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Comparative Effects of Coffee Pulp and Citrus Pectins on Safety, Nutrient Digestibility, and Fecal Microbiota in Adult Dogs

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Simple Summary

Coffee pulp is a by-product of coffee processing and a promising sustainable source of dietary pectin. This study evaluated the tolerability and effects of coffee pulp pectin on nutrient digestibility and gut microbiota in healthy dogs compared with conventional citrus pectin. Thirty-two healthy dogs were fed diets supplemented with citrus pectin or coffee pulp pectin for 28 days. Neither supplement showed apparent adverse effects on feed intake, nutrient digestibility, body condition score, fecal characteristics, or health-related blood parameters. Although overall microbial diversity remained stable, both pectin sources selectively influenced specific bacterial groups. *Escherichia–Shigella* was identified by LEfSe as a bacterial marker in the coffee pulp pectin group; however, its higher abundance at baseline suggests pre-existing differences rather than a supplementation effect. These findings suggest that coffee pulp pectin was well tolerated by healthy adult dogs during the 28-day feeding period and may serve as a sustainable alternative source of dietary pectin with potential to influence the canine gut microbiota.

Abstract

Coffee pulp, a major by-product of coffee production, is a novel and sustainable source of dietary pectin. This study evaluated the safety of coffee pulp pectin (CP) compared with citrus pectin (CT) based on clinical parameters, nutrient digestibility, and fecal microbiota responses in healthy adult dogs. Thirty-two dogs were randomly assigned to four dietary treatments for 28 days: control, 1%CT, 2%CT, or 2%CP ($n = 8$/group). No adverse effects were observed following CP or CT supplementation ($p > 0.05$). Alpha diversity remained unchanged, whereas beta diversity was significantly influenced by treatment ($R^2 = 0.049$, $p = 0.006$) and sampling time ($R^2 = 0.066$, $p = 0.001$). Selected bacterial taxa showed source-dependent differences. LEfSe identified *Escherichia–Shigella* as a bacterial biomarker in the coffee pulp pectin group, with no clinical abnormalities observed; however, its higher abundance at baseline suggests pre-existing differences rather than a supplementation effect. *Bifidobacterium* was identified as a biomarker in the 1%CT group and at day 14 in the 2%CT group. Despite distinct microbial responses compared with citrus pectin, CP was well tolerated without substantial changes in alpha diversity. These findings support coffee pulp as a promising novel and sustainable source of dietary pectin for canine nutrition.

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1. Introduction

The gastrointestinal (GI) tract plays an important role in canine health through its involvement in nutrient utilization, immune function, and host–microbe interaction. Central to these functions is the gut microbiota, a complex microbial ecosystem that interacts closely with the host to regulate digestive, immune, and inflammatory responses [1,2]. Consequently, prebiotic supplementation has gained increasing attention in companion animal nutrition as a dietary strategy to promote gastrointestinal health [1,3]. In parallel, the growing trend of pet humanization has increased demand for functional and sustainable pet food ingredients [4,5]. Together, these factors have stimulated interest in exploring novel dietary fiber sources with prebiotic potential to support gut microbial balance, intestinal barrier integrity, and overall health.

The physiological effects of dietary fiber are largely determined by its physicochemical characteristics, including solubility, viscosity, and fermentability [6]. Pectin is one of the fiber sources and is recognized for its prebiotic potential [7]. It is a complex heteropolysaccharide composed primarily of homogalacturonan (HG; ~65%), rhamnogalacturonan I (RG-I; 20–30%), and rhamnogalacturonan II (RG-II; 2–10%) domains, with structural characteristics varying according to plant origin and degree of esterification (DE) [7,8]. Based on DE, pectin is generally classified as high-methoxyl pectin (HMP; DE > 50%) or low-methoxyl pectin (LMP; DE < 50%). LMP is considered more susceptible to microbial fermentation, making it particularly suitable for prebiotic applications [9,10]. Commercial pectin is primarily derived from citrus peels (85.5%) and apple pomace (14.0%), and its DE may vary depending on the source and processing conditions [8,11]. Previous studies have demonstrated that citrus pectin supplementation enhances gut fermentation, increases fecal moisture and output, and promotes short-chain fatty acid (SCFA) production, particularly butyrate [12–16]. It has also been shown to stimulate beneficial bacteria such as *Bifidobacterium* spp. In contrast, apple pectin has been associated with enrichment of *Faecalibacterium* spp. and *Parabacteroides* spp., alongside reductions in potentially harmful bacteria such as *Clostridium perfringens*. However, pectin supplementation may slightly reduce the digestibility of crude protein, ether extract, and organic matter by approximately 3% on an as-fed basis [13]. Therefore, it is important to balance the functional benefits with nutritional outcomes.

Alongside the growing interest in functional dietary fibers, agricultural by-products have gained increasing attention as sustainable sources of dietary ingredients, offering opportunities for waste valorization and resource efficiency [17]. Agricultural production generates substantial quantities of waste annually, with approximately 1.05 billion tons of food wasted worldwide [18]. Many of these by-products remain rich in dietary fiber and bioactive compounds, providing opportunities as functional feed ingredients. Fruit processing residues (e.g., apple pomace, orange peel, and mango peel) have been widely used as pectin sources with prebiotic potential [19].

Among these agricultural by-products, coffee-processing residues have attracted considerable attention as an alternative pectin source. Coffee production generates substantial waste streams, accounting for nearly 60% of the coffee fruit [20–23]. Coffee pulp, one of the most abundant fractions (approximately 9.4 million tons annually), is a rich source of pectin and has also been reported to contain bioactive compounds, including caffeine, polyphenols, and chlorogenic acids, which may contribute to prebiotic and antioxidant activities [24,25]. These characteristics suggest that coffee pulp pectin may represent a sustainable functional

ingredient for supporting gut health. However, the prebiotic potential of coffee pulp pectin in dogs remains largely unexplored.

The objective of this study was to compare the effects of coffee pulp pectin and commercial citrus pectin on safety, nutrient digestibility, and fecal microbiota composition in healthy adult dogs. We hypothesized that coffee pulp pectin would be safe and well tolerated while promoting gut microbiota modulation, thereby supporting its potential use as a sustainable alternative pectin source in canine nutrition.

2. Materials and Methods

2.1. Diets and Pectin Preparation

The dietary composition of the basal diet (Ole dog food liver flavor®; Greatest Pet Care Co., Ltd., Bangkok, Thailand) contained beef and bone meal, corn, soybean meal, cassava, defatted rice bran, broken rice, tuna oil, chicken fat, flavoring agents, choline chloride, vitamins, minerals, preservatives, and yucca. The proximate composition included 20% crude protein, 8.9% crude fat, and 6.7% crude fiber on a dry matter basis (DM), with a metabolizable energy content of 3360 kilocalories per kilogram (kcal/kg) DM.

Citrus pectin (CT; CAS No. 9000-69-5; Thermo Fisher Scientific®, Waltham, MA, USA) was commercially obtained. The coffee pulp pectin (CP) used in this study was supplied by the holder of Thai Petty Patent No. 20718 and was prepared according to the method described in the Patent. Briefly, coffee pulp from wet-processed coffee cherries was frozen at −20 °C and treated with sodium sulfite solution (0.5–2%, *w*/*v*) for 2–24 h to prevent browning. Pectin was extracted using citric acid (0.1–1.0 M) at pH 1.0–3.5, followed by homogenization at 5000–10,000 rpm for 20–30 s. The mixture was heated at 80–100 °C for 90–300 min and filtered through a nylon filter (0.45–1.0 µm) to obtain the first liquid extract. The remaining solid residue was subjected to a second extraction using sodium hydroxide solution (0.1–1.0%, *w*/*v*) for 30–90 min. The two liquid extracts were combined at a 1:1 ratio, and the pH was adjusted to 7.0. Pectin was precipitated by adding 85–100% ethanol (1:1–1:3, *v*/*v*) and refrigerating the mixture for 12–24 h. The precipitate was filtered, dried at 60 °C for 12–24 h, and ground into a light brown powder with a mild coffee odor. The degree of esterification (DE) was 6.7% for CT and 33% for CP, with CP containing 2.5% caffeine (*w*/*w*). The extraction conditions described above represent the ranges specified in the patent, as the exact processing parameters used for the supplied batch were not available.

