Title: Efficacy of prebiotic, probiotic and synbiotics in improving growth in children under age five years in Africa: a protocol for a systematic review

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Numéro PROSPERO 343138

Abstract

Background: Stunting is among main obstacles to human development affecting millions of children worldwide and particularly in the sub-Saharan Africa region. Randomized clinical trials have shown positive effects of prebiotics, probiotics, and synbiotics in improving growth in children and toddlers. However, although the global mobilization to tackle its challenges in their different aspects is visible, it remains to define effective large-scale up interventions and strategies to obtain long lasting impacts.

Objective: The objective of this review is to re-evaluate the effectiveness of prebiotics, probiotics, and/or synbiotics on the growth of children in children 0 to 5 years in Africa including recently published studies.

Methods: Systematic search will be carried out in Pubmed, science direct, clinicaltrial.org, and google scholar. Both randomized and observational studies that assess the association between prebiotics, probiotics, and synbiotics, and health benefits and growth in children under 5 years of age will be included in the review. PRISMA-P (preferred reporting items for systematic review and meta-analysis protocols) has been used for this protocol, and PRISMA will be used for the systematic review. The Cochrane Risk Assessment Tool will be used to assess the quality of eligible studies. If the compiled data are appropriate and sufficient, we will perform a meta-analysis using RevMan software.

Conclusion: This review will provide up-to-date and reliable information on the effectiveness of prebiotics, probiotics, and synbiotics on the growth of children under 5 years of age especially in developing countries. PROSPERO registration number CRD42022343138.

Key words: prebiotics, probiotics, synbiotics, Growth, Children.

Introduction

Stunting is the impaired linear growth and development that children experience due to poor nutrition, repeated infections, and inadequate psychosocial stimulation(WHO, 2018). In 2021 149.2 million children under 5 years of age worldwide were too short for their age (stunting), 45.4 million were too thin for their height (wasting) and 38.9 million were too heavy for their height (overweight)(UNICEF/WHO/ World Bank Group, 2021). Among the 5 continents, the sub-Saharan Africa region shows the highest number with 61.4 million children stunted in 2020 (Bank, 2021), and a pooled prevalence of 35%, among the highest worldwide. Of the sub-regions, the East Africa region shows the highest with 37%, followed by Central Africa with 35%. Predictors such as being male (AOR = 1.27, 95% CrI 1.25, 1.30), small birth size (AOR = 1.29, CrI 1.25, 1.32), home delivery (AOR = 1.17, CrI 1.14, 1.20), and no education of mothers (AOR = 3.07, CrI 2.79, 3.39) were some of the significant predictors of stunting of children (Takele, Gezie and Alamneh, 2022). Several efforts tackling different aspects of stunting are being made to improve health benefits and growth through supplementation with prebiotics, probiotics, and synbiotics (Lai et al., 2019). However, although the global progress in tackling the problem has been steady, it has not been fast enough to meet the targeted objectives (Global initiatives, 2020). Therefore, much remains to be done in terms of defining strategies and scaling up effective interventions to achieve long lasting impacts (W HO, 2013).

The health benefits imparted by probiotics and prebiotics as well as synbiotics have been the subject of extensive research in the past few decades. These food supplements also termed as functional foods have been demonstrated to alter, modify and reinstate the pre-existing intestinal flora, facilitate smooth functions of the intestinal environment, and activate immune-modulation (Pandey, Naik and Vakil, 2015a). In a single-blind randomized clinical trial in children with Severe acute malnutrition, both probiotic and synbiotic showed significant increase rate of weight gain, weight for age (WAZ) and length for age z score (LAZ) compared to placebo (Nuzhat et al., 2023). In other studies, administration of milk containing synbiotics and LCPUFA showed better growth in toddlers (Pandey, Naik and Vakil, 2015b), while in a systematic review and meta analysis of RCT studies, data from under-nourished children in low and middle income countries (LMIC) showed positive effects on weight and height gain (Catania et al., 2022).

