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Effects of dietary cellobiose on the intestinal microbiota and excretion of nitrogen metabolites in healthy adult dogs

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Abstract

In order to evaluate the potential prebiotic effects of cellobiose, 10 healthy adult research beagle dogs received a complete diet containing 0, 0.5 and 1 g cellobiose/kg bodyweight (BW)/day. At the end of each feeding period, faeces, urine and blood of the dogs were collected. The results demonstrated a significant increase of faecal lactate concentrations, indicating a bacterial fermentation of cellobiose in the canine intestine. Along with this, a dose-dependent linear increase of the relative abundance of *Lactobacillaceae* in the faeces of the dogs was observed ($p = 0.014$). In addition, a dose-dependent increase ($p < 0.05$) of *Alloprevotella*, *Bacteroides* and *Prevotella*, and a linear decrease for *unidentified Lachnospiraceae* ($p = 0.011$) was observed when cellobiose was added to the diet, although the relative abundance of these genera was low (<1%) among all groups. The faecal pH was not affected by dietary cellobiose. Cellobiose seemed to modulate the excretion of nitrogen metabolites, as lower concentrations of phenol ($p = 0.034$) and 4-ethylphenol ($p = 0.002$) in the plasma of the dogs were measured during the supplementation periods. Urinary phenols and indoles, however, were not affected by the dietary supplementation of cellobiose. In conclusion, cellobiose seems to be fermented by the intestinal microbiota of dogs. Although no effect on the faecal pH was detected, the observed increase of microbial lactate production might lower the pH in the large intestine and consecutively modulate the intestinal absorption of nitrogen metabolites. Also, the observed changes of some bacterial genera might have been mediated by increased intestinal lactate concentrations or a higher relative abundance of lactobacilli. Whether these results could be considered as a prebiotic effect and used as a dietetic strategy in diseased animals to improve gut function or hepatic and renal nitrogen metabolism should be evaluated in future studies.

KEYWORDS
Bacteroides, canine, diet, lactate, lactobacilli, prebiotic

1 | INTRODUCTION

Fermentable carbohydrates may exert prebiotic effects, as they are metabolized by the gut microbiota to short-chain fatty acids (SCFA) and modify the bacterial composition in the intestine (Niba et al., 2009). Short-chain fatty acids mediate several beneficial effects for the host, such as improving morphology and health of the intestinal mucosa (Niba et al., 2009), or modifying gut homeostasis and immune function (Gibson et al., 2017). With regard to the bacterial community in the intestine, fermentable carbohydrates may

promote the growth of bacteria considered to be beneficial, such as *Bifidobacteria* and *Lactobacilli*, and reduce the occurrence of undesired bacteria, such as Clostridia or *Escherichia coli* (Niba et al., 2009).

Fermentable carbohydrates might also modulate the excretion of nitrogen (N) metabolites by different mechanisms. They may stimulate the growth of certain members of the intestinal microbiota and therefore increase their requirement for N (Younes, 2012). In particular, they may use ammonia as source of N (Williams et al., 2001). As a consequence, the microbial N fixation for bacterial protein synthesis increases the faecal N excretion (Younes, 2012). The microbial fermentation of some carbohydrates may also increase the concentration of lactic acid and therefore lower the pH in the digesta of the large intestine. At low pH values, ammonia is converted to ammonium, resulting in a reduced intestinal N absorption and increased faecal N excretion (Beynen et al., 2002). Finally, fermentable carbohydrates may reduce the occurrence of coliforms, which produce ammonia and amines (Scholten et al., 1999).

Different fermentable carbohydrates have been evaluated with regard to their prebiotic potential, including fructooligosaccharides, galactooligosaccharides and inulin (Gibson et al., 2017). Besides, the disaccharide cellobiose might also act as a prebiotic, as its β-(1,4′) glycosidic bond prevents its degradation by digestive enzymes, but allows for a microbial fermentation in the large intestine (Engelking, 2015). Only a few studies have evaluated the bacterial fermentation of cellobiose so far. In vitro investigations could demonstrate an increase of SCFA, lactic acid and bifidobacteria (Sanz et al., 2005) or of the strain *Lactobacillus acidophilus* NCFM (van Zanten et al., 2012), when cellobiose was added to human faecal inoculum. Heinritz et al. (2018) found that cellobiose reduced ammonia concentrations in porcine inoculum under osmotic stress. In the caecal digesta of rats fed a diet with 6% cellobiose, higher concentrations of microbial metabolites were detected than in rats of the control group (Morita et al., 2008). Finally, a recent study in cats could demonstrate dose-dependent effects of cellobiose on bacterial metabolites and the microbiota in the faeces of cats (Paßlack & Zentek, 2019). In particular, a decrease of ammonium, i-butyric acid, i-valeric acid and *Clostridium* cluster I, and an increase of *Lactobacillus* spp. and Enterococci was detected, when the cats received up to 1 g cellobiose/kg body weight (BW)/day. To our best knowledge, no studies on the effects of cellobiose have been conducted in dogs. It was the aim of the present study to evaluate the prebiotic potential of cellobiose and its effect on the excretion of N metabolites in healthy, adult dogs.

