EFFECTS OF SHORT-CHAIN FRUCTOOLIGOSACCHARIDES AND GALACTOOLIGOSACCHARIDES, INDIVIDUALLY AND IN COMBINATION, ON NUTRIENT DIGESTIBILITY, FECAL FERMENTATIVE METABOLITE CONCENTRATIONS, AND LARGE BOWEL MICROBIAL ECOLOGY OF HEALTHY ADULT CATS

BY

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THESIS

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ABSTRACT

Short-chain fructooligosaccharides (scFOS) and galactooligosaccharides (GOS) are non-digestible oligosaccharides that result in a prebiotic effect in some animal species; however, the cat has not been well studied in this regard. This experiment evaluated scFOS and GOS supplementation on nutrient digestibility, fermentative end-product production, and fecal microbial ecology of cats. Eight healthy adult cats were fed diets containing no prebiotic, 0.5% scFOS, 0.5% GOS, or 0.5% scFOS + 0.5% GOS (scFOS+GOS) in a replicated 4x4 Latin square design. Apparent total tract crude protein digestibility was decreased ($P < 0.05$) when cats were fed a diet containing scFOS + GOS compared to the other treatments. Dry matter, OM, acid hydrolyzed fat, and GE digestibilities were not different among treatments. Cats fed scFOS-, GOS-, and scFOS+GOS-supplemented diets had greater ($P < 0.05$) fecal *Bifidobacterium* spp. populations compared to cats fed the control diet. Fecal pH was lower ($P < 0.05$) for cats fed the scFOS+GOS-supplemented diet compared to the control. Butyrate ($P = 0.05$) and valerate ($P < 0.05$) concentrations were higher when cats consumed the scFOS+GOS diet. Acetate tended to be greater ($P = 0.10$) when cats were fed the scFOS+GOS diet. Total SCFA ($P = 0.06$) and total BCFA ($P = 0.06$) concentrations also tended to be greater when cats consumed the scFOS+GOS treatment. Fecal protein catabolites, including ammonia, 4-methylphenol, indole, and biogenic amines, did not differ among treatments, nor did blood lymphocytes, neutrophils, or total white blood cell counts, or fecal DM concentration and output. Low level supplementation of scFOS, GOS, and their combination exert positive effects on select indices of gut health in cats.

Keywords: cat, short-chain fructooligosaccharides, galactooligosaccharides, microbiota, protein catabolites, nutrient digestibility

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CHAPTER 1

INTRODUCTION

Prebiotics are non-digestible food ingredients that modify the microbial ecology of the colon and improve indices of host health (Gibson and Roberfroid, 1995). Many non-digestible oligosaccharides (NDO) are resistant to enzymatic hydrolysis in the gastrointestinal tract, are fermented by the intestinal microflora, and selectively stimulate the growth and/or activity of one or more intestinal bacteria. Galactooligosaccharides are a mixture of oligomers synthesized from lactose and consist of 2 - 8 saccharide units. Tzotzis and Vulevic (2009) noted that GOS meets all of the criteria of a true prebiotic. Short-chain fructooligosaccharides, a mixture of short-chain glucose and fructose monomers, have been widely studied in humans (Roberfroid, 2007) and dogs (Flickinger et al., 2003; Middelbos et al., 2007; Barry et al., 2009); however, few studies have been done in cats to date (Sparkes et al., 1998; Hesta et al., 2001; Barry et al., 2010).

The colonic microbiota can play an important role in host animal health. *Bifidobacterium* spp. and *Lactobacillus* spp. are desirable bacterial species because of their beneficial effects, including inhibition of pathogenic bacteria (e.g., *Clostridium* spp.) and improving host immunity. Cats supplemented with 175 mg lactosucrose/d (Terada et al., 1993), 0.75% oligofructose (Sparkes et al., 1998), or 4% oligofructose (Barry et al., 2010) had modified colonic microbial populations compared to an oligosaccharide-free control.

In the canine, low level inclusion of scFOS or inulin (0.2 and 0.4% of diet) increased ileal nutrient digestibility with no effect on fecal quality or microbial ecology of the colon, whereas dogs fed an inulin-supplemented diet tended to have increased total tract crude protein digestibility (Barry et al., 2009). The moderate to high inclusion levels of inulin-

type fructans were noted to lower total tract crude protein digestibility by dogs (Middelbos et al., 2007) and cats (Hesta et al., 2001). This reduction may have resulted from the higher fecal bacterial mass produced in response to oligosaccharide supplementation.

Cats nearly always are provided with more protein than is required to meet amino acid requirements. This leads to an active population of clostridial species throughout the gastrointestinal tract of cats. Clostridia are the major microbial species that use amino acids as fermentative substrates. Consequently, several putrefactive compounds, including ammonia, biogenic amines, branched-chain fatty acids (BCFA), indole, phenol, and sulfur-containing compounds, are produced. The large quantities of putrefactive compounds may play an important role in causing disease of the large bowel, including colorectal cancer (Johnson, 1977). The objective of this study was to determine the effects of low level prebiotic inclusion (0.5% scFOS, 0.5% GOS, and 0.5% scFOS + 0.5% GOS) on nutrient digestibility, fermentative metabolite concentrations, and large bowel microbial ecology of healthy adult cats.

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CHAPTER 2

LITERATURE REVIEW

Introduction

Since the potential health benefits of prebiotics were first described by Gibson and Roberfroid (1995), these compounds have been of interest to both the human and animal food industries. Many non-digestible oligosaccharides (NDO), including inulin-type fructans, lactosucrose, lactulose, xylooligosaccharides (XOS), mannanoligosaccharides (MOS), and isomaltooligosaccharides (IMO), have prebiotic properties in dogs (Terada et al., 1992; Strickling et al., 2000; Beynen et al., 2001; Swanson et al., 2002a; Hesta et al., 2003; Middelbos et al., 2007; Barry et al., 2009); however, only inulin-type fructans, galactooligosaccharides (GOS), and lactulose are proven prebiotics (Roberfroid, 2007b).

Inulin-type fructans and GOS are commonly studied as functional food ingredients that improve human health. Ingestion of inulin-type fructans and GOS selectively stimulate growth of beneficial *Bifidobacterium* spp. in the colon. By producing acetate and lactate, *Bifidobacterium* spp. decrease luminal pH and create an unfavorable environment for growth of pathogenic bacteria. Clinical benefits include blood sugar attenuation and lipid regulation, reduction of colon cancer risk, laxation, inhibition of diarrhea, fatty liver disease prevention, inflammatory bowel disease treatment, an increase in absorption of minerals, and improvement in immune function (Roberfroid, 2007a; Kelly, 2009; Tzortzis and Vulevic, 2009). Beneficial effects of prebiotics, including inulin-type fructans and GOS, reported in humans are expected to occur in dogs and cats due to similarities in their digestive physiology and microbial ecology of the large bowel. Therefore, the objective of

this literature review was to evaluate use of prebiotics and their potential benefits in companion animal diets.

Prebiotics

A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the large intestine, and thus improves host health (Gibson and Roberfroid, 1995). Due to such effects, prebiotics must be resistant to enzymatic digestion and absorption in the gastrointestinal tract and must be fermented by intestinal bacteria (Roberfroid, 2007b). Non-digestible carbohydrates (oligo- and polysaccharides) are candidate prebiotics because they are not hydrolyzed by digestive enzymes or absorbed in the small intestine. However, not all non-digestible carbohydrates are classified as prebiotics because they do not lead to changes in bacterial populations (Gibson and Roberfroid, 1995; Roberfroid, 2007b). Among oligosaccharides, inulin-type fructans, GOS, and lactulose (Roberfroid, 2007b; Tzortzis and Velevic, 2009) are proven prebiotics due to their fulfillment of the classification criteria.

Inulin-type fructans and short-chain fructooligosaccharides (scFOS)

Inulin-type fructans are β-(2-1) linear fructans naturally found in several fruits and vegetables including wheat, onion, banana, garlic, and leek (Roberfroid, 2007a). Chemically, inulin-type fructans are linear polydisperse carbohydrates consisting of β-(2-1) fructosyl-fructose glycosidic linkages. Some of these molecules have a glucose unit as the initial moiety. Native chicory inulin is produced from hot water extraction of fresh chicory roots (De Leenheer, 1996). The degree of polymerization (DP) is between 2 to approximately 60 units and the average DP (DP_{av}) is 12 units. Oligofructose, obtained from chicory inulin by partial enzymatic hydrolysis using endoinulinase (EC 3.2.1.7), is a mixture

of fructose chains [fructopyranosyl-(fructofuranosyl)$_n$ fructose; ($F_{py}F_n$)] and contains a fructose chain with a terminal glucose [glucopyranosyl-(fructofuranosyl)$_n$ fructose; ($G_{py}F_n$)] in which the DP varies from 2 to 7 with a DP_{av} of 4. Additionally, oligofructose can be obtained by enzymatic synthesis (transfructosylation) using the fungal enzyme, β-fructosidase (EC 3.2.1.7), from *Aspergillus niger*. In that process, sucrose is used as the starting substrate to which 1, 2, or 3 additional fructose units are linked by forming new β-(2-1) linkages. Therefore, this synthetic compound consists of only fructose chains with terminal glucose units ($G_{py}F_n$) and varies in DP between 2 to 4 with a DP_{av} of 3.6. High molecular weight inulin-type fructan (inulin HP) is a mixture of $G_{py}F_n$ with 10 to 60 DP (DP_{av} = 25) that results from a physical separation technique eliminating the oligomers with DP < 10 from native inulin. A mixture of 30% oligofructose and 70% inulin HP is termed "oligofructose-enriched inulin" (Roberfroid, 2007a).

Short-chain fructooligosaccharides (scFOS), short chain lengths of synthetic β-(2-1) linear fructans, are synonymous with oligofructose (Roberfroid, 2007a). Short-chain FOS is the mixture of inulin-type oligomers synthesized from sucrose. For this mixture, scFOS have $G_{py}F_n$ structures with a maximum DP (DP_{max}) of less than 10. Because of the β-(2-1) linkages in the fructose monomers, inulin and scFOS resist hydrolysis by mammalian enzymes in the small intestine and are fermented by bacteria in the colon (Gibson and Roberfroid, 1995; Roberfroid, 2007a).

Galactooligosaccharides

Galactooligosaccharides are a mixture of NDO produced from lactose consisting of between two and eight saccharide units. These units always have glucose as the terminal unit and the remaining units are galactose and disaccharides comprised of two units of

galactose (Tzortzis and Vulevic, 2009). Galactooligosaccharides are synthesized from lactose by the transgalactosylation activity of β-galactosidases. The oligosaccharide composition varies among GOS mixtures depending on origin of the β-galactosidases. 6′-galactosyllactose, which has β-(1-6) glycosidic bonds between two galactose monomers, is the main product when yeast β-galactosidase (from *Kluyeveromyces* subsp. *lactis* or *K. fragilis*) is used. Enzymes derived from *Bacillus circulans* or *Cryptococcus laurentii* form mainly 4′-galactosyllactose. Enzymes derived from *Aspergillus oryzae* mainly form 3′- and 6′-galactosyllactose. The main products of GOS are trisaccharides (4′- or 6′-galactosyllactose) and longer oligosaccharides consisting of 4 or more monosaccharide units (Sako et al., 1999).

