Modulation of the intestinal microbiota of dogs by kefir as a functional dairy product

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ABSTRACT

Kefir is a traditional dairy product with multiple probiotic characteristics derived from its associated microorganisms, including more than 50 species of lactic acid bacteria and yeast. For centuries, many people have produced kefir for human consumption; its consumption and potential role as a probiotic supplement in companion animals have never been tested. The present study explored the potential application of kefir as a probiotic supplement for dogs. Kefir was orally administered to healthy adult dogs ($n = 6$) for 2 wk. On d 0 and 14 (before and after kefir consumption, respectively), gut microbiota was analyzed comprehensively using quantitative PCR and 16S rDNA amplicon-based community analysis using fresh fecal samples. The 16S rDNA amplicon-based community analysis showed that the relative abundance of the phylum *Fusobacteria* was significantly decreased after kefir consumption. Furthermore, the relative abundance of the families *Prevotellaceae*, *Selenomonadaceae*, and *Sutterellaceae* increased significantly, whereas that of the families *Clostridiaceae*, *Fusobacteriaceae*, and *Ruminococcaceae* decreased significantly. The quantitative PCR assay showed that kefir consumption significantly increased the population of lactic acid bacteria and the lactic acid bacteria:*Enterobacteriaceae* ratio and significantly decreased the *Firmicutes*:*Bacteroidetes* ratio. In summary, 2-wk kefir administration successfully modified the gut microbiota without causing any clinically evident adverse effects. Therefore, kefir could be further developed as a novel probiotic food supplement for dogs to improve the quality of life of dogs.

Key words: kefir, gut microbiota, probiotic, community analysis, feed supplement

INTRODUCTION

The intestinal microbiota encompasses a large variety of microorganisms in the intestinal tract, with at least 10^{14} microbial cells in mammals, originating from up to 1,000 different species (Suchodolski, 2011). To date, gut microbiota and its association with health or disease have been assessed in various animals, including dogs (Suchodolski, 2011; Hooda et al., 2012; Strompfová et al., 2014). Owing to increasing interest in the health and welfare of companion animals, especially dogs, the composition and role of canine intestinal microbiota have been actively investigated, and studies on the possibilities of its therapeutic modification with various probiotics, prebiotics, and synbiotics have increased in the past decades (Schmitz and Suchodolski, 2016). For instance, 4 individual bacterial strains—*Enterococcus faecium* NCIMB10415, *Ec. faecium* NCIMB10415, *Lactobacillus acidophilus* DSM13241, and *Bifidobacterium animalis* ssp. *animalis* NCIMB41617—have been assessed by the European Food Safety Authority for their safety and efficacy as probiotics or food supplements for dogs (Schmitz and Suchodolski, 2016). Other species, including *Lactobacillus fermentum*, *Butyrivibrio fibrisolvens*, and *Lb. acidophilus*, have been actively investigated as potential probiotic agents (Asanuma et al., 2001; Beasley et al., 2006; Tang and Saris, 2013). However, these candidates were tested individually, whereas foods often contain multiple strains, and their efficacy in trials suggests that they should be manufactured in the pharmaceutical industry to reach consumers (Schmitz and Suchodolski, 2016).

Kefir is a traditional fermented milk containing more than 50 species of microorganisms, including lactic acid bacteria, yeast, and acetic acid bacteria (Guzel-Seydim et al., 2011). According to the Codex Standard for Fermented Milks (Codex Alimentarius, 2003), kefir is defined as fermented milk prepared from a unique starter culture known as kefir grains comprising lactic acid bacteria, acetic acid bacteria, and yeast. We previously reported that kefir administration for 3 wk successfully modulated gut microbiota in mice fed a normal diet

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(Kim et al., 2015b) and that regular kefir administration prevented obesity and nonalcoholic fatty liver disease induced by a high-fat diet by improving gut micro- and mycobiota (Kim et al., 2017b). In addition, individual kefir microorganisms, such as *Lactobacillus kefirianofaciens* DN1 and *Lactobacillus kefir* DH5, and bioactive compounds such as exopolysaccharides could modulate the gut microbiota and exerted beneficial health effects (Jeong et al., 2017a,b; Kim et al., 2017a, 2018).

