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The effects of galacto-oligosaccharides on faecal parameters in healthy dogs and cats

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ABSTRACT

The aim of this study was to evaluate the effects of galacto-oligosaccharides (GOS) on faecal parameters in healthy dogs and cats. To this end, 20 dogs and 20 Domestic shorthair cats were fed a commercially available adult dog food, or cat food, respectively, with either syrup containing GOS (at 1% w galacto-oligosaccharides/w formulated feed) on top (test group) or no topping (control group) for 56 days in a cross-over design. The study consisted of 2 periods of 24 days adaptation, followed by 4 days of collection of faeces. Faecal samples were tested for moisture, nitrogen, pH, macronutrients, enzymes, and fermentation products. The faecal microbiota were analysed by 16S rDNA profiling. It appeared that GOS have different effects in dogs compared to cats. In dogs, the addition of GOS resulted in increased carbohydrate fermentation (increase of acetic and butyric acid), whereas in cats GOS resulted in increased amino acid fermentation (increase of isovaleric acid). The α-diversity of the canine faecal microbiota was reduced by dietary GOS (Inverse Simpson Index, $p = 0.063$; Shannon index, $p = 0.035$) whereas the α-diversity of cat faecal microbiota was unaffected (Inverse Simpson Index, $p = 0.539$; Shannon index, $p = 0.872$). *Lachnospiraceae* spp. and *Bifidobacterium* spp. positively responded to GOS in both cats and dogs. *Lactobacillus* spp. and *Enterobacteriaceae* spp. positively responded to GOS in dogs. In both dogs and cats, GOS may therefore improve stool microbiota and result in the production of specific metabolites that are beneficial to gut health.

Galacto-oligosaccharides (GOS) are prebiotics consisting of one glucose molecule bound to a chain of one to eight galactose molecules. GOS are derived from lactose and are a known substrate for *Bifidobacterium* spp. and *Lactobacillus* spp. in people (Giovannini et al., 2014), and for *Bifidobacterium* spp. in dogs when administered in a synbiotic combination (Ogué-Bon et al., 2010). Stimulation of *Bifidobacterium* spp. and *Lactobacillus* spp. has also been demonstrated when administering plant-derived fructo-oligosaccharides (FOS) in dogs (Adogony et al., 2007), so GOS might be a good and more natural alternative for plant-derived FOS in more carnivore-type diets to be used as a prebiotic. The benefits of GOS were demonstrated in human infants, because if they were fed with a GOS supplemented infant formula, they had significantly lower signs of colic compared to infants that were fed a control milk replacer, which was attributed to the decrease in *Clostridium* spp. and the increase of *Bifidobacterium* spp. and *Lactobacillus* spp. (Giovannini et al., 2014). Dysbacteriosis in dogs with increased Clostridium spp. is also common in dogs with diarrhoea, however, although suggested a causal role for diarrhoea in dogs, the role of Clostridium spp. is somewhat debatable, as well as the possible protective role of Bifidobacterium spp. and Lactobacillus spp. in dogs with diarrhoea (Minamoto et al., 2014). *Bifidobacterium* spp. and *Lactobacillus* spp. have been linked to increased resistance to infection and diarrhoea, increased immune function as well as protection against cancer (Macfarlane et al., 2008). *Bifidobacterium* spp. and *Lactobacillus* spp. primarily promote acetate and lactate formation (Hopkins and Macfarlane, 2003). However, the butyrigenicity of GOS has also been reported (Topping and Clifton, 2001), which can be explained by the use of lactate by butyrate forming bacteria such as *Anaerostipes caccae and Eubacterium halli* (Belenguer et al., 2006). *Bacteroides* spp. and *Clostridium* spp. are also capable of fermenting GOS (Ohtsuka et al., 1989). Therefore, it is important to evaluate the effects of GOS on the microbiota in healthy dogs, before using them as a prebiotic in dogs with diarrhoea. The aim of this study was to evaluate the effects of GOS on faecal parameters including microbiota in healthy dogs and cats.