Infants fed breast milk have higher fecal *Bifidobacterium* spp. and lower fecal *Clostridium spp.* and *Enterococcus* spp. populations compared to infants fed cow's milk. Among the variety of different oligosaccharides in human milk, lactose-derived oligosaccharides have been found in large quantities (Tzortzis and Vulevic, 2009). Rycroft et al. (2001) studied the effects of FOS and GOS on intestinal microflora *in vitro* using human fecal inoculum. Both FOS and GOS increased the number of *Bifidobacterium* spp. and GOS decreased *Clostridium* spp. populations. A "Measure of Prebiotic Effect (MPE)" was created to evaluate prebiotic efficacy *in vitro* (Vulevic et al., 2004). The rate of substrate assimilation, the change in bacterial populations, and the ratio of lactate : total short-chain fatty acids (SCFA) produced were accounted for in the equation to compute the MPE value. At 1% w/v, GOS had the highest MPE value, suggesting that it was the most robust prebiotic of all substrates tested. *In vivo*, fecal *Bifidobacterium* spp. concentrations

were increased in healthy adult populations when fed GOS and various GOS mixtures (Ito et al., 1993; Tanaka et al., 1993; Gopal et al., 2003).

Prebiotic effects in dogs and cats

Supplementation of NDO can result in beneficial effects in companion animals. Ingestion of NDO selectively stimulates the growth of *Bifidobacterium* spp. in the colon. By producing acetate and lactate, *Bifidobacterium spp.* decrease luminal pH, creating an unfavorable environment for pathogenic bacteria. During NDO fermentation, SCFA are produced. Butyrate plays an important role as an energy source for colonocytes and helps prevent bacterial translocation by increasing gut integrity (Macfarlane and Cumming, 1991). Jacob (2007) noted that other beneficial effects include immune system modulation, laxation, reduction of protein catabolite production as a result of amino acid fermentation in the large bowel, and therapeutic roles in diabetes mellitus, lipid metabolism, liver disease, and chronic renal failure.

Stool characteristics. Dietary fiber can increase fecal bulk by increasing fiber residues, fecal water, bacterial cell mass, or a combination of the three (Diez et al., 1997). However, low level inclusion of NDO may not affect fecal consistency or production. Barry et al. (2009) fed five ileal cannulated adult female hounds with either an oligosaccharide-free diet, inulin (0.2 and 0.4 %), or scFOS (0.2 and 0.4%) to determine their effects on nutrient digestion, stool metabolite concentrations, and microbiota populations. Fecal score was not affected by treatment.

Short-chain fructooligosaccharides and yeast cell wall (YCW; source of mannanoligosaccharides) were tested as potential replacements for traditional dietary fiber sources in dog diets (Middelbos et al., 2007). Six ileal cannulated adult female hounds were

fed 1) control diet (no fermentable carbohydrate supplementation); 2) as (1) + 2.5% cellulose (low fermentable fiber); 3) as (1) + 2.5% beet pulp (moderately fermentable fiber); 4) as (1) + 1% cellulose + 1.5% scFOS (CF); 5) as (1) + 1% cellulose + 1.2% scFOS + 0.3% YCW (CFY1), or 6) as (1) + 1% cellulose + 0.9% scFOS + 0.6% YCW (CFY2). Fecal consistency of dogs fed CF, CFY1, or CFY2 was not affected by treatment.

Flickinger et al. (2000) evaluated the effect of indigestible oligosaccharides (alpha-glucooligosaccharides and maltodextrin-like OS) on stool characteristics. Six ileal cannulated female adult hounds were fed either 1) an enteral formula diet (control); 2) as (1) + 6% alpha-glucooligosaccharides; 3) as (1) + 6% maltodextrin. Dogs supplemented with diet 2 had softer feces ($P < 0.05$) and greater fecal output (64.8 vs. 35.0 g/d; $P < 0.05$) compared to the control.

Diez et al. (1998) evaluated the effects of three fibers (sugar beet fiber, guar gum, and inulin) supplemented to the basal diet of adult healthy beagles at 7% (as-is basis). As compared to the control, dogs supplemented with 7% inulin excreted more wet feces (96.0 vs. 65.6 g/d; $P < 0.05$), and had lower ($P < 0.05$) fecal DM percentages (27.0 vs. 34.4%).

Hesta et al. (2001) fed adult cats diets containing 0, 3, 6, or 9% oligofructose. Fecal moisture percentage was greater ($P < 0.01$) for cats fed 6% (73.7%) or 9% (75.5%) oligofructose compared to 0% (69.3%) or 3% (70.9%) concentrations. Cats fed 9% oligofructose ($P < 0.05$) excreted more feces per day (as-is basis; 31.9 g/d) compared to 0% (22.9 g/d) or 3% (22.2 g/d) oligofructose treatments.

In a subsequent experiment, Hesta et al. (2001) fed cats diets containing inulin (0, 3, or 6% of the diet) or 3% oligofructose. Cats fed a 6% inulin-supplemented diet excreted

feces with greater ($P < 0.05$) fecal moisture (74.8%) compared to 0% and 3% inulin treatments (70.1 and 71.1 %, respectively).

Barry et al. (2010) fed healthy adult cats diets containing 4% oligofructose, 4% pectin, or 4% cellulose (as the control) in order to determine their effects on fecal protein catabolites and microbial populations. Fecal scores were greater ($P < 0.01$) in cats fed the 4% oligofructose-supplemented diet (2.8, softer feces) compared to the 4% cellulose diet (2.0), whereas fecal DM percentage and output were not affected. Data from these studies suggest that high inclusion levels alter fecal consistency in cats. Supplementation at the 0.5 - 1% level is recommended for optimal stool quality (Hesta et al., 2001).

During carbohydrate fermentation, fecal pH will be decreased as a result of SCFA and lactic acid production. Cats supplemented with 175 mg/d lactosucrose (0.12% of diet) had numerically lower fecal pH (6.1) compared to no supplementation (6.3) after 14 d of administration (Terada et al., 1993). Fecal pH was lower ($P < 0.01$) for cats fed diets supplemented with 6% (6.0) or 9% (5.7) oligofructose compared to cat fed 0% (6.4) or 3% (6.2) oligofructose (Hesta et al., 2001). However, 3 and 6% inulin (Hesta et al., 2001) or 4% scFOS (Barry et al., 2010) supplementation did not change fecal pH compared to control in cats.

Microbial ecology of the large intestine. The colonic microbiota play an important role in host animal health. *Bifidobacterium* spp. and *Lactobacillus* spp. are desirable bacterial species because of their beneficial effects, including inhibition of pathogenic bacterial (e.g., *Clostridium* spp.) growth and improving host immunity (Gibson and Roberfroid, 1995; Roberfroid, 2007a). Lower gastrointestinal tract pH as a result of lactate production creates an unfavorable environment for several pathogenic bacterial species.

Human studies reported that metabolic end-products resulting from carbohydrate fermentation (lactate and acetate) inhibited growth of gram-positive and gram-negative pathogenic bacteria (Gibson and Wang, 1994). The other benefits of *Bifidobacterium* spp. to the host include B vitamin synthesis, immune modulation, and blood ammonia reduction (Gibson and Roberfroid, 1995).

To evaluate bifidogenic effects in adult cats, Sparkes et al. (1998) studied the effect of 0.75% oligofructose on fecal microflora. As compared to no supplementation, fecal concentrations of *Lactobacillus* spp. (6.3 vs. 5.7 $\log_{10}$ cfu/g fecal DM) and *Bacteroides* spp. (9.5 vs. 8.0 $\log_{10}$ cfu/g fecal DM) were greater ($P < 0.05$). However, fecal concentrations of *Escherichia coli* were lower (6.3 vs. 7.5 $\log_{10}$ cfu/g fecal DM; $P < 0.05$), whereas *Clostridium perfringens* (4.9 vs. 6.6 $\log_{10}$ cfu/g fecal DM) tended to be lower ($P < 0.10$). Terada et al. (1993) reported that adult cats supplemented with 175 mg lactosucrose/d had higher ($P < 0.05$) fecal *Bifidobacterium* spp. and *Lactobacillus* spp. and lower ($P < 0.05$) fecal *Bacteroides* spp. and *Clostridium* spp. populations compared to no supplementation at 7 and 14 d of supplementation. In the study of Barry et al. (2010), fecal concentrations of *Bifidobacterium* spp. were greater (11.6 vs. 10.4 $\log_{10}$ cfu/g fecal DM, $P < 0.01$) in cats fed a 4% oligofructose-supplemented diet compared to a 4% cellulose-supplemented diet, whereas fecal concentrations of *E. coli* were lower (8.4 vs. 9.3 $\log_{10}$ cfu/g fecal DM, $P < 0.01$).

In dogs, the effect of prebiotics on canine microbiota concentrations were studied but with inconsistent results. Swanson et al. (2002a) conducted two experiments testing effects of scFOS and (or) *Lactobacillus acidophilus* supplementation of adult dogs. Dogs were supplemented orally via gelatin capsules with: 1) 2 g sucrose + 80 mg cellulose (as the

control); 2) 2 g FOS + 80 mg cellulose; 3) $1x10^9$ cfu LAC + 80 mg cellulose, or 4) 2 g FOS + $1x10^9$ cfu LAC twice daily. In the first experiment, fecal concentrations of *C. perfringens* tended ($P < 0.10$) to be lower in dogs supplemented with scFOS (9.6 $\log_{10}$ cfu/g fecal DM) compared to the control (9.9 $\log_{10}$ cfu/g fecal DM), whereas fecal concentrations of *Bifidobacterium* and *Lactobacillus* spp. were not affected. In the second experiment, dogs supplemented with scFOS had greater fecal concentrations of *Bifidobacterium* spp. (9.9 vs. 9.4 $\log_{10}$ cfu/g fecal DM; $P < 0.05$) and *Lactobacillus* spp. (9.8 vs. 9.1 $\log_{10}$ cfu/g fecal DM; $P < 0.10$), whereas fecal concentrations of *C. perfringens* were not affected. In another study, Swanson et al. (2002c) supplemented adult dogs twice daily with 2 g scFOS + 1 g MOS or 2 g sucrose (as the control). Dogs fed the scFOS+MOS-supplemented diet had greater ($P < 0.05$) fecal concentrations of *Lactobacillus* spp. in ileal effluent (8.7 vs. 7.6 $\log_{10}$ cfu/g fecal DM) and feces (9.8 vs. 8.3 $\log_{10}$ cfu/g fecal DM) compared to the control. Fecal *Bifidobacterium* spp. concentrations were greater ($P < 0.05$) in dogs supplemented with scFOS+MOS (10.0 $\log_{10}$ cfu/g fecal DM) compared to the control (9.4 $\log_{10}$ cfu/g fecal DM); however, fecal concentrations of *C. perfringens* and *E. coli* were not affected.

