Neutral and acidic oligosaccharides in preterm infants: a randomized, double-blind, placebo-controlled trial1–4

Elisabeth AM Westerbeek, Jolice P van den Berg, Harrie N Lafeber, Willem PF Fetter, Guenther Boehm, Jos WR Twisk, and Ruurd M van Elburg

ABSTRACT

Background: Serious infectious morbidity is high in preterm infants. Enteral supplementation of prebiotics may reduce the incidence of serious infections, especially infections related to the gastrointestinal tract.

Objective: The objective was to determine the effect of enteral supplementation of a prebiotic mixture consisting of neutral oligosaccharides (scGOS/LcFOS) and acidic oligosaccharides (AOS) on serious infectious morbidity in preterm infants.

Design: In a randomized controlled trial, preterm infants (gestational age <32 wk and/or birth weight <1500 g) received enteral supplementation of 80% scGOS/LcFOS and 20% AOS (1.5 g kg−1 d−1) or placebo (maltodextrin) between days 3 and 30 of life. Serious infectious morbidity was defined as a culture positive for sepsis, meningitis, pylonephritis, or pneumonia. The analysis was performed by intention-to-treat and per-protocol, defined as ≥50% supplementation dose during the study period.

Results: In total, 113 preterm infants were included. Baseline and nutritional characteristics were not different between groups. In the intention-to-treat analysis, the incidence of ≥1 serious infection, ≥1 serious endogenous infection, or ≥2 serious infectious episodes was not significantly different in the scGOS/LcFOS/AOS-supplemented and placebo groups. In the per-protocol analysis, there was a trend toward a lower incidence of ≥1 serious endogenous infection and ≥2 serious infectious episodes in the scGOS/LcFOS/AOS-supplemented group than in the placebo group (P = 0.09 and P = 0.07, respectively).

Conclusions: Enteral supplementation of scGOS/LcFOS/AOS does not significantly reduce the risk of serious infectious morbidity in preterm infants. However, there was a trend toward a lower incidence of serious infectious morbidity, especially for infections with endogenous bacteria. This finding suggests a possible beneficial effect that should be evaluated in a larger study. This trial was registered at isrctn.org as ISRCTN16211826. Am J Clin Nutr 2010;91:679–86.

INTRODUCTION

Preterm infants admitted to the neonatal intensive care unit (NICU) have a high risk of serious infections. Although serious infections are often due to bloodstream infections caused by coagulase-negative staphylococci (CoNS), many infections are also caused by endogenous bacteria, which often originate from the gastrointestinal tract (1). In a recent review of the literature, we found that the number of bifidobacteria and Lactobacillus is lower in the gastrointestinal tract of preterm infants than in term infants, whereas the number of potentially pathogenic bacteria is higher. Furthermore, antibiotic administration after birth causes a significant delay in the intestinal bacterial colonization (2). Intestinal microbiota have the potential to influence the maturation of the infant’s immune system (3, 4).

Breast-milk oligosaccharides have immunomodulatory, antiadhesive, and antimicrobial effects (5). The antiadhesive and antimicrobial effect is due to a direct effect of acidic human milk oligosaccharides, which act as receptor analogs to the ligands of pathogens preventing adhesion of pathogens to the epithelial surface (6). The immunomodulatory effect is thought to be related to the bifidogenic effect of breast milk on the microbiota of the infant’s gut (5). This bifidogenic effect of breast milk is attributable to the large amount of oligosaccharides in breast milk. Nonhuman milk oligosaccharides, such as small-chain galactooligosaccharides (scGOS) and long-chain fructooligosaccharides (LcFOS), are used to substitute these functions (7). In previous studies in preterm and term infants, supplementation with neutral oligosaccharides stimulated a bifidogenic intestinal flora with a decrease of pathogens (3–5). In addition, neutral oligosaccharides increased small-chain fatty acids (SCFAs) production and decreased pH in human studies (6). In an in vitro model, SCFAs stimulated the mucin-2 synthesis and improved intestinal integrity (8). Furthermore, SCFAs may decrease the growth of pathogens and has a regulatory role in intestinal motility (6).

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2 The funding source had no involvement in the analysis of the data or in the interpretation of the results.

3 Supported by Danone Research, Friedrichsdorf, Germany. Danone Research provided the preterm formula (Nenatal Start) and postdischarge formula (Nenatal 1), neutral and acidic oligosaccharides and placebo supplementation.

4 Address correspondence to RM van Elburg, Department of Pediatrics, Subdivision of Neonatology, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, Netherlands. E-mail: rm.vanelburg@vumc.nl. Received September 7, 2009. Accepted for publication December 7, 2009. First published online December 23, 2009. doi: 10.3945/ajcn.2009.28625.
In term infants, supplementation of neutral oligosaccharides to infant formula reduced the incidence of infections and atopy (9–13). Of the oligosaccharides in breast milk, ~80% are neutral and up to 20% are acidic. Nonhuman milk acidic oligosaccharides (AOS) can be derived from pectin. AOS are able to act as receptors-agonists and are known to inhibit the adhesion of pathogens on the epithelial surface (6). AOS may also directly affect the immune cells via interaction of selectins, dendritic cell specific C-type lectin, integrins, and other target receptors as Toll-like receptors (6). The combination of SCGOS/LcFOS with AOS may increase the growth of a bifidogenic flora, decrease the growth of pathogens in the intestine, have a positive effect on the mucus layer of the gastrointestinal tract, and may stimulate the maturation of the immune system (14–16). As a result of these effects, we hypothesize that preterm infants receiving a combination of SCGOS/LcFOS with AOS may have a reduced incidence of serious infections. The primary aim of this randomized, double-blind, placebo-controlled trial was to determine the effect of enteral supplementation of a probiotic mixture consisting of neutral (SCGOS/LcFOS) and acidic (AOS) oligosaccharides on serious infectious morbidity in preterm infants. In addition, we determined the effect on feeding tolerance and short-term outcome in preterm infants.

SUBJECTS AND METHODS

Subjects

Infants with a gestational age (GA) <32 wk and/or birth weight <1500 g, admitted to the level III NICU of the VU University Medical Center, Amsterdam, were eligible for participation in the study. Exclusion criteria were as follows: infants with a gestational age >34 wk, major congenital or chromosomal anomalies, death within 48 h after birth, and transfer to another hospital within 48 h after birth. The medical ethical review board of our hospital approved the study protocol. Written informed consent was obtained from all parents.

Randomization, blinding, and treatment

After assignment to 1 of 3 birth weight groups (≤799, 800–1199, and ≥1200 g), the infants were randomly allocated to treatment within 48 h after birth to receive either enteral 80% SCGOS/LcFOS and 20% AOS or placebo powder (maltodextrin). An independent researcher used a computer-generated randomization table (provided by Danone Research, Friedrichsdorf, Germany) to assign infants to treatment with SCGOS/LcFOS/AOS or placebo. Investigators, parents, medical and nursing staff were unaware of treatment allocation. The randomization code was broken after data analysis was performed. SCGOS/LcFOS/AOS and the placebo powder (maltodextrin) were prepared and packed sterile (Danone Research, Friedrichsdorf, Germany). The 2 powders were indistinguishable by appearance, color, and smell. During the study period, SCGOS/LcFOS/AOS and placebo powder were monitored for stability and microbiological contamination.

Supplementation with SCGOS/LcFOS/AOS or placebo was administered in increasing doses between days 3 and 30 of life to a maximum of 1.5 g · kg⁻¹ · d⁻¹ to breast milk or preterm formula. Two members of the nursing staff added the daily supplementation to breast milk or to preterm formula (Nenatal Start; Danone Research, Friedrichsdorf, Germany) according to the parents’ choice. Per 100 mL, the preterm formula provided 80 kcal, 2.4 g protein (casein-whey protein ratio 40:60), 4.4 g fat, and 7.8 g carbohydrate. The preterm formula did not contain oligosaccharides. When infants were transferred to another hospital before the end of the study, the protocol was continued under supervision of the principal investigator (EAMW).

