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Prebiotics in animal feeding

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Abstract
A prebiotic is defined as 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 bacteria. Fructo oligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), isomaltooligosaccharide (IMO) and lactulose alter the microflora, increasing the level of bifidobacteria and lactobacilli. They inhibit pathogens through competition with receptor sites on the gut wall and inhibit pathogen persistence and thus reduce the potential risk of infection. They may also compete effectively for nutrients with pathogens. Prebiotics manipulate the microbial intestinal environment and subsequently prevent the occurrence of infectious bowel disease. It may influence the immune system directly or indirectly as a result of intestinal fermentation and promotion of growth of certain members of the gut microbiota.

Keywords: Animal, gut, health, prebiotics

Introduction
A prebiotic is a food or dietary supplement product that confers a health benefit on the host associated with modulating the microbiota. Prebiotics are not drugs, not functioning because of absorption of the component, not due to the component acting directly on the host, and are due to changes to the resident bacteria – either changing the proportions of the resident bacteria or the activities thereof (WHO, 1994) [1]. A prebiotic may be a fibre, but a fibre is not necessarily a prebiotic. Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism, hence synbiotics. Prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare (Saarela *et al*., 2000) [2]. A prebiotic is a fibre such as fructose oligosaccharide, galactose oligosaccharide, *etc*. and is consumed that is intended to stimulate the microflora in the large intestine. Prebiotic has shown to increase the population and/or function of the probiotic, as the probiotic is an external species, whereas prebiotics stimulate the flora which is already present (Fuller, 1992) [3].

Prebiotics are substances that can promote the growth of beneficial microorganisms, mainly in the intestinal tract, and will modify the colonic microbiota. A prebiotic is defined as '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 bacteria in the colon and thus improves host health' (Gibson and Roberfroid, 1995) [4]. The stimulated bacteria should be of a beneficial nature, namely bifidobacteria and lactobacilli (Gibson *et al.*, 1999) [5]. A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health. 'A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health (ISAPP, 2008) [6].

However many food components, especially many food oligosaccharides and polysaccharides (including dietary fibre), have been claimed to have prebiotic activity without due consideration to the criteria required. These criteria for a prebiotics are:

1. Resistance to gastric acidity, hydrolysis by mammalian enzymes
2. Resistance to gastrointestinal absorption
3. Fermentation by intestinal microflora
4. Selective stimulation of the growth and/or activity of those intestinal bacteria that contribute to health and well-being. (Gibson *et al.*, 2004) [7].

Resistance, in the first criterion, does not necessarily mean that the prebiotic is completely indigestible, but it should guarantee that a significant amount of the compound is available in the intestine (especially the large bowel) to serve as a fermentation substrate.

Prebiotics are reported to be particularly suited to the growth and activities of the bifidobacteria and lactobacilli (Rowland and Tanaka, 1993) [8]. These are classed as beneficial microorganisms because species within these groups have been reported to exert therapeutic and prophylactic influences on the health of infants and adults (Goldin and Gorbach, 1992) [9]. A prebiotic must stimulate the growth of a limited number of bacteria and thus will lead to a change in the overall microbial balance in the colon. Experiments have shown that fructo oligosaccharides (FOS), galactooligosaccha rides (GOS), xylooligosaccharides (XOS), isomaltooligosacch aride (IMO) and lactulose alter the microflora, increasing the level of bifidobacteria and/or lactobacilli (Grajek and Sip, 2004) [10].

Types of prebiotics

Prebiotic oligosaccharides

The production of prebiotic oligosaccharides is usually achieved through the following three general processes (Grizard and Barthomeuf, 1999) [11].

1. Direct extraction of natural oligosaccharides from plants
2. Controlled hydrolysis of natural polysaccharides and
3. Enzymatic synthesis using hydrolases and/or glycosyl transferases from plant or of microbial origin.

Important prebiotics of oligosaccharide groups are fructo oligosaccharide, inulin type oligosaccharide, galacto oligosaccharides *etc.*

Fructans

Fructans is used to name molecules that have a majority of fructose residues, (Roberfroid, 2005) [12]. Fructans can vary with respect to the following sources- chain composition, linkage, degree of polymerisation and functions. The sources may be Plant, bacteria and fungi. The linkage found in fructans is mostly 2, 1 and 2, 6. Plant fructans do not exceed degree of polymerisation maximum up to 200 units; where as bacterial fructans can exceed 1,00,000 units of polymerisation (Roberfroid, 2005) [12].

