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Fecal Microbial Communities of Overweight and Obese Client-Owned Dogs Fed Cooked Bean Powders as Assessed by 454-Pyrosequencing

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Abstract

Dry beans are consumed around the world and contain a multitude of health benefits, some of which may be related to the gut microbiome. The objective of this study was to evaluate the effects of feeding a 25% cooked Navy Bean (NB) or Black Bean (BB) powder on the fecal microbiota of overweight and obese companion dogs undergoing calorically restricted weight loss, compared to dogs fed an iso-nutrient control diet using 454 pyrosequencing. A double-blinded, placebo-controlled, three-arm clinical trial was conducted. Thirty client-owned, clinically healthy, overweight or obese, adult, male and female dogs of diverse breeds were randomized to one of the three isocaloric, nutritionally complete weight loss diets containing either 0% bean powder (placebo-control); 25% cooked BB powder; or 25% cooked NB powder and calorically restricted to achieve weight loss of up to 2% body weight/wk. for 4 wks. Fresh fecal samples were collected from each dog immediately after completing the 4-wk diet intervention and weight loss phase. Fecal genomic DNA was extracted and used to create 16S rRNA gene amplicons, which were subjected to 454-pyrosequencing. Predominant bacterial phyla present in all dogs included Firmicutes, Fusobacteria, Actinobacteria, Proteobacteria, and Bacteroidetes. Predominant fecal bacterial genera included *Clostridium*, *Blautia*, *Fusobacterium*, and undefined *Lachnospiraceae*. Fecal undefined *Ruminococcus* were greater ($P<0.05$) in dogs fed BB compared to dogs fed the control diet. Client-owned dogs with various dietary and environmental exposures, ages, and breeds were evaluated for fecal microbiota changes during short-term weight loss on different diets. A high variability in fecal microbiota was observed in this free living dog population, leading to few differences among treatments. The gut microbiota may be an area for investigation during long-term weight loss in companion animals, but such studies require a higher level of dietary control and larger sample sizes.

Keywords: Black beans; Caloric restriction; Canines; Gut microbiota; Navy beans; Obesity; Weight loss

Abbreviations: BB: Black Bean; BW: Body Weight; GI/GIT: Gastrointestinal/Gastrointestinal Tract; NB: Navy Bean; BCS: Body condition score

Introduction

Dry common beans (*Phaseoulis vulgaris* L.), also referred to as pulse grains, are an excellent source of protein, carbohydrates, dietary fiber, vitamins, minerals, and phytochemicals [1,2]. Consumption of beans and bean-containing products have been previously studied in humans, rats, and mice [1,3-9]. Consumption of these foods are associated with numerous health benefits, primarily observed in humans, including weight loss promotion, decreased risk of cardiovascular disease, cancers, diabetes, osteoporosis, hypertension, gastrointestinal (GI) disorders, and reduced blood cholesterol [1,3-9]. The health benefits of bean consumption may be, in part, due to the non-digestible carbohydrates they contain and the consequent impact they have on the gut microbiome composition and function [7,10]. Because obesity continues to be a major issue in the U.S. for companion animals, with an estimated 30-50% of pet dogs considered overweight or obese [11], investigating the potential for bean consumption to support healthy weight control in companion dogs is warranted.

The canine colon is densely populated with microorganisms, approximately 10^{11} to 10^{12} colony forming units/mL of digesta [12]. The gut microbiome has a number of roles pertaining to overall host health including to: (1) develop and maintain GI immunity, (2) contribute to fecal biomass and ultimately aid in laxation, (3) produce organic acids, which provide energy for GI epithelial cells and induce apoptosis in pre-cancerous cells, (4) improve mineral absorption, and (5) inhibit pathogen adhesion to GI epithelia [13-16]. Predominant bacterial phyla present in the canine GI tract (GIT) include Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria and dietary intervention is known to alter GI microbiota numbers and/or activity [17-23].

Recently, we reported that consumption of navy bean- and black bean-containing diets supported body weight (BW) reduction and modulated serum lipid concentrations in calorically restricted overweight and obese dogs [24]. Because the microbiota have been linked with improved metabolic status in humans and animal models [25,26], we evaluated the fecal microbiota in companion dogs after short-term caloric restriction. The purpose of this study was to determine the effects of cooked bean powder-containing diets on the fecal microbiota of overweight and obese dogs when compared to dogs fed an isocaloric, nutrient matched control diet using 454-pyrosequencing.