Nutritional support

Protocol guidelines for the introduction of parenteral and enteral nutrition followed current practices at our NICU. Nutritional support was administered as previously described (17), except for minimal enteral feeding (defined as 12–24 mL · kg⁻¹ · d⁻¹), which was advanced from either day 2 or from day 4 in those with a birth weight <10th percentile, GA <26 wk, Apgar score <6 at 5 min, umbilical artery pH <7.10, or base deficit >10 mmol/L. For each infant in the study, a feeding schedule was proposed based on birth weight and the guidelines as mentioned above. However, the medical staff of our NICU had final responsibility for the administration of parenteral nutrition and advancement of enteral nutrition.

After discharge, all infants received breast milk or preterm formula (Nenatal Start without oligosaccharides) and a post-discharge formula (Nenatal 1 without oligosaccharides) until the corrected age of 6 mo.

Study outcomes

The primary outcome of the study was the effect of SCGOS/LcFOS/AOS supplementation to the enteral nutrition on serious infectious morbidity (1, 17) during admission in the NICU and during the 80-d follow-up. A new serious infectious episode was defined as a new positive culture after adequate antibiotic treatment of the previous infectious episode with clinical recovery. More than one positive culture with the same bacteria from different sites at the same time was registered as one serious infectious episode. In addition, an analysis was performed of serious infectious morbidity caused by CoNS, most commonly related to (the combination of) indwelling lines and parenteral nutrition and by non-CoNS (endogenous) infections, usually from microorganisms from the gastrointestinal tract. The occurrence of serious infections was independently determined by 2 investigators (EAMW and RMvE), who were unaware of treatment allocation. Secondary outcomes (during admission and the 80-d follow-up) were feeding tolerance, such as time to full enteral feeding, defined as a feeding volume >120 mL · kg⁻¹ · d⁻¹, age when parenteral feeding was discontinued, days of minimal enteral feeding, defined as 12– 24 mL · kg⁻¹ · d⁻¹, days of no enteral feeding, and the occurrence of Bell stage II and III necrotizing enterocolitis (18) and growth (19). Other secondary outcomes were need for mechanical ventilation, presence of periventricular-intraventricular hemorrhage (20), patent ductus arteriosus treated with ibuprofen, indomethacin and/or surgical ligation, bronchopulmonary dysplasia (BPD) (21), retinopathy of prematurity (22), age at discharge from NICU, age at discharge from the hospital, and death.
Statistical analysis

On the basis of the differences in incidences of infectious morbidity (76% and 50%, respectively) in a previous study (17), a 2-tailed \( z = 0.05 \), a \( \beta = 0.20 \), and a sample size of \( 2 \times (2 \times 7.85 \times 0.63 (0.37)) / (0.26)^2 = 2 \times 54 \) infants were calculated. Normally distributed and nonparametric data were presented as means ± SDs and median (range), respectively. Logistic regression analysis was performed to determine whether enteral supplementation of \( \text{ScGOS/LcFOS/AOS} \) influenced the incidence of serious infections and more than one infectious episode. Multinominal logistic regression analysis was performed to determine whether enteral \( \text{ScGOS/LcFOS/AOS} \) supplementation influenced the incidence of serious endogenous infections. In a second analysis, adjustments were made for possible confounding factors: gestational age, birth weight, Apgar score <6 at 5 min, and exclusive breastfeeding during the study period (defined as the first 30 d of life.) Secondary outcomes were analyzed by Student’s \( t \) test, Mann-Whitney \( U \) test, and chi-square test or Fisher’s exact test for continuous normally distributed, nonparametric continuous, and dichotomous data, respectively.

All statistical analyses were performed on an intention-to-treat basis. To determine the effect of sufficient dosage and time of exposure to supplementation, a per-protocol analysis was performed, excluding infants who received a mean supplementation dose <50% of the maximum supplementation dose (1.5 g · kg\(^{-1} \) · d\(^{-1} \)) during the study period and infants not completing the study period (30 d of life). For all statistical analyses, a 2-tailed \( P \) value <0.05 was considered significant. SPSS 15.0 (SPSS Inc, Chicago, IL) was used for the data analysis.

RESULTS

Between May 2007 and November 2008, 113 preterm infants entered the study. The trial profile is shown in Figure 1. Baseline patient and nutritional characteristics were not different between the 2 groups (Table 1). In the \( \text{ScGOS/LcFOS/AOS} \)-supplemented group, 23 of 55 (42%) infants had ≥1 serious infections.

