



# Active Food Scientific Monitor



## Interview

with Dr. José  
M. Saavedra

In this issue we are pleased to introduce you to Dr José M. Saavedra, Associate Professor of Pediatrics at Johns Hopkins University School of Medicine, Baltimore, and Medical & Scientific Director of the Nutrition Division of Nestlé USA.

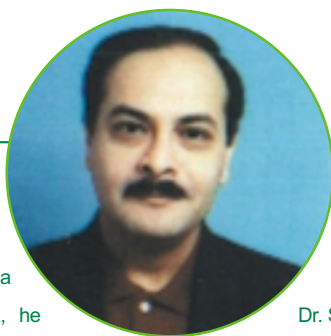
The preliminary findings of several recent human clinical trials indicate that pro- and prebiotics may prove to be useful dietary adjuncts in the management of gastrointestinal and systemic conditions that are related to an adequate interaction between our microbial environment and our immunological relationship to it. An important mechanism of pro- and prebiotic agents is the induction of immunological responses from the gut-associated lymphoid tissue.

## The immunological promises of probiotics and prebiotics

A few years ago Dr J.M. Saavedra did one of the first preventive nutritional studies in the field of probiotics. This study showed that a number of clinical effects of probiotic agents are related to their immuno-modulatory effects.

**You did a number of studies on acute diarrhoea in children and the potential beneficial effects of probiotics.**

"Most of the original interest came from the line of thinking that a number of gastrointestinal disorders are probably related to alterations in the intestinal flora. We do not know yet if there is a direct cause-and-effect between changes in flora and a number of conditions. However, there is a clear association. A number of acute conditions - from common infant diarrhoea, particularly viral diarrhoea, as well as antibiotic-associated diarrhoea, all the way to a number of intestinal altered states, particularly inflammatory conditions, whether they are primary inflammatory conditions (such as irritable bowel disease, Crohn's disease or colitis), or secondary inflammatory conditions such as necrotising enterocolitis in newborns - are associated in



### Poetry: what's in a name?

Dr. Saavedra was born in Peru and finished medical school in Lima. After a paediatric residency in New York, he became a gastroenterologist and nutritionist at Johns Hopkins University. His interest in gastroenterology comes from his original work at the Nutrition Research Institute in Peru managing children with chronic diarrhoea.

He is associated with several scientific societies, most importantly, with the Society for Clinical Nutrition and the American Society for Parenteral and Enteral Nutrition, the American Academy of Paediatrics and the North-American Society of Gastroenterology and Nutrition. Recently, he became Medical & Scientific Director of the Nutrition Division of Nestlé USA.

Dr. Saavedra is married and is the father of two children.

His main hobby is poetry, both English and Spanish. This is maybe not so surprising for someone called Saavedra. The family name of Miguel de Cervantes, one of the most famous Spanish writers and poets, author of Don Quixote, was indeed Saavedra. And some other members of the very broad Saavedra family are or were writers. "I prefer the romantic Spanish poets, such as Antonio Machado y Ruiz. But I also became a great fan of English poetry. My favourite is the American poet Carl Sandburg." Dr. Saavedra writes poetry himself, "but only for personal use", he admits.



# P R E F A C E

by Dr. Anne Franck

## Oligofructose allowed in baby food and infant formulae in Europe

In a two-step evaluation process, the Scientific Committee on Food (SCF) of the European Commission has confirmed the use of oligofructose as a safe ingredient in baby food. The ruling was made possible by a substantial scientific contribution and submissions by the food industry, which demonstrated the ingredient's safe and effective use.

The emergence on the market of non-digestible oligosaccharides, including oligofructose, in baby and infant foods prompted the European Commission to ask the SCF to evaluate the safety and efficacy of oligosaccharides in these applications. Due to the lack of information available in general literature, the food industry was asked to provide extensive scientific information. ORAFI was invited to attend the SCF hearing to explain and demonstrate the benefits of these ingredients. After examination of all the data submitted, the Scientific Committee confirmed, on September 26, 2001, that oligofructose and galacto-oligosaccharides can be used in follow-on foods in a concentration of up to 0.8 g/dl in the product ready for consumption.

After a second round of scientific submissions by the industry and the positive evaluation by the SCF, the Committee concluded on December 13, 2001 that the safe use of a mixture of oligofructose and galacto-oligosaccharides can be extended to infants, aged between 0 and 6 months.

Oligofructose is a prebiotic ingredient used world-wide in many food and drink products for its beneficial effects on gut balance and health. Already used in baby food products in other areas of the world, including Asia and Latin America, the SCF statement means that it will now be possible to develop new baby food products with additional nutritional benefits in Europe.

Additional information can be found in the European Commission documents SCF/CS/NUT/IF/35, released on September 27, 2001, and SCF/CS/NUT/IF/47, released on December 14, 2001.



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## The immunology

one way or another with changes in the intestinal flora.

We know that the intestinal flora of breast-fed babies is different from that of formula-fed children in general, with very big variations. All these elements point to the possibility or the suggestive potential of either a preventive or a therapeutic approach of a change, a modification of the intestinal flora in children as well as in adults.

That is where the interest came from. The most significant work that we did goes back to the original study, which was published in *The Lancet* a few years ago. I think it was the first preventive study in the field of probiotics. All the previous studies used probiotic agents for the management or treatment of acute intestinal conditions in children, especially diarrhoea. In our study, we demonstrated for the first time that the regular ingestion of probiotics, in this case bifidobacteria, actually did decrease the incidence of diarrhoeal disease in children that were not treated for intestinal problems, but for orthopaedic or respiratory problems. The interesting thing about that study, besides the significant decrease in diarrhoea was, to our surprise, a decrease in the shedding of rotavirus, whether the children developed diarrhoea or not.

Until recently, the concept of probiotics was: "let's give a lot of good bacteria to counteract the bad bacteria". The supposed mechanism was that these probiotic agents could compete for nutrients, for receptors, for the occupancy in the lumen of the intestines and thus prevent bacterial infections. But what we were seeing and what has now been demonstrated by others, is that these probi-



## ical promises of pro- and prebiotics

otics have the greatest effect on viral infections. What we demonstrated was thus a preventive effect of a bacterial agent on a viral agent. This has led to the hypothesis that is now generally accepted, that probiotics may induce an immunological response.

A lot of studies have since been done, in children and in animals. I am particularly struck by a number of animal studies confirming what we have shown in infants: if you give a probiotic agent prophylactically, than you will stimulate a gut-associated lymphoid immune response, particularly the secretory response for IgA. That will decrease either the occurrence or the severity of diarrhoeal disease.

A recent study done in Peru on free-living children showed, in addition, that the effects of probiotic agents were more marked in children who were not breast-fed. Again supporting the fact that the mechanism probably is a stimulation of the immunological response of the gut.

### What do you expect for the near future in this field?

We still need to learn a lot about the mechanisms, about the doses and about the specific strains of probiotics. It is very clear now that every strain of probiotic is different, which likely will lead to different types of benefit, depending on what strains we use and depending on what conditions we are trying to ameliorate. For example, it is very clear that different strains of bifidobacteria will have different degrees of activity in the amount of immunological secretory IgA response in the gut. It is very clear also that some strains of lactobacilli will have a greater effect on modulating the pro- and anti-inflammato-

ry families of lymphocytes in the gut-associated lymphoid tissue. Some probiotics may have a better effect on the cellular response of the gut. A lot of studies are now focusing on decreasing the chances of inflammation following sensitisation with food allergens. Some Scandinavian studies show, for instance, that there is at least some effect on decreasing the allergic response, which means that some bacterial strains have an effect on the cellular response and potentially prevent or decrease allergic symptoms in the gut, while others have a more secretory immune response and prevent or decrease the chance of secretory IgA.

