

Probiotics and prebiotics in infant nutrition

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The human colonic microflora has a central role in health and disease, being unique in its complexity and range of functions. As such, dietary modulation is important for improved gut health, especially during the highly-sensitive stage of infancy. Diet can affect the composition of the gut microflora through the availability of different substrates for bacterial fermentation. Differences in gut microflora composition and incidence of infection exist between breast-fed and formula-fed infants, with the former thought to have improved protection. Historically, this improvement has been believed to be a result of the higher presence of reportedly-beneficial genera such as the bifidobacteria. As such, functional food ingredients such as prebiotics and probiotics could effect a beneficial modification in the composition and activities of gut microflora of infants by increasing positive flora components. The prebiotic approach aims to increase resident bacteria that are considered to be beneficial for human health, e.g. bifidobacteria and lactobacilli, while probiotics advocates the use of the live micro-organisms themselves in the diet. Both approaches have found their way into infant formula feeds and aim to more closely simulate the gut microbiota composition seen during breast-feeding.

Probiotics: Prebiotics: Gut microflora

At birth the gastrointestinal (GI) tract is essentially germ-free, with initial colonisation occurring during birth or shortly afterwards. The GI tract of newborns is inoculated primarily by organisms that originate from the maternal microbial flora of the genital tract and colon and from the environment (e.g. through direct human contact and hospital surroundings; Holzapfel *et al.* 1998; Mountzouris *et al.* 2002). Bacterial populations develop during the first few days of life (Collins & Gibson, 1999) and the intestinal flora develops as a result of the influence of intestinal physiology and diet on the acquired bacteria (Drasar & Barrow, 1985).

Bacteria such as facultative Gram-positive cocci, enterobacteria and lactobacilli are the first colonisers. These micro-organisms rapidly consume any O₂ that is present and subsequently create a more reduced environment, which then allows the growth of obligate anaerobic species (Rotimi & Duerden, 1981). Marked differences in the composition of the gut flora have also been recognised in response to infant feeding regimens. The microflora of breast-fed infants is dominated by populations of bifidobacteria, which may explain the purported healthier outlook of breast-fed infants compared with their formula-fed counterparts (Harmsen *et al.* 2000). Formula-fed infants

have a more complex microbiota, with bifidobacteria, bacteroides, lactobacilli, clostridia and streptococci all being prevalent (Stark & Lee, 1982; Benno *et al.* 1984; Harmsen *et al.* 2000). It is thought that the presence of certain glycoproteins and soluble oligosaccharides in human breast milk is selectively stimulatory for bifidobacteria (Gauhe *et al.* 1954; Petschow & Talbott, 1991). Moreover, human milk is also known to contain substantial amounts of oligosaccharides (i.e. lacto-N-tetraose and lacto-N-neotetraose). Certain of these oligosaccharides may act as soluble receptors in the mucosa for different pathogens, thus increasing the resistance of breast-fed infants (Kunz *et al.* 2000).

There is therefore a strong indication that diet can influence the relative amounts of microbial species and strains of the intestinal flora (Holzapfel *et al.* 1998). Following weaning these differences tend to disappear, the microflora increases in diversity and a community resembling the adult flora becomes established (Collins & Gibson, 1999). The colonic microflora of infants is generally viewed as being adult-like after 2 years, although the levels of facultative anaerobes are reported to be higher than those in adults. Once the climax microbiota has become established, the major bacterial groups are

Abbreviations: GI, gastrointestinal; LGG, *Lactobacillus rhamnosus* GG.

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relatively stable throughout most of adult life (Mitsuoka, 1992; Kimura *et al.* 1997; Macfarlane & McBain, 1999).

Human breast milk is always seen as the preferred choice for infant nutrition (Cuthbertson, 1999). It is a wholly nutritious complete food for infants and contains many components that have important bioactive roles (Goldman *et al.* 1997; Garofalo & Goldman, 1999). Whenever breast-feeding is not possible or not chosen, infant formulas are the alternatives. One approach to fortify the biological role of formula feeds has been to use probiotics and prebiotics as constituents. Here, the aim is to improve the gut microbiota composition to better resemble that seen with breast-feeding.

