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Review

# Probiotics and Time to Achieve Full Enteral Feeding in Human Milk-Fed and Formula-Fed Preterm Infants: Systematic Review and Meta-Analysis

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Received: 13 June 2016; Accepted: 26 July 2016; Published: 30 July 2016

**Abstract:** Probiotics have been linked to a reduction in the incidence of necrotizing enterocolitis and late-onset sepsis in preterm infants. Recently, probiotics have also proved to reduce time to achieve full enteral feeding (FEF). However, the relationship between FEF achievement and type of feeding in infants treated with probiotics has not been explored yet. The aim of this systematic review and meta-analysis was to evaluate the effect of probiotics in reducing time to achieve FEF in preterm infants, according to type of feeding (exclusive human milk (HM) vs. formula). Randomized-controlled trials involving preterm infants receiving probiotics, and reporting on time to reach FEF were included in the systematic review. Trials reporting on outcome according to type of feeding (exclusive HM vs. formula) were included in the meta-analysis. Fixed-effect or random-effects models were used as appropriate. Results were expressed as mean difference (MD) with 95% confidence interval (CI). Twenty-five studies were included in the systematic review. In the five studies recruiting exclusively HM-fed preterm infants, those treated with probiotics reached FEF approximately 3 days before controls (MD −3.15 days (95% CI −5.25/−1.05), $p = 0.003$). None of the two studies reporting on exclusively formula-fed infants showed any difference between infants receiving probiotics and controls in terms of FEF achievement. The limited number of included studies did not allow testing for other subgroup differences between HM and formula-fed infants. However, if confirmed in further studies, the 3-days reduction in time to achieve FEF in exclusively HM-fed preterm infants might have significant implications for their clinical management.

**Keywords:** probiotics; preterm infants; human milk; full enteral feeding; systematic review

## 1. Introduction

Nutrition during critical time windows in early life can affect long-term health [1]. Early provision of optimal enteral nutrition to preterm infants might improve neurodevelopmental outcome by decreasing the rate of several complications of prematurity, such as extrauterine growth restriction, necrotizing enterocolitis (NEC), sepsis, bronchopulmonary dysplasia, and retinopathy of prematurity [2].

Late introduction and slow advancement of enteral feeding may alter gastrointestinal motility and disrupt microbial colonization [3], leading to a delay in establishing full enteral feeding (FEF). The consequent prolonged need for parenteral nutrition can have serious infectious and metabolic complications, which might prolong hospital stay, increase morbidity and mortality, and affect growth and development [4].

Several clinical variables and interventions have been proposed as predictors of the time to FEF achievement in preterm and very-low-birth-weight (VLBW) infants. Among these variables, the influence of type of feeding was also documented, as FEF achievement was delayed in formula-fed infants compared to human milk (HM)-fed infants [5].

Recently, probiotic use has been associated with a reduced time to achieve FEF and better feeding tolerance [6], as well as a reduction of NEC [7,8] and late-onset sepsis [9]. Probiotics are live microorganisms which, when ingested in adequate amounts, confer a health benefit to the host, by modifying the composition and function of gut microbiota and the immunological responses in the host [10]. The role of probiotics in attaining a more rapid achievement of FEF could be related to their favorable effect on the physiological intestinal dysbiosis of preterm infants [11], which is the result of the exposure to a unique environment and to several iatrogenic manipulations, such as broad spectrum antibiotics [12]. It is well known that gut microbiota in HM-fed infants is different compared to formula-fed infants [13]; data from an observational study also suggest a feeding-dependent effect of probiotics, as in that study NEC incidence was reduced in infants treated with probiotics and receiving HM, but not in those exclusively formula-fed [14]. However, the relationship between probiotics and type of feeding in attaining a more rapid achievement of FEF has not been explored yet, even in the most recent meta-analysis on this topic [6].

Thus, the aim of the present paper was to evaluate the effect of probiotics on time to FEF achievement according to type of feeding (exclusive HM vs. formula), by performing a systematic review and meta-analysis of currently available literature on this topic.

## 2. Materials and Methods

### *2.1. Literature Search*

The study protocol was designed by the members of the Task Force on Probiotics of the Italian Society of Neonatology. PRISMA guidelines [15] were followed in order to perform a systematic review of published studies reporting the relationship between probiotic use and time to FEF achievement in preterm infants according to type of feeding.

In order to be included in the meta-analysis, studies had to meet the following inclusion criteria: randomized or quasi-randomized clinical trials involving preterm infants (gestational age (GA) <37 weeks) who received, within one month of age, any probiotic compared to placebo or no treatment, and reporting on type of feeding. The outcome of interest was time for FEF achievement (any definition). Only English-written studies and studies involving humans were included in the meta-analysis.

