Uludag Univ. J. Fac. Vet. Med.
29 (2010), 2: 71-76

Use of Fructans in Dogs

Çağdaş KARA*

Geliş Tarihi: 20.09.2010
Kabul Tarihi: 19.01.2011

Abstract: Fructans are classified as prebiotic, which is defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health. In addition to their intestinal health benefits by increasing beneficial microbial populations, fructans have been shown to decrease fecal odor components, reduce blood cholesterol, prevent or inhibit the occurrence of some types of cancer, enhance vitamin synthesis, increase mineral absorption, and stimulate the immune system. Different forms of fructans can have different physiological effects in dogs. Specific effects may vary due to fructan chain length and/or rate of fermentation. The type of diet utilized (plant-based or animal-based and level of crude protein) and variation among individual animals might greatly affect the efficacy of fructans supplementation. The full beneficial effects of fructans probably will not be experienced unless dietary concentrations are above 0.4% of dry food. In this review, the importance and effects of fructans were emphasized and the studies investigating use of fructans in dogs were summarized.

Key Words: Prebiotic, Fructans, Dog.

Köpeklerde Fruktanların Kullanımı

Özet: Fruktanlar prebiyotik niteliğinde olan maddelerdir. Prebiyotikler kalın barsakta bir veya sınırlı sayıda bakteri türünün gelişimini ve/veya aktivitesini uyarma yoluyla konağı olumlu yönde etkileyen sindirilemeyen gıda içeriği olarak tanımlanmaktadır. Fruktanların yararlı mikrobiyal popülasyonu arttırma yoluyla barsak sağlığına olan faydalarına ek olarak dışkı koksunu oluşturan bileşikleri azalttığı, kan kolesterol seviyesini düşürdüğü, bazı kanser tiplerinin oluşumunu engellediği, vitamin sentezini arttırdığı, mineral emilimini yükselttiği ve bağışıklık sistemini uyardığı tespit edilmiştir. Fruktanların farklı formları köpeklerde farklı etkilere sahip olabilmektedir. Fruktanların etkileri zincir uzunluğu ve fermantasyon oranı gibi özelliklerden dolayı değişebilmektedir. Kullanılan diyetin tipi (bitkisel veya hayvansal temelli, ham protein seviyesi) ve köpekler arasında bireysel farklılıklar fruktan ilavesinin etkinliğini değiştirebilmektedir. Fruktanların diyetteki konsantrasyonları kuru mamanın % 0.4'ünün üstünde olmadığı zaman yararlı etkileri görülmemektedir. Bu derlemede fruktanların önemi ve etkileri vurgulanmış olup köpeklerde fruktanların kullanımı ile ilgili araştırmalar özetlenmiştir.

Anahtar Kelimeler: Prebiyotik, Fruktanlar, Köpek.

* University of Uludag, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, 16059 Gorukle, Bursa, Turkey. cagdaskara@uludag.edu.tr

1. Introduction

Fructans are classified as prebiotic, which is defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health[10,34]. Although the most common prebiotics studied are fructans, the beneficial effects of mannans, lactosucrose, lactulose and others (transgalactosylated, oligosaccharides, xylooligosaccharides) have been reported[37].

Fructans include short-chain fructooligosaccharides (scFOS), inulin (long-chain fructooligosaccharides) and oligofructose (hydrolyzed inulin)[9] and are also referred to as fructooligosaccharides (FOS)[11]. Although the term FOS is often used to refer to all nondigestible oligosaccharides composed of fructose and glucose units, it refers specifically to short chains (~ 3–6 units) of fructose units bound by β-(2–1) linkages that are attached to a terminal glucose unit. Because the β-(2–1) fructose linkages are resistant to mammalian enzymes, fructans reach the colon and serve as a source of highly digestible substrate for colonic bacteria[11].

scFOS is microbially derived, and its components include kestose (GF2), nystose (GF3), and fructofuranosylnystose (GF4)[8]. Commonly, fructans are extracted chicory root in the form of inulin or synthesized from sucrose through transfructosylation by an enzyme from fungus, such as Aspergillus niger, Aurebasidum pullulans and Fusarium oxysporum[29]. Banana, barley, garlic, honey, onion, rye, brown sugar, asparagus root and Jerusalem artichoke contain fructans[32]. Hussein et al.[14] analyzed 25 pet food ingredients and found wheat by-products to contain the highest concentrations of fructans (wheat bran: 4 mg/g dry matter).