Bacterial fermentation in the large intestine

The primary role of the colonic microbiota is to salvage energy from dietary material that has escaped digestion in the upper GI tract, through the process of fermentation. Approximately 8–10% of the total daily energy requirements of the host are derived from colonic bacterial fermentation (Gibson *et al.* 2000). Most bacteria in the adult human colon are saccharolytic and so obtain their energy through the fermentation of carbohydrates. Diet is one of the principal factors that determine the type and amount of bacteria that colonise the bowel, as well as regulating metabolic processes. Principal substrates for colonic bacterial growth are dietary ingredients that have escaped digestion in the upper GI tract (Cummings *et al.* 1987).

The principal products of colonic fermentation are SCFA. It is estimated that >95% of the SCFA produced is absorbed through the colonic epithelium, indicating that SCFA can potentially be a source of energy to the host (Cummings & Macfarlane, 1991). The most predominant SCFA in the human colon are acetate, butyrate and propionate. Acetate and propionate are found in portal blood. Acetate is metabolised systemically (brain, muscle tissues), whereas propionate is cleared by the liver (Salminen *et al.* 1998). The function of propionate is still not clear; however, it may lower the hepatic synthesis of cholesterol by interfering with its synthesis in the liver (Delzenne & Kok, 1999). Butyrate is a major source of fuel for the mucosa (colonic epithelium) and has been shown to be involved in mitosis and mucosa regeneration (Cummings, 1981). Butyrate is almost completely consumed by the colonic epithelium. It plays an important role in the metabolism and normal development of colonic epithelial cells (Barcenilla *et al.* 2000). Lactate, ethanol and succinate are also important products of fermentation; however, they do not accumulate in the lumen as they are utilised by other bacterial species, i.e. act as electron sink products in anaerobic metabolism (Cummings *et al.* 1987). Another important product of fermentation in the colon is gas, with H₂ and CO₂ being predominant. Some H₂ may be further metabolised to CH₄, acetate or H₂S, while the rest is expelled from the body via flatus.

Gut flora and health

The human colonic microbiota is a complex and metabolically-active ecosystem that plays a major role in host

well-being (Gibson & Roberfroid, 1995). In addition to its role in metabolic activities that result in salvage of energy and absorbable nutrients, the large intestinal microbiota contributes towards health in a number of other ways. The colonic microbiota is suggested to play an important role in the protection against pathogens, and has important trophic effects on intestinal epithelia and immune structure and function (Guarner, 2006).

Indigenous intestinal bacteria protect the host from infection by exogenous pathogens (Mitsuoka, 1992; Tancredi, 1992) and opportunistic bacteria that are present in the gut (Guarner, 2006). This mechanism of protection is termed colonisation resistance (Macfarlane & McBain, 1999). The strictly anaerobic components of the microflora appear to be the most crucial to the maintenance of colonisation resistance (van der Waaij, 1999). This equilibrium between species of resident bacteria provides a 'balanced' gut flora that directly influences GI health.

Some indigenous colonic bacteria are thought to be beneficial to health, i.e. lactobacilli and bifidobacteria. Amongst the health-promoting actions of the colonic microbiota are colonisation resistance, facilitation of digestion, production of SCFA, antitoxigenic activity and stimulation of the immune system of the host (Guarner & Malagelada, 2003). However, a number of factors influence the composition of the microbiota, and may be related to changes in physiological conditions of the host (age, health status, stress etc.), the composition of the diet and environmental circumstances (e.g. use of pharmaceutical compounds such as antibiotics). In this way, the conditions underlying digestion (e.g. pH, substrate availability, transit time, IgA secretion etc.) may be modulated, which could result in a decline of beneficial bacteria and an increase in potentially-harmful bacteria. Some groups are considered benign, such as *Eubacterium* spp. and methanogens (Gibson, 1998). Additionally, some species are considered to be detrimental for human health. The most important colonic pathogens are probably clostridia, some bacteroides and sulphate-reducing bacteria. They have been associated with the production of toxins (Steer *et al.* 2001).

