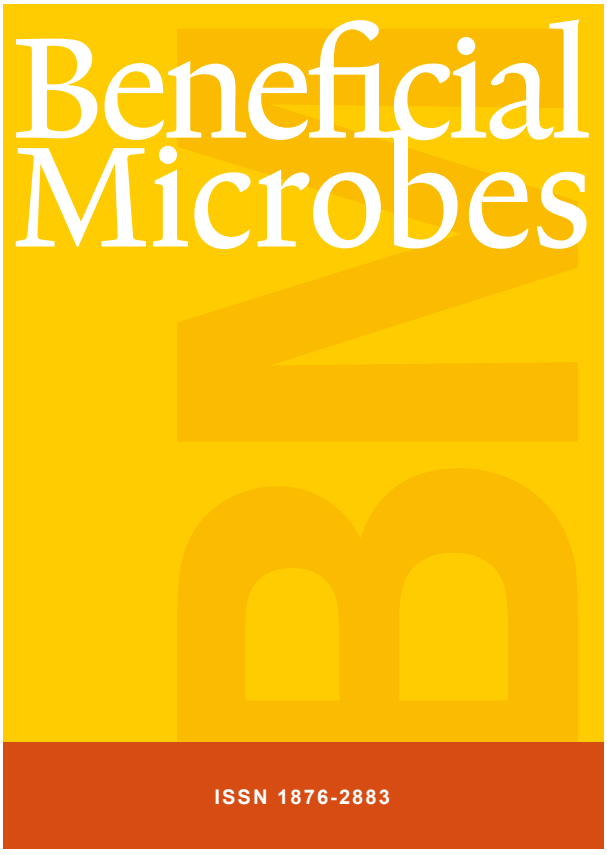

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Effectiveness of two probiotics in preventing necrotising enterocolitis in a cohort of very-low-birth-weight premature new-borns

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RESEARCH ARTICLE

Abstract

According to previous research, the incidence of necrotising enterocolitis (NEC) decreases after supplementation with probiotics. However, few studies have considered the equivalence or otherwise of different strains of probiotics in this respect. Accordingly, this prospective observational study was conducted in a cohort of 245 very-low-birth-weight (VLBW) new-borns to assess the prevalence of NEC after supplementation with the probiotic Inforan® (Berna Biotech, Madrid, Spain) 250 mg capsules containing 10^9 cfu of *Lactobacillus acidophilus* (ATCC 4356) and 10^9 cfu of *Bifidobacterium bifidum* (ATCC 15696); or with Bivos® (Ferring, Madrid, Spain) containing *Lacticaseibacillus* (formerly *Lactobacillus*) *rhamnosus* (LGG) (ATCC 53103) (10^9 cfu); or with no probiotic supplementation. Statistical analysis was performed using multivariant regression for the duration of parenteral nutrition, length of neonatal intensive care unit stay, use of oxygen therapy and presence of chorioamnionitis. Of the VLBW new-borns in the study group, 65 received Infloran, 108 received Bivos and 72 received no probiotic. A significant association was observed between a reduced presence of NEC Stage ≥2 and probiotic supplementation. The odds risk (OR) obtained was 0.174 (95% confidence interval (CI): 0.032-0.936) for Infloran and 0.196 (95%CI: 0.053-0.732) for Bivos. Therefore, both probiotics are associated with a lower prevalence of NEC in VLBW new-borns, with no significant differences.

Keywords: probiotics, premature infant, morbidity, mortality

1. Introduction

Necrotising enterocolitis (NEC) is the gastrointestinal pathology that most frequently affects very-low-birth-weight (VLBW) infants. It poses significant dangers, including neurodevelopmental disorders and increased mortality. Moreover, the younger the gestational age, the greater the risk. Among other explanations suggested, the pathogenesis of NEC has been ascribed to an excessive inflammatory response and immature innate immunity. However, according to epidemiological studies, the risk is reduced by preferential feeding of breast milk and the development by neonatal units of standardised nutrition protocols (Hwang *et al.*, 2013).

