Impact of Breed on the Fecal Microbiome of Dogs under the Same Dietary Condition

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The gut microbiome influences the health and well-being of dogs. However, little is known about the impact of breed on the fecal microbiome composition in dogs. Therefore, we aimed to investigate the differences in the fecal microbiome in three breeds of dog fed and housed under the same conditions, namely eight Maltese (8.0 ± 0.1 years), eight Miniature Schnauzer (8.0 ± 0.0 years), and nine Poodle dogs (8.0 ± 0.0 years). Fresh fecal samples were collected from the dogs and used to extract metagenomic DNA. The composition of the fecal microbiome was evaluated by 16S rRNA gene amplicon sequencing on the MiSeq platform. A total of 840,501 sequences were obtained from the 25 fecal samples and classified as Firmicutes (32.3–97.3% of the total sequences), Bacteroidetes (0.1–62.6%), Actinobacteria (0.2–14.7%), Fusobacteria (0.0–5.7%), and Proteobacteria (0.0–5.1%). The relative abundance of Firmicutes was significantly lower in the Maltese dog breed than that in the other two breeds, while that of Fusobacteria was significantly higher in the Maltese than in the Miniature Schnauzer breed. At the genus level, the relative abundance of *Streptococcus*, *Fusobacterium*, *Turicibacter*, *Succinivibrio*, and *Anaerobiospirillum* differed significantly among the three dog breeds. These genera had no correlation with age, diet, sex, body weight, vaccination history, or parasite protection history. Within a breed, some of these genera had a correlation with at least one blood chemistry value. This study indicates that the composition of the fecal microbiome in dogs is affected by breed.

Keywords: Dog, fecal microbiome, breed, dietary condition, bacterial abundance

Introduction

The canine digestive tract is colonized by millions of bacteria that exert a positive and dynamic effect on host health [1]. These bacteria are thought to greatly colonize the colon, and play an important role in numerous functions in the host. According to Honneffer *et al.* [2], the expected gut microbiota of animals and humans comprises 10^{10} – 10^{14} microbial cells; this number may be similar to or 10 times greater than the number of host cells. A number of studies show microbial composition and the relationship of specific microbes with the persistence of clinical health signs

and during a variety of disease states and conditions such as gastrointestinal inflammation, obesity, and diarrhea [3, 4].

Owing to the easy sample collection method, fecal samples are generally used to define the intestinal bacteria, hence the term fecal microbiota. However, this reflects microbial groups present in the distal part of the colon more than in the proximal parts of the intestine [5, 6]. Studies using canine models have emphasized the importance of the intestinal microbiota in harvesting energy from the diet. It has already been identified that the fecal microbiome is affected by numerous aspects. Earlier studies exposed that gut health status is one of the most robust factors influencing

the composition and diversity of the fecal microbiome [3].

The effect of host health and physiology on microbial variations was also examined in dogs [4]. Gastrointestinal (GI) diseases are related to changes in the intestinal microbiota. According to Suchodolski et al. [7], dogs which suffered from diarrhea showed significant differences in fecal microbial communities compared to healthy dogs, and the fecal dysbiosis in acute diarrhea might be related to changes in systemic metabolic states. Other GI diseases such as inflammatory bowel diseases (IBD) can be linked to modifications in the microbiota of the small intestine and feces [2]. Dogs with calcium oxalate stones also have varied diversity in intestinal microbiota when compared with healthy dogs [8].

When compared with humans and domestic livestock, dogs have a comparatively simple gastrointestinal tract (GIT) and do not depend on microbiota for energy. However, current studies have exposed that a dog's GIT microbial community is composed of some hundred microbial phylotypes [9]. The primary purpose of studying canine microbiota is to obtain a better understanding of the taxonomy, dynamics, and functions of GI microbial communities, as well as the interactions between microbial members, the substances created and consumed by microbes, the influence of diet, drugs, and housing environmental factors, relations with the host, and the variances between healthy and diseased populations [10].