Fructans have many functional and nutritional properties that may have application for companion animal nutrition. In addition to their intestinal health benefits by increasing beneficial microbial populations, fructans have been shown to decrease fecal odor components, reduce blood cholesterol, prevent or inhibit the occurrence of some types of cancer, enhance vitamin synthesis, increase mineral absorption, and stimulate the immune system[15]. However, potential adverse side-effects, such as diarrhea and flatulence, also may occur in animals consuming high levels of fructans or at moderate levels of ingestion in unadapted animals[21].

Although some information is available on the effects of feeding fructans in selected species (human, rats), there is a paucity of information in companion animals[35]. In this review, the importance and effects of fructans were emphasized and the studies investigating use of fructans in dogs were summarized.

2. Effects of Fructans

Fructans have been investigated for their intestinal health benefits at moderate to large concentrations in the canine diet. Several beneficial effects have been observed in these investigations, including increased beneficial microbial populations (Lactobacillus and Bifidobacterium) with a commensurate decrease in potentially pathogenic populations (Clostridium perfringens, Escherichia coli and Salmonella)[34,43] and decreased fecal putrefactants such as phenols, indoles, ammonia, and some biogenic amines[21,34].

While potentially pathogenic bacteria in the colon are unable to ferment fructans, beneficial microbial populations can metabolize them[2,12]. By the metabolism of fructans, short chain fatty acids (SCFA; acetate, propionate and butyrate), gases (H_2, H_2S, CO_2 and CH_4) and organic acids (lactate, succinate and pyruvate) are produced[24]. Especially butyrate can be used by the colonic epithelium, exhibiting a trophic effect. Propionate and he remaining butyrate can be metabolized by the liver into glucose. Acetate can be used by muscle and peripheral tissues[12]. Lactate and acetate are also responsible for a decreased pH in the colon. Due to the decreased pH, more NH_4^+ (ammonium ions) are formed, which can not cross the membrane[42].

The metabolism of nitrogenous compounds in the colon by bacterial fermentation results in putrefactive catabolites that are implicated as the major odor components of feces[30,34]. Fructans are thought to inhibit either the production of nitrogenous compounds-fermentative end products or the bacterial populations that produce them[9]. When energy supplies are limited in the colon, bacteria ferment amino acids to SCFA and ammonia to obtain energy. However, if a sufficient energy source such as scFOS, inulin and oligofructose are provided, the luminal concentrations of nitrogenous compounds decrease and the concentrations of fecal nitrogen (bacterial mass) increase[5,6]. By decreasing potential pathogens, putrefactants also are reduced, thereby alleviating some risk of intestinal diseases associated with increased putrefactants in the intestine[2].

Fructans supplementation demonstrated the capacity to decreased aberrant crypt foci growth, a precursor to colon cancer[22,28]. The possible anticarcinogenic activity of fructans might be accounted for, in part, by the possible anticarcinogenic action of butyrate. Butyrate is produced by bacterial fermentation of fructans in the colon. Fructans may aid in increasing the concentrations of calcium and magnesium in the colon. High concentrations of these cations in the colon may help control the rate of cell turnover. High concentration of calcium in the colon may also lead to the formation of insoluble bile or salts of fatty acids. This might reduce the potential damaging effects of bile or fatty acids on colonocytes[23,25].