The application of human probiotics to dogs showed several benefits in some studies (Aktas et al., 2007; Rossi et al., 2014). However, no studies have investigated the beneficial effects of kefir on the gastrointestinal tract of dogs, especially on the gut microbiota. Considering interspecies differences in the anatomical, metabolic, and physiological aspects among the aforementioned animals, this study aimed to investigate the modulatory effect of kefir on the gut microbiota of healthy dogs and provide experimental information regarding the possibility of its use as a novel probiotic food supplement for dogs.

MATERIALS AND METHODS

Kefir Preparation

In total, 50 g of viable kefir grain (Sensorgen Co., Seoul, South Korea) was used to inoculate 500 mL of sterilized milk (Seoul Milk Inc., Seoul, South Korea) and was cultured at 25°C for 24 h. After fermentation, the grain and milk were separated using a sterilized plastic filter (2-mm pore size). Kefir milk was prepared daily during the experimental period. Lactic acid bacteria and yeast in kefir milk were enumerated in accordance with our previous study (Kim et al., 2015a). Kefir milk contained 9.32 ± 0.23 log cfu/mL of lactic acid bacteria and 7.12 ± 0.36 log cfu/mL of yeast.

Animals and Experimental Designs

Healthy adult dogs [$n = 6$; 4 male, 2 female; mean age = 5.17 ± 2.32 yr (range: 3–8); mean BW = 28.65 ± 2.94 kg (range: 24.2–32.0)] of the following breeds were included in the present study: mongrel, Poodle, Shepherd ($n = 2$), Malinois, and Labrador. The experimental design was approved by the Sejeong College Institutional Animal Care and Use Committee (SJ2017-04). Dogs were individually housed in floor pens (2.5 m²) and provided outdoor exercise once a day. The animals were kept at ambient temperatures between 20 and 24°C in a room with a humidity of 30 to 70% and natural light. Dogs were fed extruded dry dog

food twice daily according to the recommended daily feeding portion (Natural Balance Ultra Premium, Dick Van Patten's Natural Balance Pet Foods, Burbank, CA). Water was provided ad libitum. Environmental enrichment was provided in the form of sanitized toys, food treats, and social interaction with humans. The adaptation period to this food was 4 wk before the experiment. The 14-d experiment comprised a kefir pre-consumption phase (d 0) and postconsumption phase (d 14). During the experimental period, dogs were provided with 200 mL of kefir once a day and consumed it ad libitum. Food intake, water consumption, and kefir consumption were recorded daily for individual dogs. On d 0 and 14, changes in BW, BCS, fecal quality, hematological parameters, and gut microbiota of the dogs were monitored. Body condition score was determined as follows: 1 = very thin (bones such as ribs could be easily observed and palpated with no fat cover), 2 = underweight (bones could be palpated with a little amount of fat cover), 3 = ideal (ribs can be palpated through slight fat cover), 4 = overweight (difficult to palpate ribs due to moderate fat cover), and 5 = obese (difficult to palpate ribs due to thick fat cover).

Sampling Procedures

Fresh fecal samples were collected on d 0 (before kefir consumption) and d 14 (after kefir consumption). Bristol's fecal score was determined immediately and fecal samples were stored at 4°C less than 2 h before DNA extraction (Lewis and Heaton, 1997). Blood samples were collected via venipuncture from the vena cephalica antebrachii on d 0 and 14 in heparin-coated tubes for hematological analysis.

Fecal Quality Analysis

Fecal samples were scored in accordance with Bristol's scoring guidelines (Lewis and Heaton, 1997): 1 = separate hard lumps; 2 = lumpy and sausage-like; 3 = a sausage shape with cracks on the surface; 4 = like a smooth, soft sausage or snake; 5 = soft blobs with clear-cut edges; 6 = mushy consistency with ragged edges; and 7 = liquid consistency with no solid pieces. One gram of each fecal sample was used to determine fecal water content after drying the fecal samples for 24 h in an 80°C dry oven using the following equation: $[(\text{fecal weight before drying} - \text{fecal weight after drying}) / \text{feces weight before drying}] \times 100$. Finally, fecal occult blood was assessed using a Hema-Screen Lab Pack (Stanbio Laboratory, Boerne, TX) in accordance with the manufacturer's instructions.

