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Probiotics to prevent necrotising enterocolitis and nosocomial infection in very low birth weight preterm infants

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Abstract

The aim of the study was to determine whether routine probiotic supplementation (RPS) with *Lactobacillus rhamnosus* GG (*LGG*) or *Lactobacillus acidophilus* + *Lactobacillus bifidum* is associated with reduced risk of necrotising enterocolitis (NEC) ≥ Stage II in preterm neonates born at ≤32 weeks' gestation. We conducted a retrospective cohort study on the effect of probiotic supplementation in very low birth weight infants in our neonatal unit by comparing two periods: before and after supplementation. The incidence of NEC ≥ Stage II, late-onset sepsis and all-cause mortality was compared for an equal period 'before' (Period I) and 'after' (Period II) RPS with *LGG* or *L. acidophilus* + *L. bifidum*. Multivariate logistic regression analysis was conducted to adjust for relevant confounders. The study population was composed of 261 neonates (Period I *v.* II: 134 *v.* 127) with comparable gestation duration and birth weights. In <32 weeks, we observed a significant reduction in NEC ≥ Stage II (11·3 *v.* 4·8 %), late-onset sepsis (16 *v.* 10·5 %) and mortality (19·4 *v.* 2·3 %). The benefits in neonates aged ≤27 weeks did not reach statistical significance. RPS with *LGG* or *L. acidophilus* + *L. bifidum* is associated with a reduced risk of NEC ≥ Stage II, late-onset sepsis and mortality in preterm neonates born at ≤32 weeks' gestation.

Key words: Probiotics: *Lactobacillus rhamnosus* GG: *Lactobacillus acidophillus*: *Lactobacillus bifidum*: Necrotising enterocolitis: Very low birth weight infants: Late-onset sepsis

Necrotising enterocolitis (NEC) is the most common gastrointestinal pathology in very low birth weight (VLBW) infants. It is associated with neurodevelopmental disorders[1] and an increase of 10–30 % in related mortality[2]. Strategies for preventing preterm birth and its consequences, including the use of antenatal steroids, have had very limited effect in reducing the risk of NEC, although a notable impact on lung immaturity has been reported[3]. Preferential breast-feeding and the fact that most neonatal units have developed standardised nutrition protocols have been epidemiologically associated with a reduced risk of NEC[4].

Bacteria from human milk are among the first to colonise the intestine of the infant, preventing the establishment and proliferation of pathogenic bacteria, promoting the development of innate immunity and, therefore, reducing the risk of NEC[5]. In special situations, such as VLBW infants admitted to intensive care units, there may be low levels of colonisation by *Bifidobacterium* and *Lactobacillus*, with intestinal microflora being modified towards the higher levels of *Klebsiella*, *Enterobacter*, *Citrobacter* and *Pseudomonas* that are commonly encountered in hospitals[6].

The stability of the neonatal intestine ecosystem depends on interbacterial cooperation and on the availability of a source of nutrients that is constant in composition and quantity. The bacterial members of indigenous microflora may be modulated by the varying composition of ingested nutrients. The administration of antibiotics to neonates upsets the balance of intestinal flora and may predispose them to episodes of infectious disease. In such cases, according to the available evidence[7], the administration of probiotics can restore the balance of intestinal flora.

Each probiotic strain of a species may have unique properties and different physiological functions. Opinions vary as to the optimum dosages of probiotics. The dosages on which there is greatest consensus include *Lactobacillus rhamnosus* GG (*LGG*); Lactobacillus *acidophilus* and *Bifidobacterium infantis*[8]. Recent systematic reviews on the use of probiotics in VLBW neonates have reported an OR for enterocolitis of 0·32 (95 % CI 0·17, 0·60) and that for death of 0·43 (95 % CI 0·25, 0·75). In view of these findings, the use of probiotics has become a generalised recommendation for this group of infants[9]. The Spanish Society of Neonatology, through its Neonatal

Abbreviations: *LGG*, *Lactobacillus rhamnosus* GG; NEC, necrotising enterocolitis; VLBW, very low birth weight.

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Metabolism and Nutrition Group, has issued a series of recommendations in this respect[10].

