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Original article

Effect of commercial prescription diets containing prebiotics on clinical signs and fecal miocrobiome in dogs with intestinal disease

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Abstract

Diet has emerged as a key modulator of the gut microbiota, offering a potential strategy for disease prevention and management. This study investigated the effects of the Prescription Diet Gastrointestinal Biome (GB) on 7 healthy dogs and 16 dogs with chronic gastrointestinal diseases (GI dogs). Our investigation monitored changes in body weight and the Canine Inflammatory Bowel Disease Activity Index (CIBDAI) in 16 GI dogs fed a GB diet. Additionally, we assessed the gut microbiota using 16S rRNA sequencing pre- (GI dogs) and post- (healthy dogs and GI dogs) administration of GB diet. In dogs with GI, a significant improvement in the severity of CIBDAI was observed post-feeding with the GB diet compared to the period pre-feeding, without any changes in body weight. Primary changes in the gut microbiome were marked by significant differences between healthy and GI dogs. However, post-feeding the GB diet in GI dogs, resulted in an increase in *Turicibacter* and a decrease in *Escherichia-Shigella* linked with gastrointestinal inflammation. In conclusion, the GB diet appears to positively influence the gut microbiota and clinical outcomes in dogs with GI. Future studies should explore these relationships by focusing on the long-term effects of diet on the gut health and disease management.

Keywords: canine, diarrhea, diet, fiber, inflammatory bowel disease, microbiome, prebiotics

Introduction

Gastrointestinal disorders in dogs are clinically categorized into three types: food-responsive, antibiotic--responsive and immunosuppressant-responsive. Due to the overlap in clinical signs among these enteropathies, no biomarkers have yet been established to allow definitive differentiation. Consequently, therapeutic trials play a crucial role in differentiating chronic enteropathies (Walker et al. 2013). Therapeutic diagnosis typically begins with dietary therapy, which has shown a significant response rate of at least 60% (Dandrieux and Mansfield 2019). To date, three primary categories of therapeutic diets have been identified to ameliorate the clinical signs of gastrointestinal disease: (1) highly digestible, fat-restricted diets; (2) hypoallergenic diets; and (3) high-fiber diets for chronic colitis.

A high-fiber diet is often the preferred as an initial approach (Leib 2000). Indeed, prebiotics such as oligosaccharides and fibers have attracted significant attention in recent research because of their potential to enhance the intestinal environment (Mondo et al. 2019). Prebiotics are dietary components that nourish beneficial bacteria in the intestine while suppressing the proliferation of pathogenic bacteria, thus exerting a beneficial impact on the host. A novel dietary formulation, the Prescription Diet Gastrointestinal Biome (GB; Hill Colgate, Tokyo, Japan), has also recently been introduced. This diet features a blend of soluble and insoluble fibers, including a unique combination of whole grains, omega-3 fatty acids, and prebiotic fibers. In addition, it is enriched in polyphenols derived from citrus, cranberry and flaxseed, which stimulate gut bacteria and promote digestive health. Polyphenols are further recognized for their antioxidant, anti-inflammatory, and antimicrobial properties (Rowland et al. 2018). Furthermore, polyphenols act as prebiotics, promoting the growth of beneficial bacteria and facilitating the production of short-chain fatty acids.

In the current study, we investigated the effects of the GB diet on domestic dogs with chronic gastrointestinal signs. We further monitored the changes in body weight, body condition score (BCS), and Canine Inflammatory Bowel Disease Activity Index (CIBDAI). Moreover, we explored the relationship between changes in clinical signs and alterations in the fecal microbiome, as evaluated using 16S rRNA sequencing, pre- and post-feeding GB diets.

Materials and Methods

Animals

Sixteen domestic pet dogs (3 females, 5 spayed females, 3 males, 5 castrated males; mean age: 5.4±4.8 years, mean weight: 6.5±7.0 kg) with chronic gastrointestinal signs or requiring dietary modification based on their past gastrointestinal medical history, as determined by veterinarians, were included in the gastrointestinal disease group (GI dogs). Seven healthy domestic pet dogs (2 spayed females, 2 males, 3 castrated males; mean age: 4.6±2.6 years, mean weight: 17.7±9.5 kg) without any current medication or disease were further included in the healthy group (healthy). The determination of healthy dogs was made by a veterinarian who conducted interviews and physical examinations. Additionally, the healthy dogs were required to have no gastrointestinal signs (such as soft stools, diarrhea, or vomiting) in the 4 weeks preceding the study's initiation. We were only able to gather 7 healthy dog volunteers, resulting in a discrepancy in the number of animals compared to the 16 GI dogs. No experimental animals were used in the study. GB diets were administered only after informed consent was obtained from the owners, who were fully informed about the study's purpose, nature, potential risks, and benefits. Consequently, ethical approval was not deemed necessary. The profiles of GI and healthy dogs are presented in Tables 1 and 2, respectively.

The GB diet was used in this study, which focused on the composition of nutritional ingredients. The chemical composition of the GB diet is presented in Table 3. Prior to being fed the GB diet, 16 GI dogs and seven healthy dogs were fed various diets, as chosen by their respective owners or prescribed by veterinarians. Feeding amounts were calculated and fed based on 1.0-2.0 × Resting energy requirement (RER, BW 0.75×70), which corresponds to the same caloric intake as the diet consumed prior to switching to the GB diet. All dogs were fed twice daily. The treats typically given by each household were provided and remained unchanged throughout the study period. In addition, the dogs had unrestricted access to drinking water.

Study design and sample collection

Before the start of the study, all dogs were fed each diet for four weeks. Fresh fecal samples were collected from GI dogs within 15 min of defecation during the last three days of the pre-study period. After a gradual transition period of one week, all dogs (both healthy and GI) were continuously fed the GB diet for eight weeks. Fresh fecal samples were collected from all dogs within 15 min of defecation during the last 3 days

Table 1. Profiles of the 16 gastrointestinal disease dogs used in the current study.