Prebiotics which are mostly fibers, beneficially affect the host's health by selectively stimulating the growth and/or activity of some genera of microorganisms in the colon (Oozeer et al., 2013); while probiotics, when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). Pre-, pro- and synbiotics may be added to food products such as yogurts and sold as human health promoters(Schrezenmeir and de Vrese, 2001). Probiotics may be beneficial in modulating the gut microbiota, appetite, body weight gain, abdominal pain, abdominal bloating as well as reduction in diarrhea in children (Lai et al., 2019). In addition, some studies have shown that probiotics may improve growth in children by reducing the risk of iron anemia deficiency by 45% and increasing weight gain by 0.13 kg/year (Sazawal et al., 2010). When to Synbiotics, they are combination of probiotics and prebiotics that work together to promote 'healthy microflora' in human intestine (Verma, David and Chandra, 2012). Their synergic activities beneficially affect the host in improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or activating the metabolism of one or a limited number of healthpromoting bacteria. In combinaison, they provide health benefits including antimicrobial, immunomodulatory, antidiarrhoeal, antiallergenic, hypolipidaemic, hypoglycaemic, and anti-osteoporotic activities. They also help improving mineral absorption and balance, contribute to suppresses the development of putrefactive processes in the stomach and intestines thus preventing the occurrence of a number of serious diseases: food allergies, ulcerous colitis, constipation, diarrhea, and gastrointestinal (Verma, David and Chandra, 2012). All these findings suggest that interventions with prebitoics, probiotics and synbiotics could have beneficial impacts on infant health and growth. Previous reviews have evaluated the effect of pre-pro or synbiotics on child growth in comparing children from high income countries (HICs) to low and middle income countries (LMICs). In the review of Onubi (2015), for exemple, studies that included well-nourished children showed no significant improvements in any growth outcomes measured by height, head circumference or BMI while studies that included under-nourished children showed improved weight in the probiotic group compared with the control group (Onubi et al., 2015). In the review of Catania (2022), who investigated the effects of Probiotic Supplementation for Promotion of Growth in Children, data from LMIC showed that probiotics may have a small effect on weight gain, while data from HIC did not show any clinically meaningful effect on weight (Catania et al., 2022). Therefore, in LMICs like Africa, the potential of probiotics could be greater than in HICs due to both the environment and risk factors associated to child malnutrition. So far, to our knowledge, only the review of Heuven (2021), haved shown that probiotics and synbiotics

have the potential to improve growth outcomes in both undernourished and healthy children living in LMICs (Heuven et al., 2021). With regard to the limited number of publications on the potential of pre-, pro- and/or synbiotics in improving growth outcomes in children living in LMICs particularly in Africa, additional reviews like ours will provide more detailed information on the efficacy of pre-, pro and or synbiotic on growth parameters in children aged 0 to 5 years in LMICs.

Objective

The objective of the present review is to re-evaluate the effectiveness of prebiotics, probiotics, and/or synbiotics on the growth of children in children 0 to 5 years in Africa. In case of no ssufficient studies from Africa, we will include studies from LMICs.

Ethics and registration

The review will not use primary data, so it will not deemed necessary to seek ethics committee or institutional review board for approval. This review protocol is registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the number PROSPERO 343138.

Methods and analysis

This protocol has been developed based on population, intervention, comparators and outcome (PICO) questions and the PRISMA-p guidelines (Moher et al., 2015). For the review, a 27-point checklist and the four-phase article selection flowchart (Figure 1: PRISMA diagram), which aims to improve the quality of systematic review and meta-analysis data will be used. Our study population consists of children under 5 years of age, who received either prebiotics, probiotics, and/or synbiotics and in whom growth was reported. Both randomized and observational studies will be included. The comparators correspond to the groups that did not receive an intervention (control groups). The first outcome will focus on infant growth measured by increases in height and weight. Stunting and underweight will be defined according to world health organization guidelines on child growth standards(WHO, 2009) Extensive searches on Pubmed, Web of Science, science direct, clinicaltrial.org, Cochrane Library, and Google Scholar will be performed to identify relevant studies.

Inclusion and exclusion criteria

We will include research articles on both randomized and non-randomized studies reporting the effect of prebiotics, probiotics, and/or synbiotics on growth and health benefits in children under 5 years. We will not restrict publication dates. Review articles and research articles that do not meet these criteria will be excluded from the review. There will be no language restriction and articles in other languages will be translated in English if necessary.