The bacteria generally used as probiotics are lactobacilli and bifidobacteria. After passing through the stomach and small intestine, some probiotics survive and colonise the large intestine (Gewolb *et al.*, 1999; Jacobi *et al.*, 2012). This differential finding has been addressed in many trials conducted to consider supplementation schemes with probiotics for VLBW new-borns. In the intestinal mucosa, the body's defences have two main components: on the one hand, the host's immune response, and on the other, the barrier function exerted by the intestinal microbiota, through a mechanism of competitive inhibition within the ecological niche. In special situations, such as that of VLBW infants admitted to intensive care units, there is little colonisation by bifidobacteria and lactobacillus, and the intestinal microbiota are modified, with an increased presence of proteobacteria, represented by *Klebsiela*, *Enterobacter*, *Citrobacter* and *Pseudomonas*, all of which are common within the hospital environment and are responsible for the development of late sepsis and NEC in VLBW infants.

With respect to probiotics, the effect of a bacterium is specific to the strain to which it belongs and cannot be extrapolated to other strains of the same species; therefore, each strain has unique properties, with different physiological functions. The assignment of a beneficial effect to a particular strain depends on the conditions of use, the population group to which it is directed and, very particularly, on the dose supplied (Murguia-Peniche *et al.*, 2013).

After the use of probiotics, it is important to avoid bacterial metabolic activity that might be harmful to the host, associated with the risk of excessive deconjugation or hydroxylation of bile salts in the small intestine, and excessive degradation of the mucin layer of the intestine, any of which could favour intestinal colonisation by potentially pathogenic strains (Oh, 2018).

Studies have shown that some strains of probiotics may be useful in the prophylaxis of NEC in VLBW new-borns (Alfaleh and Anabrees, 2014; Uberos *et al.*, 2017). However, very little research has been undertaken to determine the clinical equivalence of the different formulations used in neonatology. In the present study, our aim is to establish the effectiveness of the probiotics *Bifidobacterium bifidum* + *Lactobacillus acidophilus* (Infloran®) and *Lacticaseibacillus* (formerly *Lactobacillus*) *rhamnossus* (LGG) (Bivos®), which are commonly used in our neonatal unit for the prevention of NEC in VLBW new-borns.

2. Materials and methods

In this prospective observational study, we compare the effectiveness and equivalence of two probiotics commonly used for the prevention of NEC in VLBW new-borns. In our neonatal intensive care unit (NICU), supplementation with probiotics was started in December 2013 and became part of standard practice, in accordance with guidelines published by the Nutrition and Neonatal Metabolism Group of the Spanish Society of Neonatology (Narbona *et al.*, 2014). On the basis of these recommendations and after reviewing the evidence available, the hospital Pharmacy Committee proposed the use of two commercial formulations for probiotic supplementation. The protocol for the present study was approved by the hospital's Ethics Committee (Verification code: b1072175150294535cb06bd44a5ca9ddfe619d24), all current regulations regarding data confidentiality were respected and the parents/guardians of the new-borns included gave informed consent for data analysis and publication.

Morbidities

For the diagnosis of NEC, patients were classified according to the Bell criteria (Bell, 1978). The presence of any grade of bronchopulmonary dysplasia (BPD) was also recorded. According to the NIHCD definition (Ehrenkranz *et al.*, 2005) and to Jobe and Bancalari (2001), BPD is present when the oxygen requirement is >21% at 28 days of life and/or when the oxygen requirement is >21% or there is positive airway pressure at 36 weeks' corrected gestational age. The level of BPD is classed as mild, moderate or severe. Clinical sepsis is diagnosed when a score ≥8 is obtained on the NOSEP-1 scale. This score is determined as follows: PCR ≥1.4 mg/dl (5 points), neutrophils >50% (3 points), thrombocytopaenia $<150\times10^9$/l (5 points), fever >38.2 °C (5 points) and total parenteral nutrition ≥14 days (6 points) (Mahieu *et al.*, 2000). The diagnosis and staging of retinopathy of prematurity (ROP) are based on retinal examination before discharge. Severe ROP is defined as stages 3-5 (International Committee for the Classification of Retinopathy of Prematurity, 2005). In ROP, the presence of at least one of the following findings is associated with an unfavourable outcome: a retinal fold involving the macula; a retinal detachment involving zone I of the posterior pole; retrolental tissue, or 'mass' (Lad *et al.*, 2009). The diagnosis of intraventricular haemorrhage (IVH) is based on the Papile grading system (Papile *et al.*, 1978). In all new-borns, a transfontanellar ultrasound is performed on the third day of life and then every week. Persistent ductus arteriosus (PDA) was diagnosed by Doppler ultrasound and treated when clinical repercussions were observed or when the diameter was greater than 2 mm. Cholestasis was defined as an increase in direct bilirubin values >2 mg/dl (34.2 µmol/l).

