassify these occurrences as 'side effects' or 'adverse events' for better clarity. 8. Page No 9, Line no 51, “When the bilirubin level reaches a safe level, they will be ordered phototherapy to be discontinued”. Rephrase for better clarity 9. How are serious adverse events addressed, especially if causally related to the intervention/placebo/trial? Is there any provision for compensation for the parent? What is the role of IFDA in regulating the safety of the trial? 10. How do you treat the TSB levels in the study if the baby had hyperbilirubinemia after the 7th day, before 42–45 days.? 11. Who collects the data from the field? Are they trained personnel to detect the morbidities? 12. What is the Name and version of the software used for data collection and management..?

REVIEWER NAME	*Wang, Yao*
REVIEWER AFFILIATION	The Chinese University of Hong Kong, Department of Obstetrics and Gynaecology
REVIEWER CONFLICT OF INTEREST	Na
DATE REVIEW RETURNED	12-Mar-2024

GENERAL COMMENTS	The study's focus on alternative treatments for hyperbilirubinemia in VLBW infants through probiotics is innovative, aiming to enhance both neonatal and maternal health. However, to enhance the manuscript's clarity and depth, consider these polished comments: 1. The selection of L. paracasei 431 and B. lactis BB-12 requires a clearer rationale. It's crucial to elaborate on why these specific strains were chosen for the study. 2. The justification for the chosen dosage (3×10^9 CFU/g per strain) is not provided. Detailing the reasoning behind this dosage could strengthen the study's methodology. 3. The omission of biological collections from the protocol could limit understanding of the probiotics' effects on maternal and neonatal microbiomes. Incorporating samples such as blood, maternal stool, maternal milk, and fetal stool is recommended for a comprehensive assessment. 4. Given the mild nature of probiotic treatments, the adequacy of the 42-day intervention period warrants further discussion to ensure effectiveness. 5. The evaluation of postpartum depression at only two time points may not fully capture fluctuations in maternal mental health. Increasing the frequency of assessments to weekly could provide a more accurate representation. 6. Including an assessment of participants' sleep quality through relevant questionnaires would offer valuable insights into the intervention's impact on overall well-being. 7. A graphical workflow diagram would significantly enhance the presentation of the study design, making it more accessible to readers.

VERSION 1 – AUTHOR RESPONSE

Reviewers' comments

Reviewer 1

Comment 1: The strengths and limitations of the study are not well defined; they should be revised and reworded.

Response: The strengths and limitations of the study have been revised and reworded as follows:

• Low risk of selection bias due to the appropriate random assignment of participants to study groups and allocation concealment.

• Low risk of performance and detection biases through the implementation of blinding for participants, care providers, and outcome assessors.

• Conducting the trial in a tertiary hospital covering very low birth weight infants from diverse geographic areas enhances the generalizability of the study results.

• The lack of long-term assessment of intervention effects, primarily due to financial and time constraints.

• Financial constraints hindered the analysis of biological samples, restricting the understanding of

probiotics' effects on the maternal and neonatal microbiome (Page 2, lines 1 to 10)
Furthermore, we have revised and highlighted the strengths and limitations sub-section in the discussion (Page 14, lines 10 to 29 & Page 15, lines 1 to 29).

Comment 2: Page no. 6, Line no 40: “Probiotics contained only...? Looks like an incomplete sentence.
Response: Thank you for bringing to light the incomplete sentence. The sentence has been revised to read “…the administered probiotics contained only one strain (Lacticaseibacillus paracasei subsp. paracasei, 1.5×10^9 CFU/day and did not assess maternal mental health outcomes” (Page 3, lines 25 and 26).