Search strategy

In a first step, we will build three loops using the basic keywords: “prebiotic” or “probiotic” or “synbiotic” or “dietary supplements”; “children or babies or infants”; and “growth”. These three “loops” will be linked together using “AND”. In a second step, the results of the first step will be combined with a third “loop” which is the type of study: “Randomized clinical trial”, “non-randomized clinical trial” using “AND”.

1. "prebiotics or probiotics or symbiotic or dietary supplements ",
2. “children or babies or infants”
3. “growth”
4. “Randomized clinical trial”
5. “non-randomized clinical trial”

The Boolean method will use the following keywords: 1) “probiotic or synbiotic or prebiotic or dietary supplements” AND “children or babies or infants” AND “growth” AND “Randomized clinical trials”,

2) “probiotics or prebiotics or synbiotics or dietary supplements” AND “children or babies or infants” AND “growth” AND “Non-randomised clinical trials”. The initial search strategy in PubMed will be as follows: (((((probiotics) OR (prebiotic) OR (synbiotics) AND children) AND growth) AND randomized clinical trials); ((((probiotics) OR (prebiotic) OR (synbiotics) AND children) AND growth) AND non-randomized clinical trials). In the other search engines, we will use the most suitable combination of key words (see table 1).

In addition, we will conduct further search of the gray literature through the databases of the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the World Food Programme, and Action Against Hunger, Emergency Nutrition Network (ENN), Global Nutrition Cluster, United Nations System Standing Committee on Nutrition and United Nations Office for the Coordination of Humanitarian Affairs (OCHA). Finally, we will look at the bibliographical references of the selected articles to identify additional relevant articles that could be included in the review. The documentary research will be carried out

between March and June 2023. Beyond this period we will regularly update the databases in order to include new publications which could be relevant. The results of the search will be documented in an appended table.

Table 1: Search strategy for each database

Database	Search strategy
Pubmed, Embase, web of sciences, Science direct, Google Scholar, Scopus	((((probiotic) OR prebiotic OR synbiotic) AND children) AND growth) AND randomized clinical trials); ((((probiotics) OR (synbiotics) AND children) AND growth) AND non randomized clinical trials).
clinicaltrial.org	((((probiotic) OR prebiotic OR synbiotic) AND children) AND growth) AND randomized clinical trials)
Cochrane Library	Appropriate combination of key words

Data management and selection process (Study selection)

Selected studies after search from databases and other sources will be retrieved into Zotero v6 software for reference management and duplicates removal. Following duplicates removal, remaining articles will then be transferred to Excel to continue the selection. The initial selection of studies will be conducted by two independent reviewers (MD), and (MK). In case of disagreement between reviewers, the arbitration of a third reviewer (DS) will be sought. The first step of the study selection will be based on reviewing the title and abstract to check whether the article meets the selection criteria. If an abstract is not provided with the title, the full text will be reviewed and assessed. After this process, all qualified studies will pass to the second step, which will be based on reviewing the full text of selected articles to check whether they meets the selection criteria predefined in a selection protocol which further defines relevant variables between prebaotics, propbiotics and symbiotics administration and health benefits in children under five. Qualified studies will pass to the third step which will be based on assessing quality of the studies using study quality assessment tools for randomized and non-randomized studies. Eligible studies will enter the final step which consist of data extraction and analysis followed by the writing the systematic review (Figure 1). If collected data are sufficient enough and suitable for meta-analysis, we will conduct a meta-analysis to show the association between administration of prebaotics, propbiotics and symbiotics administration and health benefits in children under five.