2.2. Animals, Study Design, and Feeding

Thirty-two healthy, mixed-breed dogs (21 females, 11 males), aged 2–4 years and weighing 15–26 kg (mean ± SD: 17.51 ± 2.64 kg), with a body condition score (BCS) of 3–6/9 (3.34 ± 0.69), were randomly selected from a population of 50 experimental dogs. The dogs were individually housed in an open-housing system in a completely randomized design with *ad libitum* access to water. None had received any biotics supplements (antibiotics, prebiotics, probiotics, or postbiotics) within the three months before the study. To minimize potential confounding effects, all dogs were fed the same complete and balanced commercial diet once daily at 3:00 PM for 30 days before the experiment and throughout the 28-day intervention period. The daily food allowance was calculated according to each dog's daily energy requirement (DER), estimated as 1.4 times the resting energy requirement (RER = 70 × current body weight$^{0.75}$) [26]. Daily feed intake was monitored by recording the amount of food offered and leftover. The feeding bowls were also visually checked by a researcher after each meal to assess any remaining supplement residue.

Dogs were randomly allocated to four groups (n = 8 per group): control, 1% citrus pectin (1%CT), 2% citrus pectin (2%CT), and 2% coffee pulp pectin (2%CP). Pectin amounts were calculated at 1% or 2% of each dog's daily food allowance (as-fed basis), with meat

and bone meal added as a filler to achieve a uniform total supplement weight of 10 g per dog. For example, a dog receiving 286 g of basal diet was given 2.86 g of pectin and 7.14 g of filler in the 1% group. The control group received 10 g of meat and bone meal as a placebo. All supplements were mixed with the basal diet and offered once daily.

All experimental procedures were approved by the Kasetsart University Institute Animal Care and Use Committee (ACKU66-VET-023).

2.3. Sample Collection, Clinical Evaluation, and Laboratory Analysis

Physical examinations, body weight (BW), BCS, and fecal score (FS) were recorded on days 0 (baseline), 14, and 28, while daily feed intake was monitored throughout the study. To evaluate the safety of pectin supplementation, 3 mL of blood was collected from the cephalic vein on each sampling day and divided into an ethylene diamine tetraacetic acid (EDTA) tube for hematological analysis and a plain serum tube for serum biochemistry analysis. Hematological parameters included red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), white blood cell count (WBC), and differential counts of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), and basophils (BASO). Serum biochemistry parameters included blood urea nitrogen (BUN), creatinine (CREA), total protein (TP), albumin (ALB), globulin (GLOB), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). All analyses were performed at the Veterinary Diagnostic Laboratory, Kasetsart University Veterinary Teaching Hospital, Bangkok, Thailand.

For digestibility assessment, all feces were quantitatively collected and weighed daily for each dog from experimental days 23 to 28. Additionally, fecal samples collected on days 0, 14, and 29 were used for fecal moisture determination and bacterial microbiota analyses. The day 29 sample was collected at 10:00 AM, prior to feeding, and was considered representative of the final day (day 28) of the dietary intervention. All fecal samples were stored in double-layered polyethylene bags and kept at −20 °C to prevent moisture loss. Proximate analysis was performed according to the Association of Official Analytical Chemists (AOAC) methods [27] to determine moisture (AOAC 930.15), crude protein (AOAC 984.13), ether extract (AOAC 2003.05), and crude ash (AOAC 942.05). The resulting data were used to calculate the apparent total tract digestibility of dry matter and nutrients, with nutrient intake calculated based on the basal diet, pectin supplement, and meat and bone meal filler consumed by each dog.

2.4. Fecal Microbiota Analysis

Fecal DNA was extracted using the QIAamp® DNA Stool Kit (Qiagen GmbH, Hilden, Germany) as described by Sathitkowitchai et al. [28] protocol. The fecal microbiome was analyzed by targeting the V3–V4 region of the 16S rRNA gene. Amplicon sequencing was performed using the Illumina MiSeq platform (Illumina, San Diego, CA, USA). Sequence data were clustered into Operational Taxonomic Units (OTUs) at 99% similarity and then annotated against the SILVA 138 99% OTU database with QIIME2's naïve Bayes classifier (v2021.8) with a 70% confidence cutoff.

2.5. Statistical Analysis

The characteristics of each dog are summarized using descriptive statistics. Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Feed intake and apparent total tract digestibility of dry matter and nutrients were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple

range test for a post hoc comparison. Body condition score, fecal score, body weight, fecal moisture, and hematological and blood biochemical parameters were analyzed using two-way mixed ANOVA, with treatment group as the between-subject factor and collection time as the within-subject factor. Bonferroni and Duncan's multiple range tests were used as post hoc comparisons for within-subject and between-subject factors, respectively.

The relative abundances of microbial taxa at the phylum and genus levels were analyzed using two-way ANOVA following centered log-ratio (CLR) transformation, with treatment group as the between-subject factor and sampling time as the within-subject factor. Post hoc comparisons were performed using Duncan's multiple range test. The false discovery rate (FDR) was controlled using the Benjamini–Hochberg procedure. Analyses focused on the 15 most abundant genera and four additional genera of interest. Alpha diversity indices, including Chao1, Shannon–Weaver, and Simpson's indices, were calculated using the vegan package (version 2.5.6), and a heatmap was generated using the pheatmap package (version 1.0.12). Beta diversity was assessed using the vegan and permute packages. Differences in microbial community composition were evaluated using Bray–Curtis dissimilarity and visualized by non-metric multidimensional scaling (NMDS). The goodness-of-fit of the NMDS ordination was assessed using the stress value. Permutational multivariate analysis of variance (PERMANOVA) was performed using the adonis2 function to evaluate the effects of treatment group, sampling time, and their interaction on microbial community composition. To account for repeated measurements within individual dogs, permutations were restricted within animals (blocked by Animal_ID), with 999 permutations performed. Differentially abundant taxa among treatment groups were identified at the genus level using linear discriminant analysis effect size (LEfSe) with the lefser package (version 1.18.0). Taxa with linear discriminant analysis (LDA) scores greater than 2 were considered to have relatively large discriminatory effect sizes.

All statistical analyses and visualizations were performed using R statistical software (version 4.4.1) within the RStudio IDE (version 2024.12.0+467), with the Rcmdr (version 2.9-5) and ggplot2 (version 3.5.2) packages. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Feed Intake, Body Weight, Body Condition Score, Fecal Score, Fecal Moisture, and Apparent Total Tract Digestibility

All dogs completed the study, and the supplemented diets were generally well accepted, with overall feed intake ranging from 94.30% to 99.10% of DER across treatment groups. No adverse effects were observed during the physical examinations. The estimated daily caffeine exposure in the 2%CP group was 8.58 ± 0.43 mg/kg BW/day (mean ± SD). Body weight, body condition score, fecal score, fecal moisture, and feed intake did not differ significantly among the treatment groups ($p > 0.05$). However, fecal moisture was significantly higher on day 14 than on the other sampling days ($p < 0.05$; Table 1). Similarly, the apparent total tract digestibility of dry matter, organic matter, crude protein, and ether extract did not differ significantly among the groups ($p > 0.05$), as shown in Table 2.

Table 1. Body weight, body condition score, fecal score, fecal moisture, and feed intake of dogs fed different types and dosages of pectin over the experimental period.

