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Influence of dietary protein content and source on colonic fermentative activity in dogs differing in body size and digestive tolerance[1]

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ABSTRACT: Low-consistency, high-moisture feces have been observed in large dogs (*Canis lupus familiaris*), compared with small dogs, and particularly in sensitive breeds (e.g., German Shepherd dogs). The aim of this work was to determine if greater colonic protein fermentation is responsible for poorer fecal quality in large sensitive dogs. Twenty-seven bitches were allotted to 4 groups based on size and digestive sensitivity: small, medium, large tolerant, and large sensitive. Five experimental diets varying in protein source [highly digestible wheat gluten (WG) vs. medium digestible poultry meal (PM), and protein concentration from 21.4 to 21.6 (LP) to 38.2 to 39.2% CP (HP)] were tested. Diets were fed for 14 d and followed by a 12-d transition period. Digestive fermentation by-products were investigated in fresh stools [ammonia, phenol, indole, and short chain fatty acids including acetate, propionate, and butyrate (C2 to C4 SCFA), branched-chain fatty acids (BCFA), and valerate] and in urine (phenol and indole). Bacterial populations in feces were identified. The PM diets resulted in greater fecal concentrations of ammonia, BCFA, valerate, indole, and C2 to C4 SCFA than WG diets ($P = 0.002$, $P < 0.001$, $P = 0.039$, $P = 0.003$, and $P = 0.012$, respectively). Greater concentrations of ammonia, BCFA, and valerate were found in the feces of dogs fed HP compared with LP diets ($P < 0.001$, $P < 0.001$, and $P = 0.012$, respectively). The concentrations of ammonia, valerate, phenol, and indole in feces of large sensitive dogs were greater ($P < 0.001$, $P < 0.001$, $P = 0.002$, and $P = 0.019$, respectively) compared with the other groups. The *Enterococcus* populations were greater in feces of dogs fed with PMHP rather than WGLP diets ($P = 0.006$). Urinary phenol and indole excretion was greater when dogs were fed PM than WG diets ($P < 0.001$ and $P = 0.038$, respectively) and HP than LP diets ($P = 0.001$ and $P = 0.087$, respectively). Large sensitive dogs were prone to excrete a greater quantity of phenol in urine ($P < 0.001$). A diet formulated with highly digestible protein, such as WG, led to reduced concentrations of protein-based fermentation products in feces together with improved fecal quality in dogs, especially in large sensitive ones. Poor fecal quality in large sensitive dogs could be partly related to the pattern of protein fermentation in the hindgut.

Key words: bacteria, dietary protein, dog, fecal quality, fermentation products

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INTRODUCTION

Digestive tolerance, defined as the overall reaction of the animal to diet, can be assessed by determining fecal quality. Poor fecal quality (i.e., feces of low consistency with high moisture content), has been observed in large dogs compared with small dogs (Zentek

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and Meyer, 1995; Meyer et al., 1999; Weber et al., 2002), and in certain sensitive breeds such as German Shepherd dogs (Zentek et al., 2002; Nery et al., 2010), in the absence of pathological conditions and when fed the same diet. Previous studies in our facilities revealed that poor fecal quality in large dogs was related to greater fermentation in the hindgut (Hernot et al., 2003, 2006; Weber et al., 2004) and decreased overall absorption of electrolytes (Weber et al., 2002; Hernot et al., 2009).

Undigested protein reaching the canine colon can vary from 218 to 650 g/kg DM of ileum chyme (Zentek, 1995). The amount is dependent on protein digestibility (Wiernusz et al., 1995), protein concentration in the diet (Yamka et al., 2003), and DM intake (Hussein and Sunvold, 2000). Proteins undigested in the small intestine are subjected to putrefaction in the large intestine, leading to the accumulation of fermentation products such as ammonia, amines, phenol, indole and sulphides, branched-chain fatty acids (**BCFA**), short-chain fatty acids (**SCFA**), and gases (hydrogen, carbon dioxide, and methane), as well as intermediate products, such as lactate and succinate (MacFarlane and Cummings, 1991; Hughes et al., 2000). Some fermentation products, such as SCFA, are important energy sources for the colonic mucosa (Ichikawa and Sakata, 1998). Others like ammonia, amines, phenol, indole, and sulphides can have deleterious effects (MacFarlane et al., 1988; Gibson et al., 1989; Ichikawa and Sakata, 1998; Hussein et al., 1999; Hughes et al., 2000; Mouillé et al., 2004). The objectives of the present study were to determine whether poor fecal quality in large sensitive dogs was associated with increased protein fermentation in the hindgut.

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Use and Care Advisory Committee of the Nantes-Atlantic National College of Veterinary Medicine. Maintenance conditions complied with French Ministry of Agriculture and Fishing standards for the protection of laboratory animals.

Animals

Twenty-seven adult spayed female dogs (4.8 ± 0.5 yr old) of 6 different breeds were included in this study: 5 Miniature Poodles (4.0 ± 0.6 kg BW), 1 Jack Russell (4.0 kg), 1 Miniature Schnauzer (6.5 kg), 6 Standard Schnauzers (14.4 ± 0.3 kg), 6 Giant Schnauzers (27.2 ± 1.0 kg), and 8 German Shepherd dogs (23.1 ± 0.7 kg). The dogs were divided into 4 groups (Table 1), based on BW and propensity of the dogs to produce feces of low consistency with high moisture content. These groups were designated: small (**SMA**, i.e., Miniature Poodles, Jack Russell, and Miniature Schnauzer), medium (**MED**, i.e., Standard Schnauzers), large tolerant (**GRT**, i.e., Giant Schnauzers), and large sensitive (**GRS**, i.e., German Shepherd dogs). Sensitivity was assessed based on literature (Zentek et al., 2002), compared with Beagle dogs, and confirmed before the experimental protocol through visual observation of fecal consistency among the different groups included in the present study when fed the same diet (Size Nutrition Maxi Adult; CP 26%, fat 16%, total dietary fiber 6.9%, and ME 4180 kcal/kg on a DM basis; Royal Canin, Aimargues, France).

Table 1. Initial and final BW of dog groups

Dog group[1]	Breeds	Initial BW, kg	Final BW, kg
SMA	Miniature Poodles, Jack Russell, and Miniature Schnauzer	4.4 ± 0.5	4.8 ± 0.5
MED	Standard Schnauzers	14.4 ± 0.3	15.2 ± 0.3
GRT	Giant Schnauzers	27.2 ± 1.0	29.1 ± 1.2
GRS	German Shepherd dogs	23.1 ± 0.7	23.1 ± 0.9

[1]SMA = small dogs; MED = medium dogs; GRT = large tolerant dogs; and GRS = large sensitive dogs.

