**Supplemental Digital Content**

**Methods**

**Study population and design**

A total of 218 healthy pregnant mothers were pre-screened between June 2011 and April 2013 so that their infants could potentially join the study. Exclusion criteria were: i) fever ≥ 38.5°C during the last week before birth, ii) Blood pressure systolic ≥ 160 mm Hg and diastolic ≥ 100 mm Hg, iii) occurrence of eclampsia and pre-eclampsia during pregnancy. iv) diabetes mellitus requiring insulin treatment, v) hyperthyroidism during pregnancy, vi) pathologic birth presentation, vii) abnormal cardiotocogram for more than 2 hours at day of delivery, viii) preterm birth before 37th week of gestation, ix) treatment for infertility and placenta implementation , x) use of probiotic, prebiotic, dietary fibre supplements or supplemented food from 2 weeks prior to the expected date of delivery and for the duration of the entire study and xi) use of non-steroidal anti-inflammatory drugs and antibiotics except antibiotic treatment related to C-section delivery. In total, 199 infants born to healthy pregnant mothers were assessed for eligibility to enrol in the study. Exclusion criteria for the infant were any known congenital disease which could interfere with the study conduct and assessments, abnormal birth weight (normal ranges: 2.25-4.0 kg) and an apgar score < 7 after 5 min. There were three parallel intervention groups of infants that were delivered by C-section and randomized immediately after birth and a non-randomised fourth group of infants born vaginally (reference group) whose mothers intended to breastfeed as long as possible (Supplemental Figure 1).

**Study products**

The intervention and control formulas were regular non-hydrolysed cow’s milk based infant formulas that provide adequate nutritional support for infants in the first six months of life. The formulas were iso-caloric and contained per 100 ml a similar amount of 67 kcal energy, 1.3 g protein, 7.6 g carbohydrate, 3.5 g of lipid, LCPUFAs, vitamins and minerals (data not shown). In addition, the prebiotic formula contained 0.8g/100 ml of scGOS/LcFOS in a 9:1 ratio, the synbiotic formula contained 0.8g/100 ml of scGOS/LcFOS (9:1 ratio) and 7.5 x 108 CFU /100 ml of *B. breve* M-16V. All formulas had a similar taste, smell and appearance and were supplied by Danone Nutricia. Parents received the assigned formula along with instructions for preparation.

**Study procedure**

The study consisted of a screening visit, a visit before discharge (< week 1) and 4 subsequent hospital visits at week 8, 12, 16 and 22. At each visit, parents received a diary for 4 weeks to record formula intake and tolerance on a daily base. In addition, parents were asked to record any medication, treatment or use of other nutritional supplement during the intervention period. At the hospital visits, the physician registered, besides the anthropometric measurements and the diaries, any adverse events (AEs). Stool collection kit (including 8 stool container, plastic bags, cooling kit for transportation, gloves, labels, stool sampling instructions, pen) and the diary with instructions were given to the parents. Frozen stool samples at the parent’s home (-20C) were collected at each hospital visit and stored in the freezer (-80°C) until further analysis.

**Laboratory Analysis**

**Samples collection and preparation**

Stool samples were collected at day 3, day 5, week 2, week 4, week 8, week 12, week16 and week 22. All samples were frozen immediately by the parents and then transported to the hospital in a cold storage bag, where they were stored at -80°C until further analysis. Upon arrival to the laboratory, frozen samples were thawed on ice and 0.5 g was fixed with 4% para-formaldehyde (PFA) and stored at -80oC. For DNA extraction, fecal samples were processed using QIAGEN DNA Stool Mini-Kit (QIAGEN, Hilden, Germany) according to the manufacturer instruction with some modification by adding a bead-beating step. Approximately 200 mg of 0.1mm glass beads were added to 200mg of stool sample and re-suspended in QIAGEN ASL buffer, bead-beating was carried out using FastPrep-24 (M.P. Biomedicals, USA) for 3 repetitions of 1 minute bead-beating with 5 minutes incubation on ice. Samples were then heated at 95°C for 15 minutes before centrifugation at 20,000 x g for 1 minute. The supernatant was transferred into a clean tube containing InhibitEX tablet and vortexed to mix proceeding with manufacturer instructions. Isolated fecal genomic DNA were eluted in 50uL AE buffer, checked with NanoDrop 2000 (ThermoScientific, DE, USA) and stored at -20°C before used for further analyses.