The aim of this study was to determine whether routine probiotic supplementation (RPS) with *LGG* or *L. acidophilus* + *Lactobacillus bifidum* was associated with a reduced risk of NEC ≥ Stage II[11] in preterm neonates born at ≤32 weeks' gestation. We hypothesised that the introduction of RPS would significantly reduce NEC ≥ Stage II.

Methods

A retrospective cohort study was designed, comparing two periods – before and after the introduction of probiotic supplementation – for VLBW infants at the Neonatal Intensive Care Unit (NICU) at our hospital (Period I: November 2010–August 2013; Period II: December 2013–July 2016).

Ethical considerations

Nutritional supplementation with probiotics for VLBW neonates came into routine practice following the publication of guidelines in this respect by the Spanish Society of Neonatology, through its Neonatal Metabolism and Nutrition Group[10]. The protocol was approved by the Ethics Committee of the Hospital and all current regulations regarding data confidentiality were complied with.

Criteria for inclusion and exclusion

The study cohort included all newborns with a gestational age ≤32 weeks and/or birth weight ≤1500 g. We differentiated those who were 27–32 weeks (which is the primary focus of the data) from those who were <27 weeks. We excluded infants with severe congential anomalies and especially those with gastrointestinal conditions.

Sample size and power

The prevalence of NEC in Spain is estimated at 7·5 % of VLBW infants[12]. For the present study, assuming an α error of 5 % and a power of 90 %, 259 infants are required.

Primary outcome

The primary outcome was incidence of NEC ≥ Stage II[11].

Secondary outcomes

Secondary outcomes were death, by any cause, late-onset sepsis with positive blood culture >72 h after admission (two positive blood cultures for *Staphylococcus epidermidis*), age at which full enteral feeding is achieved (120 ml/kg per d) and days of parenteral nutrition. All outcomes were monitored until discharge or death during initial hospitalisation. The diagnosis of pneumatosis intestinalis by the attending neonatologist was verified independently by the radiologist on call. In case of disagreement, consensus was reached by group discussion between the neonatal and radiology teams during the weekly rounds, and the final diagnosis was then used for coding in the database.

Enteral and parenteral nutrition

Enteral and parenteral nutrition was provided in accordance with the recommendations of the Nutrition and Metabolism Group of the Spanish Neonatology Society[10] and the standard protocol of the hospital's Neonatal Unit. Donor breast milk was not available for routine use.

In accordance with the above, all clinically stable newborns were given trophic feeding with breast milk (or otherwise, formula, for premature neonates) at 1 ml/kg every 3 h, from the 1st day of life. Enteral nutrition was subsequently increased, as tolerated, at a rate of 15–25 ml/kg per d until full enteral nutrition was reached. Consideration was given to fortifying the breast milk after reaching feeding volumes exceeding 80 ml/kg per d; this fortification protocol did not change in Periods I and II. Tolerance to feeding and the presence or absence of bloating were recorded daily. The standard protocol of feeding did not change in the 6 years included in the study.

Protocol for the administration of probiotics

Two commercial presentations of probiotics were used (they were the products used in the Neonatal Unit at the specified times), with the following dosages: (a) Bivos® (Ferring) containing *LGG* (ATCC 53103) (10^9 colony-forming units (CFU)) – a daily dose of nine drops every 24 h was dissolved in 2 ml of (breast or formula) milk and supplied by nasogastric tube[13]; (b) Infloran® (Berna Biotech) 250 mg capsules containing 10^9 CFU *L. acidophilus* (ATCC 4356) and 10^9 CFU *Bifidobacterium bifidum* (ATCC 15696)[14] – a daily dose of one capsule every 12 h was dissolved in 2 ml of (breast or formula) milk and supplied by nasogastric tube, according to the protocol of our Unit, also used by other authors[15]. Probiotic supplementation was started at the first enteral feed of at least 1 ml/bolus and was continued until 35 weeks postmenstrual age or until discharge from the NICU.

Statistics

Study data were recorded in the e-Health record and in the Neosoft® (Spanish Society of Neonatology) program. The descriptive data were summarised using medians and interquartile ranges for continuous values and frequency distribution for categorical variables. Univariate comparisons for continuous variables were performed using the Mann–Whitney test and by the χ^2 test for categorical variables. Values for risk of NEC, mortality and late-onset sepsis were obtained by multiple logistic regression analysis, adjusting for gestational age ≤27 weeks or intra-uterine growth restriction (IUGR: birth weight <10th centile for gestation). Characteristics that differed between study periods and other parameters considered to influence neonatal outcomes (e.g. maternal antenatal antibiotics) were also assessed during modelling. The effects of the study periods were summarised as adjusted OR with 95 % CI. The analysis was conducted on all neonates with <32 weeks' gestation, and in a subset of neonates with gestational age ≤27 weeks, who were at a higher risk of NEC. The analysis was performed using IBM SPSS 20.0 for Windows (IBM).