No.	Breeds	Sex	Age (yr)	Body weight (kg)	Diagnosis	Diet fed before study	Medication initiated at the start of the study	Medication administered after 4 and 8 weeks of feeding test diets
1	Yorkshire Terrier	Spayed female	8	2.98	chronic colitis	Articular support dry		
2	Toy Poodle	Castrated male	11	4.38	chronic colitis	i/d Comfort dry		
3	Mix	Castrated male	1	2.6	chronic colitis	Digestive Support (High fiber) dry	Bioymbuster (probiotic) 1 tablet, twice a day	
4	Toy Poodle	Castrated male	13	4.8	chronic colitis	i/d (Low Fat) dry		
5	Boston Terrier	Spayed female	8	4.2	chronic colitis	Vets Plan dry		
6	Miniature Dachshund	Female	13	4.3	chronic enteropathy	w/d dry		
7	Cavalier King Charles Spaniel	Female	2	6.4	chronic colitis	i/d dry	One spoonful of psyllium, twice a day	One spoonful of psyllium, twice a day
8	Miniature Pinscher	Spayed female	11	4.1	chronic enteropathy	IAMS dry		
9	Toy Poodle	Castrated male	10	3.9	chronic colitis	The data were not acquired	metronidazole10 mg/kg/twice a day	
10	Bulldog	Male	2	19.5	chronic enteropathy	Digestive Support (Low Fat) dry	Budesonide 0.15 mg/kg/once a day	Budesonide 0.15 mg/kg/once a day
11	Mix	Castrated male	1	11.8	chronic colitis	The data were not acquired	Mito max super (probiotic), once a day	
12	White Shepherd	Spayed female	2	24	chronic enteropathy	Select Protein Duck Tapioca dry		
13	Toy Poodle	Male	4	7.8	chronic enteropathy	t/d dry, Flora care dry, Best Balance dry		
14	Toy Poodle	Male	16	3.3	chronic colitis	Science Diet dry	Bioymbuster and Diabuster 1 tablet, twice a day; metronidazole 12 mg/kg/twice a day	
15	Labrador Retriever	Female	9	22.4	chronic colitis	Digestive Support (Low Fat) dry	new biolacmin w (Priboitics) 2 tablet twice a day, Diabuster 2 tablet, twice a day; metronidazole 11 mg/kg/twice a day	new biolacmin w (Priboitics) 2 tablet twice a day
16	Mix	Spayed female	11	11.36	chronic enteropathy	Choice S dry, Gran-Deli wet		

Table 2. Profiles of the 7 client-owned healthy dogs used in the current study.

No.	Breeds	Sex	Age (yr)	Body weight (kg)
1	Mix	spayed Female	4	12.8
2	Mix	castrated Male	9	18
3	Siberian Husky	spayed Female	6	18
4	Kai Ken	Male	3	16
5	German Shepherd	Male	2	42
6	Pembroke Welsh Corgi	castrated Male	9	13
7	Pembroke Welsh Corgi	castrated Male	4	15

Table. 3 The chemical composition of the Gastrointestinal Biome diet.

	Units	
protein	%	20.9
fat	%	12.5
carbohydrate (NFE)	%	52.2
fiber	%	7.2
Calcium	%	1.18
phosphrus	%	0.68
Sodium	%	0.39
Potassium	%	0.99
Magnesium	%	0.099
DHA	%	0.175
EPA	%	0.26

of the 8-week GB diet period. Immediately after collection, the fresh fecal samples were refrigerated at 4°C, and within 4 hours, they were transferred to a chilled storage at -80°C until analysis. Fecal samples were sent to the Anicom Specialty Medical Institute Inc. (Kanagawa, Japan) for gut microbiota analysis.

All GI dogs were scored for severity according to the CIBDAI scoring system (Jergens et al. 2003) before switching to the GB diet at 4 and 8 weeks of feeding GB diets. In addition to the CIBDAI, body weight, BCS, and fecal conditions were compared. Fecal condition comparisons were evaluated by referencing photographs that depicted six levels (1: very loose and watery; 2: not maintaining a cylindrical shape; 3: soft and barely cylindrical; 4: firm and cylindrical with some breaks in the middle; 5: firm and cylindrical, broken, and easy to pick up; 6: spherical or cylindrical and hard). We considered a level of 5 as a normal fecal condition.

Sample analysis

Frozen fecal samples were thawed and lysed using bead-homogenization and enzymes. Genomic DNA was extracted from the samples using a Chemagic Kit Stool (CMG-1076; PerkinElmer Japan G.K., Kanagawa, Japan). Stool samples (200 µL) and 810 µL of the Lysis buffer (containing 224 µg/mL Protenase K) supplied with the chemagic kit were added to Precellys 2 mL Soft Tissue Homogenizing Ceramic Beads Kit (Bertin Instruments, Montigny-le-Bretonneux, France) and crushed (6,000 rpm, 20 sec crush, 30 sec interval, 20 sec crush) in the Precellys Evolution (Bertin Instruments) bead homogenizer. The specimens were then placed on a heat block at 70°C for 10 minutes for lysis with Protenase K, and then placed on a heat block at 95°C for 5 minutes to inactivate Protenase K. The lysed specimens were subjected to automated DNA extraction using chemagic 360 (PerkinElmer Japan G.K.), using the protocol for chemagic kit stool to obtain 100 µL of DNA extract.

