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Inulin and oligofructose as prebiotics in the prevention of intestinal infections and diseases

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Health and wellbeing are challenged constantly by pathogens. A number of defence mechanisms exist to protect the body from pathogen colonisation and invasion, with an important role to play for the natural intestinal bacterial flora (mainly by bifidobacteria and lactobacilli). The present paper reviews the evidence on the effects of inulin and oligofructose on colonisation and translocation of pathogens and the prevention of intestinal diseases. *In vitro* experiments have shown that lactic acid-producing bacteria have antagonistic (antibacterial) activity against pathogens partly because of the production of organic acids which are the endproducts of inulin and oligofructose fermentation. In addition, studies with epithelial layers have shown that inulin and oligofructose inhibit pathogen colonisation and that endproducts of their fermentation have the ability to support barrier function. Furthermore, studies in various animal models have shown that inulin and oligofructose accelerate the recovery of beneficial bacteria, slow down pathogen growth, decreasing pathogen colonisation and systemic translocation. Finally, data from human intervention trials either in patients with intestinal disorders or disease, or prone to critical illness, found that inulin and oligofructose restore the balance when the gut microbial community is altered, inhibit the progression of disease or prevent it from relapsing and/or developing. To conclude, the dietary use of inulin and oligofructose offers a promising approach to restore microbial communities and to support barrier function of the epithelia by their prebiotic action. This may offer the host protection against invasion and translocation of pathogens (endogenous and/or exogenous) and in the prevention of gastrointestinal diseases.

Inulin: Oligofructose: Prebiotics: Intestinal diseases: Gastrointestinal pathogens

Introduction

Man and animals are challenged constantly by pathogens that influence health and wellbeing. In order to cope with these challenges, a number of defence mechanisms have evolved, which include the physical lining of the intestinal wall and functional barriers able to defend against, and to recognise and eliminate, potential pathogens, thus preventing invasion and colonisation. The numerous bacterial populations that reside in the gastrointestinal tract provide a frontline mechanism of host defence. The use of gnotobiotic and GM animals has provided valuable insights into the interactions between those bacteria and defence functions of the host. Perhaps the most dramatic example is the lack of resistance of gnotobiotic animals to luminal pathogens. Some protection against pathogen invasion is provided simply by establishing the natural intestinal bacterial flora (Buddington *et al.* 2002*a*). Numerous gastrointestinal diseases are caused by alterations in the bacterial flora resulting in diminished strength of the mucosal barrier and even damage that increases translocation of gastrointestinal pathogens into the systemic circulation of the host, with high levels of mortality as a consequence. Therefore, strategies to increase mucosal barrier function will provide beneficial effects for health and wellbeing in the healthy individual and might be of help in the prevention of disease.

Normal colonisation of the intestine with commensal microbes has an influence on the development of disease as well as on cellular and humoral mucosal immune responses in neonatal life, and in maintaining the physiologically steady state of inflammation in the gut throughout life. This is most obvious in infants fed human milk, in which incidence of diarrhoea and illness is generally lower compared with infants that are fed with infant formula. Some time ago, the presence of non-digestible oligosaccharides in human milk was discovered. These oligosaccharides promote optimal colonisation of the neonatal gut by inducing growth of lactic acid-producing bacteria with beneficial effects for health. As changes in the gut microbial

Abbreviations: DP, degree of polymerisation; HP-inulin, high-performance inulin.

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composition occur with advancing age, this has important implications for the occurrence of disease. In older individuals, the numbers of bifidobacteria are lower at the expense of potential pathogenic bacteria (Mitsuoka & Hayakawa, 1973), which might be one of the reasons why elderly individuals are more prone to gastrointestinal disorders and illness.

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species in the colon that can improve host health (Gibson & Roberfroid, 1995). Inulin-type fructans are natural food ingredients with prebiotic activity; they can be extracted from the root of the chicory plant (*Cichorium intybus*). Chicory roots are rich sources of inulin (present as more than 70 % DM) and the amount of inulin is fairly constant from year to year. Inulin and oligofructose are β (2–1) fructans represented as GF_n and F_n, where G is glucose and *n* is the number of fructose (F) units in the oligosaccharide chain. When inulin is extracted from the chicory root, it comprises a family of identical linear structures (GF_n) that differ in their degree of polymerisation (DP), ranging from 3 to 60, with an average DP of 10. Oligofructose is obtained by partial enzymic hydrolysis of inulin by an endo-inulinase; it is composed of the same fructose monomers as inulin, but has lower DP, ranging from 2 to 8 (average DP = 4) (Franck, 2002). In high-performance inulin (HP-inulin; Orafti), the long-chain inulin fractions range in chain length from about 12 to 65 (average DP = 25). The second-generation prebiotic Synergy1 (Beneo™ Synergy 1, Orafti) is an oligofructose-enriched inulin obtained from a co-spraydried specific combination of oligofructose and HP-inulin.

The current hypothesis is that supplementing the diet with the prebiotics inulin and oligofructose increases the density and the metabolic activity of lactic acid-producing bacteria (bifidobacteria and lactobacilli), which enhances the defence mechanisms of the host. This may increase the resistance to various health challenges, an acceleration of recovery of the gastrointestinal tract after disturbances, and amelioration of disease symptoms. The present paper provides a critical review of the existing evidence available with regard to this hypothesis.

Fermentation and dietary fibre properties

Food substances that cannot be absorbed in the small intestine reach the large bowel, where they are metabolised by the endogenous microbial community. The resident microbiotia in the digestive tract is a heterogeneous microbial ecosystem containing up to 1×10^{14} colony-forming units of bacteria. The proximal part of the large bowel is highly saccharolytic and is a major site for carbohydrate fermentation. More distally, the environment becomes proteolytic, where products of protein fermentation accumulate (for example, indoles, phenols, ammonia) with potentially detrimental effects for health (for example, colorectal cancer). The microbial communities in these different parts of the colon play an important role in normal gut function and maintenance of host health. In order to stay healthy, bacteria beneficial to health and identified as lactic acid-producing bacteria (mainly bifidobacteria and lactobacilli) should be increased in numbers, while keeping the numbers of potential pathogens low. Inulin and oligofructose are not digested in the upper gastrointestinal tract as a consequence of their chemical structure and reach the colon intact (Andersson *et al.* 1999). This has been demonstrated in patients with an ileostomy, which is a reliable model to reflect absorption in the small bowel. Once inulin and oligofructose reach the colon, they are fermented selectively by the intestinal bacteria (bifidobacteria and lactobacilli).