**Fecal samples analyses**

Determination of the composition of the faecal microbiota

All 16S rRNA-targeted oligonucleotide probes, their sequence and their targeted bacterial groups are listed in supplementary Table 1. All probes were purchased from MWG (Ebersgreg, Germany) and were covalently linked at their 5’-end with Cy3. The Nucleic acid stain DAPI (Invitrogen, Leiden, the Netherlands) was used for determining total fecal cell counts. PFA-fixed fecal samples were hybridized with the specific probes and then counted using an automated Olympus AX70 epifluorescence microscope equipped with a Lang LStep13 8 slides-stage (Paes Nederland BV, Zoeterwoude, the Netherlands) and an F-View II charge-coupled device (Soft Imaging System GmbH, Münster, Germany) and image analysis software. The percentage of bacterial cells was determined at 25 randomly chosen positions on each well by counting all cells using a DAPI filter set (SP100; Chroma Technology Corp., Brattleboro, VT, USA) and by counting the targeted bacterial group using a Cy3 filter set (41007; Chroma Technology Corp.).

Determination of the *Bifidobacterium* species

The absolute gene counts of total *Bifidobacterium* and several *Bifidobacterium* species including the probiotic strain *B. breve* M-16V were assessed with quantitative real-time PCR (Q-PCR). Two Q-PCR chemistries (SYBR Green and TaqMan) were selected and carried out using ABI Prism 7900HT (Applied Biosystems, California, USA). SYBR Green PCR master mix (Applied Biosystems, Carlsbad, CA) and TaqMan universal master mix (Applied Biosystems, Austin, USA) were used for their respective assays. Primers were selected and/ or designed to amplify specific regions of the 16S rRNA gene or the 16S-23S intergenic spacer region or specific genomic region (*Bifidobacterium breve* M-16V) with their respective optimized Q-PCR condition (Supplementary Table 2). Standards for each *Bifidobacterium* target were generated from their respective genomic DNA by endpoint PCR with their respective primer pairs. The specificity of the amplified PCR product was checked using gel electrophoresis (1.5% agarose + 1x TAE buffer) prior to purification using MinElute PCR Purification Kit (QIAGEN) as per manufacturer protocol. The concentrations of the purified amplicons were measured using NanoDrop 2000 (ThermoScientific, DE, USA) and the copy number/µl were calculated (DNA concentration / (fragment length x weight of base pair)) before diluting to a range of 101-106 copies/µl of standards used in each assay. In brief, fecal genomic DNA was diluted 100, 1,000 and 10,000 times and dispensed into 384-wells optical plate using Microlab Nimbus robotics (Hamilton, Ne Vada USA). Q-PCR conditions were 1 cycle of 95°C for 20s followed by amplification at 95°C for 1s, 60°C for 20s for 40 cycles and 1 cycle of 95°C for 15s, 60°C for 15s and 95°C for 15s with readings collected at the last step for melting curve analysis in SYBR Green assays. For TaqMan assays, the conditions were set at 1 cycle of 50°C for 2 minutes, 1 cycle of 95°C for 10 minutes, followed by 40 cycles of amplification at 95°C for 15s and 60°C for 1 minute. SDS 2.4 (Applied Biosystems, USA) was used to visualize and check abnormality of curves deviating from standard amplification. Raw data were then exported into Excel, where the Ct values were recalculated for log Copy numbers/g faeces using in-house optimized macros. The final results were then used for statistical analysis.

