**Supplementary Table 8.** Trials assessing the effects of pectin hydrolysate-derived acidic oligosaccharides (pAOS) combined with GOS/FOS.

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| **Reference** | **Prebiotic** | **Dose** | **Objectives** | | **Subjects and main features of the trial** | **Outcomes** |
| Fanaro et al., 2005 [66] | GOS/FOS/pAOS | 0.6 g/100 mL (GOS/FOS);  0.2 g/100 mL (pAOS) | To investigate the effect of pAOS and GOS/FOS/pAOS on intestinal microbiota, stool characteristics as well as acceptance and tolerance | | In this prospective, randomized,double blind study, 46 term infants were fed a standard formula supplemented with either maltodextrin as control (n = 15), or with 0.2 g pAOS (n = 16), or with the latter plus 0.6 g GOS/FOS; n = 15). Fecal microbiota and pH were measured. Stool characteristics and possible side effects (crying, vomiting, and regurgitation) were recorded | There was no difference in the bifidobacteria counts between the control and the group supplemented with pAOS alone. In infants fed the combination of pAOS and GOS/FOS, bifidobacteria were increased. There was also a significant increase of the counts of lactobacilli in the group fed the combination of acidic and neutral oligosaccharides. In the group fed GOS/FOS/pAOS, the stool frequency was significantly higher. The stool consistency scores were lowest in the group fed the complete oligosaccharides mixture, but also, in the group fed the pAOS, the stool consistency was significantly softer in comparison with the group fed the standard formula |
| Magne et al., 2008 [67] | GOS/FOS  GOS/FOS/pAOS | 5.8/ 0.54 g per 100 mL  (GOS/FOS);  5.8/ 0.54/0.2 g per 100 mL  (GOS/FOS/pAOS) | | To study the effect on fecal microbiota of a formula with prebiotic oligosaccharides alone or in combination with acidic oligosaccharides in infants | A double-blind, placebo-controlled, randomised intervention trial in which 82 healthy, full-term, partially breast-fed children, from 1 week to 3 months old, were assigned to either control group, or GOS/FOS group, or GOS/FOS/pAOS group. Children were studied for the duration of the partial formula feeding period and every 2 weeks for 2 months after breast-feeding cessation. The total bacteria count and the proportion of 7 bacterial families were determined using in situ hybridisation coupled to flow cytometry | Compared with the control group, there was an increase of the *Bifidobacterium* genus and a decrease of proportions for the Bacteroides and the *Clostridium coccoides* in both oligosaccharide groups. The proportion of bifidobacteria was significantly higher in the GOS/FOS/pAOS compared with the GOS/FOS group |
| Westerbeek et al., 2008 [79];  Westerbeek et al., 2010 [69] | 80% GOS/FOS and 20% AOS | 1.5 g/kg/day maximum | To investigate the effect of enteral supplementation of a prebiotic mixture on serious infectious morbidity in preterm infants | | A double-blind placebo controlled randomised trial; 113 preterm infants were randomly allocated to receive enteral acidic and neutral oligosaccharides supplementation or placebo (maltodextrin) between day 3 and 30 of life. Primary outcome was serious infectious morbidity | Enteral supplementation with GOS/FOS/AOS did not significantly reduce the risk of serious infections in preterm infants |
| Grüber et al., 2010 [75] | GOS/FOS  (ratio 9:1)  (85 weight percent), and specific pAOS (15 weight percent) | 0.68g/100 mL (GOS/FOS) and 0.12g/100 mL (pAOS) | To assess the incidence of fever episodes in healthy term-born infants given the prebiotic mixture during the first year of life | | A double-blind, placebo-controlled, randomized prospective nutritional intervention study. Healthy, term infants age up to 8 weeks with low atopy risk. The intention-to-treat population was composed of 414 infants in the prebiotics group, 416 infants in the control group, and 300 infants in the breast-feeding group. The observation period was until the age of 1 year | Atopic dermatitis occurred in significantly fewer infants from the prebiotic group than from the control group. The cumulative incidence of atopic dermatitis in the prebiotic group was in the low range of the breast-feeding group |
| Westerbeek et al., 2011 [70] | 72% GOS/8% FOS and 20% pAOS | 1.5 g/kg/d maximum | To determine the effect of enteral supplementation of prebiotics on intestinal inflammation in preterm infants | | In this randomized controlled trial, preterm infants received enteral supplementation of prebiotics (GOS/FOS/AOS , n = 55) or placebo (maltodextrin, n = 58) between d 3 and 30 of life. Fecal IL-8 and f-calprotectin were assessed at baseline, d 7, 14, and 30 of life. In total, 113 infants were included | Enteral supplementation of prebiotics had no effect on fecal IL-8 and f-calprotectin |