![Figure 1](https://example.com/figure1.png)

**FIGURE 1.** Trial profile. GA, gestational age; BW, birth weight.
The relation of the first endogenous infection with postnatal age, birth weight, Apgar score, and exclusive breastfeeding during the 30-d study period decreased the OR compared with 31 of 58 (54%) in the placebo group [odds ratio (OR): 0.62; 95% CI: 0.30, 1.23; P = 0.16]. In the per-protocol analysis (n = 43) of subjects in the placebo group (14 of 51; 28%) compared with 11 of 55 (22%) in the placebo group (OR: 0.47; 95% CI: 0.19, 0.98; P = 0.045). The incidence of ≥2 serious infectious episodes was lower in the SCGOS/LCFOS/AOS-supplemented group (2 of 43; 5%) than in the placebo group (14 of 51; 28%) (OR: 0.20; 95% CI: 0.04, 0.97; P = 0.03) (Table 2).

In an additional analysis, adjustment for gestational age, birth weight, Apgar score <6 at 5 min, and exclusive breastfeeding during the 30-d study period decreased the OR and increased the P value to >0.05 for endogenous infections compared with 12 of 58 (21%) in the placebo group (OR: 0.49; 95% CI: 0.17, 0.98; P = 0.045). The incidence of ≥2 serious infectious episodes was lower in the SCGOS/LCFOS/AOS-supplemented group (2 of 43; 5%) than in the placebo group (14 of 51; 28%) (OR: 0.20; 95% CI: 0.04, 0.97; P = 0.03) (Table 2).
Intention-to-treat infection in the first 80 d of life.

Per-protocol group (9 of 39; 23%) (AOS-supplemented group (0 of 43; 0%) than in the placebo positive for microorganisms. OR, odds ratio.

a clear trend, enteral supplementation with SCGOS/LCFOS/AOS short-term outcomes in preterm infants. Although there was the enteral SCGOS/LCFOS/AOS-supplemented group. secondary outcomes, the incidence of mild BPD was lower in preterm infants. Interestingly we observed that, as one of the did not significantly reduce the risk of serious infections in SCGOS/LCFOS and AOS on serious infectious morbidity and DISCUSSION

To our knowledge, this was the first study to describe the effects of enteral supplementation of a prebiotic mixture of SCGOS/LcFOS and AOS on serious infectious morbidity and short-term outcomes in preterm infants. Although there was a clear trend, enteral supplementation with SCGOS/LcFOS/AOS did not significantly reduce the risk of serious infections in preterm infants. Interestingly we observed that, as one of the secondary outcomes, the incidence of mild BPD was lower in the enteral SCGOS/LcFOS/AOS-supplemented group. Preterm infants face many challenges, and performing an intervention study in this high-risk group is a challenge. Because of their increased risk of infectious morbidity with paralytic ileus and necrotizing enterocolitis, increasing enteral feeding is often difficult. Prebiotics increase the intestinal growth of Bifidobacteria and Lactobacillus and reduce the amount of potentially pathogenic bacteria (23). However, because of feeding problems and excessive antibiotic use in very-low-birth weight preterm infants, sufficient amounts of study supplements could not always be administered to accomplish a beneficial effect on the intestinal microbiota, and the gut and inhibition of adhesion of pathogens to the epithelial surface may be involved in the susceptibility to endogenous infections in preterm infants.

The incidence of endogenous infections and ≥2 serious infections episodes was lower in infants who received a mean supplementation dose of ≥50% of the maximum supplementation dose of 1.5 g · kg⁻¹ · d⁻¹ during the study period (per-protocol

TABLE 2

<table>
<thead>
<tr>
<th>Intention-to-treat [n/total n (%)]</th>
<th>Crude analysis</th>
<th>Adjusted analysis²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>≥1 Serious infection¹</td>
<td>0.63 (0.30, 1.32)</td>
<td>0.22</td>
</tr>
<tr>
<td>≥1 Serious endogenous infection²</td>
<td>0.45 (0.17, 1.16)</td>
<td>0.10</td>
</tr>
<tr>
<td>≥2 Serious infectious episodes³</td>
<td>0.47 (0.16, 1.40)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Per-protocol [n/total n (%)]

