**Supplementary Table 2.** Human trials assessing the effects of a single prebiotic ingredient.

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| **Reference** | **Prebiotic** | **Dose** | **Objectives** | **Subjects and main features of the trial** | **Outcomes** |
| Euler et al., 2005 [18] | FOS | 0.15 or 0.3 g/100 mL | Assessment of beneficial prebiotic effect on the microbiota of gastrointestinal tract of infants | Seventy-two (58 Formula fed and 14 breast fed) healthy term infants aged 2 to 6 weeks enrolled in a 5-week, prospective, randomized, crossover, single-site study with a nonrandomized human milk comparator group | Fructo-oligosaccharide at higher supplementation dosage resulted in more frequent and significantly softer stools but infant formula supplemented with 0.15 or 0.3 g/100 mL FOS had minimal effect on fecal flora *and C. difficile* toxin |
| Brunser et al., 2006 [19] | FOS | 0.2g/100 mL | To evaluate the effects of a milk formula supplemented with La1 or FOS upon the colonic microbiota of bottle-fed infants compared to breast-fed infants | Ninety infants close to 4 months of age were randomized into one of three groups to be blindly assigned to receive for 13 weeks: a) an infant formula, b) the same formula with fructo-oligosaccharides or c) with La1. At the end of this period, all infants received the control formula for 2 additional weeks. Twenty-six infants, breastfed throughout the study, were recruited to form breastfed group | The increase of bifidobacteria in the FOS group was not significant when expressed as absolute counts, but it became significant when expressed as percentage of the total bacteria population detected by FISH |
| Kapiki et al., 2007 [20] | FOS | 0.4 g/100 mL | To compare Bifidobacteria counts in the stool flora of fructo-oligosaccharides at a concentration of 0.4 g/dL or the same formula with maltodextrin as a placebo | Double-blind study in which 56 bottle-fed preterm infants were. randomized to receive for 14 days either a formula with prebiotic or placebo. Faecal samples were taken at inclusion day and one week later. The number of bifidobacteria in the stools, stool characteristics and somatic growth wererecorded during the study | Numbers of bifidobacteria and Bacteroids in the stools were significantly higher in the treated group same time, while *Escherichia coli* and enterococci were reduced. Supplementation also increased stool frequency per day |
| Kim et al., 2007 [26] | Inulin | 1.5 g/d | To investigate the effects of native inulin in formula-fed babies | In this study 3 weeks of inulin consumption were followed by 3 weeks without or vice versa. The study group consisted of 14 babies with an average age of 12.6 weeks (± 6.4 weeks). The influence of inulin on the microbial composition, pH, consistency and amount of feces, and on frequency of defecation was assessed | The consumption of inulin increased the content of *Bifidobacterium* and *Lactobacillus* in the feces of formula-fed babies, without affecting the number of Bacteroides or the total anaerobic count. With inulin there was a trend for stools to become softer and for the amount of feces to increase significantly. Frequency of defecation was not affected by the consumption of inulin |
| Shibata et al., 2009 [23] | Kestose | 1g/d (<1 year old)  2g/d (>1 year old) | To assess the clinical effect of kestose on the treatment of atopic dermatitis (AD) in infants | A randomized, double-blind, placebo-controlled trial was carried out using 15 and 14 infants with AD in the kestose group and placebo groups, respectively. One to 2 g kestose and maltose were administered to the subjects in the kestose and placebo groups, respectively, every day for 12 weeks. Clinical evaluations of AD using Severity Scoring of Atopic Dermatitis (SCORAD) and the enumeration of bifidobacteria in the feces using real-time PCR were performed at weeks 0, 6, and 12. | The medians of the SCORAD score were significantly lower in the kestose group than in the placebo group on both Week 6 and Week 12. No significant correlation was found between the improvement of the SCORAD score and the count of bifidobacteria |