2 | MATERIAL AND METHODS

The study was approved by the Animal Welfare Committee (Landesamt für Gesundheit und Soziales) in Berlin, Germany (G 0168/18). Ten healthy, adult research beagle dogs (6 female, 4 male, 58.8 ± 8.07 months old, 14.5 ± 2.38 kg BW) were included in this study. The dogs received a dry extruded complete diet ('Vet-Concept Basic plain', Vet-Concept GmbH & Co. KG, Föhren, Germany) throughout the study. The analysed nutrient concentrations are presented in Table 1 and Table S1. Daily energy requirements were calculated according to the recommendations of the NRC (2006) in order to maintain BW of the dogs (0.5 MJ metabolizable energy/kg $BW^{0.75}$/day, individually adjusted if required). The individual amount of food was offered twice a day.

The study was divided into three feeding periods of 21 days each. In the first feeding period, the dogs received the diet without cellobiose. Afterwards, cellobiose (ElsPetFeed Ingredients GmbH, Elsdorf, Germany) was fed at 0.5 g/kg BW/day (feeding period 2) and 1 g/kg BW/day (feeding period 3) to all dogs. All dogs were fed the same dose at the same time. For the supplementation, the dry extruded food was mixed with warm water, and the cellobiose was mixed in afterwards. At the end of each feeding period, fresh faeces and urine of the dogs were collected and stored at −20°C (−80°C for the faecal samples used for the determination of the microbiota) until further analysis. In addition, fasting blood of the

TABLE 1 Analysed composition of the complete diet[a] fed in the present study

Composition	Analysed
	g/kg
Dry matter	924
	g/kg dry matter
Crude protein	301
Crude fat	78.5
Crude ash	70.0
Crude fibre	21.5
Total dietary fibre	105
Insoluble dietary fibre	93.2
Soluble dietary fibre	11.8
Calcium	15.7
Phosphorus	10.8
Sodium	2.94
Potassium	5.14
Magnesium	1.32
	mg/kg dry matter
Iron	257
Manganese	40.7
Zinc	124
Copper	15.6

[a]'Vet-Concept Basic plain' (Vet-Concept GmbH & Co. KG, Föhren, Germany); Composition (according to the manufacturer): Cereals (corn, wheat), meat and animal by-products (poultry meat meal), fish and fish by-products (salt-water fishmeal), oils and fats, plant by-products (dried beet pulp), vegetables (dried carrots), minerals, dried herbs, extracted yeast (source for MOS), dried green-lipped mussel (Perna canaliculus).

dogs was collected by cephalic venipuncture. The blood was centrifuged at 4°C and 1811 g for 10 min (Heraeus Megafuge 1.0R, Thermo Scientific), and the plasma was stored at −20°C until further analysis.

Bacterial metabolites (SCFA, lactate, ammonium) were determined in the faeces of the dogs as described by Paßlack et al. (2015). Besides the measurement of the SCFA concentrations, the molar ratio [(concentration of a single SCFA/concentration of total SCFA)*100] was also calculated. The faecal pH was measured in the original undiluted faecal samples immediately after thawing (Seven Multi pH meter, Mettler-Toledo GmbH). The faecal microbiota was analysed by 16S rDNA sequencing using an Illumina NextSeq500 sequencer (LGC). For this, the DNA was extracted from the faecal samples (0.2 g) of the dogs using a commercial extraction kit (QIAamp Fast DNA stool mini kit, Qiagen; slightly modified with a lysis step at 90°C). The DNA extracts were analysed by amplicon sequencing, targeting the V3-V4 region of the 16S rDNA gene, and with two 150-base pair reads. Combination of forward and reverse reads (BBMerge tool; Bushnell et al., 2017) was followed by demultiplexing, and the resulting 16S rDNA sequences were finally analysed with the QIIME2 pipeline (Bolyen et al., 2019) and the SILVA SSU database (Yilmaz et al., 2014). For quality control and determination of sequence counts, the DADA2 was used (Callahan et al., 2016). In order to increase confidence of sequence reads and reduce bias by potential sequence errors, sequence variants with less than five counts were not considered for analysis (Huse et al., 2007). For normalization of sequence reads, rearification with an equal representation of 10,000 sequences/sample was performed (Weiss et al., 2017).

Alpha diversity indices (richness, Shannon, evenness, Simpson) and beta diversity (weighted UniFrac distances for principal coordinate analysis plot) of the faecal microbiota of the dogs was calculated using QIIME (Caporaso et al., 2010).

The complete blood count (CBC) and clinical chemistry were performed in the Clinic for Small Animals, Freie Universität Berlin, using standard laboratory methods. Plasma indoxyl sulphate was measured according to Chen et al. (2018), using the HP/Agilent 1100. For the measurement of phenols and indoles in the plasma of the dogs, 500 µl plasma were mixed with 500 µl concentrated HCl at 100°C in a water bath for 60 min. Afterwards, 500 µl of the hydrolysate was mixed with 500 µl MeOH. The phenols and indoles were measured using the HP/Agilent 1100. For the detection of indoxyl sulphate in the urine of the dogs, the method described by Curzon and Walsh (1962) was used. The indoles and phenols in the urine were measured by gas chromatography (GC 6890 N, Agilent Technologies), as described by Eisenhauer et al. (2019).

