REVIEW ARTICLE

The utilisation of prebiotics and synbiotics in dogs

Carlo Pinna, Giacomo Biagi

Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Ozzano dell'Emilia (BO), Italy

Abstract

The microbiota of the large intestine plays a fundamental role in maintaining the state of health of the gastrointestinal tract and the host. The use of specific dietary supplements such as prebiotics and synbiotics might positively influence the composition and metabolism of the intestinal microbial population. Several studies have been conducted on the use of prebiotics in dogs. Most studies have aimed to assess whether using prebiotics brings about an improvement in the canine intestinal ecosystem. Moreover, the effect of prebiotics on canine immune system has also been investigated. Among the prebiotics used in the studies present in the literature, short-chain fructooligosaccharides and oligofructose seem to be the most effective in modulating the canine intestinal ecosystem and improving intestinal absorption of minerals but with little or no effect on canine immune system. Conversely, mannanoligosaccharides may have a positive influence on the immune system of dogs. Some positive effects of prebiotics on canine intestinal microbiota might be enhanced when these are used in combination with one or more probiotic strains (synbiotic). Clinical effects of prebiotics have been investigated in humans and animal models but little evidence exists that prebiotics may be helpful in canine diseases. Finally, most studies on canine intestinal microbiota were conducted using traditional culture methods, so that more research remains to be done with modern molecular identification methods to investigate the effects of prebiotic substances. This paper presents an overview of the scientific literature dealing with the use of prebiotics and synbiotics in the canine species.

Introduction

The gastrointestinal microbiota is a complex ecosystem that influences gastrointestinal functionality and the host's health in general. Prebiotics are non-digestible carbohydrates that withstand digestion and reach the colon where they stimulate growth and/or activity of beneficial microbial species. Prebiotic substances have been the object of several studies in dogs as they may improve the composition of canine intestinal microbiota, reducing the presence of pathogens and toxins. Moreover, prebiotics may result in enhanced immune function and could be used in the treatment of specific diseases of dogs such as infections by intestinal pathogens, intestinal constipation and hepatic and renal failure.

Recently, considerable interest has arisen towards the use of fibre and prebiotic substances in the food intended for dogs. In fact, though the dog is recognised as an animal with a prevalently carnivorous diet, several studies have demonstrated that by positively modifying the intestinal microbiota, prebiotic substances (mainly of vegetable origin) are capable of exerting a large influence on the trophic and health conditions of the digestive system and, consequently, on the animal's general state of wellbeing. It is the purpose of this review to present an overview of the scientific literature dealing with the use of prebiotics and synbiotics in the canine species.

The intestinal microbiota of dogs

To date, studies on the characterisation of canine intestinal microbiota are rather scarce and little is known about which factors may determine variations in terms of the number and species of bacteria present. Like in all mammals, a dog's gastrointestinal tract is sterile at birth, but with the passing of hours it comes to be populated by numerous bacterial species originating from the birth canal and the surrounding environment, as well as from maternal milk (Buddington, 2003). At the end of the weaning period, with the transition to a diversified diet, the resident microbial population undergoes a considerable change in terms of both species and number, also showing enormous differences between one dog and another (Schaible and Kaufmann, 2005).

Similarly to what had already been observed by some authors in studies aimed at characterising human intestinal microbiota (Langendijk *et al.*, 1995; Harmsen *et al.*, 2000), Greetham *et al.* (2002) and more recently Hooda *et al.* (2012) highlighted the scant reliability of traditional culture methods for isolating and identifying dog intestinal microbiota.

Corresponding author: Prof. Giacomo Biagi, Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy.
Tel. +39.051.2097379 - Fax: +39.051.2097373.
E-mail: giacomo.biagi@unibo.it

Key words: Dog, Immune system, Intestinal microbiota, Prebiotics, Synbiotics.

Received for publication: 16 September 2013.
Accepted for publication: 28 December 2013.

Italian Journal of Animal Science 2014; 13:3107
doi:10.4081/ijas.2014.3107

Thanks to more recent studies making use of molecular identification methods (Suchodolski *et al.*, 2009; Middelbos *et al.*, 2010; Handl *et al.*, 2011; Suchodolski, 2011; Swanson *et al.*, 2011; Beloshapka *et al.*, 2013), hundreds of phylotypes have been identified in the intestine of the dog. The phyla Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria and Actinobacteria account for over 99% of the bacterial species harboured in the intestine of dogs. However, the phyla Spirochaetes, Tenericutes, Verruco-microbia, Cyanobacteria and Chloroflexi have also been identified. In the study by Beloshapka *et al.* (2013), predominant bacterial families in adult beagles fed raw meat-based diets included *Fusobacteriaceae*, *Clostridiaceae*, and *Bacteroidaceae* and predominant genera included *Fusobacterium*, *Cetobacterium*, *Clostridium*, and *Bacteroides*. Moreover, the canine intestine also hosts non-bacterial microorganisms. In their work, Swanson *et al.* (2011) identified archea (about 1% of all sequencing reads), fungi (about 0.01% of all sequences) and viruses (less than 1% of all sequencing reads).

With respect to the variability of the composition of canine intestinal microbiota, considerable intraspecies differences have been observed in dogs housed under identical conditions and receiving the same diet, with very marked variations in the microbial species residing in the large intestine compared to those found in the small intestine. Moreover, there are large differences between the bacterial species that inhabit the different sections of the intestine and faecal microbiota (Suchodolski *et al.*, 2005).

The animal's age is one of the factors influ-

encing the composition of faecal microbiota: in older animals, lecithinase-positive clostridia and bacteria belonging to the class bacilli are found in higher concentrations than lactobacilli, pepto-streptococci and bifidobacteria (Benno and Mitsuoka, 1989; Benno *et al.*, 1992; Mitsuoka, 1992); furthermore, faecal microbiota seems to be influenced not only by age, but also by the dog breed (Simpson *et al.*, 2002). Unfortunately, studies that investigated the effect of age on canine intestinal microbiota were conducted using traditional culturing techniques and no research was done using current assays.

Main effects of intestinal microbiota on the host's health

Gastrointestinal microbiota is a complex ecosystem made up of hundreds of bacterial species, some of which are potentially pathogenic, while others are considered good for the host (Roberfroid *et al.*, 1995). The beneficial microorganisms that reside in the large intestine influence gastrointestinal functionality and the host's health in general, in virtue of some principal mechanisms: i) detoxification of some toxic substances introduced through the diet or newly formed as a result of metabolic processes of the body and of intestinal microbiota (Tomomatsu, 1994); ii) barrier effect against the proliferation of potentially pathogenic bacteria and their adhesion to the intestinal mucosa, thanks to occupation of the attack sites of these microorganisms and production of selective antimicrobial substances (Liévin-Le Moal and Servin, 2006); iii) uptake of ammonia and amine used as a source of nitrogen to support microbial protein synthesis, with a consequent reduction in the intestinal absorption of these undesirable substances (Howard *et al.*, 2000); iv) interaction with the host immune system (Round and Mazmanian, 2009; Cerf-Bensussan and Gaboriau-Routhiau, 2010); and v) production of vitamins (LeBlanc *et al.*, 2012).

The short-chain fatty acids (SCFA) that derive from microbial fermentation of carbohydrates represent a source of energy that the host can use. In particular, SCFA are used as energy substrates by colonocytes (butyric acid), hepatocytes (propionic acid and lactic acid) and peripheral tissues (acetic acid; Cummings and Englyst, 1987). It has been estimated that microbial fermentations can cover between 2 and 7% of the maintenance energy requirements of an adult dog (Herschel *et al.*, 1981; Stevens and Hume, 1998). Besides creating a favourable environment for beneficial microbial species, the pH reduction caused by SCFA induces a shift from ammonia to ammonium ions, thus preventing absorption by the intestine (McQuaid, 2005).

Prebiotics and synbiotics

According to a recent definition, a prebiotic is *a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health* (Roberfroid, 2007). To be effective, a prebiotic has to withstand digestion and reach the colon where it selectively stimulates the growth and/or metabolic activity of microbial species that promote evident beneficial effects for the host.

