The Effects of Fermented and Cultured Supplements on Dog's Gut Microbiome

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ABSTRACT

The dog's microbiome has emerged as the crucial moderator in the interactions between food and the body. This study was conducted to examine the canine gut microbiome, testing the effects of probiotic supplements (fermented sauerkraut and cultured and unpasteurized Kefir) on overall gut microbiome composition. The hypothesis is that adding fermented sauerkraut and unpasteurized and cultured kefir supplements would shape the gut microbiota reflecting significant change in the alpha diversity (including *Firmicutes*:*Bacteroidetes* ratio), richness (Shannon Index), and evenness specifically while looking at the relative abundance of each dog. This study engaged seven dogs eating the same raw, dehydrated diet with protein over a 6-week period with the addition of fermented and cultured food supplements. All dogs' gut microbiomes were analyzed using 16s rRNA gene sequencing through Animal Biome test kits to gather the alpha taxonomic composition of each dog at the beginning (baseline) and 6-weeks of adding fermented and cultured supplements. The results suggest that the driving force in microbiota composition when looking at alpha levels of relative abundance, evenness, diversity, and richness in dogs is specific to the individual, with dogs presenting various representations of main phylum and major genus. The statistical significance suggests that evenness and *Firmicutes*:*Bateroidetes* ratio were significant (P< 0.001) when compared between mean control value of dogs not treated with probiotic supplements versus the seven dogs treated for 6-weeks with probiotic supplements. Data also suggests that when dogs live in the same household, they tend to have similar taxonomic gut microbiome communities. Today, society is seeing a rise in microbiome-associated disorders in dogs (animals in general) and even in humans, and understanding differing effects on the gut microbiome will shape how we treat chronic issues not just for our canines, but pets and even humans.

KEYWORDS: Gut Microbiome, Diversity, Evenness, Richness, Probiotics

INTRODUCTION

Dogs have a unique collection of hundreds of different types of single-celled microorganisms (bacteria and other microbes) that inhabit the gastrointestinal tract (GI) of cats and dogs in the digestive tract (Simpson et al., 2002). The gut microbiome is directly connected to the brain via the Vagus nerve and 80% of the immune system is controlled by the gut microbiome (Barko, 2018). The microbiome affects almost every aspect of a dog's health to include weight, allergies, digestive issues, and even mental health. When gut bacteria are out of balance in a dog, disorders such as inflammatory bowel disease (IBD), allergies, diabetes, and digestive issues can result.

This study was conducted to answer if adding fermented sauerkraut and cultured and unpasteurized Kefir (items easily found in grocery stores) have an effect on a dog's gut microbiome when added to their daily meal intake? The hypothesis if adding fermented sauerkraut and unpasteurized and cultured kefir supplements would shape the gut microbiota reflecting significant change in the alpha diversity, richness, and evenness specifically while looking at the relative abundance of each dog.

Biodiversity describes the variety and variability of all living organisms within a given ecological area. Biodiversity can be used to refer to the number of species, their genetic diversity, or habitat variety. There are two main components that contribute to biodiversity—species richness and species evenness. Species richness describes the number of different species present in an area (more species = greater richness). Species evenness describes the relative abundance of the different species in an area (similar abundance = more evenness).

Role of the canine physiological gut microbiome

The interaction between gut microbiota, its host, and other somatic cells regulates many functions, such as digestion, host metabolism, vitamins synthesis (vitamin K and complex B), biotransformation of bile acids, xenobiotics metabolism, correct maturation of gastrointestinal cells, and defense against pathogenic bacteria (Steiner and Ruaux, 2008). Therefore, the microbiota can be defined as a metabolically active "organ" (Mondo et al., 2019), a living ecosystem in itself. Serotonin, a neurotransmitter, is mostly produced in the intestine, which has led to the development of the gut-brain axis concept (O'Mahony, 2015). A healthy and stable microbiome can simultaneously act as pro-and anti-inflammatory, keeping a balance to prevent excessive inflammation while still being able to promptly respond to infections (Tizard, 2018).

The microbial communities along the tract vary to reflect the microenvironment and physiological functions of each intestinal segment. Commensal bacteria (bacteria found in the intestine and other anatomical locations of the intestine) have a fundamental role on the induction, shaping, and function of the host immune system, which in turn is important in the development of the physiological gut structure and the identification of pathogens from commensal bacteria (Mondo et al., 2019). Commensal bacteria act on the host's immune system to induce protective responses that prevent colonization and invasion by pathogens; these bacteria can directly inhibit the growth of respiratory pathogens by producing antimicrobial products/signals and competing for nutrients and adhesion sites (Kahn et al., 2019). Furthermore, commensal bacteria have a fundamental role on the induction, shaping, and function of the host immune system, which in turn is important in the development of the physiological gut structure and the identification of pathogens from commensal bacteria (Mondo, 2019). Along the GI tract, bacterial sequences typically belong to one of five phyla: *Firmicutes*, *Fusobacteria*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* (Pilla et al., 2020).

Dysbiosis is an imbalance in bacterial composition, and bacterial metabolic activities and bacterial distribution inside the gut change (Pilla, 2019). Dysbiosis is defined when the reduction of bacterial diversity, loss of beneficial bacteria, and overgrowth of pathogens (Pilla, 2019) occurs. A state of dysbiosis is found in a wide range of diseases, such as inflammatory bowel disease (IBD), obesity, allergy, and diabetes, but it is unclear if it is a cause or a consequence (Pilla, 2019). Several studies about these diseases have indicated the presence of a

microbial alteration, but no consistent pattern of microbiota changes has yet been observed (Pilla, 2019).

Many of the bacteria in a dog's microbiome is inherited from its mother after birth and other bacteria from the environments and other animals (including humans) that a dog is exposed to in early years (Barko, 2018). These bacteria influence a dog for the rest of its life. Barko (2018) states that although the foundational bacteria taxon of gut communities is established in a dog's early years, the gut microbiome changes over time with age, diet, and animal's lifestyle. If a dog is prescribed antibiotics or other medication, the gut microbiome could shift quickly and can take at least a year or more to bring balance back after the dog is taken off the medications.

Canine gut microbiome studies

Besides the diet, probiotics, prebiotics, and antibiotics administration affect, and change microbiota composition, but their efficiency is not clear. The use of pre-and probiotics is broadly spread in human medicine to preserve or restore a healthy condition (Sanders et al., 2018). The employment of these devices is new in veterinary medicine and pet treatment.

Prebiotics are more recent and, in accordance to their first definition given in 2015, they are "a non-digestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host" (Bindels et al., 2015). Nowadays, several research studies reported benefits from the addition of prebiotics in pets' diets.