### Are similar effects seen with prebiotics?

Studies on the possibility that prebiotic agents directly or indirectly induce similar immuno-modulation have begun only recently. The preliminary findings of several human clinical trials indicate that prebiotics may indeed prove to be useful in the management of gastrointestinal and systemic conditions that are related to an adequate interaction between our microbial environment and our immunological relationship to it.

We recently conducted a controlled, double-blind, randomised, longitudinal study among 123 healthy children aged between 4 and 24 months attending day-care centres. They received a regular cereal or cereal supplemented with oligofructose. Significant differences were noted in the symptoms, medical visits and day-care absenteeism associated with diarrhoea. Episodes of cold symptoms with fever, rhinorrhoea and cough were reported less frequently in the supplemented group.

Antibiotic prescription associated with respiratory illness also was significantly lower in the supplemented group.

These results suggest an ameliorating effect of the regular ingestion of a prebiotic incorporated into a weaning food in this healthy population. The effect may be different in populations with different immunological status or different intestinal flora.

Other studies have confirmed these findings. But further studies are needed to corroborate these preliminary findings.

There is a big difference in the use of probiotics and prebiotics. With prebiotics, we can be less selective about the types of agent that we are promoting the growth of. The use of prebiotics basically assumes that the native flora of the individual is going to respond and that bacteria that are beneficial will be stimulated selectively. So, the effect of prebiotics depends a lot on the type of flora that the individual already has. If you have, for example, a number of strains of bifidobacteria and lactobacilli in the colon, you would expect a better response. If the amount of native bacteria or the number of species of lactobacilli and bifidobacteria is nearly non-existent, then the effect of prebiotics will be less. The benefit of prebiotics, on the other hand, is that they are much simpler to use, they are less costly and are much easier to introduce into the food supply. Probiotics can easily be used in almost any matrix, in almost any food, whereas the way probiotics are delivered is much more complicated, as we believe that some viability is necessary.

Much has been learned, much has yet to be learned.

# Improved resistance and immune response with inulin and oligofructose

There is increasing awareness that immune functions and health are responsive to the bacteria resident in the gastrointestinal tract (GIT). Changes in the populations of GIT bacteria caused by diet, antibiotics or other means can alter enteric and systemic immune functions. For example, a decrease in bifidobacteria in the colon, especially during ageing, might be an important cause of decreased immunity. Certain components of the gut microflora may be involved in the aetiology and/or maintenance of severe gut diseases such as inflammatory bowel disease, colon cancer, colitis, etc. (2, 27).

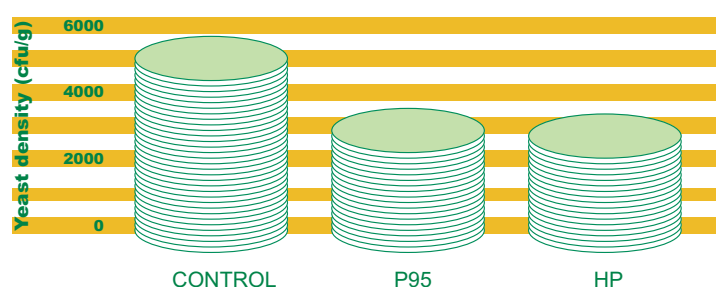
In contrast, the lactic acid-producing bacteria (LAB) are considered to provide health benefits and enhance immune functions. Increasing the densities of LAB has been associated with improved resistance to enteric and chemical pathogens. It is thought that lactic acid bacteria act as adjuvants, able to stimulate both non-specific host

defence mechanisms and some cells involved in the specific response. The result is often an increased phagocytic activity and/or an elevation of immune molecules such as secretory immunoglobulins IgA, which may affect pathogens (27). Changes in the intestinal microflora may mediate immune modulation via the direct contact of LAB or bacterial products (cell walls or cytoplasmic components) with immune cells in the intestine, the production of short-chain fatty acids (SCFA) from fermentation, or changes in mucin production (35).

It is becoming apparent that modification of intestinal flora following the ingestion of probiotics, prebiotics and synbiotics can interact with the immuno-

logical components of the intestine, and yield gastrointestinal protective effects and, given the nature of the immunologic response of the gut associated lymphoid tissue (GALT), yield systemic effects, such as the modulation of cellular response to antigens and the humoral responses that may have significance for other mucosal surfaces, such as the skin and respiratory tract, and thus provide a broad systemic benefit (33). This review examines if and how supplementing the diet with non-digestible oligosaccharides (NDO) and, more specifically, the fructans inulin and oligofructose (OF), influences the defence functions and thereby increases resistance to pathogens and other health challenges.

**Candida challenge** After Buddington et al. (2002 b).





## Probiotics and Prebiotics

The prebiotic properties of inulin and oligofructose are now well documented, both in vitro and in vivo (animal and human feeding studies). The majority of the influences of NDO on defence functions can be attributed directly or indirectly to changes in the population and metabolic activities of the bacterial populations present in the GIT. They are not considered to be immunogenic and apparently do not induce the expression of various enzymatic systems associated with xenobiotic metabolism directly (2). There are a number of possible mechanisms in operation (2,27). It is well established that the fermentation of inulin and oligofructose increases the production of SCFA, primarily acetate, butyrate and propionate, in the gut. A number of studies provide evidence for the immunomodulatory and anti-inflammatory properties of SCFA. They lower the gut pH to levels below those at which pathogens such as *Escherichia coli* are able to compete effectively. Butyrate may reduce the requirement of epithelial cells for glutamine, thereby sparing it for other cells, such as those of the immune system (35). SCFA, and particularly butyrate, enhance the defence functions of both the small and large intestine, and stimulate mucosal defences by increasing the

proliferation of enterocytes and colonocytes (2). Moreover, butyrate can exert an immunoregulatory effect on the cells of a colonic epithelial cell line. It might regulate gene expression and protein synthesis (24). Bifidobacteria bind to various cell-surface glycolipids, occupying receptor sites for pathogens (27). They are reported to produce anti-adhesive glycans and proteins inhibiting the binding of pathogens to their cellular receptors (27). They produce antimicrobial agents such as bacteriocins, active against both Gram-negative and Gram-positive pathogens.

A positive influence of inulin on the GALT, by supporting the immune system and making the luminal content less toxic, has been reported (24). It has been demonstrated that the prebiotic effects of inulin and oligofructose occur at the critical interface between the mucosa and surface-associated bacteria (12). Moreover, oligosaccharides themselves may act as anti-infective agents through the occupation of bacterial ligands for pathogen colonisation/receptor sites (27).

## Resistance to luminal pathogens

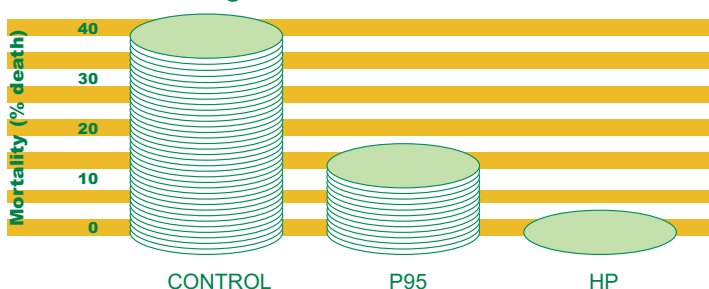
The mature GIT ecosystem is resistant to colonisation by invading species and to overgrowth by opportunistic pathogens that are already present.