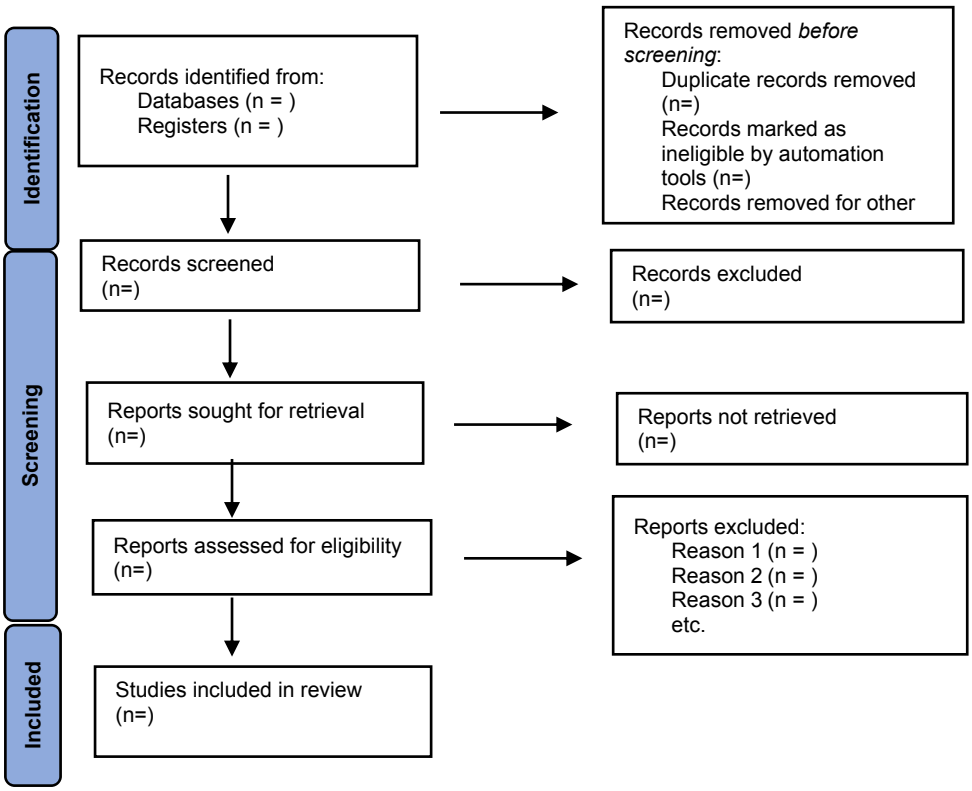

Figure 1: PRISMA Diagram displaying the studies selection

Evaluation of the quality of studies

Two reviewers (MD) and (MK) will independently assess the methodological quality of included studies. The risk of bias in randomized controlled trials (RCTs) will be assessed using the Cochrane Revised Tool for Randomized Trials (RoB 2)(Sterne et al., 2019). This includes seven domains of bias: sequence generation and allocation concealment (selection and allocation bias), blinding of participants and staff (performance bias), blinding of outcome assessors (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and an auxiliary domain: “other bias” (Sterne et al., 2019). If only one of the domains is judged to be of high quality then the study will be considered at high risk of bias. If all domains are judged to be weak, then it will be considered at low risk of bias. Otherwise, the study will be considered to present an uncertain risk. For non-randomized and/or non-controlled interventional studies, we will use the "Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies (Study Quality Assessment Tools, 2021). This tool includes 14 items that are answered with “yes,” “no,” or “not applicable.” These will then be converted into a dichotomous rating (“yes” = 1, “no” and “not applicable” = 0) (Study

Quality Assessment Tools, 2021). Each quote will receive a score by summarizing the 14 elements. Poor, fair and good study quality will correspond to a score of 0 to 5, 6 to 9 and 10 to 14 respectively (Study Quality Assessment Tools, 2021). If low quality studies are included, a sensitivity analysis will be carried out to assess the impact of lower quality studies on the conclusions of the review and will possibly be excluded in the synthesis of results. Disagreements and uncertainties regarding individual scores in each of the categories will be resolved by consensus between the two examiners.

Data extraction

Data extraction will be performed using a standardized protocol for all studies included in the final review. Data on author and year, study design, study settings, objectives, sociodemographic characteristics of the study population including (sex, age) signs and symptoms of stunting (length/height-for-age and weight-for-length/height z scores, growth percentiles, mid-upper arm circumference (MUAC)), the presence of comorbidities, intervention(s) and type of intervention used, as well as the results reported will be extracted using a standardized form. Data extraction will be conducted by two independent researchers and the results compared to ensure accuracy and completeness.

