An ORAFTI Newsletter
Nr.8 - Spring 2003

Active Food Scientific Monitor

Interview
with Professor
Dr. Y. Vandenplas

In this issue

we present an interview with

Prof. Dr. Yvan Vandenplas,

Academic Children's Hospital,

Free University of Brussels,

Belgium.

Professor Yvan Vandenplas is head of the Academic Children's Hospital of the Free University of Brussels in Belgium. He is especially interested in infant gastro-enterology.
He is involved in several research projects concerning gastro-oesophagal reflux, Helicobacter pylori, food allergy and the interaction between the intestinal flora and health.
Prof. Vandenplas would love to have more time to read, to listen to music, to see films, and to do some sport. He used to play squash, and he is still a great lover of the music of the seventies and eighties. However, traveling to scientific congresses is one of his few remaining leisure activities.

Breast-feeding is widely accepted as the "gold standard" for human infants. However, the majority of infants a few months old are fed with a cow's milk-based formula. The intestinal flora of the breast-fed infant has a higher proportion of bifidobacteria and lactobacilli. Oligosaccharides, which are growth factors for these bacteria, are absent from cow's milk, which is likely to account for the differences in colonic flora.
The oligosaccharide content of breast milk is not constant, making it impossible for industry to copy nature completely. However, although the exact composition cannot be mimicked, the function and effect of breast milk on the body can be imitated. The addition of non-digestible oligosaccharides to cow's milk-based infant formula brings this alternative one step closer to the gold standard of human milk.

Prebiotic oligosaccharides in infant nutrition

What makes human milk so unique?

In infant nutrition, breast milk and its associated physiological benefits have always been considered to be the gold standard. Apart from being a complete food for infants up to the age of 6 months, human milk contains a myriad of components that have significant bio-active and immunomodulatory roles. Infant formula provides a safe, nutritious and healthy alternative. However, infant formula cannot replicate exactly the bio-active or immunomodulatory properties of breast milk, which contains many complex biological components.

continued on page 2

P R E F A C E

by Dr. Anne Franck

Continuation of page 1

Active Food
Scientific Monitor

In the right direction

As we are keen to produce a newsletter that is valued by to our readers, we recently asked you to give us your impressions and comments about the Active Food Scientific Monitor by filling out a questionnaire on the ORAFTI website (www.ORAFTI.com). Many of you gave us your thoughts and provided numerous suggestions.

It transpired that the Scientific Monitor is read cover-to-cover by most of you, especially the State of the Art, Monitor and Frequently Asked Questions sections which you find interesting, and even very interesting. Most of you are happy with the writing style, the length and the scientific level of the articles. You usually keep the issues after reading them. However, you would like to see more illustrations and graphics in the Scientific Monitor, and this is something which we have already tried to improve in this issue. We would like to thank all of you who participated in this survey.

In this issue we present an overview of the use of prebiotic oligosaccharides, especially chicory inulin and oligofructose, in infant and childrens' nutrition products. As you know, breast-feeding remains the gold standard for feeding infants. There is, however, an increasing interest in supplementing cow's milk-based formulae with prebiotics, which contribute to some of the key physiological effects associated with breast milk. The addition of inulin or oligofructose brings this alternative one step closer to the gold standard.

Prebiotic oligo

Oligosaccharides and glycoconjugates are prominent bio-active components in human milk. Due to their non-digestibility, these oligosaccharides serve as prebiotics in the intestine of breast-fed babies by promoting selectively the predominance of bifidobacteria. Cow's milk and hence cow's milk-based infant formula have an extremely low content of oligosaccharides.

There is substantial evidence that the oligosaccharide secretion in human milk is a complex, variable and dynamic process. The highest concentration of oligosaccharides, 20 g/l milk, is reached on the fourth day of life. On days 30 and 120 of lactation, there is a decrease of 20% and 40%, respectively, in comparison to day 4. The amount of oligosaccharides in human milk changes during lactation, and the composition of oligosaccharides varies during the day and even during one feeding, and is dependent on many factors, including the geographical origin of the mother. Whether the content and composition of oligosaccharides differ in the milk of mothers who deliver at term and those who deliver pre-term is still a matter of debate.

What is the importance of these oligosaccharides in human milk?

Although breast-fed and formula-fed infants have a similar gastrointestinal flora on day 3 or 4 of their life, there is a substantial difference in the composition of their colonic

saccharides in infant nutrition

flora after several weeks of life. Lactic acid bacteria such as bifidobacteria and lactobacilli dominate the flora of the breast-fed infant, whereas the formula-fed infant has a more diverse flora, containing more bacteroides and clostridia species.

The predominance of bifidobacteria and lactobacilli in the intestinal flora of breast-fed infants is widely considered to be induced by certain components present in human milk. Although the exact mechanisms responsible for this phenomenon remain to be clarified, it has been demonstrated that human milk oligosaccharides are strongly bifidogenic, acting as a specific growth substrate.

Furthermore, some oligosaccharides and glycoconjugates in human milk may protect the infant against infections by acting as receptor homologues that inhibit the binding of entero-pathogens to their host receptors. Neutral oligosaccharides cause inhibition of bacterial adhesion to the epithelia. Ongoing research is investigating how specific carbohydrate structures could protect against specific pathogens.

What are the health benefits of a bifido-dominant flora in infants?

There is increasing awareness that the human intestinal microflora is a major factor in health and disease. The presence of a healthy, non-pathogenic intestinal microflora provides advantages to the growth, development and immunity in the first years of life. The gastro-intestinal microflora protects the host against colonisation by invasive micro-organisms, and it has a role in protection against bacterial translocation and in reduction of gastro-intestinal permeability. The gut flora regulates the gastro-intestinal transit and stimulates migrating motor complexes.

The intestinal flora has metabolic functions, like intervention in the deconjugation of bile salts and stimulation of the entero-hepatic circulation. Lactobacilli hydrolyse any lactose that reaches the colon after passage through the small bowel. The gut flora is capable of fermenting complex carbohydrates that reach the colon, and short-chain fatty acids are end products of this fermentation. The flora can hydro-lyse proteins and therefore may have a role in decreasing the allergenicity of non- digested proteins.

It has been shown that a difference in the neonatal gut microflora precedes the development of atopy, suggesting a crucial role for a balanced indigenous intestinal microflora for the maturation of human immunity to a non-atopic mode.

Whether the preferential gastro-intestinal colonisation by bifidobacteria in young breast-fed infants has clinically significant long-term consequences needs to be studied further.

Will the addition of oligosaccharides to infant formula offer the same health benefits?

Some companies add bifidobacteria to infant formula in an attempt to make the composition of the gastro-intestinal microflora of formula-fed infants mimic that of breast-fed infants. Others try to mimic the function of mother's milk by adding oligosaccharides to the formula in order to obtain the same intestinal colonisation by bifidobacteria as induced by mother's milk.

The addition of bifidobacteria as probiotics in infant formula results in a gastro-intestinal flora more comparable with that of breast-fed infants. This is accompanied by significant changes in stool frequency and consistency, which become closer to those of breast-fed infants. The addition of probiotics to infant formula has been shown to decrease the incidence and severity of episodes of infectious diarrhoea induced by rotavirus in hospitalised children. However, the probiotic concept may be regarded as "unphysiological", since probiotics are not present in human milk. Moreover, in order to exert their beneficial effects, the bacteria need to be administered in a viable way.

In line with the prebiotic concept, non-digestible oligosaccharides (inulin and/or oligofructose and galacto-oligosaccharides) are added to infant formula. This seems a "more physiological" approach, even if the dynamic changes in oligosaccharide content of human milk cannot be copied perfectly in artificial feeding. It has been shown that the addition of prebiotic oligosaccharides to infant formula results in an effect on gastro-intestinal colonisation similar to that in breast-feeding: bifidobacteria become dominant in the flora. The absolute number of bifidobacteria and the proportion of bifidobacteria as a percentage of the total micro-organisms increase significantly. The number of lactobacilli also increases significantly. Clinical data demonstrate that the addition of oligosaccharides decreases the stool pH and improves stool consistency significantly, leading to a lower incidence of hard stools.

The addition of prebiotics to infant formula is currently regarded as safe.

In conclusion, although the composition of human milk cannot be replicated exactly, the addition to infant formula of prebiotic oligosaccharides, like inulin and oligofructose, brings artificial feeding one step closer to the gold standard of breast-feeding. Carefully balanced experiments in vitro and in vivo together with robust clinical studies are needed to further investigate the potential long-term health benefits of these food components in infant nutrition.

Prebiotic oligosaccharides in infant and child nutrition

There is increasing interest in understanding the effects of diet in infancy and implications for later life. An expert group of European pediatricians focusing on infant nutrition has concluded recently that early nutrition modulates growth and functional development of the human organism and appears to exert life-long programming effects that modulate health, disease and mortality risks in adulthood, as well as neural functions, behaviour and quality of life.