| Westerbeek et al., 2011 [71] | 80% GOS/FOS and 20% | 1.5 g/kg/d maximum | To evaluate the effect of enteral supplementation of the prebiotic mixture on intestinal permeability of preterm infants | | In this randomized controlled trial preterm infants received enteral supplementation of GOS/FOS/AOS (n=55) or placebo (maltodextrin, n=58) between days 3 and 30 of life. 113 infants were included. The supplementation of GOS/FOS/AOS or placebo was administered in increasing doses between days 3 and 30 of life to breast milk or preterm formula. Intestinal permeability, reflected by the urinary lactulose/mannitol (L/M) ratio after oral ingestion of lactulose and mannitol, was assessed at three time points: before the start of the study (t0), at day 4 (t1) and at day 7 (t2) of life | GOS/FOS/AOS had no effect on the L/M ratio between t0 and t2 |
| Westerbeek et al., 2011 [80] | 80% GOS⁄FOS (ratio 9:1) and 20% pAOS | 1.5 g ⁄ kg ⁄ day maximum | To study the effects of enteral supplementation of the prebiotic mixture on stool viscosity, stool frequency and stool pH in preterm infants | | In this explorative RCT preterm infants received enteral supplementation with GOS ⁄ FOS ⁄ pAOS or placebo (maltodextrin) between days 3 and 30 of life. Stool samples were collected at day 30 after birth. 113 of 208 eligible preterm infants entered the study: prebiotics (n = 55) and placebo group (n = 58). The supplementation of prebiotics or placebo was administered in increasing doses between days 3 and 30 of life to breast milk or preterm formula in the intervention group | Stool viscosity at day 30 was lower in the prebiotics group compared with the placebo group. Stool pH at day 30 was lower in the in the prebiotics group compared with the placebo group |
| Stam et al., 2011 [74] | GOS/FOS  (ratio 9:1) and pAOS | 0.8 g/100 mL (0.68 g/100 mL neutral and 0.12 g/100 mL acidic oligosaccharides) | To evaluate the effect of study prebiotics on the specific immunoglobulin responses to *Haemophilus influenza* type b (Hib) and tetanus immunization in healthy non-atopic infants during the first year of life | | This substudy has been embedded in a multinational multicenter RCT (n=1130 children). Only data of the Dutch children, 80 in the prebiotics group and 84 in the control group, were used for this substudy. They all followed the national vaccination schedule leading to a homogeneous group. Blood was sampled at 6 and 12 months of age | No effect of prebiotics supplementation on vaccination specific antibody levels was found in children up to the age of 12 months; the vaccine specific antibody levels in infants fed the study prebiotics or a control diet were similar during the first year of life. The authors hypothesize that this specific prebiotic mixture mainly promotes Th1 and Treg dependent immune responses and induces a down regulation of IgE-mediated allergic responses, while the desired vaccine-specific serum antibody responses remain intact |
| van Stuijvenberg et al., 2011 [78] | GOS/FOS/pAOS | 0.8 g/100 mL (0.68 g/100 mL neutral and 0.12 g/100 mL acidic oligosaccharides) | To assess the effect of adding prebiotics to standard formula feeding on the number of fever episodes in the first year of life | | In the present randomised, double-blind, placebo-controlled trial 830 healthy term infants, without a first-degree family history of allergic disease, of mothers who indicated to give only formula feeding were randomised either to receive a standard non-hydrolysed cows' milk-based formula to which a mixture of specific oligosaccharides was added (prebiotics group (PG)), or to receive a similar formula without oligosaccharides (control group (CG)). A separate reference group consisted of 300 breast-fed infants. The primary outcome was the number of fever episodes prospectively documented by the parents | There was no difference in the number of fever episodes between the PG (median value 1·19; 25th-75th percentile 0·09-2·34) and CG (1·16; 25th-75th percentile 0·06-2·38). The median number of fever episodes in the separate breast-feeding reference group was 1·24 (25th-75th percentile 0·51-3·45). There was no effect of adding specific prebiotics to standard formula feeding in reducing the number of fever episodes in the present study |