Parameters	Groups												SEM [1]	*p*		
	Control			1% Citrus Pectin			2% Citrus Pectin			2% Coffee Pulp Pectin				Group	Time	Group × Time
	Time (Days)			Time (Days)			Time (Days)			Time (Days)						
	0	14	28	0	14	28	0	14	28	0	14	28				
BW (kg)	18.0	18.1	18.0	16.6	17.2	16.7	18.0	17.7	17.2	16.8	16.6	16.8	0.278	0.752	0.363	0.330
BCS [2]	3.25	3.25	3.25	3.13	3.13	3.13	3.38	3.38	3.38	3.63	3.63	3.63	0.071	-	-	-
FS [3]	2.00	2.00	2.00	2.13	2.13	2.00	2.25	2.13	2.00	2.00	2.00	2.00	0.025	0.438	0.175	0.625
Fecal moisture	66.0 b	71.3 a	67.9 ab	65.1 b	68.7 a	70.5 ab	69.9 b	71.2 a	71.5 ab	69.3 b	72.6 ab	69.5 ab	0.387	0.307	0.002	0.154
Feed Intake (%DER) [4]																
Day 1–14		92.89			92.42			98.47			95.63		1.504	0.479	-	-
Day 15–28		95.71			97.73			99.73			96.98		0.884	0.457	-	-
Day 1–28		94.30			95.08			99.10			96.31		0.882	0.237	-	-

[1] Standard error of the mean. [2] BCS body condition score; scored using the 9-point Body Condition Score System for Dogs [29]. [3] Fecal score; scored using the 7-point Purina Fecal Scoring System for Dogs and Cats (Nestlé-Purina Pet Food Co., St. Louis, MO, USA). [4] Daily energy requirement. [a,b] Values within the same row with different superscript letters are significantly different ($p < 0.05$); values sharing a common superscript letter are not significantly different.

Table 2. Apparent total tract digestibility of dry matter and nutrients of dogs fed pectin at different dosages and types.

Apparent Digestibility (%)	Groups				SEM [1]	*p*
	Control	Citrus Pectin		Coffee Pulp Pectin		
		1% [2]	2% [2]	2% [2]		
Dry matter	66.5	63.7	68.1	66.6	1.162	0.615
Organic matter	71.0	68.9	72.3	70.8	0.993	0.705
Crude protein	69.6	65.8	69.9	68.2	1.145	0.598
Ether extract	80.2	79.2	78.8	77.0	1.684	0.933

[1] Pooled standard error of mean. [2] Supplementation level based on daily intake.

3.2. Hematology and Blood Chemistry

Hematological and serum biochemical parameters were not significantly affected by treatment or group × time interactions ($p > 0.05$). However, significant changes over time were observed in several parameters (Table 3). Most values remained within their respective reference ranges. Throughout the study, EOS and RDW remained above the reference ranges, whereas PLT remained below its reference range, with platelet clumping observed on blood smears. RBC and MCH values exceeded their respective upper reference limits on days 14 and 28, while ALB exceeded its upper reference limit on day 14. The MCHC was above the upper reference limit on day 0 but decreased significantly by day 14 ($p < 0.05$), reaching values within the reference range. These changes were associated with sampling time, with no significant treatment-group effects observed. No clinical abnormalities were observed in any dog during the study.

Table 3. Hematological and blood biochemical parameters of dogs fed different types and dosages of pectin over the experimental period.

Parameters [1]	Groups												SEM [2]	*p*			Reference Range [3]
	Control			1% Citrus Pectin			2% Citrus Pectin			2% Coffee Pulp Pectin				Group	Time	Group × Time	
	Time (Days)			Time (Days)			Time (Days)			Time (Days)							
	0	14	28	0	14	28	0	14	28	0	14	28					
WBC (10^9/L)	13.3 ^{a}	13.5 ^{a}	11.8 ^{ab}	11.4 ^{a}	12.06 ^{a}	11.2 ^{ab}	13.0 ^{a}	13.1 ^{a}	12.0 ^{ab}	11.3 ^{a}	11.4 ^{a}	11.3 ^{ab}	0.263	0.434	0.043	0.703	5.00–14.100
NEU (10^9/L)	7.92 ^{a}	8.31 ^{a}	6.79 ^{b}	7.00 ^{a}	7.36 ^{a}	6.72 ^{b}	8.49 ^{a}	8.73 ^{a}	7.29 ^{b}	6.98 ^{a}	7.08 ^{a}	6.81 ^{b}	0.200	0.471	0.002	0.557	2.90–12.00
LYM (10^9/L)	2.88	2.97	3.04	2.47	2.57	2.54	2.54	2.38	2.59	2.40	2.52	2.55	0.097	0.707	0.391	0.860	0.40–2.90
MON (10^9/L)	0.54	0.60	0.54	0.44	0.50	0.49	0.48	0.49	0.51	0.41	0.43	0.41	0.015	0.115	0.912	0.818	0.10–1.40
EOS (10^9/L)	1.90	1.63	1.47	1.44	1.61	1.46	1.50	1.49	1.63	1.43	1.40	1.55	0.059	0.865	0.895	0.278	0.00–1.30
BASO (10^9/L)	0.02 ^{a}	0.02 ^{b}	0.01 ^{b}	0.02 ^{a}	0.02 ^{b}	0.02 ^{b}	0.03 ^{a}	0.02 ^{b}	0.02 ^{b}	0.03 ^{a}	0.02 ^{b}	0.02 ^{b}	0.001	0.512	0.002	0.665	0.00–0.14
RBC (10^{12}/L)	7.33 ^{b}	8.03 ^{a}	7.96 ^{a}	7.63 ^{b}	7.92 ^{a}	7.71 ^{a}	7.29 ^{b}	7.53 ^{a}	7.58 ^{a}	7.86 ^{b}	7.97 ^{a}	8.21 ^{a}	0.097	0.685	0.003	0.346	4.95–7.87
HCT (%)	48.6	50.9	50.8	50.2	50.2	50.0	48.3	48.5	49.0	50.4	50.4	52.0	0.522	0.806	0.301	0.738	35.00–57.00
HGB (g/dL)	15.3 ^{b}	16.8 ^{a}	16.9 ^{a}	16.5 ^{b}	17.1 ^{a}	16.8 ^{a}	15.5 ^{b}	16.0 ^{a}	16.2 ^{a}	16.5 ^{b}	16.8 ^{a}	17.4 ^{a}	0.183	0.611	<0.001	0.224	11.90–18.90
RDW (fL)	34.9 ^{a}	33.1 ^{b}	32.8 ^{b}	34.2 ^{a}	30.7 ^{b}	31.5 ^{b}	34.1 ^{a}	31.9 ^{b}	31.8 ^{b}	32.3 ^{a}	31.9 ^{b}	32.0 ^{b}	0.338	0.744	<0.001	0.108	9.10–19.4
MCV (fL)	64.3 ^{a}	63.5 ^{b}	63.8 ^{ab}	65.9 ^{a}	63.5 ^{b}	65.1 ^{ab}	66.5 ^{a}	64.9 ^{b}	65.1 ^{ab}	64.3 ^{a}	63.6 ^{b}	63.6 ^{ab}	0.351	0.706	0.002	0.448	60.00–77.0
MCH (pg)	20.9 ^{b}	32.9 ^{a}	33.3 ^{a}	21.7 ^{b}	34.1 ^{a}	33.6 ^{a}	21.7 ^{b}	33.1 ^{a}	33.1 ^{a}	21.1 ^{b}	33.3 ^{a}	33.5 ^{a}	0.597	0.470	<0.001	0.539	21.20–25.90
MCHC (g/dL)	32.4 ^{a}	20.9 ^{c}	21.3 ^{b}	33.0 ^{a}	21.7 ^{c}	21.9 ^{b}	32.3 ^{a}	21.5 ^{c}	21.5 ^{b}	32.8 ^{a}	21.2 ^{c}	21.3 ^{b}	0.560	0.795	<0.001	0.695	21.00–26.20
PLT (109/L)	137 ^{b}	192 ^{a}	174 ^{b}	154 ^{b}	233 ^{a}	190 ^{b}	109 ^{b}	194 ^{a}	128 ^{b}	130 ^{b}	207 ^{a}	99 ^{b}	9.282	0.423	<0.001	0.612	211.00–621.00
MPV (fL)	11.0 ^{a}	10.4 ^{b}	10.8 ^{b}	10.7 ^{a}	9.83 ^{b}	10.1 ^{b}	11.15 ^{a}	10.65 ^{b}	10.79 ^{b}	10.8 ^{a}	10.5 ^{b}	10.5 ^{b}	0.104	0.555	<0.001	0.597	8.70–13.2
TP (g/dL)	6.13 ^{c}	6.59 ^{a}	6.43 ^{b}	6.29 ^{c}	6.69 ^{a}	6.68 ^{b}	6.38 ^{c}	6.60 ^{a}	6.55 ^{b}	6.10 ^{c}	6.55 ^{a}	6.26 ^{b}	0.056	0.750	<0.001	0.496	5.40–7.50
ALB (g/dL)	2.95 ^{b}	3.11 ^{a}	3.10 ^{a}	3.05 ^{b}	3.11 ^{a}	3.08 ^{a}	3.01 ^{b}	3.09 ^{a}	3.09 ^{a}	3.01 ^{b}	3.15 ^{a}	3.08 ^{a}	0.023	0.993	<0.001	0.426	2.30–3.10
GLO (g/dL)	3.18 ^{c}	3.48 ^{a}	3.33 ^{b}	3.24 ^{c}	3.58 ^{a}	3.60 ^{b}	3.36 ^{c}	3.51 ^{a}	3.46 ^{b}	3.09 ^{c}	3.40 ^{a}	3.19 ^{b}	0.065	0.854	<0.001	0.284	2.70–4.40
BUN (mg/dL)	11.9 ^{a}	11.6 ^{b}	13.4 ^{a}	12.5 ^{a}	11.1 ^{b}	13.9 ^{a}	14.3 ^{a}	11.3 ^{b}	13.5 ^{a}	13.9 ^{a}	11.6 ^{b}	12.3 ^{a}	0.314	0.958	0.001	0.165	8.00–28.00
CREA (mg/dL)	1.26 ^{a}	0.91 ^{b}	1.03 ^{c}	1.35 ^{a}	0.97 ^{b}	1.05 ^{c}	1.19 ^{a}	0.90 ^{b}	0.91 ^{c}	1.19 ^{a}	0.92 ^{b}	0.99 ^{c}	0.022	0.625	<0.001	0.173	0.50–1.70
ALT (U/L)	30.1	29.8	41.5	32.0	26.5	25.9	28.4	23.3	22.9	25.6	25.3	23.5	1.469	0.396	0.436	0.212	10.00–109.00
ALP (U/L)	44.5 ^{b}	39.8 ^{a}	43.0 ^{a}	55.0 ^{b}	47.3 ^{a}	50.3 ^{a}	71.4 ^{b}	57.4 ^{a}	55.0 ^{a}	56.9 ^{b}	56.4 ^{a}	54.3 ^{a}	2.060	0.238	0.004	0.129	8.00–76.00