Comment 3: What is the regulatory requirement for the two strains of probiotics (Lactobacillus paracasei 431 and Bifidobacterium lactis BB-12) in Iran? Are there formulations being marketed with these strains?
Response: Lactobacillus paracasei 431 and Bifidobacterium lactis BB-12 are well-known probiotic strains commonly used in probiotic supplements and functional foods due to their documented health benefits. In this study, these strains have been sourced from Chr. Hansen and meet the necessary international standards. The formulations containing these strains are currently available in the Iranian market, having been approved by the Iranian Food and Drug Administration of the Ministry of Health and Medical Education. This approval ensures that the strains are safe, effective, and manufactured according to good manufacturing practices (GMP). Products containing these strains, which can be found in pharmacies and health food stores in Iran, are often marketed for their benefits to digestive health and immune function. We have detailed this information in the manuscript (Page 13, lines 23 to 29 & Page 14, lines 1 and 2).

Comment 4. Page No: 7: Elaborate on the Study hypothesis: Decrease of TSB and PPD by what proportion...?
Response: Thank you for your insightful query regarding the study hypothesis. As explained in the sample size sub-section, the expected proportions of decrease in the primary outcomes are a minimum of 25% in TSB levels on the 7th day after the intervention and a minimum of 30% in depression scores due to the intervention. Those reductions were considered clinically significant based on expert opinions. We have revised the hypotheses as follows:
1. Supplementing breastfeeding mothers with L. casei 431 and B. lactis BB-12 probiotics reduces the total serum bilirubin level (TSB) in VLBW infants by a minimum of 25%.
2. Supplementing breastfeeding mothers with L. casei 431 and B. lactis BB-12 probiotics reduces postpartum depression symptom severity by at least 30% (Page 4, lines 19 to 22).

Comment 5. Page no-7, incorrect English. Line no 46: "if they are eligible" or “if eligible” instead of “if are eligible”
Response: Thank you for pointing out the grammatical error. We have revised it to “if eligible” (Page 5, line 5).

Comment 6. Pg No 8, Line No 43: The bottles will be given to the participants in order of entering the study. Rephrase the sentence for better clarity.
Response: Thank you for your suggestion regarding the sentence. To clarify, we have rephrased the sentence as follows:
Participants within each stratum will receive the bottles in the sequence corresponding to their enrollment into the study (Page 6, lines 6 and 7).

Comment 7. The term 'side events' is frequently used throughout the manuscript. It would be relevant to reclassify these occurrences as 'side effects' or 'adverse events' for better clarity.
Response: We appreciate the suggestion to modify the terminology used in our manuscript. The recommendation to classify these instances as 'adverse events' is duly noted (Page 6, lines 10 and

30. Page 7, line 30. Page 8, lines 1 and 2, 15 and 17).

Comment 8. Page No 9, Line no 51, “When the bilirubin level reaches a safe level, they will be ordered phototherapy to be discontinued”. Rephrase for better clarity.
Response: We have rephrased the sentence for better clarity as follows:
Phototherapy is discontinued once the bilirubin level lowers to a safe threshold based on the guidelines (Page 7, lines 13 and 14).

Comment 9. How are serious adverse events addressed, especially if causally related to the intervention/placebo/trial? Is there any provision for compensation for the parent? What is the role of IFDA in regulating the safety of the trial?
Response: We appreciate the reviewer's insightful comments and attention to detail, which have significantly contributed to the quality and transparency of our manuscript. We have explained these aspects in the manuscript as follows:
In instance severe complications during the study leading to significant discomfort, treatment allocation will be promptly unblinded to the specialist and/or the in charge person in the ward if it requested to facilitate the immediate provision of necessary support and treatment, provided free of charge under the direct supervision of a designated perinatologist or neonatologist from the research team. To maintain transparency and accountability, any unblinding due to severe complications will be promptly reported to the research ethics committee. This communication serves to keep the committee informed of significant events and enables them to offer guidance or take necessary actions to protect the interests of all participants (Page 8, lines 18 to 26).
As previously mentioned, these two strains have been sourced from Chr. Hansen and meet the necessary international standards. In Iran, the regulatory requirements for probiotics, including strains like Lactobacillus paracasei 431 and Bifidobacterium lactis BB-12, involve approval by the Food and Drug Administration of the Ministry of Health and Medical Education. This typically includes ensuring that the strains are safe, effective, and manufactured according to good manufacturing practices (Page 13, lines 24 to 29).