A search was conducted for studies published before 2 March 2016 in PubMed [16], the Cochrane Library [17], and Embase [18]. The following search string was used for the PubMed search: ((preterm infant OR pre-term infant) OR (preterm infants OR pre-term infants) OR (preterm neonate OR pre-term neonate) OR (preterm neonates OR pre-term neonates) OR (preterm newborn OR pre-term newborn) OR (preterm newborns OR pre-term newborns) OR (premature infant OR premature infants) OR (premature neonate OR premature neonates) OR (premature newborn OR premature newborns) OR infant, extremely premature (MeSH Heading (MH)) OR premature birth (MH) OR infant, low birth weight (MH) OR infant, very low birth weight (MH)) AND (full enteral* OR feed*) AND (probiotic OR probiotics OR pro-biotic OR pro-biotics OR probio*)) NOT (animals (MH) NOT humans (MH).

The string was built up by combining all the terms related to probiotics and FEF achievement: PubMed MeSH terms, free-text words, and their combinations obtained through the most proper Boolean operators were used. The same criteria were used for searching the Cochrane Library and Embase.

Arianna Aceti and Luigi Corvaglia performed the literature search: relevant studies were identified from the abstract; full-texts of relevant studies were examined, as well as their reference lists in order to identify additional studies.

### 2.2. Data Extraction and Meta-Analysis

Study details (population, characteristics of probiotic and placebo, type of feeding, and outcome assessment) were evaluated independently by Arianna Aceti and Luigi Corvaglia, and checked by Davide Gori. Study quality was evaluated independently by Arianna Aceti and Davide Gori using the risk of bias tool as proposed by the Cochrane collaboration (Chapter 8 of the Cochrane Handbook of Systematic Reviews) [19].

The corresponding authors of the studies in which days to FEF achievement were not reported as mean ± standard deviation (SD) were contacted by email. When data were not provided, the study was not included in the meta-analysis.

The association between probiotic use and FEF achievement according to type of feeding was evaluated by a meta-analysis conducted by AA and DG using the RevMan software (Cochrane Informatics and Knowledge Management Department, version 5.3.5) downloaded from the Cochrane website [20]. Mean difference (MD) in days to achieve FEF between infants receiving probiotics and those receiving placebo or no treatment was calculated using the inverse variance method, and reported with 95% confidence interval (CI).

For the analysis, we planned to use at first a fixed effect model. Heterogeneity was measured using the $I^2$ test: if significant heterogeneity was present ($p < 0.05$ from the $\chi^2$ test) and/or the number of studies was $\leqslant 5$, a random-effects model was used instead.

## 3. Results

### Literature Search

Overall, 372 papers were identified through the literature search, 155 in PubMed [16], 73 in the Cochrane Library [17], and 144 in Embase [18].

As shown in Figure 1, 35 studies met the inclusion criteria [21–55]. Fourteen additional papers were identified from the reference lists of included studies or by "snowballing" techniques [52,56–68].

Twenty-four studies were excluded after examining the full-texts [28,29,31–33,35,42–47,51,53–55,57–59,62,63,65,69]. Twenty-five studies were then suitable for inclusion in the systematic review (Table 1) [21–27,30,34,36–41,48–50,56,60,61,64,66,68,70].

Among them, only eight studies reported FEF achievement according to type of feeding: infants were fed exclusively HM, either own mother's (OMM) or donor human milk (DHM), in six studies [22,38,50,56,60,70], while two studies reported FEF in exclusively formula-fed infants [41,61].

The corresponding authors of four of these papers were contacted by email, as data for FEF achievement were not suitable for inclusion in the meta-analysis: mean ± SD of days for FEF achievement were provided for one study [22], while data were unavailable for three studies [41,61,70]; these three studies were thus excluded from the meta-analysis.

Overall, five studies were included in the meta-analysis: in all these studies, infants were fed exclusively HM, either OMM or DHM (Figure 1) [22,38,50,56,60].