The intestinal microbiota in dogs, however, are yet to be scientifically characterized at the species level, and it is difficult to relate knowledge based on bacteria culture methods [11] to the newer high-throughput culture-independent data. Furthermore, the bottlenecks associated with the high-throughput sequencing techniques being developed are obviously due to computational capacity and biological clarifications by investigators. Over the past few years, it is clear that studying the whole microbiome is necessary to understand its role in the host rather than studying phylogeny alone. Therefore, analyses using

shotgun sequencing of all DNA in a sample provides information on the functional ability of the microbial genome. Little information is available on the relationships among intestinal microbiota and the feed, age, and sex of the canine species. To the best of our knowledge, this is the first study to evaluate the role of GI microbiota in different canine breeds. Thus, the aims of the present study were to examine the composition of the intestinal microbiota in three different dog breeds fed and housed under the same conditions, to clarify the breed-dependent transitions, and compare the composition of potentially beneficial bacteria.

Materials and Methods

Ethical Statement

This study was approved by the Institutional Animal Care and Use Committee of the National Institute of Animal Science, South Korea (No. 2017-256, 4 July 2017).

Study Animals and Diet

The experiment was performed using three breeds of clinically healthy female canines, namely eight Maltese (8.0 ± 0.1 years), eight Miniature Schnauzer (8.0 ± 0.0 years), and nine Poodle dogs (8.0 ± 0.0 years). All dogs were housed indoors in separate blocks, and given free access to water. Detailed information of canine breed name, age, sex, body weight, vaccination history, parasite protection history, and diet information is provided in Table 1. The dogs did not show any signs of disease and were generally in good condition. None of the dogs showed any signs of GI diseases or received antibiotics in the two months before fecal sample collection. All three canine breeds were fed daily with the same basal canine diet 5LL9 (LabDiet, USA). The diet comprised 27% crude protein, 10% crude fat, 5% crude fiber and included the following mineral nutrients: 7.7% ash, 1.8% Ca, 0.95% P, 0.7% K, 0.21% S, 0.16% Mg, 0.66% Cl, 0.41% Na, 13.2 ppm F, 387 ppm Fe, 130 ppm Zn, 73 ppm Mn, 14 ppm Cu, 2.13 ppm I, 0.92 ppm Co, 1.18 ppm Cr, and 0.43 ppm Se.

Blood Sample Collection and Analysis

Blood was collected from all the breeds via jugular venipuncture

Table 1. Breed type, age, gender, weight, vaccination and diets information of dogs.

Breed type ¹	Age (year)	Gender	Average weight (kg)	Vaccination for each DOG ²	Parasite protection for each DOG ³	Diet history ⁴
Poodle	8	Female	4.60	Yearly (DHLPP vaccine)	Monthly (Advocate)	LabDiet 5LL9
Maltese	8	Female	3.78	Yearly (DHLPP vaccine)	Monthly (Advocate)	LabDiet 5LL9
Miniature Schnauzer	8	Female	7.64	Yearly (DHLPP vaccine)	Monthly (Advocate)	LabDiet 5LL9

¹Poodle $n = 9$; Maltese $n = 8$; Miniature Schnauzer $n = 8$

²DHLPP vaccination protects the dogs from distemper, hepatitis, Leptospirosis, parainfluenza, and parvovirus diseases.

³Advocate injections protects from various parasites such as mites and worms.

⁴All three canine breeds were fed daily with the same Lab Diet 5LL9 commercial diet.

into an evacuated red top tube without any anticoagulant for biochemistry panel analysis. Blood biochemistry was analyzed under standard conditions on a clinical chemistry analyzer (ProCyt Dx Hematology Analyzer; IDEXX Laboratories, Inc., USA). Blood biochemistry parameters evaluated included albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, blood urea nitrogen (BUN), calcium, cholesterol, creatinine, glucose, perfusion index (Pi), gamma-glutamyl transferase (GGT), and total protein.