Prebiotics are non-digestible carbohydrates, mainly oligosaccharides with a low degree of polymerisation, obtained by extraction from vegetable raw materials (for example, hot water extraction of inulin from chicory, artichokes, bananas and wheat and of specific oligosaccharides from soybeans; Franck and Bosscher, 2009), by enzymatic synthesis [for example, the fructooligosaccharides (FOS) produced from sucrose and galactooligosaccharides (GOS) obtained from lactose (Fujita *et al.*, 1992; Spiegel *et al.*, 1994)] or else by partial enzymatic hydrolysis of oligosaccharides and polysaccharides [for example, the hydrolysis of inulin to FOS and of xylan polymers to xylooligosaccharides (XOS) under the action of xylanase (Imaizumi et al., 1991; De Bruyn et al., 1992)]. Main characteristics of some prebiotic substances are presented in Table 1. However, fructans such as inulin (a long-chain fructan, up to 60 units), oligofructose (OF; fructans chains with 8 to 10 units, often referred to as long-chain FOS) and short-chain FOS (fructans chains with 3-5 units) have been widely tested in companion animals (Hernot *et al.*, 2008) and seem to be the most frequently used prebiotic substances in the pet food industry. Moreover, pet food ingredients may contain certain amounts of natural prebiotic substances (Van Loo *et al.*, 1995; Campbell

Table 1. Main characteristics of some non-digestible oligosaccharides with prebiotic potential.

Name	Chemical composition	Production process	dp
Inulin	β(2-1) fructans with a terminal glucose	Extraction from chicory root, artichokes, bananas and wheat	11-60
Oligofructose (long-chain fructo-oligosaccharides)	β(2-1) fructans	Enzymatic (β-fructosidase) synthesis from sucrose or partial enzymatic or chemical hydrolysis from inulin	8-10
Short-chain fructo-oligosaccharides	β(2-1) fructans	Enzymatic (β-fructosidase) synthesis from sucrose or partial enzymatic or chemical hydrolysis from inulin	3-5
Galacto-oligosaccharides (oligogalactose)	Chains of galactose with some glucose	Enzymatic (β-galactosidases) synthesis from lactose	2-5
Soybean-oligosaccharides	Mainly galactose with presence of mannose, glucose, fructose, arabinose and xylose	Extraction from soybeans	3-4
Xylo-oligosaccharides	β(1-4)-linked xylose	Partial enzymatic (xylanases) hydrolysis of polyxylans polymers from vegetables and fruits	2-10
Lactitol	4-O-β-D-galactopyranosyl-D-glucitol	Hydrogenation of lactose	2
Lactulose	Galactose and fructose	Isomerisation of lactose	2

dp, degree of polymerisation.

et al., 1997; Hussein *et al.*, 1998; Moshfegh *et al.*, 1999). The fructan content of some vegetable raw materials is presented in Table 2.

The combination of a prebiotic and one or more probiotic bacterial strains is defined as a synbiotic (Schrezenmeir and De Vrese, 2001). The simultaneous combination of probiotic strains and a source of prebiotic molecules that they can metabolise might offer the administered bacterial strains greater possibilities of growing and colonising the host, thus promoting the potential beneficial effects.

Effects of prebiotic substances in healthy dogs

Effects on composition of intestinal microbiota

Flickinger *et al.* (2003b) reviewed the effects of inulin and OF in domesticated animals. The effects of prebiotics in dogs and other companion animals were recently reviewed by Swanson and Fahey (2006) and Hernot *et al.* (2008). A brief summary of the effects of prebiotics on canine intestinal microbiota is reported in Table 3.

The effects on canine intestinal microbiota which result from the administration of substances with prebiotic action have been studied by various authors, but with sometimes conflicting results. For example, in a study with adult beagles, Flickinger *et al.* (2003a) found that the use of OF [fructose chains obtained from hydrolysed inuline, degree of polymerisation (dp) of 3 to 10] at different doses (3, 6 and 9 g/kg of diet) linearly

Table 2. Fructan content of some vegetable raw materials.

Vegetable	Moshfegh *et al.* (1999)		Campbell *et al.* (1997)
	Inulin, g/100 g of *as is*	OF, g/100 g of *as is*	Short-chain FOS, g/100 g of DM
Artichoke, globe	4.4	0.4	2.2
Banana	0.5	0.5	0.6
Barley	0.8	0.8	1.9
Chicory root	41.6	22.9	2.1
Jerusalem artichoke	18.0	13.5	28.6
Oats	-	-	0.4
Onion powder	18.3	18.3	4.8
Peas	-	-	0.8
Potato, sweet	-	-	0.1
Rye	-	-	4.1
Wheat	-		1.4
Wheat bran	2.5	2.5	4.0

OF, oligofructose; FOS, fructooligosaccharides; DM, dry matter.

Table 3. Effects of prebiotic administration on composition of canine faecal microbiota.

Reference	Prebiotic used	Level of inclusion, g/kg	Method of determination	Lactobacilli	Bifidobacteria	*C. perfringens*	Total clostridia	*E. coli*/coliforms
Howard *et al.*, 2000°	scFOS	15	Selective media	↔	↔	ND	↑	↔
Strickling *et al.*, 2000	FOS	5	Selective media	↔	↔	↔	ND	↔
	MOS	5		↔	↔	↓		↔
	XOS	5		↔	↔	↔		↔
Willard *et al.*, 2000	FOS	10	Selective media	↔	↔	ND	↔	↔
Flickinger *et al.*, 2003b	OF	9	Selective media	↔	↔	↓	ND	ND
Middelbos *et al.*, 2007a	scFOS+dried yeast	12+3	DNA analysis	↑	↑	↔	ND	↔
	scFOS+dried yeast	9+6		↑	↑	↔		↔
Barry *et al.*, 2009	scFOS	2 and 4	DNA analysis	↔	↔	↔	ND	↔
	Inulin	2 and 4		↔	↔	↔		↔
Biagi *et al.*, 2010	Lactitol	10	Selective media	↔	NR	↓	ND	↓
Beloshapka *et al.*, 2012	Polydextrose	5, 10 and 15	DNA analysis	↔	↔	↓	ND	↔

scFOS, short-chain fructooligosaccharides; ND, not determined; FOS, fructooligosaccharides; MOS, mannanoligosaccharides; XOS, xylooligosaccharides; OF, oligofructose; NR, not recovered.
°Bacterial counts were conducted on intestinal digesta.

increased the aerobic population and reduced *C. perfringens* in faeces [respectively, +0.8 and -0.3 log_{10} cfu/g of faecal dry matter (DM) with 9 g/kg of OF, compared with control], without exerting any influence on the faecal population of lactobacilli and bifidobacteria. The latter result was also observed by Swanson *et al.* (2002b) when adult dogs were fed FOS at 2 g/d. In another study (Howard *et al.*, 2000), the administration of short-chain FOS (at 15 g/kg of diet) increased aerobic population in the distal colon of adult dogs (+1.8 log_{10} cfu/g of faecal DM, compared with a diet containing cellulose at 60 g/kg). In the study by Grieshop *et al.* (2004), feeding adult dogs with chicory (10 g/kg of diet) or mannanoligosaccharides (MOS; 10 g/kg of diet) increased faecal bifidobacteria (+0.4 and +0.5 log_{10} cfu/g of faecal DM, respectively) and MOS also resulted in a decrease of faecal *E. coli* concentrations.

In a study by Strickling *et al.* (2000), the use of FOS (OF from chicory root), MOS (from yeast cell wall of *Saccharomyces cerevisiae*) or XOS (mainly made of xylobiose and xylotriose, which are dimers and trimers of xylose, respectively) at a dietary concentration of 5 g/kg DM did not affect the number of faecal bifidobacteria in adult dogs; compared with animals administered FOS and XOS, those receiving the diet supplemented with MOS showed a numerical reduction in *C. perfringens* in the faeces (-0.26 and -0.68 log_{10} cfu/g of faecal DM, respectively). Although MOS are often described as prebiotic non-digestible oligosaccharides, they are not fermented by beneficial bacteria; instead, MOS act by binding and removing pathogens from the gastrointestinal tract and stimulating the immune system (Spring *et al.*, 2000). In a study by Vickers *et al.* (2001), *in vitro* fermentation of different sources of inulin and FOS increased concentrations of SCFA, whereas fermentation of MOS resulted in moderate production of SCFA.