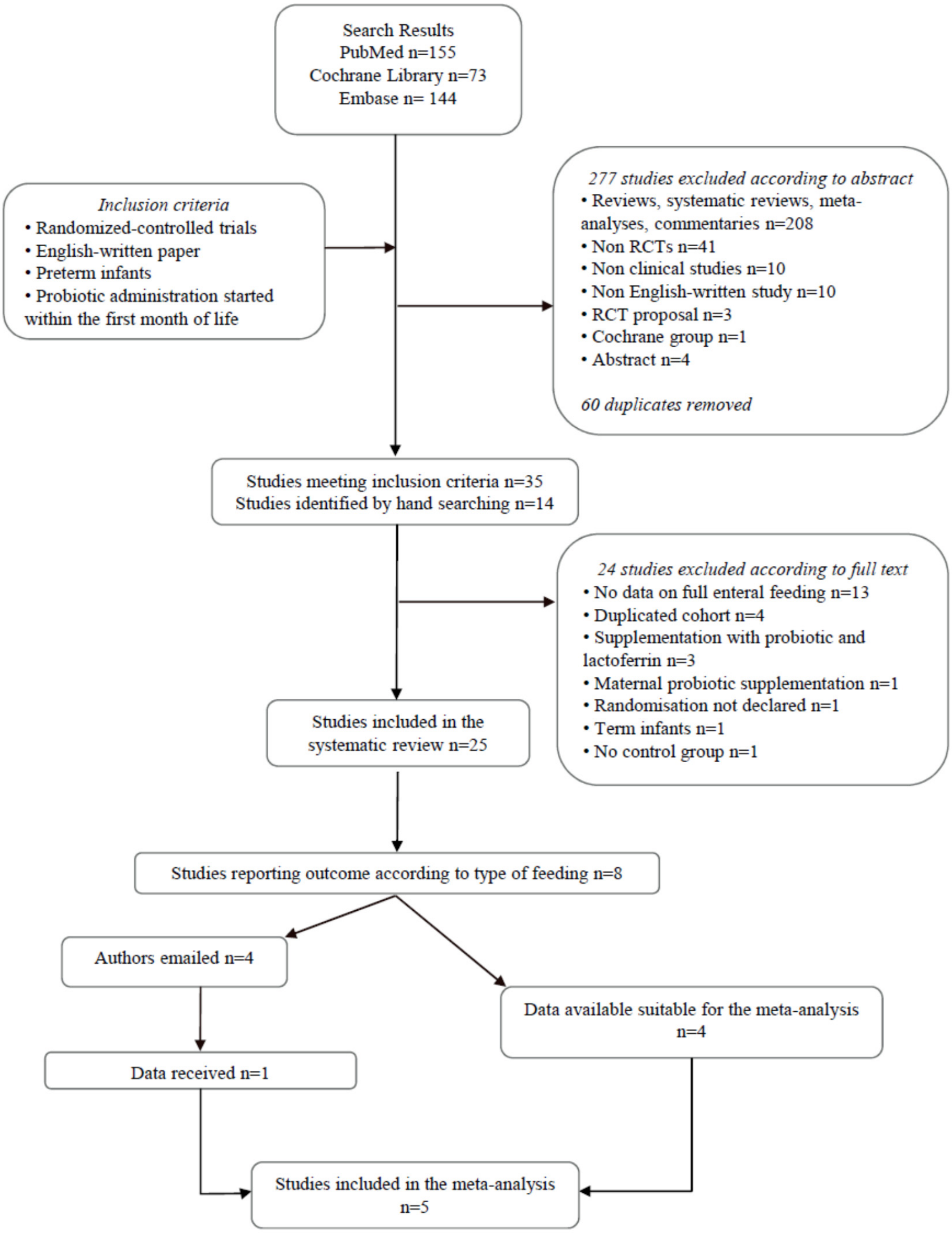


**Figure 1.** Flow chart of the search strategy used for the systematic review. The relevant number of papers at each point is given.

