The utilisation of prebiotics and synbiotics in dogs

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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 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.

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 probiotic 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 (Schäible 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.

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 phylootypes 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 harvested in the intestine of dogs. However, the phyla Spirochaetales, 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-
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 (Lévi-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 microfloral 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 microbio-

ta 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 xyloooligosaccharides (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

| 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 poyxilans 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                      | 2.5                           | 2.5                           | 1.4                           |
| Wheat bran                 |                               |                               | 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         | ↔            | ↔             | ↓              | ↑                | ↔                |
| Strickling et al., 2000    | FOS            | 5                        | ≤                        | ≤            | ≤             | ND             | ND               | ↔                |
|                            | MOS            | 5                        | Selective media         | ↔            | ↔             | ↓              | ND               | ↔                |
|                            | XOS            | 5                        | ≤                        | ≤            | ≤             | ↔              | ND               | ↔                |
| 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                     | DNA analysis            | ↑            | ↑             | ↔              | ND               | ↔                |
| Barry et al., 2009         | scFOS          | 2 and 4                  | DNA analysis            | ↔            | ↔             | ↔              | ND               | ↔                |
|                            | Inulin         | 2 and 4                  | DNA analysis            | ↔            | ↔             | ↔              | ND               | ↔                |
| Biagi et al., 2010         | Lactitol       | 10                       | Selective media         | ↔            | ↔             | ↓              | 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. (2000), 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 xylose 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 MOS, 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. (2000) 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 (Teraeda 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 microflora, 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 bacterial-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 faecal DM, respectively) and lactobacilli (+0.9 log_{10} CFU/g of faecal 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, *coli* form 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 faecal 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 the 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 mmol/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-Mazzorro 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 (Knaerreberg 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%), pyllium 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.,...
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; Fickinger 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; Propst et al., 2003; Middelbos et al., 2007). 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 aracic; 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 10^8 cfu/d) or their combination. In the second experiment, compared with control, FOS supplementation increased faecal total aerobes (+0.6 log10 cfu/g of faecal DM), bifidobacteria (+0.6 log10 cfu/g of faecal DM) and lactobacilli (+0.7 log10 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 symbiotic 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 log10 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 caninederived 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 df a commercial symbiotic containing 7 different probiotic species (Enterococcus faecium, Streptococcus salivarius ssp. thermophilus, 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 symbiotic administration. In the same study, none of the evaluated serum (cohabalan, folate, IgA, tryptophan-like immunoreactivity and pancreatic lipase immunoreactivity) or faecal (IgA and a1-proteinase inhibitor) markers of gastrointestinal and immune function were influenced by symbiotic 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 Ogú-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 symbiotic 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), inulin 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.

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