To this end, 20 healthy dogs (5 Beagles, 1 Boxer, 1 Foxhound, 12 Mongrels, 1 Spaniel, 11 male, 9 female, body weight 9.0–27.2 kg, age 2–14 years), and 20 healthy Domestic shorthair cats (12 male, 8 female, body weight 3.4–8.5 kg, age 1–18 years) were used in this study. There

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was a huge age variation to ensure that the results were representative for the whole dog and cat population. The dogs[1] and cats[2] were fed a commercially available adult dog food, or cat food, respectively (both test group and control group) with either syrup containing GOS[3] (at 1% w galacto-oligosaccharides/w formulated feed) on top (test group) or no topping (control group) (Appendix A: nutrient composition of the diets). The collection tubes were coded, so the laboratory staff did not know which sample belonged to which group.

Total duration of the study was 56 days in a cross-over design. The study consisted of 2 periods of 24 days adaptation, followed by 4 days of collection of fresh faeces. The pooled samples of these 4 consecutive days were homogenized analysed as described by Deda et al. (2015).

Faecal samples were tested for moisture, nitrogen, and pH, macronutrients and food components (fat, fatty acids, starch, muscle fibres, microscopically on a 4-point scale), enzymes (amylase, trypsine) according to Spillmann, 2003, and fermentation products (lactic acid, formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, 4-methylvaleric acid) by gas-liquid chromatography. The faecal microbiota were analysed by 16S rDNA profiling. Bacterial communities were profiled using Next-Generation Sequencing technology. Briefly, faecal content was homogenized using a FastPrep-24 5G Grinder (MP Biomedicals SARL, Illkirch-Graffenstaden, Fr.) from which gDNA was isolated using a Qiagen Powerfaecal HT DNA kit (Qiagen Ltd., Manchester, UK). 16S rDNA amplicons were amplified using primers targeting the V4 region of the 16S rDNA (Caporaso et al., 2011) and sequenced on the Illumina MiSeq platform using the dual-indexing approach described by Kozich et al. (2013). Amplicon sequences were then quality-filtered and analysed using the Mothur SOP (accessed 2019.02.12.) (Schloss et al., 2009; Kozich et al., 2013). Results were visualized and tested using R 3.5.2 (2018-12-20) (R Core Team 2019) in RStudio 1.1 (RStudio Team 2015). Inverse Simpson Index and Shannon index were calculated for comparison of relative abundances and tested by a Wilcoxon signed-rank test. Differences in faecal parameters were tested for normality by a Kolmogorov Smirnov test. Normal distributed data were tested by a *t*-test whereas not-normally distributed data were tested by a Wilcoxon test. A *p*-value of <0.05 was considered significant and a p-value of <0.10 as a trend. The study protocol was evaluated and approved by the national central committee for animal experiments and registered under number AVD1080020184847WP1–4.

The results for the moisture, nitrogen, pH, macronutrients, enzymes, and fermentation products in dogs are shown in Table 1. The test group had significantly more muscle fibres and lactic acid in their stools compared to the control group. There was a tendency towards more acetic acid and butyric acid in the test group. The results for the cats are shown in Table 1. The test group had significantly more isovaleric acid in their stools compared to the control group. There was a tendency towards more valeric acid, and trypsine activity in the test group compared to the control group, whereas the test group had a tendency towards lower fatty acids in the stool. The α-diversity of the canine faecal microbiota was reduced by dietary GOS (Inverse Simpson Index, $p = 0.063$; Shannon index, $p = 0.035$) whereas the α-diversity of cat faecal microbiota was unaffected (Inverse Simpson Index, $p = 0.539$; Shannon index, $p = 0.872$). Relative abundance of taxa is shown in Appendix B.

Lachnospiraceae spp. and *Bifidobacterium* spp. positively responded to GOS in both cats and dogs. *Lactobacillus* spp. and *Enterobacteriaceae* spp. positively responded to GOS in dogs (Fig. 1).