The dogs were housed at the Nantes-Atlantic National College of Veterinary Medicine (France) for the entire duration of the study. During adaptation to the diet, the dogs were housed either individually or in groups (from 2 to 4 animals) in boxes with outdoor access and were walked regularly whenever this was compatible with the protocol. During the test periods, the dogs were individually housed in pens with 1-way slatted floors, to permit collection of feces and urine and avoid coprophagy.

Dog health was assessed by regular clinical examination. Prophylactic treatments against intestinal worms were administered before each phase of the experimental protocol.

Diets

Five dry, nutritionally complete, extruded diets were tested. The diets were formulated to have the same energy content and similar fat, total dietary fiber (**TDF**), and ash contents, but to differ in protein source and concentration as well as starch quantity (Table 2). These diets were: i) wheat gluten as main protein source (45.5% of CP) and a low CP concentration (21.6% on a DM basis; **WGLP**), ii) wheat gluten as main protein source (72.4% of CP) and an increased CP concentration (38.2%; **WGHP**), iii) both wheat gluten and poultry meal as main protein sources (30.2% of CP originating from wheat gluten and 31.8% from poultry meal) and a medium CP concentration (28.6%; **WPMP**), iv) poultry meal as the main protein source (46.5% of CP) and a low CP concentration (21.4%; **PMLP**), and v) poultry

Table 2. Ingredients that contribute to dietary protein and nutritional analysis of diets

	Diet and protein source[1]				
Item	WGLP WG	WGHP WG	WPMP WG and PM	PMLP PM	PMHP PM
Ingredient, % of CP					
Wheat gluten	45.5	72.4	30.2	–	–
Poultry meal	–	–	31.8	46.5	74.4
Corn gluten	25.5	14.5	19.0	25.6	14.3
Corn	16.5	6.3	11.0	17.5	7.3
Yeast	2.5	1.4	1.9	2.5	1.4
Others[2]	9.9	5.3	6.0	7.9	2.7
Composition, %					
DM	92.0	89.7	91.5	90.3	89.9
Moisture	8.0	10.3	8.5	9.7	10.1
Composition, % on DM basis					
CP	21.6	38.2	28.6	21.4	39.2
Fat	19.6	16.6	17.6	17.6	17.8
NFE[3]	52.2	37.5	44.7	53.0	36.8
Ash	6.6	7.7	9.1	8.0	6.2
Na	0.42	0.47	0.37	0.38	0.39
K	0.95	1.04	1.03	0.82	0.58
Total dietary fiber	9.8	7.5	7.8	6.9	8.6
GE, kcal/g DM	5.2	5.4	5.2	5.0	5.2

[1]WGLP = low protein (LP) content with wheat gluten (WG) as main dietary protein source; WGHP = high protein (HP) content with WG as main dietary protein source; PMLP = LP content with poultry meal (PM) as main dietary protein source; PMHP = HP content with PM as main dietary protein source; and WPMP = medium protein content with WG and PM mix as dietary protein source.

[2]Others include flavoring substances, beet pulp, egg powder, and, except in PMHP diet, Lysine.

[3]NFE = calculated nitrogen-free extract.

meal as the main protein source (74.4% CP originating from poultry meal) and a high CP concentration (39.2%; **PMHP**). The main dietary protein ingredients are listed in Table 2.

The dogs were fed to maintenance with a daily energy allowance of 110 kcal ME kg/$BW^{(0.75)}$ and a protein intake of 10.21 ± 0.06 g/$BW^{(0.75)}$ for the PMHP and WGHP diets, 5.65 ± 0.03 g · kg^{-1} · $BW^{(-0.75)}$ for the PMLP and WGLP diets, and 7.38 ± 0.12 g · kg^{-1} · $BW^{(-0.75)}$ for the WPMP diet. Diet intake was recorded daily. Water was available ad libitum throughout the study. Before this study, the dogs had been fed a variety of commercial dry extruded diets (Royal Canin, Aimargues, France).

Study Design

The study was divided in 2 phases. In Phase I, the dogs were fed WGLP and PMHP diets in a cross-over design comprising a 7-d adaptation period followed by a 7-d test period. During the 12-d washout periods, dogs were fed a commercial diet (Size Nutrition Maxi Adult; Royal Canin, Aimargues, France). In Phase II, the dogs were fed WGHP, WPMP, and PMLP test diets in a modified Latin square design. Adaptation, test, and washout periods were similar to those described before.

Reagents

All reagents and standards were supplied by one company (Sigma Aldrich; Saint-Quentin Fallavier, France), except when otherwise specified. Mercury chloride (Merck S.A., Fontenay-sous-Bois, France) and Hibitane 5% (Regent Medical Overseas Ltd., Manchester, UK) were supplied by other companies.

Stool Collection and Preparation

Total feces production was recorded individually on a daily basis. During the test period, a total of 1 to 2 samples of feces were collected within 15 min of defecation to determine the concentration of fermentation products. The stools were homogenized and divided into 3 aliquots for ammonia, SCFA, indole, and phenol analysis. Feces were diluted to 1:5 (wt/vol) in perchloric acid (0.6 mol/L) for ammonia analysis, and to 1:10 (wt/vol) in mercury chloride (1 g/L) for SCFA analysis. The diluted samples were centrifuged at 855 × *g* for 20 min at 4°C. The supernatant was collected and kept at −20°C pending analysis. Samples for determination of phenol and indole concentrations were collected in test tubes and kept at −20°C until analysis. During Phase I, fecal samples were obtained from the rectum to determine bacterial counts.

Urine Collection

Urine excretion was collected individually on a daily basis. During the test period, 10% of the daily urine weight was pooled in flasks containing 2 mL of aqueous chlorhexidine gluconate solution (Hibitane 5%) and kept at 4°C. At the end of the test period, 10-mL aliquots of pooled urine were sampled and stored at −20°C until analysis for urinary phenol and indole concentrations.