For bacterial DNA amplification, polymerase chain reaction (PCR) was performed using diluted genomic DNA and primers targeting the V3-V4 region of the 16S rRNA gene with Illumina 16S Metagenomic Sequencing Library Preparation (version 15044223 B) (Table 4). Amplification products were purified using Sera-Mag™ Select (Cytiva, Freiburg im Breisgau, Germany), and eluted with 40 µL of Buffer EB (QIAGEN, Hilden, Germany). After purification, the amplified product was subjected to PCR using a Nextera XT Index Kit v2 (Illumina Inc., San Diego, CA, USA). U.S.A.), and indexed. PCR reaction solution was prepared by mixing 2.5 µL of amplified product, 2.5 µL of each primer, 12.5 µL of 2x KAPA HiFi Hot-Start ReadyMix, and 5 µL of PCR grade water. The PCR product was subjected to secondary PCR (72°C for 5 min; 30 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec) using an index-sequence primer to purify the PCR product and prepare the sequence library. The DNA concentration in the sequencing library and the sizes of the amplified products were determined using a 4200 TapeStation System (Agilent Technology, Santa Clara, CA. U.S.A.) with High SensitivityD1000. The 850 µL library prepared at a final concentration of 4pM was mixed with 150 µL of PhiX prepared at the same concentration and analyzed by MiSeq (Illumina Inc.). The purified PCR products were then sequenced using an Illumina MiSeq Reporter. Raw 2×300 bp paired-end sequence reads and were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline software, version 1.8.0 (http://qiime. org). The relative proportions of the bacterial taxonomic distributions at the phylum, class, order, family, and genus levels were then compared for each test period.

Table 4. PCR primers, reaction solution and amplification conditions used in the current study.

Primer	Sequence
Illumina_16S_341F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG
Illumina_16S_805R	GTCTCGTGGGC TCGGAGATGTGTATAAGAGACAGGGACTACHVGGGT WTCTAAT
Reaction solution	
10 μL of DNA	
0.05 μL of each primer (100 μM)	
12.5 μL of 2x KAPA HiFi Hot-Start ReadyMix (F. Hoffmann-La Roche, Ltd., Basel, Switzerland)	
2.4 μL of PCR grade water	
Amplification conditions	
72°C for 5 min	
30 cycles of 95°C for 30 sec, 55°C for 30 sec	
72°C for 30 sec	

Table 5. Chnages in body weight, body condition score, fecal condition and CIBDAI score in 16 gastrointestinal disease dogs used in the current study.

	body weight			body condition score			fecal condition			CIBDAI		
	pre	4 week	8 week	pre	4 week	8 week	pre	4 week	8 week	pre	4 week	8 week
mean	8.6	8.6	8.7	2.7	2.7	2.7	3.1	4.5	4.7	3.1	1.4	1.0
SD	7.2	7.2	7.3	0.6	0.7	0.7	1.3	0.9	0.6	1.8	1.6	1.7

Statistical analysis

Data are presented as the median and minimum–maximum values or mean ± standard deviation. The gut microbiota was compared among three groups: (1) pre-administration of the GB diet in dogs with GI, (2) post-administration of the GB diet in dogs with GI, and (3) post-administration of the GB diet in healthy dogs. For dogs with GI, questionnaires were compared during three periods: (1) before switching to the GB diet, (2) 4 weeks post-feeding the GB diet, and (3) 8 weeks post-feeding the GB diet. Statistical analyses were performed using the Kruskal-Wallis test and Dunn's multiple comparison test (Graph Pad Prism5, Graph Pad Software, La Jolla, CA, USA). Differences were considered statistically significant at $p<0.05$. To estimate the bacterial diversity of each sample, four indices (Shannon, Observed otus, Pielou's evenness, and faith_pd index) were calculated, and rarefication curves were constructed using QIIME. The microbial communities in the samples were investigated using phylogeny-based unweighted or weighted UniFrac distance matrices calculated using the Greengenes reference tree. Principal coordinate analysis (PCoA) and hierarchical dendrogram construction were performed using the QIIME software.

Results

In GI dogs, the questionnaires showed that the CIBDAI improved significantly at 4 and 8 weeks post-feeding compared to pre-feeding ($p<0.05$, $p<0.001$; Dunn's multiple comparisons test) (Table 5). Additionally, significant improvements in fecal condition were observed at 4 and 8 weeks post-feeding compared to those pre-feeding ($p<0.01$, $p<0.005$; Dunn's multiple comparisons test). There were no significant differences in body weight or BCS pre- and post-administration of the GB diet.

During the study period, the fecal microbiome of the dogs primarily comprised five bacterial phyla: Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria. Significant changes were observed in the proportion of the phylum Proteobacteria and Fusobacteria among the three groups of dogs: pre-feeding GI dogs, post-feeding GI dogs, and healthy dogs ($p<0.05$, Kruskal-Wallis test) (Table 6). Specifically, the proportion of the phylum Proteobacteria was significantly higher in pre-feeding GI dogs than in healthy dogs ($p<0.05$; Dunn's multiple comparison test), whereas the proportion of the phylum Fusobacteria was significantly lower in pre-feeding GI dogs than in healthy dogs ($p<0.05$; Dunn's multiple comparison test). The phyla Actinobacteria, Bacteroidetes, and Firmicutes did not show any significant changes.

Table 6. Relative proportions of bacterial phyla, class, order, family, and genus in the feces of 16 gastrointestinal disease (GI) dogs pre- and post-administration of the Gastrointestinal Biome (GB) diet, and in 7 healthy dogs post administration of the GB diet.