**Table 1.** Studies included in the systematic review.

| Author, Year | Study Details | Study Population | Intervention Specie / Dose (D) / Start of Treatment (S) / End of Treatment (E) | Milk | Placebo | FEF Definition |
|---|---|---|---|---|---|---|
| Bin-Nun, 2005 [40] | P | Preterm infants with BW < 1500 g, who began enteral feeding on a weekday | *B. infantis, Str. thermophilus, B. bifidus* | OMM, PFM | HM or FM | 100 mL/kg/day |
| | B | | D: 0.35 × $10^9$ CFU each, OD | | | |
| | R | | S: start of enteral feeding | | | |
| | C | | E: 36 w postconceptual age | | | |
| Braga, 2011 [60] | P | Inborn infants with BW 750–1499 g | *L. casei, B. Breve* | HM (± PFM from w3) | Extra HM | 150 mL/kg/day |
| | DB | | D: 3.5 × $10^7$ CFU to 3.5 × $10^9$ CFU OD | | | |
| | R | | S: day 2 | | | |
| | C | | E: day 30, NEC diagnosis, discharge, death, whichever occurred first | | | |
| Costalos, 2003 [41] | P | GA 28–32 w | *Saccharomyces boulardii* | PFM | MDX | Not defined |
| | R | No major GI problem | D: 1 × $10^9$ CFU BD | | | |
| | C | Not receiving antibiotics | S: non-specified | | | |
| | | Not receiving breast milk | Median duration of probiotic supplementation: 30 days | | | |
| Costeloe, 2015 [64] | P | Preterm infants with GA 23–30 + 6 weeks, without any lethal malformation or any malformation of the GI tract | *Bifidobacterium breve BBG-001* | OMM, DHM, FM | Corn starch powder | 150 mL/kg/day |
| | DB | | D: 8 · 3–8 · 8 $\log_{10}$ CFU/day | | | |
| | R | | S: as soon as possible after randomisation | | | |
| | C | | E: 36 w PMA or discharge if sooner | | | |
| | Multic. | | | | | |
| Demirel, 2013 [27] | P | Preterm infants with GA ⩽ 32 weeks and BW ⩽ 1500 g, who survived to feed enterally | *S. boulardii* | HM, FM | None | Not defined |
| | B | | D: 5 × $10^9$ CFU OD | | | |
| | R | | S: first feed | | | |
| | C | | E: discharge | | | |
| Dilli, 2015 [49] | P | Preterm infants with GA < 32 weeks and BW < 1500 g, born at or transferred to the NICU within the first week of life and fed enterally before inclusion | *B. lactis* | HM, FM | MDX powder | 100 mL/kg/day (FEF for hydration) |
| | DB | | D: 5 × $10^9$ CFU | | | 150 mL/kg/day (FEF for growth) |
| | R | | S: beyond d7 after birth | | | |
| | C | | E: death or discharge (max 8 weeks) | | | |
| | Multic. | | | | | |

**Table 1.** *Cont.*

| Author, Year | Study Details | Study Population | Intervention Specie<br>Dose (D)<br>Start of Treatment (S)<br>End of Treatment (E) | Milk | Placebo | FEF Definition |
|---|---|---|---|---|---|---|
| Fernández-Carrocera, 2013 [30] | P | Preterm infants with | *L. acidophilus* $1 \times 10^9$ CFU/g, *L. rhamnosus* $4.4 \times 10^8$ CFU/g, *L. casei* $1 \times 10^9$ CFU/g, *L. plantarum* $1.76 \times 10^8$ CFU/g, *B. infantis* $2.76 \times 10^7$ CFU/g, *Str. thermophilus* $6.6 \times 10^5$ CFU/g | OMM, PFM | None | Not defined |
| | DB | BW < 1500 g | Total D: 1 g powder OD | | | |
| | R | Infants with NEC stage IA and stage IB were excluded | S: start of enteral feeding | | | |
| | C | | E: non specified | | | |
| Hays, 2014 [66] | P | Preterm infants with GA 25–31 weeks, BW 700–1600 g, AGA, enteral feeding initiated before day 5 | Probiotic group composed of 3 subgroups: | OMM, DM or PFM | MDX | Not defined |
| | DB | Infants with NEC stage ⩾ IB, malformations or severe medical or surgical conditions were excluded | P1 *B. lactis* | | | |
| | R | | P2 *B. longum* | | | |
| | C | | P3 *B. lactis + longum* | | | |
| | Multic. | | D: $1 \times 10^9$ CFU each probiotic daily | | | |
| | | | Duration: 4 weeks for infants ⩾29 w/6 weeks for infants ⩽28 w GA | | | |
| Hikaru, 2010 [68] | P | Extremely low birth weight and very low birth weight infants | *B. breve* | OMM, PFM | None | Not defined |
| | R | | D: $0.5 \times 10^9$ CFU BD | | | |
| | C | | S: birth | | | |
| | | | E: discharge from NICU | | | |
| Jacobs, 2013 [25] | P | Preterm infants with GA <32 weeks and BW < 1500 g | *B. infantis BB-02* 300 CFU $\times 10^6$, *Str. thermophilus Th-4* 350 CFU $\times 10^6$, *B. lactis BB-12* 350 CFU $\times 10^6$ | HM, FM | MDX powder | Enteral feeds of 120 mL/kg for ⩾3 days |
| | DB | | Total D: $1 \times 10^9$ CFU $\times$ 1.5 g MDX powder OD | | | |
| | R | | S: enteral feed ⩾ 1 mL every 4 h | | | |
| | C | | E: discharge or term corrected age | | | |
| | Multic. | | | | | |

