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Apparent total tract macronutrient digestibility of mildly cooked human-grade vegan dog foods and their effects on the blood metabolites and fecal characteristics, microbiota, and metabolites of adult dogs

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Abstract

Vegan, mildly cooked, and human-grade dog foods are becoming more popular, as beliefs and views of pet owners change. To our knowledge, however, dog studies have not examined the digestibility of commercial vegan diets. Therefore, the objective of this study was to determine the apparent total tract digestibility (ATTD) of mildly cooked human-grade vegan dog foods and their effects on blood metabolites and fecal microbiota, characteristics, and metabolites of adult dogs consuming them. Three commercial dog foods were tested. Two were mildly cooked human-grade vegan dog diets, while the third was a chicken-based extruded dog diet. Twelve healthy adult female beagles (7.81 ± 0.65 kg; 7.73 ± 1.65 yr) were used in a replicated 3 × 3 Latin Square design. The study consisted of three experimental periods, with each composed of a 7 d diet adaptation phase, 15 d of consuming 100% of the diet, a 5 d phase for fecal collection for ATTD measurement, and 1 d for blood collection for serum chemistry and hematology. During the fecal collection period, a fresh sample was collected for fecal scoring and dry matter, pH, metabolite, and microbiota measurements. All data were analyzed using the Mixed Models procedure of SAS (version 9.4). All three diets were shown to be highly digestible, with all macronutrients having digestibility values above 80%. The vegan diets had higher ($P < 0.001$) ATTD of fat, but lower ($P < 0.05$) ATTD of organic matter than the extruded diet. Dogs consuming the vegan diets had lower circulating cholesterol ($P < 0.001$), triglyceride ($P < 0.001$), and platelet ($P < 0.009$) concentrations and lower ($P < 0.010$) blood neutrophil percentages than dogs consuming the extruded diet. Dogs consuming vegan diets had lower ($P < 0.001$) fecal dry matter percentages, lower ($P < 0.001$) fecal phenol and indole concentrations, and higher ($P = 0.05$) fecal short-chain fatty acid concentrations than those consuming the extruded diet. Fecal bacterial alpha and beta diversities were not different ($P > 0.05$) among diets, but dogs consuming vegan diets had altered ($P < 0.05$) relative abundances of nearly 20 bacterial genera when compared with those consuming the extruded diet. In conclusion, the mildly cooked human-grade vegan dog foods tested in this study performed well, resulting in desirable fecal characteristics, ATTD, and serum chemistries. The vegan diets tested also led to positive changes to serum lipids and fecal metabolites, and interesting changes to the fecal microbial community.

Lay Summary

Vegan, mildly cooked, and human-grade dog foods are increasing in popularity, but few studies have been performed to examine their performance. Our objective was to determine the apparent total tract digestibility (ATTD) of mildly cooked human-grade vegan dog foods and their effects on blood metabolites and fecal microbiota, characteristics, and metabolites of dogs. Two mildly cooked human-grade vegan dog diets and a chicken-based extruded dog diet were tested using 12 healthy adult dogs in a replicated 3 × 3 Latin Square design. All diets were highly digestible, with all macronutrients having digestibility values >80%. Vegan diets had higher ATTD of fat, but lower ATTD of organic matter than the extruded diet. Dogs consuming vegan diets had lower circulating cholesterol, triglycerides, platelets, and neutrophils than dogs consuming the extruded diet. Dogs consuming vegan diets had lower fecal dry matter percentages and phenol and indole concentrations, and higher fecal short-chain fatty acid concentrations than those consuming the extruded diet. Finally, ~20 bacterial genera were altered between dogs consuming vegan and extruded diets. In conclusion, the mildly cooked human-grade vegan dog foods tested performed well, resulting in desirable fecal characteristics, high ATTD, adequate serum chemistries, positive changes to serum lipids and fecal metabolites, and interesting changes to fecal microbiota.

Key words: canine nutrition, nutrient digestion, pet food

Abbreviations: AAFCO, Association of American Feed Control Officials; AHF, acid-hydrolyzed fat; ATTD, apparent total tract digestibility; BC, Bramble Cowbell diet; BCFA, branched-chain fatty acids; BR, Bramble Roost diet; CP, crude protein; CT, Chicken and Brown Rice Recipe diet; DM, dry matter; OM, organic matter; SCFA, short-chain fatty acids

Introduction

Over the past few decades, some people have decided to become vegetarian or vegan because of concerns about the environment, personal health, or animal welfare (Fox and Ward, 2008; de Boer and Aiking, 2011; Kerschke-Risch, 2015; North et al., 2021). Personal health is often the biggest driving force for those switching to a plant-based diet, with evidence showing a positive relationship between consumption of an animal-based diet and incidence of coronary heart

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disease, stroke, obesity, and a variety of cancers (US Department of Health and Human Services, 2001; Tuso et al., 2013). People are also becoming more concerned about the environmental impact of meat production, which has a greater carbon footprint than that of plant production (Mekonnen and Hoekstra, 2012; Hedenus et al., 2014; Scarborough et al., 2014).

Trends in the pet food market often follow human dietary trends and is thought to be the driver of the increased interest in vegetarian and vegan dog foods. The same concerns about animal welfare, environmental sustainability, and perceptions of what a healthy diet contains exist in regard to pet foods (Deng and Swanson, 2015; Martens et al., 2019; Alexander et al., 2020; Xu et al., 2021; Knight et al., 2022). Although commercial pet foods typically incorporate animal and plant byproducts from the human food industry, which is a sustainable practice, an increasingly common trend among pet owners is to opt for diets that do not contain byproducts (Swanson et al., 2013; Deng and Swanson, 2015; Meeker and Meisinger, 2015). Mildly cooked human-grade dog foods make up a small segment of the pet food market, but are gaining popularity due to their ingredient sourcing and the lower level of processing they undergo. Human-grade diets may only be labeled as such if the final product is human-grade and all ingredients have been processed, handled, stored, and transported in a manner that meets the standards set by Title 21 Code of Federal Regulations Part 117, as well as other federal laws that pertain to ingredients, facilities, and processing methods (AAFCO, 2022). It is possible for human-grade dog foods to take any form, but mildly cooked diets appear to be the most common format on the market.

A few recent studies have investigated mildly cooked dog foods (Algya et al., 2018; Do et al., 2021; Tanprasertsuk et al., 2021; Geary et al., 2022), but none of those studies have examined diets that were vegan. A major concern that many people share in reference to vegan dog foods is whether or not they are nutritionally adequate and highly digestible. A survey performed by Knight et al. (2022) reported that the feeding of vegan dog foods was associated with a healthier state than those fed with raw or conventional diets. However, those results were based on survey responses rather than controlled, scientific data. In another study, the nutrient concentrations of 26 plant-based diets for dogs and cats were evaluated using laboratory analyses (Dodd et al., 2022). Those findings suggested that there may be some validity to the concerns about the provision of essential nutrients. However, most of the diets lacking appropriate nutrient levels were formulated for cats (Dodd et al., 2022). In Gray et al. (2004) evaluated two vegan diets intended for cats, reporting that neither diet met minimum nutrient recommendations set by the Association of American Feed Control Officials (AAFCO). In Brazil, three vegan dog foods and one vegan cat food were analyzed recently (Zafalon et al., 2020). Although all foods tested in that study met minimum macronutrient requirements, insufficient calcium, sodium, and potassium concentrations, and excess zinc and copper concentrations were measured. Given the results, the researchers deemed all four vegan diets unhealthy.

Although owner surveys and investigations of dietary nutrient concentrations have been conducted, few have tested them in vivo (Cavanaugh et al., 2021). To our knowledge, there have been no studies to examine the digestibility of vegan pet foods. Therefore, the objective of this experiment was to determine the apparent total tract digestibility (ATTD) of commercial mildly cooked human-grade vegan dog foods, and their effects on blood metabolites, hematology, and fecal microbiota, characteristics, and metabolites of adult dogs consuming them. We hypothesized that the mildly cooked vegan diets would have a greater ATTD compared with an extruded kibble diet. We also hypothesized that the mildly cooked vegan diets would not negatively influence fecal characteristics, serum metabolites, or hematology. Lastly, we predicted that the fecal microbial populations and metabolite concentrations would be positively influenced by the mildly cooked vegan diets compared with the extruded kibble diet.

Materials and Methods

All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to experimentation. All methods were performed in accordance with the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Animals and housing

Twelve healthy adult spayed female beagle dogs (7.81 ± 0.65 kg; 7.73 ± 1.65 yr old) were used in a replicated 3 × 3 Latin square design experiment. All dogs were housed individually in pens (approximately 1.2 m wide × 2.4 m long) in an environmentally controlled facility at the University of Illinois at Urbana-Champaign. Dogs had free access to fresh water at all times. On the basis of the maintenance energy requirement for adult dogs, information from previous feeding records, and caloric estimates of each diet, an amount of food to maintain BW was offered and intake was measured twice daily (0800 and 1700 hours). Dogs were weighed and body condition scores were assessed (9-point scale) once a week prior to the 0800 hours feeding. Dogs were provided with toys for behavioral enrichment and were socialized at least twice weekly with each other and humans.

Dietary treatments and experimental periods

All three dietary treatments were commercial diets formulated to meet all AAFCO (2021) nutrient recommendations for adult dogs at maintenance. The treatments were as follows: 1) The Cowbell (BC); Bramble, Inc., New York, NY; 2) The Roost (BR); Bramble, Inc.; and 3) Life Protection Formula Chicken and Brown Rice Recipe (CT); Blue Buffalo, Wilton, CT. The human-grade vegan diets tested were mildly cooked according to U.S. Food and Drug Administration guidelines, with standard operating procedures for ingredient weights and cooking temperatures and times (target temperature of 74 °C for at least 5 min). The CT diet was selected because it is one of the most popular diets in the premium animal-based kibble category, a category that is sold to owners that may be willing to move to a human-grade and/or vegan food.

The experiment was composed of three 28 d periods, with each consisting of a 7 d diet transition phase, 15 d of consuming 100% of the diet, a 5 d fecal collection phase, and 1 d for blood collection. Dogs were adapted to the new dietary treatment at the beginning of each experimental period using the following feeding protocol: days 1 and 2: 75% kcal from prior dietary treatment + 25% kcal from new dietary treatment; days 3 and 4: 50% kcal from prior dietary treatment + 50% kcal from new dietary treatment; days 5 and 6: 25% kcal from prior dietary treatment + 75% kcal from new dietary treatment; days 7 to 28: 100% kcal from new dietary treatment.

Fecal collection, scoring, and handling

Total feces excreted were collected during the collection phase of each period. Feces were collected from each dog, weighed, and frozen in Whirlpak bags at –20 °C until analyses. During the collection phase, all fecal samples were scored using the following scale: 1 = hard, dry pellets, small hard mass; 2 = hard, formed, dry stool; remains firm and soft; 3 = soft, formed, and moist stool, retains shape; 4 = soft, unformed stool, assumes shape of container; and 5 = watery, liquid that can be poured.

During the first day of the collection phase, one fresh fecal sample (within 15 min of defecation) was collected for measurement of pH, dry matter (DM), short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA), phenols, indoles, ammonia, and microbiota. Fecal pH was measured immediately using an AP10 pH meter (Denver Instrument, Bohemia, NY) equipped with a Beckman Electrode (Beckman Instruments Inc., Fullerton, CA). Fecal aliquots for analysis of phenols and indoles were frozen at –20 °C immediately after collection. One aliquot was collected and placed in 2 N hydrochloric acid for ammonia, SCFA, and BCFA analyses. An additional aliquot was used for fresh fecal DM determination. Finally, aliquots of fresh feces were collected for microbiota analysis. These samples were immediately transferred to sterile cryogenic vials (Nalgene, Rochester, NY), quickly frozen in dry ice, and stored at –80 °C until analysis. The remainder was placed in a Whirlpak bag and stored at –20 °C.

Blood collection and analysis

On the final day of each experimental period, overnight fasted blood samples were collected via jugular puncture for serum chemistry and hematology. Samples were immediately transferred to appropriate vacutainer tubes for hematology (#367841 BD Vacutainer Plus plastic whole blood tube—Lavender with K_2EDTA additive) and serum collection (#367974 BD Vacutainer Plus plastic serum tube—red/gray with clot activator and gel for serum separation; Becton, Dickinson and Co., Franklin Lakes, NJ). The blood tube for serum isolation was centrifuged at 1,300 × *g* at 4 °C for 10 min (Beckman CS-6R Centrifuge; Beckman Coulter Inc., Brea, CA). Once serum was collected, it was transported to the University of Illinois Veterinary Medicine Diagnostics Laboratory for serum chemistry analysis. K_2EDTA tubes were cooled (but not frozen) and then transported to the University of Illinois Veterinary Medicine Diagnostics Laboratory for hematology analyses.

Chemical analyses

Diet subsamples were collected from 4 to 5 packages of each diet. Before analysis, vegan diets were lyophilized (Dura-Dry MP microprocessor-controlled freeze-dryer, FTS Systems, Stone Ridge, NY). Fecal samples were dried at 55 °C in a forced-air oven. All dried diet and fecal samples were then ground in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) through a 2-mm screen using dry ice to minimize nutrient degradation. Diet and fecal samples were analyzed for DM and ash according to AOAC (2006; methods 934.01 and 942.05) with organic matter (OM) being calculated. Crude protein (CP) was calculated from Leco (FP2000 and TruMac) total nitrogen values according to AOAC (2006; method 992.15). Total lipid content (acid-hydrolyzed fat, AHF) was determined according to the methods of the American Association of Cereal Chemists (1983) and Budde (1952). Total dietary fiber of diets was determined according to Prosky et al. (1992). Gross energy was measured using an oxygen bomb calorimeter (model 6200, Parr Instruments, Moline, IL).

Fecal metabolites

Fecal SCFA (acetate, propionate, and butyrate) and BCFA (valerate, isovalerate, isobutyrate) concentrations were determined by gas chromatography according to Erwin et al. (1961) using a gas chromatograph (Hewlett-Packard 5890A series II, Palo Alto, CA) and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100+ mesh Chromosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier with a flow rate of 75 mL/min. Oven, detector, and injector temperatures were 125, 175, and 180 °C, respectively. Fecal ammonia concentrations were determined according to the method of Chaney and Marbach (1962). Fecal phenol and indole concentrations were determined using gas chromatography according to the methods described by Flickinger et al. (2003).

Fecal DNA extraction and Miseq Illumina sequencing of 16S amplicons

Total DNA from fecal samples was extracted using DNeasy PowerLyzer PowerSoil Kit (Qiagen, Valencia, CA). Concentration of extracted DNA samples were quantified using a Qubit 3.0 Fluorometer (Life Technologies, Grand Island, NY). 16S rRNA gene amplicons were generated using a Fluidigm Access Array (Fluidigm Corporation, South San Francisco, CA) in combination with Roche High Fidelity Fast Start Kit (Roche, Indianapolis, IN). The primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) that target a 252-bp fragment of the V4 region of the 16S rRNA gene were used for amplification (primers synthesized by IDT Corp., Coralville, IA; Caporaso et al., 2012). CS1 forward tag and CS2 reverse tag were added according to the Fluidigm protocol. Quality of the amplicons was assessed using a Fragment Analyzer (Advanced Analytics, Ames, IA) to confirm amplicon regions and sizes. A DNA pool was generated by combining equimolar amounts of the amplicons from each sample. The pooled samples were then size selected on a 2% agarose E-gel (Life Technologies) and extracted using a Qiagen gel purification kit (Qiagen). Cleaned size-selected pooled products were run on an Agilent Bioanalyzer to confirm appropriate profile and average size. Illumina sequencing was performed on a MiSeq using v3 reagents (Illumina Inc., San Diego, CA) at the Roy J. Carver Biotechnology Center at the University of Illinois.