Data synthesis and analysis

Synthesis of the extracted data will focus on the following elements: the study population, the study design, the sample size, the type of malnutrition suffered by the children included, the presence of comorbidities, details of the prebiotics, probiotics, and/or synbiotics (e.g. strains and number of probiotic organisms), dosage regimen, duration of intervention, health benefits, growth, and biases. If there is too much missing data we will perform a narrative synthesis. If the compiled data are sufficient and appropriate, we will perform a meta-analysis to establish the relationship between the intervention and infant growth. We will grade the quality of evidence using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) guidelines (Balshem et al., 2011). All statistical analyzes for randomized clinical trials will be performed using Review Manager (Rev Man) V.5.3 for Windows. Heterogeneity will be quantified by I^2 and interpreted as the percentage of total variation between studies attributable to heterogeneity rather than chance. A value of 0% to 40% might not be important, 30% to 60% may represent moderate heterogeneity, 50% to 90% may represent substantial heterogeneity and 75% to 100%: considerable heterogeneity(Cochrane Handbook for Systematic Reviews of Interventions, 2023). Analyzes will be based on the random effects model if studies are clinically heterogeneous in terms of different settings (e.g. participant characteristics, countries), doses and strains of synbiotics and probiotics or types of prebiotics,

treatment durations and other factors . The source of statistical heterogeneity will be explored to assess whether the effects of the intervention will be significantly different for the following subgroups: type of intervention (pre-, pro or synbiotic), health status of participants (healthy versus undernutrition). Data on normally-distributed continuous variables will be expressed as means, while categorical variables will be described as a percentage and odd ratios (OR) associated with 95% confidence intervals (CI) calculated to estimate the association between intervention and children's growth. For observational studies the effect of heterogeneity I2 will be calculated based on Cochrane's Q, with a value of 0% indicating no heterogeneity and 100% substantial heterogeneity (Higgins et al., 2003). The degree of heterogeneity will be assessed based on the proposed Cochrane categories(Cochrane Handbook for Systematic Reviews of Interventions, 2023). Heterogeneity among included studies will be investigated by a sensitivity analysis that takes into account the results of the risk of bias assessment. In addition, we will perform subgroup analyzes to evaluate the effects of the intervention will be significantly different according to the following subgroups: type of intervention (pre-, pro or synbiotic), health status of the participants (healthy versus undernutrition). If there is too much missing data we will do a narrative synthesis.

Results and prioritization

The two primary outcomes will be growth during the intervention period, measured by weight gain and length/height gain. The secondary endpoints will be the gain in head circumference, the gain in body mass index (BMI) and the Z score for weight, height achieved at the end of the intervention, etc.

Discussion

Stunting is a public health concern affecting children worldwide particularly in developing countries such as Asia and Africa. Although the requirement for global mobilization to provide appropriate solutions is obvious, much remains to be done to identify effective interventions and how to scale up interventions in order to make them effective and achieve long lasting results. Few studies from developing countries have show weak association between pre-pro- and synbiotics and health benefit and growth. This systematic review will summarize the evidence regarding the effectiveness of the use of prebiotics, probiotics, and synbiotics in improving growth in children under five years of age. Findings will be interpreted considering the methodological challenges associated with the design of included studies. The evidence generated from this document will inform designing more effective intervention strategies to tackle malnutrition in young children.

Conclusion

In summary, the results of this systematic review could have important implications for public policy and the future direction of research in the field of child nutrition and health in Africa. Indeed, if the results of the review show that pre-, pro- or synbiotics are effective, they could be integrated into national nutrition and child health programmes in Africa.

Abbreviations

WHO: World Health Organization; PRISMA-P: Preferred Reporting Items for Systematic Review and Meta-Analysis Statement-Protocol Extension; UNICEF: United Nation of International Children's Emergency Fund; RCT: Randomized Control Trial; LCPUFA: Long Chain Polyunsaturated Fatty Acids-an overview; MUAC: Middle Upper Arm Circumference; AOR: Adjusted Odd Ratio; pre: prebiotics; pro: probiotics.

Acknowledgments

The authors would like to acknowledge UKRI GCRF- Action Against Stunding project.

Contributors

MK: PhD at Cheikh Anta Diop University in Dakar as part of the UKRI GCRF Action Against Stunting Hub research project; drafted and revised the manuscript.

MD: Research assistant at Cheikh Anta Diop University in Dakar; designed and critically revised the protocol and supervised drafting of the manuscript.