Nutritional management

In our NICU, enteral and parenteral nutrition are conducted in accordance with the recommendations of the Nutrition and Metabolism Group of the Spanish Neonatology Society (Narbona *et al.*, 2014). All clinically stable new-borns are given minimal enteral nutrition with breast milk (or formula for preterm infants) at a rate of 1 ml/kg every 3 h, from the first day of life, with subsequent increases in enteral nutrition, according to tolerance, at a rate of 15-25 ml/kg/day, until full enteral nutrition is achieved. The fortification of breast milk is considered when volumes >80 ml/kg/day are achieved.

Protocol for the administration of probiotics

Since December 2013, our NICU has used two presentations of probiotics: the first is Infloran (Berna Biotech, Madrid, Spain) 250 mg capsules containing 10^9 cfu of *L. acidophilus* (ATCC 4356) and 10^9 cfu of *B. bifidum* (ATCC 15696) (European Medicines Agency, 2014). A daily dose of one capsule every 12 h is dissolved in 2 ml of (breast or formula) milk and supplied via nasogastric tube. The second probiotic is Bivos (Ferring, Madrid, Spain), containing *L. rhamnosus* (LGG) (ATCC 53103) (10^9 cfu). In this case, a daily dose of 9 drops every 24 h is dissolved in 2 ml of (breast or formula) milk and supplied via nasogastric tube.

The probiotic supplementation is started at the first enteral feed, with at least 1 ml per bolus, and is continued until 35 weeks postmenstrual age or until the infant is discharged from the NICU. The neonatologist attending the new-born determines which, if any, probiotic should be supplied.

Statistical considerations

The descriptive data are summarised using medians and interquartile intervals for the continuous variables and distribution frequencies for the categorical variables. Univariate comparisons are made by the Mann-Whitney test for the continuous variables and by the chi-square test for the categorical ones. The association between comorbidities and probiotic supplementation is evaluated by multinomial regression analysis, adjusting for days of central catheter, parenteral nutrition, oxygen therapy and breastfeeding. The statistical analysis was performed using IBM SPSS 20.0 for Windows software (IBM, Armonk, NY, USA).

3. Results

From December 2013 to November 2020, 245 new-borns weighing <1,500 g were treated with one of the probiotics described above (65 received Infloran and 108 received Bivos), while another 72 received no such supplementation (Figure 1).

Table 1 shows the characteristics of the maternal and neonatal variables for this study group. Notably, there were significantly more cases of chorioamnionitis in the group that received no probiotics. The duration of NICU stay was longer among the new-borns who received probiotics, as was the duration of parenteral nutrition.

Table 2 shows the incidence of comorbidities in each group, revealing a significantly higher prevalence of NEC Stage ≥2 in the new-borns who did not receive probiotic supplementation. The need for surgery and mortality in the group without probiotic supplementation was slightly higher than that observed in the groups that received supplementation. There was a significant inverse association between probiotic supplementation and NEC Stage ≥2, for Infloran, odds ratio (OR) 0.174; $P<0.05$ (95% confidence interval (CI): 0.032-0.936) and for Bivos, OR 0.196; $P<0.01$ (95% CI: 0.053-0.732). However, no significant associations were observed for any of the comorbidities considered (Table 3), and there were no significant differences in the prevalence of NEC Stage ≥2 between the Infloran and Bivos groups.