Fructo- Oligosaccharides (FOS)

These are the subclass of the group fructans. These have low molecular weight and degree of polymerisation is less than 10 (Roberfroid, 2005) [12]. The oligofructose subgroup can be further subdivided into the group called short-chain fructo-oligosaccharides (scFOS). Commercially, scFOS consists of low-molecular-weight linear chains synthesized by enzymatic fermentation from sucrose; however, the short chains also exist in nature. Owing to differences in structure, it is important to characterize and understand the collective nutritional, chemical and food science properties of scFOS as a separate fructan subgroup. scFOS is present in selected foods that include onion, artichoke, garlic, wheat and banana. scFOS is manufactured by a bioenzymatic (or fermentation) process, using sucrose from sugar beet or cane sugar as the starting raw material. scFOS has been included in a wide variety of foods and supplements globally that have been marketed for animal use.

Physiological effects of sc-FOS:

- Both *in vivo* and *in vitro* models have been used to demonstrate that scFOS is not digested between the mouth and small intestine. This is because neither the pancreas nor the small intestine mucosa secretes enzymes capable of hydrolyzing the β–(1→2) fructosyl fructose linkages (Czaczyk, 2003) [13].
- Bacteria utilize FOS by the production of short chain fatty acids (Hosoya *et al.*, 1988) [14]. Selective utilization of scFOS by intestinal bacteria has been demonstrated *in vitro* using pure cultures of selected bacterial species or using mixed faecal flora inoculations and also in animal and human studies by measuring the bacterial composition of the faeces.
- Piglets fed with sc-FOS in milk replacer develop resistance against *E. coli* & don't develop diarrhoea (Bunce *et al.*, 1995) [15].
- Bouhnik *et al.* (2004) [16] demonstrated that feeding of sc-FOS at 10 g/day not only increases counts of bifidobacteria but also the percentage of the bacteria among total anaerobes.

Mechanistic studies suggest that scFOS may be a better substrate for intestinal bacteria than oligofructose or inulin due to its shorter and more specific degree of polymerisation. McKellar *et al.* (1993) [17] suggested that scFOS was a better growth factor than inulin.

Inulin type Fructans

Inulin is a carbohydrate that is extremely widespread in nature. It occurs in plants mainly as an energy reserve and as a cryoprotectant. Different plant species typically contain inulin with varying chain lengths. Wheat, onions and bananas have short-chain inulins (maximal degree of polymerisation is 10); dahlia tubers, garlic and Jerusalem artichoke have medium-chain inulins (maximal degree of polymerisation is 40); and globe artichoke and chicory typically contain long-chain inulin molecules (maximal degree of polymerisation is 100) (Van Loo *et al.*, 1995) [18]. Other plants, such as certain types of lily (*Urginea maritima*) and blue agave and certain bacteria (e.g. *Streptococcus mutans*) produce high degree of polymerisation (more than 1000).

Inulin resists hydrolysis by acid in the stomach and by human digestive enzymes. Digestive enzymes are unable to hydrolyse glucosidic bonds present in inulin (Bjorck, 1991) [19].

Physiological effect of inulin

- Inulin is bifidogenic in nature when given a rate of 15gm/day. (Bouhnik *et al.*, 2004) [16].
- Inulin does not appear to be absorbed significantly in the small intestine, except possibly the very short oligosaccharides (Ma *et al.*, 1995) [20]. However, even if such small oligosaccharides are absorbed, they are not hydrolysed inside the body and are excreted as such in the urine.
- Inulin may influence the digestion process and thus affect metabolic responses; especially digestion of disaccharides, glucose transport and absorption of leucine, proline and glycyl-sarcosine (Buddington *et al.*, 1999) [21].
- More slowly fermented long-chain inulin is fermented in distal parts of the intestine. Inulin modifies the composition of the intestinal flora, but due to the slower and less intense fermentation as compared to FOS, impact

on composition of the intestinal flora is less pronounced (Harmsen *et al.,* 2002) [22].

- Mixture of the long-chain inulin and short-chain oligofructose @ 1:1 may be more potent than oligofructose alone in promoting Ca absorption (Coudray *et al.,* 2003) [23].
- The development of chemically induced breast cancer in human was suppressed by feeding the animals a diet containing inulin (Taper and Roberfroid, 1999) [24]. This indicates that systemic effects were involved and the cancer-preventing action was carried out by continuous feeding of inulin.
- As antibiotics are being banned as growth promoters in animal feed, prebiotics such as inulin offers a good alternative. Young animals are typically fed relatively high doses of inulin [up to 2% (w/w) in feed] which directly or indirectly improves the health of the animals (Taper and Roberfroid, 1999) [24].