The dogs in test group had significantly more muscle fibres in their stools compared to the control group. This can be explained by a decreased fermentation of protein, which is in line with the study by Jackson and Jewell (2019), demonstrating decreased proteolysis with increased saccharolysis. The increase in lactic acid can be explained by an increase in anaerobic fermentation of glucose by the increased number of *Bifidobacterium* spp. and *Lactobacillus* spp., and a tendency towards more acetic acid in the test group in line with the findings of Hopkins and Macfarlane (2003). The tendency towards more butyric acid in the test group can be explained by the increase of butyrate forming bacteria which also may benefit from the more abundant lactate to use as a substrate for butyrate production (Belenguer et al., 2006). The increase in acetic acid is indicative of increased aerobic and anaerobic fermentation, whereas the increase in butyric acid is the result of increased anaerobic fermentation by the increased numbers of *Lachnospiraceae* spp., which are suggested to play an important role in maintaining gastrointestinal health in dogs and cats (Suchodolski, 2011). As feeding a high protein diet (60.9% crude protein) to dogs revealed lower numbers of *Lachnospriaceae* spp. (Hang et al., 2012), which is associated with IBD in dogs (Honneffer et al., 2014), supplementation of GOS to high protein diets might be of interest.

In cats, the test group had significantly more isovaleric acid and a tendency towards more valeric acid and trypsine activity in their stools compared to the control group. This can be explained by increased amino acid fermentation by *Lachnospiraceae* spp., which was also demonstrated in pigs (Tran et al., 2016), and is in contrast to our findings in dogs. The increase in amino acid formation has also been demonstrated in cats on supplementation with FOS (Barry et al., 2010). In cats, the test group had a tendency towards lower fatty acids in the stool, which might be explained by increased fatty acid fermentation. The α-diversity in dogs decreased after supplementation of GOS. This can be explained by the increase of the 4 taxa by GOS that probably had an effect on the survival of several other spp..

It can be concluded that GOS have different effects in dogs compared to cats. In dogs, the addition of GOS resulted in increased carbohydrate fermentation, whereas in cats GOS resulted in increased amino acid fermentation. In both dogs and cats, GOS may therefore improve stool microbiota and results in the production of substrates that are beneficial to gut health.

CRediT authorship contribution statement

Ronald Jan Corbee: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The author declares no conflict of interest, the study was funded by Saputo Dairy UK LTD.

Appendix A. Appendices

Nutrient profile dog food:

Crude Protein 23.0%, Crude Fat 12.0%, Crude Fiber 2.5%, Crude Ash 6.5%, Moisture 10.0%, Calcium 1.5%, Phosphorus 1.0%.

Ingredients dog food:

Mixed meats (25%), Animal protein extract (24%), Corn, Wheat, Poultry (8%), Animal fat (beef, poultry), Rice bran, Rice, Wheat meal, Beet pulp, Hydrolysed chicken liver, Hemoglobulin, Minerals, cellulose, Linseed, Yeast.

Nutrient profile cat food:

Crude Protein 33.0%, Crude Fat 18.5%, Crude Fiber 2.2%, Crude Ash 6.1%, Moisture 10.0%, Calcium 1.1%, Phosphorus 0.9%.

Ingredients cat food:

[1] Carocroc original 23/12 manufactured by Vobra Special Petfoods, Veghel, the Netherlands.

[2] Carocroc with chicken 33/19 manufactured by Vobra Special Petfoods, Veghel, the Netherlands.

[3] Nutrabiotic® GOS 64% Syrup batch no. AQ8035 supplied by Dairy Crest Ltd.

Table 1
Faecal parameters in dogs and cats in the test group and the control group.