Fermentation

Fermentation of inulin and oligofructose by lactic acid-producing bacteria results in an increase in bacterial biomass and the production of SCFA (acetate, propionate and butyrate), lactic acid and the gases CO_2 and H_2. The SCFA are utilised rapidly by the microbial flora or are absorbed, mainly through the mucosal surface of the caecum and ascending colon, and used as energy sources by the host. Mucosal cells use butyrate efficiently for their maintenance. In this way, endproducts of inulin and oligofructose fermentation can help to strengthen the intestinal wall by providing valuable sources of energy to the intestinal epithelium. Other SCFA (acetate and propionate) cross the intestinal wall without being metabolised to a significant extent and are delivered to the systemic circulation, where they reach the portal blood and enter the liver. The estimated energy value is about 4·2 kJ (1·0 kcal)/g for inulin and about 6·3 kJ (1·5 kcal)/g for oligofructose (Roberfroid *et al.* 1993).

Effect of chain length. Inulin-type fructans with different chain lengths are fermented at different rates according to their DP. The fermentation time of oligofructose in batch cultures *in vitro* inoculated with faecal slurries is about 5 h; fermentation time of the long-chain inulin fractions (HP-inulin) is about 15 h. Inulin-type fructans with a low DP are fermented rapidly in the proximal part of the colon. Their intensive fermentation modifies drastically the composition of the intestinal flora (bifidogenic effect) in the more proximal part of the large intestine. HP-inulin, on the other hand, which is fermented more slowly, is able to reach the more distal parts of the colon. In this part of the intestine, easily fermented carbohydrates are scarce, so bacterial catabolism shifts towards proteolysis, which results in the production of toxic putrefactive products. HP-inulin is able to reduce the proteolytic activity in favour of a beneficial saccharolytic activity in the distal parts of the colon (Van Loo, 2004*a*,*b*).

Dietary fibre properties

Through their fermentation and stimulation of bacterial growth, both inulin and oligofructose increase bacterial biomass and therefore have stool-bulking properties. In man, inulin and oligofructose have a bulking index (g stool weight increase per g fibre ingested) of about 1·5–2, which is comparable with soluble dietary fibre components, such as pectins and gums (Den Hond *et al.* 2000). A description of the dietary fibre properties of inulin and oligofructose is

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beyond the scope of the present paper and the reader is referred to Cherbut (2002) and Nyman (2002).

Prebiotic effect: evidence of increase in bifidobacteria and lactobacilli levels

To be an effective prebiotic, a compound has to display several characteristics. First, there should be neither hydrolysis nor absorption of the compound in the upper part of the gastrointestinal tract. Second, the compound should be fermented selectively by the gut flora. Third, it should stimulate the growth and/or activity of a limited number of beneficial bacteria in the colon in such a way that the composition of the intestinal microbiota is altered towards a healthier one and, as such, induces effects beneficial to health (Van Loo, 2004*a*,*b*; Gibson *et al.* 2004). In a recent review of the evidence for the prebiotic nature of several compounds (including galacto-oligosaccharides and lactulose), inulin and oligofructose were confirmed as the most extensively studied prebiotic compounds with major prebiotic efficacy (Gibson *et al.* 2004).

Fermentation by bifidobacteria and lactobacilli in cultures

Batch-culture experiments with isolated bacteria *in vitro* have demonstrated that bifidobacteria (Wang & Gibson, 1993, 1994; Gibson & Wang, 1994*a*; Hopkins *et al.* 1998; Bielecka *et al.* 2002; Van der Meulen *et al.* 2004) as well as certain lactobacilli, such as *Lactobacillus plantarum* (Cebeci & Gürakan, 2003), are able to grow well in culture medium with inulin or oligofructose as the single source of carbon. The unique characteristic of bifidobacteria to grow on inulin and oligofructose can be ascribed to the presence of an inducible β-fructofuranosidase enzyme able to hydrolyse the β (2–1) glycosidic linkages between the fructose moieties. In addition, it appears that bifidobacteria are equipped with specific transport systems for oligofructose uptake when the DP is below 8 (Janer *et al.* 2004). This suggests that bifidobacteria hydrolyse inulin and oligofructose at the cell surface, degrade the shorter fractions intracellularly, and at the same time metabolise the released fructose moieties. In this way, bifidobacteria prevent the loss of digestion products to nearby microbial competitors. This specific transport system provides bifidobacteria with the ability to compete efficiently with other commensal bacteria in the gut for this growth substrate (Van der Meulen *et al.* 2006). Fructosidases able to hydrolyse fructose moieties have been identified in certain lactobacilli, enabling them to degrade and metabolise inulin-type fructans efficiently (Barrangou *et al.* 2005).

Prebiotic effect in vitro

The selective growth of bifidobacteria on inulin and oligofructose in more complex media was demonstrated first by Wang & Gibson (1993). In batch cultures inoculated with faecal slurries, a selective increase in numbers of viable populations of bifidobacteria was seen solely when inulin and oligofructose were added to the medium. This effect could not be obtained when other carbohydrates (pectin, polydextrose or starch) that had a more general effect upon bacterial genera were used as substrates. Further studies *in vitro* were able to reproduce the positive effects that inulin and oligofructose have on the numbers of bifidobacteria. In addition, a parallel decrease in faecal pathogens, bacteroides, clostridia and coliforms, was observed with inulin and oligofructose (Gibson & Wang, 1994*b*). Studies *in vitro* with more sophisticated, three-stage continuous-culture systems configured so as to reproduce the conditions in the three major regions of the human bowel also demonstrated the bifidogenic effects of oligofructose, inulin, and a combination of both (Synergy1), and this effect was observed even along the different parts of the colon (proximal, transverse and distal). Oligofructose stimulated bifidobacteria growth mostly in the proximal colon, whereas Synergy1 and HP-inulin proved to be particularly efficient for improving bifidobacteria growth towards the more distal regions of the colon (GR Gibson and KM Tuohy, unpublished results).