Galacto Oligosaccharides (GOS)

The commercial GOS products are composed of oligosaccharides ranging from disaccharides to octasaccharides (Shin and Yang, 1998) [25]. GOS have attracted particular attention because they have certain similarities to oligosaccharides occurring in human breast milk and modulate the microbial population in the gut. Thus, they affect different gastrointestinal activities and have the potential to influence inflammatory and immunological processes (Sharon and Ofek, 2000) [26]. The non-glucose and lactose components in GOS are considered non digestible oligosaccharides as judged by *in vitro* digestibility studies (Burvall *et al.,* 1980) [27].

Physiological effects of GOS

- GOS is potential to stimulate the growth of bifidobacteria and lactobacilli at 1% (w/v) when fed over 24h (Tanaka *et al.,* 1983) [28].
- GOS also supported growth of several enterobacteriaceae and streptococci.
- Hopkins *et al.* (1998) [29] studied the growth of a range of isolates of bifidobacterium and found significantly increased growth rates on GOS as compared with a range of other carbohydrates.
- GOS has been shown to be protective against the development of induced colorectal tumors in rats (Wijnands *et al.,* 1999) [30].

Functional disaccharides

Lactulose

Lactulose is the first true prebiotic recognized for its effects on the gut flora. It has also been shown that small amounts (10 to 15 g twice a day) may induce tonic contractions of the colon leading to the known anti constipating effect (Jouet, 2006) [31]. Lactulose has also been used to reduce the rate of *Salmonella* carriage in chronic carriers (Cherrington *et al*., 1991) [32]. Mineral absorption, particularly calcium and magnesium, have been shown to be enhanced by ingestion of lactulose. Continuous and over feeding of lactulose may lead to obstructive Jaundice and Cirrhosis (Schumann, 2002) [33].

Lactitol

Lactitol is also derived from lactose through hydrogenation of the parent compound. Feeding of Lactitol increased both bifidobacteria and lactobacilli, but decreases the no. of bacteroids and Clostridia species (Ouwehand and Vesterlund, 2004) [34]. In a randomized clinical trial, combinations of sucrose and lactitol were evaluated at different doses for effect on faecal flora and short-chain fatty acids. While total bacteria remained constant, at the highest intake of lactitol a significant increase in bifidobacteria was observed (Balongue *et al*., 1997) [35]. Feeding of Lactitol also increases the Ca absorption.

Effect of prebiotics

Effect on Gut

- Prebiotics help in selective growth of some selective bacteria like lactobacillus (Nousiainen *et al*., 2004) [36].
- They may also inhibit pathogens through competition with receptor sites on the gut wall and inhibit pathogen persistence and thus reduce the potential risk of infection. They may also compete effectively for nutrients with pathogens (Messens and de Vuyst, 2002) [37].
- It is also acknowledged that it may reduce the risk of colon cancer through stimulating apoptosis (Scheppach, 1996) [38].
- Feeding of Prebiotics develop resistance to gastric acidity, hydrolysis by mammalian digestive enzymes and GI absorption. (Gibson *et al.,* 2004) [7].

Effect on Immune System

- Prebiotic effects may influence the immune system directly or indirectly as a result of intestinal fermentation and promotion of growth of certain members of the gut microbiota.
- Presence of increased numbers of a particular microbial genus or species or a related decrease of other microbes, may change the collective immuno-interactive profile of the microbiota (Caplice and Fitzgerald, 1999) [39].
- Microbial products such as short chain fatty acids (SCFA) may interact with immune cells and enterocytes and modify their activity (Isolauri *et al*., 2001) [40].

Effect on Gastro intestinal Disorder

- Prebiotics are able to manipulate the microbial intestinal environment and subsequently prevent the occurrence of infectious bowel disease (Sartor, 2005) [41].
- A number of prebiotics have been demonstrated to be effective in the manipulation of the microbiota. These include inulin, germinated barley foodstuff (GBF) and oligosaccharides such as oligofructose. (Guarner, 2007) [42].
- A mixture of various prebiotics decreased the incidence of bloody diarrhoea and mucosal injury (Kanauchi *et al*., 1998) [43].
- Gut microbiota may interfere with the process of carcinogenesis. Increasing the proportion of lactic acid bacteria in the gut may lead to decrease in certain bacterial enzymes involved in the synthesis or activation of carcinogens (Alakomi *et al*., 2000) [44].

Effect on Mineral Absorption

Prebiotic increases Ca, Mg, Fe and Zn absorption in various species of animals. Prebiotics are resistant to hydrolysis by small intestinal digestive enzymes. The colonic fermentation produces SCFA and other organic acids that contribute to lower luminal pH in the large intestine which, in turn improves the passive diffusion (Lopez *et al*., 2000) [45].