Sampling and Processing

The fecal microbiota composition was examined in all three canine breeds (or 25 dogs). After feeding the animals for three weeks, fresh individual fecal samples were collected aseptically into polythene zip bags and immediately transferred to the laboratory.

DNA Extraction and 16 rRNA Gene Sequencing

Total community DNA was extracted from the 25 fecal samples using the RBB+C bead-beating method [12], and stored at -20°C before sequencing. From each DNA sample, a library was generated using the 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') primers to amplify 16S rRNA gene amplicons, which produced approximately 450 bp products for targeting the V3-V4 region, sequenced on the MiSeq platform (Illumina, USA). Paired reads were collected using the FLASH program [13]. The assembled fecal microbiome sequences were demultiplexed and microbiome analyses were performed using QIIME v.1.9.1 software [14]. Finally, the subsequent high-quality sequences were classified into taxa using BLASTN v.2.4.0 against the National Center for Biotechnology Information (NCBI)

Reference Sequence (RefSeq) database (<http://www.ncbi.nlm.nih.gov/RefSeq/>). The alpha diversity was analyzed through number of OTUs, Chao1, Shannon diversity index, and Simpson diversity index, while the beta diversity was analyzed through the unweighted principal coordinate analysis (PCoA) plot. Microbial enterotypes were analyzed using Dirichlet multinomial mixture models [15] with Mothur v.1.42.3 software [16].

Statistical Analysis

Taxa demonstrating on average $\geq 0.2\%$ of the total sequences were considered major taxa, and used for statistical analysis. The taxonomic composition of fecal bacteria was compared among the three canine groups based on the means of the relative taxonomic abundances (*i.e.*, reads of a taxon/total reads in a sample), as mentioned in our previous study [17]. The mean proportion of each taxon, blood chemistry value, and diversity index was compared among the three canine breeds using analysis of variance (ANOVA), followed by Duncan's test using XLSTAT statistical software (Addinsoft, USA). A p value of ≤ 0.05 was considered statistically significant. Correlations between bacterial genera and other factors such as body weight and health conditions were analyzed by Spearman's correlation analysis using XLSTAT statistical software (Addinsoft).

Results

Blood Biochemistry Results in Different Breeds

Table 2 shows the median range of whole-blood parameters for three different dog breeds. Albumin, glucose, and triglycerides were significantly different, and the remaining

Table 2. Concentrations of selected blood Biochemistry in the clinically healthy Poodle, Maltese, and Miniature Schnauzer breeds.

Analyte ¹	Normal range ²	Poodle	Maltese	Miniature Schnauzer	p -value
Albumin	2.5–4.0 g/l	2.92 $\pm$ 0.06 ^a	2.69 $\pm$ 0.87 ^b	3.04 $\pm$ 0.06 ^a	0.04
ALP	10–150 u/l	92.60 $\pm$ 12.81	83.64 $\pm$ 16.37	135.17 $\pm$ 27.83	0.30
ALT (GPT)	5–107 u/l	45.21 $\pm$ 3.54	54.62 $\pm$ 5.76	72.02 $\pm$ 15.48	0.19
AST (GOT)	5–55 u/l	41.04 $\pm$ 2.03	37.48 $\pm$ 2.18	66.08 $\pm$ 12.81	0.08
Total Bilurubin	0.1–0.3 mg/dL	0.14 $\pm$ 0.01	0.14 $\pm$ 0.01	0.15 $\pm$ 0.01	0.58
BUN	6–25 mg/dL	15.15 $\pm$ 0.35	15.69 $\pm$ 0.49	16.36 $\pm$ 0.94	0.59
Calcium	8.9–11.4mg/dL	9.50 $\pm$ 0.23	9.36 $\pm$ 0.12	10.21 $\pm$ 0.24	0.06
Cholesterol	92–324 mg/dL	168.93 $\pm$ 11.41	161.84 $\pm$ 9.37	170.23 $\pm$ 11.50	0.82
Creatinine	0.5–1.6 mg/dL	0.72 $\pm$ 0.02	0.63 $\pm$ 0.02	0.77 $\pm$ 0.04	0.18
Glucose	70–150 mg/dL	80.24 $\pm$ 2.65 ^b	94.17 $\pm$ 2.49 ^a	78.60 $\pm$ 3.89 ^b	0.01
Pi	0.14–9.7 mg/dL	4.84 $\pm$ 0.25	4.91 $\pm$ 0.20	4.25 $\pm$ 0.11	0.18
GGT	0–14 μ /l	7.57 $\pm$ 0.42	7.06 $\pm$ 0.34	6.66 $\pm$ 0.48	0.33
Total protein	5.0–7.4 mg/dL	7.07 $\pm$ 0.13	6.82 $\pm$ 0.16	7.11 $\pm$ 0.14	0.50