Flickinger et al. (2003) fed sixteen adult female hounds a corn-based diet supplemented with 0 or 1.9 g oligofructose/d. Fecal concentrations of *Bifidobacterium* spp. concentrations were not affected by oligofructose supplementation. In a subsequent study, ileal cannulated adult female hounds were fed a meat-based kibble diet supplemented with 0, 1, 2, or 3 g scFOS/d via gelatin capsule. Short-chain fructooligosaccharides linearly increased ($P < 0.05$) fecal populations of total aerobes (8.5, 8.7, 8.9, and 9.3 $\log_{10}$ cfu/g fecal DM, respectively) and linearly decreased ($P < 0.05$) fecal *C. perfringens* (10.0, 10.0, 9.8, and 9.7 $\log_{10}$ cfu/g fecal DM, respectively) compared to the control.

Middelbos et al. (2007) found that dogs fed a 1% cellulose + 1.5% scFOS-supplemented diet had greater ($P < 0.05$) fecal concentrations of *Bifidobacterium* spp. (8.7 vs. 7.7 $\log_{10}$ cfu/g fecal DM) and *Lactobacillus* spp. (12.2 vs. 11.3 $\log_{10}$ cfu/g fecal DM) compared to dogs fed a 2.5% cellulose diet.

Barry et al. (2009) fed dogs low-level additions of scFOS or inulin (0.2 and 0.4% of diet). Colonic microbial populations were not affected by treatments.

Data from these studies support the bifidogenic effect of inulin-type fructans, MOS, and (or) lactosucrose in dogs and cats. However, magnitude of response varies depending on a host of conditions, some of which can be controlled and some of which cannot.

Short-chain fatty acids (SCFA). Bacterial fermentation of carbohydrates produces SCFA and gases, including methane, carbon dioxide, and hydrogen (Macfarlane and Cumming, 1991). The main SCFA are acetate, propionate, and butyrate. Butyrate, a major energy source of colonocytes, plays an important role in gastrointestinal tract health by regulating the balance of cell maturation, cell differentiation, and apoptosis (Blottiére et al., 2003).

Hesta et al. (2001) noted that fecal total SCFA concentrations were greater ($P < 0.05$) in cats fed a 6% inulin-supplemented diet (7632 μmol/d) compared to control-fed cats (4329 μmol/d). Cats fed a diet supplemented with 3% oligofructose had numerically greater total SCFA (5892 μmol/d) compared to cats fed a control (4329 μmol/d) and a 3% inulin-supplemented diet (5116 μmol/d). Barry et al. (2010) noted that cats fed a 4% oligofructose-supplemented diet had greater ($P < 0.01$) fecal butyrate concentrations compared to those fed a 4% cellulose-supplemented diet (97.3 vs. 39.2 μmol/g fecal DM).

In dogs, oral supplementation of 1, 2, or 3 g/d scFOS did not change fecal concentrations of SCFA. In the same study, dogs fed 1.9 g/d hydrolyzed inulin (oligofructose) had greater ($P < 0.05$) fecal concentrations of propionate (31.5 vs. 20.3 mmol/g fecal DM), whereas fecal total SCFA concentrations tended ($P < 0.10$) to be greater (82.7 vs. 63.1 mmol/g fecal DM) compared to the control (Flickinger et al., 2003). Swanson et al. (2002a) noted that a diet supplemented with 4 g scFOS/d increased fecal concentrations of lactate (41.7 vs. 2.7 μmol/g fecal DM; $P < 0.05$), propionate (119.6 vs. 83.6 μmol/g fecal DM; $P < 0.05$), and butyrate (58.2 vs. 40.8 μmol/g fecal DM; $P < 0.10$) compared to the control diet fed to adult dogs. In another experiment, fecal concentrations of butyrate (63.4 vs. 48.2 μmol/g fecal DM; $P < 0.05$) and lactate (70.2 vs. 17.3 μmol/g fecal DM; $P < 0.10$) were greater in dogs fed a scFOS-supplemented diet compared to a control (Swanson et al., 2002a).

Propst et al. (2003) fed adult hounds kibble diets supplemented with 0, 0.3, 0.6, or 0.9% oligofructose and inulin (as-is basis). Compared to the control, fecal concentrations of acetate (348.9, 358.0, and 382.8 vs. 274.6 μmol/g fecal DM), propionate (127.1, 129.1, and 132.1 vs. 92.7 μmol/g fecal DM), butyrate (53.9, 51.2, and 53.3 vs. 39.2 μmol/g fecal DM), and total SCFA (529.9, 538.3, and 568.8 vs. 406.4 μmol/g fecal DM) were increased linearly ($P < 0.05$) in dogs supplemented with oligofructose. In the same study, dogs supplemented with inulin had a linear increase ($P < 0.05$) in fecal concentrations of propionate (110.6, 111.2, and 109.7 vs. 92.7 μmol/g fecal DM) and butyrate (45.5, 46.5, and 48.4 vs. 39.2 μmol/g fecal DM). Middelbos et al. (2007) noted that fecal butyrate concentrations were greater ($P < 0.05$) in dogs fed a 1% cellulose + 1.5% scFOS-supplemented diet (40 μmol/g fecal DM) compared to a 0% cellulose (28 μmol/g fecal DM) and 2.5% cellulose-

supplemented diet (21 μmol/g fecal DM). Barry et al. (2009) noted that a quadratic increase ($P < 0.05$) in fecal butyrate concentrations occurred in dogs fed 0.2 and 0.4% scFOS-supplemented diets (63.3 and 40.2 μmol/g fecal DM) compared to the control (40.1 μmol/g fecal DM). Data presented from these studies provide evidence that low level inclusion of prebiotic can increase fecal SCFA concentrations in dogs and cats.

Fecal odor components. Substances resulting from fermentation by proteolytic bacteria of both endogenous and undigested protein influence fecal malodor. These putrefactive compounds include ammonia, aliphatic amines (e.g., agmatine, cadavarine, histamine, phenylethylamine, putrescine, and tyramine), branched-chain fatty acids (e.g., isobutyrate and isovalerate), phenols (e.g., phenol, p-cresol, and 4-ethylphenol), indoles (e.g., indole, 3-methylindole, 2-methylindole, 2,3-methylindole, 2,5-methylindole, and 5-chloroindole), and volatile sulfur–containing compounds (e.g., dimethyl disulfide, diethyl disulfide, and di-n-butyl disulfide) (Hussein, 1998).

Cats and dogs ingest more protein than the requirement to enhance diet digestibility. This leads to an active population of clostridial species throughout the gastrointestinal tract. Lubbs et al. (2008) noted that *C. perfringens* populations were greater (12.39 vs. 10.83 cfu/g; $P < 0.05$) in cats fed a high protein diet (approximately 50% of diet) compared to a moderate protein diet (approximately 30% of diet). Because *Clostridium* spp. are the one of major microbiota that use amino acids as fermentative substrates (Macfarlane and Cummings, 1991), putrefactive compound concentrations are increased and become a critical concern in dog and cat health. High concentrations of putrefactive compounds may play an important role in causing or exacerbating many types of cancer, including colorectal

cancer (Johnson, 1977). Additionally, ammonia can stimulate and cause tumorogenesis (Lin and Visek, 1991).

In cats, Terada et al. (1993) noted that after 7 and 14 d of 175 mg lactosucrose/d supplementation, fecal concentrations of ammonia (159.7 and 161.5 vs. 338.8 μg/g wet feces), ethylphenol (10.9 and 7.6 vs. 19.6 μg/g wet feces), and indole (17.8 and 29.7 vs. 48.3 μg/g wet feces) were decreased ($P < 0.05$) compared to no supplementation. Hesta et al. (2005) fed adult cats with a reduced protein diet (28.9% DM) supplemented with oligofructose (3.11% DM) or no oligofructose (as the control). In this study, fecal concentrations of volatile sulfur-containing compounds were not different among dietary treatments. However, Barry et al. (2010) noted that cats fed a 4% oligofructose-supplemented diet had greater fecal concentrations of ammonia (0.2 vs. 0.1 μmol/g fecal DM; $P < 0.01$), indole (2.4 vs. 1.4 μmol/g fecal DM; $P = 0.01$), 4-methyl phenol (3.7 vs. 1.6 μmol/g fecal DM; $P < 0.01$), isobutyrate (12.6 vs. 8.2 μmol/g fecal DM; $P < 0.05$), isovalerate (21.0 vs. 13.3 μmol/g fecal DM; $P < 0.05$), valerate (29.8 vs. 22.5 μmol/g fecal DM; $P < 0.05$), total BCFA (63.3 vs. 44.0 μmol/g fecal DM; $P < 0.05$), cadaverine (55.68 vs. 15.26 μmol/g fecal DM; $P < 0.01$), putrescine (13.28 vs. 2.07 μmol/g fecal DM; $P < 0.01$), tryptamine (5.77 vs. 1.17 μmol/g fecal DM; $P < 0.01$), and total amines (76.10 vs. 20.69 μmol/g fecal DM; $P < 0.01$) compared to cats fed a 4% cellulose control diet, whereas tyramine was lower (0.24 vs. 1.38 μmol/g fecal DM; $P < 0.05$) The authors stated that these increases might be the result of rapid fermentation and absorption of the oligosaccharides in the proximal colon, with proteolytic bacteria continuing to ferment amino acids as an energy source to survive in the descending colon, resulting in greater fecal putrefactive compound

production. Also in this study, these carbohydrates were the main dietary fiber sources rather than low level supplements as was the case for most of the previous studies.

Swanson et al. (2002a) noted that 4 g scFOS/d-supplemented dogs had lower ($P < 0.01$) fecal concentrations of isobutyrate (6.0 vs. 7.6 μmol/g fecal DM), isovalerate (9.8 vs. 12.2 μmol/g fecal DM), and total BCFA (16.5 vs. 20.4 μmol/g fecal DM) compared to no supplementation. Swanson and co-workers (2002b) supplemented adult dogs with 0 (no FOS or MOS), 1 g FOS, 1 g MOS, or 1g FOS + 1 g MOS twice daily via gelatin capsule. Dogs supplemented with 2 g/d FOS had lower ($P < 0.05$) fecal concentrations of total phenol and indole (1.50 μmol/g fecal DM) compared to no supplementation (3.03 μmol/g fecal DM). Additionally, dogs supplemented with 1.9 g/d oligofructose tended ($P = 0.06$) to have lower fecal ammonia concentrations (2.0 mg/g fecal DM) compared to no supplementation (4.4 mg/g fecal DM) but not fecal BCFA and biogenic amine concentrations (Flickinger et al., 2003). Barry et al. (2009) noted that dogs fed a scFOS (18.7 and 21.7 vs. 39.5 μmol/g fecal DM)- or inulin (38.15 and 15.7 vs. 39.5 μmol/g fecal DM)-supplemented diet (0.2 and 0.4% of diet) had lower ($P < 0.05$) fecal phenol concentrations, but fecal biogenic amine concentrations were not affected.