In another study, Barry *et al.* (2009) did not observe any effect on the faecal microbiota of dogs after administering short-chain FOS or inulin at relatively low doses (2 and 4 g/kg of diet). Vanhoutte *et al.* (2005), in contrast, highlighted the positive role played by a combination of OF (4.5 g/d) and inulin (5.6 g/d) on the intestinal microbiota of dogs; their findings included, in particular, an increase in the populations of streptococci; interestingly, no bifidobacteria were detected in any of the seven dogs that were involved in the study. Similarly, Willard *et al.* (2000) reported that bifidobacteria and lactobacilli were inconsistently isolated from faeces of dogs during a study in which FOS were used as a dietary supplement at 10 g/kg of diet.

When administered to a group of adult shepherd dogs, lactosucrose (1.5 g/d), a bifidogenic fibre enzymatically synthesised from D-galactose, D-fructose and D-glucose, showed to be effective in increasing bifidobacteria (+0.5 log_{10} cfu/g of faeces) and decreasing *C. perfringens* (-1.6 log_{10} cfu/g of faeces) in the faeces (Terada *et al.*, 1992).

In a recent study (Beloshapka *et al.*, 2012), the utilisation of polydextrose (a polysaccharide synthesised by random polymerisation of glucose and sorbitol with an average dp of 12) at dietary concentrations of 5, 10 and 15 g/kg of diet linearly reduced faecal concentrations of *C. perfringens* in healthy adult dogs (-0.3, -0.4 and -0.8 log_{10} cfu/g of faecal DM with polydextrose at 5, 10 and 15 g/kg of diet, respectively), without affecting the faecal concentrations of *E. coli*, lactobacilli and bifidobacteria.

In a study by Biagi *et al.* (2010), various fibre sources and prebiotic substances were tested in the dog, both *in vitro* and *in vivo*. Among the substrates used in the *in vitro* test, FOS (two different sources: from partially hydrolysed inulin from chicory with dp 3 to 7 and from chicory with dp 2 to 10), lactitol, inulin from chicory (two sources: one with dp>20 and the other one with dp 2 to 60), citrus and apple pectins and psyllium fibre showed to be rapidly fermentable by canine intestinal microbiota, resulting in a reduction in the pH of faecal inocula. Among the substances tested, lactitol had an evident positive effect on undesirable bacterial populations, as reflected in an *in vivo* reduction in the concentrations of coliform bacteria and *C. perfringens* (-2.2 and -1.0 log_{10} cfu/g of faeces, respectively) when lactitol was fed to adult dogs at 10 g/kg of diet for 30 d. Middelbos *et al.* (2010) reported that supplementing the diet with beet pulp had the effect of significantly increasing the clostridia count in dog faecal specimens (from 83 to 90% of bacteria-assigned sequences obtained using pyrosequencing). In the previously cited study by Biagi *et al.* (2010), compared with control, beet pulp induced an increase in the pH (+0.13) and counts of coliforms (+0.5 log_{10} CFU/mL) of faecal inocula. Moreover, Middelbos *et al.* (2010) observed that beet pulp also induced a reduction in the concentration of bacteria belonging to the phylum *Actinobacteria* (from 1.4 to 0.8% of total sequences), which includes bifidobacteria, without, however, affecting the number of bacilli, among which we find numerous bacterial species considered to be beneficial and belonging to the genus *Lactobacillus* spp.

According to what emerged during the study conducted by Middelbos *et al.* (2007a), the dietary inclusion of two combinations of short-chain FOS and dried yeast as a source of MOS (12 g/kg FOS+3 g/kg yeast cell wall and 9 g/kg FOS+6 g/kg yeast cell wall, respectively) resulted in an increase in the faecal populations of bifidobacteria (+1.0 and +1.4 log_{10} CFU/g of feacal DM, respectively) and lactobacilli (+0.9 log_{10} CFU/g of feacal DM for both treatments) and, consequently, a lowering of the faecal pH (-0.3 and -0.4, respectively). The authors also highlighted a tendency toward a reduction in the faecal counts of *E. coli*, hypothesising that MOS had the ability to bind to the fimbriae of some pathogenic bacteria, such as, precisely, coliform bacteria and *Salmonella* spp., thus preventing them from colonising the intestinal wall. In another study with adult dogs (Middelbos *et al.*, 2007b), a yeast cell wall preparation fed at different levels of inclusion (0.5, 2.5, 4.5 and 6.5 g/kg of diet) as a source of MOS linearly reduced faecal counts of *E. coli* (-0.9 log_{10} CFU/g of feacal DM in dogs receiving 6.5 g/kg of yeast cell wall).

Beloshapka *et al.* (2013) investigated the effect of inulin (14 g/kg of diet) or a yeast cell wall extract (14 g/kg of diet) in adult dogs receiving raw meat-based diets [containing approximately per kg of diet 250 to 300 g of crude protein (CP) and 450 to 500 g of fat]. Administration of inulin resulted in lower faecal abundance of *Enterobacteriaceae* and *Escherichia* and higher faecal lactobacilli while the yeast cell wall extract increased faecal bifidobacteria. Moreover, inulin reduced faecal abundance of some *Bacteroides* and *Fusobacterium* species.

Based on results from the cited studies, despite some discrepancies, the use of prebiotic substances seems to represent an efficient way to manipulate the gastrointestinal ecosystem of dogs, increasing the abundance of beneficial bacteria and reducing the presence of undesired microbes. Among the prebiotics that have been tested, short-chain FOS and OF, when used at concentrations higher than 10 g/kg of diet, seem to be the most effective in modulating the canine intestinal ecosystem.

Effects on intestinal ammonia and other bacterial catabolites

In general, limitation of carbohydrates as a source of energy in the hindgut leads to microbial proteolysis with consequent release of toxic substances, such as ammonia and amines (Russell *et al.*, 1983).

Prebiotics might reduce concentrations of ammonia in the animal intestine, as increased fermentation leads to higher amounts of nitrogen converted into bacterial protein (Howard *et al.*, 2000). Moreover, the use of prebiotics might reduce intestinal proteolysis and pro-

duction of putrefactive compounds by increasing the number of beneficial bacteria, enhancing SCFA production and reducing luminal pH (Terada *et al.*, 1992). In the study by Biagi *et al.* (2010), compared with control, the ammonia concentration was lower in the inocula to which citrus pectin was added (-23%) and higher in those treated with gluconic acid and inulin (+17 and 20%, respectively). In the same study, FOS and lactitol had no effect on ammonia concentrations. In a previous *in vitro* study that used swine cecal inoculum, gluconic acid instead brought about a reduction in the ammonia concentration (Biagi *et al.*, 2006).

Terada *et al.* (1992) reported a significant decrease of faecal ammonia (-60%) and bad smell of faeces in dogs receiving 1.5 g/d of lactosucrose. Similarly, in the study by Flickinger *et al.* (2003a), faecal ammonia concentrations tended to be reduced by OF but concentrations of other protein catabolites remained unaffected. In contrast, ammonia intestinal and faecal concentrations were not influenced by diet in the already cited studies by Strickling *et al.* (2000) and Barry *et al.* (2009); the same result was observed in the study by Beynen *et al.* (2002) in which adult dogs received a diet supplemented with OF (dp from 3 to 20) at 10 g/kg for 30 d. Similarly, the use of FOS at 30 g/kg in adult dogs did not reduce faecal ammonia (Hesta *et al.*, 2003). In the study by Swanson *et al.* (2002b), the use of FOS reduced faecal concentrations of indole (a protein catabolite of bacterial origin) by 1.2 mol/g of faecal DM but faecal ammonia and biogenic amines were unaffected. Beloshapka *et al.* (2012) reported that faecal indole concentrations tended to decrease with increasing dietary levels of polydextrose but other protein catabolites (ammonia, phenol and branched-chain fatty acids) were not influenced by treatment.

Finally, Propst *et al.* (2003) reported that the use of different concentrations (3, 6 and 9 g/kg of diet) of OF or inulin in adult dogs resulted in increased faecal ammonia and isovalerate (a branched-chain fatty acid that derives from protein fermentation) concentrations. In the same study, OF also resulted in increased faecal concentrations of total biogenic amines and SCFA and reduced faecal phenols. Zentek *et al.* (2002) found that the intake of MOS (1 g/kg BW/d) had the effect of lowering both the pH (-0.3) and the ammonia concentration (-38 µmol/g of faeces) in canine faeces. The same authors also noted that the utilisation of MOS significantly decreased faecal unbound water content (from 165 to 55 g/kg of faeces) and apparent digestibility of protein, the latter result presumably as a consequence of the increased viscosity of the intestinal chyme.