					Median % (min-max %)			
phylum	class	order	family	genus	pre GI dogs	post GI dogs	healthy dogs	p-value
Actinobacteriota					1.3(0.0-25.6)	4.0(0.0-34.7)	5.5(0.0-15.8)	0.3588
	Actinobacteria				0.1(0.0-4.9)	0.1(0.0-34.7)	0.0(0.0-0.4)	0.4555
		Actinomycetales			0.0(0.0-0.5)	0.0(0.0-1.1)	0.0(0.0-0.0)	0.2891
			Actinomycetaceae		0.0(0.0-0.5)	0.0(0.0-1.1)	0.0(0.0-0.0)	0.2891
				Actinomyces	0.0(0.0-0.5)	0.0(0.0-1.1)	0.0(0.0-0.0)	0.2891
		Bifidobacteriales			0.0(0.0-3.9)	0.0(0.0-34.6)	0.0(0.0-0.4)	0.7196
			Bifidobacteriaceae		0.0(0.0-3.9)	0.0(0.0-34.6)	0.0(0.0-0.4)	0.7196
				Bifidobacterium	0.0(0.0-3.9)	0.0(0.0-34.6)	0.0(0.0-0.4)	0.7196
	Coriobacteriia				0.7(0.0-24.5)	1.4(0.0-19.6)	5.4(0.0-15.4)	0.3616
		Coriobacteriales			0.7(0.0-24.5)	1.4(0.0-19.6)	5.4(0.0-15.4)	0.3616
			Coriobacteriaceae		0.4(0.0-24.5)	1.2(0.0-19.6)	5.1(0.0-13.7)	0.2742
				Collinsella	0.4(0.0-24.5)	1.2(0.0-19.6)	5.1(0.0-13.7)	0.2742
			Eggerthellaceae		0.0(0.0-0.5)	0.0(0.0-4.8)	0.0(0.0-1.7)	0.6238
				Slackia	0.0(0.0-0.3)	0.0(0.0-3.7)	0.0(0.0-1.3)	0.4805
Bacteroidota					26.0(0.0-49.7)	13.6(0.0-50.6)	25.2(16.6-29.2)	0.7591
	Bacteroidia				26.0(0.0-49.7)	13.6(0.0-50.6)	25.2(16.6-29.2)	0.7591
		Bacteroidales			26.0(0.0-49.7)	13.6(0.0-50.6)	25.2(16.6-29.2)	0.7591
			Bacteroidaceae		20.0(0.0-47.9)	4.7(0.0-43.4)	16.5(0.2-26.2)	0.3377
				Bacteroides	20.0(0.0-47.9)	4.7(0.0-43.4)	16.5(0.2-26.2)	0.3377
			Prevotellaceae		0.0(0.0-19.9)	0.0(0.0-39.8)	3.9(0.0-24.8)	0.2019
				Alloprevotella	0.0(0.0-0.0)	0.0(0.0-2.2)	0.0(0.0-0.0)	0.4874
				Prevotella	0.0(0.0-19.9)	0.0(0.0-39.8)	3.9(0.0-24.8)	0.2019
			Tannerellaceae		0.0(0.0-0.7)	0.0(0.0-8.9)	0.0(0.0-4.2)	0.3451
				Parabacteroides	0.0(0.0-0.7)	0.0(0.0-8.9)	0.0(0.0-4.2)	0.3451
Firmicutes					45.8(17.1-83.4)	51.3(15.1-89.0)	39.8(33.4-55.5)	0.6767
	Bacilli				14.3(0.3-64.7)	19.0(0.8-53.2)	4.2(.2-26.8)	0.1803
		Erysipelotrichales			1.4(0.1-9.9)	4.6(0.1-15.9)	4.1(0.2-11.5)	0.0611
			Erysipelatoclostridiaceae		0.6(0.0-9.7)	1.1(0.0-11.3)	0.8(0.0-11.0)	0.7585
				Catenibacterium	0.0(0.0-0.0)	0.0(0.0-8.4)	0.0(0.0-2.4)	0.3325
				Erysipelatoclostridium	0.6(0.0-9.7)	1.1(0.0-11.3)	0.7(0.0-11.0)	0.9757
			Erysipelotrichaceae		0.4(0.0-3.5)	2.2(0.1-13.0)*	1.6(0.2-4.1)	0.0137
				Allobaculum	0.0(0.0-1.5)	0.0(0.0-1.1)	0.0(0.0-0.0)	0.368
				Faecalitalea	0.0(0.0-3.1)	0.1(0.0-12.9)	1.5(0.0-4.1)	0.0731
				Holdemanella	0.0(0.0-0.7)	0.0(0.0-5.1)	0.0(0.0-0.7)	0.8333
				Solobacterium	0.0(0.0-0.0)	0.0(0.0-2.1)	0.0(0.0-1.1)	0.2133
				Turicibacter	0.0(0.0-0.2)	0.2(0.0-2.4)*	0.0(0.0-0.1)#	0.0107
		Lactobacillales			8.9(0.0-61.9)	12.0(0.3-48.2)	0.0(0.0-24.5)*#	0.017
			Enterococcaceae		0.9(0.0-23.4)	0.0(0.0-27.6)	0.0(0.0-0.5)	0.0878
				Enterococcus	0.9(0.0-23.4)	0.0(0.0-27.6)	0.0(0.0-0.5)	0.0878
			Lactobacillaceae		0.0(0.0-45.9)	0.0(0.0-45.4)	0.0(0.0-0.0)	0.8177
				Lactobacillus	0.0(0.0-45.9)	0.0(0.0-45.4)	0.0(0.0-0.0)	0.6278