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Dog ID	BCS[1]	Age	Sex	Baseline Breeds[2]
O_C1	6	6	F	Dalmatian
O_C2	7	6	M	Labrador Retriever Mix
O_C3	7	4	F[3]	Saint Bernard
O_C4	7	7	F	Labrador Retriever
O_C5	7	7	M	Corgi
O_C6	6	5	F	Australian Shepherd
O_C7	7	3	M	Mixed - unknown
O_C8	8	7	F	Labrador Retriever Mix
O_C9	9	6	F	Golden Retriever
O_C10	8	7	F	Border Collie
O_BB1	8	5	F	Keeshond
O_BB2	6	3	F	Basset Hound
O_BB3	8	7	M	Australian Cattle Dog
O_BB4	9	5	F	Border Collie Mix
O_BB5	7	2	F	Boston Terrier Mix
O_BB6	9	3	M	Shih Tzu
O_BB7[4]	7	5	F	Pit Bull
O_BB8	7	5	M	Australian Cattle Dog
O_BB9	8	6	F	Australian Cattle Dog
O_BB10	8	2	M	Australian Shepherd Mix
O_NB1	7	6	M	Airedale mix
O_NB2	7	2	M	Border Collie Mix
O_NB3	7	4	F	Boston Terrier
O_NB4	7	8	F	Labrador Retriever
O_NB5	8	4	M	Dachshund
O_NB6	9	4	M	Dachshund
O_NB7	6	5	M	Australian shepherd
O_NB8	7	6	F	Australian shepherd
O_NB9	7	2	F	Boxer
O_NB10	8	5	M	Karelian Bear Dog Mix

[1]BCS, body condition score
[2]As reported by owner
[3]Intact female
[4]Only provided a fecal sample at wk. 0 and was not included in the microbiome analysis

Table 1: Body condition score, age, sex, and breed of overweight or obese dogs prior to beginning study diets for weight loss

Materials and Methods

Animals and diets

The Colorado State University (CSU) Institutional Animal Care and Use Committee approved all animal procedures prior to experimentation. The study design, animal procedures, and complete inclusion/exclusion criteria are described in detail by Forster et al. [24]. Briefly, thirty client-owned, clinically healthy, overweight or obese, male and female adult dogs were recruited for the study and written informed owner consent was obtained. Each dog was assessed by the study clinician and assigned a BCS on a 9 point scale [27]. Dogs were classified as overweight with BCS of 6-7/9 and obese with BCS of 8-9/9. A summary of the baseline characteristics for each dog, including BCS, age, sex and breed can be found in Table 1. These dogs were monitored by a veterinarian at the CSU Veterinary Teaching Hospital throughout the study, including weekly BW checks and biweekly physical exams, BCS monitoring, and blood collection as reported in Forster et al. [24]. Additionally, a medical observation form was completed by the owner at weeks 2 and 4 of the study in order to report any adverse effects or changes in the dogs' health and behavior, including vomiting, diarrhea, flatulence, fecal score (5-point scale), energy level, and diet acceptance [24].

Dogs were randomized based on BCS to one of three isocaloric, macro- and micronutrient matched weight loss diets: (1) placebo-control diet (0% bean powder); (2) BB diet; or (3) NB diet. The BB and NB diets included cooked BB or NB powder at 25% of the diet (Vegefull, ADM Edible Bean Specialties, Decatur, IL, USA). Before being ground into powder, the beans are washed, soaked, cooked, and dehydrated. Diets were formulated to meet nutritional recommendations and regulatory standards [28,29]. All 3 diets were manufactured and processed in the same location under the same conditions (ADM Alliance Nutrition Feed Research Pilot Plant, Quincy, IL, USA; Applied Food Biotechnology Plant, St. Charles, MO, USA). Canines were calorically restricted with all study diets for a target weight loss of 2% BW/wk. Dog owners and study clinician were blinded to the assigned diet group during the study. Dogs were equally distributed and randomized to diet groups by sex and BCS (Figure 1).

Fecal DNA extraction and Pyrosequencing

One fresh fecal sample was collected from each dog at baseline and after 4 wks. of consuming the study diet. Owners were instructed to collect each sample within 5 hrs. of defecation. Samples were immediately frozen at -20°C and stored at -80°C until analysis. Genomic DNA was extracted from fecal samples using the PowerLyzer™ PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Extracted DNA was quantified using a Qubit® 2.0 Fluorometer (Life Technologies, Invitrogen, Grand Island, NY, USA) and DNA quality was measured using E-Gel® electrophoresis (EX 2% agarose, Invitrogen, Life Technologies™, Grand Island, NY, USA). Amplification of a 600-bp sequence in the V4-V6 variable region of the 16S rRNA gene was done using barcoded primers as previously described [30,31]. PCR amplicons of all samples were further purified using AMPure XP beads (Beckman-Coulter, Inc., Indianapolis, IN, USA) to remove smaller fragments. The quality of the DNA was assessed before sequencing using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Finally, the DNA amplicons were combined in equimolar ratios to create a DNA pool that was used for pyrosequencing. Pyrosequencing of the PCR amplicons was performed on a GS PicoTiterPlate (PTP) at the W. M. Keck Center for Biotechnology at the University of Illinois using a 454 Genome Sequencer and FLX titanium reagents (Roche Applied Science, Indianapolis, IN, USA).