The conflicting results that are observed when prebiotics are fed to dogs are not easy to explain. First, different basal diets might cause some discrepancy: diets that are rich in wheat, barley or oats are likely to contain significant amounts of soluble fiber (including short-chain fructans; Van Loo *et al.*, 1995) that might mask the effect of prebiotics. Another cause of conflicting results might reside in the type of prebiotics that are used: in fact, within the same category, prebiotic substances may differ based on their origin, degree of polymerisation, *etc*. Obviously, the level of dietary inclusion of prebiotics also has an influence on their effects: in particular, FOS seem to be effective at concentrations higher than 10 g/kg of diet. Moreover, in many studies the effects of prebiotics on intestinal microbiota were evaluated based only on faecal analyses. This is very common today, as most scientists try to avoid for ethical reasons any type of invasive methods of analysis when a study is conducted with dogs. Unfortunately, it is well known that the concentration of bacterial metabolites able to cross the intestinal mucosa can vary while digesta move along the intestine (Stevens and Hume, 1998). Therefore, faeces might not reflect the changes in the concentration of ammonia, SCFA and other molecules that the prebiotic may have induced in the colon. Finally, another reason for discrepancies in the effect of prebiotics might reside in the inconsistent presence of their target bacteria in dogs, namely bifidobacteria and lactobacilli. In fact, while some authors reported high average concentrations of bifidobacteria in the faeces of experimental dogs (Flickinger *et al.*, 2003a; Middelbos *et al.*, 2007a), bifidobacteria were inconsistently isolated from canine faeces in the studies by Willard *et al.* (2000), Greetham *et al.* (2002), Apanavicius *et al.* (2007), Biagi *et al.* (2007, 2010), Lamendella *et al.* (2008). Similarly, Willard *et al.* (2000) reported that lactobacilli were inconsistently isolated from dog faeces.

In the study by Beloshapka *et al.* (2013), according to 454 pyrosequencing, bifidobacteria were not detectable and lactobacilli were present at less than 0.05% of total sequences. In the same study, when faecal samples were analysed by quantitative polymerase chain reaction both *Bifidobacterium* and *Lactobacillus* were detectable, even if at low levels. Authors concluded that bifidobacteria and lactobacilli might be underestimated using these 16S rRNA gene-based sequencing approaches due to the presence of several sources of bias, as already highlighted by other authors (Garcia-Mazcorro *et al.*, 2011; Handl *et al.*, 2011).

Effects on intestinal short-chain fatty acids

Short-chain fatty acids (acetate, propionate, lactate and butyrate) are the main products of carbohydrate fermentation. There is evidence that butyric acid is the preferred fuel substrate of the terminal ileal mucosa (Chapman *et al.*, 1995) and the epithelial cells of the large intestine (Roediger, 1980). Moreover, these organic acids possess antibacterial properties (Knarreborg *et al.*, 2002) and reduce luminal pH thus improving animal intestinal health.

In the *in vitro* study by Biagi *et al.* (2010), total SCFA in the inocula were increased by lactitol (+18%), inulin from chicory (+17%), pectins from apple (+15%), psyllium fiber (+21%) and partially hydrolysed guar gum (+69%). The higher SCFA concentrations lead to significantly lower pH values of inocula. Similarly, faecal propionic acid and total SCFA were increased in dogs fed OF at 6 g/kg of diet (+55 and +31%, respectively; Flickinger *et al.*, 2003a). These results agree with those obtained by Propst *et al.* (2003), who reported higher faecal concentrations of acetic, propionic and butyric acids in dogs receiving inulin or OF at different levels of inclusion (3, 6 and 9 g/kg of diet). Similarly, the utilisation of polydextrose at 5, 10 and 15 g/kg of diet in adult dogs resulted in lower faecal pH and higher faecal concentrations of acetate, propionate and total SCFA (Beloshapka *et al.*, 2012).

Conversely, Barry *et al.* (2009) reported decreased faecal concentrations of acetic acid, propionic acid and total SCFA in dogs fed inulin at 2 and 4 g/kg of diet. In a study by Twomey *et al.* (2003), the administration of FOS at 30 or 60 g/kg of diet to dogs resulted in higher faecal lactate concentrations (85, 143 and 289 mmol/kg of faeces for control, FOS at 30 g/kg and FOS at 60 g/kg, respectively) and lower faecal pH (5.4, 5.3 and 5.0 for control, FOS at 30 g/kg and FOS at 60 g/kg, respectively).

Effects on faecal quality

It has been observed that the utilisation of prebiotics can result in humans in increased stool weight and decreased stool transit time (Macfarlane *et al.*, 2006). Prebiotics are sometimes proposed in humans as mild laxatives despite the fact that a clear scientific evidence of this effect is still lacking (Cummings and Macfarlane, 2002).

Twomey *et al.* (2003) observed that administration of FOS at 30 or 60 g/kg of diet to dogs resulted in decreased faecal dry matter (DM; 327, 303 and 296 g/kg of faeces for control, FOS at 30 g/kg and FOS at 60 g/kg of diet, respectively). Conversely, several authors (Strickling *et al.*, 2000; Swanson *et al.*, 2002b; Flickinger *et al.*,

2003a; Beloshapka *et al.*, 2012) did not observe any effect of prebiotic administration on canine faecal dry matter when prebiotics were administered at lower doses than those used by Twomey *et al*. (2003). Based on the literature, it seems evident that high dietary concentrations of prebiotics may have an influence on faecal volume and consistency. Because, in general, pet owners expect their dogs to excrete small and firm faeces, prebiotics should be administered to dogs at relatively low concentrations (less than 20 g/kg of dietary DM).

Effects on nutrient digestibility

Several authors reported that prebiotic consumption by dogs resulted in lower organic matter (OM) and CP total tract apparent digestibility (Diez *et al.*, 1997, 1998a, 1998b; Zentek *et al.*, 2002; Flickinger *et al.*, 2003a; Hesta *et al.*, 2003; Propst *et al.*, 2003; Middelbos *et al.*, 2007a; Beloshapka *et al.*, 2012). Conversely, Middelbos *et al.* (2007b) reported higher ileal digestibility of OM (79.4 *vs* 74.5%) and CP (72.4 *vs* 65.3%) in dogs fed spray-dried yeast cell wall at 6.5 g/kg of diet. In the study by Twomey *et al.* (2003), total tract apparent digestibility of protein in dogs receiving FOS at 30 or 60 g/kg of diet was unaffected.

In general, prebiotic consumption leads to higher faecal nitrogen concentrations because faecal bacterial mass is increased (Hesta *et al.*, 2003; Karr-Lilienthal *et al.*, 2004) thus leading to lower apparent digestibility of OM and CP. As already mentioned, conversion of ammonia nitrogen into bacterial protein is a positive effect that can improve intestinal health.

Another possible effect of prebiotics is an improved intestinal absorption of minerals. In a study with adult dogs, Beynen *et al.* (2002) found that the use of OF at 10 g/kg increased apparent calcium (+86%) and magnesium (+67%) absorption, whereas phosphorus absorption was unaffected. Similarly, the use of lactulose at 1 or 3 g/MJ of metabolisable energy in adult dogs increased apparent calcium (+63 and +83%, respectively) and magnesium (+27 and +52%, respectively) absorption in a dose-dependent manner, but not phosphorus (Beynen *et al.*, 2001). Results from these two studies seem to confirm that, similarly to what has been observed in other animal species and humans (Scholz-Ahrens *et al.*, 2007), prebiotics may have a positive influence on calcium and magnesium intestinal absorption in dogs. The positive effects of prebiotics on mineral absorption have been attributed to several mechanisms, including increased solubility of minerals and enterocytes proliferation because of increased bacterial production of SCFA and increased expression of calcium binding proteins (Scholz-Ahrens *et al.*, 2007).

Effects on immune system

The gastrointestinal tract is the main site of interaction between the immune system and microorganisms. While the immune system plays a very important role in maintaining homeostasis with intestinal microbiota, bacteria residing in the gut influence development of gut-associated lymphoid tissues and shape animal immunity (Round and Mazmanian, 2009).