Microbial data analysis

Forward reads were trimmed using the FASTX-Toolkit (version 0.0.14) and QIIME 2.0 (Bolyen et al., 2019) was used to process the resulting sequence data. Briefly, high-quality (quality value ≥ 20) sequence data derived from the sequencing process were demultiplexed. Data were then denoised and assembled into amplicon sequence variants (ASV) using DADA2 (Callahan et al., 2016). The SILVA 132 database (Quast et al., 2013) was used to assign taxonomy. An even sampling depth (sequences per sample) was used for assessing alpha- and beta-diversity measures. Beta-diversity was assessed using weighted and unweighted UniFrac (Lozupone and Knight, 2005) distance measures and presented using principal coordinates analysis (PCoA) plots.

Statistical analyses

All data were analyzed using the Mixed Models procedure of SAS (version 9.4, SAS Institute, Cary, NC) with treatment as a fixed effect and dog as a random effect. Differences among dietary treatments were determined using a Fisher-protected least significant difference with a Tukey adjustment to control for experiment-wise error. A PROC NPAR1WAY was utilized for nonparametric data, which was based on the simple linear rank statistics with Wilcoxon scores. The PROC NPAR1WAY computes a one-way ANOVA statistic, which for Wilcoxon scores, is known as the Kruskal–Wallis test (SAS Institute). A probability of $P \leq 0.05$ was accepted as being statistically significant.

Results

One dog was removed from the study in experimental period #1 due to the development of an inguinal hernia and need for surgery, which was unrelated to dietary treatments. All other dogs completed all experimental periods of the study. Therefore, all analyses were based on $N = 11$. The analyzed

Table 1. Ingredient and analyzed chemical and energy composition of commercial kibble or mildly cooked human-grade vegan diets fed to dogs

Item	CT[1]	BC[2]	BR[3]
Dry matter, %	92.46	28.51	32.82
	Dry matter basis		
Organic matter, %	92.36	94.47	94.47
Ash, %	7.64	5.53	5.53
Crude protein, %	26.69	32.50	34.20
Acid-hydrolyzed fat, %	17.28	21.71	20.82
Total dietary fiber, %	12.88	12.24	11.50
Total insoluble fiber, %	7.70	6.72	6.69
Total soluble fiber, %	5.17	5.52	4.81
Starch			
Total starch, %	39.94	39.13	40.74
Gelatinized starch, %	34.85	33.81	33.32
Resistant starch[4], %	5.09	5.32	7.42
Oligosaccharides			
Raffinose, %	0.26	0.11	0.17
Stachyose, %	0.44	6.33	3.33
Verbascose, %	0.16	0.26	0.09
Total oligosaccharides, %	0.86	6.71	3.59
Gross energy, kcal/g	5.16	5.66	5.48
Metabolizable energy (ME) estimates			
ME (Atwater factors)[5], kcal/g	4.04	4.37	4.36
ME (modified Atwater factors)[6], kcal/g	3.65	3.96	3.94
ME (NRC equation)[7], kcal/g	4.05	4.37	4.38

[1]CT, Life Protection Formula Chicken and Brown Rice, Blue Buffalo, Wilton, CT. Ingredients: deboned chicken, chicken meal, brown rice, barley, oatmeal, pea starch, flaxseed, chicken fat, dried tomato pomace, natural flavor, peas, pea protein, salt, potassium chloride, dehydrated alfalfa meal, potatoes, dried chicory root, pea fiber, alfalfa nutrient concentrate, calcium carbonate, choline chloride, DL-methionine, preserved with mixed tocopherols, dicalcium phosphate, sweet potatoes, carrots, garlic, zinc amino acid chelate, zinc sulfate, vegetable juice for color, ferrous sulfate, vitamin E supplement, iron amino acid chelate, blueberries, cranberries, barley grass, parsley, turmeric, dried kelp, *Yucca schidigera* extract, niacin (vitamin B_3), glucosamine hydrochloride, calcium pantothenate (vitamin B_5), copper sulfate, biotin (vitamin B_7), L-ascorbyl-2-polyphosphate, L-lysine, L-carnitine, vitamin A supplement, copper amino acid chelate, manganese sulfate, taurine, manganese amino acid chelate, thiamin mononitrate (vitamin B_1), riboflavin (vitamin B_2), vitamin D_3 supplement, vitamin B_{12} supplement, pyridoxine hydrochloride (vitamin B_6), calcium iodate, dried yeast, dried *Enterococcus faecium* fermentation extract, dried *Trichoderma longibrachiatum* fermentation extract, dried *Bacillus subtilis* fermentation extract, folic acid (vitamin B_9), sodium selenite, oil of rosemary.
[2]BC, The Cowbell, Bramble Inc., New York, NY. Ingredients: organic pea protein, lentil, sweet potato, carrots, organic sunflower oil, organic flax oil, peas, apples, malt extract, potato starch, nutrient mix [choline chloride, potassium chloride, L-methionine, tricalcium phosphate, taurine], vitamins [D-calcium pantothenate, riboflavin, niacin, vitamin B_{12}, vitamin A acetate, vitamin E supplement, folic acid, thiamin mononitrate, pyridoxine hydrochloride, vitamin D_2 supplement], trace minerals [zinc proteinate, iron proteinate, copper proteinate, manganese proteinate, calcium iodate, selenium yeast], nutritional yeast, caramel color, tricalcium phosphate, potassium chloride, sodium phosphate, magnesium, salt.
[3]BR, The Roost, Bramble Inc. Ingredients: organic pea protein, long grain brown rice, potato, garbanzo beans, carrots, organic sunflower oil, peas, butternut squash, blueberries, malt extract, potato starch, nutrient mix [choline chloride, potassium chloride, L-methionine, tricalcium phosphate, taurine], vitamins [D-calcium pantothenate, riboflavin, niacin, vitamin B_{12}, vitamin A acetate, vitamin E supplement, folic acid, thiamin mononitrate, pyridoxine hydrochloride, vitamin D_2 supplement], trace minerals [zinc proteinate, iron proteinate, copper proteinate, manganese proteinate, calcium iodate, selenium yeast], nutritional yeast, tricalcium phosphate, potassium chloride, sodium phosphate, magnesium, salt.
[4]Resistant starch = total starch—gelatinized starch.
[5]ME (Atwater factors; kcal/g) = [(4 × CP in DM) + (9 × AHF in DM) + (4 × NFE in DM)]/100.
[6]ME (modified Atwater factors; kcal/g) = [(3.5 × CP in DM) + (8.5 × AHF in DM) + (3.5 × NFE in DM)]/100.
[7]ME (NRC 2006 equation):
-Gross energy (GE; kcal) = (5.7 × % CP in DM) + (9.4 × % AHF in DM) + [4.1 × (% NFE + % TDF in DM)]
-Energy digestibility (ED; %): 96.6 – (0.95 × % TDF in DM)
-Digestible energy (DE): (kcal GE × ED)/100
-ME (NRC; kcal/g) = [kcal DE – (1.04 × % CP in DM)]/100

chemical composition of the test diets is shown in Table 1. As expected, the CT diet had a higher DM content than BC or BR diets. On a DM basis, CP and AHF concentrations and gross energy contents were higher in the BC and BR diets than the CT diet, but total dietary fiber, total insoluble fiber, and total soluble fiber concentrations were similar across diets.

Food intake, fecal output, and ATTD data are reported in Table 2. Dogs fed with BC or BR had higher ($P < 0.0001$) daily food intakes (g/d, as-is) than those fed with CT. On a DM basis, however, food intake was higher ($P < 0.05$) in dogs fed with the CT diet than those fed with the BC or BR diets. Dogs fed with the CT diet also had higher ($P < 0.05$) OM intake than those fed with BR. Daily CP, fat, and caloric intakes were not different among treatments. Daily fecal output (g/d, as-is) was not different among diets, but dry fecal output (g/d, DM) was higher ($P < 0.05$) in dogs fed the CT diet than those fed with the BR diet. The wet fecal output (g/d, as-is): DM intake ratio was different across all three diets, with dogs fed the BC diet having a greater ($P < 0.05$) ratio than those fed with the CT or BR diets. Dogs fed with the BR diet also had a greater ($P < 0.05$) wet fecal output: DM intake ratio than those fed with the CT diet. ATTD of OM was greater ($P < 0.05$) in dogs fed with the CT diet than those fed with the BC or BR diets. Dogs fed with the BR diet also had greater ($P < 0.05$) ATTD of OM than those fed with the BC diet. Dogs fed with the BC or BR diets had greater ($P < 0.05$) ATTD of fat than those fed with the CT diet. ATTD of DM, CP, and energy were not different among diets.

Serum metabolite data are presented in Table 3. All metabolites were within the standard reference ranges for adult dogs, with the exception of globulin (BR: 2.61 g/dL, BC: 2.64 g/dL, CT: 2.67 g/dL; reference range: 2.7 to 4.4 g/dL). Interestingly, dogs fed with the BC or BR diet had lower serum cholesterol ($P < 0.001$), triglyceride ($P < 0.001$), and calcium ($P < 0.05$) concentrations than those fed with the CT diet. Dogs fed with the BC diet had greater ($P < 0.05$) blood bicarbonate concentrations than those fed with the BR or CT diets. Lastly, dogs fed with the CT diet had greater ($P < 0.05$) serum anion gap than those fed with BC or BR. Dogs fed with BR also had greater ($P < 0.05$) serum anion gap than those fed with the BC diet.

Blood hematology data are presented in Table 4. All measures were within the standard reference ranges for adult dogs with the exception of mean corpuscular hemoglobin concentrations (BR: 32.85 g/dL, BC: 32.98 g/dL, CT: 33.02 g/dL; reference range: 33.0 to 38.6 g/dL), white blood cell concentrations (BC: 4.12 × 10^3/μL, CT: 4.74 × 10^3/μL, BR: 4.84 × 10^3/μL; reference range: 6.00 × 10^3/μL to 17.00 × 10^3/μL), and neutrophil concentrations (BC: 2.54 × 10^3/μL, BR: 2.75 × 10^3/μL, CT: 3.35 × 10^3/μL; reference range: 3.00 × 10^3/μL to 11.50 × 10^3/μL). Dogs fed with the BC or BR diets had higher ($P < 0.001$) mean platelet volume, lower ($P < 0.05$) platelet concentrations, and lower ($P < 0.05$) neutrophil percentages than dogs fed with the CT diet. Dogs fed with the CT or BC diets had lower ($P < 0.05$) monocyte concentrations than those fed with the BR diet. Dogs fed with the BR diet had greater ($P < 0.05$) eosinophil concentrations and percentages than those fed with the CT or BC diets. Dogs fed with the BC diet also had greater ($P < 0.05$) eosinophil concentrations and percentages than those fed the CT diet. All other hematological variables were not different among diets.

Fecal characteristics and metabolite concentrations are presented in Table 5. Fecal DM percentage was lower ($P < 0.001$) in dogs fed with the BC or BR diets than those fed with the CT diet, but fecal scores and pH were not different among groups. Fecal acetate concentrations were greater ($P < 0.05$) in dogs fed with the BC diet than those fed with the CT or BR diets. Fecal total SCFA concentrations were greater ($P =$

Table 2. Food intake, fecal output, and ATTD of dogs fed commercial kibble or mildly cooked human-grade vegan diets

Item	CT[1]	BC	BR	SEM[2]	*P*-value
Food intake					
g food/d (as-is)	147.9[b]	346.7[a]	280.9[a]	27.9	<0.001
g dry matter/d	136.7[a]	98.8[b]	92.2[b]	11.4	0.021
g organic matter/d	126.3[a]	93.4[a,b]	87.1[b]	10.7	0.031
g crude protein/d	36.9	32.1	31.5	3.47	0.495
g fat/d	23.6	21.5	19.2	2.21	0.378
kcal/d[3]	705.9	559.2	504.9	61.2	0.071
Fecal output					
Fecal output, as-is (g/d)	64.7	64.7	57.4	8.02	0.762
Fecal output, dry matter (g/d)	23.0[a]	16.6[a,b]	14.6[b]	2.28	0.035
As-is fecal output (g/d)/dry matter intake (g/d)	0.468[z]	0.654[x]	0.595[y]	0.033	0.002
Nutrient and energy digestibility, %					
Dry matter	83.39	83.20	84.72	0.762	0.209
Organic matter	87.06[x]	85.00[z]	86.31[y]	0.685	0.049
Crude protein	84.74	84.41	85.85	0.820	0.378
Fat	91.73[b]	94.76[a]	94.20[a]	0.363	<0.001
Energy	86.98	86.51	87.21	0.635	0.582

[1]CT, Life Protection Formula Chicken and Brown Rice, Blue Buffalo; BC, The Cowbell, Bramble Inc.; BR, The Roost, Bramble Inc.
[2]Pooled standard error of the mean.
[3]kcal/d = total calories consumed per day.
[a,b,c]Means lacking a common superscript differ ($P < 0.05$).
[x,y,z]Means lacking a common superscript differ using Wilcoxon ($P < 0.05$).

Table 3. Serum metabolites of dogs fed commercial kibble or mildly cooked human-grade vegan diets

Item	Reference range	CT[1]	BC	BR	SEM[2]	*P*-value
Creatinine, mg/dL	0.5–1.5	0.582	0.500	0.609	0.07	0.542
BUN[3], mg/dL	6–30	12.9	13.7	16.1	1.61	0.680
BUN/creatinine ratio		23.6	28.5	27.7	2.32	0.250
Total protein, g/dL	5.1–7.0	5.56	5.42	5.43	0.10	0.143
Albumin, g/dL	2.5–3.8	2.89	2.78	2.82	0.08	0.087
Globulin, g/dL	2.7–4.4	2.67	2.64	2.61	0.10	0.735
Albumin/globulin ratio	0.6–1.1	1.1	1.06	1.1	0.06	0.662
Calcium, mg/dL	7.6–11.4	9.79[a]	9.50[b]	9.53[b]	0.13	0.013
Phosphorus, mg/dL	2.7–5.2	4.01	3.72	3.99	0.20	0.296
Sodium, mmol/L	141–152	147	148	147	0.29	0.275
Potassium, mmol/L	3.9–5.5	4.38	4.49	4.46	0.06	0.289
Sodium/potassium ratio	28–36	33.5	32.8	33	0.44	0.494
Chloride, mmol/L	107–118	115	115	115	0.51	0.080
Glucose, mg/dL	68–126	84.6	84.2	84.9	2.51	0.885
ALP[3], U/L	7–92	41.8	47.4	40.2	8.43	0.525
CALP[3], U/L	0–40	14.4	20.8	16.3	7.39	0.203
ALT[3], U/L	8–65	36.2	39.8	36.3	7.01	0.151
GGT[3], U/L	0–7	3.82	3.45	4.00	0.48	0.657
Total bilirubin, mg/dL	0.1–0.3	0.17	0.19	0.16	0.01	0.169
Creatine kinase, U/L	26–310	94.1	105	110	7.43	0.081
Cholesterol, mg/dL	129–297	184[a]	133[b]	130[b]	10.17	<0.001
Triglycerides, mg/dL	32–154	56.2[a]	36.8[b]	34.2[b]	2.89	<0.001
Bicarbonate, mmol/L	16–24	21.2[b]	23.3[a]	21.5[b]	0.41	<0.001
Anion Gap	8–25	15.6[x]	14.2[z]	15.1[y]	0.40	0.020

[1]CT, Life Protection Formula Chicken and Brown Rice, Blue Buffalo; BC, The Cowbell, Bramble Inc.; BR = The Roost, Bramble Inc.
[2]Pooled standard error of the mean.
[3]BUN, blood urea nitrogen; ALP, alkaline phosphatase; CALP, corticosteroid-induced alkaline phosphatase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.
[a,b,c]Means lacking a common superscript differ ($P < 0.05$).
[x,y,z]Means lacking a common superscript differ using Wilcoxon ($P < 0.05$).

0.05) in dogs fed with the BC diet than those fed with the CT diet. Fecal propionate and butyrate concentrations were not different among diets. Fecal isobutyrate and isovalerate concentrations were greater ($P < 0.05$) in dogs fed with the BC diet than those fed with the BR diet. Fecal valerate and total BCFA concentrations were not different among diets. Dogs fed with the CT diet had greater ($P < 0.05$) fecal phenol, 4-methylphenol, 4-ethylphenol, indole, 3-methylindole, 2,3-dimethylindole, and total phenol and indole concentrations than dogs fed with the BC or BR diets. Also, dogs fed with the BC diet had greater ($P < 0.05$) fecal phenol, indole, 3-methylindole, 2,3-dimethylindole, and total phenol and indole concentrations than dogs fed with the BR diet. Fecal ammonia concentrations were not different among diets.

Assessment of fecal bacterial alpha diversity (observed OTU, Faith's PD, and Shannon Diversity Index) and beta diversity showed no differences among diets (Figures 1 and 2). However, the relative abundances of 5 bacterial phyla and 18 bacterial genera were affected by dietary treatment (Table 6). At the phylum level, dogs fed with the BR diet had greater ($P < 0.05$) relative abundance of fecal Actinobacteria than dogs fed with the CT or BC diets. Dogs fed with the CT diet also had greater ($P < 0.05$) relative abundance of fecal Actinobacteria than dogs fed with the BC diet. Dogs fed with the BC diet had greater ($P < 0.05$) relative abundance of fecal Bacteroidota than dogs fed with the CT diet. Dogs fed with the CT diet also had greater ($P < 0.05$) relative abundance of fecal Firmicutes and lower ($P < 0.05$) relative abundance of fecal Proteobacteria than dogs fed with the BC or BR diets. Lastly, dogs fed with the BC diet had greater ($P < 0.05$) relative abundance of fecal Fusobacteriota than dogs fed with the CT diet.

The relative abundances of 18 bacterial genera were altered by diet (Table 6.). Specifically, the relative abundances of fecal *Adlercreutzia* and *Clostridium sensu stricto 1* were higher ($P < 0.05$), while the relative abundance of fecal *Megamonas* was lower ($P < 0.05$) in dogs fed CT than those fed BC or BR. The relative abundances of fecal *Parvibacter*, *Peptoclostridium*, *Ruminococcus-Gnavus*, and Lachnospiraceae were higher ($P < 0.05$), while the relative abundance of fecal *Escherichia-Shigella* was lower ($P < 0.05$) in dogs fed with CT than those fed with BR. The relative abundances of fecal *Turicibacter* and *Peptococcus* were higher ($P < 0.05$), while the relative abundances of fecal Muribaculaceae and *Fusobacterium* were lower ($P < 0.05$) in dogs fed with the CT diet than those fed with the BC diet. The relative abundance of fecal *Bifidobacterium* was higher ($P < 0.05$), while the relative abundance of fecal *Blautia* was lower ($P < 0.05$) in dogs fed with the BR diet than those fed with the CT or BC diets. The relative abundances of fecal uncultured Erysipelotrichaceae

Table 4. Hematology of dogs fed commercial kibble or mildly cooked human-grade vegan diets

Item	Reference range	CT[1]	BC	BR	SEM[2]	*P*-value
RBC[3], 10^6/μL	5.50–8.50	6.30	6.43	6.66	0.24	0.578
Hemoglobin, g/dL	12.0–18.0	14.12	14.22	14.78	0.52	0.632
Hematocrit, %	35.0–52.0	42.71	43.09	44.95	1.44	0.508
Mean cell volume, fl	58.0–76.0	67.87	67.15	67.64	0.65	0.464
MCH[3], pg	20.0–25.0	22.42	22.14	22.22	0.26	0.114
MCHC[3], g/dL	33.0–38.6	33.02	32.98	32.85	0.17	0.612
Mean platelet volume, fl	N/A	10.25[b]	11.23[a]	11.15[a]	0.24	<0.001
Platelets, 10^3/μL	200–700	280.64[a]	244.45[b]	246.36[b]	15.76	0.009
WBC[3], 10^3/μL	6.00–17.00	4.74	4.12	4.84	0.26	0.057
Neutrophil, 10^3/μL	3.00–11.50	3.35	2.54	2.75	0.24	0.073
Lymphocyte, 10^3/μL	1.00–4.80	1.12	1.07	1.06	0.13	0.396
Monocyte, 10^3/μL	0.20–1.40	0.26[b]	0.26[b]	0.34[a]	0.04	0.039
Eosinophil, 10^3/μL	0.10–1.00	0.15[z]	0.2[y]	0.61[x]	0.07	0.001
Basophil, 10^3/μL	0.00–2.00	0.02	0.018	0.017	0.005	0.184
Neutrophil, %	N/A	67.68[a]	59.89[b]	56.41[b]	3.12	0.010
Lymphocyte, %	N/A	23.44	26.01	21.63	2.41	0.073
Monocyte, %	N/A	5.38	6.45	7.14	0.54	0.059
Eosinophil, %	N/A	2.88[c]	7.39[b]	12.08[a]	1.31	<0.001
Basophil, %	N/A	0.37	0.46	0.37	0.096	0.732

[1]CT = Life Protection Formula Chicken and Brown Rice, Blue Buffalo; BC = The Cowbell, Bramble Inc.; BR = The Roost, Bramble Inc.
[2]Pooled standard error of the mean.
[3]RBC, red blood cells; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells.
[a,b,c]Means lacking a common superscript differ ($P < 0.05$).
[x,y,z]Means lacking a common superscript differ using Wilcoxon ($P < 0.05$).

and *Enterococcus* were higher ($P < 0.05$) in dogs fed with the BC diet than those fed with the CT or BR diet. The relative abundance of fecal uncultured Lachnospiraceae was higher ($P < 0.05$) in dogs fed with the BC diet than those fed with the BR diet. Lastly, the relative abundance of fecal *Parasutterella* was higher ($P < 0.05$) in dogs fed with BC than those fed with CT or BR and higher ($P < 0.05$) in dogs fed with BR than those fed with CT.

Discussion

With the number of pets in households expanding, the pet food market is flourishing. There are many aspects of pet ownership, but a survey of 900 dog owners suggested that the most difficult duty was choosing the correct dog food (Schleicher et al., 2019). A wide array of diet options are available, with differences in ingredient profile, guaranteed analysis targets, and diet format. Research has revealed that pet owners demonstrating the highest levels of anthropomorphism prioritize freshness, taste, and quality in their pet's diet (Boya et al., 2015; Schleicher et al., 2019). For dog owners with these priorities, popular diet options are typically fresh and human-grade foods. Fresh and human-grade foods are typically minimally processed and contain high-quality ingredients, which make them increasingly desirable to dog owners. Another potential option that is also steadily gaining traction with this demographic are vegan dog foods, which may also be minimally processed and human grade.

Previous feeding trials have been performed to analyze the effects of various fresh and human-grade dog foods. A study done by Algya et al. (2018) reported that mildly cooked diets were highly digestible and palatable, reduced blood triglycerides, and maintained adequate fecal quality. Another study performed by Do et al. (2021) analyzed human-grade dog foods. The human-grade foods tested maintained adequate fecal characteristics and were highly digestible, reduced fecal output, and modified fecal microbiota. Another study of mildly cooked, human-grade dog foods identified dramatic modifications to the fecal microbiome as a result of consuming the human-grade diets (Geary et al., 2022). The current study was performed using similar methods and measurements to test commercial mildly cooked human-grade dog foods that were formulated to be vegan.

To date, vegan pet foods have been poorly studied. Primary concerns pertain to their nutritional adequacy and protein quality. The amount of protein, although important, is arguably less important than the quality of the protein (i.e., amino acid profile). Our laboratory recently measured the amino acid concentrations and used the cecectomized rooster assay to determine the amino acid digestibilities of the mildly cooked human-grade vegan diets tested in the current study (Roberts et al., 2022). Our analyses demonstrated that all indispensable amino acids in both vegan diets exceeded concentrations recommended by AAFCO (AAFCO, 2022) and all indispensable amino acid digestibility values were above 80%. Those were promising results, but the execution of an in vivo study in dogs was also of interest to confirm adequate palatability, stool quality, nutrient digestibility, and markers of metabolism in the target species.