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Reporting

The Strengthening the Reporting of Observational Studies in Epidemiology checklist for reporting observational studies was used[16].

Results

A total of 461 newborns were admitted to the NICU at our hospital during the two periods considered. Of these infants, 261 were <32 weeks' gestational age and/or <1500 g birth weight (Period I *v.* II: 134 *v.* 127) (Fig. 1). Probiotics, in either of the two commercial formulations used, were given to eighty-six newborns during Period II. Period II infants who were not dependent on O_2, with birth weight close to 1500 g, without antibiotic or infectious risk factors did not receive probiotic supplementation, according to the protocol of our neonatal unit. A total of 20/259 (7·7 %) newborns in Period I and 16/226 (7·0 %) in Period II died. Causes of death included extreme prematurity, brain defects, sepsis and HIV.

Their gestational ages and birth weights were comparable (Table 1). The use of antenatal maternal antibiotics (ampicillin or erythromycin) and the number of births with gestational age ≤27 weeks were comparable in the two periods considered. In all, 84·3 and 85·8 % (Period I *v.* Period II) of the mothers received antenatal steroids, and the rate of twin births (36·6 and 39·4 % in Periods I and II, respectively) was also comparable between the two periods. We also did not observe differences in the days of umbilical channeling between Periods I and II. During Period II, the median birth weight was slightly higher than that recorded during Period I, although within the limits of statistical significance. During Period II, fewer hours of O_2 therapy were supplied; although the difference is not statistically significant, this did result in a statistically significant decrease in episodes of mild bronchopulmonary dysplasia and retinopathy of prematurity Stages I and II (Table 1)[17]. Of the VLBW infants with NEC ≥ Stage II, eleven received breast-feeding compared with nine who received formula milk for premature infants; there were no statistically significant differences.

Outcomes for neonates 27–32 weeks

Although mortality was slightly lower in Period II (14·9 *v.* 12·6 %), there was a very significant difference between newborns who received probiotics and those who did not (10·6 *v.* 2·4 %, without probiotics *v.* with probiotics) after adjusting for IUGR, late-onset sepsis and intraventricular haemorrhage (Table 2). NEC ≥ Stage II among the infants who received probiotics decreased significantly (5·3 *v.* 1·4 %, without probiotics *v.* with probiotics), which highlights the protective effect of probiotics, after adjustment for IUGR and ventilatory support. Likewise, NEC stage IV decreases among VLBW infants receiving probiotics, although without achieving significant differences (Tables 2 and 3). Similar effects were observed for late-onset sepsis; in this case too, probiotics exerted a protective effect, after adjustment for IUGR, ventilatory support and days of admission to the NICU. No significant differences were observed in the age of achieving full enteral nutrition, in the days of parenteral nutrition administered (Table 2) or in breast milk nutrition between the study periods (Table 1).

The incidence of patent ductus arteriosus (left atrium:aortic root ratio >1·4 or ductal diameter >1·5 mm with a left–right shunt) and the proportion of those who needed treatment did not differ between the two study periods (Table 1).

Outcomes for neonates ≤27 weeks

In all, 32·4 and 34·5 % of the infants with a gestational age ≤27 weeks died during Periods I and II. However, among those who received probiotics, the figure was significantly lower (45·5 *v.* 5·3 %) (Table 2). At the limits of statistical significance, the rates of NEC ≥ Stage II also decreased (20·5 *v.* 15·8 %) (Table 2). We observed no difference between Periods I and II, or between infants who received or did not receive supplementation with probiotics, as regards the age of achieving full enteral nutrition or the days of parenteral nutrition (Table 2).