**Measurements of gut microbiota metabolic activity**

SCFA content (acetic, propionic, n-butyric, iso-butyric, n-valeric and iso-valeric acids) in faecal samples were quantitatively determined by a Varian 3800 gas chromatograph (GC) (Varian Inc., Walnut Creek, CA, USA) equipped with a flame ionization detector. Lactate was measured enzymatically using a D-lactic acid/ L-lactic acid detection kit containing D- and L-lactate dehydrogenase (Boehringer Mannheim, Mannheim, Germany). Faecal samples were thawed and pH was measured directly at room temperature using a Handylab pH meter (Schott Glas,Mainz, Germany) equipped with an Inlab 423 pH electrode (Mettler-Toledo, Columbo, Schwerzenbach, Switzerland).

**Randomisation and Blinding**:

Randomization table with treatment codes and a block size of 6 was generated by the sponsor (Nutricia Research, The Netherlands) using SAS 9.2 (SAS Institute, Inc, Cary, NC). The detail of the randomization sequence was unknown to the investigator and site staff, and was contained in a set of opaque sealed envelopes each bearing on the outside only the name of the site and a number. After eligibility of the subject was assessed and informed consent was obtained, the participant was enrolled in the study. Based on the order in which subjects entered the study and country, they were assigned a randomisation number after which the correspondingly numbered, opaque, sealed randomisation envelope was opened, revealing the code of the study product (A, B, C, D, E or F) that was assigned to the randomisation number beforehand. These randomisation envelopes had been prepared by the clinical studies supplies manager of Nutricia Research and were kept in an agreed location on each site. The assigned randomisation number and study product code were documented in the CRF and in the subject’s file. Randomization was secured until data were analyzed.

# Statistical Analysis

All the statistical analyses were performed on both the modified intention-to-treat (mITT) and Per Protocol (PP) populations. The mITT consisted of all randomized subjects who provided at least one baseline and post-baseline stool sample. For the safety data, the All Subjects Treated (AST) population was used to report the results (Supplementary Figure 1).

All statistical analyses were performed using SAS 9.2 (SAS Institute, Inc, Cary, NC). If the percentage of values below the lower limit of detection (LOD) was at most 30% in samples (at any time-points) for a specific parameter, the values were replaced with LOD/√2. Else if the percentage of values below lower LOD was greater than 30%, the values were converted to binary and the analyses were adapted for this outcome. A generalized linear mixed model procedure (PROC GLIMMIX) with lognormaldistribution and identity link function was used to evaluate the treatment effect on primary and secondary parameters. The model was fitted with core factors (treatment, time as categorized by week numbers, treatment-by-time interaction, and country) and selected covariates (mean daily formula feeding and mean number of daily breast-feeding servings). The model was performed using an unstructured covariance structure followed by auto-regressive compound symmetry and variance components covariance structure until the model converged. For the parameters that were log-transformed, the estimates of the interventions were back-transformed to original measurement units using mean and variance formula for the lognormal distribution. The intervention effects were estimated as the difference of estimated means of the interventions at each time-point. The corresponding 95% confidence interval was constructed using the pooled variance. The intervention effect was compared with 0 using z-test. For all endpoints, two-sided p-values <0.05 were considered statistically significant.**Supplementary Table 1: 16S rRNA-gene targeted oligonucleotide probes used for FISH**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Sequence from 5’ to 3’-end** | **Targeted bacterial groups** | **References** |
| Ato291 | GGTCGGTCTCTCAACCC | *Atopobium* group | [1](#_ENREF_1) |
| Bdis656 | CCGCCTGCCTCAAACATA | *Bacteroides distasonis* group | [2](#_ENREF_2) |
| Bfra602 | GAGCCGCAAACTTTCACAA | *Bacteroides fragilis* group | [2](#_ENREF_2) |
| Bif164-mod | CATCCGGYATTACCACCC | *Bifidobacterium* group | [3](#_ENREF_3) |
| Chis150 | TTATGCGGTATTAATCTYCCTTT | *Clostridium histolyticum* group | [2](#_ENREF_2) |
| Clit135 | GTTATCCGTGTGTACAGG | *Clostridium lituseburense* group | [2](#_ENREF_2) |
| Ec1531 | CACCGTAGTGCCTCGTCATCA | Enterobacteriaceae (*E.coli*, *Shigella*, *Salmonella*, *Klebsiella*) | [4](#_ENREF_4) |
| Erec482 | GCTTCTTAGTCARGTACCG | *Eubacterium rectale*–*Clostridium coccoides* cluster | [2](#_ENREF_2) |
| Lab158 | GGTATTAGCAYCTGTTTCCA | *Lactobacillus*–*Enterococcus* group | [5](#_ENREF_5) |