Prebiotic effect in human subjects

Healthy subjects. The prebiotic properties of inulin and oligofructose have been demonstrated in numerous, well-controlled randomised intervention trials (Gibson *et al.* 1995; Menne *et al.* 2000; Rao, 2001; Tuohy *et al.* 2001*a*,*b*). As a result, evidence is emerging about the physiological effects of prebiotics at extra-intestinal sites, such as modulation of serum lipid and cholesterol levels (Pereira & Gibson, 2002) and the immune system. The rapid fermentation of oligofructose by bifidobacteria induces a significant bifidogenic effect at a dosage as low as 5 g/d (Rao, 2001). HP-inulin is fermented somewhat more slowly and studies have demonstrated a significant increase in bifidobacteria with a dosage of 8 g/d (Tuohy *et al.* 2001*a*). This relatively slow fermentation of HP-inulin allows the substrate to be used by populations of bifidobacteria in the more distal part of the colon, inducing a bifidogenic effect there. In the study by Langlands *et al.* (2004), equal amounts of oligofructose (7·5 g/d) and HP-inulin (7·5 g/d) were given to subjects undergoing multiple endoscopic biopsies from the caecum, transverse colon, descending colon and rectum. Prebiotic administration increased the numbers of mucosal bifidobacteria significantly ($P < 0{\cdot}05$) in both the proximal and distal parts of the colon compared with the non-supplemented group. In a similar way, lactobacilli counts were significantly higher ($P < 0{\cdot}05$) in the proximal as well as in the distal colonic regions compared with individuals not receiving prebiotic supplements. The bifidogenic properties of oligofructose and inulin are combined in Synergy1. The short-chain fructan fraction in Synergy1 has a bifidogenic effect in the proximal part of the colon. The long-chain fraction maintains the metabolic activity in more distal parts of the large intestine (Van Loo, 2004*a*,*b*). Increases in bifidobacteria counts upon prebiotic ingestion are generally not dose dependent but, rather, correlate with the endogenous presence of bifidobacteria in the colon of individuals at baseline. Roberfroid *et al.* (1998) indeed found a clear correlation between the initial levels of bifidobacteria and the magnitude of the bifidogenic effect. Volunteers who possessed low initial population levels of bifidobacteria

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experienced the greatest increase in bifidobacteria with inulin and oligofructose supplementation.

In agreement with findings from *in vitro* studies, the bifidogenic effect of inulin and oligofructose in human subjects is accompanied by a decrease in the numbers of other bacteria. This was seen clearly in the study by Gibson *et al.* (1995), in which numbers of bacteroides, clostridia and fusobacteria decreased significantly ($P < 0{\cdot}01$) when subjects were fed oligofructose (15 g/d) for 2 weeks, whereas the numbers of Gram-positive cocci decreased significantly ($P = 0{\cdot}0002$) with supplementation of the diet with inulin (15 g/d) for 2 weeks, compared with the control group (sucrose). Similar findings were obtained in the study by Harmsen *et al.* (2002). Daily supplementation with HP-inulin (9 g/d) increased the level of bifidobacteria significantly ($P < 0{\cdot}05$), whereas the numbers of eubacteria and clostridia were decreased significantly ($P < 0{\cdot}05$) compared with the non-supplemented period. Very recently it was observed that when feeding infants (6–24 months of age) with oligofructose (2 g/d) this led to a rise in bifidobacteria levels (up to 9·5 $\log_{10}$ colony-forming units/g faeces) at the expense of clostridia levels, which were significantly lower in the oligofructose group *v.* controls ($P < 0{\cdot}05$) (Waligora-Dupriet *et al.* 2005).

Patients. Human intervention trials on the prebiotic effects of inulin and oligofructose have been focused primarily on healthy individuals. Modulating the intestinal microbial ecology in a beneficial way indeed improves gastrointestinal functions, with improvement of general wellbeing and health. On the other hand, evidence is emerging about the role of an unbalanced microbial community with altered functioning of the bowel and its role in the aetiology of many gastrointestinal disorders and diseases, among which best studied are inflammatory bowel diseases (Swidsinski *et al.* 2002; Seksik *et al.* 2003). The use of prebiotics might be one way to prevent these changes and modify the development or state of disease. Many studies have shown the prebiotic effect of inulin and oligofructose to be associated with a beneficial impact on the progression of disease, either by prevention or by improvement of the symptoms. In children with chronic idiopathic constipation, the administration of Synergy1 (5–15 g/d) for a period of 8 weeks induced a clear prebiotic effect when compared with the control (maltodextrin) group (R Henschkel, unpublished results). Also, elderly constipated individuals showed beneficial effects on their bowel habits (stool frequency) and colonic bifidobacteria counts after 1 week of supplementation with inulin (20 g/d) compared with their baseline levels ($P < 0{\cdot}05$) (Kleessen *et al.* 1997). The bifidogenic effect was associated with decreased numbers of enterococci ($P < 0{\cdot}01$) and enterobacteria (NS) compared with the levels at the start of the intervention. Clear bifidogenic effects after treatment with inulin and oligofructose in more specific disease conditions have been demonstrated, and these were always associated with improvements in the patients' wellbeing and the prevention and/or management of disease. Examples are hospitalised patients with *Clostridium difficile*-associated diarrhoea (Lewis *et al.* 2005), patients suffering from pouchitis (Welters *et al.* 2002), patients with active ulcerative colitis (Furrie *et al.* 2005) and patients treated for colonic cancer or at high risk for developing the disease (Van Loo *et al.* 2005).