Analysis of Fermentation Products

Samples for ammonia analysis were thawed, re-diluted to 1/5 (vol/vol), and centrifuged at 2,124 × *g* for 10 minutes at 4°C. The supernatant was collected and analyzed for ammonia content according to the method of Chaney and Marbach (1962). Briefly, 5 mL of Solution A (aqueous solution containing 10g/L of phenol, and 0.05g/L of sodium nitroprusside), and 5 mL of Solution B (aqueous solution containing 5 g/L of sodium hydroxide, and 0.42 g/L of sodium hypochlorite) were added to

0.5 mL of supernatant, and absorbance was read by spectrophotometry at 625 nm. Calibration curves were obtained using standard ammonium sulphate dilutions.

Short chain fatty acids, including acetate (**C2**), propionate (**C3**), and butyrate (**C4**; **C2 to C4 SCFA**), which are originated mainly from fermentation of carbohydrate and protein, and SCFA originated mainly from protein fermentation, including BCFA (isobutyrate, and isovalerate, and 2-methyl butyrate) and valerate, were analyzed by gas chromatography. Samples were thawed, and centrifuged at 2,124 × *g* for 10 min at 4°C, and the supernatant was collected for analysis. The internal standard was 4-methyl valerate. Samples were analyzed using a gas chromatograph (Hewlett Packard 6890; Hewlett Packard, Palo Alto, CA) equipped with a hydrogen flame ionization detector (**FID**) and a HP-FFAP polyethylene glycol TPA column (30 m × 530 µm ID, 1.0 µm film thickness; HP19195F-123, Hewlett Packard). The inlet temperature was 200°C and injection by pulsed splitless mode. The oven temperature program was 85°C initial temperature maintained for 0.1 min, increased by 25°C per min until 140°C and maintained for 3.5 min, and increased by 30°C per minute until 170°C maintained for 7 min. The carrier gas was helium and the FID temperature was 250°C.

Phenol (phenol, p-cresol, and 4-ethyl phenol) and indole (indole and skatole) were extracted according to Flickinger et al. (2003). Briefly, 2 g of feces were mixed with 5 mL of methanol, covered with parafilm, and incubated for 1 h at 4°C with frequent mixing. After centrifuging at 2,124 × *g* for 10 min at 4°C the supernatant was recovered and 5 mL of methanol were added to the pellet, mixed thoroughly, and kept for 1 h as described previously. A combination of both supernatants was then analyzed by gas chromatography for phenol and indole using a gas chromatograph as described before. The internal standard was 5-chloro indole. Phenol and indole were separated with an Rtx-5 amine column (30 m × 250 µm ID, 1.0 µm film thickness; Restek No. 12353; Restek, Lisses, France). Initial temperature of the inlet was 200°C and injection by splitless mode. Initial temperature of the oven was 85°C maintained for 2.0 min, and the temperature program included an increase by 10°C per min until 250°C maintained for 4.0 min. The carrier gas was helium and the FID temperature was 220°C.

Urine Analysis

Urinary phenol and indole were quantified by gas chromatography. The phenol and indole extraction method was similar to the method described for feces, with 5 mL of methanol being added to 5 mL of urine.

Bacterial Counts

Samples of at least 0.5 g of feces were mixed thoroughly in Ringer solution and diluted to 1:10 (wt/vol). The solution was then filtered and re-diluted to 10^{-3}, 10^{-5}, and 10^{-7}. Media were kept at 48°C and counting was done at 38°C. *Enterococcus* was determined on D-coccose gelose (bile esculine agar), *Escherichia coli* was determined on MacConkey agar without crystal violet solution, *Lactobacillus* was determined on rogosa agar, and *Clostridia* was determined on tryptone sulfite neomycin agar (**TSN**).

Statistical Analysis

The experimental unit was the individual dog. The WGLP and PMHP diets were tested in Phase I, whereas WGHP, WPMP, and PMLP diets were tested in Phase II as a crossover design. Data obtained from Phase I and Phase II were pooled. The independent variables were diet (WGLP, WGHP, WPMP, PMLP, and PMHP), protein source (wheat gluten, poultry meal, and a mixture of both), protein concentration (low, medium, and high), and dog group (SMA, MED, GRT, and GRS). The dependent variables included fecal score; fecal moisture content; fecal concentrations of ammonia, C2 to C4 SCFA, BCFA, valerate, indole, and phenol; urinary concentrations of phenol and indole; and bacterial counts. Statistical analyses were performed with a software (XLSTAT, v. 2007.5; Addinsoft, New York, NY). Results were subjected to the non-parametric Kruskal Wallis test. Dunn's procedure for multiple pairwise comparisons was applied whenever statistical significance was observed. Statistical significance was considered at $P < 0.05$ and a trend was considered at $P < 0.10$. Data are presented as mean ± SE.

Whenever an effect of diet was observed, the effect of protein source and protein concentration and the effect of each diet on each group of dogs were analyzed. When an effect of dog was observed, a statistical analysis was performed on each diet.

RESULTS

All dogs remained healthy throughout the study. The entire daily ration was consumed during the test periods. Weight variations between the beginning and end of the experimental protocol are shown in Table 1. Some dogs were replaced between Phase I and Phase II for non-nutritional reasons independent of the treatment in the present study. For this reason, the total number of animals was greater than that required by the study design (6 animals/group). For practical reasons, fresh feces could not be collected from all the animals in this

study. Therefore, the number of dogs fed each diet was 6 in all instances, except for SMA dogs fed WGHP, PMLP, and PMHP, and GRT and GRS fed WGHP (n = 5), and for SMA dogs fed WPMP (n = 4).

Fermentation Products in Feces

The concentrations of fermentation products in the feces on a DM basis are shown in Table 3. Ammonia, BCFA, valerate, phenol, indole, and C2 to C4 SCFA

Table 3. Fecal concentration of fermentation products for each diet and dog group