DS: Professor at the Gaston Berger University in Saint Louis; revised the protocol.

SA: Professor of paediatrics, Liverpool School of Tropical Medicine; revised the protocol.

BF: Professor at Cheikh Anta Diop University in Dakar; revised the protocol.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This work is supported by RSTMH (Royal Society of Tropical Medicine and hygiene) as part of our 2022 Early Career Grants programme, member number 0022827.

Ethical approval and consent to participate

Not applicable.

Data availability statement

No data are available. Data sharing does not apply to this manuscript as it describes a protocol. The systematic review is ongoing at the time of submission.

BIBLIOGRAPHY

Balshem, H. *et al.* (2011) 'GRADE guidelines: 3. Rating the quality of evidence', *Journal of Clinical Epidemiology*, 64(4), pp. 401–406. Available at: https://doi.org/10.1016/j.jclinepi.2010.07.015.

Bank, A.D. (2021). African Development Bank Group. Available at: https://www.afdb.org/fr/news-and-events/press-releases/la-banque-africaine-de-developpement-et-ses-partenaires-renouvellent-lengagement-mettre-fin-au-retard-de-croissance-chez-les-enfants-africains-44673 (Accessed: 26 January 2022).

Catania, J. *et al.* (2022) 'Probiotic Supplementation for Promotion of Growth in Children: A Systematic Review and Meta-Analysis', *Nutrients*, 14(1), p. 83. Available at: https://doi.org/10.3390/nu14010083.

Cochrane Handbook for Systematic Reviews of Interventions (2023). Available at: https://training.cochrane.org/handbook/current (Accessed: 7 May 2024).

Heuven, L.A. *et al.* (2021) 'Gut Microbiota–Targeted Nutritional Interventions Improving Child Growth in Low- and Middle-Income Countries: A Systematic Review', *Current Developments in Nutrition*, 5(11), p. nzab124. Available at: https://doi.org/10.1093/cdn/nzab124.

Higgins, J.P.T. *et al.* (2003) 'Measuring inconsistency in meta-analyses', *BMJ : British Medical Journal*, 327(7414), pp. 557–560.

Initiatives mondiales *et al.* (2020) 'Estimations communes sur la malnutrition infantile - édition 2020 : niveaux et tendances', *SUN*, 9 April. Available at: https://scalingupnutrition.org/fr/news/estimations-communes-sur-la-malnutrition-infantile-edition-2020-niveaux-et-tendances/ (Accessed: 22 December 2021).

Lai, H.-H. *et al.* (2019) 'Probiotic Lactobacillus casei: Effective for Managing Childhood Diarrhea by Altering Gut Microbiota and Attenuating Fecal Inflammatory Markers', *Nutrients*, 11(5), p. 1150. Available at: https://doi.org/10.3390/nu11051150.

Moher, D. *et al.* (2015) 'Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement', *Systematic Reviews*, 4(1), p. 1. Available at: https://doi.org/10.1186/2046-4053-4-1.

Nuzhat, S. *et al.* (2023) 'Effects of probiotic and synbiotic supplementation on ponderal and linear growth in severely malnourished young infants in a randomized clinical trial', *Scientific Reports*, 13(1), p. 1845. Available at: https://doi.org/10.1038/s41598-023-29095-w.

OMS (2013) 'OMS. Le retard de croissance chez l'enfant : défis et perspectives. Rapport sur un colloque du projet Promouvoir une croissance saine et prévenir le retard de croissance chez l'enfant. Genève : Organisation mondiale de la Santé ; 2014.', in. *Le retard de croissance chez les enfants défis et perspectives*, Geneve. Available at: https://apps.who.int/iris/bitstream/handle/10665/148526/WHO_NMH_NHD_GRS_14.1_fre.pdf.

Onubi, O.J. *et al.* (2015) 'Effects of probiotics on child growth: a systematic review', *Journal of Health, Population and Nutrition*, 34(1), p. 8. Available at: https://doi.org/10.1186/s41043-015-0010-4.

Oozeer, R. *et al.* (2013) 'Intestinal microbiology in early life: specific prebiotics can have similar functionalities as human-milk oligosaccharides', *The American Journal of Clinical Nutrition*, 98(2), pp. 561S–71S. Available at: https://doi.org/10.3945/ajcn.112.038893.