A large number of publications have demonstrated that fructans added to the daily diet significantly increase the absorption of both calcium and magnesium in growing animals. Numerous animal studies have shown that fructans significantly increase bone mineralization[26]. The SCFA formed from the bacterial fermentation of fructans may facilitate the colonic absorption of calcium and, possibly, also magnesium ions. This could be beneficial in preventing osteoporosis and osteopenia[19,20]. Propionate and butyrate can act independently to stimulate fluid absorption of calcium, magnesium, and other cations in the colon. This action also may be enhanced with the help of acetate. Propionate may enhance the absorptive capacity of the colon by stimulating proliferation of the colonic epithelium[38].

Fructans are able to regulate lipemia and triglyceridemia in both humans and animals[7]. Propionate, a product of fructans fermentation in the colon, inhibits hepatocyte lipids synthesis[41]. Fructans can decrease triacylglycerol and phospholipid serum concentrations. This is almost exclusively due to a decrease in the concentration of plasma VLDL (very low density lipoproteins). This effect is likely to result from a decrease in the hepatic synthesis of triacylglycerol rather than from a high catabolism of triacylglycerol-rich lipoproteins. The triacylglycerol-lowering action of fructans is due to a reduction of de novo fatty acid synthesis (de novo lipogenesis) in the liver through reduction of the activity of all lipogenic enzymes, namely acetyl-CoA carboxylase, fatty acid synthase, malic enzyme, ATP citrate lyase and glucose-6-phosphate dehydrogenase[7].

Fructan, such as oligofructose, administration in the diet of rats increases the plasma and intestinal concentration of glucose-dependent insulinotropic polypeptide and of glucagon-like peptide 1. These peptides are released by the endocrine cells of the intestine and they enhance postprandial insulin secretion[3]. Glucagon-like peptide 1 plays an important role in lowering blood glucose levels, primarily through its ability to potentiate the stimulatory effect of glucose on insulin secretion from pancreatic β-cells. It also affects blood glucose levels through its inhibitory effects on gastric emptying, suppression of appetite and inhibition of glucagon secretion from pancreatic α-cells. The effects of fructans on blood glucose and insulin levels are not yet fully understood[13,16,18].

As a result of intestinal fermentation and growth of beneficial intestinal microbial populations, fructans may influence host defence[17]. Fructans beneficially affect the immune system, especially the intestinal immune functions by targeting the gut-associated lymphoid tissue (Peyer's patches), and consequently they have been shown to reduce the risk of diseases related to dysfunction of the gastrointestinal defence's functions[27]. Beneficial microbial populations in the colon may cross the intestinal barrier into the Peyer's patches and activate immune cells there. Butyrate, a product of fructans fermentation in the colon, may alter epithelial cell gene expression, for example IL–8 and monocyte chemoattractant protein 1, and this in turn would alter the signalling of the epithelial cell to the mucosal immune system[17].

3. Use of Fructans

One philosophy regarding the treatment of certain gastrointestinal diseases has been to attempt to eliminate intestinal pathogens by administering antibiotics. While this method may be effective in certain cases, a side effect of this therapy can be the development of antibiotic-resistant strains of intestinal bacteria. Alternatively, one can attempt to work with the existing gut flora and change it in a way that is more favorable to the host animal. This is made possible by the "prebiotic" effect of certain fibers such as fructans[33].

Small intestinal overgrowth (SIBO) is a serious problem in dogs with IgA deficiency. Therefore, Willard et al.[40] supplemented scFOS (1 % of the diet) to IgA-deficient German shepherds to decrease small intestinal microbial density and ameliorate the clinical signs associated with SIBO. This experiment suggested that scFOS supplementation may be helpful in prevention or treatment of SIBO in dogs.

Barry et al.[2] have reported that concentrations of ileal IgA were not modified with inulin (0.2 or 0.4 % of the diet) or scFOS (0.2 or 0.4 % of the diet) supplementation. Traditionally, ileal IgA is used as a marker of intestinal immunity. In healthy adult dogs, no change would be expected to occur because they have fully developed immune systems.