The same group underlined the fact that, concerning growth, maturation, regulation and functioning of the intestine, some oligosaccharides play an important role in infant nutrition. This applies particularly to non-digestible oligosaccharides, like inulin and oligofructose, which can modulate the physiology of the large bowel by improving the composition of the microbial ecosystem that colonizes that part of the gastro-intestinal tract (Roberfroid, 2002). The “gold standard” for infant feeding is, and always will be, human breast milk (Hamburger et al., 1997). However, the majority of infants a few weeks old are fed with a cow’s milk-based formula. With that in mind, there is an intensive search for food constituents, including probiotics and prebiotics, that contribute to a balanced gastro-intestinal flora, excellent physical and mental growth and development, and levels of immunity, as well as morbidity and mortality equivalent to those seen in breast-fed infants. Although it seems impossible to replicate exactly the complex composition of human breast-milk, it appears that the beneficial health effects of the oligosaccharides in human milk can be mimicked. Moreover, some oligosaccharides, such as inulin and oligofructose, modulate key physiological functions like calcium absorption and possibly lipid metabolism, which may be of importance for children and adolescents.

The infant intestinal microflora

Before birth, the gastro-intestinal tract of the fetus is sterile. Birth leads to a rapid colonization by the mother's intestinal and vaginal flora and by bacteria from the environment during delivery. Within the first week of life, initial colonizers of the infant gut are thought to be enterobacteria (e.g. Escherichia coli) and streptococci, followed by bifidobacteria and bacteroides (Mountzouris et al., 2002; Walker, 2002).

In breast-fed infants, the appearance of clostridia and bacteroides is transient, occurring only during the early weeks of life, resulting in a predominance of Bifidobacterium species (Hamburger et al., 1997; Bauraind, 2000). After one week, breast-fed infants develop a flora comprising mainly bifidobacteria, whereas formula-fed infants acquire a flora with bifidobacteria, bacteroides, streptococci and clostridia. At one month of age, bifidobacteria are the most prevalent organism in both groups, but the number and frequency of these organisms in the stool of bottle-fed infants is approximately one-tenth that of breast-fed infants. Depending on the type of infant formula used, there are cases reported for bottle-fed infants where bifidobacteria were not the dominant micro-organisms (Mountzouris et al., 2002).

The introduction of solid food changes the composition of the intestinal flora in ways that depend on the nature of the diet. Formula-fed infants have a flora more like that of adults, and their ability to ferment complex carbohydrates develops more quickly than that of breast-fed children (Edwards, 2001). Parrett et al. (1997, 1998) studied the development of the fermentation capacity for simple and complex carbohydrates in breast-fed and formula-fed infants during weaning by incubations of fecal cultures in vitro. Pre-weaned breast-fed infants were less able to ferment oligosaccharides and carbohydrates than were weaned infants.

The ability to ferment oligofructose (Raftilose®) increased in early weaning, whereas the ability to ferment soyabean polysaccharides did not develop until late weaning. In formula-fed infants there were no significant differences between any stage of weaning for any substrate.

By 12 months of age, the anaerobic bacterial population of the large bowel of breast-fed and formula-fed infants begins to resemble that of adults in numbers and composition. Most of the bacteria present are strict anaerobes.

The relatively simple microflora profile in a breast-fed infant during the first two years of life may contribute significantly to the child's resistance to intestinal infections and improve defence systems. Several properties of bifidobacteria may contribute to this protective effect. Bifidobacteria are able to secrete substances that inhibit the growth of pathogenic bacteria. The flora has metabolic functions, since it is capable of hydrolysing or fermenting carbohydrates and proteins that reach the colon. Generally, breast-fed children tend to have a more acidic stool (ranging from pH 5 to 6) compared with the neutral pH found in the

continued on page 6

Oligosaccharides in human breast-milk

The predominance of bifidobacteria in the intestinal flora of breast-fed infants is due to certain components of human milk, which contains a wide variety of carbohydrates, many of which occur in small quantities but have significant physiological functions. These carbohydrates occur free and in complexes, and include nucleotide sugars, glycolipids, glycoproteins and oligosaccharides (Hamburger et al., 1997).

Prominent among postulated defence agents are the human milk oligosaccharides and glycoconjugates. More than 130 different oligosaccharides have been identified, and they represent quantitatively the third largest component of human milk (12–14 g/l), after lactose and lipids. In contrast, cow's milk has a very low content of oligosaccharides (< 1.0 g/l) and these have a structure that is different from that of the oligosaccharides in human milk (Coppa et al., 2002; Mountzouris et al., 2002).

Some of the oligosaccharides in human milk are linear and others are branched, some being composed of simple sugars like galactose and others containing sugar derivatives like uronic acids or uronic esters, some being acidic and others being neutral (Vandenplas, 2002). Several investigations have shown that the bifidogenic effect of human milk is essentially due to the presence of these oligosaccharides (Coppa et al., 2002; Mountzouris et al., 2002).

Some of these oligosaccharides are known to be potent inhibitors of bacterial adhesion to epithelial cells by acting as receptor analogues to mucosal adhesion molecules. This may contribute towards the lower incidence of gastrointestinal, respiratory and urinary infections seen in breast-fed infants compared with those who are formula-fed (Mountzouris et al., 2002; Vandenplas, 2002; Hamburger et al., 1997).

Oligosaccharides having the same structure as those of human milk are not available industrially. However, some of the available non-digestible oligosaccharides, mainly inulin, oligofructose and galacto-oligosaccharides, are able to influence the composition of the human intestinal flora by selective stimulation of bifidobacteria (Coppa et al., 2002).

As there is substantial evidence that oligosaccharide secretion in human milk is a complex, variable and dynamic process, it should be the goal of an infant formula containing non-digestible oligosaccharides to mimic the physiological effect, but not the exact composition, of human milk (Vandenplas, 2002).

Continuation of page 5

feces of formula-fed infants. This may have a protective effect against the growth of pathogenic micro-organisms (Van Laere et al., 2001; Mountzouris et al., 2002). The flora of breast-fed children produces mainly acetate and lactate as the end products of fermentation of carbohydrates, whereas formula-fed infants produce mainly acetate and propionate. Lactic and acetic acids have a direct antimicrobial effect. Furthermore, a small amount of acetate has been shown to be associated with infantile diarrhoea and upper respiratory tract infections (Mountzouris et al., 2002).

On the other hand, bifidobacteria have been attributed with a role in modulating the immune system. Several studies indicate a difference in the intestinal flora of atopic (more clostridia and fewer bifidobacteria) and non-atopic infants during the first year of life (Vandenplas, 2002). Bifidobacteria may help to balance the response between Th1 and Th2 cells, and stimulate the induction of oral tolerance, presumably via the stimulation of other subclasses of T helper cells (producing transforming growth factor beta (TGF ß) and interleukin 10). In addition, the appropiate colonization of the gut leads to an accelerated maturation of the secretory IgA response and possibly to a maturation of innate immunity to microbial stimulation (Walker, 2002). An improved gut maturation with earlier “gut closure” may be an additional benefit (Hamburger et al.,1997).

Improvement of gut microflora with prebiotics

In the past, several attempts have been made to increase the bifidogenicity of infant formula in order to provide formula-fed infants with a gut flora dominated by bifidobacteria. An increase in lactose content, the addition of bovine lactoferrin and a reduced concentration of phosphate had no effect on the numbers of bifidobacteria. The addition of probiotics, e.g. bifidobacteria or lactobacilli, to infant formula has proven to be more successful. The addition of probiotics results in a ‘breast-fed like’ flora and changes the nature of stools accordingly (Vandenplas, 2002; Mountzouris, 2002). The use of probiotics in infants with atopic dermatitis and cow’s milk protein allergy has shown positive outcomes (Saavedra & Tschernia, 2002). Several studies have shown that supplementation with bifidobacteria or lactobacilli can decrease the incidence, duration and/or severity of infant and childhood acute diarrhoea disease, particularly of viral origin (Bauraind, 2000; Robberecht, 2002; Saavedra & Tschernia, 2002).

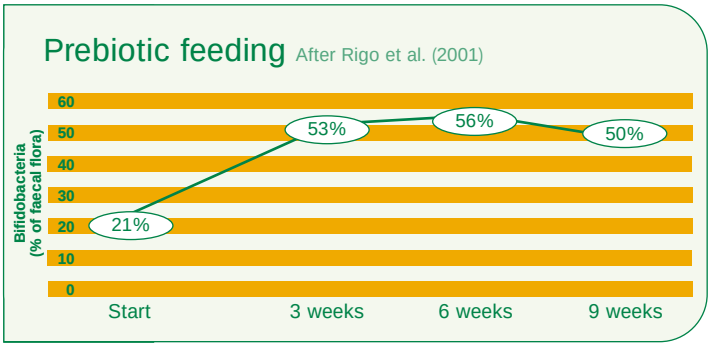
However, the use of probiotics in infant formula is limited by the fact that the bacteria need to be administered in such a way that they can survive transit through the stomach and small intestine. Another approach is the addition of prebiotic oligosaccharides, like inulin and oligofructose, to infant formula in order to improve the composition of the colonic microflora. Prebiotics represent a physiological way of increasing the counts of bifidobacteria and lactobacilli in the intestinal flora using a mechanism similar to that found in human milk. A further advantage is that the growth of the beneficial bacteria is promoted only in the lower parts of the gastrointestinal tract, where they occur naturally (Roberfroid, 2000).