Comment 10. How do you treat the TSB levels in the study if the baby had hyperbilirubinemia after the 7th day, before 42–45 days?
Response: We value the reviewer's insightful feedback which has greatly enhanced the quality and transparency of our manuscript.
The research team will not intervene in the management of hyperbilirubinemia during the study period but will diligently record the procedures undertaken. We have detailed this aspect in the manuscript as follows:
“A checklist has been developed to meticulously document the commencement and cessation of each phototherapy type and any blood exchange transfusions administered during the 42-45 days following the intervention. In initiating and discontinuing phototherapy, the neonatologists at the hospital follow the Queensland Maternity and Neonatal Clinical Guidelines while consulting the Plot TSB levels on the nomogram, considering gestational age, weight, and age appropriateness” (Page 7, lines 8 to 12).

Comment 11. Who collects the data from the field? Are they trained personnel to detect the morbidities?
Response: Thank you for this insightful comment. We explicitly explained it in the manuscript as follows:
“All data will be collected by the PI, a doctoral candidate in midwifery who has received thorough training from supervisors, including the neonatologist within the study team. The neonatologist, a highly experienced faculty member, who is present in the study setting every working day, will directly oversee the data collection process” (Page 9, Lines 12 to 15).

Comment 12. What is the Name and version of the software used for data collection and

management?
Response: We appreciate the reviewer's insightful comments and attention to detail.
A level of less than 0.05 will be considered as statistically significant, and all analyses will be conducted using IBM SPSS (version 24) (Page 12, lines 21 and 22).

Reviewer 2
Comment 1. The selection of L. paracasei 431 and B. lactis BB-12 requires a clearer rationale. It's crucial to elaborate on why these specific strains were chosen for the study.
Response: Thank you for your insightful comment. The choice of L. paracasei 431 and B. lactis BB-12 was based on several key considerations as follows:
In our study, we will utilize two strains, L. paracasei 431 and B. lactis BB-12, well-studied strains with documented safety and effectiveness in various health aspects.40 41 Numerous clinical trials involving these strains have demonstrated their positive impact on gut microbiota, immune function, and gastrointestinal health.42 43 L. paracasei 431 has been noted for its ability to modulate immune responses and enhance barrier function in the gut,44 while B. lactis BB-12 is known for its positive effects on microbial balance and its potential to reduce pathogenic bacteria.45 Our research team and others have already successfully used these strains in previous studies, which provided us with a foundation of experience and data to build upon.24 46-48 (Page 13, lines 17 to 24).

Comment 2. The justification for the chosen dosage is not provided. Detailing the reasoning behind this dosage could strengthen the study's methodology.
Response: We appreciate the opportunity to provide a detailed justification for the chosen dosage of 1×10^9 CFU/g per strain. We have described in the manuscript as follows:
There are no strict recommended guidelines for the dosage. However, the dosage of 1×10^9 CFU/day from each strain falls within the range commonly used in clinical trials and is in line with guidelines for probiotic administration. This dosage has been effective in various studies without causing adverse effects.49 Additionally, the promising results in our previous study, where only Lacticaseibacillus paracasei subsp. Paracasei at 1.5×10^9 CFU/day was used,24 provided the rationale for selecting the dosage in the current investigation. (Page 14, lines 3 to 8)

Comment 3. The omission of biological collections from the protocol could limit understanding of the probiotics' effects on maternal and neonatal microbiomes. Incorporating samples such as blood, maternal stool, maternal milk, and fetal stool is recommended for a comprehensive assessment.
Response: Thank you for your constructive feedback and for highlighting the potential value of incorporating additional biological collections into our study protocol. However, due to budgetary limitations, we were unable to include them in this trial. We explained this aspect in the limitation sub-section as follows:
“Due to budgetary limitations, in this trial, we are unable to incorporate the collection and analysis of biological samples such as blood, maternal stool, maternal milk, or fetal stool, which could have provided valuable insights into the probiotic effects on the maternal and neonatal microbiome. This omission limits our ability to fully understand the mechanisms behind any observed effects on neonatal and maternal outcomes. Future research should prioritize integrating microbiome analysis to advance our understanding of the role of probiotics in maternal and neonatal health, particularly in the context of VLBW infants” (Page 14, lines 22 to 28).

Comment 4. Given the mild nature of probiotic treatments, the adequacy of the 42-day intervention period warrants further discussion to ensure effectiveness.
Response: Thank you for your insightful comment. We have explained this aspect in the manuscript as follows:
Since most VLBW infants admitted to the hospital from which we will be recruiting participants come from other cities, including other provinces, it may be challenging to retain participants in the long-term post-discharge due to limited physical access. However, previous studies have demonstrated

the positive effects of short-term direct probiotic administration to newborns, including a reduction in the duration of phototherapy and total serum bilirubin (TSB) levels.39 In a trial, administering Bifidobacterium breve and Lactobacillus rhamnosus orally at a concentration of 2×10^6 CFU/day starting from the first hour of life resulted in rapid and substantial colonization by days 5 and 6.52 To our knowledge, no studies have investigated the effects of postpartum probiotic administration on PPD. Nevertheless, a trial has shown a positive effect of 4 weeks of probiotic supplementation on certain cognitive functions in patients with major depressive disorder.53 Therefore, based on the evidence supporting the effectiveness of short-term interventions and the practical challenges involved, our research team chose a 42-45-day intervention period. This duration strikes a balance between providing sufficient treatment time and addressing the logistical challenges of the study. Long-term interventions, coupled with extended assessments of maternal and infant outcomes, in future studies, could provide deeper insights into the intervention impacts (Page 15, lines 14 to 29).

Comment 5. The evaluation of postpartum depression at only two time points may not fully capture fluctuations in maternal mental health. Increasing the frequency of assessments to weekly could provide a more accurate representation.
Response: Thank you for your invaluable comment. We have explained this aspect in the manuscript as follows:
The primary outcome of postpartum depression scores will be assessed once post-intervention, i.e. at the end of the 42-45-day intervention period, aligning with the conventional time frame for assessing postpartum depression. This timeframe allows for examining the immediate impact of probiotic supplementation on maternal mental health.51 Assessing postpartum depression at two time points (baseline and 42-45-day post-delivery) may not capture fluctuations adequately and more frequent evaluations, coupled with clinical assessments, could yield comprehensive results. However, due to the vulnerability of women with VLBW infants, frequent assessments could burden participants. Therefore, a more frequent assessment of PPD is recommended in future studies on less vulnerable women (Page 15, lines 5 to 13).

Comment 6. Including an assessment of participants' sleep quality through relevant questionnaires would offer valuable insights into the intervention's impact on overall well-being.
Response: The authors appreciate the valuable feedback from the reviewer regarding the potential benefits of assessing participants' sleep quality as part of the study. We mentioned it in the limitation sub-section as follows:
In this trial, we are going to assess the short-term intervention’s effects on a few maternal short-term outcomes. Thus, it may not capture the intervention's impact on the overall well-being of women. Future studies should assess broader maternal outcomes, such as sleep quality, to provide a more holistic view of the probiotic intervention's influence on maternal health (Page 15, lines 1 to 4).

Comment 7. A graphical workflow diagram would significantly enhance the presentation of the study design, making it more accessible to readers.
Response: The authors appreciate the valuable feedback from the reviewer regarding the drawing of the graphical workflow diagram. It was added to the manuscript as follows (Page 5, Figure 1).

Figure 1 Flowchart of the trial process