Data are presented as mean ($\pm$ SEM).

¹ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; AST, Aspartate transaminase; BUN, Blood Urea Nitrogen; Pi, Perfusion Index; GGT, Gamma glutamyl transferase; HDL, High density lipoprotein; LDL, Low density lipoprotein.

²Normal ranges are from published reports.

^{a,b}Values with different letters within the same row are different ($p < 0.05$).

parameters did not show any significant differences between Poodle, Maltese, and Miniature Schnauzer breeds under the same dietary conditions. The characteristics of the blood biochemistry panel of the three breeds were determined to be within normal ranges.

The Composition of Intestinal Microbiota in Different Breeds

A total of 840,501 cleaned ribosomal rRNA sequences with a 500-nucleotide average read length were obtained from the total fecal sample (including all three breeds). Of these, 288,608 were obtained from the Poodle group (average sequence number 32,067; range 28,019–39,548), 264,559 from the Maltese group (average sequence number 33,069; range 28,950–42,403), and 287,334 from the Miniature Schnauzer group (average sequence number 35,916; range 28,798–45,278). Of the total 840,501 sequences, Poodle, Maltese, and Miniature Schnauzer dogs shared 34.34%, 31.48% and 34.19% sequences, respectively.

As shown in Table 3 and Fig. 1A, Firmicutes and Bacteroidetes were the most abundant phyla and occupied more than 90% of the total sequences in all three canine breeds. Firmicutes was the most abundant phylum in Miniature Schnauzer, Poodle, and Maltese breeds, occupying 80.17%, 78.26%, and 57.64% of the total sequences, respectively (Fig. 1B). The second most dominant phylum was Bacteroidetes with abundances of 33.41%, 15.79%, and 14.88% in Maltese, Poodle, and Miniature Schnauzer breeds, respectively (Fig. 1C). The third greatest abundant phylum is Proteobacteria (Fig. 1D). The subsequent most abundant phyla were Actinobacteria, and Fusobacteria of all cleaned sequences. Actinobacteria showed greater abundances in the Maltese (4.56%) and Poodle (3.32%)

breeds than that in the Miniature Schnauzer breed (1.98%). Protobacteria and Fusobacteria abundances were 1.75% (Maltese); 1.48% (Miniature Schnauzer); and 1.07% (Poodle) and 2.63% (Maltese); 1.55% (Poodle); and 1.49% (Miniature Schnauzer), respectively. In addition, the gut microbial taxonomic compositions at the phylum and family level from each dog of the three canine breeds are shown in Fig. 2.