			Pediococcus	0.0(0.0-1.6)	0.0(0.0-0.0)	0.0(0.0-0.0)	0.4874
		Leuconostocaceae		0.0(0.0-7.6)	0.0(0.0-0.0)	0.0(0.-0.0)	0.7983
			Weissella	0.0(0.0-7.6)	0.0(0.0-0.0)	0.0(0.-0.0)	0.4874
		Streptococcaceae		2.0(0.0-61.9)	2.6(0.0-33.9)	0.0(0.0-24.5)	0.0459
			Streptococcus	2.0(0.0-61.9)	2.6(0.0-33.9)	0.0(0.0-24.5)	0.0459
Clostridia				22.4(3.8-62.4)	18.5(3.6-81.6)	23.7(7.7-36.9)	0.9897
	Clostridiales			3.7(0.0-24.8)	0.7(0.0-36.4)	0.7(0.0-4.3)	0.4648
		Clostridiaceae		3.7(0.0-24.8)	0.7(0.0-36.4)	0.7(0.0-4.3)	0.4648
			Clostridium sensu stricto 1	3.7(0.0-24.8)	0.7(0.0-36.4)	0.7(0.0-4.3)	0.4648
	Lachnospirales			9.8(0.0-37.4)	8.6(0.4-48.5)	12.3(4.8-20.9)	0.5016
		Lachnospiraceae		9.8(0.0-37.4)	8.6(0.4-48.5)	12.3(4.8-20.9)	0.5016
			Blautia	2.9(0.0-7.3)	1.6(0.0-8.7)	3.5(1.4-9.2)	0.2166
			Cellulosilyticum	0.0(0.0-2.8)	0.0(0.0-0.3)	0.0(0.0-0.9)	0.5717
			Epulopiscium	0.0(0.0-3.2)	0.0(0.0-1.1)	0.0(0.0-0.1)	0.7414
			Howardella	0.0(0.0-0.0)	0.0(0.0-2.7)	0.0(0.0-0.0)	0.7983
			Lachnoclostridium	0.5(0.0-3.6)	0.2(0.0-23.9)	0.7(0.0-2.2)	0.826
			Roseburia	0.0(0.0-7.1)	0.0(0.0-8.9)	0.1(0.0-1.0)	0.0896
			Tyzzerella	0.5(0.0-32.8)	0.1(0.0-4.5)	0.0(0.0-2.6)	0.8845
	Oscillospirales			0.2(0.0-4.5)	0.8(0.0-8.9)	1.6(0.1-9.5)	0.0967
		Butyricicoccaceae		0.0(0.0-0.4)	0.0(0.0-3.9)	0.2(0.0-1.2)	0.1543
			Butyricicoccus	0.0(0.0-0.1)	0.0(0.0-0.7)	0.2(0.0-1.2)	0.0695
		Oscillospiraceae		0.0(0.0-1.3)	0.0(0.0-0.8)	0.0(0.0-7.6)	0.7716
			Colidextribacter	0.0(0.0-0.0)	0.0(0.0-0.2)	0.0(0.0-1.3)	0.4391
			Flavonifractor	0.0(0.0-1.3)	0.0(0.0-0.8)	0.0(0.0-7.6)	0.7941
		Ruminococcaceae		0.0(0.0-4.2)	0.2(0.0-8.9)	0.3(0.1-4.3)*	0.0457
			Faecalibacterium	0.0(0.0-0.4)	0.0(0.0-3.4)	0.0(0.0-1.3)	0.7872
			Fournierella	0.0(0.0-0.2)	0.0(0.0-0.0)	0.0(0.0-1.2)	0.0539
			Negativibacillus	0.0(0.0-0.0)	0.0(0.0-0.2)	0.0(0.0-2.2)	0.0539
			UBA1819	0.0(0.0-0.0)	0.0(0.0-8.9)	0.0(0.0-0.1)	0.1278
	Peptococcales			0.0(0.0-0.0)	0.0(0.0-0.4)	0.0(0.0-1.9)	0.1015
		Peptococcaceae		0.0(0.0-0.0)	0.0(0.0-0.4)	0.0(0.0-1.9)	0.1015
			Peptococcus	0.0(0.0-0.0)	0.0(0.0-0.4)	0.0(0.0-1.9)	0.1015
	Peptostreptococcales-Tissierellales			4.7(0.0-12.4)	2.1(0.3-40.7)	2.5(0.0-5.5)	0.3079
		Anaerovoracaceae		0.0(0.0-0.0)	0.0(0.0-0.0)	0.0(0.0-1.7)	0.8943
		Peptostreptococcaceae		0.0(0.0-5.0)	0.0(0.0-11.8)	0.0(0.0-0.0)	0.2276
			Clostridioides	0.0(0.0-3.4)	0.0(0.0-1.8)	0.0(0.0-1.2)	0.4119
			Paeniclostridium	0.0(0.0-1.4)	0.0(0.0-1.9)	0.0(0.0-0.0)	0.2008
			Peptoclostridium	0.0(00-5.2)	0.0(0.0-2.8)	0.8(0.0-5.5)	0.308
			Romboutsia	0.1(0.0-1.9)	0.2(0.0-3.7)	0.0(0.0-0.1)	0.0752
			Terrisporobacter	0.0(0.0-7.1)	0.0(0.0-35.1)	0.0(0.0-1.4)	0.4586
Negativicutes				0.0(0.0-5.5)	0.0(0.0-11.8)	16.8(2.6-27.8)*#	0.0011
	Acidaminococcales			0.0(0.0-4.2)	0.0(0.0-11.8)	0.0(0.0-2.6)	0.8231
		Acidaminococcaceae		0.0(0.0-4.2)	0.0(0.0-11.8)	0.0(0.0-2.6)	0.8231
			Phascolarctobacterium	0.0(0.0-4.2)	0.0(0.0-11.8)	0.0(0.0-2.6)	0.6937
	Veillonellales-Selenomonadales			0.0(0.0-5.5)	0.0(0.0-5.8)	16.8(0.1-27.8)*#	0.0003