Despite the variations of taxa along the GI tract, samples from specific regions of the tract are difficult to obtain, and therefore most clinical studies focus on the fecal microbiota. Canine fecal samples reliably present most of the relevant taxa, unlike humans, in which most significant taxa are closely associated with the mucosa (Vázquez-Baeza et al., 2016). Recently, the development of new molecular technologies, such as next-generation sequencing (NGS), has allowed understanding the complexity and diversity of gut-microbial communities (Kim et al., 2017). Molecular-phylogenetic analysis of the bacterial 16S rRNA gene has created a more detailed inventory of bacteria groups present in the bowel. (Mondo et al., 2019) To date, there are limited comprehensive reviews or scientific work of the intestinal microbiome specifically regarding the importance of the intestinal microbiome in dogs and cats.

There are some studies about the use of probiotics in the domestic canine diet. Zentek et al, (2003) found that in dogs, an intake of 1.5% inulin could reduce fecal pH and increase *Bifidobacteria* population. Using 16S rRNA sequencing, it has been shown that dogs fed with a relatively small amount of dietary fiber change the structure of gut microbiota, increasing the density of *Firmicutes* and decreasing that of *Fusobacteria* (Middlebos et al, 2010). Another study underlined how a dietary supplementation of fructo-oligosaccharides (FOS) induces beneficial effects, such as the growth of *Bifidobacteria*, and it improves the digestibility of several minerals in the entire GI tract in the dog (Pinna et al, 2018).

Another study, chicory root (a source of inulin), improved fecal scores, increased *Bifidobacterium*, and decreased *C. perfringens* in the feces of healthy dogs (Zentek et al, 2003). A meta-analysis (several different studies on the same topic to review trends) of 15 stud-

ies including 65 different treatment conditions showed that fecal shorty chain fatty acids (SFCA) concentrations increase linearly with prebiotic doses (Patra, 2011). Furthermore, it also revealed that fecal *Bifodobacteria* and *Lactobacillus* increase with prebiotic doses and no changes for pathogenic *C. perfringens* or *E. coli*. The prebiotics were not related to the composition of the dog's diet, suggesting that prebiotic therapies can provide benefits independent of the diet (Patra, 2011).

Probiotic supplementation studies have shown benefits in small animals in several clinical trials. A small clinical trial with a probiotic strain of *Saccharomyces boulardii* improved clinical signs in dogs with IBD and protein losing enteropathy (Mustafa et al., 2016). In dogs with food-responsive diarrhea treated with lyophilized *Lactobacillus* for 21 days along with diet change, there were increased *Lactobacilli* and decreased *Enterobacteria* in the feces accompanied by improved clinical signs (Sauter, 2006). In another study of 36 dogs with acute gastroenteritis, a probiotic combination improved clinical signs compared to a placebo (Herstad et al., 2010). In a shelter-based study, this probiotic, administered with metronidazole, improved fecal scores compared to dogs treated with metronidazole alone (Fenimore, et al., 2017).

While variations in composition are observed between different studies, it is important however to note that regardless of the methods used, key bacterial species are consistently present in fecal samples of healthy dogs indicating the presence of a core fecal bacterial community. The fecal microbiome of healthy dogs is co-dominated by three phyla: *Fusobacterium*, *Bacteroidetes*, and *Firmicutes* (Middelbos et al., 2010) When reviewing the literature, a wide variation in percentages of specific bacterial taxa can be seen. It is important to remember that the methods for sequencing and data analysis are in constant evolution, and much of those variations can be attributed to different sequencing and data analysis methods.

By understanding the relationship between a dog's microbiome and digestibility of the food consumed, we can gain insights into the manipulation of diet on the gut microbiome and treating the problem of the gut microbiome versus prescribing medication because of digestive issues, diabetes, skin allergies, and other diseases in veterinarian medicine.

METHODS

Materials

Seven dogs (Table 1 in Appendix A) were selected by Volhard Dog Nutrition based on an already consistent, fresh dehydrated Volhard diet which uses raw protein as the common baseline for feeding and their regional living location. Either the Volhard AM Porridge/PM Crumble or NDF2 raw diet was distributed to each dog directly from the raw dog food nutrition company. Cultured with probiotic Wildbrine sauerkraut and Answers raw goat milk was used for the whole food supplement feeding each day, with each owner given locations to purchase the same items to administer to their dogs each day. Two complete Animal Biome test kits were used per dog ($75 per kit) for non-invasive fecal samples collections and were funded by Volhard Dog Nutrition.

Controls and variables of study

The experimental controls were the amount and type of Volhard raw diet used, amounts of fermented and cultured sauerkraut (1 Tbsp/10 pounds), and unpasteurized and cultured Answers raw goat milk (1 Tbsp/10 pounds). Independent variables identified were fermented and cultured sauerkraut and the unpasteurized and cultured raw goat milk. Variables dependent to this research were the age of dog, breed of dog, medications before and during the study, type of water dog ingests, health of dog prior and during research, activity level of each dog, whether the dog was spayed or neutered, length of time outdoors, and process of dog's birth.

Supplemental dog feeding protocol for study

Each dog owner was asked to follow the following supplemental feeding protocol (with no changes to the diet) each day during the 6-week testing period set by a certified nutritionist at Volhard Dog Nutrition: (1) In the morning, add one tablespoon for every ten pounds the dog weighs of fermented and cultured Wildbrine sauerkraut to their morning NDF2 or AM Porridge feeding and (2) In the evening, add one tablespoon for every ten pounds the dog weighs of unpasteurized and cultured Answers raw goat milk to NDF2 or PM Crumble feeding. Each meal also contained adding any type of meat protein.

Procedure

The research was conducted over a 6-week period (collection times set by Volhard Dog Nutrition) gathering information from a beginning baseline of dogs not on fermented and cultured supplements to a 6-week period of adding fermented and cultured supplements to daily diet. Volhard Nutritional Dog Food Company assisted in recruiting their own canine clients to participate in the study, utilizing dogs who were on Volhard's raw food diet for more than two years. I used a digital survey that was completed by each owner to collect background demographic data on each dog participating in the study; and a participant form was given to each owner outlining whole food supplemental feeding protocols for each meal, timeline of fecal collections for the study, and research summary plan that outlined the research being conducted. Animal Biome collection kits were ordered and shipped to each owner by Volhard Dog Nutrition. Owners used the Animal Biome testing kit and directions to collect a non-invasive pea-sized fecal sample from their dog before adding the whole food supplements to their dog's daily diet. The fecal samples were registered online with Animal Biome and shipped for testing. Each dog owner was asked to follow a supplemental whole food feeding protocol each day during the 6-week testing period after baseline fecal collection. After 6-weeks of daily whole food supplements, the Animal Biome kit was used to collect another pea-sized fecal sample and shipped for testing. Animal Biome extracts the DNA from all the bacteria in the sample (16s rRNA gene sequencing) then amplifies a small region from each cell (like a bacteria barcode) and sequences thousands of them to then provides raw data on taxon, phylum, family, and class of bacteria found in each fecal sample. Pre-probiotic and post-probiotic results from Animal Biome were provided to each dog owner and then given to me for analysis.