Conclusion

Prebiotics improve the performance of an animal by improving feed conversion and daily weight gain. It can improve digestive problems and general health of an animal.

References

1. WHO. Environmental health criteria, No.136. Environmental aspects, Geneva, 1994.
2. Saarela M, Mogensen G, Fonden R, Matto J, Mattila-Sandholm T. Probioticbacteria: safety, functional and technological properties. Journal of Biotechnology. 2000; 84(3):197-215.
3. Fuller R. Probiotics. The scientific basis, Chapmann & Hall, London, 1992.
4. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition. 1995; 125:1401-1412.
5. Gibson GR, Rastall RA, Roberfroid MB. Prebiotics. In: Colonic Microbiota: Nutrition and Health. Gibson GR and Roberfroid MB (Eds). Kluwer Academic Press, Dordrecht. 1999; 101-124.
6. ISAPP. 6th Meeting of the International Scientific Association of Probiotics and Prebiotics, London, Ontario, 2008.
7. Gibson GR, Probert HM, Van Loo JAE, Roberfroid MB. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. Nutrition Research Review. 2004; 17:257-259.
8. Rowland IR, Tanaka R. The effects of transgalactosylated oligosaccharides on gut flora metabolism in rats associated with human faecal micro flora. Journal of Applied Bacteriology. 1993; 74:667-674.
9. Goldin BR, Gorbach SL. Probiotics for humans. In: Probiotics. The Scientific Basis. Fuller R. (Ed.). Chapman and Hall, London. 1992, 355-376.
10. Grajek W, Sip A. Biological fixation food with utilization of lactic acid bacteria metabolites In: Lactic acid bacteria. Classification, metabolism, genetiics, application. Eds. Z. Libudzisz, P. Walczak, J Bardowski. Politechnikalodzka. 2004, 103-197.
11. Grizard D, Barthomeuf C. Enzymatic synthesis and structure determination of NEO-FOS. Food Biotechnology. 1999; 13:93-105.
12. Roberfroid M. Inulin: A fructan, in Inulin-Type Fructans: Functional Food Ingredients, Roberfroid M. Ed. CRC Press, Boca Raton, FL, USA.
13. Czaczyk K. The creating biofilms of bacteria - the creature the phenomenon and mechanisms of influences. Biotechnology. 2003; 3:180-192.
14. Hosoya N, Dhorranintra B, Hidaka H. Utilization of [U-^{14}C] fructo oligosaccharides in man as energy resources, Journal of Clinical Biochemistry and Nutrition. 1988; 5:67-74.
15. Bunce TJ, Howard MD, Kerley MS, Allee GL, Pace LW. Protective effect of fructo oligosaccharide (FOS) in prevention of mortality and morbidity from infectious E. coli K:88 challenge, Abstract for the American Society of Animal Science & American Dairy Science Association Annual Meeting, 1995.
16. Bouhnik Y, Raskine L, Simoneau G, Vicaut E, Neut C, Brouns F, Bornet F. The capacity of non-digestible carbohydrates to stimulate fecal bifidobacteria in healthy humans: A double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. American Journal of Clinical Nutrition. 2004; 6:1658-1664.
17. McKellar RC, Modler HW, Mullin J. Characterization of growth and inulinase production by Bifidobacterium spp. on fructo oligosaccharides, Bifidobacteria. Microflora. 1993; 12(2):75-86.
18. Van Loo J, Coussement P, De Leenheer L, Hoebregs H, Smits G. On the presence of inulin and oligofructose as natural ingredients in the Western diet. Critical Review on Food Science and Nutrition. 1995; 35:525-552.
19. Bjorck I. on the possibility of acid hydrolysis of inulin in the rat stomach. Food Chemistry. 1991; 41:243-250.
20. Ma TY, Hollander D, Erickson RA, Truong H, Nguyen H, Krugliak P. Mechanism of colonic permeation of inulin: is rat colon more permeable than small intestine? Gastroenterology. 1995; 108:12-20.
21. Buddington RK. Influence of fermentable fibre on small intestinal dimensions and transport of glucose and proline in dogs. American Journal of Veterinary Research. 1999; 60(3):354-358.
22. Harmsen HJ, Raangs GC, Franks A, Wildeboer-Veloo AC, Welling GW. The effect of the prebiotic inulin and the probiotic *Bifidobacterium longum* on the fecal microflora of healthy volunteers measured by FISH and DGGE. Microbial Ecology, Health and Diseases. 2002; 14: 219-237.
23. Coudray C, Demigne C, Rayssiguier Y. Effects of dietary fibres on magnesium absorption in animals and humans. Journal of Nutrition. 2003; 133:1-4.
24. Taper HS, Roberfroid M. Influence of inulin and oligofructose on breast cancer and tumor growth. Journal of Nutrition. 1999; 129:1488S-1491S.
25. Shin HJ, Yang JW. Enzymatic production of galactooligosaccharide by Bullera singularis β-galactosidase. Journal of Microbiology and Biotechnology. 1998; 8:484-489.
26. Sharon N, Ofek I. Safe as mother's milk: Carbohydrates as future anti-adhesion drugs for bacterial diseases, Glycoconj J. 2000; 17(7-9):659.
27. Burvall A, Asp NG, Dahlqvist A. Oligosaccharide formation during hydrolysis of lactosewith S. lactis lactase (Maxilact): Part III: Digestibility by human intestinal enzymes in vitro. Food Chemistry. 1980; 5:189-194.
28. Tanaka R, Takayama H, Morotomi M, Kuroshima T, Ueyama S, Matsumoto K, *et al*. Effects of administration of TOS and Bifidobacterium breve 4006 on the human fecal flora. Bifidobacteria Microflora. 1983; 2:17-24.
29. Hopkins MJ, Cummings JH, Macfarlane GT. Inter-species differences in maximum specific growth rates and cell yields of bifidobacteria cultured on oligosaccharides and other simple carbohydrate sources. Journal of Applied Microbiology. 1998; 85:381-386.
30. Wijnands MVW, *et al*. A comparison of the effects of dietary cellulose and fermentable galacto-oligosaccharide, in a rat model of colorectal carcinogenesis: Fermentable fibre confers greater protection than non-fermentable fibre in both high and low fat backgrounds, Carcinogenesis, 20(4):651-658.
31. Jouet P, Sabate JM, Cuillerier E, Coffin B, Lemann M, Jian R. Low-dose lactulose produces a tonic contraction in the human colon, Neurogastroenterology & Motility. 2006; 18:45.
32. Cherrington CA, Hinton M, Pearson GR, Chopra I. Short-chain organic acidsat pH 5,0 kill Escherichia coli and Salmonella spp. without causing membrane perturbation.