Pandey, Kavita.R., Naik, Suresh.R. and Vakil, Babu.V. (2015a) 'Probiotics, prebiotics and synbiotics- a review', *Journal of Food Science and Technology*, 52(12), pp. 7577–7587. Available at: https://doi.org/10.1007/s13197-015-1921-1.

Pandey, Kavita.R., Naik, Suresh.R. and Vakil, Babu.V. (2015b) 'Probiotics, prebiotics and synbiotics- a review', *Journal of Food Science and Technology*, 52(12), pp. 7577–7587. Available at: https://doi.org/10.1007/s13197-015-1921-1.

'Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria' (2001).

Sazawal, S. *et al.* (2010) 'Effects of Bifidobacterium lactis HN019 and Prebiotic Oligosaccharide Added to Milk on Iron Status, Anemia, and Growth Among Children 1 to 4 Years Old', *Journal of Pediatric Gastroenterology and Nutrition*, 51(3), pp. 341–346. Available at: https://doi.org/10.1097/MPG.0b013e3181d98e45.

Schrezenmeir, J. and de Vrese, M. (2001) 'Probiotics, prebiotics, and synbiotics—approaching a definition', *The American Journal of Clinical Nutrition*, 73(2), pp. 361s–364s. Available at: https://doi.org/10.1093/ajcn/73.2.361s.

Sterne, J.A.C. *et al.* (2019) 'RoB 2: a revised tool for assessing risk of bias in randomised trials', *BMJ*, p. l4898. Available at: https://doi.org/10.1136/bmj.l4898.

Study Quality Assessment Tools | NHLBI, NIH (2021). Available at: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools (Accessed: 7 May 2024).

Takele, B.A., Gezie, L.D. and Alamneh, T.S. (2022) 'Pooled prevalence of stunting and associated factors among children aged 6–59 months in Sub-Saharan Africa countries: A Bayesian multilevel approach', *PLOS ONE*, 17(10), p. e0275889. Available at: https://doi.org/10.1371/journal.pone.0275889.

UNICEF. WHO/The World Bank Group joint child malnutrition estimates: key findings of the 2021 edition. (no date). Available at: https://www.who.int/data/gho/data/themes/topics/joint-child-malnutrition-estimates-unicef-who-wb (Accessed: 31 May 2023).

Verma, S.K., David, J. and Chandra, R. (2012) 'Synbiotics in Functional Foods.', *Indian Dairyman (India)* [Preprint]. Available at: https://scholar.google.com/scholar_lookup?title=Synbiotics+in+Functional+Foods.&author=Verma%2C+Sachin+Kumar&publication_year=2012 (Accessed: 28 June 2022).

World health Organisation 2015 (no date) *Stunting in a nutshell*. Available at: https://www.who.int/news/item/19-11-2015-stunting-in-a-nutshell (Accessed: 8 June 2023).

World Health Organization (2009) *WHO child growth standards : growth velocity based on weight, length and head circumference : methods and development*. World Health Organization. Available at: https://apps.who.int/iris/handle/10665/44026 (Accessed: 8 June 2023).

World Health Organization (2018) *Reducing stunting in children: equity considerations for achieving the global nutrition targets 2025*. Geneva: World Health Organization. Available at: https://apps.who.int/iris/handle/10665/260202 (Accessed: 8 June 2023).

Appendix

Appendix 1 : RoB 2: a revised tool for assessing risk of bias in randomised trials

Table 1 | Version 2 of the Cochrane risk-of-bias assessment tool for randomised trials: bias domains, signalling questions, response options, and risk-of-bias judgments