Nutrient digestibility. The effects of inulin (3 and 6%, DM basis) and oligofructose (3%, DM basis) on nutrient digestibility by healthy adult cats were studied (Hesta et al., 2001). As compared to no supplementation, total tract crude protein digestibility was decreased ($P < 0.01$) in cats fed 3% (82.8 vs. 87.0%) and 6% (77.3 vs. 87.0%) inulin or 3% (83.1 vs. 87.0%) oligofructose. Total tract fat digestibility was decreased (93.1, 90.1, and 93.2%, respectively, vs. 96.1%; $P < 0.01$) in cats supplemented with either concentration of inulin or oligofructose compared to the control. However, once the total tract crude protein

digestibility was corrected for bacterial nitrogen, no differences among treatments were noted. In contrast, Barry et al. (2010) reported that 4% oligofructose supplementation (88.0%) did not affect total tract crude protein digestibility compared to the 4% cellulose treatment (90.5%) in cats.

In dogs, total tract crude protein (84.9 vs. 86.9%) and total dietary fiber (15.3 vs. 27.3%) digestibility values were lower ($P < 0.05$), but no difference in ileal digestibility was observed in dogs fed a 1.5% scFOS-supplemented diet compared to no supplementation (Middelbos et al., 2007). Additionally, Flickinger et al. (2003) noted that dogs fed a 1.9 g/d oligofructose-supplemented diet had lower total tract OM (73.6 vs. 78.7%; $P < 0.05$), DM (79.2 vs. 83.0 %; $P < 0.05$), lipid (78.3 vs. 86.7 %; $P < 0.01$), and crude protein (78.4 vs. 81.5%; $P < 0.15$) digestibility values compared to no supplementation. The authors suggested that the decrease in nutrient digestibility resulted from faster intestinal transit time due to the presence of fermentable fiber in the diet. The tendency for oligofructose to decrease total tract crude protein digestibility was due to an increase in fecal bacterial biomass. Additionally, oligofructose might form a complex with dietary fat, thus, affecting lipid digestibility. In the same study, Flickinger et al. (2003) noted that ileal digestibility of crude protein (59.4, 61.0, 68.9, and 72.0%) and lipid (92.3, 92.6, 93.9, and 94.7%) tended to be linearly increased ($P < 0.10$) with increasing scFOS concentration (0, 1, 2, and 3 g/d), but no trend was observed in total tract nutrient digestibility. Barry et al. (2009) noted that ileal crude protein digestibility was linearly increased ($P < 0.01$) in dogs fed 0, 0.2, or 0.4 % inulin (78.4, 80.3, and 81.5%, respectively) and scFOS (78.4, 80.4, and 81.1%, respectively). In the same study, total tract crude protein digestibility tended ($P < 0.10$) to be linearly increased in dogs supplemented with 0, 0.2, or 0.4% inulin (88.7, 88.9, and

89.6%, respectively); however, this response was not affected by scFOS supplementation. The authors hypothesized that peptide tyrosine tyrosine (PYY) affected intestinal transit time, thus affecting crude protein digestibility. Short-chain fatty acid production stimulates PYY activity, delaying gastric emptying and increasing intestinal transit time, thereby leading to higher digestibility values. Additionally, glucagon like peptide-1 (GLP-1) has been noted to increase intestinal transit time in response to fermentable substrate (ileal brake effect), resulting in greater digestibility coefficients.

Mineral absorption. Non-digestible oligosaccharides may stimulate apparent mineral absorption (Beynen et al., 2002). It has been hypothesized that production of SCFA and lactate as a result of carbohydrate fermentation in the colon increases mineral solubility and absorption (Beynen et al., 2001). The absorption of Ca, Mg, and Fe by rats was increased by oligofructose supplementation (Otha et al., 1995). In dogs, the effect of lactulose on the apparent absorption of Ca and Mg was studied by Beynen et al. (2001). Six healthy adult dogs were fed a diet containing either 0, 1, or 3 g lactulose/MJ metabolizable energy. A linear increase ($P < 0.05$) in Ca (11.5, 18.7, and 21.1%, respectively) and Mg (23.3, 29.7, and 35.5%, respectively) absorption was noted. Beynen et al. (2002) fed five dogs a dry food with either 1% w/w oligofructose or no supplementation (control). Dogs fed 1% oligofructose had greater ($P < 0.05$) apparent Ca (16.0 vs. 8.6%) and Mg (23.4 vs. 14.0%) absorption compared to the control. However, P absorption was not affected because it is not sensitive to a pH change in the ileal digesta (Beynen et al., 2002). Apparent absorption rates of Ca, P, Mg, Na, and K were not changed with 1g/kg BW (approximately 5%) of transgalactooligosaccharides (TGOS), mannanoligosaccharides (MOS), lactose, or lactulose in adult dogs (Zentek et al., 2002).

Immune function. *Bifidobacterium* spp. can act as immunomodulators (Gibson and Roberfroid, 1995) and may have many effects on immune function including mitogenic activity, promotion of macrophages, stimulation of antibody production, and anti-tumor effects (Bornet and Brouns, 2002). Swanson et al. (2002b) noted that blood lymphocytes (as a percentage of total WBC) were greater ($P < 0.05$) in dogs fed a 2 g/d MOS-supplemented diet (16.8%) compared to no supplementation (15.6%), whereas ileal IgA concentrations were greater ($P < 0.10$) in dogs fed a combination of 2 g FOS/d + 2 g MOS/d (4.9 vs. 3.4 mg/g DM or 12.22 vs. 8.22 mg/g crude protein). The authors suggested that this combination might increase local immunity and enhance protection against pathogens. In another study, sixteen bitches at 35 d of gestation were fed 0 (control) or 1% scFOS (Adogony et al., 2006). In this study, IgM concentrations in milk were consistently increased ($P < 0.01$) during lactation in bitches fed a 1% scFOS-supplemented diet compared to the control, while serum IgM was not increased in blood. Additionally, from whelping to weaning, the same diets were fed ad libitum to the mother and her puppies. At 21 d of age, puppies were intranasally inoculated with a vaccine against *Bordetella brochiseptica*. The concentrations of *B. bronchiseptica*-specific IgM were not significantly different between treatments (280.3 vs. 314.7 ng/ml).

Middelbos et al. (2007) reported that there was no significant difference in white blood cell counts or ileal and blood Ig concentrations in dogs fed 1.5% scFOS or combinations of scFOS + YCW (1.2% + 0.3% or 0.9% + 0.6%) compared to no supplementation or to the 2.5% cellulose negative control treatment. The authors stated that the higher supplement concentrations might be necessary to alter immunological indices. These data indicate that scFOS, YCW (MOS), and their combination may help promote

systemic and local immune responses in dogs. Due to inconsistent results, the proper inclusion level is not currently established. There is no research regarding prebiotic effects on immune status of cats.

Glucose metabolism. Inulin-types fructans may regulate glycemia and insulinemia by delaying gastric emptying, increasing intestinal transit time, and decreasing hepatic gluconeogenenesis due to SCFA production, especially propionate, and/or stimulation of glycolysis (Roberfroid, 2007a). Diez et al. (1997) fed dogs a corn-based diet supplemented with a blend of scFOS and sugar beet fiber (SBF) in a 4:1 ratio at 0, 5, or 10% (DM basis). Postprandial plasma glucose concentrations were lower ($P < 0.01$) in dogs fed the 8% FOS + 2% SBF-supplemented diet compared to no supplementation, whereas preprandial plasma glucose concentrations were not affected. Hesta et al. (2003) fed healthy adult dogs 1) a low fiber control diet; 2) a high fiber diet (HF); and 3) as (1) + 10% isomalto-oligosaccharides. Dogs fed diet 3 had lower postprandial plasma glucose concentrations at 20 ($P < 0.01$), 60 ($P < 0.01$), 90 ($P = 0.05$), 150 ($P < 0.05$), and 360 ($P < 0.10$) min after eating compared to the control. Respondek et al. (2008) noted that the rate of glucose infusion was increased ($P < 0.05$) in obese dogs supplemented with 1% scFOS during the euglycemic hyperinsulinemic clamp, suggesting a greater insulin sensitivity compared to the obese dogs fed the control diet (7.77 vs. 4.72 $mg \cdot kg^{-1} \cdot min^{-1}$). In the study of Verbrugghe et al. (2009), eight non-obese and eight obese cats were randomized and allotted to food (control extruded diet [46% CP, 15% fat, and 27% carbohydrate] or control + 2.5% of a mixture of oligofructose and inulin). Glucose tolerance was not affected by treatment in healthy, normal weight or obese cats. The authors stated that the absence of an effect of inulin and oligofructose on glucose and insulin metabolism in cats might be explained by low carbohydrate concentrations compared

to dogs and to slow fermentation due to the relatively short large intestine and to the high degree of polymerization of the prebiotic tested. Additionally, cats are prone to insulin resistance, especially obese cats; therefore, this unique feature of the carnivore might affect glucose and insulin responses. However, greater propionylcarnitine ($P = 0.03$) and lower methylmalonylcarnitine ($P = 0.07$) and aspartate amino transferase ($P = 0.025$) concentrations were observed in healthy, normal weight or obese cats fed 2.5% of a mixture of inulin and oligofructose, suggesting that they may help modulate glucose metabolism by inhibiting gluconeogenesis and amino acid catabolism. Therefore, feeding 2.5% of a mixture of inulin and oligofructose may be helpful in treating feline insulin resistance and diabetes (Verbrugghe et al., 2009).

Lipid metabolism. Modulation of either the digestion/absorption or the metabolism of lipids may be impacted by inulin-type fructans, thus affecting blood triglyceride and cholesterol concentrations. Inulin-type fructans may affect lipid homeostasis through 1) modification of glucose and/or insulin concentrations; 2) modification of macronutrient absorption by either delaying gastric emptying and (or) decreasing intestinal transit time; 3) increasing propionate, an inhibitor of fatty acid synthesis; 4) increasing concentrations of biogenic amines, especially putrescine; and 5) increasing production of enteroendocrine peptide (Roberfroid, 2007a). Plasma triglyceride concentrations were reduced ($P < 0.05$) with 8% scFOS + 2% beet pulp supplementation during a 6 h postprandial period in non-hyperlipidemic dogs, whereas prepandial triglycerides and cholesterol concentrations were lower ($P < 0.05$) compared to the control (Diez et al., 1997). In the same study, 4% scFOS + 1% beet pulp decreased ($P < 0.05$) preprandial triglyceride concentrations (Diez et al., 1997). However, Respondek et al. (2008) noted that 1% scFOS did not affect plasma

triglyceride and cholesterol concentrations after an overnight fast of obese dogs compared to the control. The authors suggested that the lack of difference might be attributed to the low concentration of scFOS used or to the initial blood cholesterol concentrations.