**Supplementary Table 2: 16S rRNA gene, 16S-23S intergenic spacer region or specific genomic region targeted primers used for Q-PCR analysis**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Target** | **Primer Label** | **Sequence (5' to 3' )** | **Amplicon length (bp)** | **Probe (if applicable)** | **References** |
| **Total *Bifidobacterium*** | F-bifido | CGC GTC YGG TGT GAA AG | 244 | FAM-NFQ-MGB | [6](#_ENREF_6) |
|  | R-bifido | CCC CAC ATC CAG CAT CCA |  |  |  |
|  | MGB-bifido | AAC AGG ATT AGA TAC CC |  |  |  |
| ***B. adolescentis*** | F\_adol\_IS | ATA GTG GAC GCG AGC AAG AGA | 71 | FAM-NFQ-MGB | [7](#_ENREF_7) |
|  | R\_adol\_IS | TTG AAG AGT TTG GCG AAA TCG |  |  |  |
|  | P\_adol\_IS | CTG AAA GAA CGT TTC TTT TT |  |  |  |
| ***B. angulatum*** | F\_angul\_IS | TGG TGG TTT GAG AAC TGG ATA GTG | 117 | FAM-NFQ-MGB | [7](#_ENREF_7) |
|  | R\_angul\_IS | TCG ACG AAC AAC AAT AAA CAA AAC A |  |  |  |
|  | P\_angul\_IS | AAG GCC AAA GCC TC |  |  |  |
| ***B. breve*** | F\_breve\_IS | GTG GTG GCT TGA GAA CTG GAT AG | 118 | FAM-NFQ-MGB | [7](#_ENREF_7) |
|  | R\_breve\_IS | CAA AAC GAT CGA AAC AAA CAC TAA A |  |  |  |
|  | P\_breve\_IS | TGA TTC CTC GTT CTT GCT GT |  |  |  |
| ***B. catenulatum*** | F\_cate\_IS | GTG GAC GCG AGC AAT GC | 67 | FAM-NFQ-MGB | [7](#_ENREF_7) |
|  | R\_cate\_IS | AAT AGA GCC TGG CGA AAT CG |  |  |  |
|  | P\_cate\_IS | AAG CAA ACG ATG ACA TCA |  |  |  |
| **Total bacteria** | Nadkarni-F | TCC TAC GGG AGG CAG CAG T | 449 | 6FAM-TAMRA | [8](#_ENREF_8) |
|  | Nadkarni-R | GGA CTA CCA GGG TAT CTA ATC CTG TT |  |  |  |
|  | Nadkarni-P | CGT ATT ACC GCG GCT GCT GGC AC |  |  |  |
| ***B. bifidum*** | BiBIF-1 | CCA CAT GAT CGC ATG TGA TTG | 278 | SYBR | [9](#_ENREF_9) |
|  | BiBIF-2 | CCG AAG GCT TGC TCC CAA A |  |  |  |
| ***B. longum* group** | LonU7 | GCC GTA TCT CTA CGA CCG TCG | 567 | SYBR | [10](#_ENREF_10) |
|  | LonU8 | TAT CGG GGA GCA AGC GAG AG |  |  |  |
| **Total Bacteria** | 338F | ACT CCT ACG GGA GGC AGC | 178 | SYBR | This study |
|  | 533R | TTA CCG CGG CTG CTG GCA C |  |  |  |
| **B. *breve* M-16V** | JLa1-F | GGC CAC CAG TAT GGT CTT ATC C | 63 | FAM-NFQ-MGB | This study |
|  | JLa1-R | TCG TGC CAT TCG CTA TTG C |  |  |  |
|  | JLa1-P | CTT GGG CGC CAT GAT |  |  |  |