Outcomes for Lactobacillus rhamnosus *GG* and Lactobacillus bifidum + Lactobacillus acidophilus

A total of fifty-three VLBW newborns received *LGG* and thirty-three received the combination of *L. bifidum* + *L. acidophilus*, in accordance with the dosing schedule described in the 'Methods' section. Tolerance was similar in both groups, and no side effects related to administration of the probiotics were recorded. Although our comparison of the two groups revealed no

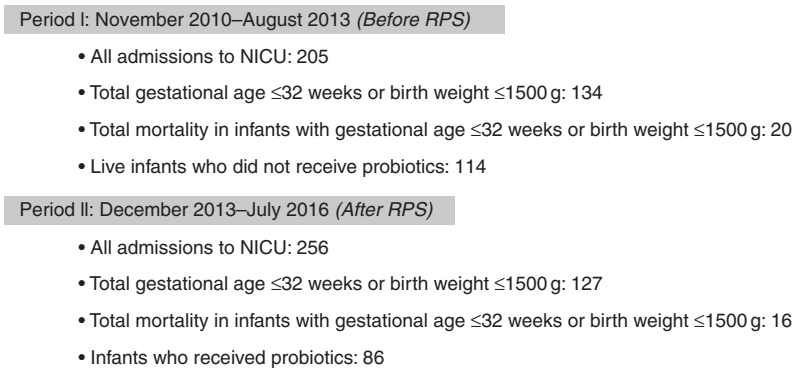

Fig. 1. Patient flow diagram. RPS, routine probiotic supplementation; NICU, Neonatal Intensive Care Unit.

Table 1. Pregnancy and neonatal characteristics
(Numbers and percentages; medians and interquartile ranges (IQR))

	Period I (*n* 134)		Period II (*n* 127)		
Characteristics	*n*	%	*n*	%	*P*
Maternal					
PIH	8	6·0	7	5·5	0·32
Chorioamnionitis	24	17·9	16	12·6	0·23
Antibiotics	54	40·3	59	46·5	0·31
Glucocorticoids	113	84·3	109	85·8	0·73
PPROM	32	23·9	29	22·8	0·84
Gestation (weeks)					0·06
Median	29		30		
IQR	27–31		28–32		
Gestation ≤27 weeks	34	25·4	29	22·8	0·63
Twin birth	49	36·6	50	39·4	0·64
Mode of delivery					
Caesarean section	106	79·1	98	77·2	0·70
Vaginal	28	20·9	29	22·8	
Neonatal					
Birth weight (g)					0·05
Median	1157		1291		
IQR	995–1408		910–1477		
Male	73	54·5	79	62·2	0·20
Apgar <7 at 5 min	39	32·0	41	35·0	0·61
IUGR	31	23·1	24	24·0	0·87
Umbilical channeling (d)					0·64
Median	3·5		4·0		
IQR	0·25–6·0		0–6·0		
Respiratory support					
O_2	104	90·4	98	88·3	0·60
CPAP	86	74·8	86	77·5	0·63
Ventilation	61	53·0	57	51·8	0·85
Duration (h)					
O_2					0·19
Median	552		336		
IQR	168–1104		96–1008		
CPAP					0·61
Median	72		72		
IQR	0–192		48–126		
Ventilation					0·93
Median	24		24		
IQR	0–114		0–120		
Bronchopulmonary dysplasia					
Mild	24	20·9	13	11·9	0·03
Moderate	17	14·8	14	12·8	0·33
Severe	11	9·6	6	5·5	0·13
PDA	24	17·9	16	12·7	0·24
Treated	12	9	12	9·5	0·89
IVH					
Grade I–II	18	13·5	11	9·1	0·20
Grade III–IV	14	10·5	7	5·8	0·13
ROP					
Stage I–II	15	12·0	2	1·6	0·001
Stage III	9	7·6	5	4	0·23
Early onset sepsis	15	11·2	13	10·2	0·80
Milk breast-feeding	84	63	69	55	0·12
Length of NICU stay (d)					0·66
Median	23·5		24		
IQR	13·7–36		12–35·5		

PIH, pregnancy-induced hypertension; PPROM, preterm pre-labour rupture of membranes; IUGR, intra-uterine growth restriction; CPAP, continuous positive airway pressure; PDA, patent ductus arteriosus; IVH, intraventricular haemorrhage; ROP, retinopathy of prematurity; NICU, Neonatal Intensive Care Unit.

significant differences regarding mortality, NEC or late-onset sepsis, mortality fell and late-onset sepsis was present among those who received the *L. bifidum* + *L. acidophilus* combination. The small number of cases available for the subgroup analysis made this question hard to resolve (Table 3).