In the current study, serum chemistry profiles were maintained within reference ranges for dogs fed all diets. Interestingly, serum cholesterol and triglyceride concentrations were lower in dogs fed the vegan diets than those fed with the extruded kibble diet. While the cholesterol response may have

Table 5. Fecal characteristics and metabolite concentrations (μmol/g; dry matter basis) of dogs fed commercial kibble or mildly cooked human-grade vegan diets

Item	CT[1]	BC	BR	SEM[2]	*P*-value
Fecal characteristics					
Fecal score[3]	2.59	2.82	2.73	0.113	0.317
Fecal pH	6.28	6.16	6.29	0.268	0.925
Fecal DM%	32.13^{a}	23.36^{b}	24.18^{b}	0.649	<0.001
Fecal metabolites					
Acetate	686^{b}	836^{a}	597^{b}	57.2	0.002
Propionate	359	463	366	34.1	0.062
Butyrate	360	453	550	80.6	0.074
Total SCFA[4]	1405^{b}	1752^{a}	1513^{a,b}	99.9	0.052
Isobutyrate	15.0^{a,b}	19.4^{a}	12.6^{b}	1.77	0.024
Isovalerate	20.4^{a,b}	26.5^{a}	16.4^{b}	2.74	0.024
Valerate	11.8	12.1	14.1	1.71	0.441
Total BCFA[4]	47.2	58.0	43.1	5.20	0.094
Phenol	1.8^{x}	0.5^{y}	0.4^{z}	0.142	<0.001
4-methylphenol	1.3^{a}	0.6^{b}	0.3^{b}	0.169	<0.001
4-ethylphenol	10.0^{a}	1.19^{b}	0.9^{b}	0.711	<0.001
Indole	7.7^{x}	1.1^{y}	0.9^{z}	0.767	<0.001
3-methylindole	1.02^{x}	0.14^{y}	0.03^{z}	0.067	<0.001
2,3-dimethylindole	0.61^{a}	0.15^{b}	0.02^{c}	0.037	<0.001
Total P/I[4]	22.4^{a}	3.7^{b}	2.5^{c}	1.56	<0.001
Ammonia	199.7	165.9	182.1	20.33	0.509

[1]CT = Life Protection Formula Chicken and Brown Rice, Blue Buffalo; BC = The Cowbell, Bramble Inc.; BR = The Roost, Bramble Inc.
[2]Pooled standard error of the mean.
[3]Fecal score: 1 = hard, dry pellets, small hard mass; 2 = hard formed, dry stool, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool, assumes shape of container; 5 = watery, liquid that can be poured.
[4]Total SCFA = acetate + propionate + butyrate; total BCFA = valerate + isovalerate + isobutyrate; total P/I = phenol + 4-methylphenol + 4-ethylphenol + indole + 3-methylindole + 2,3-dimethylindole.
[a,b,c]Means lacking a common superscript differ ($P < 0.05$).
[x,y,z]Means lacking a common superscript differ using Wilcoxon ($P < 0.05$).

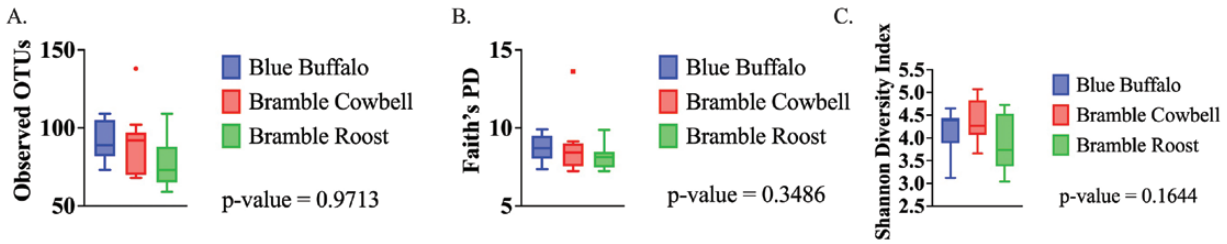

Figure 1. Alpha diversity measures of fecal samples collected from healthy adult dogs fed commercial kibble or mildly cooked human-grade vegan diets.

been due to differences in dietary cholesterol (i.e., cholesterol is not present in plant-based ingredients), it would not appear to account for the triglyceride response because dietary fat concentrations were greater in the vegan diets. The form of the diets tested may have contributed in these responses, as reduced blood cholesterol and/or triglycerides have been reported in previous studies testing fresh (Algya et al., 2018) or human-grade (Geary et al., 2022) diets. Even though the mechanism of action for such a response is not known, these data suggest that this diet format may be useful in overweight pets that are known to have elevated blood lipids.

Apparent total tract macronutrient digestibility was one of the main focus areas of the current study due to its importance as a measure of diet quality. Nutrient digestibility is affected by a number of factors, some of which include nutrient source, methods of processing, ingredient profile, and the physiological state of the animal, which can vary greatly between individuals. A 4 × 4 Latin square design experiment performed by Algya et al. (2018) evaluated the ATTD of an extruded diet, a raw diet, or two mildly cooked fresh diets and how they impacted serum chemistry and fecal characteristics, metabolites, and microbiota of adult dogs consuming them. Nutrients were highly digestible in all diets tested, but the mildly cooked diets had the highest digestibility for all nutrients except for fat digestibility that was highest in the raw diet. The nutrient digestibility values of the mildly cooked diets tested by Algya et al. (2018) were higher than those of the current study (CP: 94.6% and 92.0%; energy: 92.7%

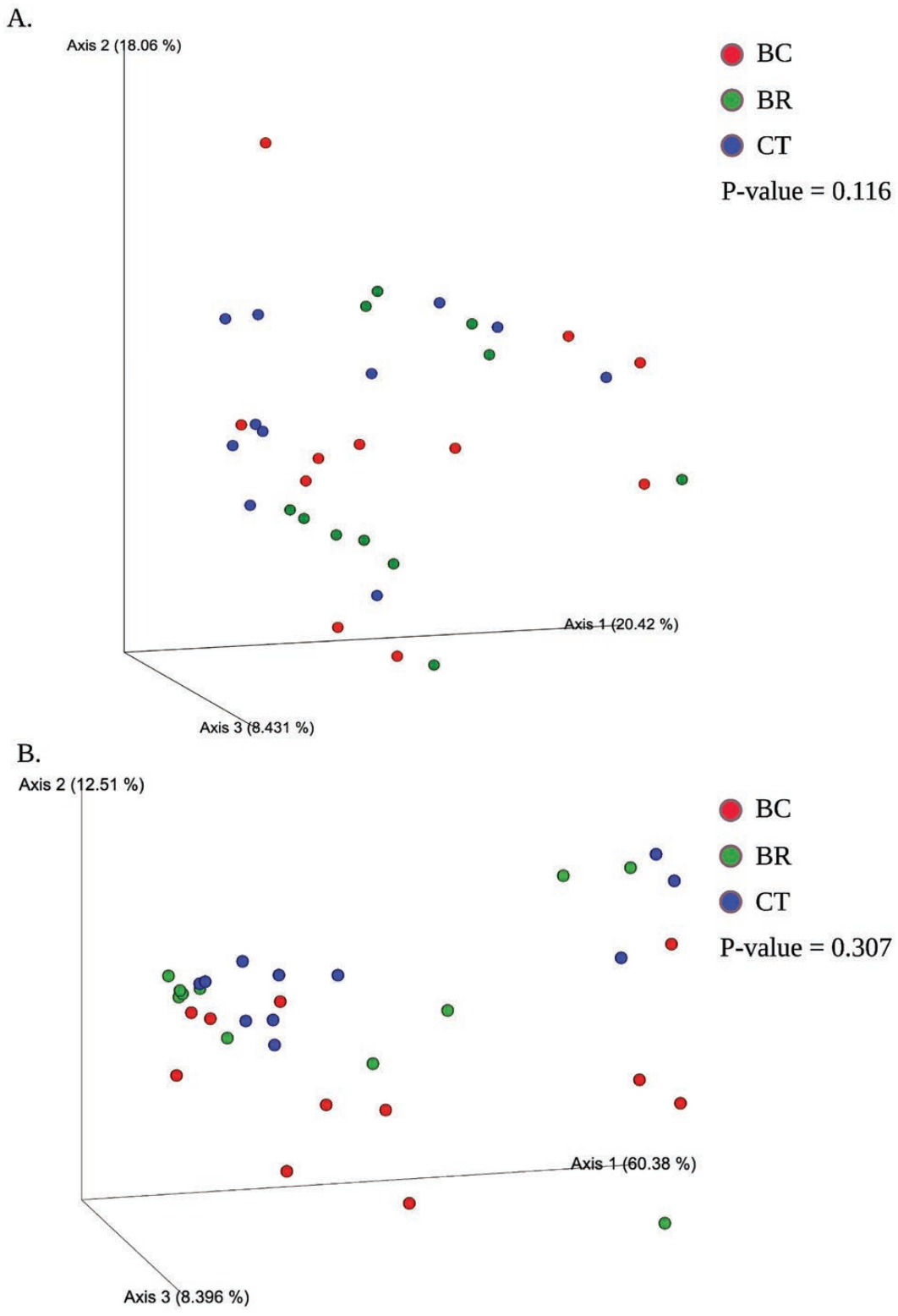

Figure 2. Beta diversity of fecal samples collected from healthy adult dogs fed commercial kibble or mildly cooked human-grade vegan diets. Principal coordinate analysis plots for unweighted (A) and weighted (B) UniFrac distances of fecal microbial communities were not altered by dietary treatments. CT = Life Protection Formula Chicken and Brown Rice, Blue Buffalo, Wilton, CT; BC = The Cowbell, Bramble Inc., New York, NY; BR = The Roost, Bramble Inc.