Apanavicius et al.[1] have reported that inulin or scFOS supplementation (1 % of the diet) in weanling puppies decreased enterocyte sloughing, an indicator of intestinal damage due to infection, suggesting a protective role during infection. In addition, Apanavicius et al.[1] observed that the total SCFA and acetate concentration and intestinal microbial populations were increased by inulin supplementation.

In the study conducted by Flickinger et al.[9], three concentrations of scFOS (1, 2, and 3 g/day) were tested. Ileal nutrient digestibilities tended to increase with increasing concentrations of scFOS. On a DMI basis, fecal output tended to decrease in response to increasing scFOS supplementation. Fecal concentrations of acetate, propionate, butyrate, and total SCFA were not affected by scFOS supplementation. scFOS is rapidly fermented in the proximal colon and approximately 95% of SCFA produced in the large bowel are absorbed from that site and therefore are not present in feces. Total anaerobes, bifidobacteria, and lactobacilli concentrations in feces were not altered by supplemental scFOS. Supplementation of scFOS decreased fecal concentrations of Clostridium perfringens.

In the experiment performed by Propst et al.[21], dogs were administered 1.5 g, 3 g or 4.5 g of oligofructose/day or 1.9 g, 3.8 g or 5.6 g of inulin/day to achieve dietary concentrations of 0.3, 0.6, or 0.9 % active ingredients based on the amount of diet offered per day. Oligofructose was purer than inulin (75% pure). Therefore, doses of inulin were increased by a factor of 1.25 in order to compare similar concentrations of the active ingredients of both fructans. Oligofructose and inulin supplementations did not affect ileal digestibilities of dry matter, organic matter, crude protein, and fat. Propst et al.[21] have reported that fecal scores (1- □hard, dry pellets; small, hard mass; 2- □hard, formed, dry stool; remains firm and soft; 3- soft, formed moist; softer stool that retains shape; 4- □soft, unformed; stool assumes shape of container; and 5- □watery; liquid that can be poured) were not affected by fructan supplementations. Concentrations of fecal acetate, propionate, butyrate, and total SCFA tended to be higher when dogs were supplemented with oligofructose and inulin. The results obtained by Propst et al.[21] have indicated that oligofructose and inulin are highly fermentable substrates that increase fecal SCFA concentrations at relatively low levels of inclusion in diets.

Strickling et al.[31] have reported that 0.5% dietary oligofructose increased the fecal moisture content in dogs.

Swanson et al.[36] have reported that 2 g/day scFOS did not significantly alter ileal digestibility of dry matter, organic matter and crude protein and fecal score, fecal pH and fecal concentrations of ammonia in dogs fed a poultry by-product meal and rice-based diet. In addition, Swanson et al.[36] have reported a decrease in fecal concentrations of total phenols and indole (odor components). Swanson et al.[36] have also found no change in fecal bifidobacteria or lactobacilli due to supplementation of 2 g/d scFOS to adult dogs.

Beynen et al.[4] observed that 1 % oligofructose supplementation increased Ca and Mg absorption in dogs. In experimental animals (mostly the rat), a large number of publications demonstrated that fructans significantly increase mineral absorption, essentially Ca and Mg[39].

Swanson et al.[35] have reported that the combination of scFOS (1 g/day) and mannanoligosaccharides (prebiotic) (1 g/day) tended to enhance local and systemic immune characteristics and decreased the concentrations of putrefactive compounds found in feces. Swanson et al.[34] have reported that the supplementation of scFOS (4 g/day) and Lactobacillus acidophilus (probiotic) ($2*10^9$ cfu/day) together as a synbiotic decreased the concentration of several fecal putrefactive compounds (biogenic amines, phenols, indoles).