| Khoshoo et al., 2010 [21] | FOS | 0.35 g/100 mL | To evaluate the tolerance of a peptide-based formula with insoluble and prebiotic fiber in children with compromised gut function | A 6-week randomized, double-blind, cross-over clinical study. Fourteen children with gastrointestinal dysmotility (n9), Crohn’s disease (n3), or mild short bowel syndrome (n2) were randomized to receive a formula with or without FOS and 3.8 g insoluble fiber/L.for 2 weeks followed by a 5-day washout period and then the second diet for another 2 weeks. Stool frequency, stool consistency, and tolerance were evaluated | Stool frequency did not differ by formula. Stool consistency did differ with less hard stools occurring with use of fiber and more watery stools occurring with control formula. No significant differences were observed in tolerance between the two formulas |
| Xia et al., 2012 [22] | FOS | 0.2 or 0.3 g/100 mL | To examine the effect of supplementation of an infant formula with FOS on select groups of intestinal bacteria in term infants | A randomized, controlled, and multicenter study. A total of 101 healthy term infants were fed human milk, a commercially available milk-based infant formula, or infant formula supplemented with FOS for approximately 4 weeks. Dietary intake, stool, and tolerance events were recorded. Fresh stool samples were collected approximately 27 days after feeding the diets. Bacterial populations were quantified by real-time PCR | There were no differences among treatment groups in the predominant stool consistency and average daily stool number during the entire study. Similarly, there was no significant difference in the frequency of feedings with spit-up or vomit during the entire study. The FOS supplementation at either dose did not significantly increase the bifidobacterial or lactobacilli populations, or decrease the populations of *C. difficile*, *E. coli*, or Bacteroides |
| López-Velázquez et al., 2013 [24];  López-Velázquez et al., 2015 [25] | Fructans derived from *Agave tequilana* (Metlin® and Metlos®) | 0.3 or 0.5 g/100 mL (Metlin®);  7 g/100 mL (Metlos®) | To demonstrate the safety and efficacy as prebiotics of Metlin® and Metlos® in newborns | A prospective, randomized, controlled, double blind study, with a pilot study design. 600 healthy term babies were allocated into five groups: Group 1: Formula added with probiotics (*Lactobacillus rhamnosus* was the probiotic used in all the groups) + Metlin + Metlos; Group 2; Formula added with probiotics + Metlin; Group 3: Formula added with probiotics + Metlos;Group 4; Formula added with probiotics, and Group 5: Formula without probiotics and prebiotics. A  reference group of breast milk feeding was included too. Biological samples were taken at 20 ± 7 days, and three months of age. In the first study, stools frequency, stools consistency, gastrointestinal intolerance (frequency of abdominal distension, flatulency, regurgitations, vomiting) were evaluated. Efficacy outcomes include the changes on gut microbiota, levels of saliva IgA, C-reactive protein and serum ferritin, triglycerides, cholesterol, and lipoproteins, and urine deoxipyridinoline (DPD) | Fructans derivate from agave and added to infant formula are safe and well tolerated by healthy term babies.The changes on abundance of Bifidobacteria showed a tendency to increase in those groups fed with breast milk and with fructans and probiotics. IgA saliva levels difference were significant only in the Probiotics +Metlin +Metlos group when comparing the values at the end of the monitoring with the value obtained at baseline. serum concentrations of total cholesterol, triglycerides and lipoproteins were significant different among the groups at the end of the study, with significantly lower values in the group supplemented with Metlin + Metlos + probiotics |