	Dog group[2]			
Item[1]	SMA	MED	GRT	GRS
Ammonia, mg/g DM				
WGLP	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.1^{A}	1.0 ± 0.2^{A}
WGHP	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.2^{A}	1.4 ± 0.3^{AB}
WPMP	0.9 ± 0.2^{ab}	1.1 ± 0.1^{ab}	0.7 ± 0.1^{AB,a}	1.5 ± 0.2^{AB,b}
PMLP	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.2^{A}	1.2 ± 0.2^{AB}
PMHP	1.2 ± 0.1^{a}	1.6 ± 0.2^{ab}	1.6 ± 0.1^{B,ab}	2.1 ± 0.2^{B,b}
BCFA, μmol/g fecal DM				
WGLP	4.8 ± 0.9^{A}	7.6 ± 2.2^{A}	7.5 ± 0.8^{A}	5.9 ± 1.0^{A}
WGHP	14.5 ± 1.7^{AB}	12.8 ± 1.4^{AB}	19.6 ± 1.9^{AB}	17.8 ± 1.6^{AB}
WPMP	17.1 ± 2.5^{B}	19.3 ± 0.9^{B}	22.2 ± 1.0^{B}	22.1 ± 2.1^{B}
PMLP	16.3 ± 2.1^{B}	21.0 ± 1.7^{B}	21.9 ± 2.4^{B}	20.2 ± 1.8^{B}
PMHP	12.4 ± 1.8^{AB}	21.3 ± 3.4^{B}	20.6 ± 1.4^{AB}	16.3 ± 4.1^{AB}
Valerate, μmol/g fecal DM				
WGLP	4.7 ± 0.2^{AB,ab}	5.0 ± 0.3^{AB,ab}	4.6 ± 0.2^{a}	9.8 ± 1.7^{AB,b}
WGHP	1.0 ± 0.3^{A,ab}	0.8 ± 0.3^{BC,a}	8.6 ± 5.3^{ab}	4.3 ± 1.7^{A,b}
WPMP	1.0 ± 0.1^{A}	0.9 ± 0.3^{BC}	3.0 ± 2.0	10.0 ± 3.5^{AB}
PMLP	0.7 ± 0.3^{A,ab}	0.7 ± 0.2^{C,a}	3.5 ± 1.7^{ab}	5.6 ± 2.2^{A,b}
PMHP	5.6 ± 0.3^{B,a}	5.5 ± 0.1^{A,a}	8.2 ± 2.0^{a}	22.0 ± 1.1^{B,b}
Phenol,[3] μmol/g fecal DM				
WGLP	0.0 ± 0.0	0.3 ± 0.2	0.0 ± 0.0	0.4 ± 0.2
WGHP	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
WPMP	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
PMLP	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5
PMHP	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2
Indole,[4] μmol/g fecal DM				
WGLP	0.9 ± 0.2	1.1 ± 0.3^{AB}	0.6 ± 0.1	1.2 ± 0.2
WGHP	0.8 ± 0.2	0.4 ± 0.2^{A}	0.9 ± 0.3	1.2 ± 0.4
WPMP	1.6 ± 0.6	1.3 ± 0.4^{AB}	0.6 ± 0.2	1.7 ± 0.5
PMLP	1.3 ± 0.4	1.2 ± 0.3^{AB}	0.7 ± 0.2	1.3 ± 0.5
PMHP	1.8 ± 0.2	2.0 ± 0.3^{B}	1.2 ± 0.2	1.7 ± 0.3
C2−C4 SCFA,[5] μmol/g fecal DM				
WGLP	232.5 ± 26.0	265.4 ± 41.4^{A}	270.4 ± 17.5	299.0 ± 60.0
WGHP	345.6 ± 41.8	381.0 ± 26.8^{AB}	367.4 ± 51.8	307.7 ± 21.2
WPMP	306.6 ± 22.3	392.2 ± 39.0^{AB}	364.0 ± 24.4	412.5 ± 23.3
PMLP	344.3 ± 38.4	433.1 ± 26.6^{B}	362.9 ± 31.8	389.2 ± 50.7
PMHP	251.7 ± 25.1	319.6 ± 20.8^{AB}	330.8 ± 22.0	265.2 ± 31.1

$^{A-C}$ Within a column, means without a common superscript letter differ ($P < 0.05$).

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]Diets: WGLP = low protein (LP) content with wheat gluten (WG) as main dietary protein source; WGHP = high protein (HP) content with WG as main dietary protein source; PMLP = LP content with poultry meal (PM) as main dietary protein source; PMHP = HP content with PM as main dietary protein source; and WPMP = medium protein content with WG and PM mix as dietary protein source.

[2]Dog groups: SMA = small dogs (mean BW, 4.4 ± 0.5 kg; n = 6 except for WGHP, PMLP, and PMHP, in which n = 5, and WPMP, in which n = 4); MED = medium dogs (14.4 ± 0.3 kg; n = 6); GRT = large tolerant dogs (27.2 ± 1.0 kg; n = 6 except for WGHP, in which n = 5); and GRS = large sensitive dogs (23.1 ± 0.7 kg; n = 6 except for WGHP, in which n = 5).

[3]Sum of phenol, p-cresol, and 4-ethylphenol concentrations. Concentrations considered as equal to zero were below the detection limit of the method used: 0.107 μmol/mL for phenol, 0.096 μmol/mL for p-cresol, and 0.080 μmol/mL for 4-ethylphenol.

[4]Sum of indole and skatole concentrations.

[5]Sum of acetate (C2), propionate (C3), and butyrate (C4) concentrations.

varied with diet ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.022$, $P = 0.002$, and $P < 0.001$, respectively), whereas ammonia, BCFA, valerate, phenol, and indole varied with dog group ($P < 0.001$, $P = 0.014$, $P < 0.001$, $P = 0.002$, and $P = 0.019$, respectively).

Greater concentrations of ammonia, BCFA, valerate, indole, and C2 to C4 SCFA were observed when dogs were fed poultry meal diets ($P = 0.002$, $P < 0.001$, $P = 0.039$, $P = 0.003$, and $P = 0.012$, respectively) compared with wheat gluten diets. High protein diets induced greater concentrations of ammonia, BCFA, and valerate ($P < 0.001$, $P < 0.001$, and $P = 0.012$, respectively) compared with low protein diets.

Fecal ammonia concentration was greater when dogs were fed the PMHP diet (1.6 ± 0.1 mg/g) compared with all the other diets (0.8 ± 0.1, 0.9 ± 0.1, 1.0 ± 0.1, and 0.9 ± 0.1 mg/g for WGLP, WGHP, WPMP, and PMLP, respectively). Total fecal BCFA concentration was lower when dogs were fed WGLP compared with all the other diets (6.5 ± 0.7 for WGLP, 16.0 ± 1.0 for WGHP, 20.5 ± 0.9 for WPMP, 20.0 ± 1.0 for PMLP, and 17.9 ± 1.6 μmol/g for PMHP). Fecal valerate concentration was greater in dogs fed PMHP (10.5 ± 1.6 μmol/g feces DM) than all the other diets (6.0 ± 0.6, 3.5 ± 1.4, 4.0 ± 1.3, and 2.7 ± 0.8 μmol/g for WGLP, WGHP, WPMP, and PMLP, respectively). Fecal indole concentration was greater when dogs were fed poultry meal diets (1.4 ± 0.1 μmol/g) compared with wheat gluten diets (0.9 ± 0.1). The fecal C2 to C4 SCFA concentration was greatest when dogs were fed WPMP and PMLP diets (374.5 ± 15.8 and 384.0 ± 19.0 μmol/g, respectively) compared with other diets (266.8 ± 19.1, 351.9 ± 18.0, and 293.6 ± 13.7 μmol/g for WGLP, WGHP, and PMHP, respectively).

Fecal ammonia concentration was greater in GRS (1.4 ± 0.1 mg/g) than in SMA and GRT dogs (0.8 ± 0.1 and 0.9 ± 0.1 mg/g). The GRT group presented a greater fecal BCFA concentration than SMA dogs (18.3 ± 1.3 and 12.5 ± 1.2 μmol/g, respectively), whereas fecal BCFA concentrations of MED and GRS dogs did not differ from those of the other groups (16.4 ± 1.3 and 16.4 ± 1.5 μmol/g). Fecal valerate concentration was greater in GRS dogs (10.5 ± 1.5 μmol/g) than in all other dog groups (2.7 ± 0.4, 2.6 ± 0.4, and 5.5 ± 1.1 μmol/g for SMA, MED, and GRT, respectively). No phenol were found in SMA and GRT feces, but phenol concentration was greater in GRS than in MED dogs (0.3 ± 0.1 and 0.1 ± 0.0 μmol/g, respectively). Differences in fecal indole concentrations were found between GRT and GRS dogs (0.8 ± 0.1 and 1.4 ± 0.2 μmol/g, respectively). However, fecal indole concentration did not differ between SMA, MED (1.2 ± 0.1 and 1.2 ± 0.2 μmol/g, respectively), and the other groups.

Fermentation Products in Urine

The urinary phenol and indole concentrations in relation to diet and dog group are shown in Table 4. The urinary

Table 4. Urinary excretion of fermentation products for each diet and dog group

Item[1]	Dog group[2]			
	SMA	MED	GRT	GRS
Phenol,[3] μmol · kg^{-1} · BW$^{(-0.75)}$				
WGLP	29.2 ± 10.3^{AB,ab}	18.8 ± 10.7^{AB,ab}	0.0 ± 0.0^{A,a}	92.0 ± 14.3^{AB,b}
WGHP	2.5 ± 2.5^{A}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	12.2 ± 7.8^{A}
WPMP	0.0 ± 0.0^{A}	5.2 ± 5.2^{A}	0.0 ± 0.0^{A}	25.0 ± 18.2^{A}
PMLP	10.7 ± 10.7^{A}	4.8 ± 4.8^{A}	0.0 ± 0.0^{A}	63.2 ± 20.3^{AB}
PMHP	143.4 ± 35.7^{B,ab}	83.4 ± 10.8^{B,a}	83.6 ± 7.8^{B,a}	306.3 ± 41.5^{B,b}
Indole,[4] μmol · kg^{-1} · BW$^{(-0.75)}$				
WGLP	10.1 ± 2.2	10.9 ± 5.9	42.1 ± 31.0	20.7 ± 11.0
WGHP	12.3 ± 1.8	22.2 ± 17.1	4.2 ± 4.2	1.2 ± 1.2
WPMP	23.3 ± 10.9^{a}	20.0 ± 11.1^{ab}	0.0 ± 0.0^{b}	0.2 ± 0.2^{b}
PMLP	54.9 ± 40.2^{a}	16.2 ± 9.5^{ab}	0.0 ± 0.0^{b}	3.8 ± 2.4^{ab}
PMHP	39.0 ± 6.6	50.2 ± 10.7	35.1 ± 12.0	37.7 ± 12.4

^{A,B} Within a column, means without a common superscript letter differ ($P < 0.05$).

^{a,b} Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]Diets: WGLP = low protein (LP) content with wheat gluten (WG) as main dietary protein source; WGHP = high protein (HP) content with WG as main dietary protein source; PMLP = LP content with poultry meal (PM) as main dietary protein source; PMHP = HP content with PM as main dietary protein source; and WPMP = medium protein content with WG and PM mix as dietary protein source.

[2]Dog groups: SMA = small dogs (mean BW, 4.4 ± 0.5 kg; n = 6 except for WPMP and PMLP, in which n = 5); MED = medium dogs (14.4 ± 0.3 kg; n = 6); GRT = large tolerant dogs (27.2 ± 1.0 kg; n = 6); and GRS = large sensitive dogs (23.1 ± 0.7 kg; n = 6).

[3]Sum of phenol, p-cresol and 4-ethylphenol concentrations. Concentrations considered as equal to zero were below the detection limit of the method used: 0.107 μmol/mL for phenol, 0.096 μmol/mL for p-cresol, and 0.080 μmol/mL for 4-ethylphenol.

[4]Sum of indole and skatole concentrations. Concentrations considered as equal to zero were below the detection limit: 0.092 μmol/mL for indole, and 0.079 μmol/mL for skatole.

excretion of phenol and indole varied with diet ($P < 0.001$) and dog group ($P < 0.001$ and $P = 0.001$, respectively).

Total phenol excretion varied with protein source ($P < 0.001$), being greater with poultry meal diets [88.5 ± 15.8 μmol · kg^{-1} · BW$^{(-0.75)}$] than with a mixed diet and wheat gluten diets [7.9 ± 5.1 and 19.3 ± 5.0 μmol · kg^{-1} · BW$^{(-0.75)}$, respectively]. Urinary phenol excretion also varied with dietary protein concentration ($P = 0.001$), being greater in high protein diets [78.9 ± 15.9 μmol · kg $^{-1}$· BW$^{(-0.75)}$] than in medium and low protein diets [7.9 ± 5.1 and 27.7 ± 5.9 μmol · kg^{-1} · BW$^{(-0.75)}$, respectively]. Urinary indole concentration was greater for poultry meal [29.1 ± 5.5 μmol · kg^{-1} · BW$^{(-0.75)}$] than with the mixed and wheat gluten diets (10.3 ± 4.2 and 15.4 ± 4.7; $P = 0.038$), and a trend towards greater indole excretion ($P = 0.087$) was observed for high protein [25.2 ± 4.0 μmol · kg^{-1} · BW$^{(-0.75)}$] compared with medium and low protein diets [10.3 ± 4.2 and 19.1 ± 6.2 μmol · kg^{-1} · BW$^{(-0.75)}$, respectively], independently of dog group.

Phenol excretion was greater in GRS dogs [99.7 ± 22.1 μmol · kg^{-1} · BW$^{(-0.75)}$] than in other dog groups [39.4 ± 13.1, 22.4 ± 6.6, and 16.7 ± 6.4 μmol · kg^{-1} · BW$^{(-0.75)}$ in SMA, MED, and GRT dogs, respectively]. Urinary indole excretion was greater in small dogs [27.1 ± 7.6 and 23.9 ± 5.4 μmol · kg^{-1} · BW$^{(-0.75)}$, for SMA and MED dogs, respectively] than in large dogs [16.3 ± 7.1 and 12.7 ± 4.1 μmol · kg^{-1} · BW$^{(-0.75)}$, for GRT and GRS dogs, respectively]. Both the fecal concentration and total urinary excretion of phenol were minimal in GRT dogs compared with GRS dogs of similar BW.

Bacterial Counts

The bacterial counts are shown in Table 5. *Enterococcus* counts were influenced by diet ($P = 0.006$) and *Clostridium perfringens* counts by dog group ($P < 0.001$). The *Enterococcus* population on an as-is basis was greater with PMHP (7.0 ± 0.3 log cfu/g feces) compared with the WGLP diet (5.9 ± 0.3 log cfu/g feces). Differences in fecal *Enterococcus* counts were apparent in GRT and GRS dogs fed the 2 diets ($P = 0.016$ and $P = 0.004$, respectively), but not in SMA and MED dogs. Fecal counts of *Clostridium perfringens* differed between SMA and GRT dogs (5.9 ± 0.3, and 3.8 ± 0.3 log cfu/g feces, respectively), but not when compared with MED and GRS (5.1 ± 0.2, and 4.7 ± 0.3 log cfu/g feces, respectively).

Comparative variation and relative *P*-values of fecal quality, fecal and urinary fermentation products, and bacterial counts are presented in tables 6 and 7.

DISCUSSION

Large dogs and certain breeds, such as German Shepherd dogs, are prone to producing soft feces with high moisture content (Zentek and Meyer, 1995; Meyer et al., 1999; Zentek et al., 2002; Nery et al., 2010). Previous studies in our facilities revealed that poor fecal quality in large dogs, in particular Great Danes, was related to: greater fermentation in the hindgut, partly explained by the greater relative surface and volume of the large intestine (Hernot et al., 2003), longer transit time in the large intestine (Hernot et al., 2006), and greater concentration of fermentation products (SCFA

Table 5. Bacteria counts (log CFU/g feces as-is) on selective media for each diet and dog group

	Dog group[2]			
Item[1]	SMA	MED	GRT	GRS
Clostridium perfringens				
WGLP	6.2 ± 0.3^{a}	5.2 ± 0.3^{ab}	3.6 ± 0.3^{b}	4.4 ± 0.5^{ab}
PMHP	5.6 ± 0.5	5.0 ± 0.4	3.9 ± 0.5	5.1 ± 0.4
Lactobacillus				
WGLP	6.6 ± 0.9	7.0 ± 0.9	5.8 ± 0.8	6.4 ± 0.8
PMHP	5.2 ± 1.3	7.7 ± 0.4	6.2 ± 0.8	8.7 ± 0.4
Enterococcus				
WGLP	7.2 ± 0.6	6.3 ± 0.7	5.0 ± 0.5^{A}	5.0 ± 0.5^{A}
PMHP	6.1 ± 0.9	7.0 ± 0.4	6.8 ± 0.5^{B}	7.8 ± 0.3^{B}
Escherichia coli				
WGLP	5.9 ± 0.6	7.0 ± 0.3	5.7 ± 0.6	5.9 ± 0.4
PMHP	6.2 ± 0.6	6.3 ± 0.3	6.4 ± 0.5	6.4 ± 0.3

^{A,B}Within a column, means without a common superscript letter differ ($P < 0.05$).

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]Diets: WGLP = low protein (LP) content with wheat gluten (WG) as main dietary protein source; WGHP = high protein (HP) content with WG as main dietary protein source; PMLP = LP content with poultry meal (PM) as main dietary protein source; PMHP = HP content with PM as main dietary protein source; and WPMP = medium protein content with WG and PM mix as dietary protein source.

[2]Dog groups: SMA = small dogs (mean BW, 4.4 ± 0.5 kg; n = 6 except for PMHP, in which n = 5); MED = medium dogs (14.4 ± 0.3 kg; n = 6); GRT = large tolerant dogs (27.2 ± 1.0 kg; n = 6); and GRS = large sensitive dogs (23.1 ± 0.7 kg; n = 6).

and lactate) in the feces (Weber et al., 2004), and (ii) lower overall absorption of electrolytes because of greater permeability in the small (Weber et al., 2002) and large intestine (Hernot et al., 2009).

Many hypotheses on how fermentation affects fecal quality have been proposed. Previous studies indicated that large dogs have greater fecal osmolarity, which could induce a net secretion of water into the gut lumen through osmotic pressure (Weber et al., 2004). Another hypothesis concerns immune responses to activity of the colonic microflora, which could affect colonic permeability and, thus, water absorption. This has been observed in pathological conditions, such as inflammatory bowel disease (Swerdlow et al., 2006). Finally, other alterations of mucosa integrity and structure might influence the absorption of water and electrolytes. For example, ammonia infusion into the colonic mucosa (Ichikawa and Sakata, 1998) and high protein diets and increased sulfur and sulfate consumption (Blachier et al., 2007) were found to affect mucosa integrity and structure.

Thus, nutritional strategies to decrease fermentative activity in the colon should improve fecal quality in large and sensitive dogs. This could be achieved by decreasing the quantity of undigested nutrients, such as polysaccharides and proteins, reaching the colon (Zentek, 1995). Previous studies in our laboratory, using a range of dietary fiber contents (from 8.3 to 10.5% of DM) and sources (fermentable to non-fermentable fiber ratio from 0.15 to 0.48) within the limits of nutritionally complete, commercially available diets for adult dogs, showed that changing the dietary fiber content had little effect on fecal score and moisture content in dogs differing in body size (Hernot, 2005). Nevertheless, these studies confirmed that fecal score provided an appropriate indication of fecal consistency and this score is widely used to determine fecal quality.

Therefore, decreasing the protein content or feeding highly digestible proteins could represent further solutions to improve fecal quality in large sensitive dogs. The TDF content of the experimental diets in this study ranged from 6.9% in PMLP to 9.8% in WGLP, which is similar to the range of dietary fibers studied previously (Hernot, 2005). The results obtained in the present study were, therefore, considered not to be influenced by dietary fiber content.

The aim of the present work was to determine the effect of protein source and dietary concentration on fermentative activity in animals that are prone to producing feces of poor quality. Fermentation products in feces and urine and bacterial populations in the feces of dogs fed different protein sources and amounts were examined in the present study to determine whether poorer fecal quality might be due to greater fermentation activity. As numerous fermentation products derived from colonic fermentation are partially absorbed by the colonic mucosa, the fecal concentration of fermentation products is determined by both fermentation activity and mucosal capacity for absorption and permeability. This limitation of the present study could have been overcome by using in situ measurements or post-mortem examinations, but would have required invasive procedures.

Effect of Protein Source

Wheat gluten is a highly digestible protein source with an apparent digestibility of 93.8% (Wiernusz et al., 1995). The protein digestibility of the wheat gluten diets used in the present study ranged between 87.9 ± 0.5 and 91.9 ± 0.3% for WGLP and WGHP (Nery et al., 2010). The well described wheat gluten-sensitive enteropathy concerns only a family of Irish Setters (Hall and Batt, 1992; Garden et al., 2000), which makes wheat gluten an adequate protein source for most dogs, provided that AA supplementation of diets is adequate to meet dog nutritional requirements. The high digestibility of wheat gluten protein makes it an interesting alternative protein source to decrease the amount of protein reaching the colon in dogs. Poultry meal is a common protein source in commercial pet food and its CP digestibility varies considerably, ranging from 77 to 90% depending on the process and producer (Zuo et al., 1996; Murray et al., 1997).

The dogs in the present study had been previously fed a variety of dry and canned diets, including the test protein sources, without presenting wheat gluten intolerance, diarrhea, or adverse reactions to any protein source used, and were therefore considered to not be allergic to these proteins. Corn gluten was the second most important protein source in the test diets. We considered that this protein source did not influence the obtained results because: i) its concentration was lessthan that of the main protein sources, and ii) corn gluten is a highly digestible protein (CP apparent digestibility of corn gluten = 93.8 ± 0.2%; Zentek, 1995).

Previous studies in our facilities, in which the same diets were fed to the same animals as in the present study, revealed that fecal score and moisture content were greater in dogs fed the PMHP (3.51 ± 0.07 and 66.6 ± 0.5%, respectively) than the WGLP diets (2.75 ± 0.09 and 61.5 ± 0.7%, respectively; Tables 6 and 7; Nery et al., 2010). Briefly, fecal score was measured using a 5-point visual scale, in which a score of 1 corresponded to hard and dry feces, 5 to liquid diarrhea, and 2.5 to optimal fecal score with well-formed stools that are easy to collect, but not too dry.

The fecal concentrations of most fermentation products (ammonia, BCFA, valerate, indole, and C2 to C4 SCFA), urinary phenol and indole excretion, and the bacterial counts of *Enterococcus* were also greater when dogs were fed poultry meal compared with wheat

Table 6. Effect of dog size and dietary protein source and content on fecal and urinary concentrations of fermentation and bacterial counts

	P-values		
Item	Source	Content	Dog size
Fecal quality[1]			
Fecal score	<0.001	0.004	<0.001
Fecal moisture	<0.001	ns[2]	<0.001
Fermentation products in feces			
Ammonia	0.002	<0.001	<0.001
BCFA[3]	<0.001	<0.001	0.014
Valerate	0.039	0.012	<0.001
Phenol	ns	0.080	0.002
Indole	0.003	ns	0.019
C2−C4 SCFA[4]	0.012	0.047	0.082
Fermentation products in urine			
Phenol	<0.001	0.001	<0.001
Indole	0.038	0.087	0.001
Bacterial counts			
Clostridium perfringens	ns		<0.001
Lactobacillus	ns		ns
Enterococcus	0.006		ns
Escherichia coli	ns		ns

[1]Nery et al. (2010).

[2]ns = not statistically different ($P > 0.10$).

[3]BCFA = branched-chain fatty acids.

[4]C2−C4 SCFA = sum of acetate (C2), propionate (C3), and butyrate (C4) concentrations of short-chain fatty acids.

Table 7. Main variations of fecal quality, fecal and urinary fermentation products and bacterial counts[1]

	Protein source			Protein content			Dog group			
Variable[2]	WG	WP	PM	LP	MP	HP	SMA	MED	GRT	GRS
Fecal quality[3]										
Score		↑↑			↑↑				↑↑	
Moisture		↑↑			↑				↑↑	
Fermentation products in feces										
Ammonia		↑↑			↑↑				↑↑	
BCFA[4]		↑↑			↑↑				↑↑	
Valerate		↑↑			↑↑				↑↑	
Phenol		⇔			⇔				↑↑	
Indole		↑↑			⇔				↑↑	
C2 to C4 SCFA[5]		↑↑			⇔				⇔	
Fermentation products in urine										
Phenol		↑↑			↑↑				↑↑	
Indole		↑↑			⇔				↓↓	
Bacterial counts										
Clostridium perfringens		⇔							↓↓	
Lactobacillus		⇔							↑	
Enterococcus		↑↑							⇔	
Escherichia coli		⇔							⇔	

[1]WG = wheat gluten diets; WP = wheat gluten and poultry meal diet; PM = poultry meal diets; LP = diets with low protein content (21.4 to 21.6% on a DM basis); MP = diets with medium protein content (28.6% on a DM basis); HP = diets with high protein content (38.2 to 39.2% on a DM basis); SMA = small dogs (mean BW, 4.4 ± 0.5 kg); MED = medium dogs (14.4 ± 0.3 kg); GRT = large tolerant dogs (27.2 ± 1.0 kg); and GRS = large sensitive dogs (23.1 ± 0.7 kg).

[2]↑↑ or ↓↓ indicates increase or decrease in variable ($P < 0.05$) from WG to PM, LP to HP, and SMA to GRS dogs, or for bacterial count, from WGLP to PMHP and SMA to GRS dogs; ⇔ indicates no variation of variable; ↑ indicates trend of variable increase from WG to PM, LP to HP, and SMA to GRS dogs, or for bacterial count, from WGLP to PMHP and SMA to GRS dogs.