Dietary modulation of the gut microbiota

The concept of a healthy microflora is not a new one and probably originates with Metchnikoff (1907), who suggested that the long and healthy life of Bulgarian peasants was a result of their consumption of fermented milk products. He hypothesised that the complex microbial population in the colon was having an adverse effect on the host through the so-called 'auto-intoxication' effect. During the last few years the role of the intestinal microflora in health and disease has become increasingly recognised. Much interest exists in modulating the composition of the gut towards a potentially more beneficial community. This outcome may be achieved by using targeted dietary supplementation with functional foods (Collins & Gibson, 1999). A functional food is a dietary ingredient that has a cellular or physiological effect above basic nutritional value (Gibson, 1998; Playne *et al.* 2003). Recognition of the health-promoting properties of specific commensal

micro-organisms has encouraged modulation of the human intestinal microflora towards a more beneficial composition and metabolism, by using probiotics, prebiotics and synbiotics (Gibson & McCartney, 1998).

Probiotics

The word 'probiotic' means 'for life' and is derived from the Latin 'pro', which means 'for', and the Greek 'biotikos', which means 'living'. The earliest definition of the term probiotic was given by Parker (1974): 'organisms and substances which contribute to intestinal microbial balance'. This definition was refined by Fuller (1989) to: 'live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance'. Bifidobacteria and lactobacilli are the most popular target micro-organisms for probiotic application. To a lesser extent, certain Gram-positive cocci and yeasts (*Saccharomyces*) are also used (Rycroft *et al.* 1999). The most effective probiotic strains for human use have proved to be of human origin (Dunne *et al.* 2001).

A number of criteria are used to select for probiotic strains. An effective probiotic must be non-pathogenic and non-toxic and exert a beneficial effect on the host. Moreover, they should: be capable of surviving passage through the GI tract, particularly the harsh environmental conditions in the human stomach and small intestine (e.g. gastric acid, bile acid and digestive enzymes), and compete along with a highly-diverse and competitive environment presented by the human gut microflora (Bezkorovainy, 2001; Dunne *et al.* 2001); adhere to the intestinal epithelial cell lining (as it may increase persistence in the gut) (Guarner & Schaafsma, 1998); produce antimicrobial substances towards pathogens; remain viable during storage and use; have good sensory properties; be isolated from the same species as its intended use (Goldin, 1998; Collins & Gibson, 1999). It has been suggested that for a probiotic micro-organism to have any effect in the presence of the human gut microflora it should be ingested at a daily dose of $\geq 10^7$ colony-forming units/ml (Donnet-Hughes *et al.* 1999). However, the quantity required may be strain dependent, target dependent or specific to certain health outcomes or applications.

Probiotics and gastrointestinal health effects

Lactose intolerance

Numerous clinical studies have reported positive effects of probiotics in the prevention and/or treatment of several GI diseases and disorders (Nobaek *et al.* 2000; Wullt *et al.* 2003; Yamano *et al.* 2006). Alleviating symptoms of lactose malabsorption was one of the first clinical effects of probiotics to be demonstrated (Gilliland, 1985). Lactose intolerance is common throughout the world and is related to a deficiency of the enzyme β -galactosidase in the intestinal mucosa. Undigested lactose is clinically manifest by abdominal distension, excessive flatulence and/or profuse watery diarrhoea. Probiotic bacteria (such as lactobacilli and bifidobacteria) increase the production of

β -galactosidase (lactase) concentrations, which can then improve lactose digestibility in the small intestine (Rastall *et al.* 2000), thus alleviating the symptoms of lactose malabsorption (Marteau *et al.* 1990; de Vrese *et al.* 2001).

Constipation

There is some evidence suggesting that probiotics might relieve constipation. Constipation is a common condition characterised by a slow transit time that results in infrequent bowel movements, small hard faeces or difficult painful defecation, in addition to discomfort, distension and abdominal bloating (Salminen *et al.* 1998). Children and the elderly are the most affected individuals; however, it can occur as a symptom-based disorder and/or disease (Ouweland *et al.* 2002; Banaszkiwicz & Szajewska, 2005; Fernández-Bañares, 2006). An unbalanced diet is the usual cause of constipation, such as high-risk diets including low-fibre, low-residue and gluten-free diets. Probiotics have been suggested to increase the metabolic activity of colonic microflora in order to improve intestinal motility and reduce faecal enzyme activity (Fernández-Bañares, 2006). Studies have shown that milk or yoghurt fermented with different probiotics may reduce intestinal transit time and increase the daily stool number in constipated patients (Marteau *et al.* 2002).