Hematological Analysis

Hematological parameters were analyzed using an Advia 2120 hematology analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) in accordance with the manufacturer's instructions.

DNA Extraction from Fecal Samples

Genomic DNA was extracted using a NucliSENS easyMAG instrument (bioMérieux, Marcy l'Etoile, France) in accordance with the manufacturer's instructions. Briefly, each fecal sample was inoculated in 1 mL of lysis buffer containing guanidine thiocyanate, Triton X-100, and EDTA and was homogenated vigorously by vortexing and chopping. The mixture was incubated overnight at 25°C. The lysed sample was then transferred to the well of a plastic vessel with 50 µL of magnetic silica and subjected to automatic magnetic bead separation. The DNA was then resuspended in 50 µL of elution buffer.

Gut Microbiota Analysis

Each sequenced sample was prepared in accordance with the Illumina (San Diego, CA) 16S Metagenomic Sequencing Library protocols. The DNA was quantified and its quality was assessed using PicoGreen (Molecular Probes, Eugene, OR) and Nanodrop (Thermo Fisher Scientific, Waltham, MA). The 16S rDNA was amplified using 16S V3-V4 primers. The primer sequences are as follows: 16S amplicon PCR forward primer: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3'; 16S amplicon PCR reverse primer: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'. The PCR reaction was performed with Herculase II Fusion DNA Polymerase (Agilent Technologies, Santa Clara, CA). The reaction for amplicon PCR was performed at 95°C for 3 min, followed by 25 cycles each at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and then at 72°C for 5 min. For index PCR, the cycle was at 95°C for 3 min, followed by 10 cycles each at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and then at 72°C for 5 min. Thereafter, a subsequent limited-cycle amplification step (301 cycles × 2) was performed to add multiplexing indices and Illumina sequencing adapters. The final products were normalized and pooled using PicoGreen, and the size of libraries was verified using the LabChip GX HT DNA High Sensitivity Kit (PerkinElmer, Waltham, MA). Thereafter, the DNA fragment (120–160 bp) was sequenced using the MiSeq platform (Illumina) from both ends.

After sequencing analysis of MiSeq raw data, a FASTQ file was generated using MCS (MiSeq Control Software version 2.2) and bcl2fastq (version 1.8.4), and a PhiX sequence was obtained through Burrows-Wheeler Aligner (BWA). High-quality sequences with a Phred quality score of 25 or better were selected. By merging paired-end MiSeq forward and reverse reads (~300 bp, respectively), the single long reads (~440 to 465 bp) were obtained using FLASH (1.2.11; Magoč and Salzberg, 2011). The obtained sequence was processed using the CD-HIT-EST-based operational taxonomic unit (OTU) analysis program CD-HIT-OTU (Li et al., 2012) to eliminate low-quality, ambiguous, and chimeric sequences, which were considered sequencing errors, clustering to form species-level OTU at clustering cutoff of 97%.

The representative sequence of each OTU was determined using BLASTN (version 2.4.0; Zhang et al., 2000) in Reference DB (NCBI 16S Microbial), and taxonomic assignment was performed using information regarding the most similar subject. If the query coverage of the best hit that matched the database was <85% and the identity of the matched domain was <85%, the taxon was considered undefined. The sequences were identified to phylum level at ≥85%, genus level at ≥94%, and species level at ≥97% similarity.

Various comparisons within the fecal community were performed using QIIME (version 1.8; Caporaso et al., 2010) and considered OTU information. Shannon and inversed Simpson indices were obtained to confirm species diversity and evenness of microbial communities in fecal samples. Alpha diversity was determined via a rarefaction curve and Chao1 value. The principal coordinate analysis based on weighted UniFrac distance was conducted to visualize the community diversity. The hierarchical relationship between samples was visualized via construction of an unweighted pair group method with arithmetic mean phylogenetic tree.