| Underwood et al., 2004 [17] | GOS | 0.25g/100mL (week 1);  0.5 g/100mL (week 2);  1.0 g/100mL (week 3);  1.5 g/100mL (week 4);  2.0 g/100mL (week 5) | To determine the impact of increasing doses of prebiotic oligosaccharides and of an ‘‘all-human diet’’ on the intestinal microbiota of premature infants | Twelve premature infants receiving formula feedings were randomly assigned to receive either galacto-oligosaccharide (F+GOS) or a pooled concentrated donor human milk product containing human milk oligosaccharides (F+HMO) in increasing doses during a 5-week period. Serial stool specimens from each infant were analyzed by terminal restriction fragment length polymorphism and quantitative polymerase chain reaction for bacterial composition. Asecond group of 15 premature infants received their mother’s own milk fortified with either a concentrated donor human milk product (H+H) or a bovine powdered fortifier (H+B) | All of the infants studied had relatively low levels of bifidobacteria and no measurable Lactobacilli. None of the prebiotic interventions resulted in significant increases in bifidobacteria compared with baseline specimens or the H+B group. Infants from the F+GOS and F+HMO groups demonstrated an increase in relative numbers of Clostridia with increasing doses. |
| Closa-Monasterolo et al., 2013 [27] | Oligofructose-enriched inulin | 0.8 g/100 mL | To demonstrate the efficacy, safety and tolerance of the prebiotic supplemented infant formula during the first 4 months of life | In this double-blind, randomized, placebo-controlled and parallel trial, formula fed healthy term newborns were randomized to receive a control (controls) or SYN1 supplemented infant formula (SYN1). Breastfed newborns (BF) were also followed for comparison. After 4 months 68 controls, 63 SYN1 and 57 BF completed the study. Anthropometry, water balance, blood parameters, adverse events, stool frequency and characteristics and fecal microbiota were assessed | SYN1 infants showed a microbiota composition closer to that of BF infants, with a trend towards higher *Bifidobacterium* cell counts, softer stools and a higher deposition frequency compared to controls. There were no differences between formulas in anthropometry and relevant adverse events, water balance or blood parameters |
| Giovannini et al., 2014 [12] | GOS | 0.4 g/100 mL | To investigate the effects of a GOS-supplemented formula on the intestinal microbiota in healthy term infants, with a specific consideration for gastrointestinal symptoms as colic, stool frequency and consistency, regurgitation | A total of 199 breastfed infants and 163 formula-fed infants (80 in the control and 83 in the study group) were enrolled by the 15th day of life and grouped according to the type of feeding: breast milk, GOS- supplemented infant milk formula (study formula), and standard infant milk formula (control formula) | In the GOS supplemented group the incidence of colic was lower with respect to the control group. A significantly lower count of *Clostridium* and a higher count of *Bifidobacterium* were found when comparing study formula and control formula in infants with colic. In children with colic the ratio between *Clostridium* count and *Bifidobacterium* and *Lactobacillus* counts was in favor of the latter two when considering the GOS-supplemented formula group with respect to the control one |
| Williams et al., 2014 [13] | GOS | 0,4 g (EF4) or 0.88 g (EF8) GOS/100 mL or a Control formula until day of life (DOL) 119 | To evaluate the tolerance of formulas supplemented with 2 different levels of GOS versus a control formula (CF) or human milk | 175 healthy, full-term infants were enrolled for a 3-group controlled, double-blind, multicenter study, and a concurrently enrolled, nonrandomized human milk–fed group (HM) by 8 days of age. Infants were randomized to be fed formula supplemented with either 4 g (EF4) or 8 g (EF8) GOS/L or a CF until day of life 119 | Intake, tolerance to feedings, stool patterns and consistency was recorded by parent each day from enrollment to DOL 35, and for 3 days before DOL 56, 84, and 119. There was a significantly higher percentage of watery stools in the higher dosage versus the CF group from study day 1 to Day of life 14 |
| Nowacki et al., 2014 [28] | Oligofructose | 0.3 g/100 mL | To investigate the effect of high sn-2 palmitate alone and in combination with oligofructose (OF) on the stool concentrations of fatty acid soaps and calcium as well as stool consistency and frequency in formula fed infants | This double-blind trial randomized 165 healthy term infants 25–45 days old to receive Control formula (n = 54), formula containing high sn-2 palmitate (sn-2; n = 56), or formula containing high sn-2 palmitate plus 3 g/L OF (sn-2+OF; n = 55). A non-randomized human milk (HM)-fed group was also included (n = 55). The primary endpoint, stool composition, was determined after 28 days of feeding. Stool consistency, GI tolerance and hydration were assessed at baseline, day 14 (GI tolerance only) and day 28 | Infants fed sn-2+OF had reduced stool palmitate soaps compared to both control and sn-2. Stool total soaps and calcium were lower in the sn-2+OF group than either control or sn-2. The stool consistency score of the sn-2+OF group was lower than control and sn-2, but higher than the HM-fed group. GI tolerance was similar in all groups |