Data were analysed using IBM SPSS Statistics 25 (SPSS Inc, 2013) and are presented as mean ± standard deviation. A general linear model for repeated measures was used, and polynomial contrasts (linear and quadratic) were calculated to evaluate the relationship between the test variables and the increasing doses of cellobiose (within-subject factor: cellobiose, number of levels: three). The level of significance was $\alpha = 0.05$ ($\alpha < 0.1$ for trends).

3 | RESULTS

3.1 | Animal health, feed intake and body weight

All dogs were clinically healthy throughout the study. The CBC and clinical chemistry results demonstrated only minor differences among the groups, and most variables were within the reference range (Table 2). Only the monocyte count was slightly above the reference value in the group that received 1 g cellobiose/kg BW/day.

The cellobiose was well accepted and tolerated by the animals. The daily amount of feed (276 ± 43.5 g) was always completely ingested by the animals. A slightly decreased BW of the dogs was observed in the second feeding period (14.4 ± 2.21 kg, 14.3 ± 2.08 kg, 14.5 ± 2.13 kg in the first, second and third feeding period respectively; linear contrast: $p = 0.313$; quadratic contrast: $p = 0.036$).

3.2 | Faecal pH, dry matter and bacterial metabolites

The faecal pH and the faecal dry matter (DM) were not affected by the dietary inclusion of cellobiose (Table 3). With increasing doses of cellobiose, the L- and D-lactate concentrations in the faeces of the dogs significantly increased (linear contrast $p = 0.042$ and $p = 0.035$ respectively). A trend for increased faecal propionic acid concentrations could be detected with increasing doses of dietary cellobiose (linear contrast; $p = 0.068$). When the molar ratio of the faecal SCFA was calculated (% of total SCFA), decreased acetic acid concentrations (linear contrast; $p = 0.013$) and a trend for increased propionic acid concentrations (linear contrast; $p = 0.065$) were observed with increasing amounts of dietary cellobiose. The ammonium concentrations in the faeces of the dogs were not affected by the dietary supplementation of cellobiose.

3.3 | Phenol and indole concentrations in plasma and urine

Dietary cellobiose linearly reduced the concentrations of phenol ($p = 0.034$) and 4-ethylphenol ($p = 0.002$) in the plasma of the dogs (Table 4). The urinary concentrations of phenols and indoles did not differ among the treatments (Table 5).

3.4 | Faecal microbiota

Dietary cellobiose had no impact on the alpha diversity indices or the beta diversity of the faecal microbiota of the dogs (Table 6 and Figure 1).

TABLE 2 Complete blood count and clinical chemistry of dogs (n = 10) fed without or with cellobiose (0 g/kg body weight (BW)/day; 0.5 g/kg BW/day; 1 g/kg BW/day)

	Cellobiose			Polynomial contrasts (p value)	
	0 g/kg BW/day	0.5 g/kg BW/day	1 g/kg BW/day	Linear	Quadratic
WBC (G/L)	8.74 ± 1.21	9.18 ± 2.35	8.82 ± 1.79	0.878	0.450
RBC (T/L)	7.32 ± 0.65	7.18 ± 0.62	6.87 ± 0.42	**0.023**	0.506
Neutrophilic granulocytes (G/L)	5.01 ± 0.77	5.15 ± 1.30	5.24 ± 0.97	0.629	0.953
Lymphocytes (G/L)	2.38 ± 0.50	2.48 ± 0.51	2.29 ± 0.57	0.554	0.168
Monocytes (G/L)	0.46 ± 0.12	0.50 ± 0.16	0.52 ± 0.14	**0.005**	0.680
Eosinophilic granulocytes (G/L)	0.50 ± 0.29	0.45 ± 0.23	0.41 ± 0.24	0.070	0.375
Basophilic granulocytes (G/L)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	**0.045**	0.343
Calcium (mmol/L)	2.67 ± 0.11	2.79 ± 0.11	2.70 ± 0.10	0.100	**0.010**
Phosphate (mmol/L)	1.07 ± 0.26	1.25 ± 0.16	1.16 ± 0.19	0.286	0.052
Sodium (mmol/L)	147 ± 2.17	147 ± 1.97	145 ± 1.83	0.301	0.242
Chloride (mmol/L)	111 ± 2.27	108 ± 1.89	110 ± 1.57	0.244	0.057
Potassium (mmol/L)	3.75 ± 0.17	3.81 ± 0.20	3.71 ± 0.21	0.423	0.290
Urea (mmol/L)	4.53 ± 1.06	4.93 ± 1.14	4.96 ± 0.74	0.071	0.424
Creatinine (µmol/L)	71.8 ± 9.62	70.3 ± 17.1	70.6 ± 13.0	0.610	0.766
ALT (U/L)	39.9 ± 6.31	43.1 ± 8.63	39.3 ± 5.64	0.591	0.062
AP (U/L)	50.4 ± 16.5	56.2 ± 13.8	52.4 ± 13.4	0.653	**0.009**
AST (U/L)	26.1 ± 3.67	26.4 ± 4.06	26.7 ± 4.57	0.758	1.000
Protein (g/L)	59.9 ± 3.01	62.3 ± 2.61	61.3 ± 3.18	0.383	0.093
Albumin (g/L)	31.6 ± 1.27	32.5 ± 1.55	31.1 ± 1.71	0.339	**0.002**
Bilirubin (µmol/L)	3.07 ± 0.64	2.97 ± 0.38	2.86 ± 0.44	0.235	0.971