The quantity of total bacteria, yeast, *Firmicutes*, *Bacteroidetes*, lactic acid bacteria, *Bifidobacterium*, *Enterobacteriaceae*, and *Clostridium* cluster I was determined from fecal-derived DNA samples using quantitative PCR (qPCR) analysis per our previous reports (Supplemental Table S1, <https://doi.org/10.3168/jds.2018-15639>; Kim et al., 2015b, 2017a,b; Jeong et al., 2017b).

Statistical Analysis

All data are presented as the mean ± standard deviation. Statistical analyses were conducted using SPSS version 18.0 (SPSS Inc., Chicago, IL). All data obtained from samples including relative abundances of fecal microbiota were tested for normality using the

Shapiro–Wilk normality test. A paired *t*-test was used to evaluate statistical significance between d 0 and 14. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

Change in BW and BCS

No significant changes were observed in BW and BCS before and after kefir consumption (Table 1). Weight loss greater than 15% of initial BW was not observed in any dogs during the experimental period, suggesting that kefir is not detrimental to canine health.

Food and Water Intake

During the experimental period, dogs consumed more than 94% of the provided kefir between 1 and 3 min actively and spontaneously, suggesting that kefir is a potentially suitable natural food supplement or can be used as a treat to attract attention (Supplemental Table S2, <https://doi.org/10.3168/jds.2018-15639>). In addition, kefir consumption did not affect the dietary habits of the experimental dogs (Supplemental Table S2). All dogs consumed the entire amount of provided food except for dog 2, who consumed 89.4% of the provided food. Dogs drank water normally throughout the experimental period, which was demonstrated by dehydration pinch test.

Fecal Quality

Two weeks of kefir consumption significantly increased fecal water content, and Bristol fecal scores remained unchanged (Table 1). In addition, occult blood was absent in the feces of all dogs, suggesting that kefir is not harmful to the gastrointestinal tract of dogs (Table 1).

Hematological Parameters

Hematological parameters of the experimental dogs were within the physiological reference ranges before and after kefir consumption, indicating that kefir consumption is not associated with the risk of subsequent anemia, systemic inflammation, or coagulative disorders (Supplemental Table S3, <https://doi.org/10.3168/jds.2018-15639>).

Modulation of Gut Microbiota

Figure 1 illustrates the results of next-generation sequencing (NGS) analysis of canine gut microbiota

Table 1. Physical examination and fecal parameters of dogs on d 0 and 14¹

Parameter	d 0	d 14
BW (kg)	28.65 ± 2.94 ^a	28.28 ± 2.63 ^a
BCS ²	3 ^a	3 ^a
Fecal water contents (%)	62.51 ± 4.14 ^a	70.70 ± 1.73 ^b
Bristol score	3.5 ± 0.84 ^a	3.67 ± 0.52 ^a
Occult blood in feces	0/6	0/6

^{a,b}Different letters in a row indicate a significant difference (*P* < 0.05 by paired and 2-way Student’s *t*-test).

¹Data are expressed as mean ± SD.

²1 = very thin; 2 = underweight; 3 = ideal; 4 = overweight; 5 = obese.

assessed via 16S rDNA amplicon-based community analysis before and after 2 wk of kefir consumption. In principal coordinate analysis based on weighted UniFrac distance, the fecal microbiota after kefir consumption was not fully separate from that before kefir consumption, although fecal microbiota of dogs after kefir consumption was roughly clustered (Figure 1A). Similarly, hierarchical clustering analysis via construction of an unweighted pair group method with arithmetic mean tree indicated an insignificant difference in fecal microbiota before and after kefir consumption (Figure 1B).

To evaluate the richness of gut microbiota, we compared the Chao1, Shannon, and Simpson diversity indices of the canine fecal samples obtained before and after kefir consumption (Figure 1C). There were no significant differences in the Chao1, Shannon, and Simpson indices, indicating that richness and evenness were similar before and after kefir consumption. Good’s coverage values were >0.999 for fecal samples obtained before and after kefir consumption, indicating negligible undetected diversity (Figure 1C).