<table>
<thead>
<tr>
<th></th>
<th>Crude analysis</th>
<th>Adjusted analysis²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>≥1 Serious infection¹</td>
<td>0.45 (0.19, 1.10)</td>
<td>0.06</td>
</tr>
<tr>
<td>≥1 Serious endogenous infection²</td>
<td>0.31 (0.10, 0.98)</td>
<td>0.045</td>
</tr>
<tr>
<td>≥2 Serious infectious episodes³</td>
<td>0.20 (0.04, 0.97)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

¹ Serious infectious morbidity was defined as sepsis, meningitis, pyelonephritis, or pneumonia diagnosed by a combination of clinical signs and a culture positive for microorganisms. OR, odds ratio.
² Adjusted for gestational age, birth weight, Apgar score <6 at 5 min, and exclusive breastfeeding during the 30-d study period.
³ Logistic regression analysis.
⁴ Multinomial logistic regression analysis.

TABLE 3

<table>
<thead>
<tr>
<th>Prebiotic mixture</th>
<th>Placebo</th>
<th>Adjusted analysis²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis [n/total n (%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>20/28 (71)</td>
<td>27/39 (69)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>4/28 (14)</td>
<td>2/39 (5)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1/28 (4)</td>
<td>2/39 (5)</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>2/28 (7)</td>
<td>2/39 (5)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>0/28 (0)</td>
<td>2/39 (5)</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>2/28 (7)</td>
<td>0/39 (0)</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>0/28 (0)</td>
<td>1/39 (3)</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0/28 (0)</td>
<td>2/39 (5)</td>
</tr>
<tr>
<td>Pyelonephritis [n/total n (%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus¹</td>
<td>0/2 (0)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0/0 (0)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>1/2 (50)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Enterobacter²</td>
<td>0/0 (0)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>1/2 (50)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Pneumonia [n/total n (%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0/0 (0)</td>
<td>1/3 (33)</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>0/0 (0)</td>
<td>1/3 (33)</td>
</tr>
<tr>
<td>Ureaplasma urealyticum</td>
<td>0/0 (0)</td>
<td>2/3 (66)</td>
</tr>
</tbody>
</table>

¹ Concurrent sepsis and meningitis at the same time listed once.
² Concurrent sepsis at the same time listed only once.

FIGURE 2. Kaplan-Meier curve for the first developed endogenous infection in the first 80 d of life.
supplementation with SCGOS/LCFOS/AOS may influence the
associated with BPD (25–27). We hypothesize that enteral
is multifactorial, inflammation is considered an important factor
serious infectious morbidity. Although the development of BPD
justment for chorioamnionitis, gestational age, birth weight, and
supplemented group than in the placebo group, also after ad-
(24), who showed a dose dependent effect of SCGOS/LCFOS (0.8
analysis). This is in line with the results of the study by Moro et al
entered supplementation of SCGOS/LCFOS on BPD.
Some limitations of the study need to be addressed. First, the
immune system and the systemic inflammatory response in the
respiratory tract, improving the balance between proin-
flammatory and inflammatory cytokines. Previous studies have
shown that neutral and acidic human milk oligosaccharides can
be absorbed and cross the border membrane of the intestine,
which suggests that human milk oligosaccharides may act sys-
emically and that their effect is not restricted to the intestine
(28). However, because serious infectious morbidity was the
primary aim of our study, future studies including a larger
number of preterm infants are needed to confirm the effect of
SCGOS/LCFOS/AOS on BPD.
In our study, the incidence of necrotizing enterocolitis was not
different between groups. Approximately 50% of the infants who
developed necrotizing enterocolitis did not reach a mean sup-
plementation dose of ≥50% during the study period or died
before the end of the study period. Therefore, we cannot draw
cclusions about the effect of SCGOS/LCFOS/AOS supple-
mentation on the incidence of necrotizing enterocolitis.