Resistance to colonisation and translocation: consequences on pathogens

Many mechanisms have been postulated by which prebiotics stimulate lactobacilli and bifidobacteria in their inhibitory activity against pathogens; a process called resistance to colonisation. Priming the activity of the immunological defences in the human body is certainly one mechanism. However, description of the immunomodulation properties of prebiotics and probiotics falls beyond the scope of the present paper. For an extensive review, the reader is referred to Watzl *et al.* (2005) and Servin (2004). Lactic acid-producing bacteria, especially bifidobacteria, are specifically equipped to ferment inulin and oligofructose. The ability to compete efficiently for available nutrients determines their survival and growth, competing-out potential pathogens because of limiting substrate availability. In addition, the fermentation of inulin and oligofructose by lactic acid-producing bacteria induces the production of acids that alter the pH of the intestinal lumen, compromising the growth of less acid-resistant species, which are often pathogenic. Moreover, bifidobacteria and lactobacilli have the capacity to produce antibacterial substances that inhibit the growth and survival of pathogens. In addition to this, since inulin and oligofructose stimulate the growth of bifidobacteria and lactobacilli in the intestine, their abundance can competitively inhibit the epithelial mucosal adherence of pathogens.

Close association with the mucosal surface is a prerequisite for pathogenic bacteria to withstand the flow of the intestinal chyme. If they cannot do this, they are eliminated rapidly from the gut. The advantage that prebiotics have over the use of probiotics is that a maximum effect can be achieved only if the probiotic organisms adhere to the intestinal mucosal cells, whereas prebiotics stimulate the growth of endogenous lactic acid-producing bacteria. Recent evidence of the prebiotic effect of inulin and oligofructose on the mucosa-associated flora in both the proximal and distal parts of the colon was presented by Langlands *et al.* (2004). There is little evidence that exogenous lactobacilli and bifidobacteria administered as probiotics have the same effect.

In order for a pathogen to invade and to translocate to extra-intestinal tissues, it must pass the epithelial barrier and enter the bloodstream. In optimal (healthy) conditions, the intestinal lining functions as a critical barrier that prevents invasion and translocation of micro-organisms. Intestinal permeability is modulated by specific tight junctions present between epithelial cells that regulate paracellular permeability. The integrity of the intestinal barrier is essential for optimal health. Inflammation or the use of antibiotics can alter the integrity of the gut barrier and it has been postulated that this, in part, might be because of changes in the microbial flora. One approach to strengthen the barrier is the use of prebiotics (Anderson *et al.* 2004). Through their stimulation of the commensal lactic acid-producing bacteria, prebiotics may contribute substantially to the gut mucosal barrier through diverse means; these are

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competition for nutrients and ecological niches (attachment sites on the intestinal mucosa), production of acids, antibacterial proteins (bacteriocins) and mucus.

Antagonistic activities in vitro

Data from batch cultures have shown that when oligofructose is added to pure cultures of *Bifidobacterium infantis* and two potential harmfully organisms (*Escherichia coli* and *C. perfringens*), a rapid decline in counts of both pathogens can be obtained as compared with conditions where glucose is present in the fermentation medium (Wang & Gibson, 1993). The fermentation of oligofructose by bifidobacteria led to an increase in the concentration of acetate and lactate in the medium, which lowered the pH of the culture. At a pH of 4·5–5·0, the numbers of *E. coli* and *Clostridium* fell to zero whereas the growth of bifidobacteria was unaffected (Wang & Gibson, 1993). Subsequent co-culture experiments done by the same researchers (Gibson & Wang, 1994*b*) showed that in the presence of oligofructose, several species of bifidobacteria are able to excrete antimicrobial substances with a broad spectrum of activity. In this way, the growth of several species belonging to the genera *Salmonella*, *Listeria*, *Campylobacter* and *Shigella* as well as *Vibrio cholerae* was affected. Fig. 1 illustrates the degree of inhibition of pathogenic micro-organisms by bifidobacteria (Gibson & Wang, 1994*b*). This antagonistic activity of bifidobacteria against the growth of *Salmonella enterica* ser. *Typhimurium* and *E. coli* was confirmed recently by Makras & De Vuyst (2006). According to these authors, the antagonistic activity of bifidobacteria against pathogenic Gram-negative bacteria appears to be widespread among the bifidobacteria. Other co-culture experiments performed by Fooks & Gibson (2002) with bifidobacteria or lactobacilli in the presence of enteropathogens such as *E. coli*, *Campylobacter jejuni* and *S. enteritidis* again showed an inhibition of the growth of these pathogens when either oligofructose or inulin was added to the medium. Particularly, the combination of *B. bifidum* Bb12 or *L. plantarum* with oligofructose was very effective, with the latter causing a 6-log decrease in the numbers of *E. coli* and compromising the growth of *C. jejuni* and *S. enteritidis* to undetectable levels (Fooks & Gibson, 2002). Fig. 2 shows the inhibition of enteropathogens by *L. plantarum* in co-culture experiments in the presence of oligofructose or starch (data from the latter are added for comparison) (Fooks & Gibson, 2002).

These results show that lactic acid-producing bacteria have antibacterial activity against pathogens. The production of organic acids (acetic acid and lactic acid) and antimicrobial substances that are active against virulent micro-organisms might be two of the underlying inhibitory mechanisms. The presence of increasing concentrations of organic acids acidifies the medium. The lower pH is of benefit for their growth, since lactic acid-producing bacteria are very acid-tolerant and, hence, their growth is less affected. This characteristic provides bifidobacteria and lactobacilli with the advantage to survive acidic environments that inhibit the growth of certain noxious bacteria. Bacteriocins are bactericidal proteinaceous molecules produced by bacteria. The bacteriocin family includes a wide variety of peptides and proteins in terms of their size, microbial targets and mechanisms of action and immunity. More research is needed to characterise these molecules and to elucidate their mechanisms of action with regard to their inhibitory potential against pathogen viability (Servin, 2004).

Data from epithelial layer experiments in vitro

An *in vitro* model of the small-intestinal tissue of pigs was used by Naughton *et al.* (2001) to study the effect of non-digestible oligosaccharides on intestinal colonisation by *S. enterica* ser. *Typhimurium* and non-pathogenic *E. coli*.

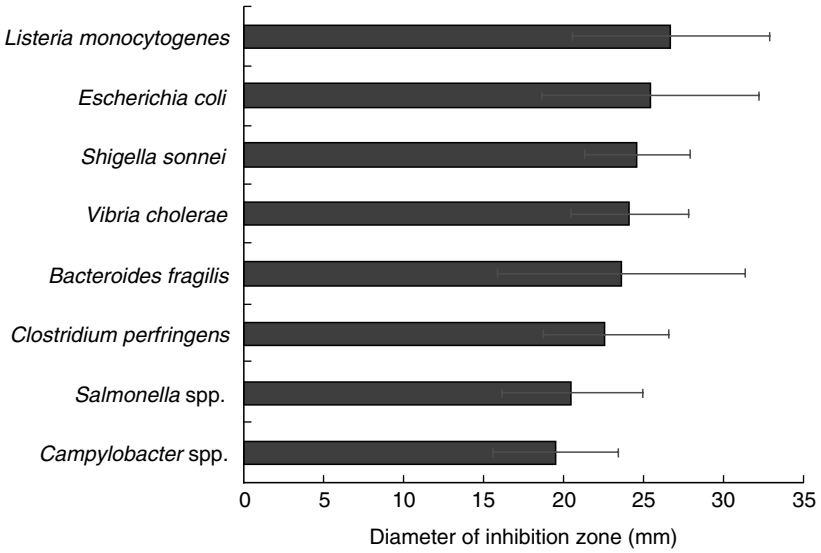

Fig. 1. Antimicrobial effect of bifidobacteria (*Bifidobacterium infantis*) measured by the clear areas on agar plates (inhibition zone) inoculated with the test bacteria and surrounding discs containing methanol–acetone extracts of *B. infantis*. Values are means of triplicate determinations, with their standard errors represented by horizontal bars (Gibson & Wang, 1994*b*).

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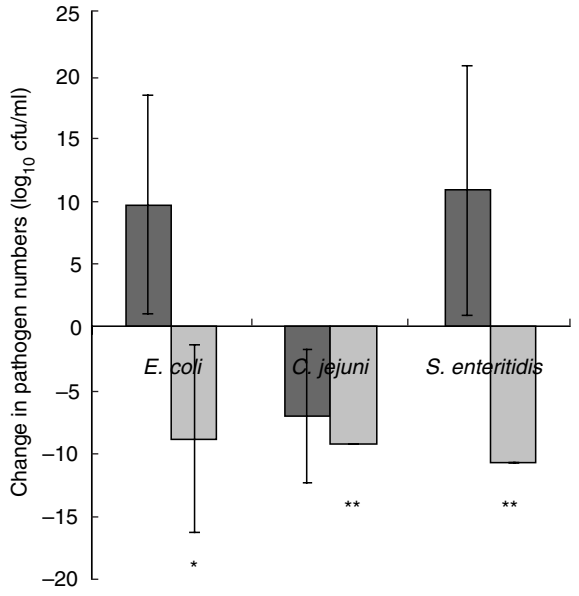

Fig. 2. Changes in the numbers of the pathogens *Escherichia coli*, *Campylobacter jejuni* and *Salmonella enteritidis* (colony-forming units (cfu)/ml) in co-culture experiments with *Lactobacillus plantarum* with the addition of oligofructose (□) or starch (■) after 24 h incubation. Values are means of three determinations, with standard errors represented by vertical bars. There was a significant change in pathogen numbers from the baseline (start of the fermentation): * $P < 0{\cdot}01$, ** $P < 0{\cdot}001$ (Fooks & Gibson, 2002).

Since the small intestine is the main site of invasion for both of these bacteria, the model is very suitable. Pigs were fed a diet containing inulin (4 %) for 4 weeks. Sections of jejunum and ileum were taken from the pigs and challenged with *Salmonella* and *E. coli*. Fig. 3 illustrates the numbers of *E. coli* associated with intestinal piglet tissue after challenge (Naughton *et al.* 2001). The numbers of *Salmonella* sp. associated with the ileal sections from animals fed the inulin diet were lower (although not significantly) compared with the control diet. Jejunal cultures from animals fed inulin showed a significant reduction ($P < 0{\cdot}05$) in the *E. coli* numbers associated with the tissue *v.* the controls (Naughton

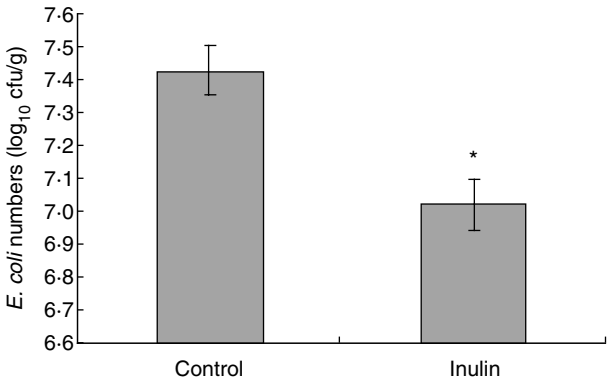

Fig. 3. Numbers of *Escherichia coli* (log_{10} colony-forming units(cfu)/g wet weight) in the jejunal tissue from pigs fed a diet with or without (control) inulin (4 %) after 1 h incubation (n 20 organ cultures). * Mean value was significantly different from that in the control pigs ($P < 0{\cdot}05$) (adapted from Naughton *et al.* 2001).

et al. 2001). Other *in vitro* models are confluent T-84 intestinal epithelial monolayers and intestinal epithelial–mesenchymal cell co-culture models that mimic spatial and functional interactions of these cell types *in vivo*. It appeared that SCFA, which are products of oligosaccharide fermentation, improved barrier function and had mucoprotective effects (Van Tol, 2005).

