The human intestine is colonised by a huge number of bacteria. From the stomach to the colon the number of bacteria increases throughout the gastro-intestinal tract (Simon and Gorbach, 1984). The highest bacterial counts are found in the colon with up to 1013 colony forming units (CFU) per ml gut content.

In general, the intestinal bacteria can be divided into species that exert either harmful or beneficial effects or both on the host. Bifidobacteria and Lactobacilli are species that exert mainly health promoting effects.

The development of the intestinal flora in newborn infants starts during birth when the aseptic gastro-intestinal tract of the fetus is inoculated by bacteria from the mother’s intestinal and vaginal flora. This leads to a diverse flora comprising Bifidobacteria, Clostridia, and gram positive Cocci (Heine et al., 1998). After this first inoculation, the flora changes rapidly, presumably under the influence of the diet. In entirely breast-fed infants the flora is predominately Bifidobacteria whereas in formula-fed infants the flora is more diverse (Harmsen et al., 2000; Figure 1). The introduction of solid food leads to the disappearance of the predominance of Bifidobacteria in breast-fed infants and at 12 months of age the flora starts to resemble that of adults in number and composition (Stark and Lee, 1982).

The following health-promoting and protective properties have been attributed to Bifidobacteria:
- Activation of the immune system;
- Inhibition of pathogens by the secretion of substances which are directly inhibitory towards several bacteria;

![Figure 1: Development of the intestinal flora in breast– and formula-fed infants (according to Harmsen et al., 2000)](image-url)
- Lowering the pH by the production of acids such as acetate and lactic acid, leading to an antibacterial environment;  
- Production of digestive enzymes such as casein phosphatase and lysozyme;  
- Production of vitamins, largely of the B-group. (Gibson and Roberfroid, 1995)

The overall benefit of these effects is to protect infants from pathogenic micro-organisms in the intestine during a phase of insufficient immune response (Koletzo et al., 1998; Heine, 1998). It has also been suggested that a Bifidus-flora promotes the induction of oral tolerance towards dietary antigens (Hanson and Telemo, 1997).

For these reasons, it seems desirable to also increase the numbers of Bifidobacteria in the intestinal flora of formula-fed infants.

Two main methods have been used to provide a bifidogenic effect with infant formula. It has been demonstrated that prebiotic oligosaccharides (OS) and probiotic supplements appear to be the most effective way to increase the numbers of Bifidobacteria in the intestine of formula-fed infants (Boehm et al., 2000; Bennet et al., 1992).

A probiotic is defined as "a microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance" (Fuller, 1989). Prebiotics are "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and this improves host health" (Gibson and Roberfroid, 1995).

Human milk OS are well known to have a prebiotic effect in promoting the predominance of Bifidobacteria in the colon of breast-fed infants (Gibson and Roberfroid, 1995). In addition compared to probiotics, prebiotics are likely to have distinct advantages such as the in situ stimulation of the growth of certain resident bacteria with beneficial effects.

Due to these findings the concept arose to add prebiotic oligosaccharides to infant formulas.

As human OS have a very complex structure and they are not commercially available, a prebiotic mixture for infant formulas was developed which has a high content of galactose. The mixture contains galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS), both of which are natural components of the human diet. This GOS/FOS mixture does not replicate the complex chemical structure of human milk OS but mimics the prebiotic effect of human milk OS.

This GOS/FOS mixture has been investigated clinically with both preterm and term infants (Boehm et al., 2000; Moro et al., in press; Knol et al., in press).

In preterm infants the bifidogenicity of the GOS/FOS mixture was investigated in a double-blind, randomised, controlled study with parallel group design (Boehm et al., 2000). Formula fed preterm infants received either a standard preterm formula (n=9) or a preterm formula supplemented with 1g / 100ml of the GOS/FOS mixture (n=11). The investigators also studied a reference group of infants who received fortified pasteurised human milk (n=10). The study period was 28 days. After a feeding period of 28 days the infants who received the formAula with the prebiotic mixture had significantly higher numbers of Bifidobacteria in their faeces than the infants in the control group (Figure 2). In addition the prebiotic
GOS/FOS mixture resulted in a stool consistency which was significantly different, i.e. softer, from the control infants and closer to the group fed human milk (Figure 3). The stool frequency of infants fed with the supplemented formula was significantly higher than in the control group and closer to infants fed with fortified breast milk (Figure 4). The infants in the three feeding groups had similar growth characteristics.

These data demonstrate that the OS mixture can be used as an ingredient to establish a Bifidus flora in formula-fed preterm infants.

Another study, this time with term infants, investigated the effects of different concentrations of the prebiotic mixture. In this double-blind, randomised, controlled clinical trial with parallel group design, 63 infants were fed for 28 days with two different dosages (33 infants with 0.4g / 100ml and 30 infants with 0.8g / 100ml) of the GOS/FOS mixture added to infant milk.
formulas (Moro et al., in press). The results of the two groups fed with supplemented formula were compared to those of an unsupplemented control group (n=27). The results of the stool microflora analyses at the end of the study showed a dose-dependent response in the bifidogenicity of the prebiotic mixture. The numbers of Bifidobacteria and Lactobacilli were significantly higher in the groups who received the supplemented formulas than in the control group. Both groups fed the supplemented formulas showed significantly softer stools compared to the control group.

Finally, a prospective multi-centre study, with a randomised and double-blind design, investigated the effects on stool microflora of feeding a prebiotic formula (Omneo, containing 0.8g OS/100ml) or standard formula fed to term infants for 12 weeks (Knol et al., in press). The results, based on stool samples collected at enrolment and when the infants were aged 6 weeks, showed that the Bifidobacteria expressed as a percentage of the total number of faecal micro-organisms increased significantly in the prebiotic group (p<0.01). Also, the percentage of Bifidobacteria in the stools of the supplemented group was significantly higher at the age of 6 weeks than that found in the control group (59% and 32% respectively) (p<0.01).

In conclusion, a bifidus-dominated flora is seen to be beneficial for infants mainly for its protective properties against enteral infections but also for the other health-related properties attributed to Bifidobacteria. Human milk OS are mainly responsible for the prebiotic effects of breast milk. A new and highly efficient mixture of prebiotic OS has been developed with the objective of providing a predominance of Bifidobacteria in the intestinal flora of formula-fed infants. Data from clinical studies reveal that this GOS/FOS mixture has a strong bifidogenic effect. Therefore it can be concluded that prebiotic OS are an effective method of providing formula-fed infants with the benefits of the bifidus-dominated flora found in breast-fed infants.
REFERENCES:


