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Influence of dietary protein content and source on fecal quality, electrolyte concentrations, and osmolarity, and digestibility in dogs differing in body size¹

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ABSTRACT: When fed the same diet, large-breed dogs tend to produce feces of poorer quality compared with small-breed dogs. Moreover, German shepherds, although having a BW similar to Giant Schnauzers, are particularly prone to digestive intolerance, producing feces of poor consistency and increased moisture. Digestive tolerance reflects the reaction of the animal to the diet, and it can be assessed by determining fecal quality (consistency, moisture, volume, odor, and color). This study was conducted to assess the effect of protein source and content on fecal quality, and to determine whether greater digestibility and lesser fecal osmolarity and electrolyte concentrations are associated with improved fecal quality in dogs differing in body size and digestive tolerance. Twenty-seven healthy female dogs were divided into 4 groups according to BW and digestive tolerance: small, medium, large tolerant, and large sensitive. Five diets, varying in protein source (wheat gluten, poultry meal, and a 50:50 mixture of both sources) and concentration (22, 29, and 39% CP)on a DM basis for low, medium, and high, respectively) were tested. The present study was divided in 2 phases: 2 diets were studied in a crossover design in phase I,

and 3 diets were studied in a Latin square design in phase II. Diets were fed for 14 d, followed by a 12-d transition period. Fecal score (1 = dry and hard feces,to 5 = liquid diarrhea), moisture, electrolytes (Na and K), and osmolarity, and digestibility of DM, energy, fat, CP, and ash were determined. Fecal score and moisture (P < 0.001) were less and overall digestibility (P < 0.001)0.001 for DM, CP, fat, ash, and energy) was greater for wheat gluten than for poultry meal diets. Large dogs had the greatest fecal score and moisture (P < 0.001), together with the greatest overall digestibility (P <0.001 for DM, P = 0.054 for CP, P = 0.005 for ash, and P = 0.003 for energy). Osmolarity was less for wheat gluten-based diets (P < 0.001), and was not affected by dog size. Fecal electrolyte concentration varied mainly with dog group (P = 0.005 for Na, and P < 0.001for K), being greater in large sensitive dogs compared with small dogs. Wheat gluten was proved to be a suitable protein source for modulating fecal quality in dogs, particularly in sensitive breeds. Poorer fecal quality in large sensitive dogs can be related to greater digestibility and greater fecal electrolyte concentrations, but not to fecal osmolarity.

Key words: diarrhea, dog, fecal quality, feces, potassium, sodium

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INTRODUCTION

Digestive tolerance reflects the reaction of the animal to the diet, although different authors may have

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used different terms such as gastrointestinal tolerance (Knapp et al., 2008) or nonspecific dietary sensitivity (Pascher et al., 2008). Digestive tolerance can be assessed by determining fecal quality (consistency, moisture, volume, odor, and color), and several studies have shown that there is a wide range of digestive tolerance levels among dogs, especially related to body size, breed, or both (Meyer et al., 1999; Weber et al., 2002). Large-breed dogs are prone to producing feces of poor quality (Zentek and Meyer, 1995; Meyer et al., 1999), which are typically not well formed, contain more water, and seem to be diarrheal in some instances. Two main explanations have been proposed for this: reduced overall absorption of electrolytes, or greater fermenta-

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tion in the hindgut (Weber et al., 2002, 2004; Hernot et al., 2003, 2005b). Additionally, colonic fermentation is related to water and electrolyte absorption because of the greater osmotic potential of its products (Weber et al., 2004). Protein constitutes a large proportion of the diet, and the ileal digestibility of different sources varies widely (Wiernusz et al., 1995; Zentek, 1995; Murray et al., 1997). Protein flow in the colon depends on DM and CP intakes and on CP digestibility (Hussein and Sunvold, 2000). Using highly digestible protein sources would therefore allow a reduction in dietary CP, resulting in decreased protein entering the colon.

The objectives of this study were 1) to determine whether the amount and source of dietary protein affects fecal quality in dogs differing in body size and digestive tolerance, and 2) to confirm whether better fecal quality is related to greater apparent digestibility, decreased fecal electrolyte concentration, and decreased fecal osmolarity in large and digestively sensitive dogs fed a diet containing high protein quality, low protein content, or both.

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Use and Care Advisory Committee of Nantes Veterinary School before the study began. Maintenance conditions complied with French Ministry of Agriculture and Fisheries standards for the protection of laboratory animals.

Animals

Twenty-seven healthy adult spayed female dogs of 6 different breeds, including 5 Miniature Poodles (initial BW, 4.0 ± 0.6 kg), 1 Jack Russell (4.0 kg), 1 Miniature Schnauzer (6.5 kg), 6 Standard Schnauzers (14.4 \pm 0.3 kg), 6 Giant Schnauzers $(27.2 \pm 1.0 \text{ kg})$, and 8 German Shepherds $(23.1 \pm 0.7 \text{ kg})$, were included in this study. Mean age at the beginning of the study was 4.8 ± 0.5 yr. Previous studies (Zentek et al., 2002) and observations in our facilities revealed that German Shepherds are particularly prone to digestive intolerance, despite having BW similar to Giant Schnauzers. For this reason, dogs were divided into 4 groups based on their BW and propensity to produce feces of poor quality: small (SMA, including Miniature Poodles, the Jack Russell, and the Miniature Schnauzer; 4.4 ± 0.5 kg), medium (MED, including Standard Schnauzers; 14.4 ± 0.3 kg), large tolerant (**GRT**, including Giant Schnauzers; 27.2 \pm 1.0 kg), and large sensitive (**GRS**, including German Shepherds; 23.1 ± 0.7 kg). Comparison between small and large dogs consisted of SMA and MED vs. GRT and GRS.

Clinical examinations were performed at the beginning of the study to ensure that the dogs were in good health, and health status was monitored throughout the study. Prophylactic treatments against intestinal worms were routinely performed according to the health management schedule of the kennel. Before the study, dogs were fed a variety of canned and dry extruded, nutritionally complete, commercially available diets (Royal Canin, Aimargues, France). Water was available ad libitum throughout the study. The dogs were housed at the National Veterinary School of Nantes for the entire duration of the study. During the adaptation periods, dogs were housed in groups, whereas they were housed individually during the test periods. As consistently as possible with the protocol, dogs were walked regularly and had access to outdoor pens.

Diets

Five dry extruded, nutritionally complete diets varying in protein source (wheat gluten, WG; poultry meal, PM; and a 50:50 mixture of both sources, WP) and protein concentration [low (LP), 22% CP; medium (MP), 29% CP; and high (HP), 39% CP on a DM basis], were tested (Tables 1 and 2): 1) WGLP with WG (45.5% of CP), and LP (21.6%); 2) WGHP with WG (72.4% of CP), and HP (38.2%); 3) PMLP with PM (46.5% of CP), and LP (21.4%); 4) PMHP with PM (74.4% of CP), and HP (39.2%); and 5) WPMP, which contained a mixture of WG (30.2% of CP) and PM (31.8% of CP), and MP (28.6%).

The main ingredients contributing to the dietary CP content are summarized in Table 1. The dogs were fed to maintenance, with a daily allowance of 110 kcal of ME/kg of BW^{0.75}; mean daily protein intake (g/kg of BW^{0.75}) was 10.21 \pm 0.06 for the PMHP and WGHP diets, 5.65 \pm 0.03 for the PMLP and WGLP diets, and 7.38 \pm 0.12 for the WPMP diet. Daily Na intake was 111 \pm 0, 124 \pm 1, 96 \pm 2, 99 \pm 1, and 103 \pm 0 mg/kg of BW^{0.75} for diets WGLP, WGHP, WPMP, PMLP, and PMHP, respectively. Daily K intake was 249 \pm 1, 274 \pm 3, 265 \pm 4, 215 \pm 2, and 152 \pm 0 mg/kg of BW^{0.75} for diets WGLP, WPMP, PMLP, and PMHP, respectively. Food intake was recorded daily for each individual dog.

Study Design

The study was divided into 2 phases. In phase I, the dogs were divided into 2 groups, each including 3 dogs of each category (SMA, MED, GRT, and GRS); the WGLP and PMHP diets were fed in a crossover design for 14 d each. Between diets, there was a transition period of 12 d, during which the dogs were fed a commercially available MP diet (Size Nutrition Maxi Adult, Royal Canin; 26% CP, 16% fat, 6.9% total dietary fiber, and 4,180 kcal of ME/kg on a DM basis). In phase II, the dogs were divided into 6 groups of 1 dog for each category (SMA, MED, GRT, and GRS), and the WGHP, WPMP, and PMLP test diets were fed in a modified Latin square design, each for 14 d, with 12-d transition periods as before. Because some dogs were replaced between phase I and II, the total number

Table 1. Proportion of ingredients (%) contributing to CP of diets¹ (DM basis)

| Item | WGLP | WGHP | WPMP | PMLP | PMHP |
|-------------|------|------|------|------|------|
| CP, % | 21.6 | 38.2 | 28.6 | 21.4 | 39.2 |
| Ingredient | | | | | |
| WG | 45.5 | 72.4 | 30.2 | | |
| PM | | | 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 | 10.0 | 5.3 | 6.0 | 7.9 | 2.7 |

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

of animals was greater than required according to the study design.

Collection of Feces

During fecal collection periods, the dogs were individually housed in pens with a 1-way slatted floor equipped with a collection tray to reduce contamination of samples and to avoid coprophagy. Throughout the second week of each 14-d experimental period (with the first week being considered the diet adaptation period), feces were individually collected. Fecal samples were pooled daily and stored at 4°C; feces voided during the night were collected immediately the next morning. At the end of each collection period, feces were homogenized and stored at -20° C until analysis (within 1 wk) for digestibility calculations. Additionally, 1 to 2 samples of fresh feces (5 g) were collected within 15 min of defecation during the day for fecal osmolarity and electrolyte concentration analyses. These samples were prepared (as described later), stored immediately at -20° C, and analyzed separately.

Analysis of Food and Feces

The DM content of the different foods was determined after oven drying to a constant weight at 103°C. The DM content of feces was determined by freeze drying. Diets and feces were analyzed for OM and ash concentrations by using standard methods (Association Française de Normalisation, 1981). Crude protein was calculated from Kjeldahl N (Association Française de Normalisation, 1981). Fat content was determined by acid hydrolysis, followed by ether extraction (Association Française de Normalisation, 1981). The Na and K contents were measured by flame photometry (Jenway, Essex, UK). Gross energy content of food and feces was determined by adiabatic bomb calorimetry (Sanyo Gallenkamp, Leicester, UK). Apparent digestibility values of the different nutrients and energy were calculated using the following formula: apparent digestibility, % =[(intake of nutrient or energy - fecal output of nutrient or energy)/intake of nutrient or energy] \times 100.

Fecal Electrolytes and Osmolarity

To determine fecal Na and K contents, fresh stool samples (within 15 min of defecation) were diluted to 1:10 (wt/vol) with a solution of mercury chloride (1 g/L of water; Merck SA, Fontenay-sous-Bois, France) and centrifuged at $855 \times g$ for 20 min at 4°C. The supernatant was frozen at -20° C pending analysis. Before analysis, samples were centrifuged at $2,124 \times g$ for 10 min at 4°C. Concentrations of Na and K were measured

| | <i>v</i> | (| | , | / |
|---------------------|----------|------|------|------|------|
| Item | WGLP | WGHP | WPMP | PMLP | PMHP |
| DM | 92.0 | 89.7 | 91.5 | 90.3 | 89.9 |
| CP | 21.6 | 38.2 | 28.6 | 21.4 | 39.2 |
| Fat | 19.6 | 16.6 | 17.6 | 17.6 | 17.8 |
| $\rm NFE^2$ | 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 |
| К | 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 of DM | 5.2 | 5.4 | 5.2 | 5.0 | 5.2 |

Table 2. Nutritional analysis of diets¹ (% unless otherwise states; DM basis)

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

 2 NFE = calculated N-free extract.

by flame photometry (Jenway, Essex, UK). Osmolarity of the supernatant was determined using an osmometer (Osmette A, Precision Systems, Natick, MA).

Fecal Score

Fecal quality was assessed for every defecation by using a 5-point visual scale, ranging from 1 (hard and dry feces) to 5 (liquid diarrhea). A score of 2.5 represented a well-formed stool that was easy to collect but was not too dry; this was considered optimum.

Statistical Analysis

The data from phases I and II were pooled, and the individual dog was considered the experimental unit. The independent variables were diet (WGLP, WGHP, WPMP, PMLP, and PMHP), protein source (WG, PM, and WP), protein concentration (LP, MP, and HP), and dog group (SMA, MED, GRT, and GRS). The variables measured were fecal score, fecal moisture, apparent digestibility, fecal osmolarity, and concentrations of Na and K in feces. Data were analyzed using XLSTAT procedures (Addinsoft, Version 2007.5, NY) and S-PLUS 6.2 (Tibco Software Inc., Palo Alto, CA). Mean, SD, and SE of variables were calculated for each group of dogs fed each diet. Nonparametric tests were used because the data were not normally distributed. Data were analyzed using the Kruskal-Wallis test, and, when statistically significant, the Dunn procedure was used for multiple pairwise comparisons. A P-value of 0.05 was considered significant, and a *P*-value less than 0.10 was considered a trend. Results are presented as mean \pm SE. Whenever a diet effect was observed (P <0.05), protein source (WG, PM, and WP) and protein content (LP, MP, and HP) were analyzed as factors influencing the aforementioned variables. Moreover, whenever an effect of diet or dog size was observed, the effect of diet was analyzed for each group of dogs, and the effect of dog size was analyzed for each diet, respectively. Additionally, linear regressions were developed to describe the relationship between fecal score or moisture and DM and CP digestibility, fecal osmolarity, and Na and K concentrations. The effects of the independent variables, diet and dog group, on the different variables measured (fecal score, fecal moisture, apparent digestibility, fecal osmolarity, and concentrations of Na and K in feces) were analyzed using a mixed-effects model (Pinheiro and Bates, 2000): VAR = diet + dog group, where VAR refers to variable.

RESULTS

Given the absence of clinical signs at examination, all dogs were considered healthy throughout the experimental period. Some dogs were replaced in the second phase of this study for nonnutritional reasons that were independent of the present experiment. Therefore, the mixed-effects model statistical approach included 3 SMA, 6 MED, 5 GRT, and 3 GRS dogs. Because of the small number of animals within dog groups, no statistically significant effect of dog size using the mixedeffects model was observed on fecal score, moisture, osmolarity, and digestibility of DM, CP, fat, ash, and energy.

All dogs consumed their entire daily ration, and BW was constant during the duration of the study. Mean BW before prefeed and after testing periods were 4.4 \pm 0.5 and 4.8 \pm 0.5 kg for SMA, 14.4 \pm 0.3 and 15.2 \pm 0.3 kg for MED, 27.2 \pm 1.0 and 29.1 \pm 1.2 kg for GRT, and 23.1 \pm 0.7 and 23.1 \pm 0.9 kg for GRS. Fecal score, moisture, osmolarity and electrolytes, and apparent digestibility coefficients are presented in Tables 3 and 4. Statistical information on the effects of dog size, dietary protein source, and dietary protein content on the aforementioned data sets is presented in Table 5.

Fecal Quality

Fecal score and fecal moisture are presented in Table 3 and Figure 1. The result of mixed-effect model analysis revealed an overall effect of diet (P < 0.001) on fecal score (data not shown). Fecal score varied with both dietary protein source (P < 0.001) and dietary protein content (P = 0.004; Table 5), and was greater (indicating poorer feces quality) when dogs were fed the PM and HP diets (Figure 1). Fecal score was greater for the PMHP diet (3.51 ± 0.07) and lesser for the WGLP diet (2.75 ± 0.09) than for the other diets (P < 0.001).

Based on the results of mixed-effect model analysis, diet affected fecal moisture (P < 0.001; data not shown). Fecal moisture (Figure 1) followed the same pattern as fecal score; it varied with dietary protein source (P < 0.001), but was not affected by dietary protein content. The least fecal moisture content was observed for WG diets, whereas the PMHP diet resulted in the greatest fecal moisture content, regardless of dog size ($61.5 \pm 0.7\%$ for WGLP, $61.0 \pm 0.7\%$ for WGHP, $63.7 \pm 0.7\%$ for WPMP, $64.5 \pm 0.7\%$ for PMLP, and $66.6 \pm 0.5\%$ for PMHP). No relationships were observed between fecal score or moisture and DM and CP digestibility values, fecal osmolarity, and Na and K concentrations (data not shown).

Independently of diet, the GRS dogs had the greatest fecal score (3.58 ± 0.05) and fecal moisture content ($66.1 \pm 0.5\%$), whereas the SMA dogs had the least fecal score and moisture content (2.59 ± 0.09 and $60.8 \pm 0.9\%$, respectively; P < 0.001). No difference was detected between MED and GRT dogs in fecal score (3.11 ± 0.05 and 3.19 ± 0.07 , respectively) or moisture content ($64.3 \pm 0.4\%$ and $62.5 \pm 0.6\%$, respectively).

Apparent Digestibility

Apparent digestibility coefficients are presented in Table 4 for each diet and group of dogs. Both source and quantity of dietary protein affected DM, CP, fat, and energy digestibility values (Table 5). Dog group

| Table 3. Fecal score, mois | sture, osmolarity, and | concentrations of Na | a and K for each | diet and dog group ¹ (mean |
|----------------------------|------------------------|----------------------|------------------|---------------------------------------|
| \pm SE) | | | | |

| Item^2 | SMA | MED | GRT | GRS |
|------------------------------------|---------------------------------|--------------------------------|--------------------------------|---------------------------------|
| Fecal score, ³ 1 to 5 | | | | |
| WGLP | $2.27 \pm 0.10^{\mathrm{a,A}}$ | $2.77\pm0.05^{\rm ab,A}$ | $2.71 \pm 0.10^{ m ab,A}$ | $3.27 \pm 0.12^{\mathrm{b,A}}$ |
| WGHP | $2.43\pm0.21^{\rm a,AB}$ | $3.02\pm0.12^{\mathrm{ab,AB}}$ | $3.13\pm0.13^{\rm ab,AB}$ | $3.56 \pm 0.07^{ m b,AI}$ |
| WPMP | $2.52 \pm 0.11^{\mathrm{a,AB}}$ | $3.14\pm0.04^{\mathrm{ab,AB}}$ | $3.30\pm0.09^{\mathrm{ab,AB}}$ | $3.57 \pm 0.06^{\mathrm{b,AI}}$ |
| PMLP | $2.58\pm0.12^{\rm a,AB}$ | $3.16\pm0.04^{\rm ab,AB}$ | $3.26\pm0.11^{\rm ab,AB}$ | $3.62\pm0.07^{\rm b,AI}$ |
| PMHP | $3.16 \pm 0.20^{\mathrm{a,B}}$ | $3.45 \pm 0.07^{\rm ab,B}$ | $3.55\pm0.09^{\mathrm{ab,B}}$ | $3.88 \pm 0.03^{ m b,B}$ |
| Fecal moisture, % | | | | |
| WGLP | 59.2 ± 1.4 | $63.0\pm0.8^{\rm AB}$ | $59.7 \pm 1.2^{\rm A}$ | $64.1 \pm 1.0^{\text{A}}$ |
| WGHP | 58.6 ± 2.0 | $62.0 \pm 0.8^{\mathrm{A}}$ | $60.1 \pm 1.1^{\text{A}}$ | $63.6\pm0.4^{\rm A}$ |
| WPMP | $60.4 \pm 2.0^{\mathrm{a}}$ | $64.2\pm0.4^{\mathrm{ab,AB}}$ | $63.5\pm0.9^{ m ab,AB}$ | $66.1\pm0.7^{\rm b,AB}$ |
| PMLP | $60.5\pm1.6^{\rm a}$ | $65.8\pm0.5^{\rm ab,B}$ | $63.6\pm0.9^{ m ab,AB}$ | $67.4 \pm 1.2^{\mathrm{b,AB}}$ |
| PMHP | $65.5 \pm 1.6^{\rm ab}$ | $66.5\pm0.6^{\rm ab,B}$ | $65.4 \pm 0.5^{ m a,B}$ | $69.1 \pm 0.4^{\mathrm{b,B}}$ |
| Fecal osmolarity, mOsm/L | | | | |
| WGLP | 483 ± 13 | $466 \pm 27^{\mathrm{AB}}$ | $481 \pm 10^{\rm AB}$ | 464 ± 20 |
| WGHP | 470 ± 23 | $466 \pm 13^{\mathrm{A}}$ | $492\pm27^{\rm AB}$ | 443 ± 27 |
| WPMP | 462 ± 13 | $506 \pm 22^{\mathrm{AB}}$ | $470 \pm 14^{\mathrm{A}}$ | 509 ± 22 |
| PMLP | 489 ± 10 | $509 \pm 18^{\mathrm{AB}}$ | $498 \pm 18^{\rm AB}$ | 504 ± 18 |
| PMHP | 518 ± 22 | $566 \pm 24^{\mathrm{B}}$ | $576 \pm 20^{\mathrm{B}}$ | 499 ± 16 |
| Fecal Na concentration, mg/g of DM | | | | |
| WGLP | 1.9 ± 0.2 | 2.1 ± 0.2 | $2.2\pm0.4^{ m AB}$ | $2.7\pm0.4^{\rm AB}$ |
| WGHP | $2.0 \pm 0.3^{\mathrm{a}}$ | $2.3\pm0.3^{ m ab}$ | $2.7\pm0.3^{ m ab,A}$ | $3.6\pm0.4^{ m b,A}$ |
| WPMP | 1.5 ± 0.4 | 2.4 ± 0.3 | $2.1\pm0.4^{ m AB}$ | $2.5\pm0.1^{\mathrm{AB}}$ |
| PMLP | 1.4 ± 0.2 | 1.9 ± 0.3 | $2.1\pm0.2^{ m AB}$ | $2.6\pm0.6^{\rm AB}$ |
| PMHP | 1.3 ± 0.4 | 1.0 ± 0.2 | $0.9\pm0.1^{ m B}$ | $1.7 \pm 0.3^{\mathrm{B}}$ |
| Fecal K concentration, mg/g of DM | | | | |
| WGLP | 1.8 ± 0.2 | 1.8 ± 0.4 | 1.8 ± 0.2 | $2.5 \pm 0.1^{\mathrm{A}}$ |
| WGHP | 1.8 ± 0.3 | 1.7 ± 0.1 | 2.0 ± 0.3 | $2.8\pm0.4^{\rm AB}$ |
| WPMP | 2.0 ± 0.3 | 2.1 ± 0.3 | 2.2 ± 0.1 | $3.7\pm0.3^{ m B}$ |
| PMLP | $2.0\pm0.3^{ m ab}$ | $1.9 \pm 0.1^{\mathrm{a}}$ | $2.5\pm0.2^{ m ab}$ | $3.3\pm0.2^{ m b,AB}$ |
| PMHP | $2.3\pm0.1^{ m ab}$ | $2.1\pm0.2^{ m a}$ | $2.6 \pm 0.1^{\mathrm{ab}}$ | $3.0\pm0.1^{ m b,AB}$ |

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

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

¹SMA = 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); GRS = large sensitive dogs (23.1 ± 0.7 kg). The number of animals was 6 for each group and each diet [except for SMA dogs fed WPMP, and PMLP for fecal score and moisture (n = 5); for SMA dogs fed WGHP, PMLP, and PMHP, and GRT and GRS dogs fed WGHP for fecal osmolarity and Na and K concentrations (n = 5); and for SMA dogs fed WPMP for fecal osmolarity and Na and K concentrations (n = 4)].

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

 3 Five-point numerical scale, from hard and dry feces (1) to liquid diarrhea (5), with 2.5 considered optimum.

affected the digestibility of DM, ash, and energy, and a trend was observed for CP (P = 0.054). In general, digestibility tended to be greater for WG than PM diets, for LP compared with HP diets (except for protein digestibility), and in GRT and GRS than in SMA dogs.

Diet affected DM digestibility according to the results of mixed-effects model analysis (P < 0.001; data not shown). Digestibility of DM was greater for WG (86.6 ± 0.3%) than PM (84.0 ± 0.3%) diets, considering all groups of dogs (P < 0.001). The same was observed for LP diets (86.4 ± 0.3%), which had greater DM digestibility (P < 0.001) than HP diets (84.3 ± 0.3%). The DM digestibility of WGLP (P = 0.002) and WPMP diets (P = 0.008) was greater in large than in small dogs. Tendencies for greater DM digestibility of WGHP (P = 0.073) and PMHP diets (P = 0.075) were also observed in large compared with small dogs.

According to mixed-effects model analysis, CP digestibility was affected by diet (P < 0.001; data not shown). Digestibility of CP was greater (P < 0.001) for WG (89.9 \pm 0.4%) than PM (82.9 \pm 0.3%) diets and was increased with greater protein content (85.2 \pm 0.5% for LP diets, and 87.7 \pm 0.7 for HP diets; P =0.029). Crude protein digestibility of the WGLP (P =0.001), WPMP (P = 0.006), and PMLP (P = 0.027) diets was greater in GRT and GRS than in SMA dogs. No differences among dog groups were observed for the other diets.

The results of mixed-effects model analysis indicated an effect of diet on fat digestibility (P < 0.001; data not shown). Fat digestibility was greater for WG (96.3 ± 0.2%) than PM (95.6 ± 0.1%; P < 0.001) diets and for LP (96.6 ± 0.1%) than HP (95.3 ± 0.1%; P < 0.001) diets for all groups of dogs. Fat digestibility of the WGLP diet tended to be greater (P = 0.095) in large dogs compared with small dogs, but no other significant effect of dog size was observed.

Digestibility of ash was likewise affected by diet according to the results of mixed-effects model analysis (P = 0.016; data not shown). Ash digestibility was af-

| Table 4. | Digestibility | (%) |) of DM, | CP. | fat, | ash, | and | energy for | each | diet | and | dog | group | o ¹ (r | $mean \pm SE$ |) |
|----------|---------------|-----|----------|-----|------|------|-----|------------|------|------|-----|-----|-------|-------------------|---------------|---|
|----------|---------------|-----|----------|-----|------|------|-----|------------|------|------|-----|-----|-------|-------------------|---------------|---|

| Item^2 | SMA | MED | GRT | GRS |
|-------------------|------------------------------|--------------------------|--------------------------|---------------------------|
| DM | | | | |
| WGLP | $85.5\pm0.7^{\rm a,A}$ | $86.4\pm0.4^{\rm ab,A}$ | $88.9\pm0.5^{\rm bc,A}$ | $89.0\pm0.5^{\rm c,A}$ |
| WGHP | $84.5\pm1.0^{\rm AB}$ | $85.0\pm0.9^{\rm AB}$ | $87.5\pm0.6^{\rm A}$ | $86.1\pm0.7^{\rm AB}$ |
| WPMP | $83.5\pm0.4\mathrm{A^{a,B}}$ | $85.2\pm0.5^{\rm ab,AB}$ | $86.4\pm0.5^{\rm b,AB}$ | $85.5\pm0.5^{\rm ab,AB}$ |
| PMLP | $84.1\pm0.3^{\rm AB}$ | $84.4\pm0.7^{\rm AB}$ | $86.3\pm0.7^{\rm AB}$ | $85.9\pm0.7^{\rm AB}$ |
| PMHP | $81.7\pm0.7^{\rm B}$ | $82.4\pm0.5^{\rm B}$ | $83.4\pm0.3^{\rm B}$ | $83.2\pm0.4^{\rm B}$ |
| CP | | | | |
| WGLP | $85.7\pm0.8^{\rm a,AB}$ | $86.6\pm0.7^{\rm ab,AB}$ | $89.9\pm0.5^{\rm c,A}$ | $89.3\pm0.5^{\rm bc,AB}$ |
| WGHP | $91.1\pm0.6^{ m A}$ | $91.4\pm0.7^{ m A}$ | $92.8\pm0.8^{ m A}$ | $92.2\pm0.4^{\rm A}$ |
| WPMP | $84.1\pm0.4^{\rm a,AB}$ | $86.2\pm0.6^{\rm ab,AB}$ | $87.6 \pm 0.4^{ m b,AB}$ | $85.8\pm0.6^{\rm ab,ABC}$ |
| PMLP | $81.2\pm0.1^{\rm a,B}$ | $81.3\pm1.0^{\rm ab,B}$ | $84.2\pm0.7^{\rm b,B}$ | $82.8\pm1.1^{\rm ab,C}$ |
| PMHP | $82.5\pm1.0^{\rm B}$ | $83.1\pm0.6^{\rm B}$ | $84.0\pm0.3^{\rm B}$ | $84.1\pm0.5^{\rm BC}$ |
| Fat | | | | |
| WGLP | $97.0\pm0.2^{\rm A}$ | 96.5 ± 0.6 | $97.5\pm0.1^{\rm A}$ | $97.5\pm0.3^{\rm A}$ |
| WGHP | $95.4\pm0.5^{ m AB}$ | 95.7 ± 0.3 | $96.0\pm0.1^{\rm AB}$ | 95.0 ± 0.5^{B} |
| WPMP | $95.5\pm0.2^{\rm AB}$ | 96.0 ± 0.2 | $95.8\pm0.2^{\rm B}$ | $95.1 \pm 0.3^{ m B}$ |
| PMLP | $95.9\pm0.4^{\rm AB}$ | 96.3 ± 0.4 | $96.1\pm0.2^{\rm AB}$ | $95.7\pm0.3^{\rm AB}$ |
| PMHP | $94.7\pm0.5^{\rm B}$ | 95.5 ± 0.4 | $95.4 \pm 0.1^{\rm B}$ | $94.9\pm0.2^{\rm B}$ |
| Ash | | | | |
| WGLP | 34.2 ± 3.7 | 38.7 ± 1.7 | 43.8 ± 2.3 | 45.5 ± 2.6 |
| WGHP | 34.5 ± 3.6 | 33.4 ± 3.7 | 42.9 ± 4.3 | 36.7 ± 3.2 |
| WPMP | 30.1 ± 1.6 | 34.9 ± 2.2 | 37.2 ± 3.4 | 35.8 ± 2.8 |
| PMLP | 29.4 ± 1.4 | 30.0 ± 2.6 | 34.5 ± 3.6 | 35.5 ± 3.5 |
| PMHP | 30.2 ± 2.0 | 30.2 ± 2.0 | 32.6 ± 1.7 | 31.8 ± 1.3 |
| Energy | | | | |
| WGLP | $89.8 \pm 0.5^{ m a,A}$ | $90.3\pm0.5^{\rm ab,A}$ | $92.4\pm0.3^{\rm bc,A}$ | $92.3\pm0.3^{\rm c,A}$ |
| WGHP | $88.9\pm0.8^{\rm A}$ | $89.6\pm0.7^{\rm A}$ | $91.3\pm0.4^{\rm A}$ | $90.2\pm0.5^{\rm A}$ |
| WPMP | $87.8\pm0.4^{\rm a,AB}$ | $89.2\pm0.4^{\rm ab,AB}$ | $90.1\pm0.4^{\rm b,AB}$ | $89.2\pm0.4^{\rm ab,AB}$ |
| PMLP | $88.1\pm0.3^{\rm AB}$ | $88.3\pm0.6^{\rm AB}$ | $89.9\pm0.5^{\rm AB}$ | $89.3\pm0.6^{\rm AB}$ |
| PMHP | $85.3\pm0.6^{\rm B}$ | $85.9 \pm 0.6^{ m B}$ | $86.8\pm0.2^{\rm B}$ | $86.6\pm0.3^{\rm B}$ |

^{a-c}Within a row, means without common superscripts differ (P < 0.05).

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

¹SMA = 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); GRS = large sensitive dogs (23.1 ± 0.7 kg). The number of animals was 6 for each group and each diet, except for SMA dogs fed WPMP, PMLP, and PMHP (n = 5).

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

fected by dietary protein source (P < 0.001), but not by protein concentration. The WG diets had greater ash digestibility ($38.7 \pm 1.2\%$) than the PM diets (31.8 \pm 0.9%). The greatest ash digestibility was observed when MED (P = 0.068), GRT (P = 0.046), and GRS (P = 0.079) dogs were fed the WGLP diet. No effect

Table 5. Effect of dog size, dietary protein source, and dietary protein content on fecal score and moisture, nutrient digestibility, fecal content of Na and K, and fecal osmolarity

| Item | $Source^1$ | <i>P</i> -value | $\operatorname{Content}^2$ | <i>P</i> -value | Dog size^3 | <i>P</i> -value |
|---------------------------|------------|-----------------|----------------------------|-----------------|---------------------|-----------------|
| Fecal score | WG < PM | < 0.001 | LP < HP | 0.004 | SMA < GRS | < 0.001 |
| Fecal moisture | WG < PM | < 0.001 | | NS^4 | SMA < GRS | < 0.001 |
| Digestibility | | | | | | |
| DM | WG > PM | < 0.001 | LP > HP | < 0.001 | SMA < GRS | < 0.001 |
| CP | WG > PM | < 0.001 | LP < HP | 0.029 | SMA < GRS | 0.054 |
| Fat | WG > PM | < 0.001 | LP > HP | < 0.001 | MED > GRS | 0.123 |
| Ash | WG > PM | < 0.001 | | NS | SMA < GRS | 0.005 |
| Energy | WG > PM | < 0.001 | LP > HP | < 0.001 | SMA < GRS | 0.003 |
| Fecal electrolyte content | | | | | | |
| Na | WG > PM | < 0.001 | | NS | SMA < GRS | 0.005 |
| K | WG < PM | 0.002 | | NS | SMA < GRS | < 0.001 |
| Fecal osmolarity | WG < PM | < 0.001 | | NS | | NS |

 ${}^{1}WG =$ wheat gluten diets; PM = poultry meal diets.

 $^{2}LP = diets$ with low protein content (22% on a DM basis); HP = diets with high protein content (39% on a DM basis).

³SMA = small dogs (mean BW, 4.4 ± 0.5 kg); MED = medium dogs (14.4 ± 0.3 kg); GRS = large sensitive dogs (23.1 ± 0.7 kg). ⁴NS = not statistically different (P > 0.05).

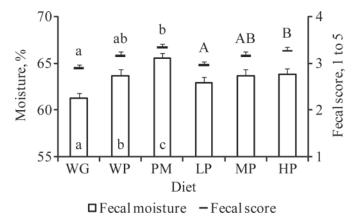


Figure 1. Fecal score and moisture according to dietary source and quantity of protein. ^{a-C}Within dietary protein sources, means without common superscripts differ (P < 0.05). ^{A,B}Within dietary protein content, means without common superscripts differ (P < 0.05). Fecal score: 1.0 = hard and dry; 2.5 = optimal; 5.0 = liquid diarrhea. WG = wheat gluten diets; WP = wheat gluten and poultry meal mix diet; PM = poultry meal diets; LP = diets with low protein content (22% on a DM basis); MP = diet with medium protein content (39% on a DM basis).

of diet on ash digestibility was detected for SMA dogs. Ash digestibility of the WGLP diet was greater (P = 0.039) for large than small dogs. No effect of dog size on ash digestibility was detected for the other diets.

According to mixed-effects model analysis, diet affected energy digestibility (P < 0.001; data not shown). Digestibility of energy was greater for WG (90.6 ± 0.2%) than PM (87.6 ± 0.3%; P < 0.001) diets and for LP (90.1 ± 0.3%) compared with HP (88.2 ± 0.4%; P < 0.001) diets. Digestibility of energy was therefore decreased for the PMHP diet in all dog groups (P = 0.006 for SMA, P = 0.003 for MED, and P < 0.001 for GRT and GRS dogs) compared with the WGLP and WGHP diets. Digestibility of energy followed the same pattern as DM, being greater for large than for small dogs (P = 0.001 for WGLP, P = 0.132 for WGHP, P = 0.007 for WPMP, P = 0.055 for PMLP, and P = 0.074 for PMHP).

Fecal Osmolarity

Fecal osmolarity results are presented in Table 3 and in Figure 2. Results of mixed-effects model analysis revealed an effect of diet on fecal osmolarity (P < 0.001; data not shown). Fecal osmolarity was greater (P < 0.001) for PM diets ($520 \pm 8 \text{ mOsm/L}$) than for WG diets ($471 \pm 7 \text{ mOsm/L}$), but there was no effect of protein content ($487 \pm 6 \text{ mOsm/L}$ for LP, 489 ± 10 mOsm/L for MP, and $506 \pm 10 \text{ mOsm/L}$ for HP diets). In MED and GRT dogs fed HP diets, fecal osmolarity was greater (P = 0.040 and P = 0.046, respectively) with PM than with WG diets. In MED and GRT dogs fed PM diets, greater fecal osmolarity was observed with HP than LP diets. No effect of diet on the SMA and GRS groups was observed. Overall, no effect of dog size was detected for fecal osmolarity, irrespective of

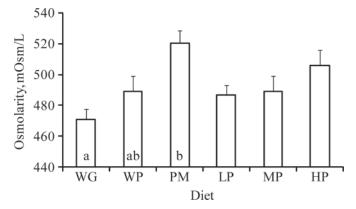


Figure 2. Fecal osmolarity according to dietary source and quantity of protein. ^{a,b}Within dietary protein sources, means without common superscripts differ (P < 0.05). WG = wheat gluten diets; WP = wheat gluten and poultry meal mix diet; PM = poultry meal diets; LP = diets with low protein content (22% on a DM basis); MP = diet with medium protein content (29% on a DM basis); HP = diets with high protein content (39% on a DM basis).

diet: 485 ± 8 mOsm/L for SMA, 503 ± 11 for MED, 504 ± 10 for GRT, and 485 ± 10 for GRS dogs (data not shown).

Fecal Electrolyte Concentrations

Fecal electrolyte concentrations are presented in Table 3 and in Figures 3 and 4. An effect of diet was detected for fecal Na (P = 0.031 in MED, P = 0.007 in GRT, and P = 0.059 in GRS), and K (P = 0.033 in GRT and P = 0.046 in GRS) concentrations. Fecal Na and K concentrations were affected by diet (P < 0.001) and by dog group (P = 0.061 and P = 0.008, respectively; data not shown), according to mixed-effects model analysis. Overall, fecal Na concentration was greater (P < 0.001) and K was decreased (P = 0.002) for WG (2.4 ± 0.1 and 2.0 ± 0.1 mg/g of DM, respectively) compared with PM (1.6 ± 0.1 and 2.5 ± 0.1 mg/g of DM, respectively) diets, especially for HP diets. No differences were detected for dietary protein content.

Both Na (P = 0.005) and K (P < 0.001) concentrations in feces increased with dog size. Fecal Na concentration was greater in GRS dogs, but this did not reach significance except when they were fed the WGHP diet (P = 0.038). Fecal K concentration was greater in GRS than in MED dogs (P = 0.041 for WGLP, P = 0.011for WPMP, P = 0.008 for PMLP, and P = 0.006 for PMHP diets), except when they were fed the WGHP diet (P = 0.123). The GRS dogs had the greatest mean concentration of both electrolytes, regardless of diet.

DISCUSSION

Large dogs are prone to producing feces of poorer quality compared with small breeds. This could be explained by anatomical and physiological differences attributable to dog size (Weber et al., 2004): 1) decreased overall electrolyte absorption, which can be explained, in part, by greater permeability in both the small (Weber et al., 2002) and the large (Hernot et al., 2005b) intestine; and 2) greater fermentative activity, which can be explained, in part, by a greater relative surface area and volume of the intestine (Hernot et al., 2003) and a longer colonic transit time (Hernot et al., 2005b). Fermentation could have an indirect impact on electrolyte absorption because it would increase intraluminal osmotic pressure, leading to increased water, Na, and K secretion into the colon (Weber et al., 2004). Therefore, minimizing fermentation could be a way of improving fecal quality (Weber et al., 2004; Hernot et al., 2005b).

Polysaccharides (approximately 120 to 440 g/kg of DM in ileum chyme) and protein (218 to 650 g/kg of DM in ileum chyme) are the main fermentation substrates for colonic microflora (Zentek, 1995). Therefore, a nutritional strategy to increase the quality of feces in large dogs could consist of reducing the quantity of undigested nutrients reaching the colon. This could be achieved by decreasing dietary fiber intake or through the incorporation of highly digestible protein sources. Within the range of fiber contents usually reported in commercially available maintenance diets, we observed no improvement in fecal score and moisture, based on previous results of fecal quality obtained in dogs differing in body size (Hernot et al., 2004, 2005a). Protein represents a major proportion of the diet (20 to 40%)and different sources vary widely in ileal digestibility (Wiernusz et al., 1995; Zentek, 1995; Murray et al., 1997); thus, its fermentation affects fecal consistency and DM content (Hussein and Sunvold, 2000; Zentek et al., 2003). The quantity of protein reaching the colon depends on DM and protein intakes and on protein digestibility (Hussein and Sunvold 2000). Using highly digestible protein sources can reduce dietary protein concentration and protein flow into the colon, minimizing protein fermentation.

Effect of Protein Source

Poultry meal is the most common protein source used in commercially available dog foods, and WG is one of the most digestible sources of protein available (Wiernusz et al., 1995). The dogs used in the current study had been fed a variety of WG- and PM-containing diets throughout their lives without specific adverse effects, and, as far as can reasonably be ascertained, they did not have food allergy, WG-sensitive enteropathy (Hall and Batt, 1992; Garden et al., 2000), or diarrhea when fed these proteins. It was considered that corn gluten meal (14.3 to 25.6% of CP in experimental diets) did not affect the results concerning protein source because its apparent CP digestibility was previously observed to be greater (93.8%; Zentek, 1995) than the values obtained in the present study for WG diets.

Compared with PM, WG was superior in terms of fecal score, fecal moisture, and CP digestibility (Table 6). A similar observation was made in a previous study (Wiernusz et al. 1995), in which greater CP digestibili-

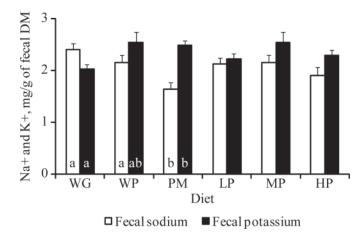
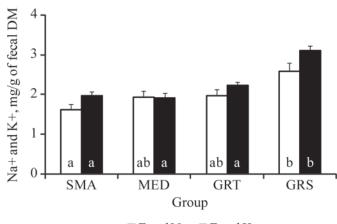


Figure 3. Fecal Na and K concentrations according to dietary source and quantity of protein. ^{a,b}Within dietary protein sources, means without common superscripts differ (P < 0.05). WG = wheat gluten diets; WP = wheat gluten and poultry meal mix diet; PM = poultry meal diets; LP = diets with low protein content (22% on a DM basis); MP = diet with medium protein content (29% on a DM basis); HP = diets with high protein content (39% on a DM basis).

ty in WG-based diets (93.8%) than in soy protein-based diets (86.7 to 89.7%) was associated with improvements in fecal score [4.23 and 3.40 to 3.83, respectively, on a]scale from 0 (liquid diarrhea) to 6 (constipation)] and fecal moisture (61.5 and 67.5 to 76.5%, respectively).This beneficial effect of WG on fecal quality is likely due to reducing the flow of undigested protein into the colon and decreasing protein fermentation; this has previously been associated with improved fecal consistency and less moisture content (Zentek, 1995; Hussein and Sunvold, 2000; Zentek et al., 2003). Because colonic fermentation is greater in large-breed dogs (Weber et al., 2003b; Hernot, 2005), the improvement in their fecal quality observed with WG diets, especially in the sensitive dogs, would support the beneficial effect of greater ileal protein digestibility to minimize colonic fermentation.



□FecalNa ■FecalK

Figure 4. Fecal Na and K concentrations according to dog size. ^{a,b}Within dog groups, means without common superscripts differ (P < 0.05). 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); GRS = large sensitive dogs (23.1 ± 0.7 kg).

| Variable | Pr | otein sour | rce ² | Protein content ³ | | | | $\operatorname{Dog}\operatorname{group}^4$ | | | | |
|---------------------------------|------------------------|------------------------|------------------|------------------------------|--------------------|----|---------------------|--|----------|-----|--|--|
| | WG | WP | PM | LP | MP | HP | SMA | MED | GRT | GRS | | |
| Fecal quality | | | | | | | | | | | | |
| Score | $\uparrow\uparrow$ | | | | $\uparrow\uparrow$ | | $\uparrow\uparrow$ | | | | | |
| Moisture | $\uparrow\uparrow$ | | | \uparrow | | | $\uparrow \uparrow$ | | | | | |
| Digestibility | | | | | | | | | | | | |
| DM | $\downarrow\downarrow$ | | | $\downarrow\downarrow$ | | | $\uparrow \uparrow$ | | | | | |
| CP | | $\downarrow\downarrow$ | | $\uparrow \uparrow$ | | | \uparrow | | | | | |
| Fat | | $\downarrow\downarrow$ | | $\downarrow\downarrow$ | | | \leftrightarrow | | | | | |
| Ash | | $\downarrow\downarrow$ | | \leftrightarrow | | | $\uparrow \uparrow$ | | | | | |
| Energy | | $\downarrow\downarrow$ | | $\downarrow\downarrow$ | | | $\uparrow \uparrow$ | | | | | |
| Fecal electrolyte concentration | | | | | | | | | | | | |
| Na | $\downarrow\downarrow$ | | | \leftrightarrow | | | $\uparrow \uparrow$ | | | | | |
| К | | $\uparrow\uparrow$ | | \leftrightarrow | | | $\uparrow\uparrow$ | | | | | |
| Fecal osmolarity | | $\uparrow\uparrow$ | | | \uparrow | | | + | <i>→</i> | | | |

Table 6. Main variations of fecal quality, nutrient digestibility, fecal content of Na and K, and fecal osmolarity according to dietary protein source, dietary protein concentration, and dog size¹

¹Double upward arrows ($\uparrow\uparrow$) indicate an increase in the variable (P < 0.05) from WG to PM, from LP to HP, and from SMA to GRS dogs; double downward arrows ($\downarrow\downarrow$) indicate a decrease in the variable (P < 0.05) from WG to PM, from LP to HP, and from SMA to GRS dogs; a horizontal arrow (\leftrightarrow) indicates no variation in the variable between protein source, protein concentration, and dog group; a single upward arrow (\uparrow) indicates a numerical increase in the variable from WG to PM, from LP to HP, and from SMA to GRS dogs.

²WG = wheat gluten diets; WP = wheat gluten and poultry meal mix diet; PM = poultry meal diets.

 ${}^{3}LP$ = diets with low protein content (22% on a DM basis); MP = diet with medium protein content (29%); HP = diets with high protein content (39%).

 4 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); GRS = large sensitive dogs (23.1 ± 0.7 kg).

Fecal osmolarity, and fecal score and moisture were greater for PM than WG diets (Table 6). Osmolarity is characterized by solute concentration (fermentation products and electrolytes), and can be responsible for less water absorption from the colon (Nishinaka et al., 2004) and, consequently, increased fecal score and moisture. Greater fecal osmolarity could indicate greater fermentative activity in dogs fed PM diets.

Fecal Na was greater, and K was decreased, for WG compared with PM diets, particularly for HP diets. Fecal K concentration was greater in the presence of high fecal scores and moisture, partially confirming the hypothesis that increased fecal electrolytes result in poor fecal quality (Table 6). The greater fecal Na concentration with WG diets partially contradicts this hypothesis. Diet formulation was focused on protein source and content, inducing differences in electrolyte intake among dogs fed various diets (as evidenced by the differing Na and K intakes). Moreover, chloride-coupled absorption and excretion of Na and K (Kunzelmann and Mall, 2002), hormonal effects, and colonic mucosal functional capacities [e.g., paracellular permeability or expression of electrolyte and water transporters; Ma and Verkman (1999); Kunzelmann and Mall (2002)] affect fecal electrolyte concentration. Therefore, these results cannot be considered independently of these factors.

Effect of Protein Content

Decreased dietary protein concentrations led to improved fecal scores and decreased fecal moisture, except for fecal moisture in WG diets. Fecal score is a good measure of fecal quality, and it is widely used to evaluate fecal consistency (Hernot, 2005). In this context, and given the results obtained, an increased protein intake can affect the quantity of substrate available for colonic fermentation and, consequently, lead to a greater fecal score. This additionally indicates that, within the range observed in commercially available diets for normal healthy dogs, protein concentration has a limited effect on fecal moisture. Overall, during diet formulation, it is therefore relevant to consider not only protein quantity, but also protein quality to improve fecal score and moisture.

High-protein diets resulted in greater protein apparent digestibility, along with greater fecal scores and moisture (Table 6). The LP diets can result in a decreased protein flow into the colon and, consequently, lead to less fermentation and improved fecal quality. Apparent digestibility is affected by protein flow into the colon, which can be of dietary and endogenous origins (digestive secretions and exfoliated cells). Given that endogenous sources account for 30 to 60% of total luminal protein in humans (Meunier et al., 1976), during reduced protein flow in the colon (which could be affected by both protein source and content), endogenous protein loss becomes proportionally greater (Darragh and Hodgkinson, 2000). It is also known that increasing dietary protein intake results in an increase in endogenous N loss. Nevertheless, it has been shown that, even if the ratio between endogenous N loss and protein intake decreases, it remains greater with decreased protein intake in pigs (Hodgkinson et al., 2000) and dogs (Yamka et al., 2003). Additionally, microbial metabolism in the hindgut, protein hydrolysis, and de novo synthesis will lead to major changes in fecal protein content (Yamka et al., 2003). Therefore, greater protein digestibility was observed for the HP diets, resulting in greater fecal scores and moisture. This was in agreement with the results of Yamka et al. (2003), who reported the total tract apparent digestibility of PM diets in dogs fed increasing concentrations of dietary CP.

Effect of Dog Size

The propensity for large-breed dogs to produce poorquality feces is well known (Zentek and Meyer, 1995; Meyer et al., 1999; Weber et al., 2003b; Hernot, 2005). However, body size is not the only factor; some large dogs are particularly sensitive, whereas others are not. Despite having a BW similar to German Shepherds, Giant Schnauzers produce feces of better consistency, compared with the much larger Great Dane (Weber et al., 2003b; Hernot, 2005). Several studies have shown that, even in the absence of exocrine pancreatic insufficiency, German Shepherds are particularly prone to digestive intolerance and produce feces of poor consistency and increased moisture (Zentek et al., 2002). Therefore, German Shepherd dogs free of exocrine pancreatic insufficiency were included in this study to increase our understanding of the pathophysiology of their dietary sensitivity.

In the current study, GRS dogs had greater fecal scores and fecal moisture than GRT dogs. This supports previous work showing that German Shepherds are sensitive (Zentek et al., 2002, 2004), and confirms that on the same diet, sensitive dogs have a decreased digestive tolerance compared with other dogs of similar BW. This could be explained by mechanisms such as less overall electrolyte absorption (Weber et al., 2002; Hernot, 2005) and greater fermentative activity in the hindgut (Hernot et al., 2003; Weber et al., 2004). Fecal score varied greatly between diets in the SMA group (from 2.27 ± 0.10 to 3.16 ± 0.20) and, to a lesser extent, in the GRS group (from 3.27 ± 0.12 to $3.88 \pm$ (0.03). Given the aforementioned mechanisms, we expected that a reduced protein flow into the colon of GRS dogs would induce a more significant decrease in fermentation and, consequently, a better absorption of electrolytes from the colonic lumen. This should have led to a larger variation in fecal scores between diets in GRS dogs. Even though the improvement in fecal score of sensitive dogs was less than expected, reducing protein content, together with modulating protein sources, could be a satisfactory nutritional strategy to improve their fecal consistency.

Confirming previous findings (Weber et al., 2003b), digestibility values for DM, CP, and energy were greater in large dogs, which produced feces of poorer quality (Table 6). Large dogs had the greatest differences in digestibility between the 2 protein sources. Because previous studies did not report differences in orocecal transit time (Weber et al., 2003a) or absorptive function (Weber et al., 2002) in adult dogs differing in body size, digestibility differences among diets in large dogs would be due to greater fermentation in the colon. Microbial activity in the hindgut includes degradation of nutrients arriving from the small intestine. These nutrients are used either for syntheses or as an energy source by the gut bacteria, which can lead to the formation of end products that are either partially absorbed (e.g., ammonia, indoles, and phenols), partially utilized by the colonic mucosa (butyrate), or excreted (as is or as bacterial mass in feces). Therefore, our results would indicate that fecal score is not only related to fermentation in the hindgut, but also that other factors might affect fecal quality in large dogs. Because ileal digestibility in dogs differing in body size, breed, or both was not measured in the present study or, to our knowledge, in previous studies, it is not possible to conclude whether the difference in protein digestibility was due only to a difference in colonic fermentation.

The greater fecal concentration of electrolytes observed for the GRS group could have actively contributed to their decreased fecal quality (Table 6). This observation could be explained, in part, by greater permeability in both the small (Weber et al., 2002) and the large intestine (Hernot et al., 2005b) and by greater fermentative activity, leading to greater secretion of electrolytes into the colon lumen (Weber et al. 2004).

Fecal osmolarity did not differ significantly among the dog groups. Osmolarity has previously been reported to be partially responsible for decreased fecal quality in large-breed dogs (Weber et al., 2004), although these were Great Danes, not German Shepherds. In our study, a numerically greater osmolarity was observed for the MED and GRT groups compared with the SMA group. This would confirm the previously reported effect of size. The finding that GRS dogs did not have a greater osmolarity than SMA, MED, and GRT dogs was unexpected. This might be due, in part, to the variability observed within groups. The mechanisms influencing fecal quality could also be different between Great Danes and German Shepherds.

Summary and Conclusions

In support of the main hypothesis of this study, fecal score and moisture varied with dietary protein, mainly with protein source and, to a lesser extent, with concentration. Decreased fecal score and moisture were obtained with diets based on highly digestible protein sources and reduced protein concentrations, indicating that digestibility affects fecal quality. Moreover, fecal quality obtained with highly digestible protein sources was affected by fecal osmolarity.

We reported poorer fecal quality in large dogs, supporting the results of previous studies. This could be due to both decreased protein ileal digestibility and greater colonic fermentative activity. Within large dogs, sensitive breeds were particularly prone to producing feces of decreased quality compared with tolerant dogs of similar BW. This could be explained partially by increased fecal electrolyte concentrations in their feces, which could cause water retention in the colonic lumen. Further research on the functional capacities of the colonic mucosa would allow a better understanding of its absorptive and excretive processes; there may well be differences among the dog groups that are not evident in feces. The WG was proved to be an efficient protein source for modulating fecal quality. It is therefore reasonable to use highly digestible protein sources at decreased protein concentrations (depending on the source) to improve the fecal quality of large sensitive dogs.

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