4. Conclusion

Fructans can be added to diets in their natural form (chicory) or as partially purified ingredients (short chain fructooligosaccharides, oligofructose and inulin). In addition, fructans can be used in combination with other prebiotics or probiotics. It is important to consider the type of diet to which the fructan is supplemented and whether the diet contains natural sources of fructans (wheat, wheat by-products) or other oligosaccharides (galactooligosaccharides in soy products). The type of diet utilized (plant-based or animal-based and level of crude protein) and variation among individual animals might greatly affect the efficacy of fructans supplementation. Different forms of fructans can have different physiological effects in dogs. Specific effects may vary due to fructan chain length and/or rate of fermentation. These factors indicated above may lead to differences among studies, making it difficult to identify an appropriate dosage of fructan to be included in diets both from physiologic and economic standpoints. The full beneficial effects of fructans probably will not be experienced unless dietary concentrations are above 0.4 % of dry food. With recommended lower levels (0.5-1 %) there are no problems be expected with regard to fecal moisture and loose stools. Future studies should carefully examine the individual effects of fructan form, basal diet composition and animal attributes. In the future, experiments must test fructans supplementation on animals of different life stages (weanlings, gestation, lactation, geriatric animals) and disease states.

References

1. Apanavicius, C.J., Powell, K.L., Vester, B.M., Karr-Lilienthal, L.K., Pope, L.L., Fastinger, N.D., Wallig, M.A., Tappenden, K.A., Swanson, K.S., 2007. Fructan supplementation and infection affect food intake, fever, and epithelial sloughing from Salmonella challenge in weanling puppies. *J. Nutr.,* 137, 1923–1930.
2. Barry, K.A., Hernot, D.C., Middelbos, I.S., Francis, C., Dunsford, B., Swanson, K.S., Fahey, G.C., 2009. Low-level fructan supplementation of dogs enhances nutrient digestion and modifies stool metabolite concentrations, but does not alter fecal microbiota populations *J. Anim. Sci.,* 87, 3244-3252.
3. Beylot, M., 2005. Effects of inulin-type fructans on lipid metabolism in man and in animal models. *Br. J. Nutr.,* 93(suppl), S163–S168.
4. Beynen, A.C., Baas, J.C., Hoekemeijer, P.E., 2002. Faecal bacterial profile, nitrogen excretion and mineral absorption in healthy dogs fed supplemental oligofructose. *J. Anim. Physiol. Anim. Nutr.,* 86, 298-305.
5. Cummings, J.H., Hill, M.J., Bones, E.S., Branch, W.J., Jenkins, D.J.A., 1979. The effect of meat protein and dietary fiber on colonic function and metabolism. II. Bacterial metabolites in feces and urine. *Am. J. Clin. Nutr.,* 32, 2094–2101.
6. Cummings, J.H., Bingham, S.A., 1987. Dietary fiber, fermentation and large bowel cancer. *Cancer Surv.,* 6, 601–621.
7. Delzenne, N.M., Kok N., 2001. Effects of fructans-type prebiotics on lipid metabolism. *Am. J. Clin. Nutr.,* 73(suppl), 456S–458S.
8. Flickinger, E.A., Van Loo, J., Fahey, G.C., 2003. Nutritional responses to the presence of inulin and oligofructose in the diets of domesticated animals: A review. *Crit. Rev. Food Sci. Nutr.,* 43, 19–60.
9. Flickinger, E.A., Schreijen, E.M.W.C., Patil, A.R., Hussein, H.S., Grieshop, C.M., Merchen, N.R., Fahey, G.C., 2003. Nutrient digestibilities, microbial populations, and protein catabolites as affected by fructan supplementation of dog diets. *J. Anim. Sci.,* 81, 2008-2018.
10. Gibson, G.R., Roberfroid, M.B., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.,* 125, 1401–1412.
11. Gilliland, S.E., 1990. Health and nutritional benefits from lactic acid bacteria. *FEMS Microbiol. Rev.,* 87, 175–188.
12. Hesta, M., Janssens, G.P.J., Debraekeleer, J., De Wilde, R., 2001. The effect of oligofructose and inulin on faecal characteristics and nutrient digestibility in healthy cats. *J. Anim. Physiol. Anim. Nutr.,* 85, 135-141.
13. Holz, G.G., Kuhtreiber, W.M., Habener, J.F., 1993. Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide–1 (7–37). *Nature,* 361, 362–365.
14. Hussein, H., Campbell, J., Bauer, L.L., Fahey, G.C., Hogarth, A., Wolf, B., Hunter, D., 1998. Selected Fructooligosaccharide Composition of Pet-Food Ingredients. *J. Nutr.,* 128, 2803S-2805S.
15. Jenkins, D.J.A., Kendall, C.W.C., Vuksan, V., 1999. Inulin, oligofructose and intestinal function. *J. Nutr.,* 129, 1431S–1433S.
16. Komatsu, R., Matsuyama, T., Namba, M., Watanabe, N., Itoh, H., Kono, N., Tarui, S., 1989. Glucagonostatic and insulinotropic action of glucagon-like peptide 1-(7–36)-amide. *Diabetes,* 38, 902–905.
17. Lomax, A.R., Calder, P.C., 2009. Prebiotics, immune function, infection and inflammation: a review of the evidence *Br. J. Nutr.,* 101, 633–658.
18. Nauck, M.A., Niedereichholz, U., Ettler, R., Holst, J.J., Orskov, C., Ritzel, R., Schmiegel, W.H., 1997. Glucagon-like peptide 1 inhibition of gastric emptying outweight its insulinotropic effects in healthy humans. *Am. J. Physiol. – Endocrinol. Met.,* 273, E981–E988.