Several studies have shown that the supplementation of infant formula with inulin or oligofructose (Raftiline® or Raftilose®), or with a mixture of galacto-oligosaccharides (GOS) and inulin promotes selectively the growth of intestinal bifidobacteria and lactobacilli. A change in fecal consistency towards softer stools and a higher stool frequency is observed. Knol et al. (2001, 2002) and Knol (2002) compared the proportion of bifidobacteria in the stool flora of term infants fed with a standard infant formula (SF) or a formula (PBF) with prebiotics (GOS + Raftiline®HP). In the PBF group, the numbers of bifidobacteria increased from 31% at 0–2 weeks to 59% at 6 weeks, whereas no significant change was observed in the SF group (28% at 0–2 weeks, 32% at 6 weeks). At 6 weeks, the percentage of bifidobacteria in the PBF group was closer to the level reported for breast-fed infants (79%).

Knol et al. (2002) and Knol (2002) showed that the flora in infants aged 4–12 weeks that have been formula-fed for at least four weeks, can still be modified by a prebiotic-containing formula (Raftiline®HP + GOS). After 4 and 6 weeks, they found an increase in the percentage of bifidobacteriato to levels typical for breast-fed infants, and the species of bifidobacteria found in the prebiotic group were highly comparable with those present in breast-fed infants.

Rigo et al. (2001) compared a new prebiotic formula (NF), containing Raftiline®HP and GOS (0.4g/dl), with data from breast-fed infants (BF) and infants fed a standard formula (SF). Fourteen term infants received NF from birth to the age of 2 months. The growth of the NF-infants was similar to that seen in BF-infants and SF-infants. The average percentage of bifidobacteria in fecal samples from NF-infants was 21% at day 3, 53% at 3 weeks, 56% at 6 weeks and 50% at 9 weeks, indicating a rapid and significant bifidogenic effect of NF and its ability to maintain a stable bifidobacteria-rich gut flora.

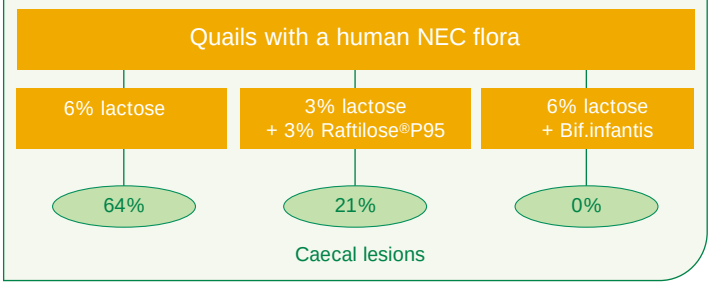
Supplementing a standard pre-term formula with 1 g/dl of oligosaccharides (Raftiline®HP + GOS) stimulated the growth of intestinal bifidobacteria selectively and resulted in stool characteristics similar to those found in pre-term infants fed human milk (Boehm et al., 2002, 2001). The difference in the numbers of bifidobacteria between the supplement and non-supplement groups was statistically highly significant ($p = 0.0008$). The study was conducted in healthy pre-term infants, who tolerated complete enteral nutrition and were not receiving antibiotic treatment. In another study in term infants, Moro et al. (2001, 2002) showed a dose-dependent increase in the numbers of bifidobacteria and a change of the fecal pH after supplementation of a standard infant formula with 0.4 g/dl or 0.8 g/dl of prebiotics (inulin + GOS). In the group fed the formula supplemented with 0.8 g/dl of prebiotics, the numbers of bifidobacteria were similar to those found in breast-fed infants.



This was confirmed in studies by Moro et al. (2001 & 2002) in term infants who received a standard formula supplemented with 0.4g/dl or 0.8 g/dl of prebiotics (Raftiline®HP + GOS) for 28 days. The influence on the fecal flora and stool characteristics (pH, frequency and consistency) was more pronounced ($p < 0.01$) with the higher dose of supplement.
In general, daily doses of about 2g or more were administrated in these studies with babies.

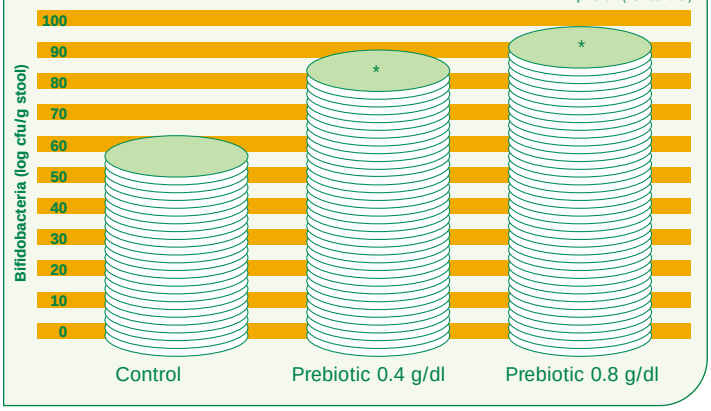
Protection against Necrotising Enterocolitis (NEC)

After Catala et al. (1999) and Danan et al. (2000)



Prebiotic effect

After Moro et al. (2001 & 2002)



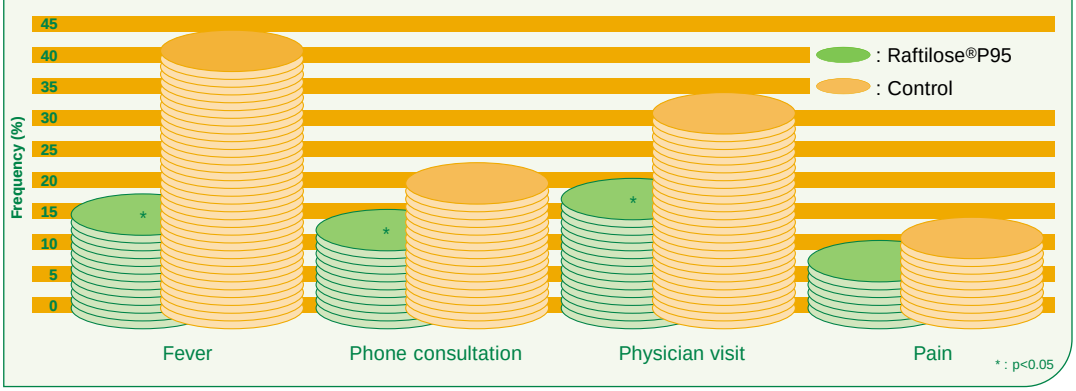
Interesting health benefits

Animal and human studies indicate that prebiotics contribute to gastrointestinal and systemic conditions that are related to a desirable balance between our microbial environment and our immunological relationship to it (Bauraind, 2000; Robberecht, 2002; Saavedra & Tschernia, 2002).
Prebiotic oligosaccharides are able to protect infants and children from infection. Using gnotobiotic quails as an experimental model of neonatal necrotizing enterocolitis (NEC), Butel et al. (1998) have shown that the onset of intestinal lesions requires a combination of low endogenous actase activity, lactose in the diet, and colonization by lactose-fermenting bacteria such as clostridia. In this model, bifidobacteria protected against NEC via the inhibition of growth of Clostridium butyricum or Clostridium perfringens.
A number of studies with germ-free quails inoculated with the fecal flora from healthy or sick human premature neonates investigated the effect of a supplementation with oligofructose. Catala et al. (1999) and Danan et al. (2000) demonstrated that oligofructose (Raftilose®P95) promotes the growth of endogenous bifidobacteria and helps to prevent the overgrowth of pathogenic bacteria (E. coli, C. perfringens, C. ramosum, C. butyricum, C. paraputrificum, C. difficile, Klebsiella) implicated in NEC. It was shown that oligofructose acts as an anti-infective agent and decreases the occurrence or severity of the lesions (Butel et al., 2001 & 2002). Supplementation with oligofructose led to the complete inhibition of NEC-like lesions in quails with C. perfringens and Klebsiella. In quails with C. perfringens and C. difficile, the prebiotic did not decrease the number of sick quails but it reduced the severity of the disease (less extensive tissue necrosis and a decrease in haemorrhages). Saavedra et al. (1999) and Tschernia et al. (1999) examined the effects of a pediatric weaning food supplemented with oligofructose (Raftilose®P95) in 123 non-breast-fed infants aged 4 to 24 months and attending day-care centres. One group received a standard infant cereal for six months, the second group received the same cereal supplemented with 0.55 g of oligofructose per 15 g of cereal. The consumption of the prebiotic-supplemented cereal was associated with a decrease in severity of diarrhoea disease. General gastro-intestinal status was improved with decreased bowel movement discomfort, vomiting and regurgitation. Furthermore, the consumption of oligofructose was associated with adequate growth and a significant reduction in febrile events and cold symptoms, antibiotic prescription (associated with respiratory illness) and day-care absenteeism.

continued on page 8

Symptoms associated with diarrhoea

After Saavedra & Tschernia (2002)



Continuation of page 7

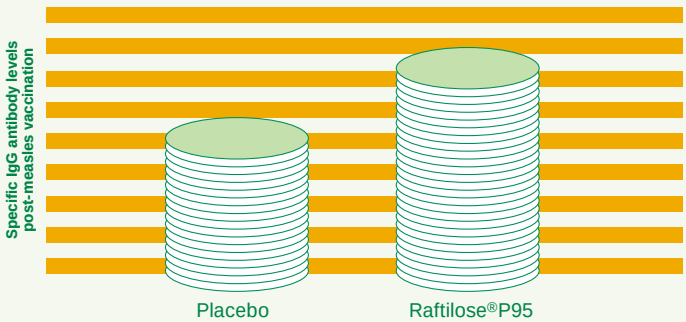
Fisberg et al. (2000) evaluated the incidence and duration of sickness in mild to moderately malnourished children who received a nutritional supplement with and without synbiotics (Lactobacillus acidophilus, Bifidobacterium infantis and oligofructose). A total of 626 children from 1 to 6 years of age and from Brazil, Mexico, Spain and Portugal participated in the study. The number of sick days decreased significantly in both feeding groups. The decrease was more pronounced for younger children (1–2 years) than for older children. Both groups experienced catch-up growth, with a significant increase in normalized percentiles for weight and height. There were no significant differences between the groups for sick days, but the number of sick days for children 3–5 years of age was lower in the group receiving synbiotics. Also constipation days were fewer in the synbiotic group.