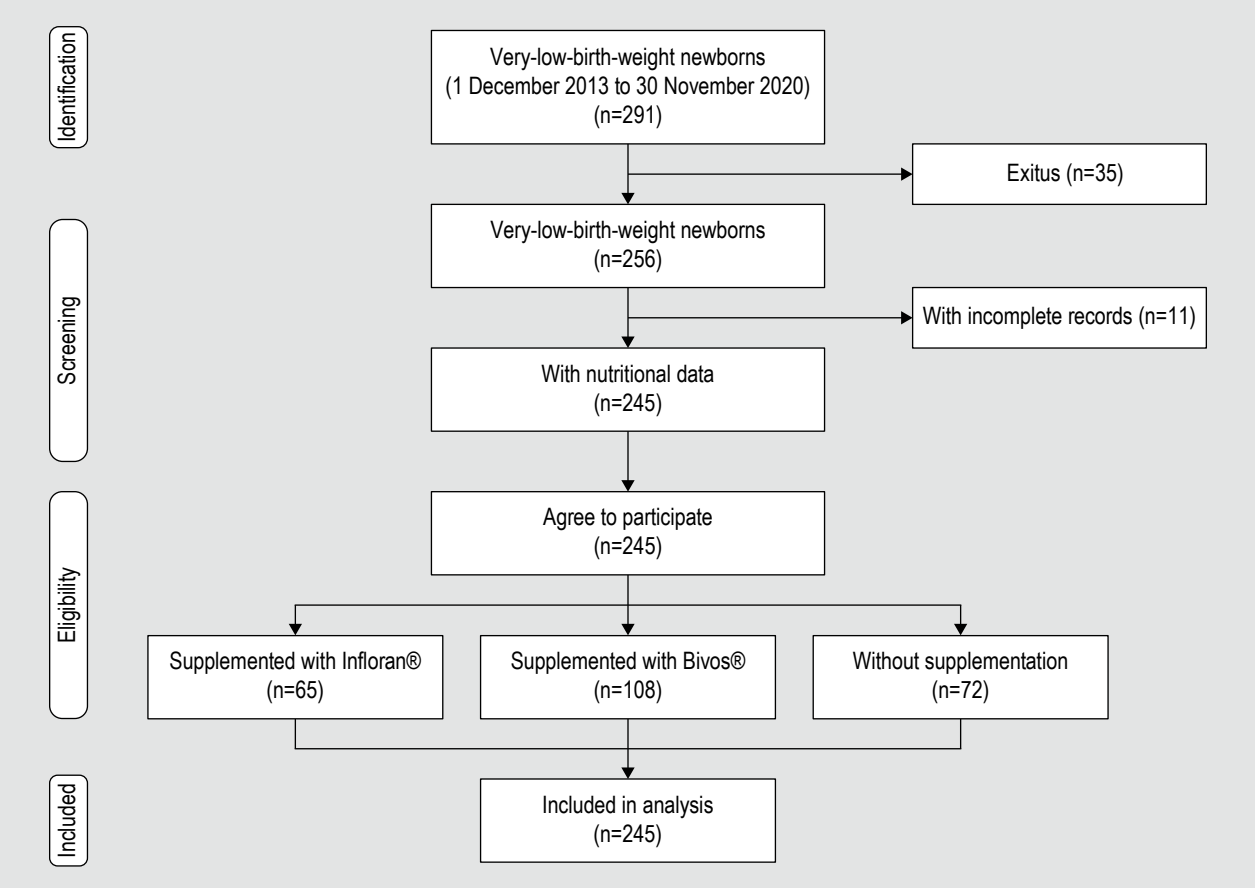

Figure 1. Flow diagram for the very-low-birth-weight new-borns included in the study (Moher *et al.*, 2009).