Additional protection can be obtained by encouraging higher densities of LAB by supplementing the diet with either probiotics or prebiotics. Conversely, disturbances or abnormalities in the populations and metabolic activities of the GIT bacteria lead to a greater sensitivity to a number of pathogens and a greater risk of colorectal cancers.

This has been demonstrated clearly by studies on neonatal necrotising enterocolitis (NEC). NEC is a devastating gastrointestinal disease of preterm infants. In these infants, intestinal colonisation with bifidobacteria is delayed for several weeks. This promotes colonisation by potentially pathogenic bacteria, including *Clostridium* spp. Using gnotobiotic quails as an experimental model of NEC, it has been shown (6) that the onset of intestinal lesions requires a combination of low endogenous lactase activity, lactose in the diet, and colonisation by lactose-fermenting bacteria such as clostridia. Bifidobacteria protected against NEC via the inhibition of growth of *Clostridium butyricum* or the disappearance of *Clostridium perfringens*. The main effect of bifidobacteria on lactose fermentation was a decrease or disappearance of butyric acid. The protective role was not associated with changes in H<sub>2</sub> production. Similar results were obtained in rats. Exogenous bifidobacterial supplementation of newborn rats resulted in intestinal colonisation by 24 hours. The bifidobacteria-supplemented rats had a significant reduction in the incidence of NEC. Plasma endotoxin and intestinal phospholipase A<sub>2</sub> expression were lower, supporting

the role of bacterial translocation and activation of the inflammatory cascade in the pathophysiology of NEC (9). A number of studies with germ-free quails inoculated with fecal flora from healthy or sick premature neonates investigated whether supplementation with the prebiotic oligofructose (RAFTULOSE®P95) also influenced the development of intestinal bifidobacterial colonisation. (10,13) The authors demonstrated that the use of oligofructose promoted the growth of endogenous bifidobacteria and helped to prevent the overgrowth of pathogenic bacteria (*E. coli*, *C. perfringens*, *Clostridium ramosum*, *C. butyricum*, *Clostridium paraputrificum*, *Clostridium difficile*, *Klebsiella* sp.) implicated in NEC (7,8,10,13). However, when bifidobacteria were initially absent, the regular ingestion of OF did not induce bifidobacterial colonisation. Nevertheless, it was shown that OF can act as an anti-infective agent and decrease the occurrence or severity of the NEC lesions depending on the species of bacteria involved (7,8). Supplementation with OF led to the complete inhibition of NEC-like lesions in quails with *C. perfringens* and *Klebsiella*. In quails with *C. perfringens* and *C. difficile*, and in quails with *C. perfringens*, *C. difficile* and *C. paraputrificum*, OF did not decrease the number of sick quails but reduced the severity of the disease significantly (less extensive tissue necrosis and decrease in haemorrhages). Bifidobacteria and OF showed a symbiotic effect that led to the complete disappearance of caecal lesions concomitant with a sharp decrease of clostridia species and caecal

## Listeria challenge After Buddington et al. (2002 b).



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butyrate. A study with antibiotic-compromised mice (18) showed that dietary inclusion of OF influences immune cell populations (macrophages) residing in the lamina propria of the caecum and the colon, the sites at which *C. difficile*-induced lesions are localised. This suggests that OF may affect toxin production from *C. difficile* indirectly, possibly through altered fermentation patterns with increased SCFA production and decreased pH. In another study, although OF and inulin augmented the effect of antibiotic on faecal microbiotas in vitro, partial suppression of *C. difficile* was donor-specific (20).

OF has been reported to protect hamsters against *C. difficile* colitis (39). Studies in calves (4) and in neonatal pigs (5) have demonstrated that the supplementation of OF can increase bifidobacteria populations in the large intestine and decrease the numbers of *E. coli* and clostridia, and therefore would help to prevent intestinal infections. Corresponding with this, supplementing the diets of pigs, chickens and rats with OF or inulin can reduce fecal densities of salmonella. This was observed in a study that evaluated different strategies to reduce carriage of *Salmonella* spp in pigs: probiotics, prebiotics, vaccination, immunoglobulins and acidification of drinking water (23). A reduction in the shedding of *S. typhimurium* was observed after supplementation with OF in drinking water, but not when given in feed. Changes were observed in microbial composition of selected sections of the intestinal tract. The combination of OF with a probiotic was not effective. The combined administration of *Lactobacillus paracasei* and OF to newborn pigs had no significant effect on the immune system, whereas in the critical period

of weaning OF improved the ability of lactobacilli to survive in the gut considerably and to adhere in higher numbers, leading to enhanced stimulation of the immune system (19). Using real-time PCR, OF and lactulose were found to be effective in stimulating lactobacilli and suppressing salmonella in rats (34).

It has been shown in vitro that some bifidobacteria exert powerful antagonistic effects towards *E. coli* O157 (27). Inclusion of OF and coconut endosperm fibre in a model of the canine large intestine resulted in the elimination of viable *Campylobacter jejuni* cells (1). An OF-supplemented diet was shown to be beneficial in dogs with small intestinal bacterial overgrowth (29, 38). Providing OF electrolyte solutions (OES) to pigs with acute secretory diarrhoea induced by cholera toxin did not cause a reduction in the duration of diarrhoea or the associated loss of water (25). However, adding OF accelerated the recovery of bacteria perceived as beneficial (lactobacilli), while slowing the recovery of pathogenic forms. This should accelerate recovery from diarrhoea and reduce the risk of secondary infections.

## Mucosal defence

The multilayered mucosal barrier acts as a selective filter to exclude pathogens, hazardous chemicals and other potential challenges of health. The mucus secreted by epithelial cells is the first layer of mucosal defence and consists of a combination of IgA, antimicrobial peptides, and a complex mixture of glycosylated proteins that are coded for several genes. IgA are secreted by B-lymphocytes (plasma cells) in the lamina propria of the mucosa and are directed to specific antigens present in the lumen of the GIT, and even to antigens from commensal bacteria.

The antimicrobial peptides secreted by the GIT are an important component of the innate immune system; however, the relationships between the antimicrobial peptides, the GIT bacteria and diet are poorly understood. The increased activity of other innate defences (e.g. macrophages and NK cells) in response to pre- and probiotics, suggests that constitutive expression of antimicrobial peptides may be modulated by changes in the GIT bacteria (2).

There is some evidence that the addition of fermentable fibres to the diet can increase mucin production, which might contribute to the lower incidence of bacterial translocation across the gut barrier. One study has reported that feeding inulin increased sulphomucin production in germ-free and heteroxenic rats (17). The patterns of immune function modulation in the GIT vary between sources of fibre, and the regions and tissue layers of the GIT. Several studies suggest that diets with fermentable fibre do not change the types and functions of immune cells, but alter their distribution, relative abundance and specific responses (2).