A randomized placebo-controlled trial (Juffrie, 2002) in Indonesia involved 118 children between 1 and 14 years old who had acute diarrhoea from various causes: 93 children ingested 2.5 to 5g/day of oligofructose, depending on age, 25 children consumed a placebo. The duration of diarrhoea was shorter in children who ingested oligofructose (2.6 days versus 4.2 days). A decrease in the production of intestinal putrefactive substances in children who ingested the prebiotic was found as well as a reduction of their stool pH (5.6 versus 6.0). In a study by Ahmed et al. (2000), 58 healthy infants aged 3–12 months with acute diarrhoea received a low-lactose formula with or without a combination of Lactobacillus rhamnosus and oligofructose. The duration of diarrhoea was shortened significantly in the supplemented group (from 2.5 to 1.6 days).

Firmansyah et al. (2000) examined the effects on the immune response after measles vaccination of an infant cereal with milk supplemented with a prebiotic mixture of oligofructose and inulin. Post-vaccination specific IgG antibody levels were significantly higher in the supplement group (96% IgG positivity rate versus 88% in the control group). No differences in growth or overall health status were observed.

Effect on the immune response

After Firmansyah et al. (2000)



Effect of Raftilose®Synergy1 on calcium absorption

After Griffin et al. (2002)

Enhancement of calcium absorption

An adequate calcium intake at or near puberty is essential for the development of an optimal peak bone mass. Griffin et al. (2002) examined the effect of a relatively modest intake (8g/d) of oligofructose (Raftilose®P95) or a mixture of oligofructose and inulin (Raftilose®Synergy1) during 3 weeks on the calcium absorption of two groups of 30 girls at or near menarche, with calcium intakes approximating the recommended dietary intake (1200–1300 mg/d). Calcium absorption was significantly higher (+ 18%) in the group receiving Raftilose®Synergy1, whereas no significant difference was seen between the oligofructose and the placebo groups.

In another study (Van den Heuvel et al., 1999), 12 healthy male adolescents aged 14–16 years consumed orange juice supplemented with 15 g/d of oligofructose (Raftilose®P95) or sucrose (control). The experiment was conducted in two 9-day periods separated by a wash-out period of 19 days. An increase of 26% in true fractional calcium absorption was observed with the group fed oligofructose. These data confirm the results obtained in animal models, where an increase in calcium absorption and in bone mineralization has been observed repeatedly.

Tolerance and safety

Oligosaccharides are, in general, considered as very safe. In none of the trials with children were there any significant signs of gastrointestinal intolerance such as flatulence, abdominal discomfort or increased purge (Saavedra & Tschernia, 2002). Supplementation of a formula for term infants with a mixture of inulin and GOS has not influenced weight gain or length increment, nor the incidence of vomiting, regurgitation and crying. Boehm et al. (2002) found no effect of a prebiotic supplement in pre-term infants on the incidence of crying, regurgitation, vomiting, or on weight and length gain.

A study by Cadranel (1994) has shown that daily dosesof up to 9 g of oligofructose (Raftilose®P95) are well tolerated by children aged 10–13y. No case of diarrhoea, abnormal stools or flatulence was recorded. Prebiotic oligosaccharides thus appear to be well tolerated by infants and children when given at doses required to obtain a better-balanced gut microflora and associated physiological benefits.

References

Ahmad R.A., Lukito W., Firmansyah A., Gliwitzki M., Suhardjo H.N. & Suhermawan R. (2000). Effect of a combined probiotic, prebiotics and micronutrients supplementation in reducing duration of acute infantile diarrhea, J Paediatr Gastroenterol Nutr, 31, A984.

Bauraind O. (2001). Prébiotiques et Probiotiques en pédiatrie. Dieta, 23, 18-21.

Boehm G., Casetta P., Lidestri M., Negretti M., Jelinek J., Stahl B. & Marini A. (2001). Effect of dietary oligosaccharides (OS) on faecal bifidobacteria in formula fed preterm infants. J Pediatr Gastroenterol Nutr, 32, 393.

Boehm G., Lidestri M., Casetta P., Jelinek J., Negretti M., Stahl B. & Marini A. (2002). Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal bifidobacteria in preterm infants. Arch Dis Child Fetal Neonatal, 86, F178-F181.

Boehm G. (2002). Prebiotic Oligosaccharides - a New Ingredient in Infant Nutrition. International Symposium - The infant Gut Flora : Health Implications and the Role of Prebiotics. November 23, 2002, Amsterdam (NL).

Bruzzese E. (2001). Clinical applications of functional foods for pediatric intestinal diseases. International Symposium - Probiotics & Prebiotics. September 2-4, 2001, Rome (I).

Butel M.J., Roland N., Hibert A., Popot F., Favre A., Tessedre A.C., Bensaada M., Rimbault A. & Szylit O. (1998). Clostridial pathogenicity in experimental necrotising enterocolitis in gnotobiotic quails and protective role of bifidobacteria. J Med Microbiol, 47, 391-399.

Butel M-J., Catala I., Waligora-Dupriet A-J., Taper H., Tessedre A-C., Durao J. & Szylit O. (2001). Protective Effect of Dietary Oligofructose against Cecitis Induced by Clostridia in Gnotobiotic Quails. Microbial Ecology in Health and Disease, 13, 166-172.

Butel M-J., Waligora-Dupriet A-J. & Szylit O. (2002). Prebiotics and experimental model of neonatal necrotising enterocolitis, Brit J Nutr, 87 (S2), S213-S219.

Cadranel S. (1994). The acceptability of oligofructose in children. Confidential Report to ORAFTI.

Caplan M.S., Miller-Catchpole R., Kaup S., Russell T., Lickerman M., Amer M., Xiao Y. & Thomson R. (1999). Bifidobacterial Supplementation Reduces the Incidence of Necrotizing Enterocolitis in a Neonatal Rat Model, Gastroenterology, 117, 577-583.

Catala I., Butel M.J., Bensaada M., Popot F., Tessedre A.C., Rimbault A. & Szylit O. (1999). Oligofructose contributes to the protective role of bifidobacteria in experimental necrotising enterocolitis in quails, J Med Microbiol, 48, 89-94.

Coppa G.V., Bruni S., Zampini L., Galeazzi T. & Gabrielli O. (2001). Prebiotics of human milk and infant formulas. International Symposium - Probiotics & Prebiotics. September 2-4, 2001, Rome (I).

Coppa G.V., Bruni S., Zampini L., Galeazzi T. & Gabrielli O. (2002). Prebiotics in infant formulas: biochemical characterisation by thin layer chromatography and high performance anion exchange chromatography. Digest Liver Dis, 34 (S), S124-S128.

Danan C., Huret Y., Tessèdre A-C., Bensaada M., Szylit O. & Butel M-J. (2000). Could Oligosaccharide Supplementation Promote Gut Colonization With a Beneficial Flora in Preterm Infants? J Pediatr Gastroenterol Nutr, 30, 217-219.

Edwards C.) (2001). Intestinal flora during the first months of life: new perspectives. Yakult International Conference - Probiotics and Health 2001. September 13-14, 2001, London (UK).

Fanaro S. & Vigi V. (2002). Prebiotics in infant nutrition: clinical results. 20th International Symposium on Neonatal Intensive Care. November 6-9, 2002, Venice (I).

Firmansyah A., Pramita G.D., Carrié Fässler A.L., Haschke F., Link-Amster H. (2000). Improved humoral immune response to measles vaccine in infants receiving infant cereal with fructooligosaccharides. J Pediatr Gastroenterol Nutr, 31 (S2), Abstract 521.

Fisberg M., Maulen I., Vasquez E., Garcia J., Comer G.M. & Alarcon P.A. (2000). Effect of oral supplementation with and without synbiotics on catch-up growth in preschool children. J Pediatr Gastroenterol Nutr, 31 (S2), Abstract 987.

Griffin I.J., Davila P.M. and Abrams S.A. (2002). Non-digestible oligosaccharides and calcium absorption in girls with adequate calcium intakes, Brit J Nutr, 87 (S2), S187-S191.

Guesry P.R., Bodanski H., Tomsit E. & Aeschlimann J.M. (2000). Effect of 3 doses of fructo-oligosaccharides in infants. J Pediatr Gastroenterol Nutr, 31 (S2), S252.

Hamburger R.N., Clemes R.A. & Brown C. (1997). The Roles of Probiotics and Prebiotics in Infants. In: Bellanti J.A., Bracci R., Prindell G. & Xanthou M. (ed). Neonatal Hematology and Immunology III, Elsevier Science (USA).

Juffrie M. (2002). Fructooligosaccharide and Diarrhea. Bioscience Microflora, 21, 31-34.