and 90.7%). Do et al. (2021) also conducted a 4 × 4 Latin square design experiment to evaluate two human-grade dog foods and an extruded kibble diet and a fresh diet that were not human-grade. The human-grade diets had higher nutrient digestibilities than the fresh and extruded diets tested, with digestibilities of CP (92.8% to 93.6%) and energy (92.0% to 94.8%) being quite high. The differences in CP and energy digestibilities between the previous and current studies may

Table 6. Predominant fecal bacterial phyla and genera (% of sequences) of dogs fed commercial kibble or mildly cooked human-grade vegan diets

Phyla	Genera	CT[1]	BC	BR	SEM[2]	*P*-value
Actinobacteria		11.6^{y}	10.8^{z}	15.7^{x}	1.65	0.048
	Bifidobacterium	7.52^{y}	7.59^{z}	12.10^{x}	1.69	0.036
	Adlercreutzia	0.166^{a}	0.071^{b}	0.073^{b}	0.028	0.002
	Parvibacter	0.049^{a}	0.017^{a,b}	0.010^{b}	0.006	0.010
Bacteroidota		1.96^{b}	7.53^{a}	4.63^{a,b}	1.41	0.008
	Muribaculaceae	0.341^{b}	4.02^{a}	1.24^{a,b}	0.859	0.012
Firmicutes		84.3^{a}	72.8^{b}	74.1^{b}	2.38	0.003
	Peptoclostridium	5.48^{a}	4.65^{a,b}	2.91^{b}	1.35	0.004
	Uncultured Erysipelotrichaceae	3.19^{b}	6.71^{a}	3.20^{b}	1.23	0.014
	Ruminococcus—gnavus	3.23^{a}	3.01^{a,b}	1.79^{b}	0.991	0.037
	Turicibacter	5.12^{a}	0.700^{b}	2.39^{a,b}	1.23	0.012
	Blautia	4.90^{a}	6.46^{a}	2.76^{b}	1.54	0.007
	Megamonas	0.586^{b}	2.80^{a}	3.55^{a}	1.42	0.017
	Enterococcus	0.010^{b}	3.69^{a}	0.101^{b}	1.28	<0.001
	Lachnospiraceae	1.55^{a}	1.22^{a,b}	0.599^{b}	0.383	0.025
	Clostridium sensu stricto 1	1.14^{a}	0.666^{b}	0.405^{b}	0.183	0.001
	Uncultured Lachnospiraceae	0.454^{a,b}	0.809^{a}	0.364^{b}	0.136	0.012
	Peptococcus	0.220^{a}	0.065^{b}	0.133^{a,b}	0.092	0.045
Fusobacteriota		1.67^{b}	5.95^{a}	3.07^{a,b}	1.19	0.013
	Fusobacterium	1.61^{b}	5.87^{a}	3.07^{a,b}	1.18	0.010
Proteobacteria		0.46^{b}	2.90^{a}	2.45^{a}	0.73	0.002
	Parasutterella	0.222^{z}	1.60^{x}	1.25^{y}	0.433	0.047
	Escherichia-Shigella	0.004^{b}	0.115^{ab}	0.806^{a}	0.218	0.036

[1]CT = Life Protection Formula Chicken and Brown Rice, Blue Buffalo; BC = The Cowbell, Bramble Inc.; BR = The Roost, Bramble Inc.
[2]Pooled standard error of the mean.
^{x,y,z}Means lacking a common superscript differ using Wilcoxon ($P < 0.05$).

be attributed to the differences in ingredient/protein source, higher dietary fiber concentrations of the vegan diets vs. those tested by Algya et al. (2018; 6.9%, 7.3%, and 11.8%) and Do et al. (2021; 7.0%, 7.1%, and 11.6%), higher galactooligosaccharide concentrations of the vegan diets, or other factors.

The amount of feces excreted has relevance to the gastrointestinal health of the pet (e.g., laxation) and is important to pet owners (e.g., management, convenience). Fecal output can be influenced by a variety of factors, but food intake, nutrient digestibility, and water-holding capacity of the dietary fibers consumed are likely those impacting it the greatest. In the study performed by Do et al. (2021), the human-grade diets tested lowered fecal output on an as-is (39.4 to 47.7 g/d vs. 95.9 g/d) and DM basis (11.2 to 11.8 g/d vs. 31.9 g/d) compared with an extruded diet. In the current study and that conducted by Algya et al. (2018), however, as-is fecal output was not different from that of dogs consuming extruded vs. fresh or human-grade foods.

In addition to fecal scores, which were of adequate quality for all diets fed in this study, stool quality can be assessed by the concentrations of fecal metabolites and microbiota present. Very little research has been done to study the effects of human-grade diets on fecal metabolite concentrations and the fecal microbiota contributing to their production. The primary fecal metabolites derived from carbohydrate fermentation include the SCFA, namely acetate, propionate, and butyrate. These organic acids are considered beneficial because they provide energy to colonocytes, regulate intestinal motility, strengthen tight junctions to maintain the epithelial barrier, stimulate production of anti-inflammatory compounds, and reduce growth of pathogenic species by lowering luminal pH (Smith et al., 2013; Barko et al., 2017). Factors affecting fecal SCFA concentrations include the type and amount of substrates entering the colon, which is largely dependent on nutrient intake and digestibility, as well as gastrointestinal transit time and the microbial groups present in the colon. In the studies conducted by Algya et al. (2018) and Do et al. (2021), fecal SCFA were not different among dogs fed with the human-grade or extruded diets tested. In the current study, fecal SCFA concentrations were higher in dogs fed with vegan diets compared with those fed with the extruded diet. These results suggest that the dietary fibers, resistant starches, and/or nondigestible oligosaccharides present in the vegan diets (e.g., main sources include lentils, garbanzo beans, and peas) were more fermentable than those present in the extruded diet (e.g., main sources include grains, tomato pomace, chicory root, and pea fiber).

Products of protein fermentation, including BCFA, ammonia, phenols, and indoles, are considered putrefactive compounds (Miner and Hazen, 1969). The production of these compounds may be affected by protein quality, concentration, and digestibility. The BCFA include isobutyrate and isovalerate, which are derived from the branched-chain amino acids, valine and leucine (Smith and Macfarlane, 1998; Aguirre et al., 2016). Valerate is technically a SCFA, but is derived from the other branched-chain amino acid (i.e., isoleucine). Ammonia is produced by various processes, including peptide degradation, deamination, and deamidation (Vince and Burridge,

1980). Ammonia is toxic and its presence in feces is usually inversely related to the amount of carbohydrate fermentation (Cummings and Macfarlane, 1991). Phenols and indoles are deaminated forms of aromatic amino acids and high concentrations have been shown to be toxic in vitro and in animal models (Hughes et al., 2000).