Note: Reference values (Clinic for Small Animals, Freie Universität Berlin): WBC: 5.6–14 G/L, RBC: 5.9–8.3 T/l, Neutrophilic granulocytes: 3–9 G/L, lymphocytes: 1–3.6 G/L, monocytes: 0–0.5 G/L, eosinophilic granulocytes: 0–0.6 G/L, basophilic granulocytes: 0–0.1 G/L, calcium: 2.5–2.9 mmol/L, phosphate: 0.96–1.6 mmol/L, sodium: 140–150 mmol/L, chloride: 98–118 mmol/L, potassium: 3.6–4.8 mmol/L, urea: 3.5–10 mmol/L, creatinine: 53–106 µmol/L, ALT: 0–76 U/L, AP: 0–97 U/L, AST: 0–41 U/L, protein: 54–66 g/L, albumin: 28–36 g/L, bilirubin: 0–5.1 µmol/L.
The bold values were statistically significant ($p < 0.05$).

The relative abundance (%) of dominant bacterial phyla, families and genera in the faeces of the dogs are presented in Tables 7–9. In order to facilitate relevant data presentation, values <1% in all treatment groups are not presented, with the exception of significant differences among groups. The data on bacterial families and genera with a relative abundance <1% and with no effect of the dietary treatment are presented in the Table S2.

The relative abundance of *Lactobacillaceae* markedly increased with increasing doses of cellobiose (p = 0.014), although the standard deviation was high among all groups. A linear increase was also observed for sequences belonging to the genera *Alloprevotella* (p = 0.046), *Bacteroides* (p = 0.003) and *Prevotella* (p = 0.039), and a linear decrease for unidentified *Lachnospiraceae* (p = 0.011), however, the relative abundance of these genera was <1% in all feeding periods. Quadratic effects were detected for *Proteobacteria* (p = 0.042), *Burkholderiaceae* (p = 0.039) and unidentified *Lachnospiraceae* (p = 0.016), but also only with a relative abundance of 1%–2% of these bacteria. The relative abundance of *Clostridiaceae* was low in general (<1%), but the number of positive samples decreased with increasing amounts of cellobiose in the diet (n = 9, n = 8 and n = 2 in the first, second and third feeding period respectively).

4 | DISCUSSION

In the present study, the potential prebiotic effects of cellobiose and its impact on the excretion of N metabolites were evaluated in dogs.

Cellobiose is a disaccharide, consisting of two β-1,4′-glykosidic bound glucose molecules (Ouellette & Rawn, 2015). According to the definition of a prebiotic, the substance should not be degraded by digestive enzymes in the small intestine, but microbially fermented, contributing to a health benefit for the host (Gibson et al., 2017). Contradicting data do exist for the potential enzymatic degradation of cellobiose by the host, as it had been digested by β-galactosidase (lactase) in the small intestine of rats (Morita et al., 2008), but not in humans (Nakamura et al., 2004). The latter might be explained by the fact that the study by Nakamura et al. (2004) was performed with human subjects in Japan, possibly lacking lactase activity (Morita et al., 2008).

TABLE 3 Faecal pH, dry matter and bacterial metabolites of dogs (n = 10) fed without or with cellobiose (0 g/kg body weight (BW)/day; 0.5 g/kg BW/day; 1 g/kg BW/day)

	Cellobiose			Polynomial contrasts (p value)	
	0 g/kg BW/day	0.5 g/kg BW/day	1 g/kg BW/day	Linear	Quadratic
pH	6.46 ± 0.42	6.38 ± 0.28	6.29 ± 0.28	0.427	0.981
Dry matter (g/kg)	316 ± 30.7	315 ± 26.7	300 ± 29.2	0.325	0.499
µmol/g					
L-lactate	0.16 ± 0.23	11.3 ± 9.50	11.9 ± 15.7	**0.042**	0.260
D-lactate	0.44 ± 1.21	8.39 ± 7.42	8.43 ± 9.86	**0.035**	0.198
Ammonium	25.0 ± 4.64	30.6 ± 9.35	28.1 ± 6.36	0.364	0.192
Acetic acid	91.7 ± 17.0	89.5 ± 24.5	92.4 ± 15.2	0.916	0.712
Propionic acid	49.8 ± 7.94	51.0 ± 14.5	61.7 ± 15.5	0.068	0.294
i-butyric acid	2.69 ± 0.39	2.80 ± 0.95	3.03 ± 0.87	0.120	0.792
n-butyric acid	21.4 ± 7.27	19.9 ± 7.02	26.4 ± 16.1	0.236	0.342
i-valeric acid	2.73 ± 0.49	3.08 ± 1.13	3.35 ± 1.05	0.060	0.903
n-valeric acid	1.78 ± 1.18	1.39 ± 1.23	2.13 ± 2.78	0.618	0.244
BCFA	5.42 ± 0.86	5.87 ± 2.08	6.38 ± 1.92	0.079	0.952
SCFA	170 ± 25.8	168 ± 39.6	189 ± 25.7	0.139	0.369
% of total SCFA					
Acetic acid	53.7 ± 3.31	52.9 ± 4.90	49.1 ± 6.22	**0.013**	0.304
Propionic acid	29.3 ± 2.51	30.3 ± 3.93	32.4 ± 4.84	0.065	0.710
i-butyric acid	1.62 ± 0.35	1.68 ± 0.47	1.60 ± 0.42	0.924	0.374
n-butyric acid	12.7 ± 3.96	12.3 ± 4.53	14.0 ± 8.27	0.516	0.596
i-valeric acid	1.64 ± 0.38	1.86 ± 0.60	1.77 ± 0.50	0.534	0.247
n-valeric acid	1.08 ± 0.75	0.93 ± 0.88	1.13 ± 1.44	0.893	0.234
BCFA	3.26 ± 0.72	3.54 ± 1.07	3.37 ± 0.91	0.751	0.286

Abbreviations: BCFA, branched-chain fatty acids; SCFA, short-chain fatty acids.
The bold values were statistically significant ($p < 0.05$).