| Sierra et al., 2015 [14] | GOS | Starter formula = 0.44 g/100 mL GOS; Follow-on formula = 0.50 g/100  mL GOS | Assessment of the effects of a GOS containing starter formula and then a subsequent feeding of a GOS-containing follow-on formula on healthy full term infants | 365 healthy term infants enrolled before 8 weeks of age and randomLy assigned to a formula with or without GOS, until 12 months of age. The incidence of infections and allergy manifestations, the antibiotics prescribed and faecal characteristics were recorded up to 12 months of age, while faecal samples were collected up to 4 months for the measurement of secretory immunoglobulin A, short-chain fatty acids and microbiota | The feeding of GOS-containing infant formula produced a definite prebiotic effect consisting of changes in faecal composition and microbiota, and in faecal consistency and the frequency of defaecation. No changes in the incidence of infection or allergic manifestation during the first year of life were observed |
| Boženský et al., 2015 [15] | GOS | 0.5 g/100 mL | To evaluate the effects of a hypoallergenic (HA) formula supplemented with prebiotic galacto-oligosaccharides on the severity of atopic manifestations | One hundred and three children were randomised (age at recruitment 6–8 weeks) into two groups.The first group received formula with hydrolysed protein (HA); the second group received an identical formula with a supplement of GOS. The duration of the study was six months. The primary outcome of the study was a difference in the severity of atopic dermatitis measured using SCORAD (Scoring Atopic Dermatitis) criteria. Secondary outcomes were anthropometry together with the tolerance and incidence of infections | No statistically significant difference between the evaluated groups was observed. There were no statistically significant differences in anthropometry, or the tolerance or incidence of infections. Although there is no evidence, that consumption of a hypoallergenic infant formula enriched with prebiotic GOS had any effect on SCORAD, it was safe and well tolerated |
| Dilli et al., 2015 [29] | Inulin | 900 mg/d | To test the efficacy of prebiotic and probiotic, alone or combined (synbiotic), on the prevention of necrotizing enterocolitis (NEC) in very low birth weight (VLBW) infants | Prospective, randomized, double-blind, controlled trial in which VLBW infants (n = 400) were assigned to a control group and 3 study groups that were given probiotic (*Bifidobacterium lactis*), prebiotic (inulin), or synbiotic (*B. lactis* plus inulin) added to breastmilk or formula for a maximum of 8 weeks before discharge or death. The primary outcome was NEC. Secondary outcomes were time to reach full enteral feeding, late-onset sepsis, length of NICU stay, and death | In VLBW infants, probiotic and synbiotic decreased NEC but not prebiotic (inulin) alone. The times to reach full enteral feeding were faster, the rates of clinical nosocomial sepsis were lower, stays in the neonatal intensive care unit were shorter, and mortality rates were lower for infants receiving probiotics, prebiotics, or synbiotic than controls |
| Paganini et al., 2017 [16] | GOS | 7.5 g/d | To evaluate the efficacy and safety of a formula added with GOS combined with a low dose (5 mg/day) of highly bioavailable iron | A total of 155 Kenyan infants aged 6.5–9.5 months received daily (1) a formula without iron (control); (2) the identical formula but with 5 mg iron or (3) the identical formula as (2) but with 7.5 g GOS (FeGOS group) | No significant differences in gut microbiota composition when control and FeGOS groups were compared, with the exception that the abundance of virulence and toxin genes of all pathogens was significantly lower in the FeGOS group compared with the control and Fe groups |