	Dog Test group	Dog Control group	P-value	Cat Test group	Cat Control group	P-value
Moisture g/100 g	68 [60–71]	69 [65–74]	0.970*	64 [57–73]	69 [56–72]	0.926*
Nitrogen mg/100 g	142 [87–234]	152 [87–261]	0.931*	320 [159–607]	317 [185–604]	0.931*
Lactic acid mmol/kg	58 ± 83	28 ± 20	**0.040**†	1.6 ± 2.4	ND	
Formic acid mmol/kg	ND	ND		ND	ND	
Acetic acid mmol/kg	125 [30–2235]	97 [38–173]	*0.079**	121 [62–195]	110 [67–127]	0.287*
Propionic acid mmol/kg	85 [54–179]	68 [53–140]	0.296*	118 [47–745]	85 [28–325]	0.370*
Iso-butyric acid mmol/kg	1.1 [1.1–10.5]	1.1 [1.1–4.5]	0.500*	1.1 [1.1–8.1]	1.1 [1.1–6.8]	0.465*
Butyric acid mmol/kg	564 ± 308	448 ± 137	*0.060*†	ND	ND	
Isovaleric acid mmol/kg	3.7 [1.0–20.5]	5.8 [1.0–12.4]	0.542*	11.1 ± 5.3	8.8 ± 4.2	**0.008**†
Valeric acid mmol/kg	ND	ND		10.0 ± 6.3	7.5 ± 5.1	*0.060*†
4-methyl valeric acid mmol/kg	ND	ND		ND	0.9 [0.9–190]	
pH	6.22 ± 0.27	6.23 ± 0.34	0.832†	6.2 [5.6–7.4]	6.2 [5.6–7.4]	0.445*
Fat (0–4)	0	0	1.000	2 [1–4]	2 [0–3]	0.808*
Fatty acids (0–4)	0	0	1.000	2 [0–4]	2 [0–4]	0.137*
Starch (0–4)	0.8 ± 0.5	0.8 ± 0.5	1.000	1 [0–4]	1 [0–4]	0.813*
Muscle fibres (0–4)	1.0 ± 0.8	0.6 ± 0.8	**0.040**†	0 [0–2]	0 [0–1]	0.593*
Amylase mm	5 [1–10]	4 [2–10]	0.689*	8 [5–12]	8 [5–15]	0.981*
Trypsine mm	11 [7–22]	11 [5–21]	0.570*	17.25 ± 2.81	16.45 ± 3.44	*0.090*†

Values are median [range] for non-parametric data, and mean ± standard deviation for normal distributed data, * = Wilcoxon, † = t-test, significant differences ($p < 0.05$) are given in bold, and trends ($p < 0.10$) in italic. Fat, fatty acids, starch, and muscle fibres were microscopically evaluated on a 4-point scale. ND = not detectable, 100 mg/kg was the detection limit for fermentation products.

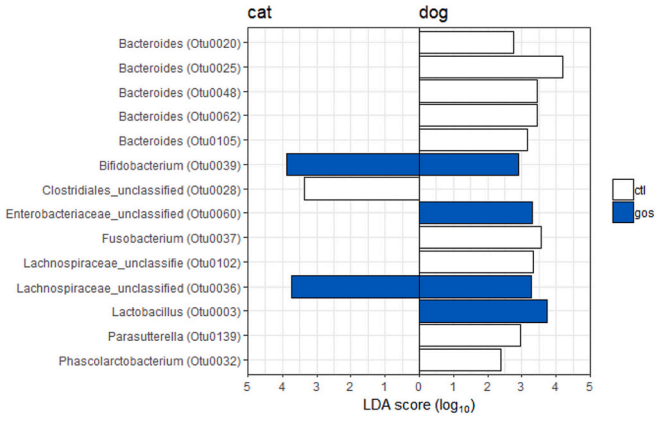

Fig. 1. Galacto-oligosaccharides responsive taxa in cats and dogs.
Linear discriminant analysis (LDA) score for different operational taxonomic units (OTUs).