Studies *in vitro* show that inulin and oligofructose inhibit pathogen colonisation of the intestinal epithelial tissues and that endproducts of oligosaccharide fermentation can support the intestinal barrier function.

Data from animal studies

Evidence on the role of bifidobacteria against the development of disease came from inoculation of germ-free quails with a flora from patients with necrotising enterocolitis (which contained *C. butyricum* and *C. perfringens*). Lesions occurred rapidly after inoculation with the necrotising enterocolitis flora (thickening of the caecal wall with gas cysts, haemorrhagic ulcerations, and necrotic areas), whereas they did not in the presence of *B. infantis* and *B. longum*. Colonisation with bifidobacteria suppressed the growth of *C. butyricum* and led to the complete disappearance of *C. perfingens* (Butel *et al.* 1998). Additional effects of supplementation with oligofructose upon those seen with bifidobacteria alone were demonstrated in subsequent investigations in similar models. Quails were harvested in either a gnotobiotic environment or an ordinary environment permitting post-colonisation by exogenous bacteria. In both environments, oligofructose (3 %) in the diet significantly increased the level of bifidobacteria and this increase was associated with a significant decrease ($P < 0{\cdot}05$) in pathogen numbers (*E. coli*, *C. perfringens*, *C. difficile*, and *C. ramosum*) *v.* the control diet (lactose) (Catala *et al.* 1999). The research group confirmed their findings in a subsequent study (Butel *et al.* 2001). According to the authors, oligofructose inhibited bacterial adhesion by acting as a cell-receptor analogue, promoting the clearance of bacteria (or their toxins). Pathogen numbers of several clostridia strains in the caecal contents of the birds fed the oligofructose diet (3 %) *v.* the control (lactose) diet are given in Fig. 4 (Butel *et al.* 2001). Further evidence on the efficacy of inulin and oligofructose at enhancing enteric protection against challenge by an intestinal pathogen was provided by Buddington *et al.* (2002*a,b*). Mice were administered orally with *Candida albicans*, and their ability to clear the pathogen from the mid-small intestine was measured. The yeast densities in the contents of the small intestine were similar in mice fed oligofructose or inulin diets (10 %) but both were significantly lower ($P < 0{\cdot}05$) than values in the control mice (cellulose). Bomba *et al.* (2002) studied the administration of oligofructose together with *L. paracasei* in weaning pigs. Pigs were selected because they provide a model relevant to man for studies on gastrointestinal physiology. The administration of the synbiotic resulted in a significant increase in both lactobacilli ($P < 0{\cdot}01$) and bifidobacteria ($P < 0{\cdot}05$) in faeces compared with the controls. These changes were associated with a significant decrease in clostridia ($P < 0{\cdot}05$), enterobacteria ($P < 0{\cdot}01$)

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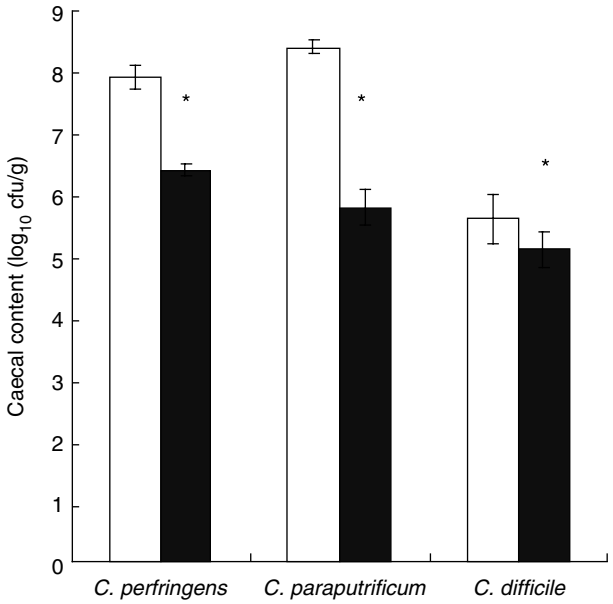

Fig. 4. Influence of oligofructose (3 %; ■) on the bacterial composition (*Clostridium perfringens*, *C. paraputrificum* and *C. difficile*; log_{10} colony-forming units(cfu)/g) in the caecum of gnotobiotic quails after inoculation with a faeces from preterm infants suffering from necrotising enterocolitis. Values are means, with standard deviations represented by vertical bars. * There was a significant change in pathogen numbers compared with the control diet (□) ($P < 0{\cdot}05$) (adapted from Butel *et al.* 2001.)

and enterococci counts ($P < 0{\cdot}001$), as well as with a decrease in coliforms (NS) *v.* the control group (Bomba *et al.* 2002). Further studies in pigs were performed by Oli *et al.* (1998), who added oligofructose to an oral electrolyte solution (5 g/100 ml) to study changes in the microbiota in pigs with acute secretory diarrhoea due to the administration of a cholera toxin. This toxin was chosen because it can induce secretory diarrhoea in man as well. The pigs receiving the oligofructose solution had significantly more lactobacilli in their small intestine ($P < 0{\cdot}05$) and caecum ($P < 0{\cdot}05$), whereas densities of Enterobacteriaceae in the mucosa of the small intestine ($P < 0{\cdot}05$), caecum ($P < 0{\cdot}05$) and colon ($P < 0{\cdot}05$) were significantly lower compared with the non-supplemented pigs. Bacterial densities of lactobacilli and enterobacteria at different sites of the pig intestine (small-intestinal lumen and mucosa, caecum and colon) in the oligofructose *v.* the non-supplemented group (after 24 h) are given in Fig. 5 (Oli *et al.* 1998).