Taxonomic Composition of Fecal Bacteria

Among all phylum groups, Firmicutes was the most abundant phylum ($p < 0.05$) in the three fecal sample groups (Table 3, Fig. 1B). The abundance of Firmicutes varied among the canine groups, and comprised 11 genera, indicating at least $\geq 0.2\%$ of the sequences in the total reads. Among these genera, only *Streptococcus* and *Turicibacter* showed significant differences ($p \leq 0.05$) among the three breeds. *Streptococcus* was the most abundant in Miniature Schnauzer (17.83%), moderate in Poodle (9.38%), and the least abundant in Maltese (3.83%) fecal samples. The abundance of *Turicibacter* was significantly greater ($p \leq 0.05$) in Maltese (2.60%) and Poodle (2.46%) fecal samples than that in Miniature Schnauzer (0.38%) samples. Among the 11 genera in the phylum Firmicutes, *Lactobacillus* was the most dominant in all three breeds, but there were no significant differences among them. In particular, *Lactobacillus* showed the greatest abundance in Poodle (34.14%) fecal samples than that in Miniature Schnauzer (18.75%) and Maltese (17.50%) samples. Residual genera in Firmicutes, such as *Megamonas*, *Streptococcus*, *Blautia*, and *Catenibacterium* also showed high abundances but there were no significant differences among the breeds. *Megamonas* showed high abundances in the Miniature Schnauzer (17.81%) breed, but there was little difference between

Table 3. The relative abundance of major taxa in three canine breeds.

Classification	Collective data average ¹	Percentage of total sequences in different breed ²					No. of dogs with detectable taxon
		Poodle dogs (n = 8)	Maltese dogs (n = 8)	Miniature Schnauzer dogs (n = 9)	SEM	p-value	
Firmicutes	72.02	78.26 ^a	57.64 ^b	80.17 ^a	0.039	0.009	25
<i>Streptococcus</i>	10.35	9.38 ^{ab}	3.83 ^b	17.83 ^a	0.486	0.042	24
<i>Turicibacter</i>	1.81	2.46 ^{ab}	2.60 ^a	0.38 ^b	0.305	0.009	24
Proteobacteria	1.43	1.07	1.75	1.48	0.211	0.478	25
<i>Succinivibrio</i>	0.20	0.01 ^b	0.02 ^b	0.57 ^a	0.321	0.003	15
<i>Anaerobiospirillum</i>	0.19	0.37 ^a	0.17 ^{ab}	0.02 ^b	0.356	0.003	17
Fusobacteria	1.89	1.55 ^{ab}	2.63 ^a	1.49 ^b	0.170	0.050	25
<i>Fusobacterium</i>	1.90	1.55 ^{ab}	2.63 ^a	1.53 ^b	0.170	0.050	25

¹Sequences obtained from all 25 fecal samples

²Values represent means.

^{a,b}Within a row, means with a different subscript were different ($p < 0.05$).

Fig. 1. Stacked histograms showing, the relative abundances of bacterial diversity for the phylum, family and genus-level in Poodle, Maltese and Miniature Schnauzer breeds. Breed name shown on the X axis and percentage reads classified on the Y axis. (A) Showed the relative abundances of taxa within all phyla, (B) showed the abundance of Firmicutes, (C) shown abundance of Bacteroidetes, and (D) shown the abundance of Proteobacteria.

Maltese (10.51%) and Poodle (9.63%) breeds. *Blautia* showed rich abundance in the Poodle (8.33%) and Miniature Schnauzer (7.12%) breeds compared to the Maltese (5.17%) breed. There were non-significant differences in abundance ($p > 0.05$) in the following genera among the three canine fecal samples: *Turicibacter* (1.81%), *Allobaculum* (1.63%), *Phascolarctobacterium* (0.94%), *Faecalibacterium* (0.83%), *Peptococcus* (0.46%), and *Clostridium* (0.28%). However, some of the Firmicutes sequences could not be classified into a recognized genus in all three breeds; here, the abundances of five unclassified groups ($p > 0.05$) in the collective data were *Peptostreptococcaceae* (5.42%), *Lachnospiraceae* (1.60%), *Erysipelotrichaceae* (0.67%), *Ruminococcaceae* (0.34%), and *Clostridiaceae* (0.17%).