## Systemic immune functions

There is increasing evidence that fermentable carbohydrates and especially non-digestible oligosaccharides (NDO) can modulate various parameters of the immune system, including an increase in serum, mesenteric lymph nodes, mucosal immunoglobulin production, the number of Peyer's patches, altered cytokine production in mesenteric lymph nodes, as well as altered leucocyte and lymphocyte numbers in tissues such as the spleen, blood and intestinal mucosa (35).

An in vitro study into the effect of carbohydrate treatment on

macrophage proliferation (24) showed that various carbohydrates were able to stimulate macrophage proliferation, although the effect diminished at higher concentrations. It was found that more stimulation of proliferation was obtained with a higher chain length. It has been shown that fructans can bind to the active site of immunoglobulins.

Another study (22) measured selected systemic immune variables in B6C3F1 mice fed for six weeks diets with 10% cellulose, 10% inulin, 10% OF, or 2.5% OF (22). White blood cell counts were within normal ranges, but were higher in the mice fed cellulose compared to the other diets. Spleen mass, CD4/CD8 and T/B ratios from suspensions of spleen and thymus, and fecal IgA concentrations did not differ. Mice fed 10% OF or inulin had higher natural killer cell activity of splenocytes and greater phagocytic activity of unactivated peritoneal macrophages. These results indicate that OF and inulin do not elicit an increase or redistribution of the lymphocyte populations tested (CD4/CD8 and T/B populations), but up-regulate macrophage-dependent (T-helper 1 type) immune responses, and act in a dose-dependent manner. This was confirmed in another study with B6C3F1 mice infected systemically with virulent strains of *Listeria monocytogenes* and *Salmonella typhimurium*. After being fed a diet with inulin or OF (10%) mice had significantly lower mortality than mice fed a diet with cellulose (3). Interestingly, inulin provided greater resistance than oligofructose to these systemic infections.

The higher densities of LAB after feeding rodent diets supplemented with fructans are associated with reduced conversion of the procarcinogen dimethylhydrazine (DMH),



deactivation of the active carcinogen azoxymethane (AOM) and lower incidences of colonic aberrant crypt foci (28), even if fed after exposure to AOM. This indicates that changes in the GIT bacteria influence the promotional phase of carcinogenesis.

Supplementing rodent diets with NDO reduces the growth of transplantable tumours (28,36) and tumour incidence in Min mice,

which are susceptible to spontaneous intestinal tumours (26). This study showed that OF may provide an immunocompetent host with a mechanism of tumour surveillance in which T-cells participate. It has been shown in vivo that, when injected into the bloodstream, gamma-inulin leads to the production of macrophages that destroy cancer cells (24).

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## Resistance to infections in humans

Human studies on this topic have begun only recently. Although most of these studies are preliminary, they indicate that prebiotics may prove to be a useful dietary adjunct in the management of gastrointestinal and systemic conditions that are related to an adequate interaction between our microbial environment and our immunologic relationship to it (33).

One study examined the effects of a pediatric weaning food supplemented with OF in 123 non-breast-fed infants aged 4 to 24 months attending daycare centres (32,37). One group received a standard infant cereal for six months, the second group received the same cereal supplemented with 0.55g OF (Raftilose®P95) per 15g cereal. There were no significant differences between the groups in frequency of diarrhoea, but consumption of the OF-supplemented cereal was associated with a decrease in severity of diarrhoeal disease. General GI status was improved with decreased perceived bowel movement discomfort, vomiting and regurgitation. Furthermore, consumption of OF resulted in adequate growth and was associated with reduction in febrile events and cold symptoms with less fever, rhinorrhea and cough, antibiotic prescription associated with respiratory illness and daycare absenteeism.

In a 16-week study with children aged between 10 and 24 months it was demonstrated that the prophylactic feeding of OF could reduce the duration and number of recurrent episodes of diarrhoea (14).

Another study (16) evaluated the incidence and duration of sickness in mild to moderately malnourished children who received a nutritional supplement with and without synbiotics (*L. acidophilus*, *B.*

*infantis* and OF). A total of 626 children aged between 1 and 6 years of age in 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 subjects. Both groups experienced catch-up growth, with a significant increase in normalised percentiles for weight and height. There was no significant difference between the groups for sick days, but the number of sick days for children aged 3-5 with at least one sick episode was lower in the group fed with synbiotics. Also, constipation days were fewer in the synbiotic-fed group.

The effects on the immune response after measles vaccination of an infant food supplemented with a prebiotic mixture of OF (Raftilose®P95) and inulin (Raftiline®GR) was examined (15). Post-vaccination IgG antibody levels were significantly higher in the supplement group (96% IgG positivity rate versus 88% in the control group). Mild reactions to vaccination were observed more often in the supplement group. No difference in growth or overall health status was observed.

The effectiveness of OF in preventing travellers' diarrhoea was tested in a randomised, double-blind, placebo-controlled study, with 244 healthy subjects, travelling to destinations of high and medium risk for diarrhoea (11). They received 10g OF (Raftilose®P95) or placebo daily during two weeks before the holiday and during the 2-week holiday. They recorded bowel habit by diary and completed a post-study questionnaire. The consumption of OF led to a small (6%) increase in stool frequency in the pre-holiday period. There was no significant decrease in episodes of diarrhoea, with 20% on placebo and 11% on OF recording episodes in the post-study questionnaire. However, it is interesting that the OF-fed group reported a significantly better sense of "well-being" during the holiday, in comparison with the placebo-group.

	Placebo	Oligofructose	P
Diarrhoea (%)	19.5	11.2	0.08
Feeling well (%)	4.7	12.9	0.04

After Cumming et al. (2001).

# STATE OF THE ART

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# MONITOR

## Prebiotic effect of oligofructose and inulin at low intake levels

The purpose of this study (Rao, 2001) was to investigate the effect of ingesting a low dose of oligofructose (OF; 5 g/day of Raftilose®P95) on the fecal microflora in humans (n=8) compared with the ingestion of a placebo (sucrose). Fecal samples were taken at the beginning of the study, at the end of the 3 weeks on placebo, after 11 days and after 3 weeks on OF, and finally 2 weeks post ingestion of OF. The samples were enumerated for bifidobacteria, bacteroides, coliforms, total anaerobes and total aerobes. Ingestion of sucrose was without effect on all fecal bacteria enumerated, whereas consumption of OF for 11 days resulted in close to one log cycle increase in numbers of bifidobacteria. No further increase was observed after the next 10 days. Two weeks after ingestion of OF, numbers of bifidobacteria had decreased to almost that of the period before treatment. Increases in

numbers of bacteroides and total anaerobic bacteria but not in aerobic bacteria occurred. This study shows that even a low dose of OF has a bifidogenic effect.

In another study, by Tuohy et al. (2001), 10 human subjects received 8g/day of inulin (Raftiline®HP) for 14 days. This study used fluorescent in situ hybridisation for enumerating the bacteria in feces in order to overcome difficulties with culture-based techniques. A statistically significant increase in bifidobacteria was observed after 7 days. The bifidogenic effects were most marked in those subjects with low starting levels of bifidobacteria. This confirms the suggestion that the initial count of bifidobacteria is an influential factor in determining the relative increase in bifidobacteria.

Rao V.A. (2001). The prebiotic properties of oligofructose at low intake levels. *Nutr. Res.* 21, 843-848.

Tuohy K.M., Finlay R.K., Wynne A.G. & Gibson G.R. (2001). A human volunteer study on the prebiotic effects of HP-inulin - faecal bacteria enumerated using fluorescent in situ hybridisation (FISH). *Anaerobe*, 7, 113-118.

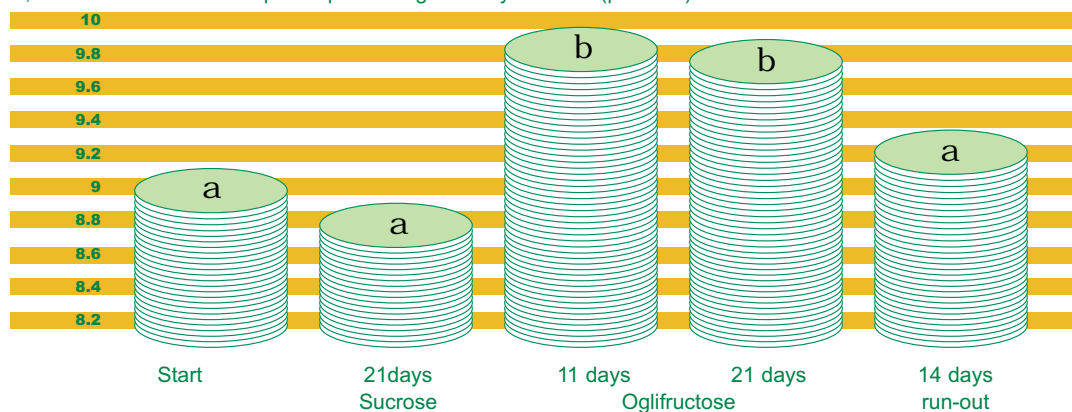
## Fermentation of prebiotics

The wide variety of new candidate prebiotics becoming available for human use requires that a manageable set of in vitro tests be agreed on, so that their non-digestibility (resistance to gastric juice, to pancreatic enzymes and to brush-border enzymes) and fermentability can be established without recourse to human studies. Short-chain fatty acids are a major product of prebiotic breakdown. Through stimulation of bacterial growth, increase in bacterial cell mass and thus stimulation of peristalsis, prebiotics affect bowel habit and are mildly laxative. They also affect microbial enzyme activity. Some are a potent source of hydrogen in the gut, and mild flatulence is common. Experiments to produce different chain lengths, degrees of branching and degrees of polymerisation might lead to prebiotics with further benefits to health.

Cummings, J.H., Macfarlane G.T. & Englyst H.N. (2001). Prebiotic digestion and fermentation. *Am. J. Clin. Nutr.* 73 (suppl.), 415S-420S.

## Bifidobacteria (log CFU/g wet feces) After Rao (2001)

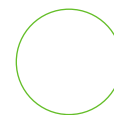
a, b: Data with different superscripts are significantly different ( $p < 0.001$ )



## Lipid lowering effects of dietary fructans

This review summarises a number of studies relevant to the effects of dietary fructans on lipid metabolism in humans and animals. In rats, the addition of OF to the diet is able to counteract triglyceride accumulation in the liver and/or in the serum,

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depending on the type of diet or patho-physiological status of the animals. In humans, attempts to reproduce similar lipid-lowering effects have generated conflicting results. Whereas studies have shown significant reductions in total cholesterol and/or triacylglycerol in normolipidemic subjects, studies in individuals with raised blood lipids showed significant decrease in fasting total cholesterol and LDL-cholesterol, but without significant changes in triacylglycerol levels. In subjects with type 2-diabetes, consumption of fructans resulted in either lowered or unchanged concentrations of fasting plasma glucose and serum total cholesterol. The differences between the results obtained in rats and humans may be explained by differences between species, physio-pathological states and doses of fructans.

Recent data showing a protective effect of dietary fructans on hepatic steatosis (fat accumulation) in animals would be interesting if confirmed in humans, since steatosis is one of the most frequent liver disorders, occurring together with syndrome X, in overweight and obese people.

The elucidation of key mediators that allow such fructans to exert systemic effects is essential in order to identify target functions, and patients who might benefit from an increase of fructans in their diet. Here, three hypotheses concerning the mechanism of these mediators are formulated: (1) a modulation of glucose or insulin homeostasis; (2) the regulation of hepatic fatty acid metabolism

by the end-products of fructan fermentation; (3) and a modulation of the intestinal production of incretins.

Delzenne N.M. & Daubioul C. (2000). Dietary fructans and lipid metabolism: Building a bridge from the colon to the liver. *Recent Res. Devel. Nutr.* 3, 227-238.

## Resistant oligosaccharides are functional food ingredients

This review article discusses the current state of knowledge on the fate and the physiological effects of resistant oligosaccharides in the gastrointestinal tract and their systemic physiological effects. As the resistant oligosaccharides share characteristics of dietary fibre, and are model "prebiotics", they are obvious candidates for recognition as functional food ingredients for which new health claims may become authorised. We discuss the scientific evidence for functional claims (bifidogenic effect, fecal bulking, increased calcium bioavailability, hypotriglyceridemic effect) and for disease risk reduction claims (constipation, infectious diarrhoea, osteoporosis, atherosclerosis, obesity, colon cancer).

Besides their positive effects on human health, resistant oligosaccharides contribute organoleptic properties to processed foods (they share the characteristics of soluble dietary fibres, and are used to replace fat and sugar). Because many consumers depend on processed foods as the mainstay of their diets, efforts should be made to increase the

resistant oligosaccharide content of popular foods.

Roberfroid M. & Slavin J.L. (2001). Resistant oligosaccharides. In: Cho S.S. & Dreher M. L. (ed), *Handbook of Dietary Fiber*. Marcel Dekker Inc., New York, USA, 125-145.

## Use and labeling of inulin and oligofructose

Franck et al. (2001) have given an overview of the key prebiotic ingredients identified today and produced industrially. Those containing fructose (inulin and OF) or galactose are fermented selectively by the colonic bacteria and are referred to as bifidogenic. Their potential nutritional and physiological benefits as well as their technological properties and applications are summarised here.

Inulin and OF are the only prebiotics available commercially and used in industrial quantities by the food industry worldwide. They are used either as supplements to foods to increase the dietary fibre content, or as macronutrient substitutes to replace fat and sugars. In most countries they can be used without specific limitations as ingredients in foods and drinks.

As reviewed by Coussement et al. (2001), inulin and OF are legally classified as food or food ingredients, and not as additives. As a consequence, neither inulin nor OF is listed as an accepted food additive in the standard lists from the European Union or Codex Alimentarius. In the USA, a committee of experts declared both substances as "Generally Recognized as Safe" (GRAS).



The name inulin is a legally accepted name for the ingredients list. For OF, either fructo-oligosaccharides or oligofructose can be used. It is not acceptable for inulin to be labelled as oligofructose.

Both substances comply with the Codex Alimentarius definition of dietary fibre, and they meet the AOAC definition of fibre as 'remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man'. It seems most logical to include inulin and OF in the soluble dietary fibre group.

Inulin and OF can be analysed using the AOAC fructan method 997.08. This method is very specific and accurate. It can be combined with the AOAC total dietary fibre methods. OF can be measured separately using HPLC or GC techniques.

Claims regarding the dietary fibre and bifidogenic effects of inulin and oligofructose are being made in many countries. In some countries a specific authorisation has been obtained for specific claims.

Franck A. & Coussement P. (2001). Prebiotics. In: Young J. (ed), Guide to Functional Food Ingredients, Leatherhead Publishing, Leatherhead, England, 1-19.

Coussement P. & Franck A. (2001). Inulin and oligofructose. In: Cho S.S. & Dreher M. L. (ed), Handbook of Dietary Fiber, Marcel Dekker Inc., New York, USA, 721-735.

### Health benefits of prebiotics and probiotics

An ad hoc committee of Swedish scientists was formed in 2000 by the Expert Group on Diet and Health of the National Food Administration and the Research Board of the Swedish

Nutrition Foundation, to review and evaluate all human studies available on health benefits of probiotics and prebiotics. The main results relating to prebiotics are:

- Inulin and OF are prebiotics showing significant bifidogenic effects in humans.
- Animal studies showing lipid-lowering effects of inulin and OF still need confirmation in man.
- Inulin and OF have dose-related bulking/ laxative effects.
- Despite clear experimental facts, evidence is still missing that prebiotics would decrease the risk of colon cancer in humans. Further identification and validation of biomarkers for risk of cancer is a prerequisite for these studies.
- Long-term studies with well-controlled diets are needed to confirm the potential of prebiotics such as fructans not only to increase calcium absorption but also to improve bone health.
- Prebiotics such as fructans have no significant side-effects, other than dose-dependent gastrointestinal discomfort.

Andersson H., Asp N.-G., Bruce A., Roos S., Wadström T. & Wold A.E. (2001). Health effects of probiotics and prebiotics. A literature review on human studies. Scand. J. Nutr./Näringsforsk. 45, 58-75.

### Synbiotic milk improves the human intestinal ecosystem

To investigate the effect of acute and prolonged consumption of a synbiotic product (2% fat milk with 2 g/100 ml of inulin (Raftiline®), *L. acidophilus* and *Bifidobacterium* spp) on the

colonic ecosystem in humans, two studies were carried out. In the first study with 28 healthy volunteers, the synbiotic enhanced breath-hydrogen excretion significantly. The second randomised, double-blind study examined the effect of prolonged consumption of the synbiotic (500 ml/day during 4 weeks) on the colonic ecosystem. No change in dietary habits, intestinal habits, fecal pH or concentrations of bile acids was observed. The synbiotic decreased fecal dry weight and fecal concentration of L-lactate significantly, it increased fecal counts of bifidobacteria and lactobacilli significantly.

Casiraghi M.C., Canzi E., Zanchi R., Garsetti M., Bonfiglio A., Brighenti F. & Testolin G. (2001). Effects of the consumption of a symbiotic milk on human colonic ecosystem. Proceedings and Abstracts Book of the Symposium on 'Probiotics & Prebiotics - New Foods', Rome, Italy, 2-4 September 2001, 230.

### Oligofructose has an excellent safety profile

To evaluate the safety of oligofructose (OF) derived from inulin hydrolysis, 4 study groups of rats (n=20 rats/gender per group) were fed OF-containing diets (5.5 g/kg, 16.5 g/kg, 49.5 g/kg and 99.1 g/kg) for 13 weeks. A control group without OF was included. Clinical chemistry and hematology parameters were measured after weeks 1, 6 and 13, macroscopic and microscopic examination of 55 tissues was performed, and cecal levels of bifidobacteria and total bacteria were determined. Small decreases in body weight and

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food consumption occurred during the first 4 weeks. Cecal weights and levels of bifidobacteria increased in a dose-related manner. No pathological abnormality in the cecal samples or other macroscopic or microscopic observations was made.

Lien E.L., Boyle F.G., Anderson W., Jacqueline W., Perry R., McCartney A., Finlay R., Wilson J.L. & Gibson G.R. (2001). Evaluation of safety and bifidogenic effect of fructooligosaccharides in a 13-week rat study. *FASEB J.* 15, A288.

## The effects of inulin and oligofructose in the small intestine

The colonic bacteria and the dimensions and transport functions of the small intestine were compared among mice (B6C3F1 strain) fed diets with 10% cellulose (control) or with the cellulose replaced entirely with inulin or with oligofructose, or partially with OF (2,5% oligofructose).

Mice fed diets containing 10% inulin (Raftiline®HP) or OF (Raftilose®P95)

had higher densities of anaerobes, aerobes, bacteroides and lactobacilli, and lower proportions of enterics than mice fed the 10% cellulose diet. The small intestine was longer and weighed more when mice were fed 10% inulin, with intermediate values for 10% OF. Rates of glucose transport and absorption of leucine, proline and glycyl-sarcosine were lower when mice were fed diets with 10% OF and inulin, whereas only leucine was lower with the 2,5% OF diet. This and previous studies show that the structural components (physical, chemical and biotic features) and functional elements (transfer of energy and materials) are affected when the diet is supplemented with OF or inulin. It changes the numbers and types of colonic bacteria, influences the dimensions and absorptive functions of the small intestine, and may be useful for managing the gastrointestinal ecosystem. The specific responses vary among the types and

amounts of non-digestible oligosaccharides and among animal models.

Buddington R.K., Donahoo J.B. & Williams C.H. (2000). The colonic bacteria and rates of small intestinal nutrient transport of mice fed diets with inulin and oligofructose. *Microbiol. Health Dis.* 12, 233-240.

## The effects of fuctans with different chain lengths on the intestinal flora

Germ-free rats associated with a human fecal flora received: 1, a control diet; 2, a diet supplemented with 50g/kg per day of short-chain oligofructose (Raftilose®P95); 3, 50g/kg per day of long-chain inulin (Raftiline®HP); or 4, 50g/kg per day of a 1:1 (w/w) mixture of OF and inulin. Changes in bacterial populations were investigated with 16 S rRNA-targeted probes applied in situ hybridisation, which made it possible to enumerate bacterial populations without prior cultivation. Diets 3 and 4 resulted in larger numbers of

cecal, colonic and fecal bacteria of the Clostridium coccoides-Eubacterium rectale cluster, whereas diet 2 did not affect this bacterial group. This is the first study to show that fructan-containing diets increase the numbers of a bacterial population other than bifidobacteria or lactobacilli. The organisms of this cluster may have health-promoting properties beyond their ability to produce butyrate.

A significant bifidogenic effect was observed in the colon and feces of the group fed diet 2. More lactobacilli were found in cecal and colonic contents of the group fed diet 4 and in the feces of the group fed diet 2. Diets 2 and 4 led to significantly smaller numbers of cecal, colonic and fecal bacteria of the Clostridium histolyticum and Clostridium lituseburense groups. A high proportion of these latter organisms may be pathogenic. Counts of total bacteria, Bacteroides-Prevotella

## Inulin and oligofructose are dietary fibre

For the purpose of communicating nutritional information to the consumer, the term dietary fibre is of great value because it clearly distinguishes between this nondigestible class of carbohydrates and digestible, glycemic carbohydrates such as sugars and starches. The importance of distinguishing between these two classes stems from the need for a balance between the two in a healthy diet. The digestible carbohydrates that are hydrolysed and metabolised are an important source of energy. Dietary fibres are resistant to the digestive processes and may be fermented in the colon. In addition fibres contribute to fecal bulking.

Inulin and oligofructose are not digested in the upper part of the gastrointestinal tract, are not absorbed or metabolised in the glycolytic pathway, or directly stored as glycogen. None of the molecules of fruc-

tose or glucose that form inulin and oligofructose appear in the portal blood. These substances are fermented by the microflora of the colon. This fermentation leads to the selective stimulation of the growth of the bifidobacteria population.