[1] WBC, White blood cell count; NEU, Neutrophil; LYM, Lymphocyte; MON, Monocyte; EOS, Eosinophil; BASO, Basophil; RBC, Total number of erythrocytes; HCT, Hematocrit value; HGB, Hemoglobin concentration; RDW, Red blood cell distribution width; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PLT, Platelets; MPV, Mean platelet volume; TP, Total protein; ALB, Albumin; GLO, Globulin; BUN, Blood urea nitrogen; CREA, Creatinine; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase. [2] SEM = Standard error of mean. [3] Reference intervals were derived from the Veterinary Diagnostic Laboratory, Kasetsart University Veterinary Teaching Hospital, Bangkok, Thailand. [a–c] Values within the same row with different superscript letters are significantly different ($p < 0.05$); values sharing a common superscript letter are not significantly different.

3.3. Fecal Bacterial Microbiota

Alpha diversity indices (Chao1, Shannon–Weaver, and Simpson's) did not differ significantly among groups, and no significant interaction between time × treatment was observed ($p > 0.05$; Table 4). However, all indices changed significantly over time ($p < 0.05$). Beta diversity analysis showed no distinct clustering among treatment groups (Figure 1). Nevertheless, PERMANOVA detected significant effects of treatment ($R^2 = 0.049$, $p = 0.006$) and time ($R^2 = 0.066$, $p = 0.001$) on microbial community composition. However, interaction between treatment and time was not significant ($p = 0.527$).

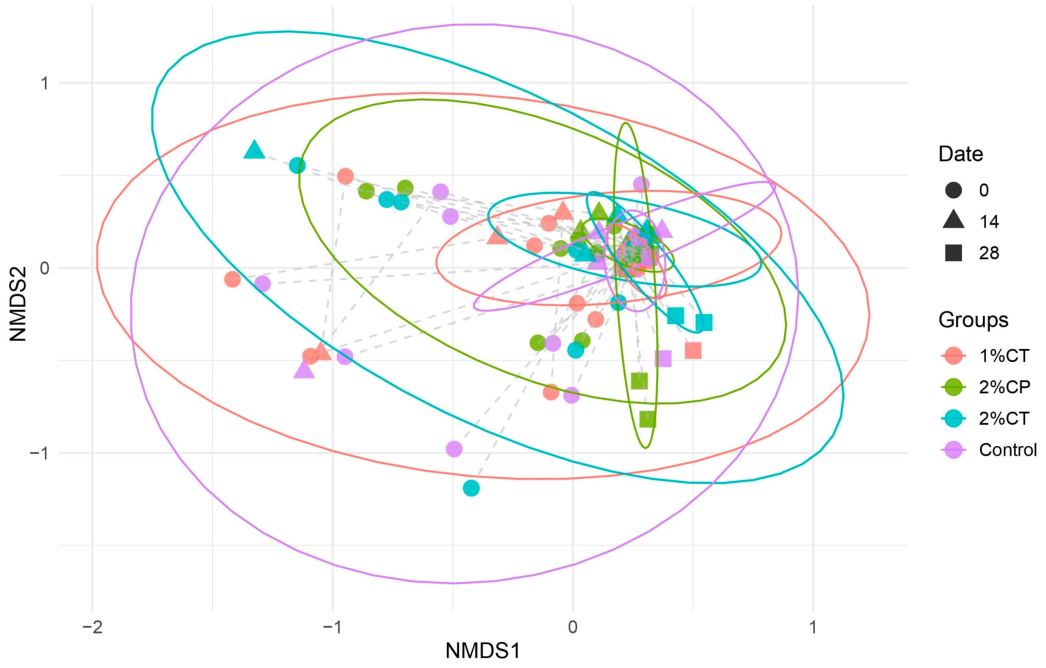

Figure 1. Non-metric multidimensional scaling (NMDS) plot based on Bray–Curtis dissimilarity showing beta diversity of the fecal microbiome across different treatments and time points. Treatments include control, 1%CT (citrus pectin), 2%CT (citrus pectin), and 2%CP (coffee pulp pectin). Each point represents an individual sample, with shapes indicating the sampling time points (days 0, 14, and 28). The clustering of samples illustrates treatment- and time-dependent shifts in gut microbial community structure.

At the phylum level, *Firmicutes*, *Actinobacteriota*, *Proteobacteria*, *Bacteroidota*, and *Fusobacteria* were the dominant phyla (Figures 2 and 3; Table A1). Most supplements did not significantly affect phylum-level relative abundances, and no interaction between group and time was observed ($p > 0.05$).

At the genus level, most genera changed significantly over time but did not differ between groups (Figures 4 and 5; Table A1). Among the analyzed genera, only *Escherichia–Shigella* showed significant effects of treatment group ($p = 0.003$) and sampling time ($p = 0.009$), with no significant group × time interaction ($p = 0.917$). The 2%CP group had a significantly higher relative abundance than the other groups at baseline ($p < 0.05$). Across treatment groups, relative abundance was significantly lower on day 14 than on day 0 ($p < 0.05$), while day 28 did not differ significantly from either time point. *Lactobacillus* showed a tendency towards differences between groups ($p = 0.085$) and over time ($p = 0.065$), with a decrease on day 14 followed by recovery by day 28, particularly in the 2%CP group. *Bacteroides*, *Blautia*, *Ruminococcus torques*, *Terrisporobacter*, *Peptoclostridium*, and *Enterococcus* also changed significantly over time ($p < 0.05$). *Bifidobacterium* and the Firmicutes/Bacteroidota ratio were not significantly affected by treatment or time ($p > 0.05$).

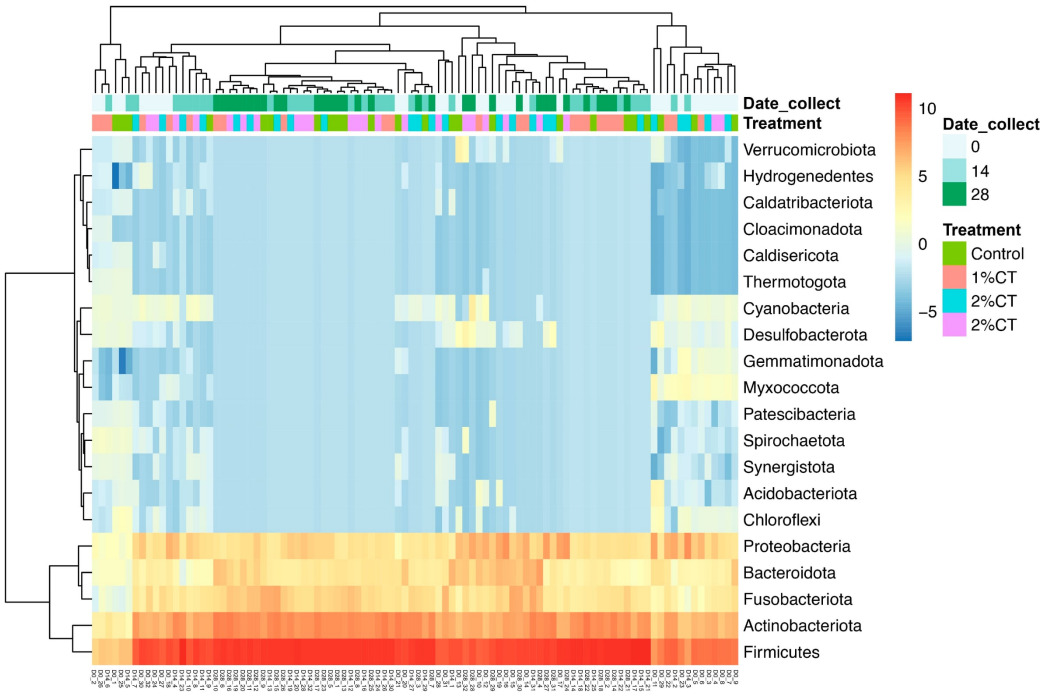

Figure 2. Heatmap representing the relative abundance of fecal microbiota at the phylum level under different dietary treatments on days 0, 14, and 28. Groups include 1%CT (citrus pectin at 1%), 2%CT (citrus pectin at 2%), and 2%CP (coffee pulp pectin at 2%). Color gradients indicate the proportional presence of each phylum.

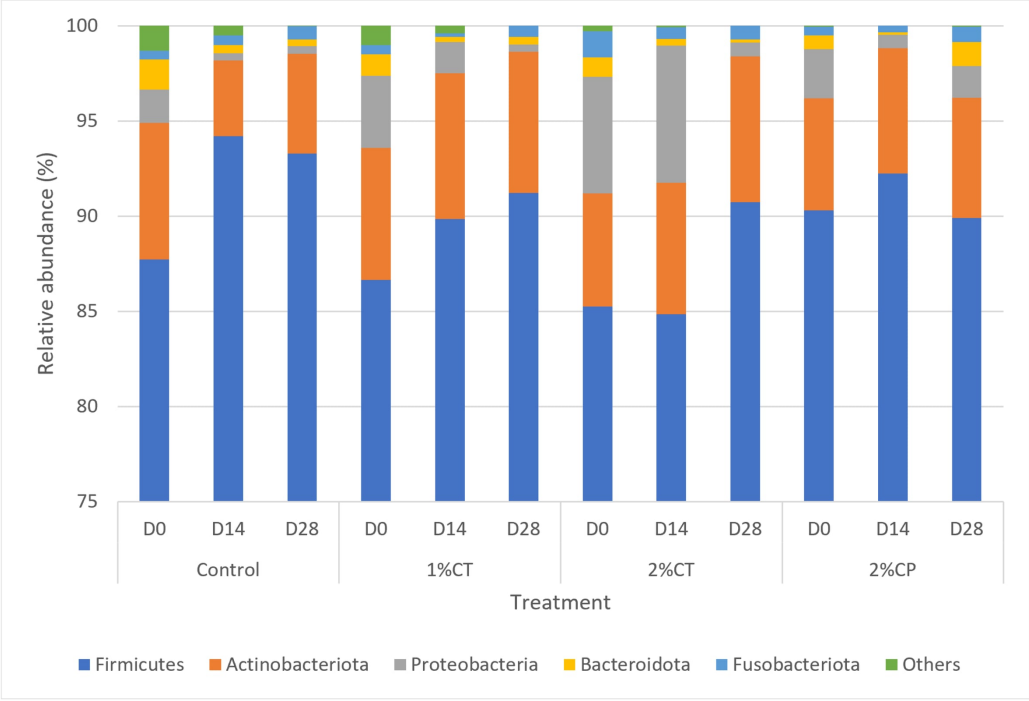

Figure 3. Stacked bar graph illustrating the relative abundance (%) of bacterial phyla in fecal samples from dogs supplemented with different pectin sources and dosages: 1% citrus pectin (1%CT), 2% citrus pectin (2%CT), and 2% coffee pulp pectin (2%CP). Data are presented at three points: prior to supplementation (D0) and following 14 (D14) and 28 (D28) days of treatment. Each bar represents the mean composition at the indicated time points, highlighting shifts in microbial community structure in response to supplementation.

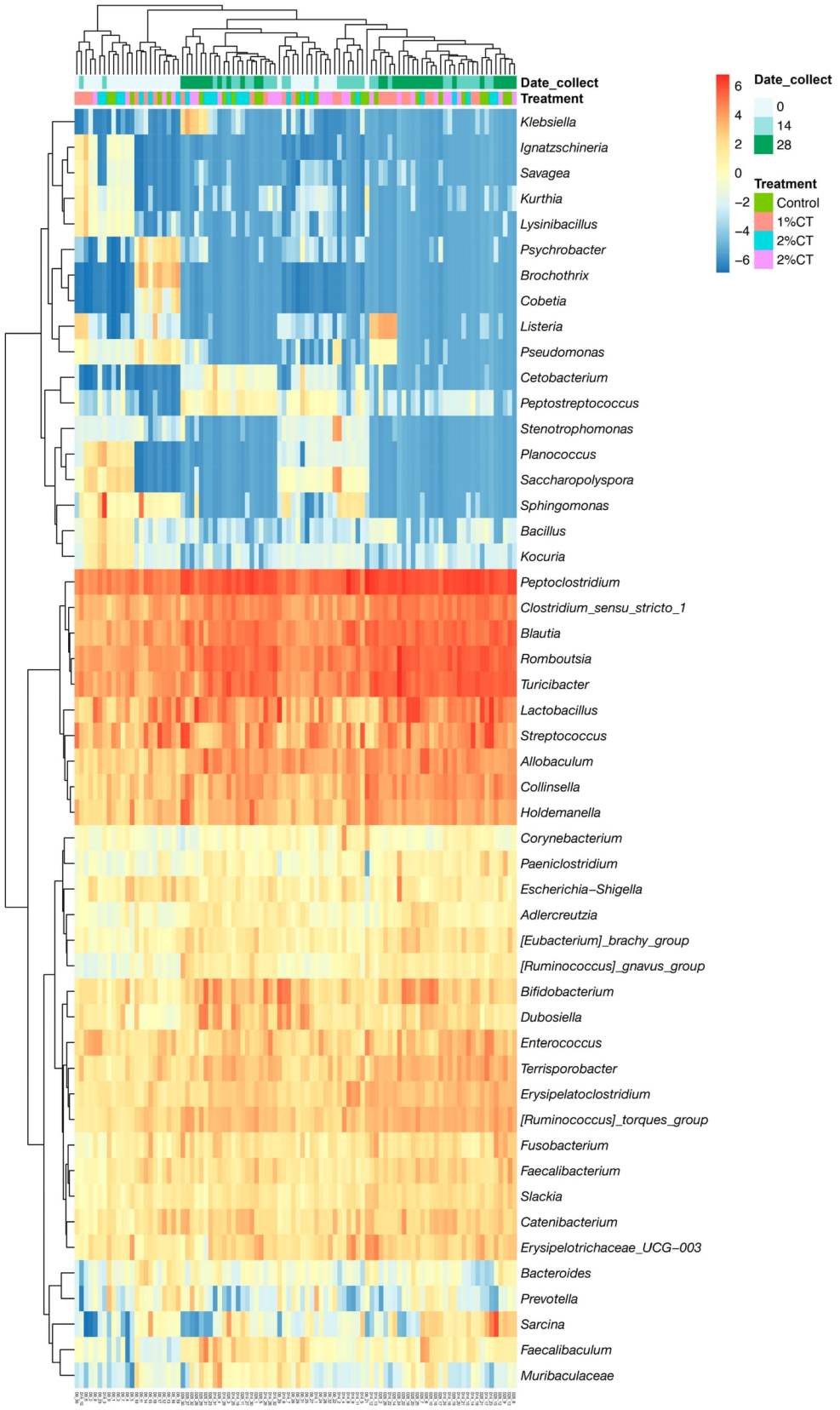

Figure 4. Heatmap representing the relative abundance of fecal microbiota at the genus level under different dietary treatments on days 0, 14, and 28. Groups include 1%CT (citrus pectin at 1%), 2%CT (citrus pectin at 2%), and 2%CP (coffee pulp pectin at 2%). Color gradients indicate the proportional presence of each genus.

Table 4. Alpha diversity indices of fecal microbiota in dogs receiving different dietary treatments.

Diversity Index [1]	Groups				SEM [2]	*p*		
	Control	1% Citrus Pectin	2% Citrus Pectin	2% Coffee Pulp Pectin		Group	Time	Group × Time
Chao1								
Day 0 [a]	671.3	655.0	605.8	543.8	14.58	0.761	<0.001	0.234
Day 14 [b]	429.0	460.9	452.1	405.0				
Day 28 [b]	466.6	424.8	437.7	502.6				
Shannon–Weaver								
Day 0 [a]	3.807	3.963	3.929	3.750	0.03	0.944	<0.001	0.427
Day 14 [b]	3.623	3.677	3.587	3.565				
Day 28 [a]	3.894	3.755	3.750	3.872				
Simpson's								
Day 0 [a]	0.081	0.065	0.060	0.061	0.00	0.955	0.038	0.394
Day 14 [a]	0.071	0.069	0.093	0.081				
Day 28 [b]	0.052	0.060	0.059	0.065				

[1] Different superscript letters between days indicate significant differences ($p < 0.05$). [2] Pooled standard error of means. [a,b] Values within the same row with different superscript letters are significantly different ($p < 0.05$); values sharing a common superscript letter are not significantly different.

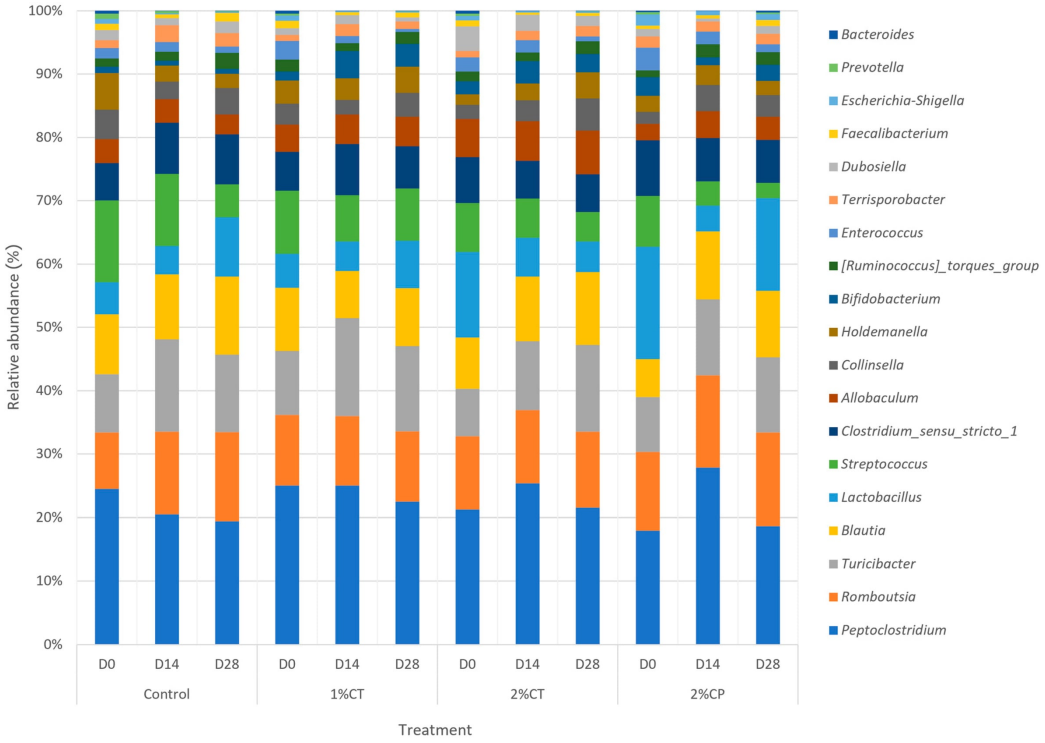

Figure 5. Stacked bar graph illustrating the relative abundance (%) of the top 15 bacterial genera and 4 selected genera in fecal samples from dogs supplemented with different pectin sources and dosages: 1% citrus pectin (1%CT), 2% citrus pectin (2%CT), and 2% coffee pulp pectin (2%CP). Data are presented at three points: prior to supplementation (D0) and following 14 (D14) and 28 (D28) days of treatment. Each bar represents the mean composition at the indicated time points, highlighting shifts in microbial community structure in response to supplementation.

LEfSe analysis identified treatment-associated microbial biomarkers on days 14 and 28 compared with the control group (Figure 6). In the 1%CT group, *Adlercreutzia* and *Parasutterella* were identified on day 14, whereas *Alloprevotella* and *Pseudomonas* were identified on day 28. *Bifidobacterium* was consistently detected as a biomarker throughout the experimental period (Figure 6A,B).