Nitrogen metabolism. During oligosaccharide fermentation, intestinal bacteria require a N source for protein synthesis to maintain maximal bacterial growth. Consequently, blood urea N may be reduced by increased N diversion to the gastrointestinal tract. This reduction may have benefits for renal patients. Dogs fed a scFOS + beet fiber (4% + 1% or 8% + 2%, DM basis)-supplemented diet had lower ($P < 0.05$) pre- and postprandial plasma urea concentrations compared to control dogs (Diez et al., 1997). Adult dogs fed diets containing 0, 1, or 3 g lactulose/MJ metabolizable energy exhibited linear increases ($P = 0.13$) in fecal N concentrations (0.86, 0.87, and 1.05 g/d), whereas urinary urea excretion was linearly decreased (264.6, 261.4, and 248.2 mmol/d; $P < 0.05$) (Beynen et al., 2001). However, 1% w/w oligofructose supplementation did not affect fecal ammonia and urinary urea concentrations compared to the control (Beynen et al., 2002), perhaps due to low level supplementation.

Fecal N excretion was greater ($P < 0.05$) when cats were fed a 2% inulin-supplemented diet compared to a control, whereas the decrease in urinary N excretion was not observed (Groenveld et al., 2001). This study suggested that N balance was not altered by FOS supplementation. Hesta et al. (2005) found that dogs fed 3.11% oligofructose in a lower protein diet (28.9%, DM basis) increased ($P < 0.10$) fecal N excretion in adult cats compared to no supplementation. Supplementation of oligofructose also tended ($P < 0.10$) to increase fecal ^{15}N excretion, and tended ($P < 0.10$) to decrease urinary ^{15}N excretion compared to no supplementation. The lower urinary N excretion might be explained by 1)

lower ammonia absorption due to lower luminal pH; 2) excess N used for bacterial protein synthesis; and/or 3) increased urea transport to the gut.

Prebiotic mixtures

Prebiotic mixtures, primarily inulin-type fructans combined with GOS, have been investigated in humans. A galactooligosaccharide and inulin-type fructan (i.e., inulin HP) mixture (9:1) has been studied with the intent of mimicking, in part, the oligosaccharide composition found in human breast milk (Boehm and Moro, 2008). Studies in pre-term and term infants have shown that a formula supplemented with this GOS/inulin HP prebiotic mixture results in an intestinal microbiota similar to that found in breast-fed infants. Kelly (2009) noted that a GOS/inulin HP combination contributes to: 1) a reduction in the incidence of atopic disease; 2) a reduction in recurrent wheezing and allergic urticaria in infants from parents with a history of atopic disease; 3) a decrease in episodes of upper respiratory tract infections, fever, and antibiotic prescriptions; 4) a decrease in the incidence of acute diarrhea; 5) improved fecal consistency and stool frequency; 6) accelerated gastro-intestinal transit time; 7) increased fecal secretory IgA; and 8) decreased bilirubin concentrations during the first 72 h of life in formula-fed infants.

There are few studies testing prebiotic mixtures in dogs. Swanson et al. (2002b) noted that a combination of 2 g scFOS/d + 2 g MOS/d increased ($P < 0.10$) ileal IgA concentrations and decreased ($P < 0.05$) fecal total indole and phenol concentrations compared to a prebiotic-free diet. Swanson et al. (2002c) reported that 4 g scFOS/d + 2 g MOS/d tended ($P < 0.10$) to increase plasma lymphocytes (19.95%) compared to the control (17.29%), whereas ileal IgA, plasma IgA, IgG, and IgM, and fecal IgA concentrations were not different among treatments. Therefore, dietary supplementation with scFOS+MOS may

have beneficial effects on colon health and immunity in dogs. Middelbos et al. (2007) reported that a combination of 1.2% scFOS and 0.3% MOS in the diet increased ($P < 0.05$) fecal butyrate concentrations (41 vs. 21 μmol/g fecal DM) and fecal populations of *Bifidobacterium* spp. (9.1 vs. 7.8 cfu/g fecal DM), whereas total tract crude protein digestibility was decreased (84.7 vs. 86.7%; $P < 0.05$) compared to the oligosaccharide-free diet. Dogs fed a combination of 0.9% scFOS and 0.6% MOS had greater ($P < 0.05$) fecal butyrate concentrations (42 vs. 21 μmol/g fecal DM) and fecal populations of *Bifidobacterium* spp. (8.7 vs. 7.8 cfu/g fecal DM) and *Lactobacillus* spp. (12.1 vs. 11.2 cfu/g fecal DM), whereas total tract crude protein digestibility was lower (84.8 vs. 86.7%; $P < 0.05$) compared to the oligosaccharide-free treatment. A combination of FOS and MOS may provide health benefits, including microbial modification, decreased putrefactive compound production, and (or) improved systemic and local immunity.

Conclusion

Data presented in this literature review indicate that prebiotic supplementation has beneficial outcomes in dogs and cats. The strongest beneficial effect is improvement in indices of gastrointestinal tract heath. Studies showed increased fecal concentrations of beneficial bacteria (*Bifidobacterium* spp. and (or) *Lactobacillus* spp.) and SCFA (i.e., butyrate and (or) acetate), whereas fecal protein catabolite concentrations were almost always decreased. Prebiotics also appear to modulate both local and systemic immunological indices in dogs, but with inconsistent results; however, no such study has been conducted in cats. Low level prebiotic inclusion appears to improve nutrient digestibility and mineral absorption in dogs. Only two studies evaluated the effects of fructans on nutrient digestibility in cats, and one study noted a decrease in total tract crude

protein and fat digestibility values when cats were supplemented with moderate to high concentrations of fructans. Due to inconsistent results and limited studies, weak evidence for immunological and nutrient digestibility responses exist in cats. The weakest data regarding prebiotic supplementation is that dealing with regulation of glucose, lipid, and N metabolism in dogs and cats, because these effects were evaluated only in normal dogs and cats as compared to those that were health-compromised.

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CHAPTER 3

EFFECTS OF SHORT-CHAIN FRUCTOOLIGOSACCHARIDES AND GALACTOOLIGOSACCHARIDES, INDIVIDUALLY AND IN COMBINATION, ON NUTRIENT DIGESTIBILITY, FECAL FERMENTATIVE METABOLITE CONCENTRATIONS, AND LARGE BOWEL MICROBIAL ECOLOGY OF HEALTHY ADULT CATS

Abstract

Short-chain fructooligosaccharides (scFOS) and galactooligosaccharides (GOS) are non-digestible oligosaccharides that result in a prebiotic effect in some animal species; however, the cat has not been well studied in this regard. This experiment evaluated scFOS and GOS supplementation on nutrient digestibility, fermentative end-product production, fecal microbial ecology. Eight healthy adult cats were fed diets containing no prebiotic, 0.5% scFOS, 0.5% GOS, or 0.5% scFOS + 0.5% GOS (scFOS+GOS) in a replicated 4x4 Latin square design. Apparent total tract crude protein digestibility was decreased ($P < 0.05$) when cats were fed a diet containing scFOS + GOS compared to the other treatments. Dry matter, OM, acid hydrolyzed fat, and GE digestibilities were not different among treatments. Cats fed scFOS-, GOS-, and scFOS+GOS-supplemented diets had greater ($P < 0.05$) fecal *Bifidobacterium* spp. populations compared to cats fed the control diet. Fecal pH was lower ($P < 0.05$) for cats fed the scFOS+GOS-supplemented diet compared to the control. Butyrate ($P = 0.05$) and valerate ($P < 0.05$) concentrations were higher when cats consumed the scFOS+GOS diet. Acetate tended to be greater ($P = 0.10$) when cats were fed the scFOS+GOS diet. Total SCFA ($P = 0.06$) and total BCFA ($P = 0.06$) concentrations also tended to be greater when cats consumed the scFOS+GOS treatment.

Fecal protein catabolites, including ammonia, 4-methylphenol, indole, and biogenic amines, did not differ among treatments, nor did blood lymphocytes, neutrophils, or total white blood cell counts, or fecal DM concentration and output. Low level supplementation of scFOS, GOS, and their combination exert positive effects on select indices of gut health in cats.

Introduction

Prebiotics are non-digestible food ingredients that modify the microbial ecology of the colon and improve indices of host health (Gibson and Roberfroid, 1995). Many non-digestible oligosaccharides (NDO) are resistant to enzymatic hydrolysis in the gastrointestinal tract, are fermented by the intestinal microflora, and selectively stimulate the growth and/or activity of one or more intestinal bacteria. Galactooligosaccharides are a mixture of oligomers synthesized from lactose and consist of 2 - 8 saccharide units. Tzotzis and Vulevic (2009) noted that GOS meets all of the criteria of a true prebiotic. Short-chain fructooligosaccharides, a mixture of short-chain glucose and fructose monomers, have been widely studied in humans (Roberfroid, 2007) and dogs (Flickinger et al., 2003; Middelbos et al., 2007a; Barry et al., 2009); however, few studies have been done in cats to date (Sparkes et al., 1998; Hesta et al., 2001; Barry et al., 2010).

The colonic microbiota can play an important role in host animal health. *Bifidobacterium* spp. and *Lactobacillus* spp. are desirable bacterial species because of their beneficial effects, including inhibition of pathogenic bacteria (e.g., *Clostridium* spp.) and improving host immunity. Cats supplemented with 175 mg lactosucrose/d (Terada et al., 1993), 0.75% oligofructose (Sparkes et al., 1998), or 4% oligofructose (Barry et al., 2010) had modified colonic microbial populations compared to oligosaccharide-free controls.

In the canine, low level inclusion of scFOS or inulin (0.2 and 0.4% of diet) increased ileal nutrient digestibility with no effect on fecal quality or microbial ecology of the colon, whereas dogs fed an inulin-supplemented diet tended to have increased total tract crude protein digestibility (Barry et al., 2009). The moderate to high inclusion levels of inulin-type fructans were noted to lower total tract crude protein digestibility by dogs (Middelbos et al., 2007a) and cats (Hesta et al., 2001). This reduction may have resulted from the higher fecal bacterial mass produced in response to oligosaccharide supplementation.

Cats nearly always are provided with more protein than is required to meet amino acid requirements. This leads to an active population of clostridial species throughout the gastrointestinal tract of cats. Clostridia are the major microbial species that use amino acids as fermentative substrates. Consequently, several putrefactive compounds including ammonia, biogenic amines, branched-chain fatty acids (BCFA), indole, phenol, and sulfur-containing compounds, are produced. The large quantities of putrefactive compounds may play an important role in causing disease of the large bowel, including colorectal cancer (Johnson, 1977). The objective of this study was to determine the effects of low level prebiotic inclusion (0.5% scFOS, 0.5% GOS, and 0.5% scFOS + 0.5% GOS) on nutrient digestibility, fermentative metabolite concentrations, and large bowel microbial ecology of healthy adult cats.

Materials and Methods

Animals and diets

All animal care procedures were approved by the University of Illinois Institutional Animal Care and Use Committee before initiation of the experiment. Eight domestic shorthair male cats 2.8 yr of age (4.94 ± 0.3 kg BW) at the start of the study were utilized.

All cats were individually housed in stainless steel cages in a temperature controlled room with a 16 h light: 8 h dark cycle. Food offered and refused was weighed daily to assess food intake. Body weight and body condition score were determined weekly.