TABLE 4 Phenol, indole and indoxyl sulphate concentrations[a] in the plasma of dogs (n = 10) fed without or with cellobiose (0 g/kg body weight (BW)/day; 0.5 g/kg BW/day; 1 g/kg BW/day)

	Cellobiose			Polynomial contrasts (p value)	
	0 g/kg BW/day	0.5 g/kg BW/day	1 g/kg BW/day	Linear	Quadratic
Phenol (µg/ml)	2.56 ± 0.32	2.53 ± 0.13	2.35 ± 0.21	**0.034**	0.369
4-ethylphenol (µg/ml)	0.17 ± 0.06	0.13 ± 0.05	0.11 ± 0.02	**0.002**	0.068
Indoxyl sulphate (µg/ml)	5.15 ± 5.73	4.97 ± 3.83	4.78 ± 2.63	0.790	0.999

[a]Indole, 3-methylindole and p-cresol: all values were below the detection limit.
The bold values were statistically significant ($p < 0.05$).

In cats, a significant increase of faecal lactate concentrations was observed following the dietary supplementation of cellobiose, which might indicate a bacterial fermentation of cellobiose in the feline intestine (Paßlack & Zentek, 2019). Similarly, increasing lactate concentrations were observed in the faeces of the dogs of the present study, when cellobiose was supplemented to the diet. Furthermore, the trend for increased faecal propionate concentrations, a metabolite of bacterial lactate fermentation (Seeliger et al., 2002), also points to a strong increase of lactate in the canine intestine.

An increase of intestinal lactate concentrations might be of practical interest for dietetic interventions, in particular in dogs suffering from kidney or liver disease. Enhanced lactate concentrations would lower the pH in the large intestine, causing a conversion of ammonia to ammonium (Beynen et al., 2002). As ammonium is not well absorbed in the large intestine, but excreted with the faeces (Beynen et al., 2002), the need for hepatic ammonia detoxification and renal urea excretion would be reduced.

It has been demonstrated that fermentable carbohydrates can modify the N excretion in patients with chronic kidney disease, as

TABLE 5 Phenol, indole and indoxyl sulphate concentrations[a] in the urine of dogs (n = 10) fed without or with cellobiose (0 g/kg body weight (BW)/day; 0.5 g/kg BW/day; 1 g/kg BW/day)

	Cellobiose			Polynomial contrasts (p value)	
	0 g/kg BW/day	0.5 g/kg BW/day	1 g/kg BW/day	Linear	Quadratic
Phenol (µg/ml)	0.96 ± 0.50	1.05 ± 0.37	0.82 ± 0.36	0.449	0.094
2,3-dimethylindole (µg/ml)	17.9 ± 5.81	18.5 ± 3.52	12.6 ± 4.24	0.076	0.060
Indoxyl sulphate (µg/ml)	193 ± 79.3	184 ± 46.3	159 ± 79.9	0.254	0.632

[a]p-cresol, 4-ethylphenol, indole, 3-methylindole, 7-methylindole, 2-methylindole: all values were below the detection limit.

TABLE 6 Alpha diversity indices of the faecal microbiota of dogs (n = 10) fed without or with cellobiose (0 g/kg body weight (BW)/day; 0.5 g/kg BW/day; 1 g/kg BW/day)

	Cellobiose			Polynomial contrasts (p value)	
	0 g/kg BW/day	0.5 g/kg BW/day	1 g/kg BW/day	Linear	Quadratic
Richness	54.3 ± 11.7	53.1 ± 8.16	57.2 ± 8.48	0.498	0.520
Shannon	2.53 ± 0.45	2.31 ± 0.44	2.46 ± 0.51	0.702	0.361
Evenness	0.64 ± 0.09	0.58 ± 0.10	0.61 ± 0.12	0.508	0.401
Simpson	0.07 ± 0.06	0.03 ± 0.04	0.03 ± 0.02	0.127	0.130