**Table 1.** *Cont.*

| Author, Year | Study Details | Study Population | Intervention Specie<br>Dose (D)<br>Start of Treatment (S)<br>End of Treatment (E) | Milk | Placebo | FEF Definition |
|---|---|---|---|---|---|---|
| Lin, 2008 [39] | P<br>B<br>R<br>C<br>Multic. | Preterm infants with GA < 34 weeks and BW ⩽ 1500 g, who survived to feed enterally | *L. acidophilus NCDO 1746, B. bifidum NCDO 1453* $10^9$ CFU<br>D: 1 × $10^9$ CFU each probiotic (= 125 mg/kg) BD<br>S: day 2 of age<br>Duration: 6 weeks | HM, FM | None | Oral intake of 100 mL/kg/day |
| Manzoni, 2006 [56] | P<br>DB<br>R<br>C | Infants with BW < 1500 g, ⩾3 day of life, who started enteral feeding with HM | *L. casei* subspecies *rhamnosus LGG*<br>D: 6 × $10^9$ CFU/day<br>S: day 3 of life<br>E: end of the 6th week or discharge | OMM, DM | None | Not defined |
| Mihatsch, 2010 [36] | P<br>R<br>C | Preterm infants with GA < 30 weeks and BW ⩽ 1500 g | *B. lactis BB12*<br>D: 2 × $10^9$ CFU/kg 6 times a day<br>S: start of enteral feeding<br>E: non specified | OMM, PFM | Indistinguishable powder | 150 mL/kg/day |
| Oncel, 2014 [24] | P<br>DB<br>R<br>C | Preterm infants with GA ⩽ 32 weeks and BW ⩽ 1500 g, who survived to feed enterally | *L. reuteri DSM 17938*<br>D: 1 × $10^8$ CFU OD<br>S: first feed<br>E: death or discharge | HM, FM | Oil base | Not defined |
| Patole, 2014 [23] | P<br>DB<br>R<br>C | Preterm infants with GA < 33 weeks and BW < 1500 g | *B. breve*<br>D: 3 × $10^9$ CFU OD (1.5 × $10^9$ CFU OD for newborn ⩽ 27 w until they reached 50 mL/kg/day enteral feeds)<br>S: start of enteral feed<br>E: corrected age of 37 w | HM, FM | Dextrin | 150 mL/kg/day enteral feeding |
| Rougé, 2009 [37] | P<br>DB<br>R<br>C<br>Bic. | Preterm infants with GA < 32 weeks and BW < 1500 g, ⩽2 weeks of age, without any disease other than those linked to prematurity, who started enteral feeding before inclusion | *B. longum* BB536, *L. rhamnosus* GG BB536-LGG<br>Total D: 1 × 108 CFU/day<br>S: start of enteral feeding<br>E: discharge | OMM, DM or PFM | MDX | Not defined |

**Table 1.** *Cont.*

| Author, Year | Study Details | Study Population | Intervention Specie / Dose (D) / Start of Treatment (S) / End of Treatment (E) | Milk | Placebo | FEF Definition |
|---|---|---|---|---|---|---|
| Roy, 2014 [50] | P | Preterm infants (GA < 37 weeks) and BW < 2500 g, with stable enteral feeding within 72 h of birth | *L. acidophilus* 1.25 $\times$ $10^9$ CFU $\times$ 1 g, *B. longum* 0.125 $\times$ $10^9$ CFU $\times$ 1 g, *B. bifidum* 0.125 $\times$ $10^9$ CFU $\times$ 1 g, *B. lactis* 1 $\times$ $10^9$ CFU $\times$ 1 g | HM | Sterile water | 120 mL/kg/day for $\geqslant$3 d |
| | DB | | D: half a 1 g sachet | | | |
| | R | | S: from 72 h of life | | | |
| | C | | E: after 6 w or at discharge | | | |
| Saengtawesin, 2014 [48] | P | Preterm infants with GA $\leqslant$ 34 weeks and BW $\leqslant$ 1500 g | *L. acidophilus* 1 $\times$ $10^9$ CFU, *B. bifidum* 1 $\times$ $10^9$ CFU | HM, PFM | None | 150 mL/kg/day |
| | R | | D: 125 mg/kg BD | | | |
| | C | | S: start of feeding | | | |
| | | | E: 6 w of age or discharge. | | | |
| Samanta, 2008 [38] | P | Preterm infants with GA < 32 weeks and BW < 1500 g, who started enteral feeding and survived beyond 48 h of age | *B. infantis, B. bifidum, B. longum, L. acidophilus* | HM | None | Not defined |
| | DB | | | | | |
| | R | | D: 2.5 $\times$ $10^9$ CFU each probiotic, BD | | | |
| | C | | S: start of enteral feeding | | | |
| | | | E: discharge | | | |
| Sari, 2011 [34] | P | Preterm infants with GA < 32 weeks or BW < 1500 g, who survived to feed enterally | *L. sporogenes* | HM, FM | None | Not defined |
| | B | | D: 0.35 $\times$ $10^9$ CFU OD | | | |
| | R | | S: first feed | | | |
| | C | | E: discharge | | | |
| Serce, 2013 [26] | P | Preterm infants with GA $\leqslant$ 32 weeks and BW $\leqslant$ 1500 g, who survived to feed enterally | *S. boulardii* | HM, FM | Distilled water | 100 mL/kg/day |
| | M | | D: 0.5 $\times$ $10^9$ CFU/kg BD | | | enteral feeding |
| | R | | S: non specified | | | |
| | C | | E: non specified | | | |
| Stratiki, 2007 [61] | P | Preterm infants with GA 27–32 weeks, formula-fed, without major congenital anomalies | *Bifidobacterium lactis* | FM | None | 150 mL/kg/day |
| | B | | D: 2 $\times$ $10^7$ CFU/g of milk powder | | | |
| | R | | S: start of enteral feeding | | | |
| | C | | E: not specified | | | |