### TABLE 4
Secondary outcomes in preterm infants

<table>
<thead>
<tr>
<th></th>
<th>Prebiotic mixture (n = 55)</th>
<th>Placebo (n = 58)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding tolerance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to full enteral feeding (d)</td>
<td>10 (4–48)</td>
<td>11 (7–50)</td>
<td>0.47</td>
</tr>
<tr>
<td>Age at finishing parenteral nutrition (d)</td>
<td>10 (3–45)</td>
<td>10 (4–34)</td>
<td>0.89</td>
</tr>
<tr>
<td>Feeding withheld during study period (d)</td>
<td>0 (0–17)</td>
<td>0 (0–15)</td>
<td>0.52</td>
</tr>
<tr>
<td>Necrotizing enterocolitis [n/total n (%)]²</td>
<td>10/55 (18)</td>
<td>6/58 (10)</td>
<td>0.23</td>
</tr>
<tr>
<td>Growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight SD score at birth</td>
<td>−0.36 (1.72)</td>
<td>−0.36 (1.33)</td>
<td>1</td>
</tr>
<tr>
<td>Weight SD score at day 30</td>
<td>−1.22 (1.36)</td>
<td>−1.42 (0.93)</td>
<td>0.36</td>
</tr>
<tr>
<td>Weight SD score at discharge</td>
<td>−1.19 (1.24)</td>
<td>−1.34 (0.92)</td>
<td>0.50</td>
</tr>
<tr>
<td>General outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDA [n/total n (%)]</td>
<td>4/55 (7)</td>
<td>10/58 (17)</td>
<td>0.11</td>
</tr>
<tr>
<td>Duration of ventilator support (d)</td>
<td>1 (0–38)</td>
<td>1 (0–79)</td>
<td>0.91</td>
</tr>
<tr>
<td>Supplemental oxygen at PMA 36 wk [n/total n (%)]³</td>
<td>1/52 (2)</td>
<td>10/52 (19)</td>
<td>0.004</td>
</tr>
<tr>
<td>21% oxygen (mild BPD)</td>
<td>1/52 (2)</td>
<td>0/52 (0)</td>
<td>0.32</td>
</tr>
<tr>
<td>&lt;30% oxygen (moderate BPD)</td>
<td>4/52 (8)</td>
<td>3/52 (6)</td>
<td>0.70</td>
</tr>
<tr>
<td>&gt;30% oxygen (severe BPD)</td>
<td>4/51 (8)</td>
<td>3/50 (6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Retinopathy grade III or IV [n/total n (%)] ²</td>
<td>4/51 (8)</td>
<td>3/50 (6)</td>
<td>0.26</td>
</tr>
<tr>
<td>Death [n/total n (%)]</td>
<td>4/54 (7)</td>
<td>8/58 (14)</td>
<td>0.36</td>
</tr>
<tr>
<td>Death within study period [n/total n (%)]</td>
<td>2/55 (4)</td>
<td>3/58 (5)</td>
<td>0.97</td>
</tr>
<tr>
<td>Death after study period [n/total n (%)]</td>
<td>2/55 (4)</td>
<td>5/58 (9)</td>
<td>0.46</td>
</tr>
<tr>
<td>Age at discharge from NICU (d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants who survived</td>
<td>15 (1–90)</td>
<td>17 (3–87)</td>
<td>0.97</td>
</tr>
<tr>
<td>Infants who died</td>
<td>36 (26–104)</td>
<td>24 (3–167)</td>
<td>0.46</td>
</tr>
<tr>
<td>Age at discharge from hospital (d)</td>
<td>52 (30–111)</td>
<td>54 (30–181)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

¹ PDA, patent ductus arteriosus treated with ibuprofen, indomethacin, or ligation; PMA, postmenstrual age; BPD, bronchopulmonary dysplasia; NICU, neonatal intensive care unit.
² Student’s t test, Mann-Whitney U test, chi-square test, or Fisher’s exact test.
³ Median; range in parentheses (all such values).
⁴ Reference 17.
⁵ Reference 18.
⁶ Reference 20.
⁷ Reference 21.
⁸ Reference 21.
⁹ Reference 21.