The effects of prebiotics on immune system of dogs and other animal species were recently reviewed by Lomax and Calder (2009). While the utilisation of 8.7 g/kg of diet of a combination of high fermentable fiber sources (containing a mixture beet pulp, FOS and gum arabic; Field *et al.*, 1999), short-chain FOS at 9.1 g/kg of diet (Adogony *et al.*, 2007) or inulin (30 g/kg of diet; Verlinden *et al.*, 2006) had only little or no effect on canine immune system, the utilisation of FOS in combination with MOS increased ileal IgA concentrations (+1.5 mg/g DM; both FOS and MOS were used at 1 g/d; Swanson *et al.*, 2002b). In another study (Swanson *et al.*, 2002c), dietary supplementation with FOS at 2 g/d plus MOS at 1 g/d increased blood lymphocytes and reduced blood neutrophils in adult dogs. Conversely, in the study by Grieshop *et al.* (2004), a combination of chicory (10 g/kg of diet) and MOS (10 g/kg of diet) reduced peripheral blood lymphocyte concentration with no other effect on immune system. Similarly, Middelbos *et al.* (2007b) observed only limited effects on canine immune system when a yeast cell wall preparation was fed to adult dogs. At present, the prebiotic effects on canine immunity have not been well studied and more research is needed. Nevertheless, based on the cited studies, despite some discrepancies, the utilisation of MOS seems to enhance immune system in dogs. Conversely, based on current knowledge, the influence of FOS and inulin on canine immune system might be negligible.

Effects of synbiotics in healthy dogs

Ogué-Bon *et al.* (2010) conducted *in vitro* tests to assess the synergistic potential of some prebiotic substrates (FOS, GOS and inulin) and several probiotic strains (*Bifidobacterium bifidum, B. longum, Lactobacillus plantarum, L. acidophilus* and *L. rhamnosus*). In this study, a specificity between bacterial strain and fermentable substrate emerged clearly; for example, GOS were rapidly used by bifidobacteria (thanks to their ability to synthesise -galactosidase), as had already been highlighted by other authors (Gopal *et al.*, 2001; Rada *et al.*, 2008; Zanoni *et al.*, 2008). FOS, on the other hand, were shown to be easily metabolised by all the test strains with the exception of *L. rhamnosus*; this latter finding confirms what had been previously observed by Kaplan and Hutkins (2000) and is probably ascribable to a deficiency of the enzyme -fructosidase in *L. rhamnosus* strains. Results from the study by Ogué-Bon *et al.* (2010) also indicated that the synbiotic combination GOS+*B. bifidum* induced greater modulation of canine faecal microbiota compared with GOS alone.

In a study by Swanson *et al.* (2002a), two experiments were performed with a group of dogs receiving for 28 d short-chain FOS (4 g/d), a strain of *Lactobacillus acidophilus* (2 x 10^9 cfu/d) or their combination. In the second experiment, compared with control, FOS supplementation increased faecal total aerobes (+0.6 $\log_{10}$ cfu/g of faecal DM), bifidobacteria (+0.6 $\log_{10}$ cfu/g of faecal DM) and lactobacilli (+0.7 $\log_{10}$ cfu/g of faecal DM). The combination of FOS and *L. acidophilus* resulted in effects of larger entity on the concentration of bacterial metabolites compared to administration of either of the two preparations alone. More specifically, the synbiotic was particularly effective in reducing the faecal concentrations of ammonia (compared with control, -5% in the second experiment) and of some catabolites (branched-chain fatty acids; compared with control, -8 and -22% in experiment 1 and 2, respectively) deriving from protein fermentation.

Tzortzis *et al.* (2004b) carried out an *in vitro* investigation into the effects on canine gut microbiota of several prebiotic substances, including a particular galactooligosaccharide [galactosyl melibiose mixture (GMM); synthesised using -galactosidase isolated from *L. reuteri*], in association with *L. acidophilus* and *L. reuteri* strains. The authors observed that all of the various tested substrates (FOS with dp 2 to 9, GMM, melibiose and raffinose) possessed prebiotic properties, but GMM showed a higher increase in bifidobacteria and lactobacilli as well as a higher decrease in clostridia compared to the other prebiotics (FOS, melibiose and raffinose). Furthermore, the increase in the counts of bifidobacteria was highest for the combination of GMM and *L. reuteri* (compared with inoculum at start, +1.3 $\log_{10}$ CFU/mL). During another *in vitro* study, Tzortzis *et al.* (2004a) investigated the effect of various carbon sources on the production of extracellular antagonistic compounds against two *Escherichia coli* strains and *Salmonella enterica* serotype Typhimurium by three canine-derived lactobacilli strains (*L. mucosae*, *L. acidophilus* and *L. reuteri*). Results showed that

production of antimicrobial compounds by lactobacilli strains was influenced by substrate in a synergistic mode of action.

In another study (Garcia-Mazcorro *et al.*, 2011), the administration for 21 d of a commercial synbiotic containing 7 different probiotic species (*Enterococcus faecium*, *Streptococcus salivarius* ssp. *termophilus*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *L. casei* ssp. *rhamnosus*, *L. plantarum* and *L. delbrueckii* ssp. *bulgaricus*) and a mixture of FOS and arabinogalactans induced a significant increase in the concentrations of *Enterococcus* and *Streptococcus* spp. (species present in the probiotic supplement) in dog faeces during synbiotic administration. In the same study, none of the evaluated serum (cobalamin, folate, IgA, trypsin-like immunoreactivity and pancreatic lipase immunoreactivity) or faecal (IgA and a_1-proteinase inhibitor) markers of gastrointestinal and immune function were influenced by synbiotic administration. The association between commercially available probiotic strains (*Lactobacillus plantarum*, two strains of *L. acidophilus*, *L. rhamnosus*, *Bifidobacterium longum* and *B. bifidum*) and commercially available fibre blends (rice bran, citrus pectin and barley and maize starch) was studied by Ogué-Bon *et al.* (2011). The authors observed that rice bran was capable of increasing SCFA production and stimulating the growth of probiotic strains. This finding is particularly interesting since rice bran is commonly used as a fibre supplement in the pet food industry and could therefore add a prebiotic effect to the known dietary effects tied to the use of this type of fibre, which include increasing the faecal mass and providing a laxative action. Finally, the authors noted that rice bran on its own had the same effect on the faecal counts of bifidobacteria and lactobacilli and concentrations of SCFA as the various synbiotic combinations, thus revealing that in this case no synergism existed between the probiotic strains and fibre source used. Despite the relatively low number of studies that have been conducted with synbiotics in the canine species, there is some evidence that the proper combination of a prebiotic with one or more probiotic strains might result in a synergistic effect on dog intestinal microbiota.

Roles of prebiotic substances in canine disease

One potential benefit from the utilisation of prebiotics in humans and monogastric animals is to reduce infection by intestinal pathogens (Callaway *et al.*, 2008). In fact, prebiotics can stimulate the growth of bacteria that compete against pathogens (Roberfroid, 2007) and also modulate activity of the immune system (Seifert and Waltz, 2007; Lomax and Calder, 2009).

By increasing the number of lactobacilli in the intestine, inulin at 10 g/kg of diet could provide positive action against *Salmonella typhimurium* infections, based on what was observed by Apanavicius *et al.* (2007) in experimentally infected puppies. The antagonist action of lactobacilli against *Salmonella typhimurium* (de Moreno de LeBlanc *et al.*, 2010) is in fact well known. In the already cited study by Apanavicius *et al.* (2007), inuline supplementation decreased enterocyte sloughing, an indicator of intestinal damage, and increased acetate intestinal concentrations whereas the utilisation of FOS at 10 g/kg of diet resulted in decreased enterocyte sloughing but did not affect lactobacilli counts.

As already mentioned, the use of prebiotics might reduce the intestinal concentrations of ammonia and, as a consequence, the amount of ammonia that is absorbed into circulation and burdens liver and kidneys (Howard *et al.*, 2000). Dogs with hepatic failure do not metabolise ammonia well and might not be able to convert ammonia into urea. On the other hand, dogs with renal functional impairment lose their ability to excrete nitrogen wastes. For these reasons, prebiotics such as lactulose (McQuaid, 2005) could be used in the treatment of dogs with renal or liver failure to prevent uremia and hepatic encephalopathy, respectively.