**Supplementary Table 3: Demographics and birth characteristics of the subjects of the mITT population**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Control  (n=45) | Synbiotic (n=45) | Prebiotic  (n=39) | Reference (n=28) |
| Gestational age,weeks | 37.9 (0.69) | 38.1 (0.75) | 38.3 (0.86) | 38.9 (1.27) |
| Gender, n (%) |  |  |  |  |
| Male | 20 (44.4) | 20 (44.4) | 20 (51.3) | 13 (46.4) |
| Female | 25 (55.6) | 25 (55.6) | 19 (48.7) | 15 (53.6) |
| Ethnicity, n (%) |  |  |  |  |
| Chinese | 23 (51.1) | 23 (51.1) | 12 (30.8) | 11 (39.3) |
| Malay | 7 (15.6) | 8 (17.8) | 10 (25.6) | 8 (28.6) |
| Indian | 2 (4.4) | 3 (6.7) | 6 (15.4) | 0 (0) |
| Other | 13 (28.9) | 11 (24.4) | 11 (28.2) | 9 (23.1) |
| Birth weight, kg | 3.12 (0.37) | 3.13 (0.32) | 3.16 (0.38) | 3.20 (0.41) |
| Birth length, cm | 48.0 (1.82) | 48.3 (1.94) | 48.1 (1.79) | 49.3 (1.89) |
| Birth head circumference,cm | 34.2 (1.09) | 34.2 (1.15) | 34.2 (1.24) | 33.5 (1.19) |
| APGAR score after minutes | 9.1 (0.34) | 9.2 (0.39) | 9.1 (0.34) | 9.1 (0.45) |

Data is reported in Mean (SD), SD: Standard deviation

Sstatistical testing of the baseline differences between the groups was not performed. This is also largely in line with the position in the literature that it is not recommended to perform such testing asdescribed by [Bland and Altman, 2011](#_ENREF_1) and [Egbewale, 2015](#_ENREF_2) 11,12.

**Supplementary Table 4: Study product intake and exposure (All subject treated population)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Control (N= 48)** | **Synbiotic (N= 51)** | **Prebiotic (N= 48)** | **Reference (N= 30)** |
| **Intervention exposure in All Subjects Treated expressed as Median (Q1-Q3**) | | | | |
| * Age at first Formula Feeding intake (days) | 1.0 (1.0, 1.0) | 1.0 (1.0, 1.0) | 1.0 (1.0, 1.0) | 1.0 (1.0, 2.0) |
| * Total days on Formula Feeding | 112.0 (5.0, 134.5 | 111.0 (45.0, 118.0) | 111.5 (16.5, 135.0) | 77.0 (3.0, 112.0) |
| * Total days on Formula Feeding without interruptions | 107.5 (53.5, 113.0) | 105.0 (28.0, 114.0) | 107.0 (7.0, 116.0) | 19.0 (3.0, 102.0) |
| * Total days exclusively Formula Feeding | 2.0 (0.5, 72.0) | 1.0 (0.0, 49.0) | 2.0 (0.5, 59.5) | 0.5 (0.0, 15.0) |
| * Total days on Breast feeding | 0.0 (0.0, 32.0) | 1.0 (0.0, 39.0) | 0.0 (0.0, 28.0) | 62.0 (0.0, 149.0) |
| **Daily formula intake (ml/subject) in the mITT population expressed as Median (Q1-Q3)** | | | | |
|  | **Control (N=45)** | **Synbiotic (N=44)** | **Prebiotic (N=39)** | **Reference (N=28)** |
| * Birth - Day 3/5, | 120.0 (75.0, 200.0) | 120.0 (70.0, 192.5) | 134.5 (65.0, 200.0) | 50.0 (0.0, 150.0) |
| * Day 4/6 - Week 1 | 150.0 (60.0, 270.0) | 180.0 (35.0, 282.5) | 162.5 (35.0, 300.0) | 60.0 (0.0, 270.0) |
| * Week 2 | 150.0 (60.0, 360.0) | 200.0 (30.0, 377.5) | 185.0 (60.0, 360.0) | 10.0 (0.0, 240.0) |
| * Week 3 | 180.0 (60.0, 420.0) | 240.0 (60.0, 580.0) | 240.0 (60.0, 380.0) | 0.0 (0.0, 270.0) |
| * Week 4 | 235.0 (70.0, 520.0) | 240.0 (60.0, 695.0) | 320.0 (30.0, 450.0) | 0.0 (0.0, 280.0) |
| * Week 5 - Week 8 | 260.0 (80.0, 630.0) | 280.0 (80.0, 720.0) | 360.0 (100.0, 600.0) | 0.0 (0.0, 420.0) |
| * Week 9 - Week 12 | 300.0 (0.0, 700.0) | 350.0 (90.0, 870.0) | 505.0 (140.0, 720.0) | 85.0 (0.0, 580.0) |
| * Week 13 - Week 16 | 360.0 (0.0, 720.0) | 400.0 (60.0, 900.0) | 520.0 (0.0, 750.0) | 100.0 (0.0, 600.0) |