Alpha diversity analysis assesses the diversity within a sample. In alpha diversity, we used two different metrics: observed species to assess richness and Shannon index to assess

evenness and diversity. The Shannon Wiener Index is a measure of diversity that combines species richness (the number of species in a given area) and their relative abundances. In the Shannon diversity index (H), p is the proportion (n_i//N) of individuals of one particular species found (n) divided by the total number of individuals found (N) and then multiplied by the natural logarithm of this proportion (ln p_i). The resulting product is summed (∑) across species (s) and multiplied by -1.

$$H = -\sum_{i=1}^{S} \frac{n_i}{N} \log_2 \left(\frac{n_i}{N} \right) \quad (1)$$

Shannon's equitability (J') or evenness can be calculated by dividing Shannon's diversity index (H') by the total number of species in the or the richness (H'_{max}). Equitability assumes a value between 0 and 1 with 1 being complete evenness and zero signifying no evenness.

$$J' = \frac{H'}{H'_{max}} \quad (2)$$

Statistical significance was assessed with 999 permutations using the two-sample t-test.

RESULTS

To assess variability and composition of dog gut microbiota, a cross-sectional study was performed with 7 dogs from 6 breeds and 14 fecal sample collections. A total of 5 phyla and 24 genera were taxonomically classified (Figure 1; Table 2 in Appendix B) from the 14 fecal samples collected at initial (baseline) and then 6-weeks after fermented and cultured supplemental feeding protocol was added for individual canines.

The relative abundance differed in each individual canine, not only at the phylum level but also at the deeper taxonomic levels such as genus (Figure 1; Table 2 in Appendix B). The most abundant genus detected was *Fusobacterium* with percentages ranging from 16.5–42.2% after 6-weeks with fermented and cultured supplements in all canines. Other abundant trends of increasing taxonomic genus levels (Figure 1; Table 2 in Appendix B): *Bacteroides* increased after 6–weeks in all seven individuals; *Collinsella* and *Sutterella* increased after 6-weeks in six of seven dogs; and *Peptoclostridium* increased or was present after 6-weeks in three dogs. On the other hand, *Lachnospiraceae* were present in all dogs at 2.6% or less for both fecal sample collections. *Blautia* percentages decreased in five of seven dogs after 6-weeks. However, some dogs presented individual-specific genus percentages after 6-weeks: *Escherichia* with 1.6% for Dog H-02; *Dialister* at less than 1.8% for Dog H-02 and Dog L-07; *Anaerobiospirillum* with 3.4% for Dog R-05; *Catenibacterium* at 1-3.5% for Dogs H-02 and R-05; *Tyzzerella* at 1.1% after 6-weeks for Dog G-01; and *Faecalitalea* at 1.1% for Dog R-05. Depending on the individuals, genus representing more than 5% (Table 2 in Appendix B) was describing from 75.4 to 98.1% of total microbiota composition.

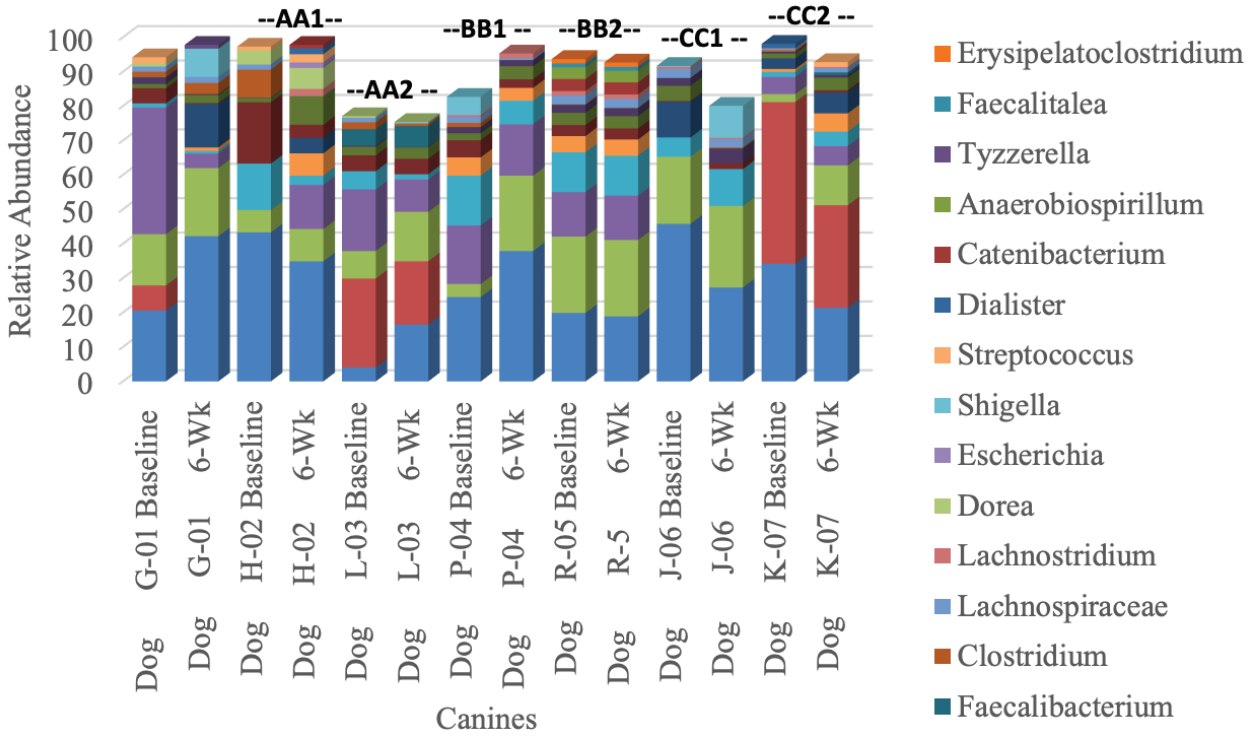

Figure 1. Bar Plot Representing Fecal Microbiome Composition at Phylum Level of Canines at Baseline and 6-Week (Treated with Fermented and Cultured Supplements). Raw data collected from each dog owner provided by Animal Biome. AA, BB, and CC show dogs that live in the same home.

The abundances of the main phyla differed for each fecal sample and individual (Figure 2; Table 3). The main phyla found in all seven dogs were: *Proteobacteri*a (1.2–9.9%), *Firmicutes* (10–45.8%), *Fusobacteria* (4.1–45.8%), *Bacteroidetes* (3.8–52.4%), and *Actinobacteria* (0–17.8%). Furthermore, none of the dogs had a predominant phylum (>50% of the total abundance) over the others. After 6-weeks on cultured and fermented supplements, four of seven dogs increased in *Fusobacteria*, and three of seven dogs increased in *Firmicutes* and *Bacteroidetes*. Dog K-7 showed little to no percentages of *Proteobacteria* or *Actinobacteria* at baseline or after 6-weeks for its taxonomic composition. Dogs living in the same household showed similar percentages in phyla, however, Dogs labeled as AA1 and AA2 (Figure 2; Table 3) showed differences in Proteobacteria and Fusobacteria percentages both at baseline and 6-week collections.

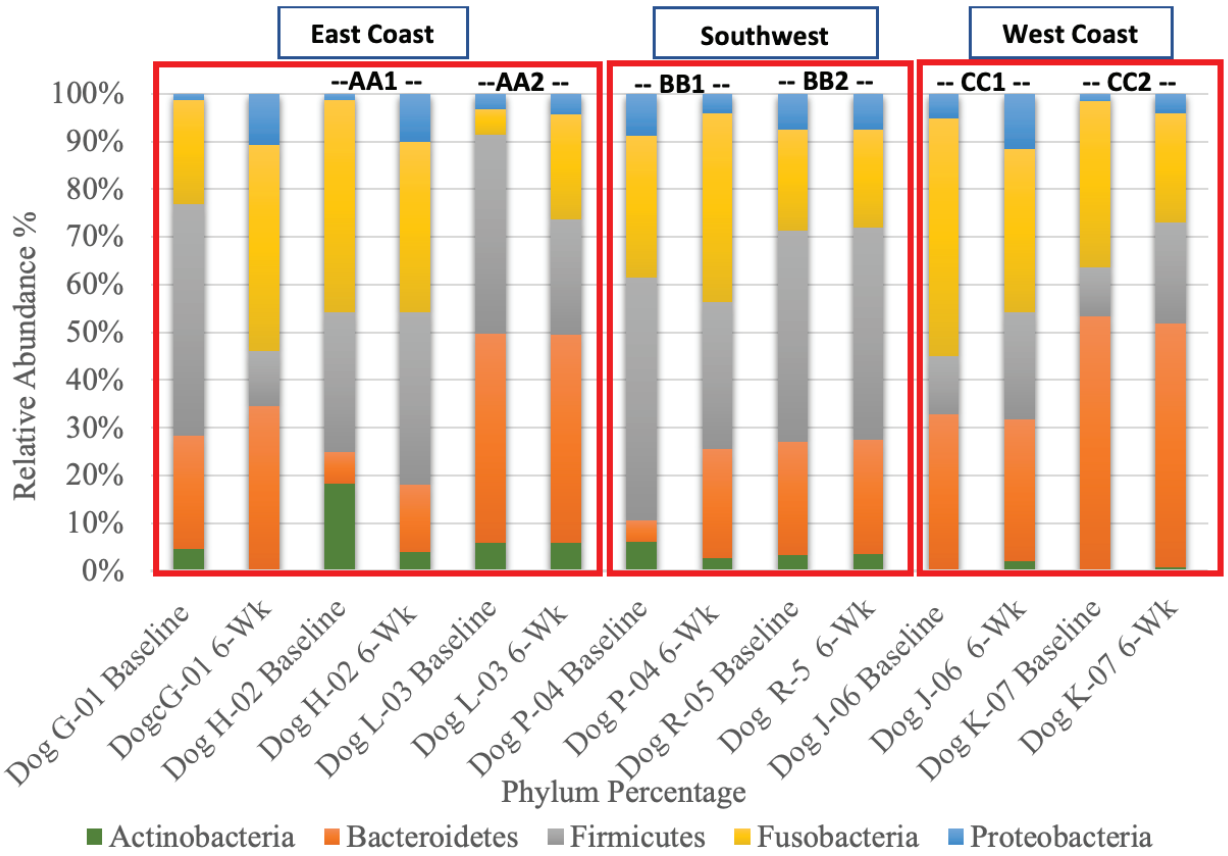

Figure 2. Alpha Diversity Percentage of Relative Abundance of Phylum Composition of Baseline and 6-Week Values of Canines. Data was collapsed for each individual dog by grouping Phylum. AA, BB, and CC show dogs that live in the same household.

Phylum	Dog G-01 Baseline	DogcG-01 6-Wk	Dog H-02 Baseline	Dog H-02 6-Wk	Dog L-03 Baseline	Dog L-03 6-Wk	Dog P-04 Baseline	Dog P-04 6-Wk	Dog R-05 Baseline	Dog R-5 6-Wk	Dog J-06 Baseline	Dog J-06 6-Wk	Dog K-07 Baseline	Dog K-07 6-Wk
Actinobacteria	4.4	0.2	17.8	3.8	4.6	4.5	5	2.5	3.2	3.2	0.2	1.7	0	0.6
Bacteroidetes	22.2	33.6	6.5	13.9	33.8	32.8	3.8	21.9	22.2	22.2	29.9	23.7	52.4	47.5
Firmicutes	45.8	11.3	28.4	35.3	32.1	18.3	42.1	29.2	41.4	41.4	11.1	18.1	10	19.6
Fusobacteria	20.6	42.2	43.3	34.9	4.1	16.5	24.5	37.9	19.9	18.9	45.8	27.3	34.2	21.4
Proteobacteria	1.2	10.5	1.3	9.9	2.5	3.3	7.3	3.8	7	7	4.7	9.3	1.5	3.7

Table 3. Alpha Diversity Percentage of Relative Abundance of Phylum Composition of Baseline and 6-week Values of Canines. Data was collapsed for each individual dog by grouping Phylum.

The alpha diversity, evenness, and richness differed for each individual dog. Alpha diversity is the mean species diversity in sites or habitats at a local scale. Figure 3 shows three of seven dogs diversity increased, and three of seven dogs diversity decreased. It is worthy to note that the dogs living in the same household (BB1, BB2, CC1, CC3) alpha diversity showed similar values after the second fecal collection and analysis was completed. The mean control of 2.2 for diversity are dogs (>1,000 sample size of dogs in Animal Biome data system) not on fermented or cultured supplements and used to compare the seven dogs in the study. When the diversity of the dogs in the study for the second fecal sample collected was averaged, this mean was 2.09.