Bioinformatics and statistical analyses

Sequence data analysis was performed with QIIME 1.8.0 [32]. Read quality was assessed and found to decrease at 420 bases, so all sequences were truncated after position 420 [33]. Sequences were then demultiplexed and quality filtered with split_libraries.py with default parameters. Resulting sequences were clustered into operational taxonomic units (OTU) using closed-reference OTU database (97% similarity threshold) then quality was filtered [34]. The dataset was rarified to 2,572 OTU for analysis of diversity and species richness. Weighted and unweighted UniFrac distances between all samples were visualized using principal coordinates analysis (PCoA) and performed to evaluate differences in microbial communities among treatment groups.

Data are presented as percentage of sequences at each taxonomic level and were analyzed using the type 3 method of the mixed models procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The main fixed effect of diet was analyzed. Proc Univariate was used to test for homogeneity of variance and normality. Means were separated using a protected least squares difference with a Tukey adjustment to

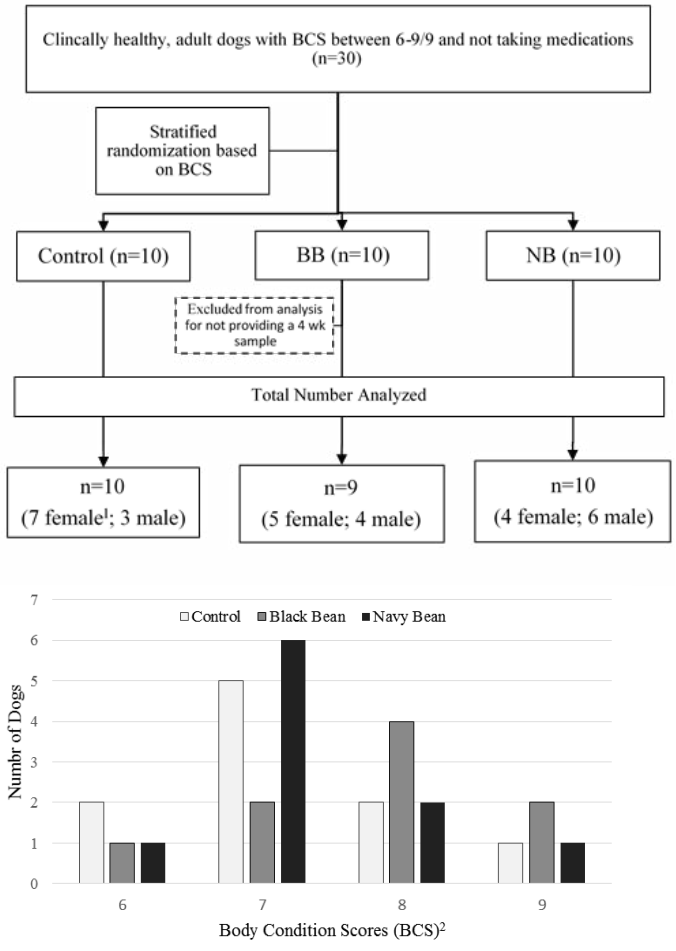
control for multiple comparisons. A probability of $P<0.05$ was accepted as being statistically significant and $P\leq0.10$ accepted as trends. Due to differences in baseline diets and consequent microbial populations among animals, statistics were applied and reported to test for dietary effects with 4 wk. data only (control vs. BB vs. NB).

Results

Dogs were randomized to one of three dietary interventions. The three diets contained approximately 28% crude protein, 8.5% fat and 3.9% fiber (16-18% total dietary fiber) [24]. Fecal samples collected from twenty-nine of the thirty dogs that completed the study contained sufficient quality for microbiota sequence analysis (Figure 1). One dog was withdrawn from the analysis due to inability to obtain a fecal sample after the 4-wk dietary intervention. No gastrointestinal discomfort or changes in flatulence or fecal consistency were reported by the owners. Pyrosequencing of 16S rRNA barcoded amplicons resulted in a total of 852,979 total sequences with a mean and standard deviation of 14,706 ± 7057 reads per sample. Sequences are available at the NCBI sequences read archive (http://www.ncbi.nlm.nih.gov/Traces/sra/) under the accession number SRP045271.

Multi-colored stacked bar graphs represent the relative abundance of bacterial genera of all dogs at baseline (Figure 2). Alpha diversity and species richness were not different among treatments (Figure 3). Based on 97% OTU composition, the PCoA of unweighted Unifrac distances (abundance of bacterial species was not accounted for; Figure 4A indicated that OTU composition was not more similar within diet*time categories than across categories (t = -3.089; P = 0.517, 2-tailed, 2-sample Monte Carlo t test with 999 iterations). Similarly, PCoA of weighted Unifrac distances between samples based on their 97% OTU composition and abundances (Figure 4B) indicated that gut microbial communities were not more similar within diet*time categories than across categories (t = -0.985; P = 1, 2-tailed, 2-sample Monte Carlo t test with 999 iterations). Predominant bacterial phyla present in all dogs at baseline included Firmicutes, Fusobacteria, Actinobacteria, Proteobacteria, and Bacteroidetes (data not shown). Firmicutes composed about 88-94% of bacterial sequences, Fusobacteria composed 0.9-9.5% of bacterial sequences, Actinobacteria composed 1.4-4.5% of bacterial sequences, and Proteobacteria and Bacteroidetes each contributed to only 0.2-0.5% and 0.06-0.3% of sequences, respectively (data not shown). Predominant bacterial genera present in all dogs at baseline included *Clostridium*, *Blautia*, undetermined *Lachnospiraceae*, undefined *Ruminococcus*, *Fusobacterium*, *Dorea*, and *Catenibacterium* (Figure 3).

Predominant bacterial phyla present in all dogs at wk. 4 included Firmicutes, Fusobacteria, Actinobacteria, Proteobacteria, and Bacteroidetes (Table 2). Firmicutes composed about 84-92% of bacterial



[1]All but one female participant in the control group was spayed and all males were castrated.
[2]Body Condition Score (BCS): 9-point scale was used, where BCS 4 and 5 are ideal, 6 and 7 are overweight, and 8 and 9 are obese[27].

Figure 1: Study design with sex and body condition score distribution of overweight or obese dogs fed control or bean based weight loss diets.

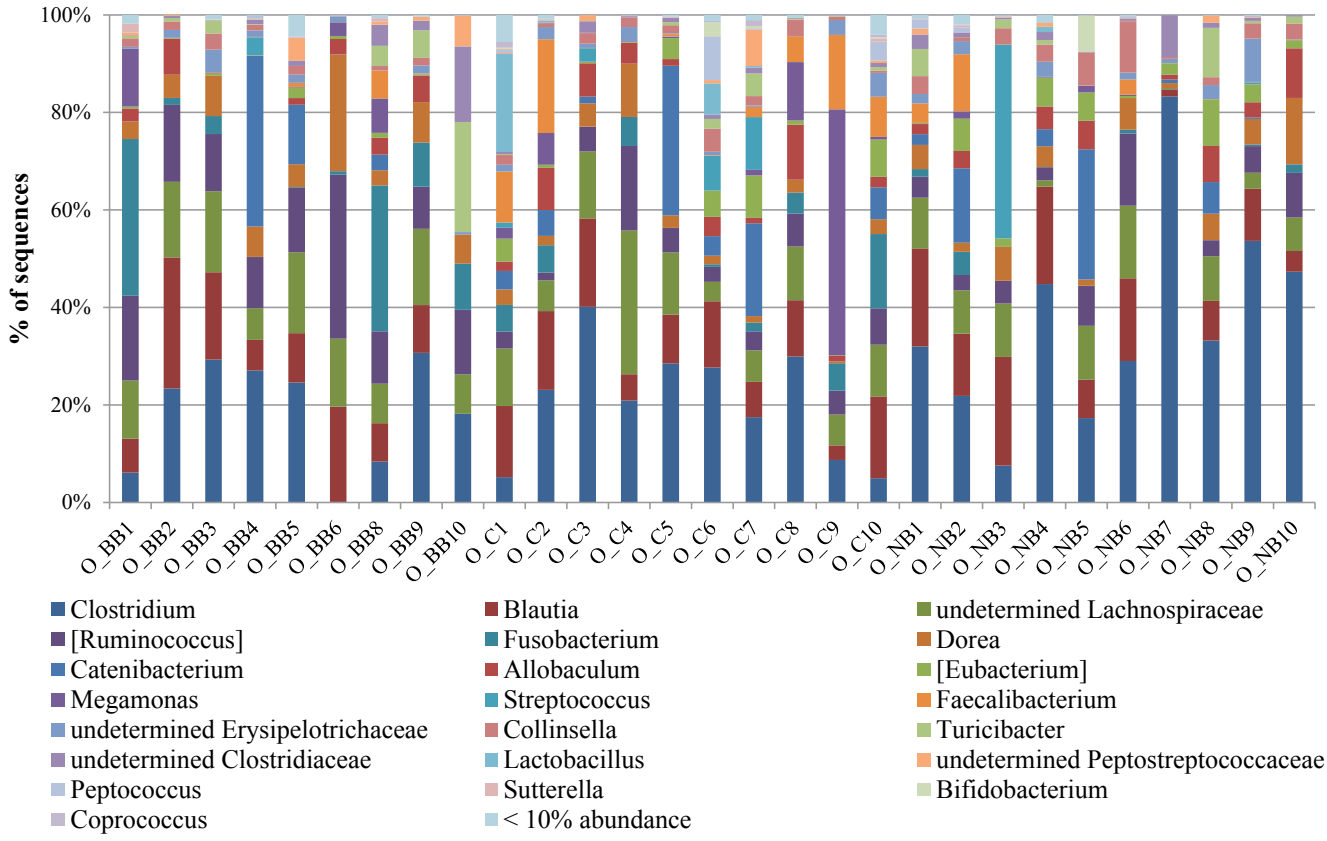


Figure 2: Multi-colored stacked bar graphs represents the relative abundance of bacterial genera (expressed as a percentage of sequences) in feces of overweight or obese dogs at baseline (wk. 0).

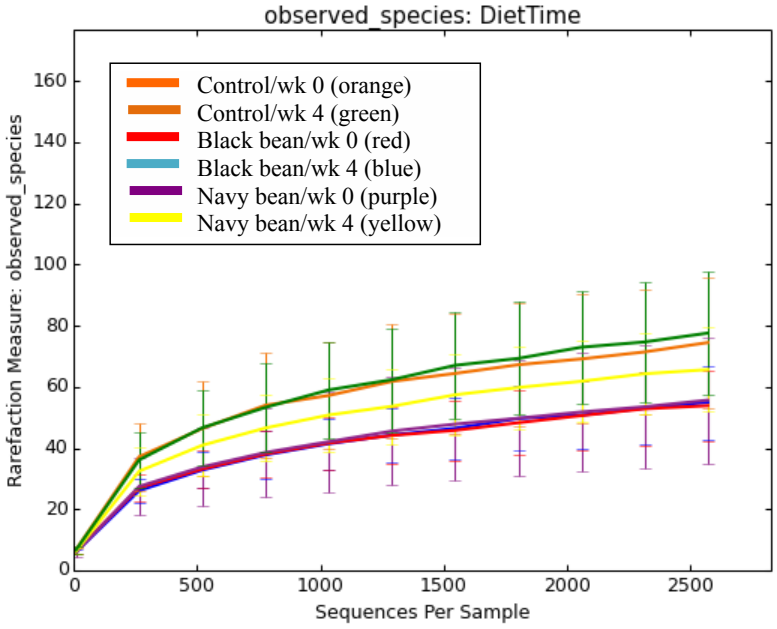


Figure 3: Rarefaction curve of 16S rRNA gene sequences from fecal bacterial samples among dogs fed bean powder-containing weight loss diets.