**Table 1.** *Cont.*

| Author, Year | Study Details | Study Population | Intervention Specie / Dose (D) / Start of Treatment (S) / End of Treatment (E) | Milk | Placebo | FEF Definition |
|---|---|---|---|---|---|---|
| Tewari, 2015 [70] | P | Preterm infants with GA < 34 weeks | *Bacillus clausii* | OMM, DHM | Sterile water | 180 mL/kg/day |
| | DB | Excluded if: NEC, congenital anomaly , outborn and >10 days of with sepsis | D: 2.4 × $10^9$ CFU/day | | | |
| | R | Stratified as extreme preterm (GA 27–30 + 6) and very preterm (GA 31–33 + 6) | S: by day 5 in asymptomatic and by day 10 in symptomatic infants | | | |
| | C | | E: 6 weeks of age, discharge or death (whichever occurred first) | | | |
| Totsu, 2014 [21] | P | Infants with BW < 1500 g | *B. bifidum* | HM, FM | Dextrin | Postnatal day at which the amount of enteral feeding exceeded 100 mL/kg/day |
| | DB | | D: 2.5 × $10^9$ CFU, divided in two doses | | | |
| | CLR | | S: within 48 h after birth | | | |
| | C | | E: body weight 2000 g | | | |
| | Multic. | | | | | |
| Van Niekerk, 2014 [22] | P | Preterm infants with GA < 34 weeks and BW < 1250 g, exposed and non-exposed to HIV (only infants unexposed to HIV are included in the meta-analysis) | *L. rhamnosus*, *B. infantis* | HM | MCT oil | "when infants no longer required the use of IV fluids" |
| | DB | | D: 0.35 × $10^9$ CFU each probiotic | | | |
| | R | | S: start of enteral feeding | | | |
| | C | | E: day 28 postconceptual age | | | |

P: prospective; B: blinded; R: randomized; C: controlled; DB: double-blinded; Multic: multicentric; M: masked; CLR: cluster-randomized; BW: birth weight; GA: gestational age; HM: human milk; L.: Lactobacillus; B.: Bifidobacterium; Str.: Streptococcus; S.: Saccharomyces; CFU: colony forming unit; OD: once daily; NEC: necrotizing enterocolitis; BD: twice daily; OMM: own mother's milk; PFM: preterm formula; FM: formula; MDX: maltodextrin; PMA: postmenstrual age; AGA: appropriate for gestational age.

Data from 359 infants in the probiotic group and 360 infants in the control group were evaluated: probiotic use was associated with a reduction in the time for FEF achievement (MD −3.15 days (95% CI −5.25/−1.05), $p = 0.003$; Figure 2a). The funnel plot did not show any clear asymmetry (Figure 2b).