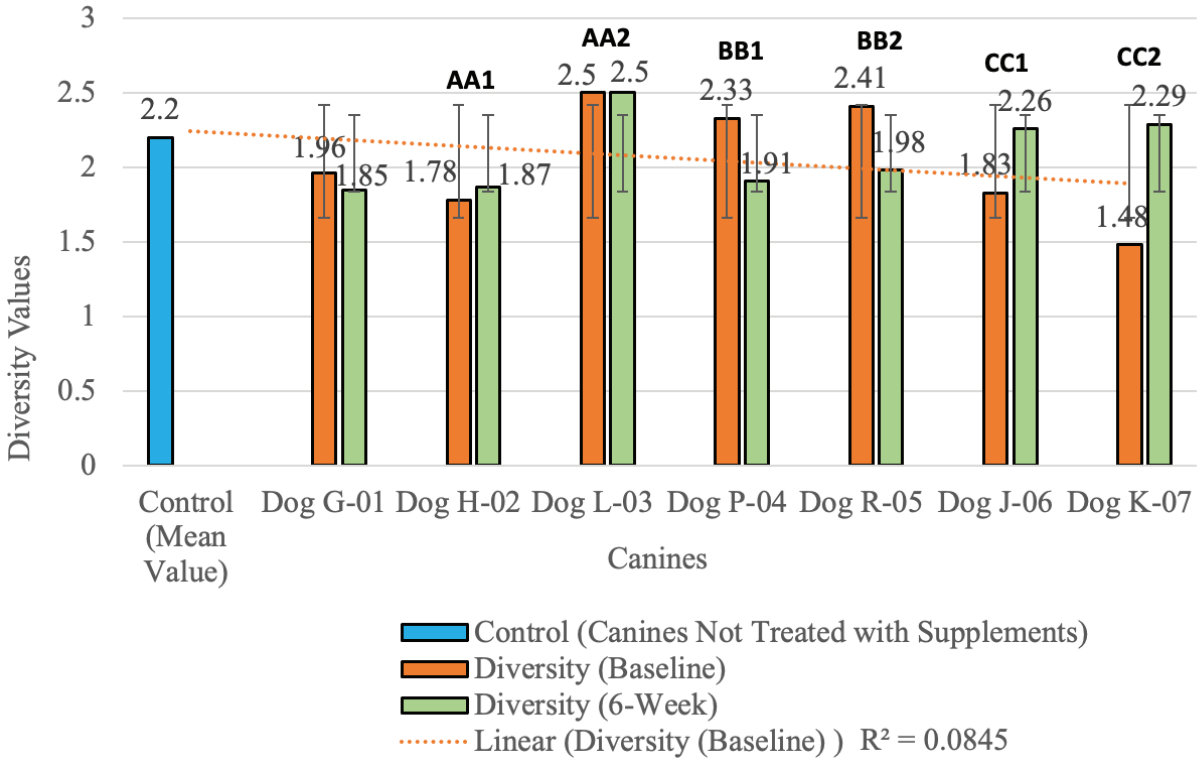

Figure 3. Alpha Diversity at Genus Level of Canines at Baseline (No Cultured and Fermented Supplements) Versus 6-Week (Treated with Fermented and Cultured Supplements). R squared is the goodness of fit based off the average mean of dogs not on cultured and fermented supplements. AA, BB, and CC show dogs in the same household.

Alpha richness is the number of species found in the sample collected. Figure 4 shows five of the seven dogs' richness in species increased when the second fecal sample was analyzed. The dogs living in the same household (BB1, BB2, CC1, CC3) alpha richness showed similar values after the second fecal collection and analysis was completed, in contrast, dogs AA1 and AA2 live in the same household and both decreased in richness. The mean control of 38 for richness are dogs (>1,000 sample size of dogs in Animal Biome data system) not on fermented or cultured supplements and used to compare the seven dogs in the study. When the diversity of the dogs in the study for the second fecal sample collected was averaged, this mean was 32.

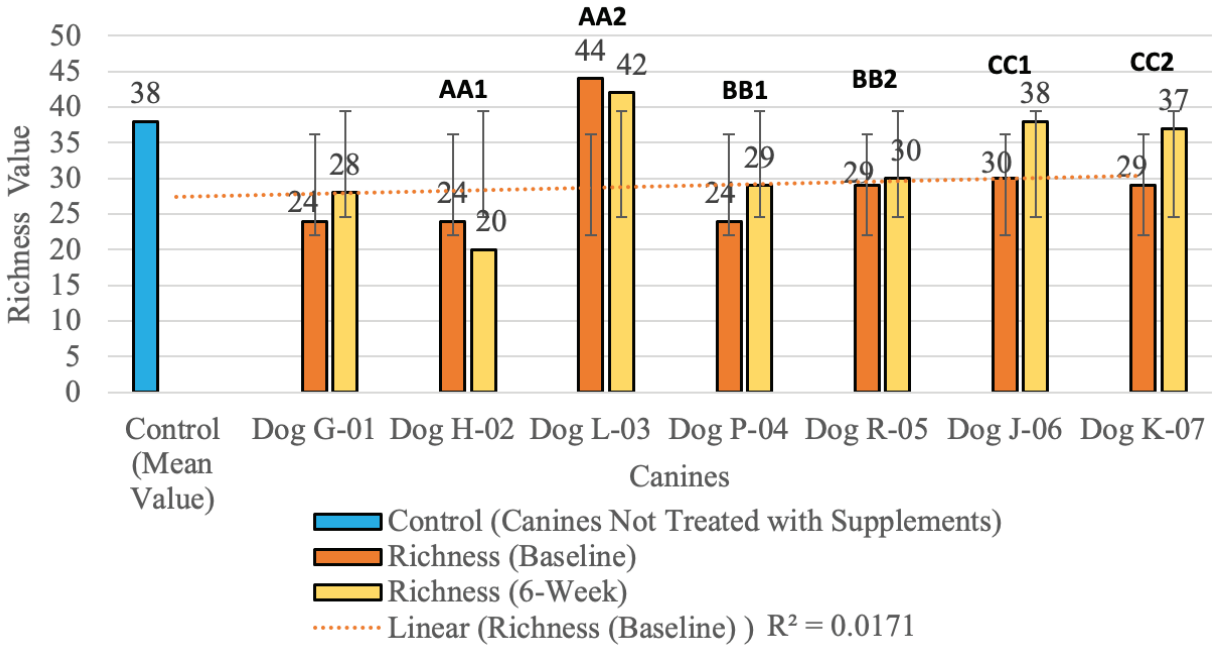

Figure 4. Alpha richness at Genus Level of Canines at Baseline (No Cultured and Fermented Supplements) Versus 6-week (Treated with Fermented and Cultured Supplements). R squared is the goodness of fit based off the average mean of dogs not on cultured and fermented supplements. AA, BB, and CC show dogs in the same household.