Table 1. Characteristics of the maternal and neonatal variables.[1]

Characteristics	Infloran® (n=65)	Bivos® (n=108)	No probiotic (n=72)	*P*-value
Maternal	**n (%)**	**n (%)**	**n (%)**	
PIH	10 (15.3)	14 (12.9)	21 (29.1)	0.62
Chorioamnionitis	5 (7.7)	15 (13.8)	28 (38.8)	0.01
Antibiotics	27 (41.5)	51 (47.2)	47 (65.2)	0.61
Glucocorticoids	47 (72.3)	84 (77.7)	66 (91.6)	0.21
PPROM	22 (33.8)	31 (28.7)	24 (33.3)	0.09
Gestation (w)[2]	30 (28, 31)	29 (28, 31)	30 (27, 32)	0.21
Gestation ≤ 27 w	13 (20)	25 (23.1)	36 (50.0)	0.24
Twin birth	29 (44.6)	50 (46.3)	44 (61.1)	0.38
Caesarean section	53 (81.5)	86 (79.6)	63 (87.5)	0.40
Neonatal				
Birth weight (g)[2]	1,219 (999, 1,426)	1,203 (915, 1,431)	1,346 (1,100, 1,500)	0.60
Birth weight (z-score)[2]	-0.64 (-1.26, 0.002)	-0.48 (-1.09, -0.02)	-0.57 (-1.40, 0.34)	0.36
Weight 7 days (z-score)[2]	-1.29 (-1.70, -0.73)	-1.24 (-1.70, -0.66)	-1.18 (-1.67, -0.55)	0.54
Male gender	35 (53.8)	60 (55.5)	45 (62.5)	0.92
Apgar ≤7 (5 min)	24 (36.9)	38 (35.1)	20 (27.7)	0.15
SGA	16 (24.6)	26 (24.1)	20 (27.7)	0.96
Breast milk	44 (67.6)	68 (62.9)	57 (79.1)	0.82
Length of NICU stay (d)[2]	29 (18-39)	29 (19-44)	20.5 (10.7, 32.7)	0.01
Central venous catheter (d)[2]	9.5 (4, 17)	9.5 (4, 17)	7 (3, 17)	0.29
Age at full feeds (d)[2]	10 (7, 16.7)	11 (8, 19.7)	10 (6, 15.5)	0.15
Parenteral nutrition (d)[2]	11.5 (7, 21)	11 (7, 19.5)	8 (4, 16)	0.02
Respiratory support				
Oxygen[2]	19 (6, 42.5)	26.5 (6.7, 48.2)	10 (3, 33)	0.03
CPAP[2]	3 (2, 5.7)	3 (1, 7.2)	2 (1, 4.5)	0.05
Mechanical ventilation[2]	1 (0, 3.5)	1 (0, 6)	0 (0, 3)	0.33

[1] CPAP = continuous positive airway pressure; NICU = neonatal intensive care unit; PIH = pregnancy induced hypertension, PPROM = preterm pre-labour rupture of membranes, SGA = small-for-gestational age.
[2] Median (IQR). *P*-value χ^2 for qualitative analysis, Mann-Whitney for quantitative analysis.

Table 2. Comorbidities for very-low-birth-weight new-borns with Infloran® or Bivos® supplementation.[1]

	Infloran (n=65) n (%)	Bivos (n=108) n (%)	No probiotic (n=72) n (%)	*P*-value
BPD	21 (32.3)	45 (41.6)	25 (34.7)	0.13
NEC ≥ Stage II	5 (7.6)	8 (7.4)	14 (19.4)	0.03
Need for surgery	0 (0)	1 (0.9)	4 (5.5)	0.13
Exitus after NEC	0 (0)	1 (0.9)	3 (4.1)	0.17
PDA	11 (16.9)	21 (19.4)	13 (18.1)	0.81
IVH				
Grade I-II	6 (9.2)	14 (12.9)	14 (19.4)	0.38
Grade III-IV	0 (0)	3 (2.7)	4 (5.5)	0.15
ROP	4 (6.1)	12 (11.1)	4 (5.5)	0.36
Sepsis	17 (26.1)	24 (22.2)	22 (30.5)	0.85
Cholestasis	11 (16.9)	15 (13.8)	7 (9.7)	0.23

[1] BPD = bronchopulmonary dysplasia; NEC = necrotising enterocolitis; PDA = patent ductus arteriosus; IVH = intraventricular haemorrhage; ROP = retinopathy of prematurity.

Table 3. Odds ratio (OR) of the comorbidities of VLBW new-borns supplemented with Infloran® or Bivos®.