Knol J., Poelwijk E.S., van der Linde E.G.M., Wells J.C.K., Brönstrup A., Kohlschmidt N., Wirth S., Schmitz B., Skopnik H., Schmelzle H. & Fusch C. (2001). Stimulation of endogenous bifidobacteria in term infants by an infant formula containing prebiotics. J Pediatr Gastroenterol Nutr, 32, 399.

Knol J., Steenbakkers G.M.A., van der Linde E.G.M., Gross S., Helm K., Klarczyk M., Schöpfer H. & Kafka C. (2002). Bifidobacterial species that are present in breast-fed infants are stimulated in formula fed infants by changing to a formula containing prebiotics. Nederlandse Darmendag - Microbiology of the GI-tract. October 11, 2002, Lelystad (NL).

Knol J. (2002). Prebiotic Oligosaccharides in Infant Nutrition - New Results from Clinical Trials. International Symposium - The infant Gut Flora : Health Implications and the Role of Prebiotics. November 23, 2002, Amsterdam (NL).

Moro G., Minoli I., Mosca F., Jelinek J., Stahl B. & Boehm G. (2001). Dosage effect of oligosaccharides (OS) on faecal flora and stool characteristics in term infants, J Pediatr Gastroenterol Nutr., 32, 401.

Moro G., Minoli I., Mosca F., Fanaro S., Jelinek J., Stahl B. & Boehm G. (2002). Dosage-Related Bifidogenic Effects of Galacto- and Fructooligosaccharides in Formula-Fed Term Infants. J Pediatr Gastroenterol Nutr, 34, 291-295.

Mountzouris K.C., McCartney A.L. & Gibson G.R. (2002). Intestinal microflora of human infants and current trends for its nutritional modulation. Brit J Nutr, 87, 405-420.

Parrett A.M., Edwards C.A. & Lokerse E. (1997). Colonic fermentation capacity in vitro: development during weaning in breast-fed infants is slower for complex carbohydrates than for sugars. Am J Clin Nutr, 65, 927-933.

Parrett A.M. & Edwards C.A. (1998). The effect of weaning on fermentation capacity for complex carbohydrates in breast-fed and formula-fed infants. Profibre Symposium-Functional Properties of Non - Digestible Carbohydrates, Nantes (F).

Rigo J., Pieltain C., Studzinski F., Knol J. & Bindels J.G. (2001). Clinical evaluation in term infants of a new formula based on prebiotics, beta-palmitate and hydrolysed proteins. J Pediatr Gastroenterol Nutr, 32, 402.

Roberfroid M. (2000). Prébiotiques et probiotiques dans la nutrition de l'enfant. Percentile, 5, 128-130.

Roberfroid M. (2002). Prebiotics and infant nutrition, 20th International Symposium on Neonatal Intensive Care. November 6-9, 2002, Venice (I).

Robberecht E. (2002). Pre- of probiotica voor de zuigeling. De Eetbrief, 94, 2-4.

Saavedra J.M. & Tschernia A. (2002). Human studies with probiotics and prebiotics: clinical implications. Brit J Nutr, 87 (S2), S241-S246.

Saavedra J., Tschernia A., Moore N., Abi-Hanna A., Coletta F., Emenhiser C., Yolken R. (1999). Gastro-intestinal function in infants consuming a weaning food supplemented with oligofructose, a prebiotic. J Pediatr Gastroenterol Nutr, 29, Abstract 95.

Tschernia A., Moore N., Abi-Hanna A., Yolken R., Coletta F., Emenhiser C., Saavedra J. (1999). Effects of long-term consumption of a weaning food supplemented with oligofructose, a prebiotic, on general infant health status, J Pediatr Gastroenterol Nutr, 29, Abstract 58.

Van den Heuvel E., Muys T., Van DokkumW. & Schaafsma G. (1999). Oligofructose stimulates calcium absorption in adolescents. Am J Clin Nutr, 69, 544-548.

Vandenplas Y. (2000). Functional Foods voor zuigelingen. 3e Voedings- en Gezondheidscongres. December 8-9, 2000, Brussels (B).

Vandenplas Y. (2002). Oligosaccharides in Infant Formula. Br J Nutr, 87 (S2), S293-S296.

Van Laere K.M.J. & Jeukens D.M.E. (2002). Mogelijkheden en toepassingen van prebiotica. VMT, 23, 51-54.

Walker A. (2002). Initial Bacterial colonization of the gut and the development of mucosal immunity in neonates. 8th Biennial Congress of the European Society for Developmental, Perinatal & Paediatric Pharmacology. October 25-28, 2002, Liège (B).

FREQUENTLY ASKED QUESTIONS

Can inulin and oligofructose be used in acid food products ?

In general, we can say yes. Inulin and oligofructose are versatile ingredients that can be used in a wide range of food applications, either for their nutritional advantages or for their technological properties or, even better, to offer a double benefit. The only real limitation for their application is their possible hydrolysis (due to the sensitivity of the ß (2,1) bonds between the fructose units) in acid conditions and at higher temperature (resulting in the formation of fructose with, as a consequence, the loss of the specific nutritional and technological benefits). An important characteristic related to this is the chain length of the fructan: a higher degree of polymerisation will result in a slower hydrolysis.

When the pH of a food product is above 4.5, as is the case for e.g. biscuits and most baked goods, cereals, chocolate, milk drinks, table spreads there is no risk of hydrolysis. A pH value below 4.5 combined with a low temperature and/or a short shelf-life will result in no, or only limited hydrolysis. This is the case for chilled or frozen products (e.g. most dairy products, ice creams and sorbets, fruit preparations for dairy products). In acid conditions and at high temperature (e.g. applied during heat treatments) or combined with a long shelf-life at ambient temperature, the fructan chains start to break down. Therefore, the only critical applications are acid products with a long shelf-life (of one year or more), e.g. non-refrigerated soft drinks and jams. In this case rather high levels of hydrolysis are noticed at the end of the shelf-life.

So, inulin and oligofructose can be used in a wide variety of food products and drinks, even in most acid applications, because the hydrolysis level can be controlled by choosing the right inulin or oligofructose type in terms of chain length, by adjusting the pH level or the other processing parameters to more beneficial conditions.

Franck A. (2002), Technological functionality of inulin and oligofructose, Br.J.Nutr., 87, pp. S287-S291.

Are inulin and oligofructose not hydrolysed in the stomach (due to acidity) and degraded (enzymatically) in the small intestine ?

Inulin and oligofructose may be used as prebiotics, they are fermented selectively by health-promoting bacteria in the gut. In order to exert their prebiotic effect, they have to reach the colon unaltered; in other words, they have to pass the barrier of the stomach (acid conditions) and the small intestine (enzymatic activity).

The pH of an empty stomach is about 1 but after the ingestion of food it increases to about 4–5, depending on the type of meal and its buffering capacity. The period that ingested foods remain in the stomach is relatively short (1 to 2 hours), too short for any significant acid breakdown of inulin or oligofructose.
Experiments in vitro and in vivo mimicking the conditions of the stomach have shown that the hydrolysis of inulin in the stomach is negligible (Okey et al., 1919).

In the small intestine, food is mixed with intestinal secretions and juices from the pancreas and the liver. Since man and many higher animals do not produce inulin-degrading enzymes (Lewis, 1912) and human intestinal enzymes are specific for à-linked monomers, the ß-linked fructose chains of inulin and oligofructose remain intact, as shown by several studies in vitro and in vivo. Experiments in vitro using homogenates of rat and human intestinal mucosa or purified intestinal enzymes have demonstrated that they have no affinity for fructans (Molis et al., 1996; Nilsson et al., 1988). Studies in vivo with ileostomy patients demonstrated that about 90% of ingested inulin and oligofructose was re recovered in the ileostomal effluent (Ellegärd et al.,1997). Intestinal aspiration studies confirmed a 90% recovery in ileal content of healthy human volunteers who ingested oligofructose (Molis et al., 1996). The small 10% loss could be attributed to analytical variation in the method and a slight breakdown at the terminal ileum where microbial colonisation may occur in ileostomy patients.

In conclusion, the available scientific data demonstrate that both inulin and oligofructose are resistant to the physiological degradation processes in the human upper gastro-intestinal tract. They are not hydrolysed significantly in the stomach or small intestine and thus enter the large intestine quantitatively and qualitatively unchanged, which makes them available for fermentation by the colonic microflora.

Ellegärd L., Andersson H., Boseus I. (1997), Inulin and oligofructose do not influence the absorption of cholesterol or the excretion of cholesterol, Ca, Mg, Zn, Fe or bile acids but increase energy excretion in ileostomy subjects. Eur.J. Clin.Nutr., 51, pp. 1-5.

Lewis H.B. (1912), The value of inulin as a foodstuff, J.Am.Med.Ass., 58, pp. 176-177.

Molis Ch., Flourié B., Ouarne F., Gailing M.F., Lartigue S. (1996), Digestion, excretion, and energy value of fructo-oligosaccharides in healthy humans. J.Am.Clin.Nutr., 64, pp. 324-328.

Nilsson U., Öste R., Jägerstad M., Birkhed D. (1988), Cereal fructans: in vitro and in vivo studies on availability in rats and humans. J.Nutr., 118, pp. 1482-1486.