[3]Nery et al. (2010).

[4]BCFA = branched-chain fatty acids.

[5]C2 to C4 SCFA = sum of acetate (C2), propionate (C3), and butyrate (C4) concentrations of short-chain fatty acids.

gluten diets, similar to the observations on fecal score and moisture. As bacterial counts were only obtained for the 2 diets tested during Phase I, it cannot be said whether the described variations were related to the dietary protein source or to protein concentration. The concentrations of protein-derived fermentation products such as ammonia, BCFA, and indole indicated that greater protein putrefaction may have occurred when dogs were fed poultry meal diets compared with wheat gluten diets. The fecal BCFA concentration is a marker of protein fermentation in the hindgut (Blachier et al., 2007). The greater protein fermentation activity in the present study is likely related to a greater flow of undigested protein in the colon of dogs fed diets based on poultry meal. Indeed, previously, researchers observed that protein sources with lower ileal CP digestibility induced a greater percentage of large intestine digestibility relative to total digestibility in dogs (Meyer et al., 1989). Although the protein content of chyme entering the colon was not measured in the present study, it was observed in another study, and the authors reported that there was less protein in the ileal chyme when dogs were fed more digestible protein such as corn gluten meal rather than greaves (Zentek, 1995). Dietary protein is a major precursor of urinary phenols (Bakke, 1969). The fecal phenol concentration, except in GRS dogs, was often very low or undetectable with the methods applied, as phenols are mostly absorbed and excreted in the urine. Other fermentation products, such as valerate or C2 to C4 SCFA, could indicate a greater fermentation of both polysaccharides and proteins, which are substrates for those fermentation products. However, the carbohydrate concentration in diets differed, whereas protein source remained constant, so the increased fecal valerate and C2 to C4 SCFA concentrations should be due to increased protein fermentation. A greater concentration of fermentation products leads to increased osmotic pressure in the colonic lumen, and possibly to osmotic diarrhea. The overall greater concentration of fermentation products produced in dogs fed poultry meal diets could, therefore, have had some effect on fecal quality, resulting in a greater fecal score and moisture content. Intestinal *Enterococci* have been linked to several negative effects,

including diarrhea, liver damage, encephalopathy, and cancer in humans (Rastall, 2004). However, the prevalence of colorectal tumors in dogs is very low (9.9/10,000 cases; Guilford and Strombeck, 1996). Using highly digestible wheat gluten as protein source led to an overall decreased concentration of fermentation products in the feces and *Enterococci* counts. Thus, when formulating a diet, the digestibility of the protein sources is an important factor to be considered in improving fecal quality in dogs of different body sizes and digestive tolerance (Nery et al., 2010), partially because it determines lower protein putrefaction in the hindgut.

Effect of Protein Concentration

Protein concentration varies from 18 to 40% in dietary formulas for healthy adult dogs. High protein diets may result in greater amounts of undigested protein reaching the colon compared with low protein diets. This could lead to greater protein putrefaction in the hindgut. However, to our knowledge, only limited data are available in the literature on the influence of protein quantity on digestive tolerance-related characteristics in dogs (Zentek, 1995; Zuo et al., 1996; Murray et al., 1997). The diets used in the present study were considered as low, medium, and high protein for CP concentrations of about 20, 30, and 40% (as-is), respectively. The protein concentration was increased by decreasing nitrogen-free extract in the diet formulation. Therefore, a potential bias might have occurred because fecal fermentation products (C2 to C4 SCFA) can be derived from both protein and carbohydrates.

Feeding high protein diets led to greater fecal concentrations of ammonia, BCFA, and valerate. Urinary phenol excretion was also greater in dogs fed a high rather than low protein diet, and urinary indole excretion varied numerically. Nery et al. (2010) observed greater fecal score in dogs fed the same diets used in the present study, which could be explained by the concentration of the aforementioned protein-derived fermentation products in the feces and urine. However, the lower impact of dietary strategy on the fecal concentrations of fermentation products in dogs fed diets with different protein concentrations indicates that, if the aim is to decrease fermentation in the hindgut, the choice of protein source used in the dietary formula is of greater importance than protein concentration.

Effect of Dog Size and Digestive Tolerance

In a previous study in our facilities (Nery et al., 2010), large sensitive dogs were found to produce feces with greater fecal score and moisture (3.58 ± 0.05 and 66.1 ± 0.5%, respectively) than the other breeds, particular in the small dogs (2.59 ± 0.09 and 60.8 ± 0.9%, respectively). Earlier studies (Weber et al., 2004) had indicated that greater concentrations of SCFA and lactate observed in the feces of Great Danes, compared with dogs of smaller BW and size, were related to poorer fecal quality in large dogs. In the present study, fecal concentration of C2 to C4 SCFA was not greater in GRS than in other dogs. This could indicate that the causes of low digestive tolerance in Great Danes were different from those observed in German Shepherd dogs. This is supported by the fact that BCFA, which is considered as a marker of protein fermentation, did not differ between the dog groups. However, fecal concentration of ammonia, valerate, phenol, and indole, and urinary concentration of phenol were greater in GRS dogs than in the other groups. Because BCFA are derived from the fermentation of specific amino acids (Leu, Ile, and Val), the greater concentrations of other less specific, protein-derived fermentation products could indicate that protein putrefaction was actually more pronounced in GRS than in GRT, MED, or SMA dogs. The greater concentration of these fermentation products in dogs producing feces of poorer quality might, therefore, indicate that protein fermentation is, at least partially, responsible for the poor feces quality observed in GRS dogs. Greater *Clostridium perfringens* counts were observed in SMA dogs. This result was unexpected because the *C. perfringens* population is known to be involved in putrefaction in the colon (Rastall, 2004). Therefore, greater *C. perfringens* counts would rather be expected in dogs excreting greater concentrations of protein-derived fermentation products.

Conclusions

It can be concluded that, to some extent, undigested protein degradation in the colon would be responsible for the decreased fecal quality in GRS dogs. Therefore, nutritional strategies based on manipulation of the protein source and concentration would be adequate to improve fecal quality in large and sensitive dogs. In particular, using highly digestible protein sources, such as wheat gluten, and decreasing the crude dietary protein content could have beneficial effects on fermentation phenomena in the hindgut, and consequently on digestive tolerance. Further investigations of the factors influencing fecal quality and the impact of colonic fermentative activity will be necessary to develop additional nutritional strategies to improve fecal quality in large and sensitive dogs.

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