	OR (CI 95%) unadjusted		OR (CI 95%) adjusted [2]	
	Infloran	**Bivos**	**Infloran**	**Bivos**
NEC ≥ Stage II	0.506 (0.174, 1.467)	0.460 (0.186, 1.137)	0.174 (0.032, 0.936)*	0.196 (0.053, 0.732)**
BPD	1.250 (0.619, 2.523)	1.830 (0.999, 3.351)	0.942 (0.373, 2.379)	1.900 (0.869, 4.153)
PDA	1.390 (0.588, 3.284)	1.568 (0.751, 3.276)	0.931 (0.283, 3.066)	1.436 (0.528, 3.907)
IVH				
Grade I-II	0.612 (0.226, 1.654)	0.848 (0.392, 1.834)	0.652 (0.216, 1.971)	0.800 (0.322, 1.990)
Grade III-IV	–	0.209 (0.058, 0.756)	–	0.478 (0.073, 3.140)
ROP	1.127 (0.288, 4.410)	2.070 (0.695, 6.168)	3.316 (0.428, 25.685)	5.552 (1.002, 30.752)
Sepsis	1.243 (0.598, 2.583)	1.074 (0.566, 2.040)	0.756 (0.314, 1.821)	0.643 (0.294, 1.407)
Cholestasis	2.420 (0.913, 6.413)	1.755 (0.709, 4.343)	2.943 (0.865, 10.005)	1,418 (0.454, 4.430)

[1] BPD = bronchopulmonary dysplasia; PDA = patent ductus arteriosus; IVH = intraventricular haemorrhage; NEC = necrotising enterocolitis; ROP = retinopathy of prematurity.
[2] Adjusted for days of parenteral nutrition, length of neonatal intensive care unit stay (days) oxygen therapy (days) and chorioamnionitis. * = $P<0.05$, ** = $P\leq0.01$.

Furthermore, the duration of oxygen therapy was shorter for the new-borns who did not receive probiotics. These variables, reflecting differences between the study groups, were used as adjustment variables in the regression models (Table 3).

4. Discussion

The results obtained show that routine supplementation with a combination of *L. acidophillus* + *B. bifidum* (Infloran) or *L. rhamnnosus* (LGG) (Bivos) is effective in preventing NEC in VLBW new-borns. According to previous research, the use of probiotics reduces mortality, and the outcomes obtained do not vary according to the type of probiotic used (Bernardo *et al.*, 2013; Uberos *et al.*, 2017). In our cohort, there were more cases of NEC Stage ≥2 in the VLBW new-borns who received no probiotic supplementation. In our cohort, we observed more cases of NEC ≥2 in VLBW new-borns without probiotic supplementation despite the fact that the birth weight in this group is slightly higher.

Although meta-analyses of experimental and observational studies have confirmed the benefits of routine probiotic supplementation in the prevention of NEC in VLBW new-borns, Kane *et al.* (2018) studied 175 VLBW new-borns who received supplementation with LGG, and reported an increase in the incidence of NEC compared to the period prior to this supplementation. However, these authors did not include in their analysis the incidence of late sepsis or the duration of central venous catheter use before and after the start of routine supplementation, and these aspects might account for the differences observed in the incidence of NEC. Furthermore, the same authors reported a higher rate of BPD and a greater need of inotropic drugs among the patients supplemented with LGG. These variables, too, may be related to the incidence of NEC. In another observational study, Meyer and Alexander (2017) measured a reduced risk of NEC after supplementation in VLBW new-borns with LGG and lactoferrin. Indeed, for many years the components of the intestinal microbiota, such as *L. acidophillus* with *B. bifidum*, have been used to treat gastrointestinal disorders. Samuels *et al.* (2016), in a quasi-experimental study, observed no significant changes in the OR for NEC in patients supplemented with *L. acidophillus* and *B. bifidum*. Multicenter observational studies on a sample of 25,821 new-borns show that after supplementation with probiotics, severe forms of NEC and mortality decrease, although the overall incidence of NEC remains stable 8.8% (Zocaya *et al.*, 2020). These results are in line with what was observed in our work. In view of these considerations, we conclude that the result of an intervention of this type, in terms of decreased OR, depends on the incidence of the condition. Therefore, in hospitals with a low incidence of NEC, determining the expected effect of an intervention on the OR will require a larger sample than where the incidence is higher. In this respect, Lin *et al.* (2008) conducted an experimental study comparing treatment with a placebo vs the combination of *L. acidophilus* and *B. bifidum*. The latter group presented reduced mortality and a lower incidence of NEC. Finally, a systematic review by Baucells *et al.* (2016) analysed the findings of nine experimental studies based on 3,521 VLBW new-borns and concluded that the combination of *L. acidophilus* with *B. bifidum* produced most benefit in the prevention of NEC and mortality. The latter findings are in line with our own observations (Table 2).

The main limitation of the present study, with its prospective observational design, arises from the absence of randomisation, which implies a greater possibility of selection bias. The main conclusions we draw from this analysis are that, for VLBW new-borns, nutritional supplementation with Infloran or Bivos effectively reduces the prevalence of NEC, and that in this respect there are no significant differences between the two treatments. We believe it would be useful to extend these research findings in a larger meta-analysis.

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Conflict of interest

The authors have no relevant conflicts of interest to declare.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

Alfaleh, K. and Anabrees, J., 2014. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database of Systematic Reviews: CD005496. https://doi.org/10.1002/14651858.CD005496.pub4

Baucells, B.J., Mercadal, H.M., Alvarez Sanchez, A.T. and Figueras, A.J., 2016. Probiotic associations in the prevention of necrotising enterocolitis and the reduction of late-onset sepsis and neonatal mortality in preterm infants under 1,500g: a systematic review. Anales de Pediatría 85: 247-255. https://doi.org/10.1016/j.anpedi.2015.07.038

Bell, M., Ternberg, J., Feigin, R., Keating, J., Marshall, R., Barton, L. and Brotherton, T., 1978. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Annals of Surgery 187: 1-7.

Bernardo, W.M., Aires, F.T., Carneiro, R.M., Sa, F.P., Rullo, V.E. and Burns, D.A., 2013. Effectiveness of probiotics in the prophylaxis of necrotizing enterocolitis in preterm neonates: a systematic review and meta-analysis. Journal of Pediatrics 89: 18-24. https://doi.org/10.1016/j.jped.2013.02.004

Ehrenkranz, R.A., Walsh, M.C., Vohr, B.R., Jobe, A.H., Wright, L.L., Fanaroff, A.A., Wrage, L.A. and Poole, K., 2005. Validation of the national institutes of health consensus definition of bronchopulmonary dysplasia. Pediatrics 116: 1353-1360. https://doi.org/10.1542/peds.2005-0249

European Medicines Agency, 2014. Orphan drug designation for *Lactobacillus acidophilus* and *Bifidobacterium bifidum* for the prevention of necrotising enterocolitis. EU/3/13/1213. European Medicines Agency, Amsterdam, the Netherlands.

Gewolb, I.H., Schwalbe, R.S., Taciak, V.L., Harrison, T.S. and Panigrahi, P., 1999. Stool microflora in extremely low birthweight infants. Archives of Disease in Childhood: Fetal and Neonatal Edition 80: F167-F173.

Hwang, Y.S., Ma, M.C., Tseng, Y.M. and Tsai, W.H., 2013. Associations among perinatal factors and age of achievement of full oral feeding in very preterm infants. Pediatrics and Neonatology 54: 309-314. https://doi.org/10.1016/j.pedneo.2013.03.013

International Committee for the Classification of Retinopathy of Prematurity, 2005. The International Classification of Retinopathy of Prematurity revisited. Archives of Ophthalmology 123: 991-997.

Jacobi, S.K. and Odle, J., 2012. Nutritional factors influencing intestinal health of the neonate. Advances in Nutrition 3: 687-696. https://doi.org/10.3945/an.112.002683

Jobe, A.H. and Bancalari, E., 2001. Bronchopulmonary dysplasia. American Journal of Respiratory and Critical Care Medicine 163: 1723-1729.