Gut microbiota composition before and after kefir consumption was compared at the phylum and family levels, respectively (Figure 1D and 1E; Supplemental Tables S4 and S5, <https://doi.org/10.3168/jds.2018-15639>). At the phylum level, the most significant differences were observed in *Fusobacteria* (*P* = 0.014; Figure 1D, Supplemental Table S4). At the family level, the relative abundances of *Prevotellaceae*, *Selenomonadaceae*, and *Sutterellaceae* increased significantly (*P* = 0.027, 0.044, and 0.028, respectively), whereas those of *Clostridiaceae*, *Fusobacteriaceae*, and *Ruminococcaceae* decreased significantly (*P* = 0.039, 0.014, and 0.034, respectively) following kefir consumption (Figure 1E and Supplemental Table S5). Species-level alterations in the gut microbiota are represented as a heatmap (Figure 1F). In total, 92.55 to 94.12% of obtained sequences were accurately identified to the bacterial species level. The relative abundance of *Bacteroides uniformis*, *Fusobacterium perfoetens*, *Fecalibacterium prausnitzii*,

In addition, group-specific qPCR analysis of gut microbiota harvested from fecal samples after 2 wk of kefir consumption was conducted for certain microbes of interest to complement the results of the NGS-based analysis (Figure 2). Kefir consumption signifi-

cantly increased the population of lactic acid bacteria ($P = 0.012$), significantly decreased the *Firmicutes*:*Bacteroidetes* ratio ($P = 0.026$), and significantly increased the lactic acid bacteria:*Enterobacteriaceae* ratio ($P = 0.044$). These were consistent with the results obtained by NGS analysis (the relative abundance of *Firmicutes* changed from 25.40 to 26.92%, that of *Bacteroidetes* changed from 42.99 to 53.01%, that of *Lactobacillaceae* changed from 0.62 to 2.18%, and that of *Enterobacteriaceae* changed from 0.25 to 0.03%). The number of total bacteria was not changed by 2 wk of kefir consumption.

DISCUSSION

The present study reports that 2 wk of kefir administration can successfully modulate the gut microbiota of healthy adult dogs without resulting in any clinically evident adverse effects. Kefir has been assessed extensively as a potential probiotic food supplement in human and animal models of disease (Bourrie et al., 2016; Kim et al., 2018); however, it has not been investigated in dogs. The probiotic attributes of beneficial microorganisms, such as survivability and functional-

ity, cannot be directly extrapolated from one species to another. Therefore, we examined, for the first time, the applicability of kefir as a novel probiotic food supplement for dogs.

For decades, numerous studies have investigated the application of probiotics in dogs to improve their health and to control diseases (Weese and Anderson, 2002; Manninen et al., 2006; Wynn, 2009; Silva et al., 2013). For instance, numerous studies isolated lactic acid bacteria from canine feces to develop novel probiotic agents for dogs, such as *Lb. fermentum* AD1, *Enterococcus faecalis* EE4, *Ec. faecium* EF01, *Lb. fermentum* LAB8, *Lactobacillus salivarius* LAB9, *Lactobacillus rhamnosus* LAB11, and *Lactobacillus mucosae* LAB12 (Strompfová et al., 2004, 2006; Manninen et al., 2006). These strains have been shown to colonize the canine gastrointestinal tract (Strompfová et al., 2004), decrease blood glucose levels (Strompfová et al., 2006), increase serum protein levels (Strompfová et al., 2006), and compete with enteropathogenic microorganisms (Rinkinen et al., 2003). Furthermore, gut microbiota is closely associated with clinical canine gastrointestinal disorders such as inflammatory bowel disease and small-intestinal bacterial overgrowth and systemic diseases such as

Figure 2. Effect of 2-wk kefir consumption on the intestinal microbiota of healthy adult dogs analyzed via quantitative real-time PCR. Highlighted taxa comprise total bacteria (used as a normalization factor), yeast, *Firmicutes*, *Bacteroidetes*, lactic acid bacteria, *Bifidobacterium*, *Enterobacteriaceae*, and *Clostridium*. Ct = cycle threshold.

obesity and immune-related disorders (Manninen et al., 2006; Suchodolski and Simpson, 2013; Schmitz and Suchodolski, 2016). To prevent these diseases, pet foods have been formulated with various probiotic bacteria, although the actual viable count of these microorganisms does not generally amount to the labeled descriptions, which prevents the food from having its intended beneficial effects (Weese and Arroyo, 2003). In this regard, additional probiotic supplements are required to maintain and improve canine health.

Kefir is a traditional dairy food product produced easily via natural fermentation of milk with kefir grain at room temperature, requiring no special instrument to acquire the final product (Guzel-Seydim et al., 2011). Hence, kefir is widely produced and consumed at home (Guzel-Seydim et al., 2011). This study indicated that all dogs consumed kefir spontaneously and that they preferred consuming kefir even in the mid and late experimental periods, which is considered a desirable feature for an optimal pet treat (Weese and Arroyo, 2003). Some studies report that probiotic microorganisms should naturally originate from the target host species; however, the present study shows that probiotics of another origin can be successfully applied to improve the gut microbiota of healthy dogs as well (Kailasapathy and Chin, 2000).

We previously reported that kefir and its associated microorganisms exerted broad-spectrum and significant modulatory effects on gut microbiota in healthy mice and in mouse models of disease (Kim et al. 2015b, 2017a,b; Jeong et al., 2017b). Concurrently, 2 wk of kefir consumption clearly altered gut microbiota of dogs at the phylum, family, and species levels. Gut microbiota compositions are unique and stable in each individual, especially in adults (Tannock, 2005). It is remarkable that although all dogs included herein were adults and varied in age and sex, kefir significantly modulated the composition of their gut microbiota. The NGS-based analysis revealed that kefir consumption significantly decreased the relative abundance of the phylum *Fusobacteria* and the families *Clostridiaceae*, *Fusobacteriaceae*, and *Ruminococcaceae* and significantly increased the relative abundance of the families *Prevotellaceae*, *Selenomonadaceae*, and *Sutterellaceae*. At the species level, the relative abundance of *Bc. uniformis*, *Fb. perfoetens*, *Fc. prausnitzii*, *Fb. mortiferum*, *Ps. capillosus*, and *Fs. saccharivorans* decreased, whereas that of *C. mitsuokai* increased. Moreover, qPCR analysis revealed that kefir consumption significantly increased the relative abundance of lactic acid bacteria, decreased the *Firmicutes:Bacteroidetes* ratio, and increased the lactic acid bacteria:*Enterobacteriaceae* ratio, which was consistent with the results obtained by NGS analysis.

A rather remarkable finding is the reduction in the relative abundance of the phylum *Fusobacteria*, revealed via NGS-based analysis. *Fusobacteria* comprises gram-negative, nonsporulating, obligate anaerobic bacilli. A recent study reported in vitro antimicrobial activity of kefir against *Fusobacterium nucleatum*, a well-known member of phylum *Fusobacteria* (Guzel-Seydim et al., 2016). In parallel, we reported a negative interaction between kefir and intestinal phylum *Fusobacteria* and family *Fusobacteriaceae* in vivo, more specifically with *Fb. perfoetens* and *Fb. mortiferum*. The phylum *Fusobacteria* appears to be a major member of commensal gut microbiota in dogs, and a recent study suggested that this microbial group was not associated with inflammatory bowel diseases in dogs, which is opposite in humans (Vázquez-Baeza et al., 2016). As the physiological roles of this bacterium in canine gut health are not yet entirely understood, future studies should find whether the decrease of *Fusobacteria* by probiotic consumption in healthy dogs is beneficial or harmful.