Safety

There were no cases of late-onset sepsis or the presence of NEC (in any grade) related to the administration of probiotics.

Table 2. Outcomes for neonates
(Numbers and percentages; odds ratios and 95 % confidence intervals; medians and interquartile ranges (IQR))

	Without probiotics		With probiotics				
	n	%	*n*	%	OR	95 % CI	*P*
Neonates 27–32 weeks							
Cases (*n*)	131		67				
Mortality*	14	10·6	1	1·4	0·098	0·015, 0·619	0·014
NEC ≥ Stage II†	7	5·3	1	1·4	0·205	0·048, 0·880	0·033
NEC Stage IV†	3	2·3	1	1·4		–	–
Late-onset sepsis‡	19	14·5	5	7·4	0·334	0·125, 0·894	0·029
Age at full feeds (d)							0·639
Median	12		10				
IQR	8–17		6–16				
Parenteral nutrition (d)							0·504
Median	11		10				
IQR	6–16		7–15				
Neonates ≤ 27 weeks							
Cases (*n*)	44	19					
Mortality*	20	45·5	1	5·3	0·081	0·009, 0·694	0·022
NEC ≥ Stage II†	9	20·5	3	15·8	0·167	0·025, 1·133	0·067
NEC Stage IV†	2	4·5	0	0		–	–
Late-onset sepsis‡	9	20·5	4	21·5	0·388	0·082, 1·835	0·232
Age at full feeds (d)						–	0·851
Median	25		26				
IQR	15–37		16–38				
Parenteral nutrition (d)						–	0·151
Median	21		25				
IQR	10–33		18–38				

NEC, necrotising enterocolitis; IUGR, intra-uterine growth restriction; CPAP, continuous positive airway pressure.
* Adjusted for IUGR, early onset sepsis, intraventricular haemorrhage.
† Adjusted for IUGR, CPAP, O_2 support.
‡ Adjusted for IUGR, CPAP, O_2 support and Neonatal Intensive Care Unit stay.

Table 3. Outcomes for *Lactobacillus rhamnosus* GG (*LGG*) or *Lactobacillus bifidum* + *Lactobacillus acidophilus*
(Numbers and percentages; medians and interquartile ranges (IQR))

	LGG		*L. bifidum* + *L. acidophilus*		
	n	%	*n*	%	*P*
Cases (*n*)	53		33		
Mortality	2	3·8	0	0	0·262
NEC ≥ Stage II	2	3·8	2	6·1	0·794
NEC Stage IV	1	1·8	0	0	0·730
Late-onset sepsis	7	13·2	2	6·1	0·295
Age at full feeds (d)					0·714
Median	11·5		10		
IQR	7·5–20		8–17		
Parenteral nutrition (d)					0·587
Median	13		11·5		
IQR	7–21		7–20·8		

NEC, necrotising enterocolitis

Discussion

Our results show that RPS for VLBW infants with *LGG* or *L. bifidus* + *L. acidophilus* is associated with a lower frequency of NEC ≥ Stage II, fewer episodes of nosocomial sepsis and lower mortality. RPS in infants with a gestational age ≤27 weeks revealed a significant decrease in mortality only in this subgroup.

Necrotising enterocolitis and death

From an epidemiological standpoint, NEC is related to prematurity (it is inversely proportional to gestational age), enteral nutrition (taking into account the daily volume of enteral feeding, the comparison between breast milk and formula, and the osmolarity of first food) and intestinal colonisation by pathogenic flora (*Escherichia coli*, *Klebsiella*, *Clostridium perfringens*, *S. epidermidis* and *Rotavirus*). Fernández-Carrocera *et al.*[18], in a randomised double-blind clinical trial, to evaluate the efficacy of a multispecies probiotic, which included strains of *L. acidophilus* and *B. bifidum*, observed no significant reduction in the risk of NEC (at any stage), although the risk of death was significantly reduced. Lin *et al.*[19], used a multicentre, randomised, double-blind clinical trial and, as in our study, observed a significant reduction in the risk of NEC ≥ Stage II or death, using a

combination of *L. acidophilus* and *B. bifidum*. In a quasi-experimental trial, Samuels *et al.*[20] observed a protective effect of *L. acidophilus* and *B. bifidum* against NEC and death in breast-fed VLBW infants and noted the low frequency of breast-feeding as the sole method. In our study, the same combination of probiotics was observed to have a protective effect against NEC and death, although here too the rate of use of breast-feeding was much lower than is desirable and below that reported elsewhere[21]. In a systematic review, Baucells *et al.*[22] reported that the best protective effects against NEC and death were obtained with the use of the combination of *L. acidophilus* and *B. bifidum*.