**Supplementary Table 5: Results of the main outcome of the interventions expressed as Estimated Mean ± SEM (PP population)**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Bifidobacteria 1 | Bifidobacteria (Log10 copies/g)2 | *Enterobacteriaceae* 1 | *Bacteroides distasonis/*  *fragilis* group1 | Atopobium group1 | *Clostridium histolyticum/ Clostridium lituseburense* group1 | *Eubacterium rectale*–*Clostridium coccoides* group1 | *Lactobacillus*–*Enterococcus* group1 | Acetate  (mmol/Kg feces) | pH |
| Reference | Day3/5 | 30.1 (6.5) | 6.6 (0.5) | 11.7 (3.5) | 1.5 (0.7) | 0.5 (0.2) | 4.0 (3.2) | 0.7 (0.2) | 3.3 (1.4) | 34.9 (5.0) | 5.9 (0.1) |
|  | Week 2 | 35.2 (8.0) | 8.1 (0.4) | 10.7 (2.8) | ND | ND | ND | ND | ND | 40.4 (5.8) | 5.8 (0.1) |
|  | Week 4 | 53.4 (6.0) | 8.0 (0.2) | 5.4 (2.0) | 1.8 (1.1) | 3.1 (1.3) | 0.6 (0.2) | 2.3 (0.8) | 3.2 (2.6) | 53.8 (7.2) | 5.9 (0.1) |
|  | Week 8 | 38.0 (4.9) | 8.1 (0.2) | 4.7 (1.4) | 1.0 (0.3) | 3.4 (1.1) | 1.1 (0.5) | 2.9 (1.1) | 1.4 (0.7) | 54.2 (6.3) | 5.9 (0.1) |
|  | Week 12 | 41.8 (8.6) | 7.8 (0.4) | 5.4 (2.4) | 1.1 (0.5) | 5.0 (2.1) | 1.0 (0.3) | 3.7 (1.0) | 0.7 (0.3) | 50.3 (7.0) | 5.9 (0.2) |
|  | Week 16 | 43.3 (7.4) | 8.4 (0.2) | 7.9 (2.7) | 2.2 (0.9) | 5.1 (2.0) | 1.3 (0.6) | 5.3 (1.8) | 3.9 (1.4) | 55.4 (7.5) | 6.0 (0.2) |
|  | Week 22 | 46.6 (4.8) | 7.9 (0.4) | 4.4 (0.9) | ND | ND | ND | ND | ND | 73.2 (10.3) | 5.7 (0.2) |
| Control | Day3/5 | 1.3 (0.6) | 3.8 (0.3) | 10.9 (4.5) | 0.6 (0.3) | 0.0 (0.0) | 0.4 (0.2) | 0.2 (0.1) | 0.9 (0.5) | 18.3 (2.7) | 6.1 (0.1) |
|  | Week 2 | 11.7 (3.6) | 5.6 (0.5) | 19.2 (4.7) | ND | ND | ND | ND | ND | 38.4 (5.6) | 6.2 (0.1) |
|  | Week 4 | 10.0 (3.8) | 6.3 (0.5) | 4.5 (1.4) | 0.2 (0.1) | 0.1 (0.1) | 0.7 (0.2) | 0.5 (0.3) | 3.6 (1.6) | 46.7 (6.5) | 6.2 (0.1) |
|  | Week 8 | 18.3 (6.8) | 7.6 (0.5) | 3.2 (1.0) | 0.1 (0.0) | 0.1 (0.0) | 0.3 (0.1) | 0.9 (0.4) | 1.8 (0.7) | 56.9 (9.0) | 6.0 (0.1) |
|  | Week 12 | 29.4 (8.6) | 7.6 (0.4) | 2.7 (0.8) | 0.2 (0.1) | 0.1 (0.1) | 0.3 (0.1) | 1.3 (0.6) | 2.5 (0.9) | 70.0 (10.1) | 6.1 (0.1) |