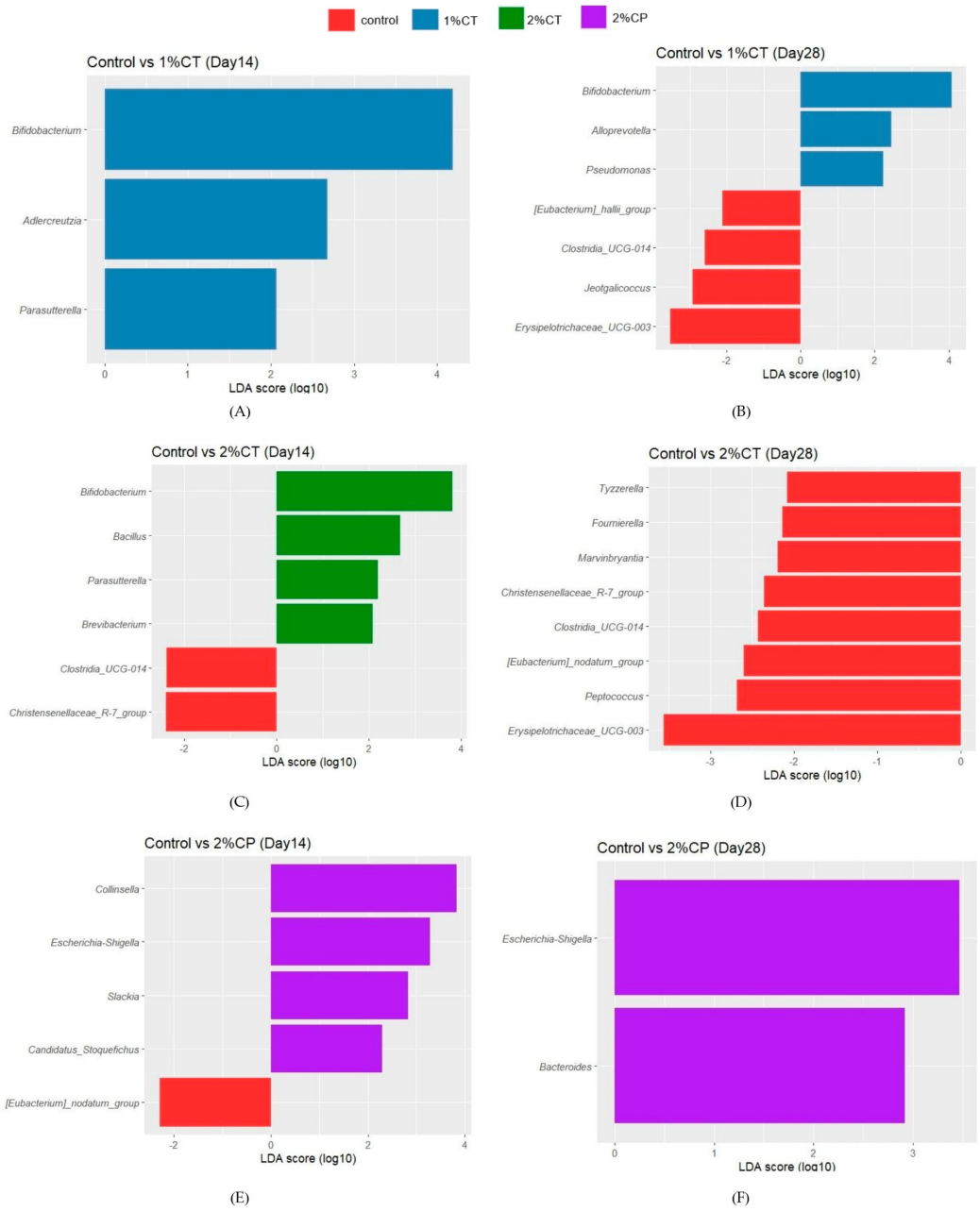

Figure 6. Linear discriminant analysis effect size (LEfSe) of the fecal microbiota at the genus level comparing the control group with dogs supplemented with citrus pectin (CT) or coffee pulp pectin (CP) at different dosages on days 14 and 28 of the experimental period. Colors indicate bacterial biomarkers associated with each treatment group: red = control, blue = 1%CT, green = 2%CT, and purple = 2%CP. (**A**) control vs. 1%CT on day 14; (**B**) control vs. 1%CT on day 28; (**C**) control vs. 2%CT on day 14; (**D**) control vs. 2%CT on day 28; (**E**) control vs. 2%CP on day 14; (**F**) control vs. 2%CP on day 28.

In the 2%CT group, *Bacillus*, *Parasutterella*, and *Bifidobacterium* were identified as biomarkers on day 14, whereas no specific biomarkers were detected on day 28 (Figure 6C,D). In contrast, the 2%CP group was characterized by *Collinsella*, *Slackia*, and *Candidatus_Stoquefichus* on day 14 and *Bacteroides* on day 28 (Figure 6E,F). *Escherichia–Shigella* remained the dominant biomarker throughout the experimental period.

4. Discussion

This study demonstrated that supplementation with 2%CP and 1–2% CT for 28 days is well tolerated in healthy adult dogs, with no apparent adverse effects on physical appearance, feed intake, BW, BCS, FS, or fecal moisture. Similarly, most hematological and serum biochemical parameters were unaffected ($p > 0.05$), consistent with previous studies evaluating prebiotic supplementation in healthy dogs [30–32]. Although the mean RBC and MCH values exceeded the upper reference limits on day 14, MCHC decreased from an initially elevated value and serum albumin showed a transient increase. These changes may reflect temporal physiological variations rather than supplementation effects. EOS counts remained above the reference ranges, and PLT counts remained below the reference ranges throughout the study; no associated clinical abnormalities were observed. Elevated EOS counts have been reported in clinically healthy dogs and may reflect normal biological variation or subclinical conditions [33]. In addition, platelet clumping was observed on blood smears, suggesting that the automated PLT counts were likely underestimated rather than representing a treatment-related effect. However, manual platelet counts were not performed and should be included in future studies to confirm this interpretation. Regarding caffeine exposure, dogs receiving 2%CP had an estimated intake of 8.58 mg/kg BW/day, with no observed anorexia or evidence of liver impairment during the study. Although previous studies have provided safety information on caffeine exposure and coffee-derived pectin-rich extracts [34,35], direct comparisons are limited by differences in experimental conditions. The absence of observed adverse clinical effects suggests that CP supplementation was well tolerated by healthy adult dogs over the 28-day study period. Nevertheless, longer-term studies incorporating more sensitive indicators of intestinal health, including SCFAs, fecal pH, inflammatory markers (e.g., calprotectin) [36–38] and intestinal barrier proteins (e.g., Zonula occludens-1) [39], are needed to further evaluate the safety and potential cumulative effects of prolonged supplementation.

Neither CP nor CT significantly altered nutrient digestibility ($p > 0.05$), likely because the inclusion level did not exceed 3%, the threshold above which increased digesta viscosity may impair digestibility [13]. Likewise, supplementation with 1–2% pectin did not affect alpha diversity, indicating that this inclusion level did not introduce broad alterations in the gut microbial community. Similar findings have been reported for 1–2%DM FOS, which exerts minimal effects on fecal microbial populations in dogs [40–42]. The effects of prebiotics on gut microbiota are influenced by their source, concentration, duration of supplementation [1,43,44], and structural characteristics [15,45]. However, the present study was limited to 28 days, and CP was tested only at a 2% inclusion level, limiting the assessment of dose-dependent responses and comparisons between CP and CT across different inclusion levels. Additionally, although the basal diet contained no added prebiotic fiber sources, its total dietary fiber and fermentable fiber contents were not determined. Therefore, the potential contribution of fermentable substrates from the basal diet to the observed microbial responses cannot be excluded. Future studies investigating longer supplementation periods, different inclusion levels of both pectin sources, and characterization of the basal diet's fiber composition may help clarify the effects of pectin sources, dose, and supplement duration on the gut microbiota.

Consistent with the alpha diversity findings, the dominant phyla remained unchanged across all groups, with Firmicutes, Actinobacteriota, Proteobacteria, Bacteroidota, and Fusobacteria representing the core gut microbiota of healthy dogs [46]. Similar observations have been reported following supplementation with pectin [15] and other prebiotics [43,47]. Although NMDS plots showed no distinct clustering among treatment groups, PERMANOVA detected significant effects of treatment and sampling time on microbial community composition. However, the significant treatment effect accounted for only

a small proportion of the total microbial variation ($R^2 = 0.049$), indicating that community composition remained largely stable. At the genus level, several genera identified in this study, including *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, *Clostridium_sensu_stricto_1*, and *Terrisporobacter*, have previously been reported to utilize galacturonic acid, a principal structural component of pectin, for the production of short-chain fatty acids (SCFAs) [15]. However, these taxa were not exclusively enriched in the pectin-supplemented groups, and their functional contribution to pectin fermentation could not be confirmed because microbial metabolites were not measured.

Among the CT supplementation groups, LEfSe analysis identified *Bifidobacterium* as a bacterial marker in the 1%CT throughout the experimental period, but only on day 14 in the 2%CT. However, no significant overall treatment effect on *Bifidobacterium* relative abundance was observed. Therefore, these findings may reflect differences in microbial composition among treatment groups rather than a clear increase in *Bifidobacterium* abundance due to supplementation. Furthermore, the relatively low DE of CT (6.7%), compared with CP (33%), may increase its susceptibility to microbial fermentation, potentially influencing substrate utilization by beneficial bacteria [9,10]. However, whether this structural characteristic contributed to the observed association with *Bifidobacterium* remains unclear, as fermentation products, including short-chain fatty acids (SCFAs), were not measured. Additional biomarkers, including *Parasutterella*, *Bacillus*, *Alloprevotella*, and *Pseudomonas*, were also associated with CT supplementation. The variation in these exploratory treatment-associated microbial signatures indicates that different CT inclusion levels may be associated with distinct microbial responses, although these patterns were not reflected in significant changes in relative abundance.