Okey R. (1919), Studies on the behavior of inulin in the animal body - II. Inulin in the alimentary tract. J.Biol.Chem., 39, pp. 149-162.

MONITOR

Oligofructose stimulates bone mineralization and prevents bone mineral loss

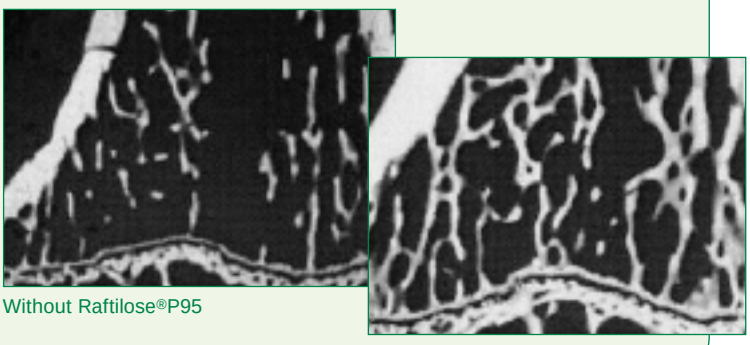
This study investigated the short-term and the long-term effects of low, medium, and high doses of oligofructose (Raftilose®P95) at the recommended intake level of dietary calcium (Ca) and at a higher intake of dietary calcium, on repeated calcium and phosphorus balances, on bone mineralization and on bone quality (tibia trabecular structure and mineral distribution between cortical and trabecular bone). Adult ovariectomized Fisher 344 female rats were fed semi-purified diets containing 5g Ca/kg with or without 25, 50 or 100g oligofructose/kg, or containing 10g Ca/kg with or without 50g oligofructose/kg for 16 weeks.

At the recommended level of Ca intake, the high dose of oligofructose increased femur mineral levels, as did the medium dose of oligofructose at a high intake of Ca. Increasing the level of Ca in the absence of oligofructose did not increase femur mineral content. At the recommended level of Ca intake, 25g oligofructose/kg diet prevented loss of trabecular area due to increased trabecular thickness, while 50 or 100g oligofructose/kg increased trabecular numbers. When the level of Ca was raised in the presence of oligofructose, trabecular area and cortical thickness were greatest, while loss of trabecular connectivity was the least of all groups. At the same time, the content of Ca in lumbar vertebra was greater. The study showed that ovariectomy-induced loss of bone structure in the tibia was prevented by oligofructose and that oligofructose was most effective when dietary calcium was high. The potential of oligofructose to stimulate bone mineralization or to preserve bone mineral content is related to the stimulated absorption of macro and micro-minerals. Supplementing diets with Raftilose®, thus may help to improve bone health in periods with high demands, e.g. after menopause when bone loss occurs following estrogen deficiency.

Scholz-Ahrens K.E., Açil Y. & Schrezenmeir J. (2002). Effect of oligofructose or dietary calcium on repeated calcium and phosphorus balances, bone mineralization and trabecular structure in ovariectomized rats. Br J Nutr., 88, 365-377.

Microradiographs of proximal tibias of ovariectomized rats

After Scholz-Ahrens et al. (2002)



Without Raftilose®P95

With 0.5% Raftilose®P95

Effect of inulin on blood lipids and spontaneous atherosclerosis

This is the first study to use an animal model of atherosclerosis to study the effect of inulin on blood lipids and aortic atherosclerosis. The study used homozygous low-density receptor knockout (LDLR -/-) mice, which develop spontaneous hypercholesterolemia with elevated levels of LDL and IDL, and with atherosclerosis, due to a genetic defect analogous to that found in humans with familial hyper-cholesterolemia.

The animals were fed isocaloric purified diets (high in carbohydrate and low in fat) with 0 or 10% inulin (Raftiline®HP) for 16 weeks. The mice receiving inulin had a lower body weight, reduced total plasma cholesterol, lower concentrations of LDL, IDL and VLDL, but not of HDL, and an increased HDL:LDL ratio.

These results support a cholesterol-lowering action of inulin in a spontaneous hyper-cholesterolemic model. Aortic atherosclerosis expressed as the ratio intima/media in mice receiving inulin was 15% lower compared to controls, but this difference was not statistically significant.

Mortensen A., Poulsen M. & Frandsen H. (2002). Effect of long-chained fructan Raftiline® HP on blood lipids and spontaneous atherosclerosis in low density receptor knockout mice. Nutr. Res., 22, 473-480.

Raftilose®Synergy1 decreases triglyceride accumulation in the liver of obese rats

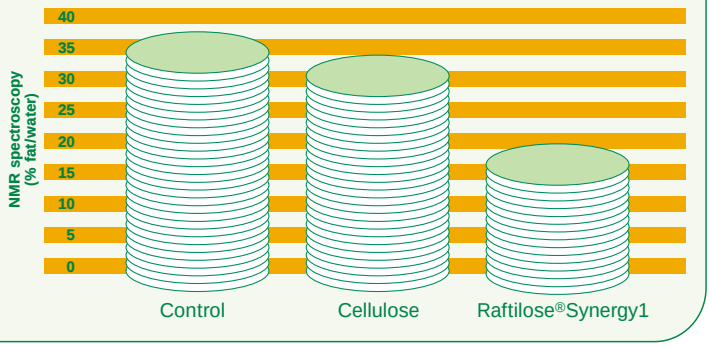
This study compared the effects of dietary supplementation with non-digestible carbohydrates, differing in their fermentability by colonic bacteria, on hepatic steatosis (fat accumulation in the liver) in growing obese Zucker fa/fa rats.

Control groups of lean and obese rats received a basal diet (19% proteins, 70% carbohydrates, 3.2% lipids, 7.3% minerals and vitamins), another group received highly fermentable inulin (Raftilose®Synergy1: 10g/100g diet) and another group received poorly fermentable microcrystalline cellulose (10g/100g of diet).

After 8 weeks of treatment, rats consuming inulin had a lower energy intake and a lower body weight compared with the control group and the cellulose group. No fat signal was found in the liver spectrum from the control lean rats, whereas a high signal intensity, indicating hepatic steatosis, was observed in obese rats fed the control and the cellulose diets. Fat signals were clearly lower in the inulin-fed group, indicating less accumulation of fat in the liver. Liver weight was less in inulin-fed rats; histological examination of the hepatic tissue revealed an altered liver struc-

continued on page 12

Continuation of page 11



ture with a huge macrovacuolar steatosis disseminated from the portal space to the centrolobular vein in both the control and the cellulose-fed rats, but very little in the inulin-fed group. The level of hepatic triacylglycerols was 48% lower in the inulin-fed rats than in the cellulose-fed group, despite the absence of any effect on the activity of the key enzymes involved in lipogenesis. The level of portal vein insulin tended to be greater in rats fed inulin than in controls. It was suggested that the higher portal concentration of insulin could promote secretion of triacylglycerols and avoid their accumulation in the hepatic tissue. The large extent of fermentation of inulin resulted in greater cecal contents compared to controls or cellulose-fed rats. The concentration of portal vein acetate was not affected by dietary treatment, whereas the concentrations of butyrate and, particularly, propionate were greater after consumption of inulin. The capacity of hepatocytes (isolated from the liver) to synthesise triglycerides or total lipids from different precursors was reduced by proprionate at the concentrations measured in the portal vein of inulin-fed rats. This phenomenon could contribute to less accumulation of triglyceride in the liver.

The beneficial effects of Raftilose® Synergy1 on liver steatosis demonstrated in this animal model could be useful, if confirmed, in overweight people.

Daubioul C., Rousseau N., Demeure R., Gallez B., Taper H., Declerck B. & Delzenne N. (2002). Dietary Fructans, but not cellulose, decrease triglyceride accumulation in the liver of obese Zucker fa/fa rats. J. Nutr., 132, 967-973.

Effect of probiotics and prebiotics on human intestinal pathogens

In this study, a range of probiotics were tested in vitro using disc/spot assays for their ability to inhibit the growth of some common enteropathogens (Campilobacter jejuni, Escherichia coli and Salmonella enteritidis). Each probiotic strain tested was combined with a range of prebiotics (lactulose, oligofructose, inulin, xylo-oligosaccharides (XOS) and mixtures of inulin/oligofructose and oligofructose/XOS) in an attempt to identify synbiotic combinations. Non-prebiotic controls included lactitol, starch and dextran. Two probiotics (Lactobacillus plantarum and Bifido-bacterium bifidum) were selected for further co-culture experiments because of their greater ability to inhibit pathogenic micro-organisms.

All of the probiotic strains tested provided some inhibition of each of the pathogenic strains used but the extent of inhibition was dependent on the probiotic strain. L. plantarum 0407 and B. bifidum Bb12 tended to inhibit pathogen growth to a greater extent, particularly with E. coli.

The highest growth rates of the bifidobacterial strain were obtained with oligofructose and XOS.

Oligofructose, inulin and XOS caused a greater inhibition of the enteropathogens than the other carbohydrate sources, suggesting a structure–function relationship (depending on the type of bond linking the monomers and the chain length). L. plantarum combined with oligofructose was the synbiotic most effective at inhibiting pathogen growth: a 6-log decrease in E. coli numbers was observed after 24h, whereas C. jejuni and S. enteritidis became undetectable. B. bifidum combined with oligofructose and XOS proved to be an effective synbiotic combination: C. jejuni and S. enteritidis became undetectable and a 2-log decrease in the number of E. coli was observed. The largest decrease in pH and increase in acetate level were observed when oligofructose or the oligofructose/XOS mixture were used as the source of carbohydrate.