This is in line with the results of the study by Moro et al
(24), who showed a dose dependent effect of SCGOS/LCFOS (0.8
g/dL compared with 0.4 g/dL during a 28-d study period) on the
growth of *Bifidobacteria* and *Lactobacillus* in the intestine. In our
study, the median time to full supplementation was 11 d, the
time to the first serious endogenous infection was 16 d, and the time
to the second serious infectious episode was 27 d. This suggests
that enteral supplementation of SCGOS/LCFOS/AOS of preterm
infants may reduce the risk of serious infectious morbidity, es-
specially endogenous infections, if supplementation is given at a dose
of ≥50% of the maximum of 1.5 g · kg⁻¹ · d⁻¹ and is given for
≥14 d. This effect is in line with the ability of AOS to act as
receptors-analogs, resulting in inhibition of the adhesion of
pathogens on the epithelial surface in the gastrointestinal tract (6).
However, our study was not designed to determine the optimal
supplementation dose and number of days required to reduce
serious infections.

We observed that the incidence of mild BPD, as defined by
Jobe et al (21), was lower in the enteral SCGOS/LCFOS/AOS-
supplemented group than in the placebo group, also after ad-
justment for chorioamnionitis, gestational age, birth weight, and
serious infectious morbidity. Although the development of BPD
is multifactorial, inflammation is considered an important factor
associated with BPD (25–27). We hypothesize that enteral
supplementation with SCGOS/LCFOS/AOS may influence the
analysis. Second, randomization was primarily based on birth weight, whereas gestational age was also an inclusion criterion. As a consequence, there was a nonsignificant difference in gestational age between both groups in favor of the SCGOS/LC-FOS/AOS group. However, adjustment for gestational age and other potential confounders defined in our study protocol did not change the results of our primary outcome. Third, study supplementation started 48 h after birth, because parents were allowed 48 h to give informed consent. Fourth, an optimal supplementation dose of 1.5 g · kg⁻¹ · d⁻¹ was reached at a median postnatal age of 11 d because of restriction of the maximal osmolarity of the enteral feeding, consistent with a maximum oligosaccharides supplementation of 1 g/60 mL feeding. Last, breast milk itself contains neutral and acidic oligosaccharides, and the effect of supplementation on infectious morbidity may be less pronounced in preterm infants who were exclusively breastfed. However, in an additional analysis, to determine the influence of type of feeding on infectious morbidity, we did not find that exclusive breastfeeding during the study period reduced the incidence of serious infections. Because breast milk is strongly promoted in our NICU, most infants received breast milk (>60%), and relatively few were exclusively formula fed (20%).

Serious infections morbidity is the most common cause of death during the neonatal period and is a major risk of neurologic impairment. The potential improvement in short- and long-term outcomes with supplementation of health-promoting nutrients is very attractive. Human milk contains, besides oligosaccharides, other beneficial nutrients such as glycoproteins. Recently, Mazoni et al (29) found that supplementation of bovine lactoferrin with or without the probiotic strain Lactobacillus rhamnosus GG reduced the risk of late-onset sepsis in very-low-birth-weight infants. In a recent review of the potential roles and clinical utility of prebiotics in newborns, Sherman et al (30) concluded that when taken in sufficient amounts, prebiotics soften stools, increase stool frequency, and increase the ratio of bifidobacteria to total fecal bacteria. Furthermore, Sherman et al concluded that the addition of a prebiotic mixture to formula is considered safe, however, additional research is needed to determine the relevance of reported outcome measures for decreasing disease and promoting health.

In summary, we found that enteral supplementation of SCGOS/LC-FOS/AOS does not significantly reduce the risk of serious infectious morbidity in preterm infants. However, there was a trend toward a lower incidence of serious infectious morbidity, especially for infections with endogenous bacteria. This finding suggests a possible beneficial effect that should be evaluated in a larger study. Because serious infectious morbidity during the neonatal period is associated with neurodevelopmental impairment (31, 32), decreasing the risk of serious infectious morbidity by nutritional supplementation, such as with prebiotics, should be investigated further.

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The authors’ responsibilities were as follows—EAMW, HNL, WPFF, GB, and RMvE: formulated the research questions and participated in the study design; EAMW, JvdPbV, and RMvE: coordinated the study; EAMW and JvWRT: analyzed the data; EAMW and RMvE: wrote the draft of this manuscript; and JvdPbV, HNL, WPFF, GB, and JvWRT: reviewed the manuscript. All authors approved the final version of the manuscript. All authors had full access to all of the data and analyses and were involved in the final decision to submit and resubmit the manuscript. None of the authors had a conflict of interest.

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