Diarrhoeal studies

A number of clinical studies have demonstrated the efficacy of probiotics in the treatment and/or prevention of diarrhoea (Guarino *et al.* 1997; D'Sousa *et al.* 2002). Diarrhoea occurs in about 20% of patients who receive antibiotics (Marteau *et al.* 2001). Antibiotics may directly affect the indigenous gut microbiota by compromising colonisation resistance and favouring the growth of pathogenic micro-organisms, such as *Clostridium difficile*. Randomised controlled trials have shown a marked therapeutic effect of probiotics to reduce the incidence of diarrhoea caused by *C. difficile* (Wullt *et al.* 2003; Plummer *et al.* 2004). In a study by Gorbach *et al.* (1987) *Lactobacillus rhamnosus* GG (LGG) was shown to reduce episodes of relapsing diarrhoea caused by *C. difficile* toxin in five patients. LGG was also found to decrease toxin levels in faeces of the patients. LGG has been shown to lower the rate of diarrhoea in Finns travelling to Turkey (Oksanen *et al.* 1990) and Americans travelling to developing countries (Hilton *et al.* 1997). LGG has also been reported to reduce the duration of acute rotaviral diarrhoea in children. In a group of children hospitalised because of severe diarrhoea and randomised into two groups, one receiving a placebo and the other given LGG (Isolauri *et al.* 1991), the duration of diarrhoea was reduced for the group given LGG compared with the placebo group. Moreover, the number of intestinal immunoglobulin-secreting cells in the group given LGG was almost twice that in the placebo group for IgM, IgA and IgG isotopes. These studies demonstrate that a probiotic approach can be effective in treating antibiotic-induced diarrhoea, traveller's diarrhoea (most likely of mixed bacterial and viral aetiology)

and diarrhoeal disease in young children (predominantly caused by rotavirus).

Atopic diseases and food allergy

Probiotics have also been reported to help prevent and/or manage atopic diseases (i.e. eczemas) and food allergies (e.g. cow's milk allergy) in infants. Immaturity of the immune system and the GI barrier may explain the peak prevalence of food allergies in infancy (Salminen *et al.* 1998). LGG and other lactobacilli are reported to hydrolyse purified casein to smaller peptides and amino acids and hence decrease the proliferation of mitogen-induced human lymphocytes compared with non-treated caseins (Sutas *et al.* 1996). Majamaa & Isolauri (1997) have studied the effects of LGG supplementation for infants with atopic eczema and have found a reduction in the extent and intensity of atopic dermatitis. Several other studies have shown the potential of probiotics in the management and prevention of atopic disease, including the use of probiotics by pregnant mothers being suggested to confer protection to the unborn infant (Isolauri *et al.* 2000; Kalliomäki *et al.* 2003).

Mechanisms of probiotic activity

Although there is good evidence for the effect of probiotic preparations in GI health, little is known about the manner in which these changes occur. Several different mechanisms by which probiotics may protect the host from intestinal disorders have been suggested:

1. production of anti-microbial substances: lactic acid bacteria produce a wide variety of anti-bacterial substances, as well as inhibitory metabolites such as organic acids, diacetyl and H₂O₂. They also produce bacteriocins and antibiotic-like substances with activity against both Gram-positive and Gram-negative bacteria (Rolfe, 2002);
2. competition for adhesion receptors or sites: the presence of some bacteria in the intestinal tract is dependent on their ability to adhere to the gut epithelium, such that they become immobilised on the gut wall and resist being flushed out by peristalsis, as well as occupying a niche at the expense of potentially-harmful organisms (Fuller, 1992; Fooks & Gibson, 2002);
3. competition for nutrients;
4. stimulation of immunity: the underlying mechanisms of immune stimulation are not well understood, but specific cell-wall components or cell layers may act as an adjuvant and increase humoral immune response;
5. degradation of toxin receptors.

Probiotic supplementation in infant formulas has shown that some strains may persist in the infant gut (Bennet *et al.* 1992; Millar *et al.* 1993) and lower stool pH (Langhendries *et al.* 1995). Supplementation with LGG (Isolauri *et al.* 1991) and with *Bifidobacterium bifidum* and *Streptococcus thermophilus* (Saavedra *et al.* 1994) have been successful in preventing rotavirus diarrhoea in infants. The LGG strain has also been well researched for

its probiotic effects in reducing atopic eczema (see Majamaa & Isolauri, 1997; Isolauri *et al.* 2000; Kalliomäki *et al.* 2003).