19. Ohta, A., Baba, S., Takizawa, T., Adachi, T., 1994. Effects of fructo-oligosaccharides on the absorption of magnesium-deficient rat model. *J. Nutr. Sci. Vitaminol.,* 40, 171-180.
20. Ohta, A., Ohtsuki, M., Hosoro, A., 1998. Dietary fructo-oligosaccharides prevent osteopenia after gastrectomy. *J. Nutr.,* 128, 106-110.
21. Propst, E.L., Flickinger, E.A., Bauer, L.L., Merchen, N.R., Fahey, G.C., 2003. A dose-response experiment evaluating the effects of oligofructose and inulin on nutrient digestibility, stool quality, and fecal protein catabolites in healthy adult dogs. *J. Anim. Sci.,* 81, 3057-3066.
22. Reddy, B.S., Hamid, R., Rao, C.V., 1997. Effect of dietary oligofructose and inuliln on colonic preneoplastic aberrant crypt foci inhibition. *Carcinogenesis,* 18, 1371–1374.
23. Pierre, F., Perrin, P., Champ, M., 1997. Short-chain fructo-oligosaccharides reduce the occurrence of colon tumors and develop gut-associated lymphoid tissue in Min mice. *Cancer Res.,* 57, 225-228.
24. Roberfroid, M.B., Van Loo, J.A.E., Gibson, G.R., 1998. The bifidogenic nature of chicory inulin and its hydrolysis products *J. Nutr.,* 128, 11 - 19.
25. Roberfroid, M.B., 1998. Dietary fructans. *Annu. Rev. Nutr.,* 18, 117-143.
26. Roberfroid, M.B., Cumps, J., Devogelaer, J.P., 2002. Dietary chicory inulin increases whole-body bone mineral density in growing male rats. *J. Nutr.,* 132, 3599–3602.
27. Roberfroid, M.B., 2007. Inulin-Type Fructans: Functional Food Ingredients *J. Nutr.,* 137, 2493S–2502S.
28. Rowland, I.R., Rumney, C.J., Coutts, J.T., Lievense, L.C., 1998. Effect of Bifidobacterium longum and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. *Carcinogenesis,* 19, 281–285.
29. Sangeetha, P.T., Ramesh, M.N., Prapulla, S.G., 2004. Production of fructo-oligosaccharides by fructosyl transferase from Aspergillus oryzae CFR 202 and Aureobasidium pullulans CFR 77. *Process Biochem.,* 39, 755-760.
30. Spoelstra, S. F., 1980. Origin of objectionable odorous components in piggery wastes and the possibility of applying indicator components for studying odor development. Agric. *Environ.,* 5, 241-260.
31. Strickling, J.A., Harmon, D.L., Dawson, K.A., Gross, K.L., 2000. Evaluation of oligosaccharide addition to dog diets: Influences on nutrient digestion and microbial populations. *Anim. Feed Sci. Technol.,* 86, 205–219.