The role of inulin and oligofructose in the defence against systemic invaders was assessed by recording survival in rats 2 weeks after intra-peritoneal infection with the pathogens *Listeria monocytogenes* and *S. typhimurium*. Systemic challenge with virulent strains of the pathogen *L. monocytogenes* resulted in 28 % mortality in the control group (cellulose), whereas none of the mice fed the inulin diet (10 %) died ($P < 0{\cdot}05$). Mortality was intermediate for mice fed the oligofructose diet (10 %), but this was still significantly lower ($P < 0{\cdot}05$) than that of the control mice. After *Salmonella* challenge, mice fed the inulin diet had significantly lower ($P < 0{\cdot}05$) mortality rates compared with the controls, whereas mortality was intermediate with oligofructose, but this difference was not significant compared with the control group (Buddington *et al.* 2002*a*). In the study reported by Bovee-Oudenhoven *et al.* (2003), oligofructose (4 %) was shown to inhibit the colonisation of *Salmonella* in rats but to increase translocation to spleen and liver compared with the control

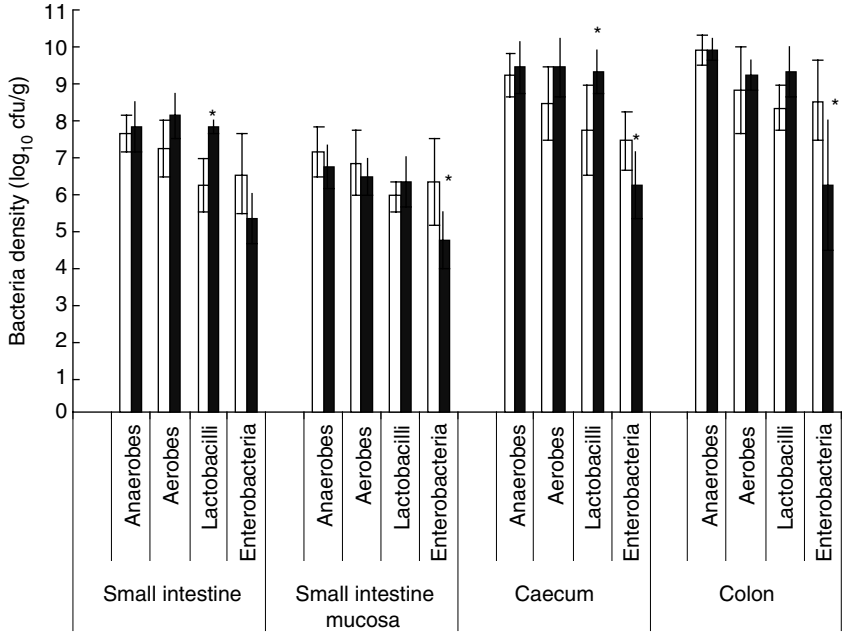

Fig. 5. Bacterial densities (log_{10} colony-forming units(cfu)/g wet weight) at different intestinal sites from the oligofructose-supplemented pigs (5 g/100 ml; ■) *v.* non-supplemented pigs (□). Values are means, with standard deviations represented by vertical bars. * There was a significant change in bacterial numbers compared with the control diet ($P < 0{\cdot}05$) (Oli *et al.* 1998).

diet (cellulose). One major drawback of this study was that rats were given diets very low in Ca, which itself can damage barrier function and enhance translocation. After increasing the Ca content of the diet, the rats recovered their resistance against translocation (Ten Bruggencate *et al.* 2004).

These observations in various animal models of disease (quails, pigs, mice and rats) indicate that inulin and oligofructose contribute in accelerating the recovery of beneficial bacteria while slowing the growth of pathogens. Moreover, the results available provide evidence for a protective effect of inulin and oligofructose against pathogen colonisation (either endogenous or from the external environment) and systemic translocation, suggesting that inulin and oligofructose could contribute to improve the barrier function of the intestinal epithelium.

Human data

The ability of inulin and oligofructose to protect against pathogen colonisation and invasion is somewhat difficult to study in human subjects, since challenge studies are ethically not approved. However, indirect proof can be obtained either by investigation of the changes in microbial composition of the faeces or biopsies in several disease states or by the relief of disease and/or accompanying symptoms. Otherwise, intestinal barrier function can be assessed by measuring intestinal permeability in human subjects. This represents the passive crossing of the intestinal epithelium by water and water-soluble inert small molecules through intercellular spaces. In the study by Den Hond *et al.* (2000), constipated subjects were administered HP-inulin (15 g/d) or placebo (sucrose) for 2 weeks. Inulin did not change intestinal permeability (determined by the measurement of urinary [^{51}Cr]EDTA excretion) compared with the control group. This means that neither inulin nor endproducts of prebiotic fermentation exerted any negative effect on intestinal permeability. However, all subjects fermented the inulin well, since it affected stool frequency positively and increased faecal bulk compared with the controls ($P < 0{\cdot}05$) (Den Hond *et al.* 2000).

In critically ill patients, the balance of the gut microbial ecology is altered in such a way that the numbers of beneficial bacteria are lower and the healthy interaction between the host and microbes is disturbed. Some of the resident bacteria are potential pathogens that can be a source of illness when the gut barrier is functionally or physically breached. In this way, patients with critical illnesses are generally known to have a high risk of bacterial translocation resulting in a worsening of the disease state. In the study by Jain *et al.* (2004), patients at high risk for developing sepsis and admitted to an intensive care unit were randomised to receive either oligofructose (15 g/d) in a synbiotic preparation or a placebo (sucrose) for 10 d. Intestinal permeability was high at baseline and lowered after therapy but the difference between the synbiotic group and the placebo group was not significant. The synbiotic changed the bacterial composition in nasogastric aspirates in such a way that significantly lower ($P < 0{\cdot}05$) potential pathogenic bacteria were found compared with the placebo. In another study, patients who had undergone major abdominal surgery and who were supplemented with oligofructose (32 g/d) and probiotics in a 2-week pre-operative course did not display any adverse effect on the barrier function when compared with the control group (sucrose). Bacterial translocation did not differ between these two groups (Anderson *et al.* 2004).