Another interesting finding is an increase in the lactic acid bacteria:*Enterobacteriaceae* ratio revealed via qPCR assay. Lactic acid bacteria acidify the host gut by producing various organic acids, creating an unfavorable environment for acid-sensitive members of family *Enterobacteriaceae* (Edelson-Mammel et al., 2006; Dharmasena et al., 2016). In addition, lactic acid bacteria associated with kefir compete with members of the family *Enterobacteriaceae* in the host for nutrition and adhesion sites and produce a bacteriocin or antimicrobial exopolysaccharides to counter them (Powell et al., 2007; Miao et al., 2014; Jeong et al., 2017a). However, the family *Enterobacteriaceae* was not significantly reduced after kefir consumption with both NGS analysis and qPCR assay in the present study. The increase of the lactic acid bacteria:*Enterobacteriaceae* ratio could be solely attributed to the increase of lactic acid bacteria population by the inflow of kefir-originated lactic acid bacteria. Given that the population of *Enterobacteriaceae* has a significant correlation with inflammatory bowel diseases in dogs (Vázquez-Baeza et al., 2016), further studies are warranted to control the *Enterobacteriaceae* population by kefir consumption with some modifications (i.e., increased amount or frequency of kefir consumption, use of kefir from other origins, and use of its sole microorganism).

A final aspect regarding the modulatory effects of kefir on canine gut microbiota is the reduction in the *Firmicutes:Bacteroidetes* ratio and the relative abundance of *Fc. prausnitzii*. Both intestinal microbial biomarkers are associated with metabolic diseases such as obesity and hepatic steatosis because these microorganisms are related to the host energy harvest from the

intestinal contents (Balamurugan et al., 2010; Kim et al., 2018). The exact roles and contribution of these microbial members in relation to host health and diseases remain a matter of debate; for example, the *Firmicutes:Bacteroidetes* ratio was first believed to be positively correlated with obesity, whereas some current studies have failed to demonstrate the correlation between *Firmicutes:Bacteroidetes* ratio and weight gain and body mass index in human (Koliada et al., 2017). The clinical significance of this microbial marker is also unclear in dogs. There are only a few studies on the association of this marker with canine health, and one study revealed that a high-protein and low-carbohydrate diet increased the *Firmicutes:Bacteroidetes* ratio in dogs (Li et al., 2017). For *Fc. prausnitzii*, it was reported that obese children harbored a higher number of this species compared with nonobese participants (Balamurugan et al., 2010), and this bacterium played a role in alleviating low-grade inflammation in metabolic diseases (Furet et al., 2010). In dogs, the reduction of *Fc. prausnitzii* was observed in dogs with nonhemorrhagic diarrhea, acute hemorrhagic diarrhea, and active inflammatory bowel diseases (Suchodolski et al., 2012). In contrast, in a fiber-deprived environment, this species is able to digest the mucin layer, which provides a more favorable environment for opportunistic pathogens to penetrate the host gut barrier (Velasquez-Manoff, 2015). Given the multifaceted roles of these gut microbial members in various situations, the results obtained in the present study need to be interpreted with caution. In addition, the effect of kefir consumption on microbiota and weight gain needs to be studied in overweight or obese dogs in the future.

Furthermore, significant changes reported in other intestinal microbial taxa including *Ruminococcaceae*, *Selenomonadaceae*, and *Sutterellaceae* and their clinical and physiological significance have rarely been studied in both human and dogs; hence, further studies are warranted to understand the significance of changes in these taxa. The relative abundance of gut yeast remained unchanged upon qPCR analysis, although kefir contains a large population of probiotic yeast (Kim et al., 2015a). Despite the total number of yeast remaining unchanged, yeast composition at lower phylogenetic levels may have been altered, which should be addressed in future studies.

We successfully demonstrated that kefir modulates the gut microbiota of healthy adult dogs within 2 wk of consumption without any harmful effects on analysis of BW, BCS, fecal occult blood, hematological parameters, and dietary habits. The 16S rDNA amplicon-based community analysis and qPCR assay of fecal samples revealed that kefir consumption clearly altered the composition of gut microbiota at different levels of

taxa. Therefore, kefir may be applied as a novel functional pet food supplement to improve the quality of life of dogs, much to their owners' satisfaction.

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