|  | Week 16 | 27.0 (9.1) | 8.0 (0.4) | 1.9 (0.6) | 0.4 (0.2) | 0.3 (0.1) | 0.3 (0.1) | 2.2 (1.0) | 2.1 (0.9) | 73.3 (10.9) | 6.0 (0.1) |
|  | Week 22 | 32.6 (8.7) | 8.1 (0.5) | 3.7 (1.0) | ND | ND | ND | ND | ND | 79.5 (12.2) | 6.0 (0.1) |
| Synbiotic | D3/5 | 17.2 (7.4) **b** | 7.3 (0.5) | 3.2 (1.4)b | 0.2 (0.1) | 0.0 (0.0) | 0.2 (0.1) | 0.3 (0.1) | 0.5 (0.2) | 35.9 (5.3)a | 5.7 (0.1) **b** |
|  | Week 2 | 33.3 (11.2) **b** | 7.9 (0.8) | 8.3 (2.2)b | ND | ND | ND | ND | ND | 49.7 (7.8) | 5.9 (0.1)**b** |
|  | Week 4 | 33.1 (13.4) **b** | 7.9 (0.6) | 3.3 (1.1) | 0.2 (0.1) | 0.1 (0.1) | 0.3 (0.1) | 0.3 (0.1) | 0.7 (0.3) | 50.4 (6.9) | 5.8(0.1)**b** |
|  | Week 8 | 32.4 (12.0) | 8.4 (0.5) | 2.2 (0.7) | 0.1 (0.0) | 0.2 (0.1) | 0.1 (0.0) | 0.3 (0.1) | 2.3 (0.8) | 47.6 (7.4) | 6.0 (0.1) |
|  | Week 12 | 38.4 (11.8) | 8.4 (0.5) | 1.9 (0.6) | 0.3 (0.1) | 0.3 (0.1) | 0.1 (0.1) | 0.8 (0.4) | 1.5 (0.6) | 65.2 (9.9) | 5.9 (0.1) |
|  | Week 16 | 37.1 (12.1) | 8.5 (0.4) | 2.2 (0.6) | 0.4 (0.1) | 0.3 (0.2) | 0.2 (0.1) | 1.6 (0.8) | 1.6 (0.6) | 71.0 (10.5) | 5.9 (0.1) |
|  | Week 22 | 36.3 (8.6) | 7.7 (0.4) | 3.8 (0.8) | ND | ND | ND | ND | ND | 82.1 (11.4) | 6.0 (0.1) |
| Prebiotic | D3/5 | 0.8 (0.5) | 3.6 (0.3) | 8.2 (4.6) | 0.3 (0.1) | 0.0 (0.0) | 0.2 (0.1) | 0.5 (0.3) | 1.1 (0.8) | 17.4 (3.0) | 6.0 (0.1) |
|  | Week 2 | 11.6 (4.1) | 6.2 (0.6) | 26.2 (7.3) | ND | ND | ND | ND | ND | 42.8 (7.2) | 6.0 (0.1) |
|  | Week 4 | 24.4 (11.3) | 6.9 (0.6) | 5.5 (2.0) | 0.7 (0.3) | 0.2 (0.1) | 0.2 (0.1) | 0.4 (0.2) | 1.7 (0.8) | 54.6 (8.4) | 5.9 (0.1)b |
|  | Week 8 | 21.1 (9.5) | 7.7 (0.6) | 1.9 (0.7) | 0.1 (0.0) | 0.1 (0.1) | 0.3 (0.1) | 0.6 (0.3) | 4.0 (1.8) | 53.5 (9.7) | 6.1 (0.1) |
|  | Week 12 | 29.2 (10.6) | 8.0 (0.6) | 2.1 (0.8) | 0.2 (0.1) | 0.3 (0.2) | 0.2 (0.1) | 1.2 (0.7) | 1.3 (0.6) | 67.9 (12.3) | 5.9 (0.2) |
|  | Week 16 | 25.8 (10.2) | 7.8 (0.5) | 2.0 (0.8) | 0.4 (0.2) | 0.7 (0.4) | 0.3 (0.2) | 1.1 (0.6) | 1.5 (0.7) | 64.2 (11.1) | 6.2 (0.2) |
|  | Week 22 | 31.6 (9.6) | 7.1 (0.5) | 3.2 (0.9) | ND | ND | ND | ND | ND | 81.0 (14.4) | 6.4 (0.2) |