The utilisation of prebiotics can produce some benefits in humans in the management of constipation by increasing stool weight and decreasing stool transit time (Macfarlane *et al.*, 2006); moreover, there is some evidence that prebiotics might be helpful in the treatment of inflammatory bowel disease in humans and animal models (Hedin *et al.*, 2007). At present, no studies have been done to verify these prebiotic effects in dogs. In general, more research needs to be done to investigate the possible role of prebiotics in canine disease.

Conclusions

A reading of the literature shows that by relying on the use of prebiotic substances it may be possible to manipulate the gastrointestinal ecosystem of dogs with the aim of improving their intestinal wellbeing and enhancing their immune function. Utilisation of prebiotics has several beneficial effects in the canine intestine, including improved composition of intestinal microbiota, reduced concentrations of protein catabolites and enhanced production of SCFA. Among prebiotics, short-chain FOS and OF seem to be the most effective in modulating canine intestinal microbiota and improving intestinal absorption of minerals but with little or no effect on canine immune system. Conversely, MOS may have a positive influence on the immune system of dogs but more research is needed on this subject. Furthermore, evidence exists that some positive effects of prebiotics in dogs might be enhanced if these are used in combination with specific probiotic strains, in the form of a synbiotic. Unfortunately, to date, most studies with prebiotics in dogs were conducted with healthy adult animals so that little is known about the interaction between prebiotic administration and factors such as age and health status.

Clinical effects of prebiotics have been widely investigated in humans but, at present, little evidence exists that prebiotics may be helpful in canine diseases such as infections by intestinal pathogens, intestinal constipation and hepatic and renal failure. More research needs to be done to investigate the possible role of prebiotics in canine disease and possibly to link prebiotic-induced changes in the intestinal microbiota to significant physiological outcomes.

Finally, most studies on canine intestinal microbiota were conducted using traditional culture methods, so that more research remains to be done with modern molecular identification methods to investigate the effects of prebiotic substances in dogs.

References

Adogony, V., Respondek, F., Biourge, V., Rudeaux, F., Delaval, J., Bind, J.L., Salmon, H., 2007. Effects of dietary scFOS on immunoglobulins in colostrums and milk of bitches. J. Anim. Physiol. An. N. 91:169-174.

Apanavicius, C.J., Powell, K.L., Vester, B.M., Karr-Lilienthal, L.K, Pope, L.L., Fastinger, N.D., Wallig, M.A., Tappenden, K.A., Swanson, K.S., 2007. Fructan supplementation and infection affect food intake, fever, and epithelial sloughing from Salmonella challenge in weanling puppies. J. Nutr. 137:1923-1930.

Barry, K.A., Hernot, D.C., Middelbos, I.S., Francis C., Dunsford, B., Swanson, K.S.,

Fahey Jr., G.C., 2009. Low-level fructan supplementation of dogs enhances nutrient digestion and modifies stool metabolite concentrations, but does not alter fecal microbiota populations. J. Anim. Sci. 87:3244-3252.

Beloshapka, A.N., Dowd, S.E., Suchodolski, J.S., Steiner, J.M., Duclos, L., Swanson, K.S., 2013. Fecal microbial communities of healthy adult dogs fed raw meat-based diets with or without inulin or yeast cell wall extracts as assessed by 454 pyrosequencing. FEMS Microbiol. Ecol. 84:532-541.

Beloshapka, A.N., Wolff, A.K., Swanson, K.S., 2012. Effects of feeding polydextrose on faecal characteristics, microbiota and fermentative end products in healthy adult dogs. Brit. J. Nutr. 108:638-644.

Benno, Y., Mitsuoka, T., 1989. Effect of advances in age on intestinal microflora of beagle dogs. Microecol. T. 19:85-91.

Benno, Y., Nakao, H., Uchida, K., Mitsuoka, T., 1992. Impact of the advances in age on the gastrointestinal microflora of beagle dogs. J. Vet. Med. Sci. 54:703-706.

Beynen, A.C., Baas, J.C., Hoekemeijer, P.E., Kappert, H.J., Bakker, M.H., Koopman, J.P., Lemmens, A.G., 2002. Faecal bacterial profile, nitrogen excretion and mineral absorption in healthy dogs fed supplemental oligofructose. J. Anim. Physiol. An. N. 86:298-305.

Beynen, A.C., Kappert, H.J., Yu, S., 2001. Dietary lactulose decreases apparent nitrogen absorption and increases apparent calcium and magnesium absorption in healthy dogs. J. Anim. Physiol. An. N. 85:67-72.

Biagi, G., Cipollini, I., Grandi, M., Zaghini, G., 2010. Influence of some potential prebiotics and fibre-rich foodstuffs on composition and activity of canine intestinal microbiota. Anim. Feed Sci. Tech. 159:50-58.

Biagi, G., Cipollini, I., Pompei, A., Zaghini, G., Matteuzzi, D., 2007. Effect of a Lactobacillus animalis strain on composition and metabolism of the intestinal microflora in adult dogs. Vet. Microbiol.124:160-165.

Biagi, G., Piva, A., Moschini, M., Vezzali, E., Roth, F.X., 2006. Effect of gluconic acid on piglet growth performance, intestinal microflora, and intestinal wall morphology. J. Anim. Sci. 84:370-378.

Buddington, R.K., 2003. Postnatal changes in bacterial populations in the gastrointestinal tract of dogs. Am. J. Vet. Res. 64:646-651.

Callaway, T.R., Edrington, T.S., Anderson, R.C., Harvey, R.B., Genovese, K.J., Kennedy, C.N., Venn, D.W., Nisbet, D.J., 2008. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Anim. Health Res. Rev. 9:217-225.

Campbell, J.M., Bauer, L.L., Fahey Jr., G.C., Lewis Hogarth, A.J.C., Wolf, B.W., Hunter, D.E., 1997. Selected fructooligosaccharide (1-kestose, nystose, and 1F- -fructofuranosylnystose) composition of foods and feeds. J. Agr. Food Chem. 45:3076-3082.

Cerf-Bensussan, N., Gaboriau-Routhiau, V., 2010. The immune system and the gut microbiota: friends or foes? Nat. Rev. Immunol. 10:735-744.

Chapman, M.A., Grahn, M.F., Hutton, M., Williams, N.S., 1995. Butyrate metabolism in the terminal ileal mucosa of patients with ulcerative colitis. Brit. J. Surg. 82:36-38.

Cummings, J.H., Englyst, H.N., 1987. Fermentation in the human large intestine and the available substrates. Am. J. Clin. Nutr. 45:1243-1255.

Cummings, J.H., Macfarlane, G.T., 2002. Gastrointestinal effects of prebiotics. Brit. J. Nutr. 87:145-151.

De Bruyn, A., Alvarez, A.P., Sandra, P., De Leenheer, L., 1992. Isolation and identification of O- -D-fructofuranosyl-(2,1)-D-fructose, a product of the enzymatic hydrolysis of the inulin from Cichorium intybus. Carbohyd. Res. 235:303-308.

de Moreno de LeBlanc, A., Castillo, N.A., Perdigon, G., 2010. Anti-infective mechanisms induced by a probiotic Lactobacillus strain against Salmonella enterica serovar Typhimurium infection. Int. J. Food Microbiol. 138:223-231.

Diez, M., Hornick, J.L., Baldwin, P., Istasse, L., 1997. Influence of a blend of fructooligosaccharides on nutrient digestibility and plasma metabolite concentrations in healthy beagles. Am. J. Vet. Res. 58:1238-1242.

Diez, M., Hornick, J.L., Baldwin, P., Van Eenaeme, C., Istasse, L., 1998a. Etude des fibres alimentaires chez le chien: présentation des résultats de 7 essais expérimentaux. Ann. Med. Vet. 142:185-201.

Diez, M., Hornick, J.L., Baldwin, P., Van Eenaeme, C., Istasse, L., 1998b. The influence of sugar-beet fibre, guar gum and inulin on nutrient digestibility, water consumption and plasma metabolites in healthy beagle dogs. Res. Vet. Sci. 64:91-96.

Field, C.J., McBurney, M.I., Massimino, S., Hayek, M.G., Sunvold, G.D., 1999. The fermentable fiber content of the diet alters the function and composition of canine gut associated lymphoid tissue. Vet. Immunol. Immunop. 72:325-341.