Journal of Applied Bacteriology. 1991; 70(2):161-165.
33. Schumann C. Medical, nutritional and technological properties of lactulose. An update. European Journal of Nutrition. 2002; 41:1-17.
34. Ouwehand AC, Vesterlund S. Antimicrobial components from lactic acid bacteria. Food Science and Technology. 2004; 139:375-396.
35. Ballongue J, Schumann C, Quignon P. Effects of lactulose and lactitol on colonic microflora and enzyme activity. Scandinavian Journal of Gastroenterology. 1997; 222:41-48
36. Nousiainen J, Jaranainen P, Setala J, von Wright A. Lactic acid bacteria as animal probiotics. Food Science and Technology. 2004; 139:547-580.
37. Messens W, de Vuyst L. Inhibitory substances produced by Lactobacilli isolated from sourdough - A review. International Journal of Food Microbiology. 2002; 72(1):31-43.
38. Scheppach W. Treatment of distal ulcerative colitis with short-chain fatty acid enemas. A placebo-controlled trial. German-Austrian SCFA Study Group. Digestive Disease Science. 1996; 41:2254-2259.
39. Caplice E, Fitzgerald GF. Food fermentations: role of micro-organisms in food production and preservation. International Journal of Food Microbiology. 1999; 50(1-2):131-149.
40. Isolauri E, Sutas Y, Kankaanpa P, Arvilommi H, Salminen S. Probiotics: effects on immunity. The American Journal of Clinical Nutrition. 2001; 73:444S-450S.
41. Sartor RB. Probiotic therapy of intestinal inflammation and infections. Current Opinion in Gastroenterology. 2005; 21:44-50.
42. Guarner F. Prebiotics in inflammatory bowel diseases, British Journal of Nutrition. 2007; 98 (1):85-89.
43. Kanauchi O, Nakamura T, Agata K, Mitsuyama K, Iwanaga T. Effects of germinated barley foodstuff on dextran sulfate sodium-induced colitis in rats, Journal of Gastroenterology. 1998; 33(2):179-88.
44. Alakomi HL, Skytta E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Applied and Environmental Microbiology. 2000; 66 (5):2001-2005.
45. Lopez HW, Coudray C, Levrat-Verny MA. Fructooligosaccharides enhance mineral apparent absorption and counteract the deleterious effects of phytic acid on mineral homeostasis in rats. Journal of Nutrition and Biochemistry. 2000; 11:500-508.