Alpha evenness refers to how close in numbers each species in an environment is. This was mathematically defined using the Shannon Wiener Index, a measure of biodiversity which quantifies how equal the community is numerically. Figure 5 shows three of the seven dogs' evenness increased by 0.07. However, three of seven dogs also decreased by 0.07 in evenness. The dogs living in the same household (AA1, AA2, BB1, BB2, CC1, CC3) evenness also showed similar values after the second fecal collection and analysis was completed. The mean control of 0.6 for evenness are dogs (>1,000 sample size of dogs in Animal Biome data system) not on fermented or cultured supplements and used to compare the seven dogs in the study. When the diversity of the dogs in the study for the second fecal sample collected was averaged, this mean was 0.61.

The ratio of *Firmicutes* to *Bacteroidetes* (F:B) was also calculated (Figure 6). F:B was calculated by dividing the abundance of *Firmicutes* and *Bacteroidetes* for each individual dog. Four of the 7 dogs F:B ratio decreased from baseline (not on supplements) to 6-weeks (on supplements). Dog P-04 had a significantly high F:B ratio at 15.15 and decreased to 1.52 after 6-weeks. The mean control of 7.9 for evenness are dogs (>1,000 sample size of dogs in Animal Biome data system) not on fermented or cultured supplements and used to compare the seven dogs in the study. When the diversity of the dogs in the study for the second fecal sample collected was averaged, this mean was 1.05.

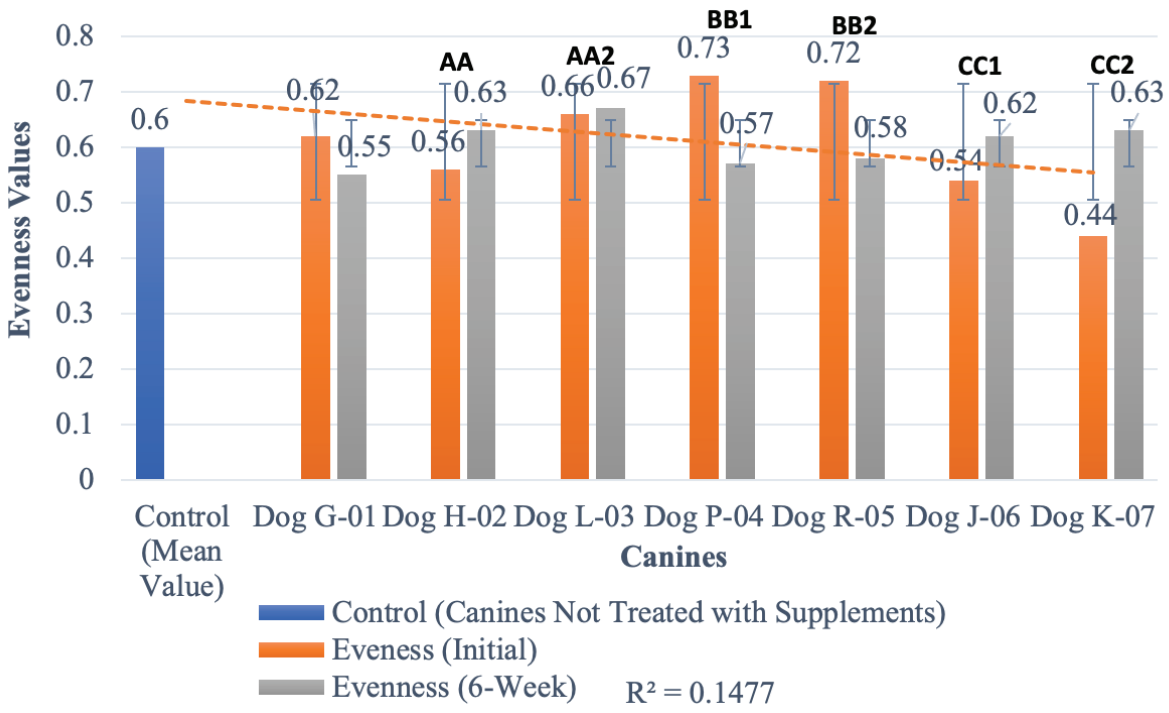

Figure 5. Alpha Evenness at Genus Level of Canines at Baseline Versus 6-week (Treated with Fermented and Cultured Supplements). R squared is the goodness of fit based off the average mean of dogs not on cultured and fermented supplements. AA, BB, and CC show dogs in the same household.

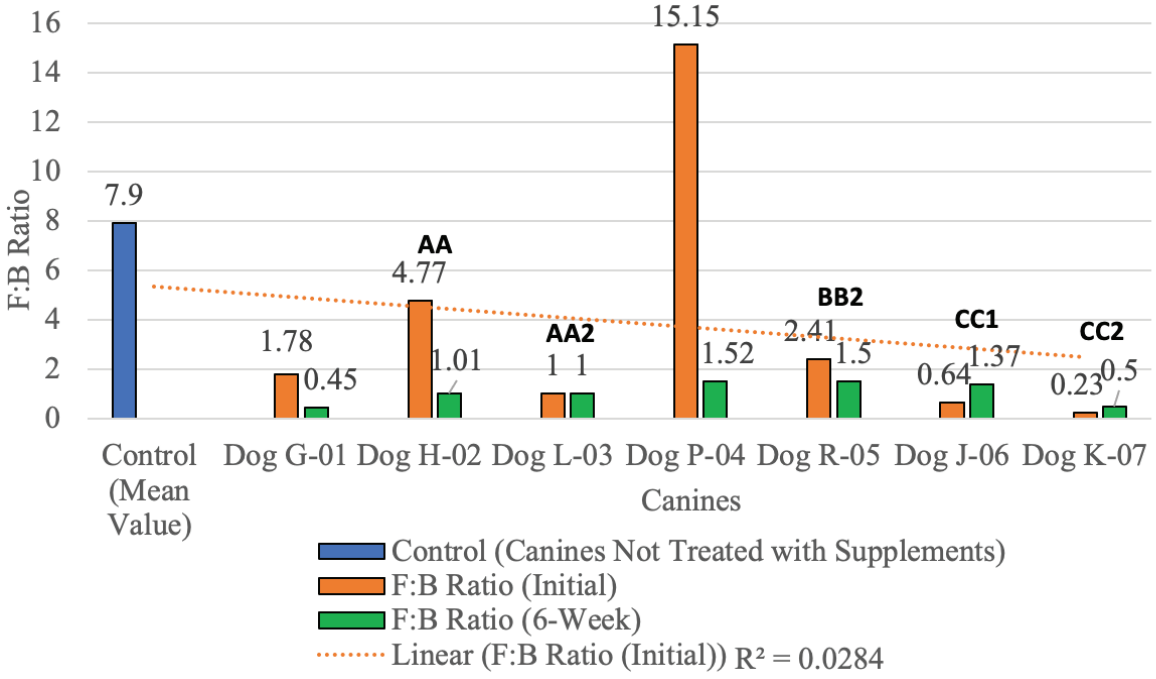

Figure 6. Alpha Firmicutes to Bacteroidetes (F:B) Ratio of Canines at Baseline Versus 6-week (Treated with Fermented and Cultured Supplements). AA, BB, and CC show dogs in the same household.