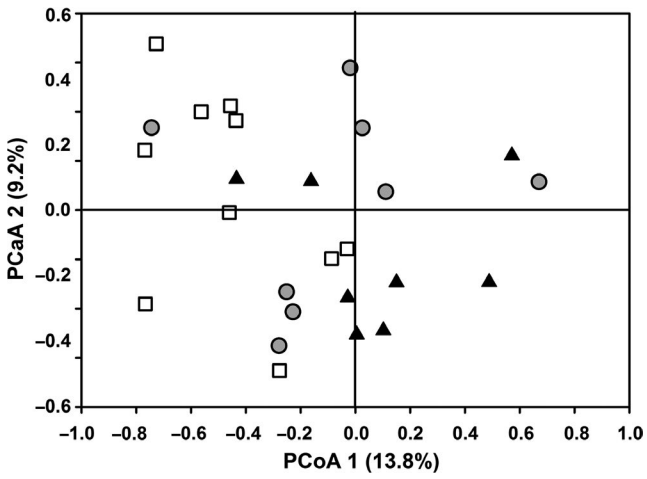

FIGURE 1 Principal coordinate analysis plot of Illumina sequence data generated from weighted UniFrac analysis. Open squares: 0 g cellobiose/kg body weight (BW)/day; grey circles: 0.5 g cellobiose/kg BW/day; filled triangles: 1 g cellobiose/kg BW/day

the faecal N excretion was increased and the renal N excretion decreased (Younes et al., 2006). In the present study, the faecal ammonium concentrations and the faecal pH were not affected by the dietary inclusion of cellobiose. However, the lower phenol and 4-ethylphenol concentrations in the plasma of the dogs indicate a reduced load of protein metabolites in the organism mediated by dietary cellobiose. It should be, however, considered that healthy dogs were included in this study. Thus, further studies with animals suffering from a liver or kidney disease would be interesting in order to investigate the potential beneficial effects of cellobiose on the N metabolism in these patients.

With regard to the prebiotic potential of cellobiose, the increased lactate concentrations in the faeces of the dogs indicate a promotion of lactic acid bacteria in the intestine. This could be supported by the analysis of the faecal microbiota, demonstrating a linear increase of *Lactobacillaceae* with increasing doses of dietary cellobiose. Nevertheless, it should be noted that the standard deviation for this bacterial family was high among all groups, indicating large individual differences in the composition of the faecal microbiota.

In contrast to lactate, the relative amount of acetic acid (% SCFA) in the faeces was reduced when cellobiose was added to the diet. Both lactate and acetic acid are fermentation products of bifidobacteria (Ruiz-Matute et al., 2011), which can be considered to be beneficial bacteria for the intestinal environment. However, the total amount of acetic acid in the faeces of the dogs was not affected by the dietary treatment, and the effects of cellobiose on the faecal lactate concentrations were markedly more pronounced than for acetic acid. As the relative abundance of *Bifidobacteriaceae* did not differ among the groups, the high faecal lactate concentrations can likely be attributed to the increase in *Lactobacillaceae*, but seem to be not associated with an increased fermentation activity of bifidobacteria.

In view of the observed effects of dietary cellobiose on lactic acid producing bacteria, the present study might indicate some prebiotic potential of cellobiose in dogs. However, besides these effects, the impact of cellobiose on the faecal bacterial composition and metabolic activity was generally low. This is also underlined by the unaffected alpha and beta diversity of the faecal microbiota of the dogs. A dose-dependent increase was observed for the relative abundance of *Alloprevotella*, *Bacteroides* and *Prevotella*, and a dose-dependent decrease for *unidentified Lachnospiraceae*. Although

TABLE 7 Relative abundance (%) of bacterial phyla in the faeces of dogs (n = 10) fed without or with cellobiose (0 g/kg body weight (BW)/day; 0.5 g/kg BW/day; 1 g/kg BW/day)

	Cellobiose						Polynomial contrasts (p value)	
	0 g/kg BW/day	n[a]	0.5 g/kg BW/day	n	1 g/kg BW/day	n	Linear	Quadratic
Firmicutes	76.3 ± 10.7	(10)	81.0 ± 11.6	(10)	78.5 ± 10.2	(10)	0.346	0.321
Bacteroidetes	15.1 ± 8.26	(10)	12.5 ± 9.20	(10)	12.6 ± 5.38	(10)	0.354	0.666
Actinobacteria	3.88 ± 1.94	(10)	4.53 ± 3.30	(10)	4.95 ± 6.01	(10)	0.585	0.884
Fusobacteria	3.83 ± 3.34	(10)	1.52 ± 1.25	(10)	2.79 ± 2.66	(10)	0.331	0.057
Proteobacteria	0.91 ± 0.63	(10)	0.49 ± 0.51	(10)	1.10 ± 0.95	(10)	0.652	**0.042**

[a]Number of positive samples.

The bold values were statistically significant ($p < 0.05$).

TABLE 8 Relative abundance (%) of dominant bacterial families in the faeces of dogs (n = 10) fed without or with cellobiose (0 g/kg body weight (BW)/day; 0.5 g/kg BW/day; 1 g/kg BW/day)