After reviewing their chemistry, origin and physiological effects, the authors state that inulin and oligofructose are dietary fibre. They indeed share the basic characteristics of dietary fibres: saccharides of plant origin, resistance to digestion and absorption in the small intestine, fermentation in the colon to produce short chain fatty acids that are absorbed and metabolised in various parts of the body, bulking effect.

Flamm G., Glinsmann W., Kritschinsky D., Prosky L. & Roberfroid M. (2001). Inulin and Oligofructose as Dietary Fiber: A review of the Evidence. *Critical Reviews in Food Science and Nutrition*, 41, 353-362.



and Enterobacteriaceae did not differ between the groups.

All supplemented diets increased the cecal and colonic concentration of butyrate and its relative molar proportion. Only diet 3 resulted in a higher concentration of fecal butyrate, whereas higher molar proportions of fecal butyrate were observed with all three diets.

The differences in rat cecal, colonic and fecal microflora in response to the diets confirm that modifications in the chain length of fructans may

have the potential to modulate the composition of the intestinal microflora.

Kleessen B., Hartmann L. & Blaut M. (2001). Oligofructose and long-chain inulin: influence on the gut microbial ecology of rats associated with a human faecal flora. *Brit. J. Nutr.* 86, 291-300.

### Inulin and oligofructose modify colonic mucosal bacteria

Adding inulin and OF to the diet causes a favourable alteration in bacterial populations associated with the colonic

mucosa. This effect was confirmed in vivo in a human feeding study during which 15 healthy men were supplemented with 15g/day of inulin/OF for 2 weeks before colonoscopy and mucosal biopsies. The effect of fructan supplementation on the mucosal flora was to increase both bifidobacteria (by 1 log colony-forming units (CFU)/g mucosa) and lactobacilli (by 0.5 log CFU/g mucosa) counts on the mucosa.

Langlans S.J., Hopkins M.J. & Cummings J.H. (2001). Inulin and FOS feeding modify colonic mucosal bacteria in vivo. *Gastroenterology*, 118 (4), A772 (4141).

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## Inulin stimulates mineral absorption

Two recent animal studies contribute to the growing evidence of the stimulating effect of inulin and oligofructose on mineral absorption.

Lopez et al. (2000) studied the influence of phytic acid (PA) and inulin on the absorption of minerals and variables of the mineral status in growing rats. PA binds mineral cations such as Ca, Zn or Fe in the gastrointestinal tract, making dietary minerals unavailable for absorption and endogenously secreted minerals unavailable for reabsorption. Previous studies have shown that the presence of fermentable carbohydrates in diets can enhance PA breakdown and facilitate divalent cations absorption.

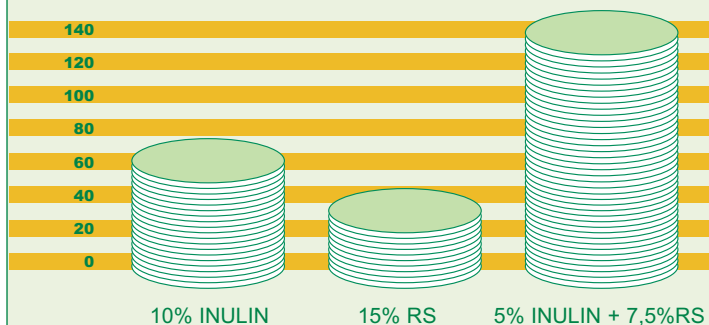
The present study indicates that inulin ingestion (100g/kg diet) improved apparent mineral absorption (Ca + 20%, Mg + 50%, Fe + 23%, Cu +45%) and mineral status (in blood, liver and bone) in the rats, whereas the antinutritional effects of PA were minor compared to the stimulatory effects of inulin. In contrast to PA, inulin fermentation can stimulate mineral absorption in the distal part of the digestive tract through decreased pH, increased mucosal mass, and bacterial

hydrolysis of PA. Thus the lowering of mineral and trace element bioavailability due to PA can be totally offset by inulin ingestion. Even though a direct extrapolation to humans may be questionable, these results show that PA-rich foods with fermentable carbohydrates may even improve mineral absorption and status.

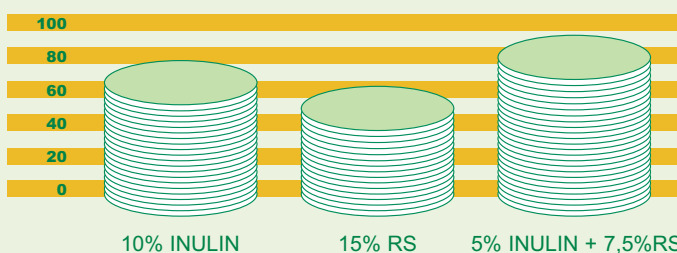
Younes et al. (2001) investigated the synergistic effect of a blend of two different fermentable carbohydrates (inulin and resistant starch) on the apparent intestinal absorption of Ca and Mg in rats. The study confirmed that inulin and resistant starch ingestion leads to considerable cecal fermentation and that both carbohydrates significantly increase the intestinal absorption and balance of Ca and Mg without altering significantly the plasma level of these two minerals. The combination of both carbohydrates has a strong synergistic effect on mineral absorption.

Younes H., Coudray Ch., Bellanger J., Demigne Ch., Rayssiguier Y., Remesy Ch. (2001). Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. *British Journal of Nutrition*, 86 (4), 479-485.

Increase in Ca absorption (%) After Younes et al. (2001).



Increase in Mg absorption (%) After Younes et al. (2001).



## Use of prebiotics in cats and dogs

Hesta et al. (2001) investigated the effects on fecal characteristics and on digestibility parameters of OF (0, 3, 6 and 9% Raftilose®P95) and inulin (3 and 6% Raftiline®GR) in 8 healthy adult cats. The 6% and 9% supplemented groups differed significantly from the control group in almost all fecal characteristics. There were no significant differences regarding the macroscopical and chemical aspects of the feces between the control and the 3% group. There was a trend for a lower pH of the feces in the 3% group, suggesting a substantial effect on the fecal composition.

With respect to digestibility parameters, there was a lower protein

digestibility in the supplemented groups as reflected by a higher bacterial nitrogen content of the feces. There were no significant differences between the 3% inulin and OF groups, although OF seemed to be more easily fermentable, as shown by a higher concentration of SCFA in the feces. The partial shift of nitrogen excretion from urine to feces may be especially interesting in relation to renal and liver insufficiency.

Vickers et al. (2001) compared the in vitro fermentation characteristics of OF, 4 inulin products and other fibre substrates (beet pulp, cellulose, soy fibre, mannanoligosaccharides) in the presence of fecal samples originating from 3 adult dogs. Total production of SCFA was higher for inulin

and OF, whereas the other substrates resulted in moderate concentrations of SCFA. Fermentation of cellulose produced the lowest concentration of total SCFA, without butyrate or lactate. Butyrate production was greatest for inulin and OF, total lactate production was greatest for OF and inulin 4 (with DP between 2 and 8). Production of SCFA increased as fermentation time increased.

Hesta M., Janssens G.P.J., Debraekeleer J. & De Wilde R. (2001). The effect of oligofructose and inulin on faecal characteristics and nutrient digestibility in healthy cats. *J. Anim. Physiol. A. Anim. Nutr.* 85, 135-141.