Bacteroidetes was the second most dominant phylum in the three breeds (21.36%), and there were three genera and

two unclassified families in this phylum (Fig. 1C), but there were no significant differences ($p > 0.05$) among the three breeds. Within Bacteroidetes, *Prevotella* was the most abundant genera ($p > 0.05$) and showed the greatest abundance in the Maltese breed (26.79%) when compared with that in the Miniature Schnauzer (8.96%) and Poodle breeds (7.42%). For the remaining known genera in the collective data, *Bacteroides* and *Paraprevotella* showed abundances of 2.83% and 0.22%, respectively. Within Bacteroidetes, unclassified *Prevotellaceae* (2.12%) and unclassified *Bacteroidaceae* (1.26%) did not show significant differences ($p > 0.05$) among the three breed samples.

Within the phylum Proteobacteria, *Sutterella*, *Escherichia*, *Succinivibrio*, and *Anaerobiospirillum* genera, and one unclassified *Sutterellaceae* family were identified (Fig. 1D). Within these genera, *Succinivibrio* showed significantly ($p <$

Fig. 2. Microbial taxonomic profiles from each dog of three canine breeds at the phylum (A), and family (B) levels, classified by the representation of >1% of the total sequences.

Taxonomic compositions of each dog among the Poodle, Maltese and Miniature Schnauzer breeds were compared based on the relative abundance.

0.05) high abundance (0.57%) in the Miniature Schnauzer breed, while Poodle (0.01%) and Maltese (0.02%) breeds showed low abundances (Table 3). *Anaerobiospirillum* showed significantly high abundance ($p < 0.05$) in the Poodle (0.37%), moderate in the Maltese (0.17%), and low abundance in the Miniature Schnauzer (0.2%) breed. Actinobacteria was the third most dominant phylum in three canine breeds (3.29%), but within this phylum *Bifidobacterium* (1.95%) and *Collinsella* (1.22%) genera did not show significant differences ($p > 0.05$) among the breeds. Within the Fusobacteria phylum, the *Fusobacterium* genus showed significant differences ($p > 0.05$) among the three breeds, where Maltese (2.63%) showed a higher abundance than the Poodle (1.55%) and Miniature

Schnauzer (1.53%) breeds (Table 3).

Two canine gut microbiome enterotypes were identified in the three breeds. Six individuals, including five Maltese dogs and one Poodle dog, were categorized as having enterotype 1, characterized by high levels of *Prevotella* but a lower abundance of *Megamonas*. Meanwhile, the other 19 individuals, including two Maltese, eight Miniature Schnauzer, and eight Poodle dogs, were categorized as having enterotype 2, which comprises high levels of *Megamonas* but low levels of *Prevotella*.

Fecal Microbial Diversity

Alpha diversity indices (number of OTUs, Chao1, Shannon diversity index, and Simpson diversity index) were not

different ($p > 0.05$) among the three breeds (Table 4). In addition, beta diversity PCoA plot showed that fecal microbiomes of the three breeds were slightly separated, indicating that the composition of fecal microbiota is different among the three canine breeds (Fig. 3). The composition of fecal microbiota in Maltese dogs was more distantly separated compared to the other two canine breeds.

Correlation Analysis

Only genera that were significantly affected by breeds were considered in the correlation analysis. No genus had a correlation with body weight in any of the three breeds (Fig. 4). *Streptococcus* did not have a correlation with blood chemistry values in the three breeds. *Fusobacterium* was positively correlated with TG ($r = 0.762$, $p = 0.017$) in the Poodle breed but was not correlated with the remaining blood chemistry values in the other two breeds. Positive correlations were found for *Turicibacter* with ALP ($r = 0.798$, $p = 0.010$) in the Poodle breed, with total bilirubin ($r = 0.784$, $p = 0.021$) in the Maltese breed, and with AST ($r = 0.819$, $p = 0.046$) in the Miniature Schnauzer breed. *Succinivibrio* was positively correlated with ALT ($r = 0.749$, $p = 0.020$) in the Poodle breed and with glucose ($r = 0.815$, $p = 0.048$) in the Miniature Schnauzer breed.