DISCUSSION

Overall, the results suggest that the driving force in microbiota composition when looking at alpha levels of relative abundance, evenness, diversity, and richness in dogs is the individual when looking at the phyla and genus structures of each dog. Studies on humans have also reported that interindividual variation is high and defines a "personal microbiome" (Human Microbiome Project Consortium, 2012). The fecal microbiome of healthy dogs is co-dominated by three phyla: *Fusobacterium, Bacteroidetes, and Firmicutes* (Middelbos, 2010; Hand, 2013) In this study, these three phyla were also seen to be more abundant in percentages.

When reviewing the literature, a wide variation in percentages of specific bacterial taxa can be seen. Within this core bacterial community, several major genera belong to the phylum *Firmicutes*. The genus consistently found in each dog were: *Megamonas*, *Blautia*, *Ruminococcus*, *Clostridium*, and *Lachnospiraceae*. *Megamonas* was more prevalent in abundance in this phylum. The phylum *Fusobacteria* was also abundant amongst all dogs by genus *Fusobacterium*. *Fusobacterium* abundance is increased in dogs with access to the outdoors (Song, 2013), and higher levels of *Fusobacterium* are also seen in other carnivore species (Bermingham, 2017). *Bacteroidetes* was also another abundant phylum in all dogs, with genus *Bacteroides* being abundant in all dogs' fecal samples collected. Wildbrines sauerkraut contained *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *L. rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus salivarius*, *L. salivarius*, and *Streptococcus thermophilus*; and the Answers kefir contained *Lactococcus lactis* and *Leuconostoc mesenteroides*. These fermented and cultured foods added to daily diet could be contributors to increased percentages in the phylum *Firmicutes* and *Actinobacteria* specifically. *Fusobacteria* had a more consistent increase in abundance over time in four of the seven dogs and may be an effect of the addition of the fermented and cultured supplements. The combined *Prevotella* and *Bacteroides* abundances seem to be inversely related to phylum *Fusobacteria* abundance, which might indicate that they occupy the same niche (Vázquez-Baeza, 2016). *Proteobacteria* and *Actinobacteria* phyla are typically colonizers of the small intestine and in physiological conditions will present in smaller numbers in fecal samples, and their increase is associated with many diseases (Pilla, 2019).

The alpha diversity, evenness, and richness were calculated using two-sample T-test calculations (Table 4) and did not show any significant changes when fermented and cultured foods were added for 6-weeks to the dogs' already established daily meal intake. However, dogs living in the same household (BB1, BB2, CC1, CC2) had similar diversity and richness values (both increased) after 6-weeks and in turn, dogs (AA1 and AA2) in the same household decreased and had similar values for diversity only. All dogs living in the same household also showed similar values for evenness after a 6-week fecal collection. Table 5 shows that there was significance in evenness ($P < 0.001$) when looking at 6-week fecal collections with fermented and cultured foods to the mean control value (>1000 sample size of dogs in Animal Biome data system) of dogs not on any cultured or fermented foods.

Bacteroidetes phylum protects against obesity and diseases due to not digesting fat well. Whereas, *Firmicutes* are a common phylum found in the gut and aids in the digestion of fat (required for energy) and linked to obesity and inflammation. The percentage of *Bacteroidetes* increased in three of the seven dogs and the *Firmicutes* decreased in six of the seven

dogs. When calculated, four of the seven dogs' overall *Firmicutes* to *Bacteroidetes* (F:B) ratio decreased after 6-weeks. A two-sample T-test (Table 5) was performed against both baselines and against a mean control (>1000 sample size of dogs in Animal Biome data system) of dogs not on cultured or fermented foods. F:B ratios showed no real statistical significance except when mean control value to 6-weeks F:B values were compared (P,0.001). Research shows *Firmicutes* abundance along with probiotics to help crowd out certain bacteria, could possibly treat obesity and weight gain (Abenavoli, 2019).

	Significance (< 0.01)	P Value	Number of Samples (N)	Degrees of Freedom (N - 1)	Mean of Baseline with No Supplements (M1)	Mean with 6-Week Supplements (M2)	Difference	Standard Error of Difference	Standard Deviation (SD)	t Ratio
Diversity	No	0.76471	7	6	2.04	2.09	-0.05	0.03	0.38	-0.3
Richness	No	0.476527	7	6	29.14	32	-2.86	15.12	6.82	-0.7
Evenness	No	0.947536	7	6	0.61	0.61	0	0	0.08	0.07
F:B Ratio	No	0.233258	7	6	3.71	0.98	2.74	4.71	2.17	1.26

Table 4. Two-sided T-Test with 99% Confidence Intervals with N=7 Alpha Level Comparing Canines Baseline Data with No Cultured and Fermented Supplements Versus Canine Data Collected After 6-weeks Adding Fermented and Cultured Supplements To Daily Meals.

	Significance (< 0.01)	P Value	Number of Samples (N)	Degrees of Freedom (N - 1)	Mean of Control Value of Canines Not on Supplements (M1)	Mean with 6-Week Supplements (M2)	Difference	Standard Error of Difference	Standard Deviation (SD)	t Ratio
Diversity	No	0.345597	7	6	2.00	2.09	-0.09	0.01	0.1	-1
Richness	No	0.054783	7	6	38	32	6	7.95	2.82	2.13
Evenness	Yes	< .00001	7	6	0.6	0.61	-0.55	0	0	-35
F:B Ratio	Yes	< .00001	7	6	8	0.98	6.95	0.03	0.17	41.3

Table 5. Two-Sided T-Test with 99% Confidence Intervals with N=7 Alpha Diversity Level Comparing Canine Baseline Data (with No Cultured and Fermented Supplements) Versus Canine Data Collected After 6-weeks (with Fermented and Cultured Supplements To Daily Meals)

In addition, individual variations in the microbiome profile exist and should be taken into account especially since this is a small sample group of seven dogs. This study is also limited to alpha diversity (variation within an individual microbiome) whereas beta diversity, the microbial variation between individuals, using PCoA and other statistical analysis could have been examined for this study. In addition, this was a 6-week study. Ideally, fecal collections at the beginning (baseline), 1 month, 2 months, 6 months, and 1 year would be more ideal to see the variations of the gut microbiome over time.