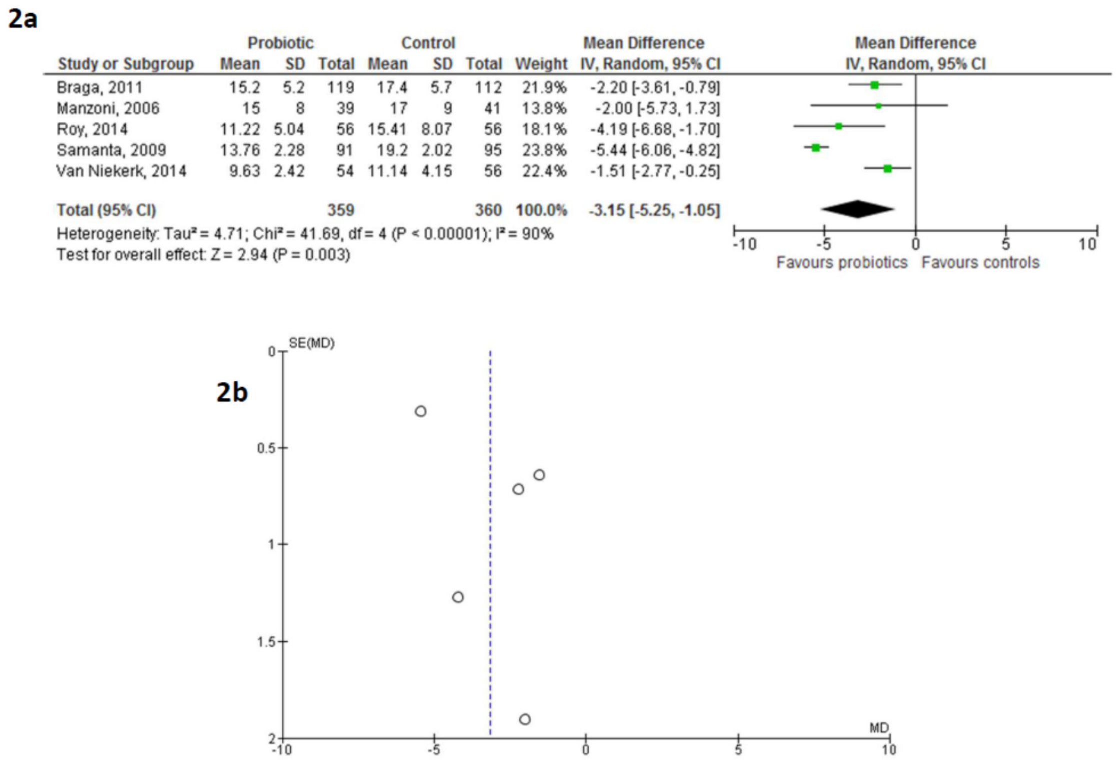


**Figure 2.** Forest plot (**2a**) and funnel plot (**2b**) showing the association between the use of probiotics and achievement of full enteral feeding in exclusively human milk-fed preterm infants. IV: inverse variance method.

Three studies were not included in the meta-analysis because data on FEF were not available as mean ± SD [41,61,70]. One study reported the use of *Bacillus clausii* in preterm infants with GA < 34 weeks, fed expressed breast milk or DHM [70] and stratified as extreme preterm (GA 27–30 + 6 weeks) and very preterm (GA 31–33 + 6 weeks). In both groups, probiotic use was associated with a reduced time to achieve FEF (risk ratio 0.82 (95% CI 0.74–0.88) and 0.67 (95% CI 0.32–0.77), respectively).

The other two studies reported probiotic use in exclusively formula-fed infants: in the study by Costalos et al., infants born at 28–32 weeks gestation and fed exclusively preterm formula received *Saccharomyces boulardii* or placebo for approximately 30 days [41]. In the study by Stratiki et al., formula-fed infants with a similar gestational age (27–32 weeks) received *Bifidobacterium lactis* vs. no treatment [61]. Neither of these two studies reported any significant difference between groups in terms of time to FEF achievement.

All the studies included in the meta-analysis, except one [50], recruited exclusively infants with birth weight <1500 g. The study by Roy et al. [50] reported specific data for extremely low birth weight (ELBW) infants: time to reach FEF in ELBW infants treated with probiotics was significantly lower than in controls (mean ± SD 13.22 ± 5.04 vs. 17.41 ± 8.07, respectively, $p = 0.014$). None of the studies included in the meta-analysis reported separate data on intrauterine growth restricted (IUGR) infants.

In all the studies, except one [56], a probiotic mix was used: the meta-analysis performed after the exclusion of the study by Manzoni et al., where a single-strain product containing Lactobacillus GG was used, confirmed the results of the overall analysis (MD −3.33 days (95% CI −5.63/−1.04), $p \leqslant 0.004$).

## 4. Methodological Study Quality

Evaluation of the quality of the studies included in the meta-analysis according to the risk of bias tool as proposed by the Cochrane Collaboration [19] is shown in Table 2.