	Selenomonadaceae	0.0(0.0-5.5)	0.0(0.0-5.8)	16.8(0.1-27.8)*#	< 0.0001
	Megamonas	0.0(0.0-5.5)	0.0(0.0-5.8)	16.8(0.1-27.8)*#	< 0.0001
	Veillonellaceae	0.0(0.0-0.0)	0.0(0.0-1.5)	0.0(0.0-2.7)	0.8251
	Dialister	0.0(0.0-0.0)	0.0(0.0-1.5)	0.0(0.0-2.4)	0.7339
Fusobacteriota		0.0(0.0-33.9)	0.9(0.0-37.6)	14.9(0.0-42.7)*	0.0142
Fusobacteriia		0.0(0.0-33.9)	0.9(0.0-37.6)	14.9(0.0-42.7)*	0.0142
Fusobacteriales		0.0(0.0-33.9)	0.9(0.0-37.6)	14.9(0.0-42.7)*	0.0142
	Fusobacteriaceae	0.0(0.0-33.9)	0.9(0.0-37.6)	14.9(0.0-42.7)*	0.0142
	Fusobacterium	0.0(0.0-33.9)	0.9(0.0-37.6)	14.9(0.0-42.7)*	0.0142
Proteobacteria		22.4(0.3-67.0)	9.8(0.5-70.2)	3.4(0.2-9.5)*	0.0483
Alphaproteobacteria		0.0(0.0-10.2)	0.0(0.0-0.1)	0.0(0.0-0.0)	0.2035
Gammaproteobacteria		22.4(0.3-67.0)	9.8(.5-70.2)	3.4(0.2-9.5)*	0.0483
Aeromonadales		0.0(0.0-0.0)	0.0(0.0-2.0)	0.0(0.0-7.4)	0.7339
	Succinivibrionaceae	0.0(0.0-0.0)	0.0(0.0-2.0)	0.0(0.0-7.4)	0.7339
	Anaerobiospirillum	0.0(0.0-0.0)	0.0(0.0-2.0)	0.0(0.0-7.4)	0.7304
Burkholderiales		0.1(0.0-3.4)	0.2(0.0-7.4)	2.1(0.1-6.2)*	0.0433
	Sutterellaceae	0.0(0.0-3.4)	0.1(0.0-7.0)	2.1(0.0-6.1)	0.1817
	Parasutterella	0.0(0.0-1.2)	0.0(0.0-7.0)	0.0(0.0-6.1)	0.7345
	Sutterella	0.0(0.0-3.4)	0.0(0.0-4.4)	0.4(0.0-3.4)	0.4807
Enterobacterales		22.3(0.0-67.0)	9.5(0.0-70.2)	0.0(0.0-6.9)*#	0.0012
	Enterobacteriaceae	22.3(0.0-65.7)	9.5(0.0-70.2)	0.0(0.0-6.6)*#	0.0017
	Escherichia-Shigella	21.5(0.0-65.7)	9.5(0.0-68.5)	0.0(0.0-6.6)*#	0.0027
	Klebsiella	0.0(0.0-7.1)	0.0(0.0-2.1)	0.0(0.0-0.0)	0.4475
	Morganellaceae	0.0(0.0-1.3)	0.0(0.0-1.4)	0.0(0.0-0.3)	0.6056
	Morganella	0.0(0.0-1.3)	0.0(0.0-1.2)	0.0(0.0-0.0)	0.5714
	Proteus	0.0(0.0-1.3)	0.0(0.0-0.0)	0.0(0.0-0.3)	0.8628
Pasteurellales		0.0(0.0-0.1)	0.0(0.0-1.7)	0.0(0.0-0.0)	0.7983
	Pasteurellaceae	0.0(0.0-0.1)	0.0(0.0-1.7)	0.0(0.0-0.0)	0.7983
	Pasteurella	0.0(0.0-0.1)	0.0(0.0-1.7)	0.0(0.0-0.0)	0.7983

* indicates significant difference ($p<0.05$; Dunn's multiple comparisons test) relative to the pre- GI dogs.
# indicates significant difference ($p<0.05$; Dunn's multiple comparisons test) relative to the post- GI dogs.

Within the class Bacillus, which includes the phylum Firmicutes, no significant changes were observed in the order Erysipelotrichales. However, within the order Erysipelotrichales in the family Erysipelotrichaceae, a significant increase was observed in post-feeding GI dogs compared to pre-feeding dogs ($p<0.05$; Dunn's multiple comparisons test), while a significant increase in the genus *Turicibacter* was further observed in post-feeding GI dogs compared to pre-feeding and healthy dogs ($p<0.05$; Dunn's multiple comparisons test). In the order Lactobacillales within the class Bacilli, a significant increase was observed in pre-feeding and post-feeding GI dogs compared to that in healthy dogs ($P<0.05$; Dunn's multiple comparisons test). The family Streptococcaceae and genus *Streptococcus* within the order Lactobacillales further showed an increase in pre-feeding and post-feeding GI dogs compared to healthy dogs ($p<0.05$; Kruskal-Wallis test). The class Clostridia comprises five orders: Clostridiales, Lachnospirales, Oscillospirales, Peptococcales, and Peptostreptococcales-Tissierellales. No significant differences were observed at the order level in this class. However, within the order Oscillospirales, the family Ruminococcaceae showed a significant increase in healthy dogs compared to pre-feeding GI dogs ($p<0.05$; Dunn's multiple comparison test). There was a significant increase in the Negativicutes-Veillonellales-Selenomonadales-Selenomonadaceae-*Megamonas,* belonging to the phylum Firmicutes, in healthy dogs compared to pre-feeding and post-feed-