1 Data obtained by Fluorescent *in Situ* Hybridization (FISH) and reported as % of total bacteria, 2 Data obtained by Q-PCR , SEM: Standard error of the mean

ND: Not determined

ap<0.0001 bp<0.05 .

A generalised linear mixed model procedure with lognormal distribution and identity link function was used and intervention effects were compared using z-test.

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**Legends:**

**Supplementary Figure 1:**

Consort Flow Chart

**Supplemental Figure 2**:

Observed proportion of infants with skin disorders derived from the adverse events (AEs). Skin disorders included AD, eczema, contact dermatitis, seborrhoea, diaper rash and skin rash. Data is reported in the All Subject Treated (AST) population and expressed in Mean (SE).

Pregnant mothers assessed for eligibility (n= 218)

Allocated to the Prebiotic formula (n=51)

Efficacy analysis (mITT) (n=39)

Missing baseline and post-baseline stool samples (n=12)

Efficacy analysis (PP) (n=20)

Use of other formula (n=9)

Missing baseline stool sample (n=13)

Study product not started (n=2)

Missing data (n=1)

Antibiotic use (n=1)

Other reasons (n=5)

Safety analysis (Treated) (n=48)

Allocated to the Synbiotic formula (n=52)

Completed the study (n=43)

Efficacy analysis (mITT) (n=45)

Missing baseline and post-baseline stool samples (n=7)

Efficacy analysis (PP) (n=27)

Use of other formula (n=11)

Missing baseline stool sample (n=5)

Study product not started (n=1)

Missing data (n=3)

Antibiotic use (n=1)

Other reasons (n=4)

Safety analysis (Treated) (n=51)

Allocated to the Control formula (n=50)

Efficacy analysis (mITT) (n=45)

Missing baseline and post- baseline stool samples (n=5)

Efficacy analysis (PP) (n=29)

Use of other formula (n=12)

Missing baseline stool sample (n=5)

Study product not started (n=1)

Antibiotic use (n=1)

Other reasons (n=2)

Safety analysis (Treated) (n=48)

New-borns screened for eligibility (n=199)

Enrolled in the study (n=183)

Not meeting inclusion-exclusion criteria (n=12)

Other reasons (n=4)

C-section delivery

Randomized (n=153)

*Vaginal delivery*

*Non-randomized* *(n=30)*

Excluded (n=19)

Withdrew informed consent (n=7)

Not meeting inclusion criteria (n=4)

Other reasons (n=8)

**Supplementary Figure 1**

Allocated

Enrolment

Analysis

Follow up

Completed the study (n=39)

Completed the study (n=45)

Completed the study (n=45

Completed the study (n=45)

Follow up

**Supplementary Figure 2**