From the results of these studies, it appears that treatment with oligofructose during critical illnesses can restore the balance in microbial communities in a beneficial way without adverse effects on intestinal permeability or bacterial translocation. The way in which this positively affects the outcome of disease is investigated further.

Proof of the positive effect of oligofructose on the outcome of disease was obtained by Saavedra & Tschernia (2002). In their study they enrolled 140 infants (age 4–24 months) attending day-care centres, which implies that they are of high risk for infectious disease. Infants supplemented with oligofructose (0·55 g/15 g dry cereal) showed significant lower events of fever, medical attention and day-care absenteeism compared with the non-supplemented group ($P < 0{\cdot}05$). In addition, the frequency of vomiting, regurgitation and abdominal discomfort was also significantly lower in the oligofructose group compared with the control group ($P < 0{\cdot}05$).

Treatment with antimicrobials changes the gut microflora in a dramatic way and disrupts the normal ecological balance. This results in an altered gut barrier function with increased susceptibility to pathogens. The antibiotic cefpodoxime is often used to treat upper and lower respiratory tract infections. Treatment is associated commonly with an overgrowth of enterococci and yeast in the intestines, and colonisation with *C. difficile* also often occurs. Orrhage *et al.* (2000) gave volunteers an antibiotic treatment together with oligofructose (15 g/d) and probiotics or a placebo for 3 weeks. Administration of the antimicrobial agent induced a marked decrease in the anaerobic microflora, mainly with a loss of bifidobacteria and an overgrowth in enterococci. Numbers of lactobacilli were significantly higher ($P < 0{\cdot}05$) in the synbiotic group compared with the placebo group. In addition, colonisation with *C. difficile* was significantly lower ($P < 0{\cdot}05$) in the synbiotic group compared with the placebo group. In patients who developed *C. difficile*-associated diarrhoea triggered by antibiotic therapy, the administration of oligofructose was efficient. Administration of oligofructose (12 g/d) indeed increased numbers of bifidobacteria significantly ($P < 0{\cdot}0001$) compared with their baseline levels. In addition, relapse of diarrhoea was much less common ($P < 0{\cdot}001$) in the patients taking oligofructose compared with the placebo (sucrose) group (Lewis *et al.* 2005).

Chronic gastrointestinal diseases such as inflammatory bowel disease (ulcerative colitis and Crohn's disease), pouchitis and colonic cancer have been linked to the composition of the colonic microbial community and its activities. The pathogenesis of inflammatory bowel disease involves multiple factors (genetic and physiological) of the host in relationship to a magnitude of complex interactions with the microbiota in the gut and the emergence of pathogens. These 'silent' pathogens are repressed when the

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gut microbiotia is functioning normally. Studies in patients with ulcerative colitis have revealed that their bifidobacterial populations are about 30-fold lower compared with that of healthy individuals (Macfarlane *et al.* 2005). Supplementation of the diet with Synergy1 (12 g/d) together with a probiotic for 4 weeks in ulcerative colitis patients was able to restore these bacterial levels and resulted in a 42-fold increase ($P < 0{\cdot}05$) in bifidobacterial colonisation in mucosal biopsies in the synbiotic group compared with the control ulcerative colitis group (maltodextrin) (Macfarlane *et al.* 2005). The restoration of the microbial populations paralleled a decrease in the severity of the symptoms when the synbiotic was administered compared with the control patients (maltodextrin), indicating modulating effects on the progression of the disease. Indeed, sigmoidoscopy scores decreased ($P < 0{\cdot}05$) in the Synergy1 group after the intervention compared with the placebo. Biopsies in the test group showed reduced inflammation and regeneration of the epithelial tissue, which did not appear in the controls. Synbiotic treatment of ulcerative colitis patients improved the full clinical appearance of chronic inflammation (Furrie *et al.* 2005). A reduction in inflammation and associated factors was observed also in patients with an ileal pouch–anal anastomosis after inulin therapy (24 g/d). Concentrations of butyric acid were increased significantly (by 62 %; $P < 0{\cdot}01$) in patients receiving inulin compared with the non-supplemented group (Welters *et al.* 2002).

Studies in treated cancer patients and patients at high risk for developing the disease revealed beneficial changes in the composition of the microbial flora after administration of Synergy1. Data from the SYNCAN project demonstrated recently that addition of Synergy1 (12 g/d) in the diet of human patients increased the numbers of bifidobacteria and lactobacilli ($P < 0{\cdot}05$), while at the same time lowering ($P < 0{\cdot}05$) the number of coliforms and clostridia compared with cancer patients receiving a placebo (maltodextrin) (Van Loo *et al.* 2005). This was paralleled by changes in several biomarkers of colorectal cancer risk, indicating preventive effects on the development of the disease.

Data from well-controlled intervention trials on the use of inulin and oligofructose in patients with intestinal disorders or disease, or prone to critical illnesses, demonstrate that inulin and oligofructose restore balance when the gut microbial community is altered, which may inhibit the progression of the disease or may even prevent the disease from developing.

Conclusion

The dietary use of inulin and oligofructose offers one promising approach to maintain health and wellbeing, and to prevent or to control disease from progressing. Inulin and oligofructose are effective prebiotics that balance the indigenous bacterial populations towards a healthy composition with abundant levels of bifidobacteria and lactobacilli. Lactic acid-producing bacteria can inhibit endogenous pathogens from multiplying and block adherence and invasion of pathogens from outside the body. In this respect, inulin and oligofructose have the potential to improve the barrier function of the intestines by their prebiotic action. Strengthening this barrier in health and disease protects the host against invasion and translocation of pathogens either from the resident flora or from outside. In this way, prebiotic therapy can be a useful adjuvant for vulnerable subjects such as formula-fed infants, the elderly, individuals taking antimicrobials, travellers to developing countries, patients with severe illness and at high risk of translocation, and patients with chronic gastrointestinal diseases, such as chronic gut inflammation and colonic cancer.

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