**Table 2.** Evaluation of the quality of the studies included in the meta-analysis according to the risk of bias tool as proposed by the Cochrane collaboration.

| Study | Random Sequence Generation | Allocation Concealment | Blinding | Incomplete Outcome Data | Selective Outcome Reporting | Other Sources of Bias |
|---|---|---|---|---|---|---|
| Braga, 2011 [60] | Low | Low | Low | Low | Unclear | Low |
| Manzoni, 2006 [56] | Low | Low | Low | Unclear | Unclear | Low |
| Roy, 2014 [50] | Low | Unclear | Low | Low | Unclear | Unclear |
| Samanta, 2008 [38] | Low | Low | Low | Unclear | Unclear | Unclear |
| Van Niekerk, 2014 [22] | Low | Unclear | Low | Unclear | Unclear | Unclear |

## 5. Discussion

The present meta-analysis shows that the use of probiotics in preterm, VLBW infants fed exclusively HM is associated with 3-days reduction in the time to FEF achievement. The only two studies included in the present systematic review in which infants were exclusively formula-fed did not report any difference between the probiotic and the control group.

The single previous meta-analysis investigating FEF as primary outcome showed an overall smaller reduction in the time to FEF achievement, but did not report separate data for HM-fed and formula-fed infants [6]. The studies included in the meta-analysis by Athalye-Jape et al. are almost the same as those included in our systematic review; quite surprisingly, in the majority of the studies included in these two reviews, both HM and formula-fed infants were recruited, but no detailed information on the relationship between type of feeding and outcome was provided.

Type of feeding might modulate the relationship between probiotics and neonatal clinical outcome [14]. It has been previously shown that HM feeding is associated with shorter time to achieve FEF compared to formula feeding [5]. Our meta-analysis, which included only studies where infants were exclusively HM-fed, showed a significant reduction in the time to achieve FEF attributable to probiotics. Despite the limitation given by the small number of studies, a probiotic-related 3-days reduction in time to achieve FEF in preterm infants fed exclusively HM has strong clinical implications and deserves further consideration. When OMM is not available or contraindicated, the use of pasteurized DHM is recommended for preterm infants: pasteurization inactivates most viral and bacterial agents, but at the same time affects some nutritional and immunological properties of HM, including endogenous probiotics [71]. It can be speculated that the beneficial effect of probiotics documented in exclusively HM-fed infants could be attributed to a synergic action exerted by the prebiotic components of HM and the exogenous probiotic, which partially restores the symbiotic properties of naïve HM [72]. In the present meta-analysis, no separate data for OMM-fed and DHM-fed infants were available; for this reason, it is not possible to clarify whether the beneficial effect of HM on FEF achievement applies both to OMM and to DHM.

Heterogeneity among included studies was high; however, given the small number of papers, our ability to explore sources of heterogeneity was limited. In the five included studies, different probiotic strains were used. We aimed to perform strain-specific sub-meta-analyses, in order to clarify whether there was any probiotic product showing a significant benefit in terms of reduction in the time to achieve FEF. However, such analyses were not feasible, as none of the studies used the same probiotic strain or mix. Similarly, it was not possible to explore additional sources of heterogeneity, such as the characteristics of probiotic administration (dose, duration, infant age at probiotic initiation, etc.). In addition, we were unable to test for subgroup differences between HM-fed and formula-fed infants, which might have partially explained the different results in terms of FEF achievement.

Apparently, studies were homogeneous in terms of included populations, as almost all of them recruited only VLBW infants. However, few data on "high-risk" infants, such as ELBW and IUGR infants, could be extrapolated from the main results of the included studies.

The use of probiotics should be weighed against their potential side effects. There are some reports about the occurrence of sepsis in preterm newborns, potentially linked to probiotic administration [73].

However, none of the studies included in the systematic review reported any side effect related to the use of probiotics.

## 6. Conclusions

According to the results of the present meta-analysis, the use of probiotics is linked to 3-days reduction in time to achieve FEF in preterm VLBW infants fed exclusively HM. If confirmed in further studies, this reduction might have strong clinical implications for this high-risk population.

**Acknowledgments:** No funding was received to produce the present paper.

**Author Contributions:** Each author listed on the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript. Specifically, all the authors, as part of the Task Force on Probiotics of the Italian Society of Neonatology, conceived and designed the study protocol. A.A. and L.C. performed the literature search and assessed study details, which were checked by D.G. A.A. and D.G. evaluated study quality and performed the meta-analyses. A.A. and L.C. wrote the first draft of the paper, which was critically revised by all the other authors.

**Conflicts of Interest:** The authors declare no conflict of interest.

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