**Supplementary Table 3.** The link between GOS/FOS administration and infant immune system.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Reference** | **Prebiotic** | **Dose** | **Objectives** | **Subjects and main features of the trial** | **Outcomes** |
| Moro et al., 2006 [29] | GOS/FOS | 0.8 g/100 mL | To evaluate the effect of a prebiotic mixture of galacto- and long chain fructo-oligosaccharides on the incidence of atopic dermatitis (AD) during the first six months of life in formula fed infants at high risk of atopy | Prospective, double-blind, randomised, placebo controlled trial. A total of 102 infants in the prebiotic group and 104 infants in the placebo group completed the study. All infants were examined for clinical evidence of atopic dermatitis. In a subgroup of 98 infants, fecal flora was analyzed | Ten infants in the intervention group and 24 infants in the control group developed AD. Prebiotic supplements were associated with a significantly higher number of fecal bifidobacteria compared with controls |
| Arslanoglu et al., 2007 [33] | GOS/FOS | 0.8 g/100 mL | To test if the prebiotics mixture could have a preventive effect on the occurrence of infections during the first 6 months of infant life | Randomized, double-blind, placebo-controlled trial. Term infants with a parental history of atopy. The primary outcome measures were infectious episodes, number of infections requiring antibiotics, and incidence of infections. A total of 206 infants (104 in placebo group, 102 in scGOS/ lcFOS group) completed the study | Infants in the GOS/FOS group had fewer episodes of all types of infections combined and the cumulative incidence of recurring infections was significantly lower than that of the placebo |
| Van Hoffen et.al, 2009 [30] | GOS/FOS  (ratio 9:1) | 0.8 g/100 mL | To analyze the effect of GOS/FOS on the immune response in infants at risk for allergy | Double-blind randomized placebo-controlled study, infants received a hypoallergenic whey formula with IMMUNOFORTIS or 8 g/l maltodextrin (placebo) for 6 months. At 3 months of age, children were vaccinated with Hexavac against diphteria, tetanus, polio (DTP). At 6 months of age, plasma samples were collected from 84 infants (verum group n = 41, placebo group n = 43). Levels of total immunoglobulins (Ig) and of cow's milk protein (CMP)- and DTP-specific Ig were measured | GOS/FOS supplementation led to a significant reduction in the plasma level of total IgE, IgG1, IgG2 and IgG3, whereas no effect on IgG4 was observed. CMP-specific IgG1 was significantly decreased. DTP-specific Ig levels were notaffected |
| Bisceglia et al., 2009 [34] | GOS/FOS  (ratio 9:1) | 0.8 g/100 mL | To evaluate the effect of prebiotics on moderate hyperbilirubinaemia in healthy, term infants. | Prospective, double-blind, clinical trial performed on seventy-six consecutive newborns. Formula containing prebiotics or maltodextrins as placebo for 28 days. Bilirubin levels were determined by the transcutaneous bilirubin measurement within 2 h after birth (T1), at 24, 48 and 72 h and at 5, 7, 10 and 28 days of life | Neonates whose formula was supplemented with prebiotics showed a lower transcutaneous bilirubin that was statistically significant from 72 h of life throughout the duration of the dietary intervention |
| Bruzzese et al., 2009 [35] | GOS/FOS | 0.4 g/100 mL | To assess if prebiotics reduce the incidence of intestinal and respiratory infections in healthy infants. | A prospective, randomized, placebo-controlled, open trial was performed. 342 healthy infants aged between 15 and 120 days fed a standard infant formula or a prebiotic-supplemented formula for 12 months. Primary outcome measures were incidence of acute diarrhea, incidence of upper and lower respiratory tract infections, and number of antibiotic courses prescribed for respiratory infections | The rate of diarrheal episode/child was significantly lower in children receiving the GOS/FOS formula than in controls. The mean rate of antibiotic courses prescribed for children fed with GOS/FOS was significantly lower compared to controls |
| Schouten et al., 2011[31] | GOS/FOS | 0.8 g/100 mL | To test the effect of supplementation of GOS/FOS on the Ig-fLC (might be involved in the pathophysiology of allergic disease) plasma concentrations in infants at risk for allergy | Double-blind, placebo-controlled, randomized trial. Hypoallergenic whey formula containing 8 g/l of the GOS/FOS (Immunofortis®)mixture (n = 34) or maltodextrin as a placebo (n=40) for 6 months | In infants receiving the prebiotic mixture, the Ig-fLC levels were significantly lower compared to the placebo-fed infants |
| MacDonald et al., 2011 [32] | GOS/FOS  (ratio 9:1) | 0.8 g/100 mL | To investigate the influence of adding a patented, specific mixture of prebiotic oligosaccharides (Danone Research) to a protein substitute suitable for infants with Phenylketonuria (PKU); PKU Anamix Infant (Nutricia) | This was an 8-week open-label, single-arm, pilot intervention study in 9 infants (8-week median age) diagnosed with PKU. On study entry, infants were prescribed PKU Anamix Infant to replace an infant phenylalanine-free protein substitute without prebiotics (IPS). Blood phenylalanine concentrations were monitored and stool samples analyzed for pH/bacterial groups | PKU Anamix infant was well tolerated and accepted with no adverse events reported. Overall, plasma phenylalanine and tyrosine concentrations were maintained within target ranges throughout the study. All infants exhibited a microbiota dominated by bifidobacteria although no statistically significant change from baseline was observed at study endpoint. No infants showed abnormally high levels of *Clostridium histolyticum/lituseburense* or potentially pathogenic enterobacteriaceae at any point during the study. A significant reduction in median stool pH versus baseline was observed at Week 4, but this significance was not present at Week 8. PKU Anamix Infant maintains phenylalanine control in line with established IPS without prebiotics and maintains levels of bifidobacteria and lowers stool pH. In exclusively breast-fed infants the latter two factors have been associated with a reduced risk of infection and may be of particular importance in infants with PKU |