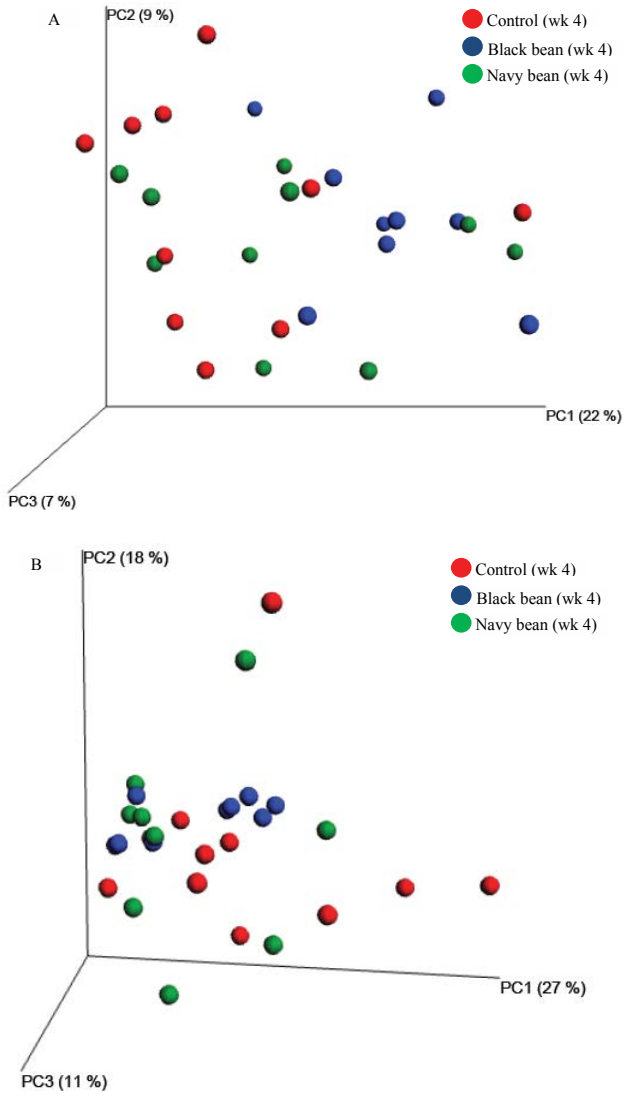


Figure 4: Principal coordinates analysis (PCoA) of unweighted UniFrac distances of fecal bacterial samples among dogs fed bean powder-containing weight loss diets after a 4-wk treatment period based on their 97% OTU composition (abundance of bacterial species was not accounted for; A). This plot indicates that OTU composition was not more similar within diet'time categories than across categories (t = -3.089; P = 0.517, 2-tailed, 2-sample Monte Carlo t test with 999 iterations). Similarly, PCoA of weighted Unifrac distances between samples based on their 97% OTU composition and abundances (B) indicates that gut microbial communities were not more similar within diet'time categories than across categories (t = -0.985; P = 1, 2-tailed, 2-sample Monte Carlo t test with 999 iterations).

sequences, Fusobacteria composed about 4-12% of bacterial sequences, Actinobacteria composed about 1-2% of bacterial sequences, and Proteobacteria, and Bacteroidetes each contributed to only 0.2-1.4% and 0.05-0.2% of bacterial sequences, respectively. No dramatic shifts related to treatment were observed in the unweighted PCoA plot, but dogs consuming BB appeared to cluster together in the weighted PCoA plot (Figures 4A and 4B).

Predominant fecal bacterial families present in all dogs at wk. 4 included *Lachnospiraceae*, *Clostridiaceae*, *Erysipelotrichaceae*, and *Fusobacteriaceae*. *Lachnospiraceae* composed about 27-43% of bacterial sequences, *Clostridiaceae* composed about 19-30% of bacterial sequences, *Erysipelotrichaceae* composed about 10-16% of bacterial sequences, and *Fusobacteriaceae* composed about 4-12% of bacterial sequences. Predominant fecal bacterial genera included *Clostridium*, *Blautia*, *Fusobacterium*, and undefined *Lachnospiraceae*. Baseline variation in the microbiota composition was significant between the control, NB, and BB groups, making differences at wk. 4 difficult to interpret (Figure 3). The relative abundance of fecal undefined *Ruminococcus* (phylum Firmicutes) was greater (P = 0.02) in dogs fed BB (8.50%) when compared to dogs fed the control diet (3.37%) at wk. 4. The relative abundance of Ruminococcaceae *Ruminococcus* tended to be higher (P = 0.05) in dogs fed the CON diet (0.10%) compared to dogs fed the BB diet (0.01%), but was similar at baseline. The relative abundance of fecal *Dorea* (phylum Firmicutes) tended to be lower (P = 0.05) in dogs fed the control or NB diet (3.53% and 3.23%, respectively) when compared to dogs fed BB (6.56%), this difference was not significant at baseline. The relative abundance of fecal *Faecalibacterium* (phylum Firmicutes) tended to be lower (P = 0.05) in dogs fed BB (0.42%) when compared to dogs fed the control diet (4.80%), and was significantly different at baseline. The relative abundance of fecal *Blautia* (phylum Firmicutes) was numerically greater (P = 0.18) in dogs fed BB or NB (18.77% and 16.03%, respectively) when compared to dogs fed the control diet (11.76%), but did not reach statistical significance.