Extruded kibble diets were formulated using oligosaccharide-free ingredients, including poultry by-product meal, brewers rice, and poultry fat (Table 1). The diets were extruded at Kansas State University's Bioprocessing and Industrial Value-Added Program Facility (Manhattan, KS) under the direction of Pet Food and Ingredient Technology, Inc. (Topeka, KS). Short-chain fructooligosaccharides (NutraFlora® P-95 short-chain fructooligosaccharides, GTC Nutrition, Golden, CO 80401) and GOS (Purimune™ galactooligosaccharides, GTC Nutrition, Golden, CO 80401) supplementation were included in the diet prior to extrusion in exchange for cellulose. Four diets were prepared:

1) Control (30% protein, 20% fat, 4% solka floc as the fiber source)

2) Control + 0.5% scFOS + 3.5% solka floc

3) Control + 0.5% GOS + 3.5% solka floc

4) Control + 0.5% scFOS + 0.5% GOS + 3% solka floc

Experimental design

A replicated 4x4 Latin square with 21-d periods was used. Cats were adapted to the diet for 14-d, followed by a 7-d total fecal collection phase. Total feces were collected, scored, weighed, and frozen at -20°C until further analysis. During the 7-d collection phase, one fresh fecal sample was collected within 15 min of defecation for measurement of pH, DM, protein catabolites, and microbiota enumeration. All fecal samples were scored for consistency based on the following scale: 1 = hard, dry pellets, small hard mass; 2 = hard, formed stool that remains firm; 3 = soft, formed, and moist stool that retains its shape;

4 = soft, unformed stool that assumes the shape of the container; and 5 = watery, liquid stool that can be poured.

Sample handling

Fecal samples were dried at 55°C in a forced-air oven. Diets and dried feces were ground through a 2 mm screen in a Wiley mill (Model#4, Thomas Scientific, Swedesboro, NJ). On d 15 of each period, one fresh fecal sample was collected during the 7-d collection phase within 15 min of defecation and an aliquot was immediately transferred into sterile cryogenic vials (Nalgene, Rochester, NY), snap-frozen in liquid nitrogen, and frozen at -80°C until DNA extraction. Additional aliquots for phenol, indole, and biogenic amine analyses were frozen at -20°C immediately following collection. Additional aliquots for ammonia, short-chain fatty acid, and branched-chain fatty acid determinations were put in 5 ml of 2 *N* hydrochloric acid and stored at -20°C. Remaining fecal samples were frozen at -20°C.

Chemical analyses

Diets and feces were analyzed for DM, organic matter (OM), and ash (AOAC, 2000). Crude protein was determined according to AOAC (2000) using a Leco Nitrogen/Protein Determinator (model FP-2000, Leco Corporation, St. Joseph, MI). Fat concentration was determined by acid hydrolysis (AACC, 1983) followed by ether extraction (Budde, 1952). Total dietary fiber was determined according to Prosky et al. (1984, 1992). Gross energy was analyzed by use of an oxygen bomb calorimeter (Model 1261, Parr Instrument, Moline, IL). Short-chain and branched-chain fatty acid concentrations were determined by gas chromatography (Erwin et al., 1961) using a Hewlett-Packard 5890A series II gas chromatograph (Palo Alto, CA) and a glass column

(180 cm x 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier with a flow rate of 75 mL/min. Oven, detector, and injector temperatures were 125, 175, and 180°C, respectively. Fecal ammonia concentrations were determined using spectrophotometry according to methods of Chaney and Marbach (1962). Phenol and indole concentrations were measured using gas chromatography according to methods described by Flickinger et al. (2003). Biogenic amine concentrations were measured by HPLC according to the methods described by Flickinger et al. (2003).

Microbial analyses

Fecal microbial populations were analyzed using the method described by Middelbos et al. (2007b). Briefly, total DNA was extracted from fresh fecal samples that had been stored at –80°C using the bead beater method (Yu and Morrison, 2004) followed by a QIAamp DNA stool mini kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Quantity and quality of DNA was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Quantitative PCR was performed for determination of *Bifidobacterium* spp., *Lactobacillus* spp., *Escherichia coli*, and *Clostridium perfringens*. Specific primers, previously used in our laboratory, were used for *Bifidobacterium* spp. (Matsuki et al., 2002), *Lactobacillus* spp. (Collier et al., 2003), *E. coli* (Malinen et al., 2003), and *C. perfrin*gens (Wang et al., 1994). Briefly, a 10-μL final volume contained 5 μL of 2 x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 15 pmol of the forward and reverse primers for the bacterium of interest, and 10 ng of extracted fecal DNA. Standard curves were obtained by harvesting pure cultures of the bacterium of interest in the log growth phase in triplicate, followed by serial dilution.

Bacterial DNA was extracted from each dilution using a QIAamp DNA stool mini-kit and amplified with the fecal DNA to create triplicate standard curves using an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). Colony-forming units in each dilution were determined by plating on specific agars; lactobacilli MRS (Difco) for lactobacilli, reinforced clostridial medium (*Bifidobacterium* spp., *C. perfringens*), and Luria Bertani medium (*E. coli*). The calculated log cfu/ml of each serial dilution was plotted against the cycle threshold to create a linear equation to calculate cfu/g dry feces.

Complete blood count

A complete blood count (CBC) was determined for each cat on d 21 of each period. Approximately 2 ml of blood was taken from the femoral vein and placed into 2.5 ml EDTA tubes for CBC analysis at the University of Illinois College of Veterinary Medicine Veterinary Diagnostic Lab.

Calculations

Apparent total tract nutrient digestibilities were calculated as nutrient intake (g/d) minus fecal nutrient output (g/d); this value then was divided by nutrient intake (g/d) and multiplied by 100%. Metabolizable energy (kcal/g) was calculated on a DM basis using the following equation (AAFCO, 2009): [(14.64 x % crude protein) + (35.56 x % acid hydrolyzed fat) + (14.6% carbohydrate)]/100, where carbohydrate is equal to 100 – (% ash) – (% crude protein) – (% acid hydrolyzed fat) – (% total dietary fiber).

Statistical analysis

The continuous variable data were analyzed by the MIXED procedure (SAS Inst., Cary, NC). For the statistical model, the random effects were animal and period, whereas the fixed effect was treatment. Least squares means were separated using least squares

differences with a Tukey adjustment. A single degree of freedom contrast was conducted to test the effect of supplementation treatments (average of three prebiotic supplemented diets) compared with the control treatment. Outlier data were removed from analysis after analyzing data using the UNIVARIATE procedure to produce a normal probability plot based on residual data and visual inspection of the raw data. Outlier data were defined as data points three or more standard deviations from the mean. Differences among treatment level least squares means with a probability of $P \leq 0.05$ were accepted as statistically significant, whereas mean differences with P-values ranging from 0.06 to 0.10 were accepted as trends.

Results

Chemical composition of diets

The chemical composition of the diets is presented in Table 2. Analyzed DM, OM, and GE concentrations, and calculated ME values, were similar among dietary treatments. Analyzed crude protein and acid hydrolyzed fat concentrations were close to the desired 30 and 20% values, respectively (as-is basis). The TDF assay cannot quantify scFOS and GOS because these oligosaccharides do not precipitate in 78% ethanol. Therefore, the TDF values for scFOS-, GOS-, and scFOS+GOS-supplemented diets were lower than for the control diet. Corrected TDF values (uncorrected TDF values + amount of oligosaccharide added to each treatment) were similar among diets (Table 2).

Food intake and apparent total tract nutrient digestibility

Food (as-is) and DM intake, fecal output and DM concentration, and apparent total tract nutrient digestibilities are presented in Table 3. Food intake, DM intake, fecal output and fecal DM concentration were not different among treatments; however, fecal output

tended (P = 0.08) to be greater for cats fed the scFOS+GOS treatment compared to the GOS treatment. Dry matter, OM, acid hydrolyzed fat, and GE digestibilities were similar among treatments. Crude protein digestibility was decreased (P < 0.05) when cats consumed the scFOS+GOS treatment compared to those fed the other treatments. Total dietary fiber digestibility is not reported because of the problem mentioned above with oligosaccharide quantification.

Fecal characteristics and fermentation metabolites

Fecal pH, score, and concentrations of ammonia, 4-methylphenol, and indole are presented in Table 4. Fecal score was not different among treatments. Fecal pH was decreased (P < 0.05) when cats were fed the scFOS+GOS treatment compared to the control and GOS treatments. Fecal pH was decreased (P = 0.05) when scFOS and (or) GOS were supplemented compared to the control. Fecal concentrations of ammonia, 4-methylphenol, and indole were not different among treatments.

Fecal SCFA and BCFA concentrations are presented in Table 5. Fecal concentrations of acetate were greater when cats consumed the scFOS+GOS treatment compared to the GOS (P = 0.05) and control (P = 0.10) treatments. Fecal concentrations of butyrate were greater (P = 0.05) for cats consuming the scFOS+GOS treatment compared to the control treatment. Fecal butyrate concentrations tended (P = 0.10) to be greater when cats were fed the scFOS and (or) GOS treatments compared to the control. Fecal concentrations of total SCFA tended (P = 0.06) to be greater when cats consumed the scFOS+GOS treatment compared to control and GOS treatments. Fecal concentrations of valerate were greater (P < 0.05) for cats fed the scFOS+GOS treatment compared to the control and scFOS treatments. Fecal valerate concentrations tended (P = 0.08) to be greater

when cats were fed the scFOS and (or) GOS treatments compared to the control. Fecal concentrations of total BCFA tended (P = 0.06) to be greater when cats were fed the scFOS+GOS treatment compared to the control. Fecal propionate, isobutyrate, and isovalerate concentrations were not different among treatments.

Fecal biogenic amine concentrations are presented in Table 6. Most fecal biogenic amine concentrations were not different among treatments; however, fecal tyramine concentration tended (P = 0.07) to be lower when cats consumed the scFOS+GOS treatment compared to the control treatment. Fecal tyramine concentrations tended (P = 0.06) to be lower when cats were fed the scFOS and (or) GOS treatments compared to the control.

Fecal microbiota

Fecal concentrations of select microbiota are presented in Table 7. Fecal *Bifidobacterium* spp. concentrations were highest (P < 0.05) in the scFOS+GOS treatment. Fecal concentrations of *Bifidobacterium* spp. were greater (P < 0.05) when cats were fed the scFOS and (or) GOS treatments compared to the control. Fecal concentrations of *Lactobacillus* spp., *E. coli, and Clostridium perfringens* were not different among treatments.

Complete blood count

White blood cell counts are presented in Table 8. There were no significant differences in neutrophils or total white blood cells concentrations among treatments, whereas blood lymphocyte concentrations (expressed as a percentage) tended (P = 0.10) to be lower for cats fed the scFOS treatment compared to those fed the control. However, the concentrations (expressed as a percentage) of lymphocytes were lower (P < 0.05) when

scFOS and (or) GOS were supplemented to cats compared to the control, whereas neutrophil concentrations were greater ($P = 0.05$).