Flickinger, E.A., Schreijen, E.M., Patil, A.R., Hussein, H.S., Grieshop, C.M., Merchen, N.R., Fahey Jr., G.C., 2003a. Nutrient digestibilities, microbial population and protein catabolites as affected by fructan supplementation of dog diets. J. Anim. Sci. 81:2008-2018.

Flickinger, E.A., Van Loo, J., Fahey Jr., G.C., 2003b. Nutritional responses to the presence of inulin and oligofructose in the diets of domesticated animals: a review. Crit. Rev. Food Sci. 43:19-60.

Franck, A., Bosscher, D., 2009. Inulin. In: S.S. Cho and P. Samuel (eds.) Fiber ingredients: food applications and health benefits. CRC Press, Taylor and Francis Group, Boca Raton, FL, USA, pp 41-60.

Fujita, K., Kitahata, S., Kozo, H., Hotoshi, H., 1992. Production of lactosucrose and its properties. In: M.A. Clarke (ed.) Carbohydrates in industrial synthesis. Verlag Bartens, Berlin, Germany, pp 68-76.

Garcia-Mazcorro, J.F., Lanerie, D.J., Dowd, S.E., Paddock, C.G., Grützner, N., Steiner, J.M., Ivanek, R., Suchodolski, J.S., 2011. Effect of a multi-species synbiotic formulation on fecal bacterial microbiota of healthy cats and dogs as evaluated by pyrosequencing. FEMS Microbiol. Ecol. 78:542-554.

Gopal, P.K., Sullivan, P.A., Smart, J.B., 2001. Utilisation of galacto-oligosaccharides as selective substrates for growth by lactic acid bacteria including B. lactis DR10 and Lactobacillus rhamnosus DR20. Int. Dairy J. 11:19-25.

Greetham, H.L., Giffard, C., Hutson, R.A., Collins, M.D, Gibson, G.R., 2002. Bacteriology of the Labrador dog gut: a cultural and genotypic approach. J. Appl. Microbiol. 93:640-646.

Grieshop, C.M., Flickinger, E.A., Bruce, K.J., Patil, A.R., Czarnecki-Maulden, G.L., Fahey Jr., G.C., 2004. Gastrointestinal and immunological responses of senior dogs to chicory and mannan-oligosaccharides. Arch. Anim. Nutr. 58:483-493.

Handl, S., Dowd, S.E., Garcia-Mazcorro, J.F., Steiner, J.M., Suchodolski, J.S., 2011. Massive parallel16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. FEMS Microbiol. Ecol. 76:301-310.

Harmsen, H.J.M., Gibson, G.R., Elfferrich, P., Raangs, G.C., Wildeboer-Veloo, A.C.M., Argaiz, A., Roberfroid, M.B., Welling, G.W., 2000. Comparison of viable counts and fluorescent in situ hybridization using specific rRNA-based probes for the quantification of human faecal bacteria. FEMS Microbiol. Lett. 183:125-129.

Hedin, C., Whelan, K., Lindsay, J.O., 2007.

Evidence for the use of probiotics and prebiotics in inflammatory bowel disease: a review of clinical trials. Proc. Nutr. Soc. 66:307-315.

Hernot, D., Ogué, E., Fahey, G., Rastall, R.A., 2008. Prebiotics and synbiotics in companion animal science. In: J. Versalovic, M. Wilson (eds.) Therapeutic microbiology: probiotics and related strategies. ASM Press, Washington, DC, pp 357-370.

Herschel, D.A., Argenzio, R.A., Southworth, M., Stevens, C.E., 1981. Absorption of volatile fatty acid, Na and H_2O by the colon of the dog. Am. J. Vet. Res. 42:1118-1124.

Hesta, M., Janssens, G.P.J., Millet, S., De Wilde, R., 2003. Prebiotics affect nutrient digestibility but not faecal ammonia in dogs fed increased dietary protein levels. Brit. J. Nutr. 90:1007-1014.

Hooda, S., Minamoto, Y., Suchodolski, J.S., Swanson, K.S., 2012. Current state of knowledge: the canine gastrointestinal microbiome. Anim. Health Res. Rev.13:78-88.

Howard, M.D., Kerley, M.S., Sundvold, G.D., Reinhart, G.A., 2000. Source of dietary fiber fed to dogs affects nitrogen and energy metabolism and intestinal microflora populations. Nutr. Res. 20:1473-1484.

Hussein, H.S., Campbell, J.M., Bauer, L.L., Fahey Jr., G.C., Lewis Hogarth, A.J.C., Wolf, B.W., Hunter, D.E., 1998. Selected fructooligosaccharide composition of pet-food ingredients. J. Nutr. 128:2803-2805.

Imaizumi, K., Nakatsu, Y., Sato, M., Sedarnawati, Y., Sugano, M., 1991. Effects of xylooligosaccharides on blood glucose, serum and liver lipids and cecum short-chain fatty acids in diabetic rats. Agr. Biol. Chem. Tokyo 55:199-205.

Kaplan, H., Hutkins, R.W., 2000. Fermentation of fructoligosaccharides by lactic acid bacteria and bifidobacteria. Appl. Environ. Microb. 66:2682-2684.

Karr-Lilienthal, L.K., Grieshop, C.M., Spears, J.K., Patil, A.R., Czarnecki-Maulden, G.L., Merchen, N.R., Fahey Jr., G.C., 2004. Estimation of the proportion of bacterial nitrogen in canine feces using diaminopimelic acid as an internal bacterial marker. J. Anim. Sci. 82:1707-1712.

Knarreborg, A., Miquel, N., Granli, T., Jensen, B.B., 2002. Establishment and application of an in vitro methodology to study the effects of organic acids on coliform and lactic acid bacteria in the proximal part of the gastrointestinal tract of piglets. Anim. Feed Sci. Tech. 99:131-140.

Lamendella, R., Santo Domingo, J.W., Kelty, C., Oerther, D.B., 2008. Bifidobacteria in feces and environmental waters. Appl. Environ. Microb. 74:575-584.

Langendijk, P.S, Schut, F., Jansen, G.J., Raangs, G.C., Kamphuis, G.R., Wilkinson, M.H.F., Welling, G.W., 1995. Quantitative fluorescence in situ hybridization of Bifidobacterium spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. Appl. Environ. Microb. 61:3069-3075.

LeBlanc, J.G., Milani, C., de Giori, G.S., Sesma, F., van Sinderen, D., Ventura, M., 2013. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. Curr. Opin. Biotech. 24:160-168.

Liévin-Le Moal, V., Servin, A.L., 2006. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. Clin. Microbiol. Rev. 19:315-337.

Lomax, A.R., Calder, P.C., 2009. Prebiotics, immune function, infection and inflammation: a review of the evidence. Brit. J. Nutr. 101:633-658.

Macfarlane, S., Macfarlane, G.T., Cummings, J.H., 2006. Review article: prebiotics in the gastrointestinal tract. Aliment. Pharm. Ther. 24:701-714.

McQuaid, T.S., 2005. Medical management of a patent ductus venosus in a dog. Canadian Vet. J. 46:352-356.

Middelbos, I.S., Fastinger, N.D., Fahey Jr., G.C., 2007a. Evaluation of fermentable oligosaccharides in diets fed to dogs in comparison to fiber standards. J. Anim. Sci. 85:3033-3044.

Middelbos, I.S., Godoy, M.R., Fastinger, N.D., Fahey Jr., G.C., 2007b. A dose-response evaluation of spray-dried yeast cell wall supplementation of diets fed to adult dogs: effects on nutrient digestibility, immune indices, and fecal microbial populations. J. Anim. Sci. 85:3022-3032.

Middelbos, I.S., Vester Boler, B.M., Qu, A., White, B.A., Swanson, K.S., Fahey Jr., G.C., 2010. Phylogenetic characterization of fecal microbial communities of dogs fed diets with or without supplemental dietary fiber using 454 pyrosequencing. PLoS ONE 5:e9768.

Mitsuoka, T., 1992. Intestinal flora and aging. Nutr. Rev. 50:438-446.

Moshfegh, A.J., Friday, J.E., Goldman, J.P., Ahuja, J.K.C., 1999. Presence of inulin and oligofructose in the diets of Americans. J. Nutr. 129:1407-1411.

Ogué-Bon, E., Khoo, C., Hoyles, L., McCartney, A.L., Gibson, G.R., Rastall, R.A., 2011. In vitro fermentation of rice bran combined with Lactobacillus acidophilus 14 150B or Bifidobacterium longum 05 by the canine faecal microbiota. FEMS Microbiol. Ecol. 75:365-376.