Discussion

Black or navy bean consumption led to few changes in the fecal microbiota of this clinically healthy, diverse breed, overweight and obese companion dog population. No gastrointestinal discomfort or changes in flatulence or fecal consistency were reported by the owners, which supports both the safety and tolerability of adding 25% cooked bean powder to dog food. This finding is consistent with our previous report demonstrating that 25% NB intake did not disrupt the fecal microflora of healthy weight companion dogs [35]. The baseline compositions of these overweight or obese dogs varied significantly with relative abundances of Firmicutes, Fusobacteria, and Actinobacteria resembling previous reports [35,36]. As such, several of the changes observed may be attributed to the variation in baseline fecal microbiota (e.g. Firmicutes undefined *Ruminococcus* and *Faecalibacterium),* however Ruminococcaceae *Ruminococcus, Dorea* (phylum Firmicutes) and *Blautia* (phylum Firmicutes) differed in at least one of the diet groups at wk. 4, and can be considered diet-modifiable. Human consumption of beans or other fermentable fibers have shown similar observational changes. For example, increased fecal *Ruminococcus* was observed in human stool following the increased consumption of resistant starch [37,38]. In dogs, fecal *Ruminococcus* numerically increased when fed fermentable fibers [23]. The dogs in the current study followed a similar pattern, which may be partially due to the resistant starch content of beans. *Blautia* are also part of the butyrate-producing bacteria group in the gut, which proliferate in response to microbial fermentation of certain carbohydrate substrates [39-41]. Increased fecal *Blautia* was observed in response to resistant starch supplementation using an *in vitro* model [42], and in this case suggests increased content of plant polysaccharides from bean powders reaching the colon for fermentation. Furthermore, Suchodolski et al. [43], who evaluated the impact of diarrhea and inflammatory bowel disease on the fecal microbiome of dogs, observed a decrease in fecal *Blautia* in dogs with acute diarrhea. These findings suggest *Blautia* has roles in maintaining a healthy canine intestinal microbiome and may be supported by the prebiotics found in common beans.

End-products of microbial fermentation were not evaluated in

			Diet				
Phylum	**Family**	**Genus**	**Control**	**Black Bean**	**Navy Bean**	**Pooled SEM**	**P-Value**
Actinobacteria			**1.36**	**2.25**	**2.33**	**0.59**	**0.44**
	Bifidobacteriaceae	*Bifidobacterium*	0.05	0.01	0.04	0.03	0.63
	Coriobacteriaceae	*Collinsella*	1.24	2.14	2.23	0.57	0.40
		Slackia	0.06	0.04	0.06	0.03	0.90
Bacteroidetes			**0.27**	**0.05**	**0.33**	**0.13**	**0.32**
	Bacteroidaceae	*Bacteroides*	0.08	0.05	0.14	0.07	0.67
	Prevotellaceae	*Prevotella*	0.15	0.00	0.15	0.07	0.27
Firmicutes			**84.07**	**88.82**	**92.14**	**3.84**	**0.33**
	Clostridiaceae	Unclassified Clostridiaceae	0.90	0.58	0.53	0.14	0.13
		Clostridium	18.36	29.76	26.75	4.65	0.22
		Sarcina	0.01	0.00	0.00	0.01	0.66
	Enterococcaceae	*Enterococcus*	0.02	0.09	0.01	0.04	0.40
	Erysipelotrichaceae	Unclassified Erysipelotrichaceae	2.78	2.46	2.99	0.69	0.87
		Allobaculum	3.22	4.11	4.01	1.08	0.81
		Bulleidia	0.39	0.01	0.00	0.23	0.41
		Catenibacterium	3.80	2.48	4.01	1.63	0.78
		Coprobacillus	0.05	0.14	0.03	0.05	0.23
		[Eubacterium]	3.65	1.73	5.41	1.90	0.41
	Lachnospiraceae	Unclassified Lachnospiraceae	8.46	9.84	9.25	1.89	0.88
		Blautia	11.76	18.77	16.03	2.58	0.18
		Coprococcus	0.24	0.10	0.30	0.10	0.35
		Dorea	3.53^{x}	6.56^{y}	3.23^{x}	1.00	0.05
		Epulopiscium	0.01	0.01	0.003	0.01	0.62
		Roseburia	0.020	0.001	0.003	0.01	0.28
		[Ruminococcus]	3.37^{a}	8.50^{b}	6.65^{ab}	1.21	0.02
	Lactobacillaceae	*Lactobacillus*	0.73	0.03	0.01	0.43	0.41
	Peptococcaceae	*Peptococcus*	1.18	0.03	0.51	0.50	0.29
	Peptostreptococcaceae	Unclassified Peptostreptococcaceae	0.46	0.10	0.26	0.12	0.11
		Clostridium	0.00	0.01	0.03	0.01	0.32
	Ruminococcaceae	*Butyricicoccus*	0.10	0.02	0.08	0.03	0.21
		Faecalibacterium	4.80^{y}	0.42^{x}	2.51^{xy}	1.17	0.05
		Ruminococcus	0.10^{y}	0.01^{x}	0.02^{xy}	0.03	0.05
	Streptococcaceae	*Streptococcus*	6.37	0.35	3.55	3.98	0.58
	Turicibacteraceae	*Turicibacter*	1.63	0.34	0.67	0.53	0.22
	[unclassified]		2.24	2.23	2.82	0.66	0.77
	Veillonellaceae	*Megamonas*	4.95	0.12	2.02	1.65	0.13
		Megasphaera	0.13	0.00	0.00	0.05	0.15
		Phascolarctobacterium	0.38	0.00	0.28	0.19	0.37
Fusobacteria			**12.90**	**8.65**	**4.49**	**3.68**	**0.28**
	Fusobacteriaceae	*Cetobacterium*	0.80	0.00	0.00	0.48	0.40
		Fusobacterium	12.11	8.65	4.49	3.70	0.35
Proteobacteria			**1.40**	**0.24**	**0.71**	**0.37**	**0.11**
	Alcaligenaceae	*Sutterella*	0.51	0.06	0.36	0.24	0.42
	Campylobacteraceae	*Campylobacter*	0.04	0.00	0.05	0.03	0.45
	Helicobacteraceae	*Helicobacter*	0.10	0.12	0.02	0.07	0.60
	Succinivibrionaceae	*Anaerobiospirillum*	0.02	0.01	0.00	0.01	0.43
	Enterobacteriaceae	*Escherichia*	0.02	0.00	0.01	0.01	0.17