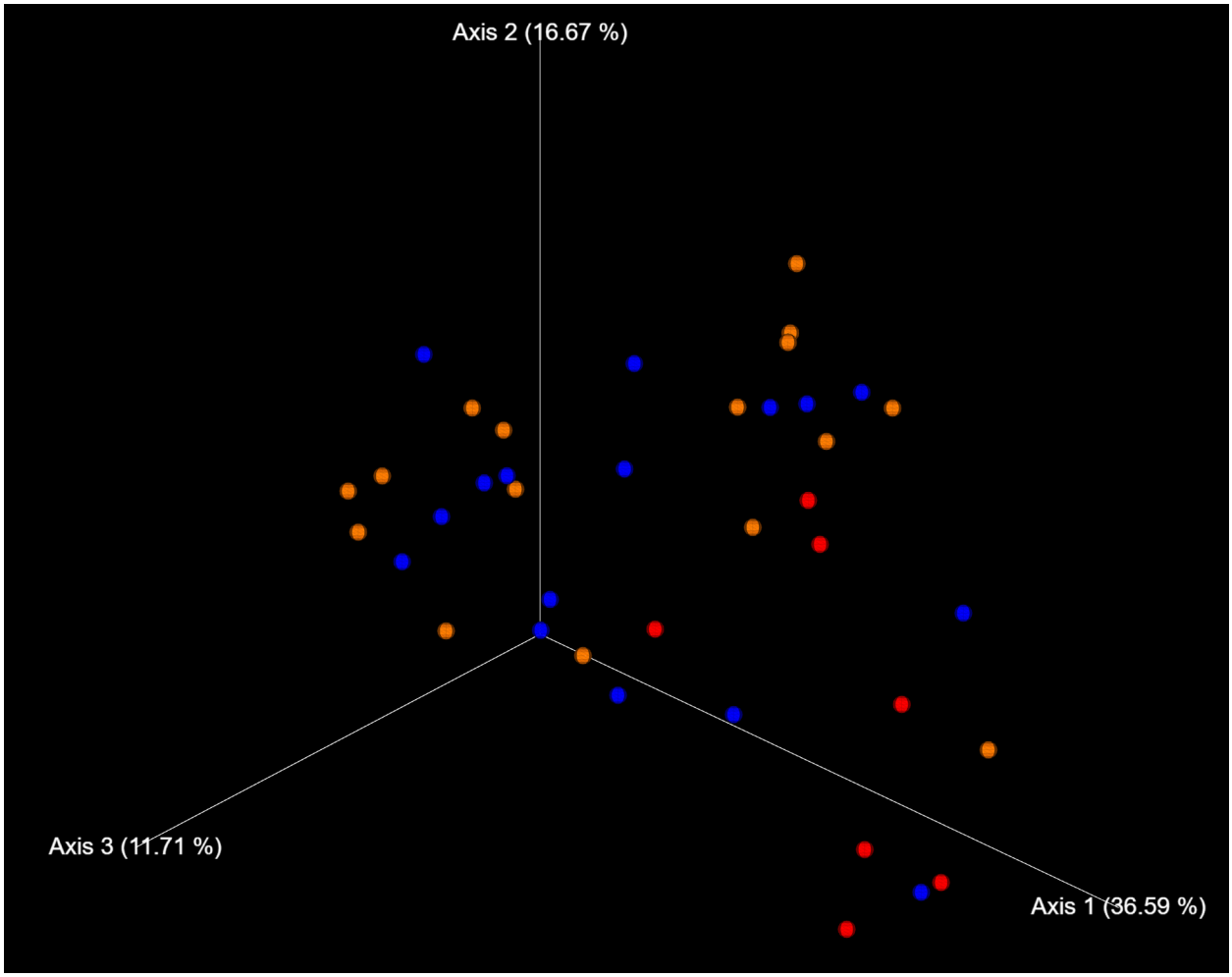

Fig. 1. Principal coordinate analysis of weighted UniFrac distance metrics of the 16S rRNA genes in 16 gastrointestinal disease dogs pre- (yellow) and post- (blue) administration of the Gastrointestinal Biome (GB) diet, and in 7 healthy dogs post-administration of the GB diet (red).

Table 7. Comparison of 16 gastrointestinal disease (GI) dogs pre- and post- administration of the Gastrointestinal Biome (GB) diet, and in 7 healthy dogs post administration of the GB diet on bacterial diversity indices.

	Shannon	Observed otus	pielou_e	faith_pd
pre GI dogs	3.78±0.75	61.3±38.5	0.65±0.09	6.93±3.17
post GI dogs	3.84±0.82	58.1±23.4	0.66±0.10	7.46±3.13
healthy dogs	4.37±1.00	68.7±38.9	0.73±0.08	7.14±1.56

ing GI dogs ($p<0.05$; Dunn's multiple comparisons test).

In the Fusobacteria-Fusobacteriia-Fusobacteriales--Fusobacteriaceae- *Fusobacterium*, significant increases were observed in healthy dogs compared to pre-feeding GI dogs ($p<0.05$; Dunn's multiple comparison test).

The phylum Proteobacteria comprises two classes: Alphaproteobacteria and Gammaproteobacteria. Within the phylum Gammaproteobacteria, significant differences were observed in the orders Burkholderiales, Enterobacterales-Enterobacteriaceae- *Escherichia--Shigella*, between the three groups ($p<0.05$; Kruskal-Wallis test). In the order Burkholderiales, a significant increase was observed in healthy dogs compared to pre-feeding GI dogs. In the Enterobacterales--Enterobacteriaceae-*Escherichia-Shigella*, significant increase was observed in pre-feeding and post-feeding GI dogs compared to healthy dogs.

The alpha diversity analysis, which evaluates species richness, did not reveal any significant changes in the bacterial diversity index among the three groups (Table 7). In the PCoA results, which assessed the similarity of the bacterial flora among individuals, the healthy dog population exhibited a cohesive distribution (Fig. 1). In contrast, the GI dog population was more scattered in the plot post-feeding than in the

pre-feeding plot. Furthermore, the populations of the GI dogs post-feeding tended to approach those of the healthy dogs.

Discussion

Our study aimed to evaluate the influence of a GB diet enriched with soluble fiber on the gut microbiome in dogs with GI disorders. Although dietary therapy is a common approach in the management of gastrointestinal diseases in canines, there is currently a paucity of research on the impact of therapeutic diets on the gut microbiota and the corresponding clinical signs in dogs. Our study hypothesized that the administration of a GB diet could precipitate alterations in the gut microbiota composition, which may, in turn, contribute to the amelioration of clinical signs.

In GI dogs, the questionnaires indicated a significant improvement in the severity of CIBDAI at 4 and 8 weeks post-feeding the GB diet compared to the pre-feeding period. The most favorable values were observed at the 8-week mark, suggesting that continuous feeding with the GB diet promoted further improvement in clinical signs. Significant improvements in fecal conditions were observed at 4- and 8-weeks post-feeding compared to pre-feeding. The GB diet, which included soluble fiber, not only improved the fecal condition, but also potentially alleviated inflammation in the intestine. However, the mean CIBDAI score in our cases was 3.1, indicating that most of the enrolled dogs had clinically insignificant disease and mild inflammatory bowel disease (IBD), according to previous studies (Jergens et al. 2003). The patients in the current study were selected based on their medical history and current clinical signs indicating a predisposition to colitis and the necessity for a high-fiber diet, as determined by veterinarians. Therefore, future research should focus solely on cases with CIBDAI scores > 4, which clearly indicate the presence of chronic enteropathy.