Ogué-Bon, E., Khoo, C., McCartney, A.L., Gibson, G.R., Rastall, R.A., 2010. In vitro effects of synbiotic fermentation on the canine faecal microbiota. FEMS Microbiol. Ecol. 73:587-600.

Propst, E.L., Flickinger, E.A., Bauer, L.L., Merchen, N.R., Fahey Jr., G.C., 2003. A dose-response experiment evaluating the effects of oligofructose and inulin on nutrient digestibility, stool quality, and fecal protein catabolites in healthy adult dogs. J. Anim. Sci. 81:3057-3066.

Rada, V., Nevoral, J., Trojanová, I., Tománková, E., Smehilová, M., Killer, J., 2008. Growth of infant faecal bifidobacteria and clostridia on prebiotic oligosaccharides in in vitro conditions. Anaerobe 14:205-208.

Roberfroid, M.B., 2007. Prebiotics: the concept revisited. J. Nutr. 137:830-837.

Roberfroid, M.B., Bornet, F., Bouley, C., Cummings, J.H., 1995. Colonic microflora: nutrition and health. Nutr. Rev. 53:127-130.

Roediger, W.E., 1980. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut 21:793-798.

Round, J.L., Mazmanian, S.K., 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. 9:313-323.

Russell, J.B., Sniffen, C.J., Van Soest, P.J., 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. J. Dairy Sci. 66:763-775.

Schaible, U.E., Kaufmann, S.H., 2005. A nutritive view on the host-pathogen interplay. Trends Microbiol. 13:373-380.

Scholz-Ahrens, K.E., Ade, P., Marten, B., Weber, P., Timm, W., Açil, Y., Glüer, CC., Schrezenmeir, J., 2007. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J. Nutr. 137:838-846.

Schrezenmeir, J., De Vrese, M., 2001. Probiotics, prebiotics and synbiotics: approaching a definition. Am. J. Clin. Nutr. 73:361-364.

Seifert, S., Watzl, B., 2007. Inulin and oligofructose: review of experimental data on immune modulation. J. Nutr. 137:2563-2567.

Simpson, J.M., Martineau, B., Jones, W.E., Ballam, J.M., Mackie, R.I., 2002. Characterization of fecal bacterial populations in canines: effects of age, breed and dietary fiber. Microb. Ecol. 44:186-197.

Spiegel, J.E., Rose, R., Karabell, P., Frankos, V.H., Schmitt, D.F., 1994. Safety and benefits of fructooligosaccharides as food ingredients.

Food Technol.-Chicago 48:85-89.
Spring, P., Wenk, C., Dawson, K.A., Newman, K.E., 2000. The effect of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. Poultry Sci. 79:205-211.
Stevens, C.S., Hume, I.D., 1998. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. Physiol. Rev. 78:393-427.
Strickling, J.A., Harmon, D.L., Dawson, K.A., Gross, K.L., 2000. Evaluation of oligosaccharide addition to dog diets: influences on nutrient digestion and microbial populations. Anim. Feed Sci. Tech. 86:205-219.
Suchodolski, J.S., 2011. Companion animals symposium: microbes and gastrointestinal health of dogs and cats. J. Anim. Sci. 89:1520-1530.
Suchodolski, J.S., Dowd, S.E., Westermarck, E., Steiner, J.M., Wolcott, R.D., Spillmann, T., Harmoinen, J.A., 2009. The effect of the macrolide antibiotic tylosin on microbial diversity in the canine small intestine as demonstrated by massive parallel 16s rRNA gene sequencing. BMC Microbiol. 9:210.
Suchodolski, J.S., Ruaux, C.G., Steiner, J.M., Fetz, K., Williams, D.A., 2005. Assessment of the qualitative variation in bacterial microflora among compartments of the intestinal tract of dogs by use of a molecular fingerprinting technique. Am. J. Vet. Res. 66:1556-1562.
Swanson, K.S., Fahey, G.C., 2006. Prebiotic impacts on companion animals. In: G.R. Gibson, R.A. Rastall (eds.) Prebiotics development and application. John Wiley and Sons, Chichester, UK, pp 217-236.
Swanson, K.S., Dowd, S.E., Suchodolski, J.S., Middelbos, I.S., Vester, B.M., Barry, K.A., Nelson, K.E., Torralba, M., Henrissat, B., Coutinho, P.M., Cann, I.K.O., White, B.A., Fahey Jr., G.C., 2011. Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. ISME J. 5:639-649.
Swanson, K.S., Grieshop, C.M., Flickinger, E.A., Bauer, L.L., Chow, J., Wolf, B.W., Garleb, K.A., Fahey Jr., G.C., 2002a. Fructooligosaccharides and Lactobacillus acidophilus modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. J. Nutr. 132:3721-3731.
Swanson, K.S., Grieshop, C.M., Flickinger, E.A., Bauer, L.L., Healy, H.P., Dawson, K.A., Merchen, N.R., Fahey Jr., G.C., 2002b. Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. J. Nutr. 132:980-989.
Swanson, K.S., Grieshop, C.M., Flickinger, E.A., Healy, H.P., Dawson, K.A., Merchen, N.R., Fahey Jr., G.C., 2002c. Effects of supplemental fructooligosaccharides plus mannanoligosaccharides on immune function and ileal and fecal microbial populations in adult dogs. Arch. Tierernahr. 56:309-318.
Terada, A., Hara, H., Oishi, T., Matsui, T., Mitsuoka, T., Nakajyo, S., Fujimori, I., Hara, K., 1992. Effect of dietary lactosucrose on faecal flora and faecal metabolites of dogs. Microb. Ecol. Health D. 5:87-92.
Tomomatsu, H., 1994. Health effects of oligosaccharides. Food Technol.-Chicago 48:61-65.
Twomey, L.N., Pluske, J.R., Rowe, J.B., Choct, M., Brown, W., Pethick, D.W., 2003. The effects of added fructooligosaccharide (Raftilose®P95) and inulinase on faecal quality and digestibility in dogs. Anim. Feed Sci. Tech. 108:83-93.
Tzortzis, G., Baillon, M.L., Gibson, G.R., Rastall, R.A., 2004a. Modulation of anti pathogenic activity in canine derived Lactobacillus species by carbohydrate growth substrate. J. Appl. Microbiol. 96:552-559.
Tzortzis, G., Goulas, A.K., Baillon, M.L.A., Gibson, G.R., Rastall, R.A., 2004b. In vitro evaluation of the fermentation properties of galactooligosaccharides synthesized by -galactosidase from Lactobacillus reuteri. Appl. Microbiol. Biot. 64:106-111.
Van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H., Smits, G., 1995. On the presence of inulin and oligofructose as natural ingredients in the western diet. Crit. Rev. Food Sci. 35:525-552.
Vanhoutte, T., Huys, G., De Brandt, E., Fahey Jr., G.C., Swings, J., 2005. Molecular monitoring and characterization of the faecal microbiota of healthy dogs during fructan supplementation. FEMS Microbiol. Lett. 249:65-71.
Verlinden, A., Hesta, M., Hermans, J., Janssens, G., 2006. The effects of inulin supplementation of diets with or without protein sources on digestibility, faecal characteristics, haematology and immunoglobulins in dogs. Brit. J. Nutr. 96:936-944.
Vickers, R.J., Sunvold, G.D., Kelley, R.L., Reinhart, G.A., 2001. Comparison of fermentation of selected fructooligosaccharides and other fiber substrates by canine colonic microflora. Am. J. Vet. Res. 62:609-615.
Willard, M.D., Simpson, R.B., Cohen, N.D., Clancy, J.S., 2000. Effects of dietary fructooligosaccharide on selected bacterial populations in feces of dogs. Am. J. Vet. Res. 61:820-825.
Zanoni, S., Pompei, A., Cordisco, L., Amaretti, A., Rossi, M., Matteuzzi, D., 2008. Growth kinetics on oligo- and polysaccharides and promising features of three antioxidative potential probiotic strains. J. Appl. Microbiol. 105:1266-1276.
Zentek, J., Marquart, B., Pietrzak, T., 2002. Intestinal effects of mannanoligosaccharides, transgalactooligosaccharides, lactose and lactulose in dogs. J. Nutr. 132:1682-1684.