32. Sunvold, G.D., Fahey, G.C., Merchen, N.R., Titgemeyer, E.C., Bourquin, L.D., Bauer, L.L., Reinhart, G.A., 1995. Dietary fiber for dogs: IV. In vitro fermentation of selected fiber sources by dog fecal inoculum and in vivo digestion and metabolism of fiber-supplemented diets. *J. Anim. Sci.,* 73, 1099-1109.
33. Sunvold, G.D., Reinhart, G.A., 1998. Use of novel fibers in canine gastrointestinal disease. Proceedings 23. Congress of the World Small Animal Veterinary Association. Buenos Aires, Argentina.
34. Swanson, K.S., Grieshop, C.M., Flickinger, E.A., Bauer, L.L., Chow, J., Wolf, B.W., Garleb, K.A., Fahey, G.C., 2002. Fructooligosaccharides and Lactobacillus acidophilus modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. *J. Nutr.,* 132, 3721–3731.
35. Swanson, K.S., Grieshop, C.M., Flickinger, E.A., Merchen, N.R., Fahey, G.C., 2002. Effects of Supplemental Fructooligosaccharides and Mannanoligosaccharides on Colonic Microbial Populations, Immune Function and Fecal Odor Components in the Canine *J. Nutr.,* 132, 1717S–1719S.
36. Swanson, K.S., Grieshop, C.M., Flickinger, E.A., Bauer, L.L., Healy, H.P., Dawson, K.A., Merchen, N.R., Fahey, G.C., 2002. Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. *J. Nutr.,* 132, 980-989.
37. Swanson, K.S., Fahey, G.C., 2004. An assessment of prebiotic use in companion animal diets. Supplement to Compendium on Continuing Education for the Practicing Veterinarian, 26, 34-42.
38. Topping, D.L., 1996. Short-chain fatty acids produced by intestinal bacteria. *Asia Pacific J. Clin. Nutr.,* 5, 15–19.
39. Weaver, C.M., Liebman, M., 2002. Biomarkers of bone health appropriate for evaluating functional foods designed to reduce risk of osteoporosis. *Br. J. Nutr.,* 88(suppl), S225–S232.
40. Willard, M.D., Simpson, R.B., Delles, E.K., 1994. Effects of dietary supplementation of fructooligosaccharides on small intestinal bacterial over-growth in dogs. *Am. J. Vet. Res.,* 55, 654-659.
41. Wright, R.S., Anderson, J.W., Briges, S.R., 1990. Propionate inhibits hepatocyte lipids synthesis. *Proc. Soc. Exp. Biol. Med.,* 195, 26–29.
42. Younes, H., Garleb, K., Behr, S., Remesy, C., Demigne, C., 1995. Fermentable fibers or oligosaccharides reduce urinary nitrogen excretion by increasing urea disposal in the rat cecum. *J. Nutr*., 125, 1010-1016.
43. Zentek, J., Marquart, B., Pietrzak, T., Ballevre, O., Rochat, F., 2003. Dietary effects on bifidobacteria and Clostridium perfringens in the canine intestinal tract. *J. Anim. Physiol. Anim. Nutr. (Berl.),* 87, 397–407.