A systematic review by Bernardo *et al.*[8], of eleven clinical trials involving 2887 patients, evaluated the benefits of using probiotics as a preventive against NEC and other morbidities associated with prematurity. Wang *et al.*[23], in a meta-analysis of twenty randomised clinical trials with a total of 3816 preterm VLBW infants, observed a decreased risk of NEC in those treated with probiotics (relative risk (RR) 0·33; 95 % CI 0·24, 0·46) and a decreased risk of death (RR 0·56; 95 % CI 0·43, 0·73). The authors did not find probiotic treatment to modify the risk of sepsis (RR 0·90; 95 % CI 0·71, 1·15). In this systematic review, although diverse probiotics were used in the different trials, most used a combination of *L. acidophilus* and *B. bifidus*.

In our study, in patients supplemented with probiotics, the incidence of NEC, mortality and late-onset sepsis is less than the Vermont Oxford international database/benchmark for the VLBW data set (NEC 3·6 %, mortality 3·7 %) and the Spanish data for NEC (10 %)[24]

Late-onset sepsis

According to other authors[25], a combination of low-dose probiotics decreases the frequency of late-onset sepsis, and thus would be the preferred approach, rather than single strains. In our view, the choice of strain is of vital importance. In this respect, Jacobs *et al.*[26], using a combination of *B. infantis*, *Streptococcus thermophilus* and *Bifidobacterium lactis* in VLBW infants, observed no protective effect against late-onset sepsis, although there was some protection against NEC ≥ Stage II. In our study, the subgroup analysis (Table 3), although lacking statistical power, suggested that fewer complications of late-onset sepsis occurred after supplementation with *L. acidophilus* and *B. bifidum* in comparison with supplementation with *LGG* alone.

Safety

In 2004, European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommended the use of probiotics in dietary supplementation for children, but until a few years ago there were doubts about the safety of this type of nutritional supplement in VLBW infants, despite the fact that experimental studies showed them to be effective in reducing mortality and the incidence of NEC[27]. Manzoni *et al.*[13], in a cohort study of 743 VLBW infants, concluded that *LGG* at a daily dose of 3×10^9 CFU during the first 4–6 weeks of life is safe and well-tolerated.

Newborns with a gestational age ≤27 weeks would benefit most from probiotic supplementation. These infants are at greatest risk of developing enterocolitis and, moreover, have a less mature immune system. Although the data available are still insufficient, clinical trials and meta-analyses have already provided sufficient evidence of their usefulness and safety[8]. In our own research, the subgroup analyses only revealed a statistical decrease in mortality after supplementation with probiotics.

Only isolated episodes have been reported of sepsis or bacteraemia related to the strains of probiotics administered in high-risk patients – specifically after nutritional supplementation with *LGG*[28,29] – but to date no references exist concerning the development of sepsis or bacteraemia related to *L. acidophilus* or *B. bifidum*.

Selection of the appropriate strain or strains would avoid possibly harmful side effects, and enable researchers to focus on the prevention of harmful metabolic activities, systemic infections and adverse effects on immunomodulation and gene transfer[30]. In the coming years, it will be necessary to select and study new strains with a better safety profile, rather than others, which, in view of the results obtained, appear to be less safe. It may also be of interest to investigate the efficacy of probiotics along with other protective factors in the prophylaxis of NEC such as donated milk[31] or lactoferrin[32].

Conclusions

We can conclude from our observations that probiotic supplementation may be indicated in preterm infants of 27–32 weeks of gestational age to reduce mortality, NEC and late sepsis. In preterm infants <27 weeks of gestational age, more studies are needed.

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J. U. designed the study and was the study coordinator; J. U. and E. N.-L. carried out the study; E. A.-R. and M. M.-O. analysed the data; E. A.-R., A. J.-C. and A. M.-C. wrote the article.

None of the authors has any conflicts of interest to declare.

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