Fooks L.J. & Gibson G.R. (2002). In vitro investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens. FEMS Microbiol. Ecol., 39, 67-75.

Synergistic effects of probiotics and prebiotics in animals

This study investigated the influence of a synbiotic combination with L. paracasei and oligofructose (Raftilose®P95), in comparison to L. paracasei alone, on fecal bacterial counts in weanling pigs under field conditions.

Significantly higher counts of Lactobacillus spp., Bifidobacterium, total anaerobes and total aerobes were found in feces of animals receiving the synbiotic mixture compared to controls and to animals receiving L. paracasei alone. Compared to the control group, significant decreases in counts of Clostridium and Enterobacterium were observed.

Enterococcus counts were reduced significantly compared to both the control group and the L. paracasei group. In animals receiving L. paracasei alone, significant decreases in counts of Clostridium and Enterobacterium were recorded as compared with the control group.

These results show that oligofructose can be used for potentiating the effects of the probiotic in the large intestine of weanling pigs.

Bomba A., Nemcova R., Gancarcikova S., Herich R., Guba P. & Mudronova D. (2002). Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. Br. J. Nutr., 88 (S1), S95-S99.

Oligofructose modifies metabolites associated with gut health status in healthy humans

The objective of this study was to determine whether supplementation with oligofructose (OFS) and/or Lactobacillus acidophilus (LAC) affects bowel function and fecal concentrations of protein catabolite in healthy adults. For this study, 62 subjects were enrolled in a random-

ized, double-blind, placebo-controlled, parallel study design. The subjects consumed, twice daily for 4 weeks: (1) 3g of sucrose; (2) 3g of OFS; (3) 3g of sucrose + 10 9 cfu of LAC; or (4) 3g of OFS + 10 9 cfu LAC.

Whereas only minor effects on bowel function were observed, important changes were seen in the concentrations of several fecal protein catabolites. There was a small decrease in fecal pH after 4 weeks with OFS, LAC, or OFS + LAC.

Supplementation with OFS tended to decrease fecal concentrations of isovalerate and ammonia after 2 weeks but not after 4 weeks, whereas LAC, surprisingly, tended to increase the concentration of ammonia. Subjects consuming OFS + LAC had significantly lower total concentrations of phenol in feces than those consuming OFS or LAC alone. After 4 weeks, OFS tended to increase fecal concentrations of putrescine.

The study researchers conclude that supplementation with OFS may improve gut health status by decreasing the concentration of some fecal protein catabolites, whereas LAC may not be as beneficial because it tended to increase fecal concentrations of indole, phenol and ammonia. Probiotics, prebiotics and synbiotics may be most beneficial for people with abnormal gut ecology, such as the elderly, pre-term infants, and people receiving antibiotics.

Swanson K.S., Grieshop C.M., Flinckinger E.A., Bauer L.L., Wolf B.W., Chow J., Garleb K.A., Williams J.A. & Fahey G.C. (2002). Fructooligosaccharides and Lactobacillus acidophilus modify bowel function and protein catabolites excreted by health humans. J. Nutr., 132, 3042-3050.

Effects of short-chain and long-chain fructans on aberrant crypt foci in rats

This study investigated the influence of chain length, dietary level (5 % or 15 %) and duration of feeding (5 or 10 weeks) of inulin-type fructans (short-chain oligofructose Raftilose®P95 and long-chain inulin Raftiline®HP) on 1,2-dimethylhydrazine dihydrochloride-induced aberrant crypt foci (ACF) in the rat colon, and on a number of intestinal parameters supposed to be involved in the development of ACF.

In general, body weight was lower in rats fed oligofructose than in those fed inulin. The cecal pools of acetate, propionate, butyrate and total short-chain fatty cids (SCFA) were significantly higher in rats consuming inulin or oligofructose. Cecal weights were increased in a dose-dependent manner in rats receiving fructans. Significantly greater relative colon wall weight was seen in animals receiving 15% inulin or oligofructose. There were large variations in the microbial data. In general, an increase in fecal and cecal anaerobic bacteria and a decrease in fecal aerobic bacteria were observed in animals given large amounts of fructans. The number of cecal enterococci was decreased in the groups given 15% fructans whereas the number of cecal bifidobacteria usually increased. Raftilose® or Raftiline® at 15% reduced the total number of ACF. The degree of inhibition was more pronounced in animals fed Raftiline®HP than in those fed Raftilose®P95. Differences between the fructans were seen, depending on the size of the ACF.

It was concluded that the effect on ACF outcome was influenced by the chain length of the fructans. Data on SCFA and weight of colon and cecum indicate a difference in site of fermentation between inulin and oligofructose, inulin being fermented in more distal parts, which most probably explains the different effect of the two fructans.

Poulsen M., Molck A-M. & Jacobsen B.L. (2002). Different effects of short- and long-chained fructans on large intestinal physiology and carcinogen-induced aberrant crypt foci in rats. Nutr Cancer, 42, 194-205.

continued on page 14

Long-chain inulin dose-dependently suppresses aberrant crypt foci and colon tumours in rats

Pre-neoplastic ACF induced by azoxymethane (AOM) have been used extensively to investigate nutritional modulation of colon carcinogenesis in rats. This study investigated the effect of 10% dietary long-chain inulin (Raftiline®HP) on the AOM-induced colonic pre-neoplastic ACF and tumours in the small intestine and colon, at the initiation (I), promotion (P) and I + P stages, in Fisher 344 male weanling rats. AOM was administered at 7 and 8 weeks of age, and rats were sacrificed at 16 weeks for the ACF experiment and at 45 weeks for the tumour experiment. Cecal weight was greater in rats fed inulin and cecal pH was lower, which may be caused by the greater level of SCFA production. The inulin group had 66% fewer aberrant crypts and 60% fewer ACF compared with the control group. Inulin inhibited tumour incidence and multiplicity, and reduced the overall size of colon tumours. The effects of inulin were much more pronounced in the promotion group. Tumour incidence in the small intestine of rats in the control, I, P and I+P groups were: 78%, 31%, 0% and 11%, and in the colon: 90%, 73%, 69% and 50%. The corresponding values for the distal portion of the colon were: 87%, 63%, 45% and 33%. Colon tumours per tumour-bearing rat were: 4.2, 3.1, 1.4 and 1.2 for the control, I, P, and I+P groups. All groups differed significantly ($p < 0.05$) from the control.

The results of this study indicate clearly that dietary Raftiline®HP suppresses AOM-induced ACF formation and colon tumours in rats, particularly at the promotion stage. Adults with pre-neoplastic lesions in their colon may therefore benefit from dietary long-chain inulin.

Verghese M., Rao D.R., Chawan C.B., Williams L.L. & Shackelford L. (2002). Dietary inulin suppresses azoxymethane-induced aberrant crypt foci and colon tumors at the promotion stage in young Fisher 344 rats. J. Nutr., 132, 2809-2813.

Continuation of page 13

Long-chain inulin suppresses aberrant crypt foci formation in mature rats

In this study, long-chain inulin (Raftiline®HP) was tested for its ability to suppress aberrant crypt foci (ACF) formation in the colon of 12 months old Fisher 344 male rats. It was the first ACF study with mature rats. Rats received two injections of 10 mg AOM/kg body weight and were fed 0, 2.5g, 5g or 10g inulin/100g for 11 weeks.

Feeding inulin at 2.5g, 5g and 10g/100g reduced total colonic ACF significantly ($p < 0.01$) as well as reducing multi-crypt foci formation. There was a clear dose-response relationship in suppression of ACF and total crypt formation. Compared with animals fed no inulin, the reductions in ACF in rats fed 2.5g, 5g and 10g inulin/100g were: 25%, 51% and 65%, and the reductions in total crypts were: 24%, 52% and 69%. These data show that long-chain inulin reduces ACF formation in mature rats, which is closer to the human age of colon cancer development.

Verghese M., Rao D.R., Chawan C.B. & Shackelford L. (2002). Dietary inulin suppresses azoxymethane-induced preneoplastic aberrant crypt foci in mature Fisher 344 rats. J. Nutr., 132, 2804-2808.

Raftilose®Synergy1 decreases colon carcinogenesis in rats

This study compared the effects of a prebiotic (Raftilose®Synergy1, 10% of diet), a probiotic (Bifidobacterium lactis Bb12 and Lactobacillus rhamnosus LGG, $5 * 10^8$ cfu/g diet), and a synbiotic (a combination of the two) on AOM-induced colon cancer in rats. The rats received the supplements from 10 days before AOM injection until their death (31 weeks after AOM). The rats were fed a high-fat diet in which the sources of carbohydrate were sucrose and maltodextrins.