Focusing on the gut microbiota in the family Erysipelotrichaceae, specifically the genus *Turicibacter* within the phylum Firmicutes, a significant increase was observed in GI dogs post-feeding compared with pre-feeding. The family Erysipelotrichaceae was also positively correlated with protein digestibility in canines, as documented in previous studies (Bermingham et al. 2017, Coelho et al. 2018). The protein content of the GB diet utilized in this study was 20.9% of a dry matter basis, aligning with the protein levels typically found in gastrointestinal therapeutic diets. Nevertheless, the GB diet is characterized by its highly digestible protein content. Given that low protein digestibility increases the risk of food hypersensitivity (Wang et al. 2022), enhanced protein digestibility may contribute to the observed increase in the Erysipelotrichaceae family in dogs with GI. Additionally, the genus *Turicibacter* has been included in the dysbiosis index due to its decreased abundance in dogs with chronic enteropathy (AlShawaqfeh et al. 2017). Consequently, the effectiveness of the GB diet in augmenting *Turicibacter* populations suggests its potential to ameliorate gastrointestinal inflammation in GI dogs.

The family Streptococcaceae and genus *Streptococcus* within the order Lactobacillales showed an increase in pre- and post-feeding GI dogs compared to healthy dogs. Given the observed increase in *Streptococcus* abundance in dogs diagnosed with inflammatory bowel disease (IBD) (AlShawaqfeh et al. 2017), quantitative analysis of the *Streptococcus* population was incorporated into the dysbiosis index assessment (AlShawaqfeh et al. 2017). Consequently, the observed decrease in *Streptococcus* levels in healthy canines suggests a correlation with lower instances of intestinal inflammation. Conversely, the absence of significant alterations in *Streptococcus* populations pre- and post--administration of a GB diet in dogs with GI indicates a negligible dietary impact on this specific bacterial group.

The family Ruminococcaceae exhibited a significant increase in abundance in healthy dogs compared to pre-feeding GI dogs. This family encompasses the genera *Butyricicoccus*, *Faecalibacterium*, and *Ruminococcus*, all of which produce short-chain fatty acids (SCFAs). SCFAs are pivotal in promoting anti-inflammatory effects within the intestine, mainly through the activation of regulatory T cells (Atarashi et al. 2013). The elevated levels of Ruminococcaceae in healthy dogs relative to pre-feeding GI dogs likely mirror the intrinsic immunological mechanisms operating within the gut of healthy dogs.

Significant increases in abundance of Fusobacteria--Fusobacteriia-Fusobacteriales-Fusobacteriaceae--*Fusobacterium* were observed in healthy dogs compared to pre-fed GI dogs. Previous studies have reported that diets high in fiber lead to an increased abundance of Fusobacterium (Mori et al. 2019). The GB diet, characterized by the presence of both soluble and insoluble fiber, likely contributes to the enhanced prevalence of Fusobacteria in healthy dogs. In humans, an elevated proportion of *Fusobacterium spp.* is associated with inflammatory bowel disease (IBD) (Allen--Vercoe et al. 2011, Tahara et al. 2014), colorectal cancer (Kostic et al. 2012), and ulcerative colitis (Ohkusa et al. 2002). Similarly, in canine populations, increased proportions of Fusobacterium have been documented in cases of acute hemorrhagic diarrhea, and in miniature

dachshunds with active inflammatory colorectal polyps (Suchodolski et al. 2012, Igarashi et al. 2016). In contrast, decreased Fusobacterium levels have been observed in dogs diagnosed with IBD (AlShawaqfeh et al. 2017). Consequently, the inverse correlation between the abundance of fusobacteria and gastrointestinal disease in dogs warrants further investigation.

A significant increase in Proteobacteria-Gammaproteobacteria-Enterobacterales-Enterobacteriaceae--*Escherichia-Shigella* was also observed in GI dogs compared to healthy dogs, both pre- and post-feeding of the GB diet. Following the feeding of the GB diet to GI dogs, a trend towards normalization was noted, with microbial levels approaching those observed in healthy canines. In dogs with Canine Idiopathic Enteropathy (CIE), alterations in fecal microbiota are characterized by an increased abundance of Proteobacteria, particularly Enterobacteriaceae (Minamoto et al. 2015). Cassmann et al. (2016) further documented variations in mucosal bacteria within the ileum and colon of dogs with CIE, revealing elevated levels of bacteria from the *Clostridium coccoides*/*Eubacterium rectale* group, *Bacteroides* spp., Enterobacteriaceae, and *E. coli* in comparison to control dogs. Furthermore, Giaretta et al. (2020) observed a higher incidence of *Escherichia coli/Shigella* spp. on the colonic surface and within crypts in dogs with CIE than in control dogs. Additionally, granulomatous colitis in dogs has been linked to infection of the colonic mucosa by *Escherichia coli* (Ishii et al. 2022, Smith et al. 2024). Consequently, the diminished proportion of proteobacteria following the administration of a GB diet in both healthy and GI dogs suggests a positive influence on host health.

In conclusion, this study found that the administration of a GB diet to dogs with GI resulted in clinical improvement and a significant reduction in CIBDAI scores. Additionally, PCoA plots indicated that the gut microbiota composition of GI dogs post-feeding the GB diet approached that of healthy dogs. Primary changes in the gut microbiome were marked by significant differences between healthy and GI dogs. However, post-feeding with the GB diet, resulted in an increase in *Turicibacter* and a decrease in *Escherichia-Shigella*. The GB diet may contribute to the amelioration of clinical signs through the modulation of this specific bacterial population.

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