	Response options		
Bias domain and signalling question*	Lower risk of bias	Higher risk of bias	Other
Bias arising from the randomisation process			
1.1 Was the allocation sequence random?	Y/PY	N/PN	NI
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Y/PY	N/PN	NI
1.3 Did baseline differences between intervention groups suggest a problem with the randomisation process?	N/PN	Y/PY	NI
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias arising from the randomisation process?			
Bias due to deviations from intended interventions			
2.1 Were participants aware of their assigned intervention during the trial?	N/PN	Y/PY	NI
2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	N/PN	Y/PY	NI
2.3 If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?	N/PN	Y/PY	NA/NI
2.4 If Y/PY/NI to 2.3: Were these deviations likely to have affected the outcome?	N/PN	Y/PY	NA/NI
2.5 If Y/PY to 2.4: Were these deviations from intended intervention balanced between groups?	Y/PY	N/PN	NA/NI
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Y/PY	N/PN	NI
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomised?	N/PN	Y/PY	NA/NI
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias due to deviations from intended interventions?			
Bias due to missing outcome data			
3.1 Were data for this outcome available for all, or nearly all, participants randomised?	Y/PY	N/PN	NI
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?	Y/PY	N/PN	NA
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	N/PN	Y/PY	NA/NI
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	N/PN	Y/PY	NA/NI
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias due to missing outcome data?			
Bias in measurement of the outcome			
4.1 Was the method of measuring the outcome inappropriate?	N/PN	Y/PY	NI
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	N/PN	Y/PY	NI
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	N/PN	Y/PY	NI
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	N/PN	Y/PY	NA/NI
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	N/PN	Y/PY	NA/NI
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias in measurement of the outcome?			
Bias in selection of the reported result			
5.1 Were the data that produced this result analysed in accordance with a prespecified analysis plan that was finalised before unblinded outcome data were available for analysis?	Y/PY	N/PN	NI
Is the numerical result being assessed likely to have been selected, on the basis of the results, from:			
5.2 ... multiple eligible outcome measurements (eg, scales, definitions, time points) within the outcome domain?	N/PN	Y/PY	NI
5.3 ... multiple eligible analyses of the data?	N/PN	Y/PY	NI
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction bias due to selection of the reported results?			
Overall bias			
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the overall predicted direction of bias for this outcome?			

Y=yes; PY=probably yes; PN=probably no; N=no; NA=not applicable; NI=no information.
*Signalling questions for bias due to deviations from intended interventions relate to the effect of assignment to intervention.

Appendix: The final AXIS tool following consensus on all components by the Delphi panel

Table 2 The final AXIS tool following consensus on all components by the Delphi panel

	Yes	No	Do not know/ comment
Introduction			
1 Were the aims/objectives of the study clear?			
Methods			
2 Was the study design appropriate for the stated aim(s)?			
3 Was the sample size justified?			
4 Was the target/reference population clearly defined? (Is it clear who the research was about?)			
5 Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?			
6 Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation?			
7 Were measures undertaken to address and categorise non-responders?			
8 Were the risk factor and outcome variables measured appropriate to the aims of the study?			
9 Were the risk factor and outcome variables measured correctly using instruments/ measurements that had been trialled, piloted or published previously?			
10 Is it clear what was used to determined statistical significance and/or precision estimates? (eg, p values, CIs)			
11 Were the methods (including statistical methods) sufficiently described to enable them to be repeated?			
Results			
12 Were the basic data adequately described?			
13 Does the response rate raise concerns about non-response bias?			
14 If appropriate, was information about non-responders described?			
15 Were the results internally consistent?			
16 Were the results for the analyses described in the methods, presented?			
Discussion			
17 Were the authors' discussions and conclusions justified by the results?			
18 Were the limitations of the study discussed?			
Other			
19 Were there any funding sources or conflicts of interest that may affect the authors' interpretation of the results?			
20 Was ethical approval or consent of participants attained?			

Downes MJ, et al. BMJ Open 2016;6:e011458. doi:10.1136/bmjopen-2016-011458

Appendix 3. Risk of bias for included studies: NIH Quality Assessment Tool for Observational Cohort and Cross-sectional Studies

Criteria	Yes	No	Other (CD, NA, NR)*
1. Was the research question or objective in this paper clearly stated?			
2. Was the study population clearly specified and defined?			
3. Was the participation rate of eligible persons at least 50%?			
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?			
5. Was a sample size justification, power description, or variance and effect estimates provided?			
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?			
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?			
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?			
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
10. Was the exposure(s) assessed more than once over time?			
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?			
12. Were the outcome assessors blinded to the exposure status of participants?			
13. Was loss to follow-up after baseline 20% or less?			
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?			

*CD, cannot determine; NA, not applicable; NR, not reported