| Piemontese et al., 2011 [68] | GOS/FOS/pAOS | 0.8 g/100 mL (0.68 g/100 mL neutral and 0.12 g/100 mL acidic oligosaccharides) | To assess the tolerance and safety of a formula containing a of oligosaccharides in early infancy | | A randomized, double-blind, placebo-controlled trial including healthy term infants. Infants were recruited before the age of 8 weeks, either having started with formula feeding or being fully breast-fed (breastfeeding group). Formula-fed infants were randomized to feeding with a regular formula containing a mixture of neutral oligosaccharides and pectin-derived acidic oligosaccharides (prebiotic formula group) or regular formula without oligosaccharides (control formula group). Growth, tolerance and adverse events were assessed at 8, 16, 24 and 52 weeks of age | The prebiotic and control groups showed similar mean weight, length and head circumference, skin fold thicknesses, arm circumference gains and stool frequency at each study point. As far as the anthropometric parameters are concerned, the prebiotic group and the control group did not attain the values shown by the breastfeeding group at any study point. The stool consistency in the prebiotic group was softer than in the control group at 8, 16 and 24 weeks and closer to that of the breastfeeding group. There was no difference in the incidence of adverse events between the two formula groups |
| Westerbeek et al., 2013 [72] | GOS/FOS/pAOS | 1.5 g/kg/d maximum | To study the effects of a prebiotic mixture on the fecal microbiota and microenvironment in preterm infants | | In this randomised controlled trial, preterm infants received enteral supplementation of prebiotics or placebo (maltodextrin) between days 3 and 30 of life. Fecal microbiota, as measured with fluorescent in situ hybridisation (FISH), and microenvironment [short-chain fatty acids (SCFAs), pH, sIgA] were measured before the start of the study and at days 7, 14 and 30 of life | Enteral supplementation of the prebiotic mixture increased the total bacteria count at day 14, but not at day 30. There was a delayed intestinal colonisation of all bacteria. Enteral supplementation of the prebiotic mixture decreased the faecal pH and there was a trend toward increased acetic acid compared to the placebo group. There was no effect on sIgA. Enteral supplementation of a prebiotic mixture of neutral and acidic oligosaccharides increases the postnatal intestinal colonisation |
| Niele et al., 2013 [77] | 80 % GOS/FOS and 20 % pAOS | 1.5 g/kg/d maximum | To determine the effect of short-term enteral supplementation of GOS/FOS/pAOS during the neonatal period in preterm infants on the incidence of allergic and infectious diseases during the first year of life | | In a randomized controlled trial, 113 preterm infants were allocated to receive enteral neutral and acidic oligosaccharide supplementation or placebo between days 3 and 30 of life. In total, 94/98 of the eligible, surviving infants participated in this follow-up study Incidence of allergic and infectious diseases was assessed by validated questionnaires | The incidence of atopic dermatitis, bronchial hyper-reactivity and infections of the upper respiratory, lower respiratory, and gastrointestinal tract was not different between the groups. Adjustment for potential confounding factors did not change the results of the primary analysis |
| van den Berg et al., 2013 [73] | 80% (GOS/FOS) in combination with 20% pAOS | 1.5 g/kg/d maximum | To determine the effect of the prebiotic mixture on antibody concentrations after DTaP-IPV-Hib immunization in preterm infants | | In this randomized clinical trial, preterm infants received enteral supplementation with GOS/FOS/pAOS or placebo (maltodextrin) between days 3 and 30 of life. In total, 113 infants were included. Blood samples were collected at 5 and 12 months of age | Baseline and nutritional characteristics were not different in both groups. Geometric mean titers were not different after prebiotic supplementation at 5 months, Ptx (37/44 EU/ml), FHA (78/96 EU/ml), Prn (78/80 EU/ml), Diphtheria (0.40/0.57 IU/ml), Tetanus (0.74/0.99 IU/ml) and Hib (0.35/0.63 µg/ml), and at 12 months Ptx (55/66 EU/ml), FHA (122/119 EU/ml), Prn (116/106 Eu/ml), Diphtheria (0.88/1.11 IU/ml), Tetanus(1.64/1.79 IU/ml) and Hib (2.91/2.55 µg/ml) |
| Boyle et al., 2016 [76] | GOS/FOS (ratio 9 : 1; 85 weight per cent) and pAOS (15 weight per cent) | 0.8 g/100 mL (0.68 g/100 mL GOS/FOS and 0.12 g/100 mL pAOS) | To evaluate whether partially hydrolysed whey formula containing oligosaccharides can prevent eczema in high-risk infants | | Randomized double‐blind controlled trial of pHF‐OS vs standard cow's milk formula. Infants with a family history of allergic disease were randomized to active (n = 432) or control (n = 431) formula until 6 months of age if formula was introduced before 18 weeks. Primary outcome was cumulative incidence of eczema by 12 months in infants randomized at 0–4 weeks (375 pHF‐OS, 383 control). Secondary outcomes were cumulative incidence of eczema by 12 or 18 months in all infants randomized, immune markers at 6 months and adverse events | Eczema occurred by 12 months in 28.7% infants allocated to pHF‐OS at 0‐4 weeks of age, vs 28.7% control, and 30.8% pHF‐OS vs 30.3% control in all infants randomized. pHF‐OS did not change most immune markers including total/specific IgE; however, pHF‐OS reduced cow's milk‐specific IgG1 (P < 0.0001) and increased regulatory T‐cell and plasmacytoid dendritic cell percentages. There was no group difference in adverse events |