Discussion

To date, no studies have compared microbiota within different canine breeds; however, a number of recent studies have investigated the effects of diet on canine gut microbiota using high-throughput DNA sequencing methods, revealing that dietary interferences may affect microbial composition and activity, and with likely effects on canine gut health. We believe that the present study is the first to investigate the effect of canine breed on gut microbiota in dogs fed and housed under the same conditions using Next-generation Sequencing (NGS) technology.

In the current study, we concluded that all three dog breeds were healthy because there were no blood biochemistry panel changes over the baseline value, and no

Fig. 3. Unweighted principal coordinate analysis (PCoA) displaying correlations among the three breeds.

The PCoA plot slightly separated fecal microbiome among the three breeds. Red symbols, Miniature Schnauzer; blue symbols, Maltese; yellow symbols, Poodle.

other abnormalities or clinical signs were observed. Since some of the genera that significantly differed among the three breeds were correlated with at least one of the blood chemistry values in one breed but had no correlation with the other two breeds, it will be difficult to conclude that canine fecal microbiota is affected by canine health status. Age was almost the same among dogs in the three breeds, while no genus showed correlations with body weight. Diet, sex, vaccination history, and parasite protection history were the same in all three breeds. Therefore, canine fecal microbiota does not seem to be affected by other factors such as age, body weight, diet, sex, vaccination history, parasite protection history, or health status. In this study, the difference between fecal microbiota seems to be affected by the breed.

The microbial separation by the PCoA plot might have also resulted from the difference in breed because canine fecal microbiota was not affected by other factors except for the breed, as described above. In addition, the PCoA plot shows the fecal microbiota in Maltese dogs to be more distantly separated compared to the other two breeds. This

Table 4. Fecal diversity statistics among three canine breeds.

Canine breed ¹	No. of observed OTUs	Chao1	Shannon	Inverse Simpson	Goods coverage
Maltage ($n = 8$)	174.125 ^a	193.621 ^a	3.616 ^a	0.812 ^a	0.999 ^a
Poodle ($n = 9$)	167.111 ^a	192.672 ^a	3.709 ^a	0.828 ^a	0.999 ^a
Miniature Schnauzer ($n = 8$)	171.500 ^a	191.580 ^a	3.346 ^a	0.761 ^a	0.999 ^a

¹Means among the three breeds were compared using ANOVA, followed by Duncan's test.

^aMeans with the same superscript letter within a column do not show a significant difference ($p > 0.05$).

Fig. 4. Correlations between genera affected by breeds and blood chemistry values.

Only genera that are significantly different among the three breeds were considered in correlations. (A) Correlations between genera and blood chemistry values in poodle dogs. (B) Correlations between genera and blood chemistry values in maltese dogs. (C) Correlations between genera and blood chemistry values in miniature schnauzer dogs.

separation might be ascribed to different enterotype classification because most of the Maltese dogs were

assigned to enterotype I, while most of the dogs of the other two breeds were assigned to enterotype II.

In the 16S rRNA gene analysis on the three dog breeds in our study, the dominant phyla were Firmicutes and Bacteroidetes, which included more than 90% of the total sequences in all three breeds. Other abundant phyla were Proteobacteria, Actinobacteria, and Fusobacteria. Earlier studies also found that Firmicutes, Bacteroidetes, Proteobacteria, and Fusobacteria were the abundant phyla, even though there were variations in the dogs and the conditions under which they were held, such as age, diet, genetic background, breeding environment, and health [10, 18, 19].