	Cellobiose						Polynomial contrasts (p value)	
	0 g/kg BW/day	n[a]	0.5 g/kg BW/day	n	1 g/kg BW/day	n	Linear	Quadratic
Atopobiaceae	0.98 ± 1.32	(10)	0.96 ± 1.73	(10)	1.08 ± 2.18	(10)	0.907	0.799
Bacteroidaceae	3.74 ± 2.80	(10)	1.85 ± 2.08	(10)	1.58 ± 1.18	(10)	0.059	0.272
Bifidobacteriaceae	2.03 ± 1.64	(10)	2.06 ± 2.18	(10)	2.03 ± 2.00	(10)	0.998	0.971
Burkholderiaceae	0.81 ± 0.68	(10)	0.36 ± 0.49	(10)	0.88 ± 1.04	(10)	0.874	**0.039**
Coriobacteriaceae	0.45 ± 0.34	(10)	1.04 ± 1.11	(10)	1.07 ± 1.12	(10)	0.099	0.245
Erysipelotrichaceae	37.0 ± 24.8	(10)	19.7 ± 21.2	(10)	19.2 ± 11.6	(10)	0.073	0.115
Fusobacteriaceae	3.19 ± 2.59	(10)	1.47 ± 1.22	(10)	2.24 ± 1.89	(10)	0.338	0.072
Lachnospiraceae	12.3 ± 7.38	(10)	14.5 ± 9.02	(10)	9.69 ± 5.13	(10)	0.452	0.138
Lactobacillaceae	7.14 ± 11.1	(10)	26.6 ± 30.3	(10)	31.6 ± 25.4	(10)	**0.014**	0.463
Peptostreptococcaceae	9.66 ± 6.47	(10)	8.64 ± 5.46	(10)	7.09 ± 4.08	(10)	0.314	0.886
Prevotellaceae	8.49 ± 6.90	(10)	7.68 ± 5.68	(10)	9.57 ± 5.22	(10)	0.529	0.614
Ruminococcaceae	0.83 ± 1.14	(10)	1.10 ± 1.19	(10)	0.68 ± 0.59	(10)	0.698	0.433
Veillonellaceae	0.36	(2)	0.39 ± 0.61	(6)	1.17 ± 1.29	(8)	0.386	0.517

[a]Number of positive samples.

The bold values were statistically significant ($p < 0.05$).

it should be noted that the relative abundance of these genera was <1% in all feeding periods, it can be speculated that the observed changes might have been triggered by higher lactate concentrations in the intestine of the dogs or induced by other competitive effects from an increased Lactobacillus abundance.

Dietary cellobiose did not affect the relative abundance of *Bifidobacteriaceae* or *Clostridiaceae* in the faeces of the dogs. Both an increase of bifidobacteria or a decrease of pathogenic clostridia might be considered beneficial in terms of a prebiotic substance (Gibson et al., 2017). With regard to the *Clostridiaceae*, it should, however, be noted that the relative abundance in the faeces of the dogs was low in general (<1%), but the number of positive samples decreased with increasing doses of dietary cellobiose. Nevertheless, as the bacterial sequencing did not include the species level in the present study, the results do not allow for further information on the effects of cellobiose on clostridia in dogs. Future studies are necessary to support the present results also in diseased dogs, evaluating the relevance of the observed changes in the microbiota for intestinal health.

For data interpretation, it should be considered that research dogs were used in this study, kept under controlled housing and feeding conditions. Although a standardization of experiments is recommended to receive reliable results (Górska, 2000), there are also reports indicating that a standardization might contribute to poor reproducibility of results (Richter et al., 2009; Voelkl et al., 2018). Thus, further studies with private-owned dogs would be interesting to verify the present results under practical conditions, particularly considering variations in the feeding regimen or in animal related factors (e.g. age and health status).

As a limitation of this study, it should finally be noted that dietary cellobiose was supplemented in increasing doses, but no washout period or cross-over design was used. Therefore, it cannot be excluded that the first supplementation period (0.5 g cellobiose/kg BW/day) might have affected the subsequent supplementation

TABLE 9 Relative abundance (%) of dominant bacterial genera in the faeces of dogs (n = 10) fed without or with cellobiose (0 g/kg body weight (BW)/day; 0.5 g/kg BW/day; 1 g/kg BW/day)