Poultry (36%), Animal protein extract (23%), Rice, Corn gluten, Corn, Wheat, Animal fat (beef, poultry), Hydrolysed chicken liver, Beet pulp, Fish oil (Salmon), Minerals.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2023.105116.

References

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

Barry, K.A., Wojcicki, B.J., Middelbos, I.S., Vester, B.M., Swanson, K.S., Fahey Jr., G.C., 2010. Dietary cellulose, fructooligosaccharides, and pectin modify fecal protein catabolites and microbial populations in adult cats. J. Anim. Sci. 88, 2978–2987.

Belenguer, A., Duncam, S.F., Calder, A.G., Holtrop, G., Louis, P., Lobley, G.E., Flint, H.J., 2006. Two routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate producing anaerobes from the human gut. Appl. Environ. Microbiol. 72, 3593–3599.

Deda, O., Gika, H.G., Wilson, I.D., Theodoridis, G.A., 2015. An overview of fecal sample preparation for global metabolic profiling. J. Pharm. Biomed. Anal. 113, 137–150.

Giovannini, M., Verduci, E., Gregori, D., Ballali, S., Soldi, S., Ghisleni, D., Riva, E., 2014. Prebiotic effect of an infant formula supplemented with Galacto-oligosaccharides: randomized multicenter trial. J. Am. Coll. Nutr. 33, 385–393.

Hang, I., Rinttila, T., Zentek, J., Kettunen, A., Alaja, S., Apajalahti, J., Harmoinen, J., de Vos, W.M., Spillmann, T., 2012. Effect of high contents of dietary animal-derived protein or carbohydrates on canine faecal microbiota. BMC Vet. Res. 8. Article number 90.

Honneffer, J.B., Minamoto, Y., Suchodolski, J.S., 2014. Microbiota alterations in acute and chronic gastrointestinal inflammation of cats and dogs. World J. Gastroenterol. 20, 16489–16497.

Hopkins, M.J., Macfarlane, G.T., 2003. Nondigestible oligosaccharides enhance bacterial colonization resistance against Clostridium difficile in vitro. Appl. Environ. Microbiol. 69, 1920–1927.

Jackson, M.I., Jewell, D.E., 2019. Balance of saccharolysis and proteolysis underpins improvements in stool quality induced by adding a fiber bundle containing bound polyphenols to either hydrolyzed meat or grain-rich foods. Gut Microbes 10, 298–320.

Macfarlane, G.T., Steed, H., Macfarlane, S., 2008. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. J. Appl. Microbiol. 104, 305–344.

Minamoto, Y., Dhanani, N., Markel, M.E., Steiner, J.M., Suchodolski, J.S., 2014. Prevalence of Clostridium perfringens, Clostridium perfringens enterotoxin and dysbiosis in fecal samples of dogs with diarrhea. Vet. Microbiol. 174, 463–473.

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

Ohtsuka, K., Benno, Y., Endo, A., Ueda, H., Ozawa, O., Uchida, T., Mitsuoka, T., 1989. Effects of 4′-galactosyl-lactose on human intestinal microflora. Bifidus 2, 143–149.

Spillmann, T., 2003. Laboratory diagnosis of pancreatic diseases in dogs - possibilities and limitations. Tierarztliche Praxis Ausgabe K: Kleintiere - Heimtiere 31 (1), 49–59.

Suchodolski, J.S., 2011. Companion animals symposium: microbes and gastrointestinal health of dogs and cats. J. Anim. Sci. 89, 1520–1530.

Topping, D.L., Clifton, P.M., 2001. Short chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol. Rev. 81, 1031–1064.

Tran, T.H.T., Boudry, C., Everaert, N., Théwis, A., Portetelle, D., Daube, G., Nezer, C., Taminiau, B., Bindelle, J., 2016. Adding mucins to an in vitro batch fermentation model of the large intestine induces changes in microbial population isolated from porcine feces depending on the substrate. FEMS Microbiol. Ecol. 92, 1–13.