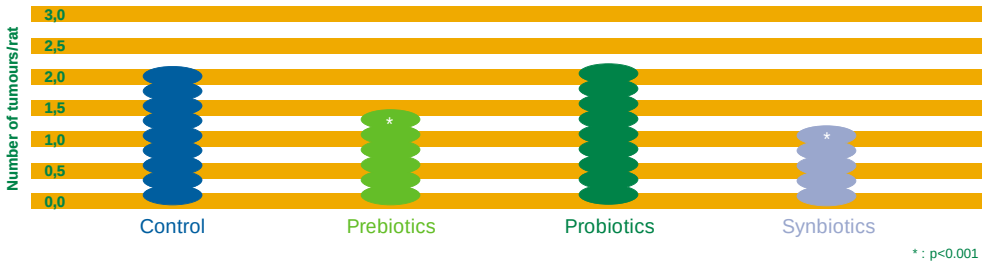
Rats treated with Synergy1 alone or in combination with the probiotic had a significantly lower ($p < 0.001$) number of total and malignant colon tumours than rats without inulin. Probiotics alone tended to reduce malignant tumours ($p = 0.079$). Cecal short-chain fatty acids were higher ($p < 0.001$) in the group treated with Synergy1. Colonic cell proliferation was lower in the Synergy1 group compared with controls. Glutathione-S-transferase placental enzyme p-type expression (associated with failure of cancer chemotherapy and poor patient survival) and, to a lesser extent, inducible NO-synthase were depressed in the tumours from rats fed Synergy1, with or without probiotics.

Cycloxygenase-2 expression was increased in tumours of controls, but not in those fed the prebiotic and/or the probiotic.

In conclusion, prebiotic (Synergy1) administration in the diet decreases AOM-induced carcinogenesis in rats significantly. It may act through a combination of mechanisms involving an increase in SCFA production, lower proliferative activity and a variation in the expression of some enzymes involved in the pathogenesis of colon cancer.

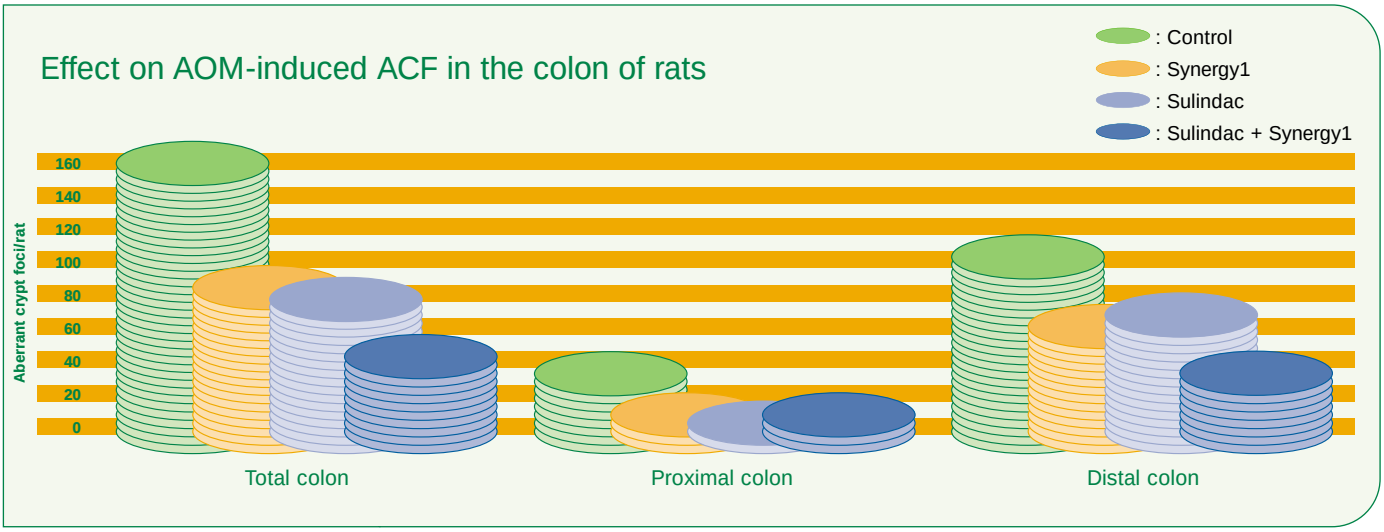
Femia A.P., Luceri C., Dolara P., Giannini A., Biggeri A., Salvadori M., Clune Y., Collins K.J., Paglierani M. & Caderni G. (2002). Antitumorigenic activity of the prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnosus and Bifidobacterium lactis on azoxymethane-induced colon carcinogenesis in rats. Carcinogenesis, 23, 1953-1960.

Effect on colon cancer in rats



* : p<0.001

Raftilose®Synergy1 enhances the inhibitory effect of non-steroidal anti-inflammatory drugs (NSAIDs) on aberrant crypt foci in rats



This study tested the possible synergistic effect of enriched inulin (Raftilose®Synergy1) and selected NSAIDs on AOM-induced ACF in the colon of Fisher 344 male rats. NSAIDs are currently being studied as potential chemopreventive agents.

In this study, 90 weanling rats were divided into 8 groups and fed for 13 weeks a control diet or a diet supplemented with either 10% Synergy1, 200ppm Sulindac, 200ppm Piroxicam, 200ppm aspirin, 200ppm Sulindac + 10% Synergy1, 200ppm Piroxicam + 10% Synergy1, or 200ppm aspirin + 10% Synergy1. All animals received two injections of 16 mg/kg body weight of AOM at weeks 7 and 8, and were sacrificed at 16 weeks of age.

There were no significant differences in weight gain or feed intake between control and experimental groups, but there was a significant ($p < 0.05$) increase in cecal weight and a decrease in cecal pH with Synergy1 (from 7.5 in control group to 6.1, 6.0, 6.8 and 6.9 in the groups fed Synergy1, Synergy1 + Sulindac, Synergy1 + Piroxicam and Synergy1 + aspirin, respectively). Administration of Synergy1 and NSAIDs) suppressed induction of colonic ACF significantly ($p < 0.05$, both in total numbers and in crypt multiplicity. The reductions in ACF in the experimental groups compared to the control group were 52.8 % (Synergy1), 53.1 % (Sulindac), 60.2% (Piroxicam), 59.1 % (aspirin), 67.1 % (Synergy1 + Sulindac), 73.8 % (Synergy1 + Piroxicam), and 70.3% (Synergy1 + aspirin). Reduction was greater when Synergy1 and NSAIDs were fed in combination rather than separately. This study demonstrates clearly that Synergy1 enhances the anti-carcinogenic effect of NSAIDs.

Verghese M., Walker L.T., Richardson J.E.W., Bonsi I.A., Shackelford L., Chawan C.B. & Van Loo J. (2002). Inhibitory effect of non-steroidal anti-inflammatory drugs (NSAIDs) on azoxymethane-induced aberrant crypt foci in rats is enhanced by prebiotic Synergy 1, Poster presented at the AAC Conference on Frontiers in Cancer Prevention Research. . October 14-18, 2002, Boston, USA.

AGENDA

Groningen, The Netherlands

April 6-9, 2003

Subject: I5th Carbohydrate Bioengineering Meeting

Content: Nature, Origins and Functions of Prebiotic Oligosaccharides

Speaker: Prof. Marcel ROBERFROID, University of Louvain

Info: Prof. Dr. L. Dijkhuizen

Tel: +31 50 3622150

E-mail: L.Dijkhuizen@biol.rug.nl

Internet: www.biol.rug.nl/cbm5

Geneva, Switzerland

May 13-15, 2003

Subject: VitaFoods International - The Global Neutraceutical Event

Content: Improved resistance and immune response with inulin and oligofructose.

Speaker: Wim CAERS, ORAFTI

Info: Victoria Emerton

Tel: +44 1372822 315

Fax: +44 1 1372 822272

Email: gangus@lfra.co.uk

Internet: www.vitafoods.eu.com

Noordwijkerhout, The Netherlands

May 18-21, 2003

Subject: Dietary Fibre 2003

Content: The role of prebiotic fibres in the process of calcium absorption

Speaker: Wim CAERS, ORAFTI

Info: Bastiaanse Communication

Tel: +31 30 229 42 47

Fax: +31 30 225 29 10

Email: DF2003@bastiaanse-communication.com

Internet: www. bastiaanse-communication.com

Brugge, Belgium

September 8-12, 2003

Subject: IDF World Dairy Congress 2003

Content: Innovation in low-fat dairy products with inulin and oligofructose

Speaker: Rudy WOUTERS, ORAFTI

Info: Rita Peys

Tel: +32 3 260 08 40

Fax: +32 3 216 06 89

Email: rita.peys@ti.kviv.be

Internet: www.fil-idf.org

COLOPHON

Active Food Scientific Monitor is published by ORAFTI, a daughter company of RAFFINERIE TIRLEMONTOISE (B), which is part of the SÜDZUCKER Group (D). ORAFTI produces inulin (Raftiline®), oligofructose (Raftilose®) and fructose syrups (Raftisweet®) from chicory roots. The commercial department of ORAFTI is based in Tienen and operates worldwide in 70 countries through own sales offices and distributors. The production units are located in Belgium and The Netherlands.
Active Food Scientific Monitor appears twice a year and is sent to researchers, nutritionists and health professionals. It will be sent free to anyone else who is interested.

We would be very pleased to receive your suggestions and reactions at the following address:
ORAFTI Active Food Ingredients
Christine Nicolay
Aandorenstraat 1
3300 Tienen - Belgium
tel.: +32 16 80 13 01
fax: +32 16 80 13 08
e-mail: afi@orafti.com
www.ORAFTI.com

Editing, design and coordination
TO.BE www.ornottobe.be

Registered publisher
Anne Franck
Aandorenstraat 1, 3300 Tienen - Belgium