	Cellobiose						Polynomial contrasts (p value)	
	0 g/kg BW/day	n[a]	0.5 g/kg BW/day	n	1 g/kg BW/day	n	Linear	Quadratic
Allobaculum 1	19.8 ± 13.9	(10)	7.47 ± 12.0	(9)	6.87 ± 6.14	(8)	0.107	0.349
Allobaculum 2	3.80 ± 1.73	(8)	2.56 ± 2.34	(4)	1.59 ± 1.01	(5)	0.132	0.631
Allobaculum 3	1.78 ± 0.61	(7)	1.46 ± 1.07	(3)	0.72 ± 0.44	(5)	0.158	0.649
Allobaculum 4	1.46 ± 0.62	(8)	0.93 ± 0.88	(4)	0.88 ± 0.67	(5)	0.533	0.990
Alloprevotella 1	0.55 ± 0.76	(6)	1.23 ± 1.14	(7)	1.24 ± 0.83	(8)	0.511	0.388
Alloprevotella 2	0.25 ± 0.11	(8)	0.61 ± 0.56	(10)	0.94 ± 0.97	(9)	**0.046**	0.918
Bacteroides 1	2.31 ± 2.02	(10)	0.92 ± 1.25	(8)	0.54 ± 0.51	(9)	0.073	0.196
Bacteroides 6	0.20 ± 0.10	(8)	0.28 ± 0.22	(10)	0.32 ± 0.16	(8)	**0.003**	0.796
Bifidobacterium 1	0.90 ± 0.36	(9)	1.62 ± 1.21	(8)	1.33 ± 1.38	(9)	0.561	0.615
Bifidobacterium 2	1.53 ± 1.35	(8)	1.26 ± 1.10	(6)	1.04 ± 0.60	(8)	0.368	0.955
Blautia 1	2.07 ± 1.13	(10)	3.28 ± 2.69	(10)	1.91 ± 1.09	(10)	0.710	0.126
Blautia 2	2.22 ± 2.36	(9)	2.19 ± 1.97	(10)	1.52 ± 0.93	(10)	0.350	0.401
Blautia 3	1.66 ± 1.08	(10)	1.83 ± 1.07	(10)	1.42 ± 1.22	(10)	0.646	0.462
Blautia 4	0.79 ± 0.56	(9)	1.18 ± 1.12	(10)	0.59 ± 0.45	(10)	0.279	0.412
Blautia 5	2.15 ± 1.83	(8)	0.72 ± 0.71	(9)	0.28 ± 0.26	(7)	0.111	0.691
Blautia 6	0.78 ± 0.38	(6)	1.03 ± 0.97	(8)	0.77 ± 0.45	(5)	0.490	0.716
Catenibacterium	2.73	(1)	0.47	(2)	5.99 ± 6.44	(7)	*	*
Fusobacterium 1	2.54 ± 2.33	(10)	1.13 ± 1.03	(10)	1.59 ± 1.53	(10)	0.283	0.125
Holdemanella	1.26 ± 2.07	(3)	1.08 ± 1.27	(8)	3.05 ± 3.57	(8)	0.673	0.668
Lactobacillus 1	7.16 ± 11.8	(6)	2.01 ± 4.33	(7)	1.89 ± 2.04	(9)	0.481	0.576
Lactobacillus 2	1.38 ± 1.18	(6)	6.84 ± 8.67	(8)	8.84 ± 9.97	(10)	0.281	0.461
Lactobacillus 3	5.05 ± 4.98	(4)	21.9 ± 24.2	(9)	23.4 ± 20.0	(9)	0.225	0.280
Megamonas	0.36	(2)	0.39 ± 0.61	(6)	1.17 ± 1.29	(8)	0.386	0.517
Peptoclostridium	8.02 ± 6.30	(10)	6.82 ± 5.23	(10)	6.30 ± 4.08	(10)	0.492	0.825
Prevotella 9_1	2.82 ± 2.33	(9)	2.33 ± 1.01	(7)	1.92 ± 1.50	(10)	0.117	0.697
Prevotella 9_3	1.49 ± 1.33	(9)	1.04 ± 0.49	(8)	1.34 ± 0.85	(10)	0.644	0.244
Prevotella 9_6	0.32 ± 0.18	(6)	0.39 ± 0.26	(8)	0.92 ± 0.62	(9)	**0.039**	0.198
Unidentified Atopobiaceae	0.90 ± 1.19	(6)	1.40 ± 1.63	(4)	1.30 ± 1.94	(5)	*	*
Unidentified Erysipelotrichaceae 1	9.60 ± 9.08	(8)	12.1 ± 12.8	(6)	6.51 ± 7.17	(6)	0.170	0.443
Unidentified Erysipelotrichaceae 2	1.32 ± 0.69	(4)	1.79 ± 0.41	(3)	1.12 ± 0.21	(3)	*	*
Unidentified Erysipelotrichaceae 3	1.33 ± 0.87	(9)	1.51 ± 2.27	(7)	0.42 ± 0.26	(7)	**0.050**	0.060
Unidentified Erysipelotrichaceae 4	1.13 ± 0.62	(4)	1.58 ± 0.51	(3)	0.92 ± 0.33	(3)	*	*
Unidentified Lachnospiraceae 1	0.66 ± 0.39	(10)	1.25 ± 0.77	(10)	0.48 ± 0.32	(9)	0.208	**0.016**
Unidentified Lachnospiraceae 2	0.71 ± 0.37	(9)	0.52 ± 0.35	(9)	0.30 ± 0.14	(10)	**0.011**	0.937
Unidentified Lachnospiraceae 5	1.45 ± 2.60	(8)	1.31 ± 1.94	(10)	1.56 ± 1.50	(10)	0.789	0.899

[a]Number of positive samples.

*Polynomial contrast could not be calculated.

The bold values were statistically significant ($p < 0.05$).

period (1 g cellobiose/kg BW/day). The intestinal microbiota might have adapted to dietary cellobiose at the lower supplementation dose to a certain degree, and the higher dose of cellobiose could have accelerated the effects induced in the preceded feeding period. Nevertheless, although linear effects were detected, many variables were already markedly changed when cellobiose was supplemented at 0.5 g/kg BW/day, and often no considerable difference was observed when cellobiose was subsequently supplemented at 1 g/kg BW/day. On one side, it can therefore be assumed that the lower dose of cellobiose was already adequate to induce changes in the composition and metabolic activity of the intestinal microbiota of dogs. On the other side, the results obtained when cellobiose was supplemented at 1 g/kg BW/day might also be considered to be reliable as long as this dose is fed for a sufficient time.

5 | CONCLUSION

Dietary cellobiose markedly increased faecal lactate concentrations and *Lactobacillaceae* in healthy adult dogs. It can be assumed that the high lactate concentrations in the large intestine could have affected the absorption of N metabolites, as reduced phenol and 4-ethylphenol concentrations were measured in the plasma of the dogs following the cellobiose treatment. Whether these effects of dietary cellobiose could be beneficial for diseased animals, particularly with intestinal disorders or impaired N metabolism, should be evaluated in future studies.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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