Prebiotics

An alternative approach to probiotics for intestinal flora modulation is the use of prebiotics. A prebiotic is 'a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon' (Gibson & Roberfroid, 1995). For a food ingredient to be classified as a prebiotic, it must: (1) neither be hydrolysed nor absorbed in the upper part of the GI tract; (2) be a selective substrate for one or a few beneficial bacteria in the colon (e.g. lactobacilli and bifidobacteria); (3) consequently be able to alter the colonic microflora towards a healthier composition (Gibson & Roberfroid, 1995). The premise behind prebiotics is therefore to stimulate certain indigenous bacteria resident in the gut rather than introducing exogenous species, as is the case with probiotics. Ingesting a diet containing non-digestible carbohydrates that are selectively fermented by indigenous beneficial bacteria is the prebiotic principle. Any dietary component that reaches the colon intact is a potential prebiotic; however, most of the interest in the development of prebiotics is aimed at non-digestible oligosaccharides. These oligosaccharides are considered to be the most important prebiotic substrates because they meet all the current criteria for prebiotic classification (Rycroft *et al.* 1999). Oligosaccharides are sugars consisting of between approximately two and twenty saccharide units, i.e. they are short-chain polysaccharides. Apart from those that occur naturally in fruits and vegetables, and are extractable, others can be commercially produced by the hydrolysis of polysaccharides (e.g. dietary fibres, starch) or through enzymic generation.

As prebiotics exploit the use of non-viable dietary components to improve gut health, the range of foods into which they can be added is much wider than that for probiotics, where culture viability needs to be maintained. The prebiotic approach has the advantage that heat stability or exposure to O₂ is not an issue and it is concentrated towards stimulation or enhancement of the indigenous probiotic flora. Hence, for practical as well as aesthetic reasons their use in formula feeds currently seems to be more widespread than the use of probiotics. The targeted health benefits are similar.

Lactulose, a bifidogenic substrate (Tuohy *et al.* 2002), was used as an adjunct to formula feeds in the 1950s (Gibson *et al.* 2000). However, today it is not used as a foodstuff because it is not licensed as such, because of its laxation effect at high doses. Two non-digestible oligosaccharides currently used in infant formulas are the fructo-oligosaccharides and galacto-oligosaccharides. There are a number of studies supporting beneficial effects on the adult human intestinal microflora of fructo-oligosaccharides (Gibson & Roberfroid, 1995; Bouhnik *et al.* 1999) and galacto-oligosaccharides (Tanaka *et al.* 1983; Bouhnik *et al.* 1997; Sako *et al.* 1999).

It is likely that inclusion of such dietary prebiotic components in moderate amounts may benefit formula-fed infants by establishing an intestinal flora with more bifidobacteria and less-harmful bacteria. The health aspects of this approach have not yet been determined.

Synbiotics

A further possibility in microflora management is the use of synbiotics, the combination of probiotics and prebiotics. A synbiotic has been defined as 'a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the GI tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare' (Gibson & Roberfroid, 1995). However, they have not yet entered the infant food market.

Conclusion

The intestinal microbiota forms a diverse and complex ecosystem. However, there is much variability in bacterial numbers and populations between the stomach, small intestine and colon. In comparison with other regions of the GI tract, the human colon is an extremely-densely-populated microbial ecosystem. The large gut microflora is acquired at birth. Initially, facultatively-anaerobic strains such as *Escherichia coli* dominate. Thereafter, differences exist in the species composition that develops, which is largely governed by the type of diet. The faecal flora of breast-fed infants is dominated by populations of bifidobacteria, and comprises only about 1% enterobacteria. It is thought that certain bifidogenic factors are present in human breast milk. In contrast, formula-fed infants have a more complex microbiota, with bifidobacteria, bacteroides, clostridia and streptococci all being prevalent. After weaning, a pattern that resembles the adult flora becomes established. This critical stage of development is likely to affect health status in later life. Microflora modulation can occur through diets that contain probiotics and/or prebiotics, and is applicable to use in infant feeds.

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