In the present study, there were significant differences in Firmicutes abundance among the three canine breeds, particularly for Miniature Schnauzer (80.17%) and Poodle (78.26%) breeds showing higher abundances than for Maltese dogs (57.64%). These dissimilarities might be due to differences in breed, individual variations in gut microbiota, or other individual variations in the animals. According to Turnbaugh *et al.* [20], specifically, gut microbiota is extremely affected by the host genotype and different environmental conditions that are difficult to standardize. Within Firmicutes, there were high abundances of genus *Lactobacillus* in all three dog breeds, but there were no significant differences among them; although, the Poodle and Miniature Schnauzer breeds had higher abundances than the Maltese dogs. In previous studies, dog fecal samples also had high abundances of *Lactobacillus* in Poodle [21] and Miniature Schnauzer dogs [22]. According to Swann *et al.* [23], *Lactobacillus* plays a key role in bile acid metabolism and affects the absorption of dietary fats and lipid soluble vitamins. Furthermore, *Lactobacillus* strains have the potential to be used in emerging probiotic food [24]; thus, *Lactobacillus* identified in the present study might also have the potential to be used as probiotics, especially in Poodle and Miniature Schnauzer dogs.

Streptococcus abundance was also high in all three dog breeds with the greatest percentage in Miniature Schnauzer dogs. *Streptococcus* plays an important role in the skin and mucosal health of canines. The abundance of these bacteria may suppress the growth of intestinal pathogens by means of competitive exclusion [25, 26], and earlier studies have shown they can improve the immune functions in humans and mice [27, 28]. The highest abundance of *Streptococcus* in Miniature Schnauzer dogs explains that gut microbiota is affected by canine breeds. *Megamonas* and *Blautia* genera were also abundant in all breeds, but no significant differences were observed among the breeds. However, we

propose that these bacteria may act as probiotics and improve intestinal health by relocating intestinal pathogens, thereby, increasing immune responses. According to Suchodolski *et al.* [7], *Blautia* is common in healthy dogs, but decreases with canine diarrhea, and may play a role in creating short-chain fatty acids.

Within Bacteroidetes, *Prevotella* was the most abundant genus with no significant difference among the three breeds. Although David *et al.* [29] showed that the type of feed plays a major role in the abundance of *Prevotella*, there are no studies on the relationship between canine breed and *Prevotella* abundance, and this requires further investigation. Observations from earlier studies indicated that *Prevotella* plays a key role in the digestive process [30]. It seems that *Prevotella* is core genus that is commonly found irrespective of different breeds.

In the phylum Proteobacteria, *Succinivibrio* was significantly higher in abundance in Miniature Schnauzer dogs than that in the Poodle and Maltese breeds. According to Petri *et al.* [31], *Succinivibrio* showed high abundance, were important bacteria in the gut microbiome of high starch fed cattle, and played a key role in propionate production through the production of upstream succinate [32]. However, there are no studies on *Succinivibrio* in dog fecal samples; therefore, little is known about the functions of *Succinivibrio*. The abundance of *Anaerobiospirillum* also differed significantly among the three dog breeds. *Anaerobiospirillum* showed higher abundances in Poodle (0.37%) and Maltese (0.17%) than that in the Miniature Schnauzer (0.2%) breed. Misawa *et al.* [33] found *Anaerobiospirillum* in a puppy with hemorrhagic diarrhea, but as extensive studies have not been conducted, the importance or pathogenic mechanisms of *Anaerobiospirillum* in the intestine remain unknown. There were also significant differences in *Fusobacterium* among the different canine breeds, with the Maltese breed showing higher abundances of *Fusobacterium* than that in the Poodle and Miniature Schnauzer breeds. *Fusobacterium* ferment carbohydrates and certain amino acids to produce butyrate, acetate, and other volatile fatty acids [34]. According to Middelbos [35], *Fusobacteria* was the codominant phylum in healthy canine feces. Thus, we propose that the significant variations found in *Fusobacterium* among the three breeds in our study may be mucosal differences associated with different canine breeds.

Knowledge on fecal microbiome composition in different dog breeds is important for understanding the relationships between the host and the fecal microbiome composition. This study shows that fecal microbiome composition differs among different dog breeds fed and housed under the

same conditions, indicating that host genetic factors may affect fecal microbiome composition in dogs. Understanding these differences may contribute to the development of different feeding strategies for maintaining a healthy gut microbiome composition in different dog breeds.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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