Vickers R. J., Sunvold G.D., Kelley R.L., Reinhart G.A. (2001). Comparison of fermentation of selected fructooligosaccharides and other fiber substrates by canine colonic microflora. *Am. J. Vet. Res.* 62, 609-615.

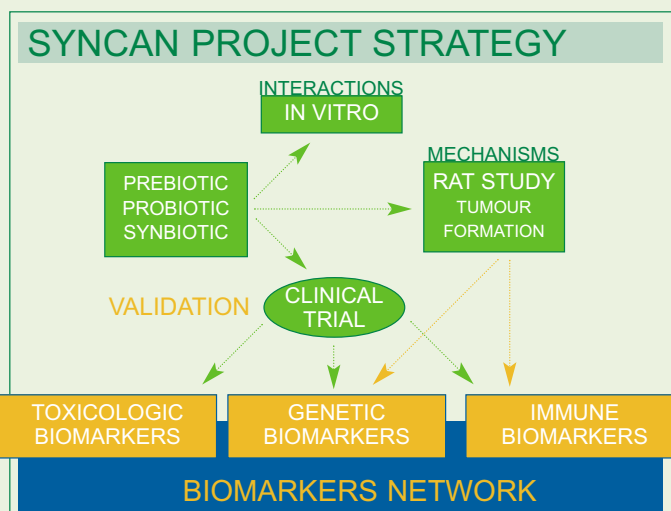
## Chemopreventive actions of synbiotics - The SYNCAN project

By means of a variety of experimental models it was demonstrated that prebiotic carbohydrates - mainly inulin and oligofructose - and probiotics - members of the group of the lactic acid-producing bacteria - consistently reduce processes of carcinogenesis and tumorigenesis. Synergistic chemopreventive actions were observed with combinations of the two, which together are called synbiotics.

These experimental animal studies used a chemopreventive model in which the animals are challenged by a carcinogenic substance to induce colon or breast cancer. Other studies used a tumour implanta-

tion model in which aggressive and invasive tumour cells are implanted into either the muscle or the peritoneum of mice. In another version of this model the tumour cells were allowed to metastasise. A third model used Apc Min mice that spontaneously develop intestinal tumours, mainly in the small intestine.

The EU-sponsored Syncan project is the first attempt to investigate the potential anticarcinogenic activities of prebiotics, probiotics and synbiotics in humans. Central to the project is a dietary intervention study with cancer patients and patients at high risk for developing colon cancer (polypectomised subjects), designed to evaluate the potential of synbiotics to reduce the development of colon cancer. A Biomarker network was set up to monitor an exhaustive series of cancer-related biomarkers (immunological, bacteriological, mucosal parameters and the composition of faecal water) which will provide useful information. In order to evaluate mechanisms in more depth, an extensive tumorigenesis study (long-term chemopreventive model) has been set up in rats. Finally, the interaction of various synbiotic combinations will be evaluated by means of separate in vitro fermentations, with the goal to identify the combinations that guarantee highly viable and metabolically active probiotics to arrive in distal parts of the colon.



Van Loo J. & Jonkers N. (2001). Evaluation in human volunteers of the potential anticarcinogenic activities of novel nutritional concepts prebiotics, probiotics and synbiotics (the SYNCAN project QLK1-1999-00346). *Nutr. Metab. Cardiovasc. Dis.* 11, Suppl. to No. 4, 87-93.



## FREQUENTLY ASKED QUESTIONS

### Do inulin and oligofructose improve the survival of probiotic cultures during the shelf-life of food products ?

There is more and more evidence that inulin and, more specifically, oligofructose do have a positive effect on the viability of probiotic cultures in fermented dairy products.

The first indication of such a positive effect was noticed in an early study (1992) done in collaboration with the Dutch dairy research institute NIZO. Oligofructose (and inulin) reduced significantly the loss of viability of *Bifidobacterium infantis* in a low-fat yoghurt system that was stored for 9 weeks at 10°C.

A second piece of evidence came from a study performed by H.-S. Shine et al. at Michigan State University. They evaluated the viability

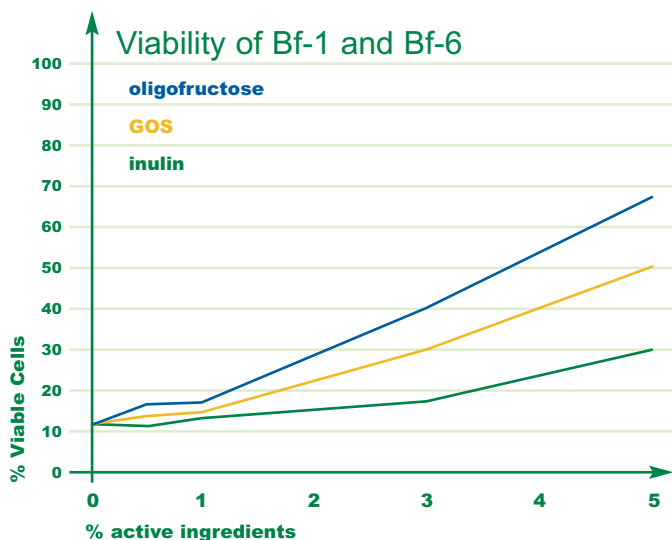
of two commercial strains of *Bifidobacterium* spp (Bf-1 and Bf-6) that were cultured in reconstituted skimmed milk containing 0, 0.5, 1.0, 3.0 and 5.0% of different oligosaccharides (inulin, oligofructose and galacto-oligosaccharides). The viability was assessed after 4 weeks of storage at 4°C. Growth promotion, enhancement of activity and retention of viability were greatest when *Bifidobacterium* Bf-1 and Bf-6 were grown in the presence of oligofructose, followed by galacto-oligosaccharides and inulin (and then the negative reference). The effects of the non-digestible oligosaccharides and inulin increased with increasing concentration of carbohydrate.

In a second study done in co-operation with NIZO, the effect of 0, 1 and 3% oligofructose on the survival of commercially used probiotic cultures (*bifidobacteria* and *lactobacilli*) was evaluated in low-fat, fermented dairy products. Five strains were investigated for their viability during storage for 10 weeks at 7°C. Four of them showed a high survival rate without significant effect from the oligofructose. The other strain of *Lactobacillus* showed only a moderate survival rate and, in this case, the addition of 3% oligofructose resulted in a higher level of viability.

Another test was set up to determine whether oligofructose could influence the survival of probiotic cultures during transit through the intestinal tract. Yoghurt containing a *Bifidus* strain was exposed in vitro to artificial intestinal juice containing pancreatin and biliary salts. An increase in viability was obtained after 90 minutes at 37°C when oligofructose was added to the fermented milk. The effect increased with increasing amounts of Raftilose®P95.

All this represents convincing evidence that inulin and, even more markedly, oligofructose have beneficial effects on the survival of probiotic cultures in dairy products (especially for cultures that exhibit a rather moderate intrinsic survival rate) and in the intestinal tract. The benefits could include a prolonged shelf-life of fermented food products, an increased number of ingested bacteria reaching the colon in a viable form, and a better stimulation of *bifidobacteria* and *lactobacilli* (both exogenous and endogenous) in the colon.

Reference  
H.-S. Shin, J.-H. Lee, J.J. Pestka, Z. Ustunol (2000) Growth and viability of commercial *Bifidobacterium* spp in skim milk containing oligosaccharides and inulin. *Journal Of Food Science*, 65(5), 884-887.



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