Discussion

A diet was formulated that was free of endogenous oligosaccharides, but was of high nutritive value. Solka floc was used as the control fiber as it is essentially inert and not subject to microbial fermentation. Test oligosaccharides were added to the diet at the 0.5% level at the expense of cellulose. A mixture of the two also was tested at the 0.5% level for each, resulting in an experimental treatment containing 1% oligosaccharide. This was done to test the potential synergies between the two oligosaccharides at the same concentrations as when fed individually, and to evaluate whether a higher dietary concentration might exert a stronger response on the outcome variables measured.

Dietary composition was similar among diets except for TDF. As expected, the differences in TDF were observed because the scFOS and GOS (alone and in combination) do not precipitate in 78% ethanol; therefore, they are unable to be quantified accurately using the TDF method. However, when TDF values were corrected for supplemental test prebiotics, values were similar among treatments.

Dry matter, OM, acid hydrolyzed fat, and GE apparent total tract digestibility values were not different among treatments; however, crude protein digestibility decreased ($P < 0.05$) with 1% scFOS+GOS supplementation, likely due to the production of greater bacterial biomass in the large bowel. This occurred only at the 1% supplementation level, indicating an effect of oligosaccharide concentration and not of individual oligosaccharides supplemented at lower dietary concentrations. This was supported by the increased fecal output data noted for the combination treatment.

Hesta et al. (2001) reported that cats fed inulin (3 and 6%)- or 3% oligofructose-supplemented diets had lower total tract crude protein digestibility values. However, after correcting the value for fecal bacterial nitrogen content, crude protein digestibility was not different among treatments. Barry et al. (2009) reported that total tract crude protein digestibility tended ($P < 0.10$) to be linearly increased for dogs supplemented with 0.2% or 0.4% inulin; however, values were not affected by 0.2 or 0.4% scFOS supplementation.

As expected, fecal DM content and score were not affected by dietary treatments. Barry et al. (2010) found that fecal DM was not affected in cats fed a 4% oligofructose-supplemented diet. In dogs, Barry et al. (2009) reported that low level inclusion (0.2 and 0.4%) of scFOS or inulin did not affect fecal quality. In the present experiment, fecal output tended to be greater in cats fed the scFOS+GOS-supplemented diet compared to the GOS diet. Hesta et al. (2005) noted that cats supplemented with 3.11% oligofructose tended to have a greater fecal moisture and output compared to the control.

Fecal pH was lower when cats were fed the scFOS+GOS treatment compared to the control and GOS treatments. Lower fecal pH likely resulted from lactic acid and SCFA production from carbohydrate fermentation. Hesta et al. (2001) reported that cats fed a 6 or 9% oligofructose-supplemented diet had lower fecal pH compared to the control and 3% oligofructose treatments.

Fecal ammonia concentrations were not different among treatments. Similar results were observed by Flickinger et al. (2003) when dogs were supplemented with scFOS at 0.5, 1.5, or 3% dietary concentrations, and by Barry et al. (2009) when dogs were supplemented with inulin or scFOS (0.2 and 0.4%).

Phenolic and indolic compounds are produced from aromatic amino acid (phenylalanine, tyrosine, and tryptophan) fermentation (Blachier et al., 2007). In the present study, only 4-methylphenol and indole were present in all fecal samples. The dietary treatments did not affect fecal concentration of phenol or indole. Flickinger et al. (2003) reported that phenol and indole were not affected by 1, 2, or 3 g/d scFOS supplementation in dogs. In contrast, Terada et al. (1993) noted that fecal ethylphenol and indole concentrations were significantly decreased in cats supplemented a 175 mg lactosucrose/d. Potentially pathogenic bacteria, including *Clostridium perfringens* and *E. coli*, are responsible for the production of putrefactive compounds. In the present study, fecal populations of *C. perfringens* and *E. coli* were not affected by oligosaccharide inclusion in diets. This might explain, in part, why fecal concentrations of phenol and indole were unaffected by dietary oligosaccharide inclusion.

Fecal acetate concentrations were greater when cats were fed the scFOS+GOS compared to the GOS ($P < 0.05$) and control ($P = 0.10$) treatments. Propst et al. (2003) reported that fecal acetate concentrations were increased when dogs consumed an oligofructose-supplemented diet (0.3, 0.6, and 0.9%, DM basis). Fecal lactate and acetate production have been shown to decrease pH, leading to development of an unfavorable environment for pathogenic bacteria (i.e., *C. perfringens* and *E. coli*) (Macfarlane and Cumming, 1991). Fecal butyrate concentrations were greater when cats consumed the scFOS+GOS treatment compared to the control. Similarly, 4% oligofructose supplementation increased fecal butyrate concentrations in adult cats (Barry et al., 2010). Additionally, Hesta et al. (2001) noted that fecal total SCFA concentrations were greater in cats fed a 6% inulin-supplemented diet compared to control cats. Butyrate serves as an

energy source for colonocytes, and high butyrate concentrations are thought to play an important role in gut health and colonocyte proliferation (Blottiére et al ., 2003). In the current experiment, scFOS or GOS alone did not affect fecal acetate, propionate, butyrate, or total SCFA concentrations. This lack of difference noted for GOS and scFOS alone appears to be a function of the dietary concentration provided. However, fecal butyrate concentrations tended to be greater when cats were fed the scFOS and (or) GOS treatments compared to the control. It appears from the data of the present study that if butyrate production is to be enhanced, a concentration between 0.5 and 1% is necessary to elicit this response.

When carbohydrate as a substrate for the microbiota of the large intestine is limiting, BCFA are produced. Short-chain fructooligosaccharides and GOS are rapidly fermented in the proximal colon, then peptides and amino acids are fermented by bacteria in the transverse and distal colon to provide energy. End-products of amino acid fermentation are BCFA, phenol, indole, and biogenic amines. Branched-chain fatty acids are generated from branched-chain amino acids (valine, leucine, and isoleucine) fermentation (Macfarlane et al., 1992). In the present experiment, the combination treatment increased fecal concentrations of valerate ($P < 0.05$) and total BCFA ($P = 0.06$). Similarly, Barry et al. (2010) noted that cats fed 4% oligofructose-supplemented diet had greater fecal concentrations of isobutyrate, isovalerate, valerate, and total BCFA compared to cats fed control. The authors stated that these increases might have resulted from the rapid fermentation and absorption of the oligosaccharides in the proximal colon; then, proteolytic bacteria in the distal colon would continue fermenting amino acids as energy sources instead of carbohydrates.

Polyamines including putrescine, spermidine, and spermine, are beneficial protein catabolites required for normal development and repair of intestinal mucosal cells (Wang and Johnson, 1990). Löser et al. (1999) noted that rats fed polyamine-deficient diets long-term had significant hypoplasia of small intestinal and colonic mucosa. Therefore, a decrease in fecal polyamine concentrations may be not desirable. In the current study, 0.5% scFOS or GOS did not affect fecal biogenic amine concentrations except for tyramine that tended ($P = 0.10$) to be lower in cats fed 1% scFOS+GOS compared to control. Also, Barry et al. (2010) noted the fecal tyramine concentrations were significant lower in cats that consumed a 4% oligofructose-supplemented diet compared to the control. Flickinger et al. (2003) noted that fecal concentrations of biogenic amines (agmatine, ethylamine, putrescine, spermidine, tryptamine, and total amines) were not different when dogs were supplemented with 0, 1, 2, or 3 g scFOS/d.

As expected, scFOS- and GOS-supplemented diets affected fecal microbial *Bifidobacterium* spp. concentrations. One important criterion to prove oligosaccharide efficacy as a prebiotic is selective fermentation by intestinal microbiota increasing beneficial bacterial concentrations, including *Bifidobacterium* spp. and *Lactobacillus* spp. (Roberfroid, 2007). While bifidobacteria populations were increased ($P < 0.05$), fecal *Lactobacillus* spp., *E. coli*, and *Clostridium perfringens* were not affected by dietary treatment. Terada et al. (1993) reported that adult cats supplemented with 175 mg lactosucrose/d had greater fecal *Bifidobacterium* spp. and *Lactobacillus* spp. and lower fecal *Clostridium* spp. Supplementation with 4% oligofructose significantly increased fecal *Bifidobacterium* spp. in adult cats compared to cellulose (Barry et al., 2010). Additionally, dogs fed 4 g FOS + 2 g MOS (Swanson et al., 2002b) or 1.5% scFOS (Middelbos et al., 2007a) had greater fecal

concentrations of *Bifidobacterium* spp. and *Lactobacillus* spp. compared to an unsupplemented control. However, low level inclusion of scFOS (0.2 and 0.4%) or inulin (0.2 and 0.4%) did not change canine gut microbial populations (Barry et al., 2009).

White blood cell, neutrophil, and lymphocyte concentrations were generally unaffected by dietary treatment. However, blood lymphocyte concentrations (expressed as a percentage) were lower ($P = 0.05$) in cats fed the scFOS and (or) GOS treatments compared to the control, whereas neutrophil concentrations (expressed as a percentage) were greater ($P < 0.05$). Values remained within the normal physiological range for cats. Previous studies with scFOS, yeast cell wall, or inulin reported inconsistent effects on immunological indices among studies, depending on type and concentration of prebiotic (Swanson et al., 2002a, 2002b; Middelbos et al., 2007a; Barry et al., 2009). Barry et al. (2009) noted that low level inclusion (0.2 and 0.4%) of scFOS and inulin did not affect ileal IgA compared to control. Additionally, Middelbos et al. (2007a) reported that there was no significant difference in white blood cell counts, ileal IgA, and serum IgA, IgG, and IgM concentrations in dogs fed 1.5% scFOS or combinations of scFOS + yeast cell wall (1.2% + 0.3% or 0.9% + 0.6%, respectively) compared to a 2.5% cellulose control. The authors stated that greater concentrations of oligosaccharide supplementation might be necessary to alter immunological indices.

Data from this experiment showed positive outcomes of scFOS and GOS on nutritional- and health-related characteristics of cats when fed alone or in combination. The effects for the scFOS+GOS treatment likely resulted from the higher concentration provided rather than from any synergy that might exist between them. These oligosaccharides could

serve as valuable nutritional interventions to improve digestive health of cats, but it is apparent that concentrations > 0.5% should be used to elicit positive responses.

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Table 1. Ingredient composition of diets containing select carbohydrates and fed to adult cats (as-fed basis).

	Treatment			
Ingredient, %	Control	scFOS[1]	GOS[2]	scFOS + GOS
Poultry by-product, low ash	36.48	36.48	36.48	36.48
Brewer's rice	27.76	27.76	27.76	27.76
Poultry fat	12.00	12.00	12.00	12.00
Yellow corn, ground	10.00	10.00	10.00	10.00
Dried egg	5.00	5.00	5.00	5.00
Solka floc[a]	4.00	3.50	3.50	3.00
Test carbohydrate[b]	0.00	0.50	0.50	1.00
Liquid digest	2.00	2.00	2.00	2.00
Sodium bisulfate	1.00	1.00	1.00	1.00
Potassium chloride	0.65	0.65	0.65	0.65
Salt	0.65	0.65	0.65	0.65
Mineral premix[c]	0.18	0.18	0.18	0.18
Vitamin premix[d]	0.18	0.18	0.18	0.18
Taurine supplement[e]	0.10	0.10	0.10	0.10

[1] scFOS: short-chain fructooligosaccharides.