[a,b]Mean values within the same row not sharing common superscript letters differ (P < 0.05) due to diet
[x,y]Mean values within the same row not sharing common superscript letters differ (P ≤ 0.10) due to diet

Table 2: Predominant bacterial phyla and genera (expressed as a percentage of sequences) in feces of overweight or obese dogs fed control or bean powder-containing weight loss diets after a 4-wk treatment period.

these dogs, yet the shifts in such bacterial populations suggest that there would be increased carbohydrate fermentation, namely for short-chain fatty acid production. Increased fecal short chain fatty acids are expected to have positive impacts on gut health in overweight dogs losing weight and merits continued evaluation. For instance, increased abundance of fecal *Lachnospiraceae* has been associated with increased butyrate production [44-46]. Furthermore, increased propionate production has been previously associated with decreased serum lipids [47], which were demonstrated in the overweight/obese dogs consuming beans in the current study [24].

It should be emphasized that the dogs in the current study not only received bean powder-dietary treatments, but also underwent a 4-wk weight loss phase. This combination of treatments may lead to differences in the microbiome not accounted for by dietary changes

alone. In humans and rodent models, researchers have observed increased fecal Bacteroidetes and decreased Firmicutes during weight loss [48-51]; though shifts in these groups were not observed in the current short-term weight loss study. Interestingly, the predominant bacterial phyla observed herein at both baseline and wk. 4 was similar to those of the lean dog group observed by Park et al. [52]. However, as the primers utilized in this study have a bias against Bacteroidetes, further research is necessary to determine if bacteria in the Bacteroidetes phyla also increase in canine populations during weight loss in the long term.

Although several fecal microbiota shifts were observed in the current study, the overall gut microbial populations remained stable in all dogs consuming bean-based or control diets. This may be due, in part, to the matched macro- and micronutrient profiles of the diets. However, bean based canine diets were shown to differ in phytochemical diversity, despite the nutrient match [53], and differences in phenolic compounds have been reported between bean cultivars that may influence metabolism by microbiota when compared to microbial composition [2,54-56]. Lin et al. [56] evaluated the polyphenolic profile of several common dry beans from different commercial market classes, and concluded that black beans and navy beans had similar hydroxycinnaminic acid derivatives. However, the black bean group had 3 identified anthocyanins (flavonoids), whereas the navy bean group had no flavonoids detected [56]. Thus, more research is necessary to identify bioactive components of the beans that may be responsible for subtle shifts in the metabolic function of the gut microbiome, for resulting health implications.

In conclusion, although the PCoA plots do not demonstrate dramatic treatment-related shifts in the fecal microbiota, the shifts in relative abundance of some taxa were associated with the consumption of cooked bean powders. The interpretation of these data is subject to several limitations. First, the dogs studied were part of a free-living population, with the potential for different exposures to environmental factors, including foods other than the assigned treatment diets. Second, the dogs were not acclimated to a control diet prior to baseline measurements, potentially masking bean diet treatment-related shifts. Because of this, it is highly recommended that future studies aiming to investigate changes in gut microbiota over time with companion dogs should incorporate a run-in period with the same placebo-control diet for at least 10 days prior to collection or utilize a Latin square design and have higher animal number due to high variability. Despite these limitations and high individual variability, this study supports that a cooked bean based canine diet only modestly shifts a healthy microbiome, and thus provides rationale for continued investigation to enhance canine gut health. Future research is warranted to better understand the impact of cooked beans on clinical obesity and obesity-related comorbidities of a free-living canine population.

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