In the current study, fecal BCFA concentrations were variable and did not respond in the same way in dogs fed with both vegan diets. Dogs fed with the BR diet had the lowest fecal isobutyrate and isovalerate concentrations, while dogs fed with the BC diet had the highest. Total BCFA concentrations were not different among diets. A lack of differences was also noted for fecal ammonia concentrations. In contrast, fecal phenol and indole concentrations were drastically reduced in dogs eating the vegan diets, with concentrations being 6 to 9 times lower than dogs eating the extruded diet. In the study conducted by Do et al. (2021), fecal BCFA, ammonia, phenols, and indoles were not affected by diet. In the study conducted by Algya et al. (2018), fecal ammonia, phenol, and indole concentrations were increased, but fecal BCFA were unchanged. These variable results across studies point out how important it is to evaluate all aspects of a diet—not just diet category (e.g., extruded, human-grade). For example, although human-grade pet foods are often sold in a minimally processed form, it is possible to manufacture human-grade kibble or canned foods as long as all ingredients and processing, storage, and handling procedures are compliant with standards in place for manufacturing human foods.

Many factors influence the abundance and activity of the gut microbiota, but diet is definitely one of the biggest influencers. As discussed above, various aspects of diet can result in microbial shifts, including ingredient profile, nutrient concentrations, processing methods, and nutrient digestibility. Consumption of strictly meat or plant-based diets in humans has been shown to dramatically alter the microbial community, leading to differences in microbiota populations and microbial gene expression (David et al., 2014). Like humans, dogs are omnivores, and adult dogs have displayed similar bacterial compositional and functional diversity, as well as long-term microbial stability and resiliency (Lozupone et al., 2012; Barko et al., 2017). Also similar to humans, the predominant bacterial phyla present in canine feces include Actinobacteria (now called Actinomycetota), Bacteroidetes (now called Bacteroidota), Firmicutes (now called Bacillota), Fusobacteria (now called Fusobacteriota), and Proteobacteria (now called Pseudomonadota; Handl et al., 2011; Swanson et al., 2011; Garcia-Mazcorro et al., 2012; Kerr et al., 2013; Barko et al., 2017). Previous human studies and dog studies testing traditional (i.e., extruded) diets have reported reductions in fecal Firmicutes relative abundance and increases in fecal Bacteroidetes, Fusobacteria, and Proteobacteria relative abundances in response to greater animal-based foods and/or protein intake (Middelbos et al., 2010; David et al., 2014). In Algya et al. (2018), dogs fed fresh diets had lower relative abundances of Actinobacteria (e.g., *Bifidobacterium*) and Firmicutes (e.g., *Turicibacter*; *Clostridium*; undefined Ruminococcaceae), but higher relative abundance of Fusobacteria (e.g., *Fusobacterium*) when compared with dogs fed with an extruded diet. In Do et al. (2021), relative abundance of fecal Fusobacteria (e.g., *Fusobacterium*) tended to be greater in dogs fed the human-grade diets when compared with those fed with an extruded diet, but relative abundances of fecal Actinobacteria, Bacteroidetes, and Firmicutes were variable. Geary et al. (2022) evaluated the same chicken and rice human-grade dog food tested by Do et al. (2021) and reported that most genera in Actinobacteria (e.g., *Adlercreutzia*;), Bacteroidetes (e.g., *Bacteroides*; *Prevotella*), and Firmicutes (e.g., *Blautia*; *Catenibacterium*; *Dorea*; *Faecalibacterium*) were decreased in the dogs consuming the human-grade diets.

In the current study, the vegan diets tested influenced all five predominant bacterial phyla. While the impact on fecal Actinobacteria was variable depending on diet, dogs fed both vegan diets had greater relative abundances of fecal Bacteroidota, Fusobacteriota, and Proteobacteria and a lower relative abundance of fecal Firmicutes. We observed interesting differences in fecal *Bifidobacterium* and *Adlercreutzia*, both members of the Actinobacteria phylum. The relative abundance of *Bifidobacterium* was expected to be greatest in dogs consuming the extruded diet because one of the ingredients is chicory, which contains inulin that this taxa is known to preferentially break down (Zentek et al., 2003). Because this taxa can also metabolize the galactooligosaccharides present in legumes (Liu et al., 2017), the greater relative abundance of *Bifidobacterium* in feces of dogs fed the vegan diets was likely due to the inclusion of lentils and garbanzo beans in those formulas. This difference would be deemed positive because *Bifidobacterium* are known to produce SCFA, have antimicrobial activity, inhibit pathogens, and stimulate the immune system (Liévin et al., 2000; LeBlanc et al., 2017). Greater vegetable consumption and omega-3 fatty acids have been shown to increase *Adlercreutzia* (Caesar et al., 2015) so greater relative abundances were expected in dogs consuming the vegan diets. The opposite was observed, however, with greater relative abundance in dogs fed with the extruded diet. This may have been due to the presence of several fruits and vegetables (e.g., tomato pomace; peas; sweet potatoes; carrots; blueberries; cranberries) in that diet. That is impossible to prove, however, because although the ingredient profiles of both vegan diets list more fruits and vegetables than the ingredient profile of the extruded diet, the actual amounts of these ingredients are unknown. A lower relative abundance of fecal *Adlercreutzia* was also observed in dogs fed with a meat-based human-grade diet (Geary et al., 2022).

Several members of the Firmicutes phylum were modified by diets of the current study. Many of these bacterial groups, including *Turicibacter*, *Blautia*, *Megamonas*, *Enterococcus*, and Lachnospiraceae, are SCFA producers and often present in higher relative abundances in dogs or people consuming higher fiber content (Jackson and Jewell, 2020; Lee et al., 2022). While some were present at highest relative abundances in dogs fed with the extruded diet (e.g., *Turicibacter*; Lachnospiraceae), others had higher abundance in dogs fed with the vegan diets (BC: *Blautia*; *Enterococcus*; BR: *Megamonas*). Blautia is thought to be a beneficial commensal member of the gut microbiota (Liu et al., 2021) and has been reported to be greater in individuals consuming chicory (Mao et al., 2018) and resistant starch (Martínez et al., 2013; Yang et al., 2013). Relative abundances of fecal *Megamonas* and Lachnospiraceae were recently reported to be greater in dogs fed diets containing prebiotics and higher dietary fiber concentrations (Lee et al., 2022).

While many dietary differences in the current study were related to carbohydrate metabolism, the greater relative abundances of *Fusobacterium*, *Parasutterella*, and Erysipelotrichaceae suggest active proteolytic fermentative activity in dogs fed with the vegan diets. Because fecal SCFA were higher

and fecal phenols and indoles were lower in dogs fed with vegan diets, however, the results suggest that the saccharolytic activity in the colon was still much greater than the proteolytic activity. As with nutrient digestibility and fecal metabolite data, differences in fecal microbiota among the diets in the current study and that of other studies are likely related to dietary ingredient profiles, nutrient concentrations, and processing methods. Further research testing specific ingredient and/or nutrient modifications is needed to more accurately study these diet-related responses and what impacts they may have on canine health.

The current study had some limitations. First, the fresh, mildly cooked, human-grade vegan dog foods were compared against a chicken-based, extruded, kibble diet. Because the dietary format of the vegan diets was dramatically different from that of the kibble diet, it is impossible to distinguish any effects of ingredients from those of diet processing that also may have played a role in the differences observed among diets. In future studies, testing vegan and meat-based foods of the same processing type and format is recommended. The study was also composed of three 28 d experimental periods. This period length is plenty of time to accurately measure ATTD and evaluate changes to the fecal microbiota. Changes to serum metabolites and hematology, however, may require a longer period of time. Even though most serum metabolites were unchanged or changed in a beneficial fashion (e.g., reduced lipids), it is unknown if any other changes may have occurred over a longer period of time. Given these uncertainties, a longer experimental period may be recommended in future studies.

In conclusion, the mildly cooked human-grade vegan diets tested in this study performed well. The vegan diets were highly palatable, highly digestible, and maintained adequate stool quality, blood metabolites within reference ranges, and other measures of health. Interestingly, the vegan diets reduced serum cholesterol and triglyceride concentrations, which may be beneficial to overweight pets. Finally, dogs consuming the vegan diets had greater fecal SCFA concentrations, lower fecal phenol and indole concentrations, and altered relative abundances of nearly 20 fecal bacterial genera.

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Conflict of Interest Statement

The authors have no conflicts of interest.

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