Kane, A.F., Bhatia, A.D., Denning, P.W., Shane, A.L. and Patel, R.M., 2018. Routine supplementation of *Lactobacillus rhamnosus* gg and risk of necrotizing enterocolitis in very low birth weight infants. Journal of Pediatrics 195: 73-79. https://doi.org/10.1016/j.jpeds.2017.11.055

Lad, E.M., Hernandez-Boussard, T., Morton, J.M. and Moshfeghi, D.M., 2009. Incidence of retinopathy of prematurity in the United States: 1997 through 2005. American Journal of Ophthalmology 148: 451-458. https://doi.org/10.1016/j.ajo.2009.04.018

Lin, H.C., Hsu, C.H., Chen, H.L., Chung, M.Y., Hsu, J.F., Lien, R.I., Tsao, L.Y., Chen, C.H. and Su, B.H., 2008. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. Pediatrics 122: 693-700. https://doi.org/10.1542/peds.2007-3007

Mahieu, L.M., De Muynck, A.O., De Dooy, J.J., Laroche, S.M. and Van Acker, K.J., 2000. Prediction of nosocomial sepsis in neonates by means of a computer-weighted bedside scoring system (NOSEP score). Critical Care Medicine 28: 2026-2033.

Meyer, M.P. and Alexander, T., 2017. Reduction in necrotizing enterocolitis and improved outcomes in preterm infants following routine supplementation with *Lactobacillus* GG in combination with bovine lactoferrin. Journal of Neonatal-Perinatal Medicine 10: 249-255. https://doi.org/10.3233/NPM-16130

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., the PRISMA Group, 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 339: b2535. https://doi.org/10.1136/bmj.b2535

Murguia-Peniche, T., Mihatsch, W.A., Zegarra, J., Supapannachart, S., Ding, Z.Y. and Neu, J., 2013. Intestinal mucosal defense system, part 2. Probiotics and prebiotics. Journal of Pediatrics 162: S64-S71. https://doi.org/10.1016/j.jpeds.2012.11.055

Narbona, E., Uberos, J., Armada, M.I., Couce, M.L., Rodriguez, G. and Saenz de Pipaon, M., 2014. Nutrition and Metabolism Group of the Spanish Neonatology Society: recommendations and evidence for dietary supplementation with probiotics in very low birth weight infants. Anales de Pediatría 81: 397-398. https://doi.org/10.1016/j.anpedi.2014.06.020

Oh, N.S., Joung, J.Y., Lee, J.Y. and Kim, Y., 2018. Probiotic and anti-inflammatory potential of *Lactobacillus rhamnosus* 4B15 and *Lactobacillus gasseri* 4M13 isolated from infant feces. PLoS ONE 13: e0192021. https://doi.org/10.1371/journal.pone.0192021

Papile, L.A., Burstein, J., Burstein, R. and Koffler, H., 1978. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. Journal of Pediatrics 92: 529-534.

Samuels, N., Van de Graaf, R., Been, J.V., De Jonge, R.C., Hanff, L.M., Wijnen, R.M., Kornelisse, R.F., Reiss, I.K. and Vermeulen, M.J., 2016. Necrotising enterocolitis and mortality in preterm infants after introduction of probiotics: a quasi-experimental study. Scientific Reports 6: 31643. https://doi.org/10.1038/srep31643

Uberos, J., Aguilera-Rodriguez, E., Jerez-Calero, A., Molina-Oya, M., Molina-Carballo, A. and Narbona-Lopez, E., 2017. Probiotics to prevent necrotising enterocolitis and nosocomial infection in very low birth weight preterm infants. British Journal of Nutrition 117: 994-1000. https://doi.org/10.1017/S0007114517000769

Zozaya, C., Garcia Gonzalez, I., Avila-Alvarez, A., Oikonomopoulou, N., Sanchez Tamayo, T., Salguero, E., Saenz de Pipaon, M., Garcia-Muñoz Rodrigo, F. and Couce, M.L., 2020. Incidence, treatment, and outcome trends of necrotizing enterocolitis in preterm infants: a multicenter cohort study. Frontiers in Pediatrics 8: 188.