CONCLUSION

The gut microbiota is essential for the health of all mammals because it participates in the host's vital physiological processes and development. Alterations of the intestinal microbial populations are associated with a variety of gastrointestinal and systemic illnesses. Therefore, understanding the gut microbiota could be useful in the diagnosis of illness and disease and change the types of therapy procedures used.

Future research studies should clarify the mechanisms that regulate the interactions between the microbiota and the host. More studies have to be done about the use of probiotics, prebiotics, and FMT in the restoration of a state of eubiosis. While recent advances in DNA sequencing and computational technology have revolutionized the field of microbiomics, many questions remain unanswered, including how long the gut microbiome takes to recover from disease, drugs, or other environmental factors by better understanding the mechanisms of action and duration of efficacy of different treatments on the gut microbiome (Arnold et al, 2016). The identification of alpha and beta bacterial taxa with bacteria-derived compounds (plants, fermented and cultured whole foods) should be investigated further to look at explaining the mechanisms underlying interactions between the microbiome and host, describing the process of microbiome maturation during host development and its impact on early-life and adult health outcomes, clarifying its role in the pathogenesis of diseased states, land assessing the viability of diagnostic tests and therapies designed to assess and treat conditions associated with underlying health issues (Kho, 2018).

Continued research beyond this will be to statistically analyze this data with beta diversity using PCoA, adding additional dogs to the study, adding additional fecal collections over the course of a year, and compare the beta diversity to gender, living location, activity level, gender, age, and weight of dogs. Do dry or wet foods to that of a raw diet with protein on fermented and cultured foods have a significant difference in relative abundance?

Today, society is seeing a rise in microbiome-associated disorders in dogs (animals in general) and even in humans, and understanding differing effects on the gut microbiome will shape how we treat chronic issues not just for our canines, but pets and even humans.

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APPENDIX A

Dog	Age (Years)	Gender	Breed	Weight (lb)	Volhard Diet (Type)	Living Location	Activity Level (Hours/Day)	Medication
G-01	4	Female	Beagle	21	AM/PM	Culpeper, VA	6 to 8	Thyroxine
H-02	10	Female	Yorkshire Terrier	7.9	AM/PM	Louisa, VA	8	
L-03	3	Female	Hound	32	AM/PM	Louisa, VA	8	
P-04	8	Male	Daschund Pug	13	NDF2	Albuquerque, NM	< 1	
R-05	5	Male	Corgi Chihuahua	10	NDF2	Albuquerque, NM	< 1	
J-06	<1	Male	Great Dane	135	NDF2	La Mirada, CA	2 to 4	Trazadone Gabapentin CBD
K-07	2	Male	Great Dane	148	NDF2	La Mirada, CA	2 to 4	

Table 1. Demographics of the Seven Canine Participants in the Study.

Phylum	Genus	Dog G-01 Baseline	Dog G-01 6-Wk	Dog H-02 Baseline	Dog H-02 6-Wk	Dog L-03 Baseline	Dog L-03 6-Wk	Dog P-04 Baseline	Dog P-04 6-Wk	Dog R-05 Baseline	Dog R-5 6-Wk	Dog J-06 Baseline	Dog J-06 6-Wk	Dog K-07 Baseline	Dog K-07 6-Wk
Actinobacteria	Collinsella	4.4	0.2	17.8	3.8	4.6	4.5	5	2.5	3.2	3.2	0.2	1.7	0	0.6
Bacteroidetes	Prevotella	7.3				25.8	18.4							47	29.8
Bacteroidetes	Bacteroides	14.9	19.8	6.5	9.4	8	14.4	3.8	21.9	22.2	22.2	20	23.7	2.3	11.6
Bacteroidetes	Alloprevotella		12.7		4.5							10	0	3.1	6.1
Bacteroidetes	Tyzzerella		1.1												
Firmicutes	Megamonas	36.8	4.2		12.8	17.9	9.4	17	14.9	12.9	12.9			5	5.6
Firmicutes	Blautia	1.2	0.8	13.5	2.7	5.3	1.5	14.5	6.9	11.6	11.6	5.6	10.7	1.5	4.2
Firmicutes	Peptoclostridium		1		6.5			5.3	3.7	4.7	4.7			0.8	5.3
Firmicutes	Ruminococcus	1.9	0.3	0.1		0.3	0.1	1.8	1.8	2.3	2.3	2.3	4.3	0.5	0.7
Firmicutes	Faecalibacterium	0.1				4.8	6.1								0.8
Firmicutes	Clostridium	1.6	3.2	8.1		2	0.6	1.2		0.1	0.1		0.3	0.4	0
Firmicutes	Lachnospiraceae	1.5	1.8	1.5	0.1	1.3	0.4	1.8	0.5	2.6	2.6	2.6	2.4	0.3	1.3
Firmicutes	Lachnostridium				2			0.5	1.4	1.3	1.3	0.6	0.4	0.2	0.3
Firmicutes	Dorea	0.9		4.1	6.1	0.5	0.2								
Firmicutes	Streptococcus	1.8		1.1	2.4										1.4
Firmicutes	Dialister				1.7									1.3	
Firmicutes	Catenibacterium				1					3.5	3.5				
Firmicutes	Faecalitalea									1.1	1.1				
Firmicutes	Erysipelatoclostridium									1.3	1.3				
Fusobacteria	Fusobacterium	20.6	42.2	43.3	34.9	4.1	16.5	24.5	37.9	19.9	18.9	46	27.3	34.2	21.4
Proteobacteria	Sutterella	1.2	2.3	1.3	8.3	2.5	3.3	2	3.8	3.6	3.6	4.4	0	1.4	3.7
Proteobacteria	Escherichia				1.6										
Proteobacteria	Shigella		8.2					5.3				0.3	9.3	0.1	
Proteobacteria	Anaerobiospirillum									3.4	3.4				
% of Microbiota Explained by Taxa >5%		94.2	97.8	97.3	97.8	77.1	75.4	82.7	95.3	93.7	92.7	91.7	80.1	98.1	92.8

Table 2. Genus and Phylum Percentages of Baseline and 6-Week Data of Canines in Study Versus Control Value of Canines Not on Fermented and Cultured Supplements.