[2] GOS: galactooligosaccharides.

[a] Solka floc: International Fiber Corporation, North Tonawanda, NY 14120.

[b] Test carbohydrate: scFOS, NutraFlora® P-95 short-chain fructooligosaccharides, GTC Nutrition, Golden, CO 80401; GOS, Purimune™ galactooligosaccharides, GTC Nutrition, Golden, CO 80401.

[c] Provided per kilogram of diet: vitamin A, 18 kIU; vitamin D_3, 2.7 kIU; vitamin E , 0.144 kIU; vitamin K, 2.16 mg; thiamin, 30.6 mg; riboflavin, 30.6 mg; pantothenic acid, 50.4 mg; nicotinic acid, 124.2 mg; pyridoxine, 30.6 mg; biotin, 0.108 mg; folic acid, 1.08 mg; vitamin B_{12}, 115 μg.

[d] Provided per kilogram of diet: manganese (as $MnCO_3$), 18.0 mg; iron (as $C_6H_8O_7$.xFe), 135.0 mg; copper (as $Cu_2(OH)_2CO_3$), 18.0 mg; zinc (as $ZnCO_3$), 180.0 mg; iodine (as KIO_3), 1.8 mg; selenium (as Na_2SeO_3), 396.0 μg; cobalt (as $CoSO_4$), 3.8 μg.

[e] Provided per kilogram of diet: taurine, 2.1 g.

Table 2. Chemical composition of extruded cat diets containing select carbohydrates.

	Treatment			
Item	Control	scFOS[1]	GOS[2]	scFOS+GOS
Dry matter, %	95.2	95.0	94.8	95.0
	----------% DM basis----------			
Organic matter	92.8	92.5	92.4	92.7
Crude protein	34.6	35.1	35.3	34.0
Acid hydrolyzed fat	21.2	20.2	20.2	21.1
Total dietary fiber – uncorrected[a]	8.2	7.6	7.8	7.3
Total dietary fiber – corrected[b]	8.2	8.1	8.3	8.3
Gross energy, kcal/g	5.4	5.4	5.5	5.4
ME[c], kcal/g (calculated)	4.0	4.0	4.0	4.0

[1]scFOS: short-chain fructooligosaccharides.

[2] GOS: galactooligosaccharides.

[a] These values were determined using the TDF assay that cannot quantify scFOS and GOS.

[b] These values were determined by adding the amount of scFOS and GOS present in each diet to the TDF (uncorrected) value.

[c] Metabolizable energy was calculated using the following equation: [(14.64 x % crude protein) + (35.56 x % acid hydrolyzed fat) + (14.6% carbohydrate)]/100, where carbohydrate is equal to 100 – (% ash) – (% crude protein) – (% acid hydrolyzed fat) – (% total dietary fiber) when all values are expressed on a DM basis (AAFCO, 2009).

Table 3. Food intake, dry matter intake, fecal output, fecal dry matter concentration, and nutrient digestibilities for cats supplemented with short-chain fructooligosaccharides (scFOS) and (or) galactooligosaccharides (GOS).

	Treatment					P-value	
Item	Control	scFOS	GOS	scFOS+GOS	SEM	Main effects	Control vs. supplementation
Food intake, g/d (as-is)	58.7	58.3	57.6	59.3	0.59	0.16	0.63
Dry matter intake, g/d	55.9	55.4	54.7	56.3	0.57	0.11	0.49
Fecal output, g (as-is)	173.1 [cd]	168.0 [cd]	158.9 [d]	183.1 [c]	7.93	0.08	0.67
Fecal DM, %	43.8	44.4	43.6	41.1	1.44	0.41	0.66
Apparent total tract digestibility, %							
Dry matter	81.4	81.7	82.7	81.2	0.56	0.25	0.43
Organic matter	84.4	84.8	85.6	84.4	0.47	0.30	0.36
Crude protein	84.2 [a]	84.0 [a]	84.7 [a]	81.9 [b]	0.52	0.01	0.28
Acid hydrolyzed fat	95.7	95.5	95.9	95.5	0.19	0.32	0.78
Gross energy	85.9	86.2	87.1	85.8	0.42	0.14	0.34

[a,b] Values lacking a common superscript letter within each row are different ($P \leq 0.05$).

[c,d] Values lacking a common superscript letter within each row are different ($P \leq 0.10$).

Table 4. Fecal pH, score, and concentrations of ammonia, 4-methylphenol, and indole for cats supplemented with short-chain fructooligosaccharides (scFOS) and (or) galactooligosaccharides (GOS).

	Treatment					P-value	
Item	Control	scFOS	GOS	scFOS+GOS	SEM	Main effects	Control vs. supplementation
Fecal							
Score[1]	2.7	2.7	2.7	2.8	0.12	0.81	0.75
pH	6.7 [a]	6.4 [ab]	6.6 [a]	6.0 [b]	0.15	0.03	0.05
Ammonia, µmol/g DM	110.0	125.7	100.6	129.3	11.88	0.16	0.46
4-Methylphenol, µmol/g DM	2.2	2.3	2.4	2.4	0.36	0.93	0.56
Indole, µmol/g DM	1.9	1.5	1.8	1.9	0.20	0.28	0.31

[a,b] Values lacking a common superscript letter within each row are different ($P \leq 0.05$).

[1] All fecal samples were scored for consistency based on the following scale: 1 = hard, dry pellets, small hard mass; 2 = hard, formed stool that remains firm; 3 = soft, formed, and moist stool that retains its shape; 4 = soft, unformed stool that assumes the shape of the container; and 5 = watery, liquid stool that can be poured.

Table 5. Fecal short-chain fatty acid (SCFA) and branched-chain fatty acid (BCFA) concentrations for cats supplemented with short-chain fructooligosaccharides (scFOS) and (or) galactooligosaccharides (GOS).

	Treatment					P-value	
Item	Control	scFOS	GOS	scFOS+GOS	SEM	Main effects	Control vs. supplementation
	----------μmol/g DM----------						
Acetate	166.5 [ab]	173.8 [ab]	159.8 [b]	219.4 [a]	15.25	0.05	0.32
Propionate	51.7	53.1	53.1	68.7	5.90	0.17	0.34
Butyrate	28.5 [b]	31.4 [ab]	35.4 [ab]	42.4 [a]	3.84	0.05	0.10
Total SCFA	246.7 [d]	258.4 [cd]	248.3 [d]	330.6 [c]	23.20	0.06	0.24
Isobutyrate	5.6	5.9	5.6	7.1	0.69	0.15	0.32
Isovalerate	9.0	9.4	9.3	10.9	1.16	0.41	0.39
Valerate	16.2 [b]	16.9 [b]	17.5 [ab]	22.3 [a]	1.77	0.01	0.08
Total BCFA	31.3 [d]	32.8 [cd]	32.4 [cd]	40.9 [c]	3.58	0.06	0.19

[a,b] Values lacking a common superscript letter within each row are different ($P \leq 0.05$).

Table 6. Fecal biogenic amine concentrations for cats supplemented with short-chain fructooligosaccharides (scFOS) and (or) galactooligosaccharides (GOS).

	Treatment					P-value	
Item	Control	scFOS	GOS	scFOS+GOS	SEM	Main effects	Control vs. supplementation
	----------µmol/g DM----------						
Cadaverine	266.1	192.2	250.8	219.4	55.55	0.86	0.78
Putrescine	44.1	36.3	48.2	33.5	8.13	0.57	0.61
Histamine	11.3	9.8	12.4	14.8	3.34	0.52	0.18
Tryptamine	20.1	24.1	19.6	45.0	6.68	0.11	0.41
Tyramine	55.5 [c]	32.9 [cd]	50.4 [cd]	28.0 [d]	8.10	0.07	0.06
Spermidine	16.3	15.4	18.9	19.4	1.90	0.40	0.48
Spermine	3.7	4.0	3.1	3.3	1.55	0.78	0.45

[c,d] Values lacking a common superscript letter within each row are different ($P \leq 0.10$).

Table 7. Fecal microbial populations for cats supplemented with short-chain fructooligosaccharides (scFOS) and (or) galactooligosaccharides (GOS).

	Treatment					P-value	
Item	Control	scFOS	GOS	scFOS+GOS	SEM	Main effects	Control vs. Supplementation
	----------CFU log_{10}/g fecal DM----------						
Bifidobacterium spp.	9.3 [c]	9.8 [b]	10.2 [ab]	10.4 [a]	0.15	0.01	0.01
Lactobacillus spp.	10.7	10.7	10.8	10.9	0.14	0.27	0.19
Escherichia coli	10.2	10.5	10.3	10.4	0.14	0.54	0.31
Clostridium perfringens	9.6	9.6	9.7	9.7	0.19	0.73	0.51

[a,b,c] Values lacking a common superscript letter within each row are different ($P \leq 0.05$).

Table 8. White blood cell counts for cats supplemented with short-chain fructooligosaccharides (scFOS) and (or) galactooligosaccharides (GOS).

	Treatment					P-value	
Item	Control	scFOS	GOS	scFOS+GOS	SEM	Main effects	Control vs. Supplementation
Total white blood cells, 10^3/µl	8.2	8.7	8.3	9.1	0.53	0.58	0.41
Neutrophils, %	52.0	58.5	59.0	56.4	2.67	0.21	0.05
Neutrophils, 10^3/µl	4.3	4.9	5.6	4.9	0.50	0.41	0.20
Lymphocytes, %	39.9 [c]	33.3 [d]	33.9 [cd]	34.9 [cd]	2.75	0.10	0.02
Lymphocytes, 10^3/µl	3.1	4.9	5.6	4.9	0.21	0.37	0.21

[c,d] Values lacking a common superscript letter within each row are different ($P \leq 0.10$).

AUTHOR'S BIOGRAPHY

Krasae Kanakupt was born on December 2, 1975, in Nakhonpathom, Thailand. He earned a Doctor of Veterinary Medicine in 1999 from Kasetsart University, Bangkok, Thailand. He then worked as a clinician in a private animal hospital located in Nonthaburi, Thailand for 1 year. He studied for the M.S. in Parasitology in the Department of Tropical Medicine, Mahidol University, Thailand. He graduated with this degree in May, 2004. After that, he began working for the pet food manufacturer, Perfect Companion Group Co., Ltd., Bangkok, Thailand. His job responsibility was technical support for the R&D department. Additionally, he provided support to customers with questions or complaints about their pet foods. His experiences in the pet food business inspired and motivated him to gain more knowledge and skill in nutrition. Fortunately, Mr. Pongthep Chiarawanon, chairman of the Perfect Companion Group Co., Ltd., gave him a good opportunity and support to study for the M.S. in companion animal nutrition in the U.S. He came to the Department of Animal Sciences, University of Illinois, in 2008 to earn the M.S. degree in companion animal nutrition under the supervision of Dr. George C. Fahey, Jr. His research focused on the effects of prebiotics on indices of gut health in cats.