In the CP group, although *Lactobacillus* showed only a tendency toward enrichment, this observation may have been influenced by bioactive compounds naturally present in coffee pulp rather than pectin alone. Previous studies have shown that most dietary phenolics reach the large intestine [48], where they are metabolized by gut microbiota and influence bacterial growth. Zhang et al. [49] demonstrated that chlorogenic acid (CGA) can enhance taxa such as *Lactobacillus*. Similarly, CGA has been reported to alter gut microbiota composition in mice [50], suggesting its potential role in modulating intestinal microbial communities. However, as CGA content in CP was not determined in the present study, its potential contribution to the observed microbial response remains unclear. Additionally, caffeine, which was quantified in CP, has been reported to reduce Proteobacteria and Actinobacteria, while increasing *Faecalibacterium* in mice [51,52]. Therefore, the microbial shifts observed in the CP group may reflect the combined effects of pectin structure and coffee-derived bioactive compounds rather than pectin alone. However, as CGA was not quantified and the specific effects of caffeine were not evaluated, the relative contribution of these compounds to the observed microbial responses remains unclear and requires further investigation.

LEfSe analysis identified *Escherichia–Shigella* as a discriminatory taxon in the 2%CP group, reflecting its higher overall abundance compared with the other groups. However, LEfSe does not establish whether this difference was caused by dietary supplementation. Notably, *Escherichia–Shigella* was already significantly more abundant in the 2%CP group at baseline before supplementation. Its relative abundance generally decreased over time across treatment groups, with a significant reduction from day 0 to day 14 ($p < 0.05$) and no significant group × time interaction. These findings suggest that the higher overall abundance in the 2%CP group may reflect pre-existing differences among dogs rather than an effect of CP supplementation. Furthermore, *Escherichia–Shigella* includes both commensal and potentially pathogenic bacteria [53]. Although no clinical abnormalities were observed, the biological significance of these differences in relative abundance remains unclear, par-

ticularly given the limited taxonomic resolution of short-read 16S rRNA sequencing, which cannot reliably distinguish potentially pathogenic from commensal strains. Moreover, the absence of clinical abnormalities during 28-day intervention supports short-term tolerability but does not establish long-term safety. Longer-term studies incorporating clinical monitoring, species- and strain-level characterization, and functional analyses are therefore warranted.

Although LEfSe identified several treatment-associated bacterial taxa, not all biomarkers were supported by the relative abundance analysis. Therefore, these findings should be considered exploratory, and their biological significance remains to be established. Collectively, the present findings suggested that CT and CP were associated with different microbial responses. These differences may be related to variations in pectin structure, including DE, side-chain sugar composition, and residual phytochemicals [9,10,24]. However, the relative contributions of these factors remain unclear.

Overall, both CT and CP appeared to be well tolerated by healthy adult dogs over a 28-day study period and were associated with differences in gut microbiota composition, without substantial changes in alpha diversity. Future studies integrating broader dose ranges, longer supplementation periods, metagenomics, microbial metabolite profiling, and host-response analysis will help clarify the functional significance of these microbiota changes and further evaluate the potential of CP as a novel pectin source.

5. Conclusions

Dietary supplementation with 1–2% citrus pectin or 2% coffee pulp pectin for 28 days was well tolerated by healthy adult dogs under the experimental conditions, with no observed adverse effects on feed intake, nutrient digestibility, or health parameters. Although alpha diversity remained largely unchanged, differences in gut microbial composition and selected bacterial taxa were observed among treatment groups. LEfSe identified *Bifidobacterium* as a microbial biomarker in the CT group and *Escherichia–Shigella* in the CP group. However, these findings should be interpreted cautiously, as they were not consistently supported by significant changes in relative abundance. Notably, the higher abundance of *Escherichia–Shigella* in the CP group was already present at baseline, suggesting pre-existing differences rather than an effect of supplementation. These microbial responses may be associated with variation in pectin structure and composition, although their function remains unclear. Overall, the findings support the potential of CP as a sustainable alternative source of dietary pectin for canine nutrition. Further studies are warranted to evaluate long-term safety and functional effects on gut microbiota and health parameters.

6. Patents

This work utilized coffee pulp pectin prepared according to Thai Petty Patent No. 20718.

Author Contributions: Conceptualization, P.P. (Pornsucha Palaseweenun), N.A. and A.K.; methodology, S.K., P.P. (Pornsucha Palaseweenun) and A.K.; formal analysis, S.K., P.P. (Pornsucha Palaseweenun), A.K. and P.P. (Preecha Patumcharoenpol); investigation, S.K., A.K., S.A., P.C., N.A., U.P. and P.P. (Pornsucha Palaseweenun); data curation: S.K., A.K. and P.P. (Preecha Patumcharoenpol); writing—original draft preparation: S.K. and P.P. (Pornsucha Palaseweenun); writing—review and editing: S.K., S.A., P.C., N.A., U.P. and P.P. (Pornsucha Palaseweenun). All authors have read and agreed to the published version of the manuscript.

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animals. All procedures were performed in accordance with relevant guidelines and regulations for the care and use of animals in research.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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Abbreviations

The following abbreviations are used in this manuscript:

16S rRNA	16S ribosomal ribonucleic acid
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BASO	Basophils
BCS	Body condition score
BUN	Blood urea nitrogen
BW	Body weight
CBC	Complete blood count
CGA	Chlorogenic acid
Co., Ltd.	Company, Limited
CP	Coffee pulp pectin
CREA	Creatinine
CRD	Completely randomized design
CT	Citrus pectin
DE	Degree of esterification
DER	Daily energy requirement
DM	Dry matter basis
EOS	Eosinophils
F/B ratio	Firmicutes/Bacteroidetes ratio
FOS	Fructooligosaccharide
FS	Fecal score
GLOB	Globulin
HCT	Hematocrit
HG	Homogalacturonan
HGB	Hemoglobin
HMP	High-methoxyl pectin
Kg	Kilogram
LDA	Linear discriminant analysis
LEfSe	Linear discriminant analysis effect size
LMP	Low-methoxyl pectin
LYM	Lymphocytes
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mL	Milliliter
MON	Monocytes
MPV	Mean platelet volume
n	Number
NEU	Neutrophils
NMDS	Non-metric multidimensional scaling

p	*p*-value
PERMANOVA	Permutational multivariate analysis of variance
PLT	Platelet count
RG-I	Rhamnogalacturonan I
RG-II	Rhamnogalacturonan II
RBC	Red blood cell count
RDW	Red cell distribution width
SEM	Standard error of the mean
SCFAs	Short-chain fatty acids
TP	Total protein
WBC	White blood cell count

Appendix A

Table A1. Relative abundance (%) of the fecal microbiome at the phylum and genus levels in dogs fed different types and dosages of pectin.

Relative Abundance (%) [1]	Groups				SEM [2]	*p*		
	Control	1% CT	2% CT	2% CP		Group	Time	Group × Time
Phylum								
Firmicutes								
Day 0	87.7	86.7	85.3	90.3	0.82	0.178	0.153	0.721
Day 14	94.2	89.8	84.8	92.2				
Day 28	93.3	91.2	90.7	89.9				
Actinobacteriota								
Day 0	7.17	6.91	5.93	5.87	0.34	0.336	0.899	0.482
Day 14	3.97	7.68	6.92	6.59				
Day 28	5.24	7.41	7.67	6.31				
Proteobacteria								
Day 0	1.76	3.80	6.13	2.59	0.68	0.179	0.285	0.822
Day 14	0.37	1.64	7.20	0.69				
Day 28	0.41	0.38	0.73	1.66				
Bacteroidota								
Day 0 [a]	1.58	1.13	1.02	0.72	0.10	0.700	0.004	0.193
Day 14 [b]	0.45	0.24	0.34	0.13				
Day 28 [ab]	0.35	0.39	0.16	1.28				
Fusobacteriota								
Day 0	0.45	0.49	1.38	0.45	0.08	0.291	0.134	0.299
Day 14	0.51	0.20	0.61	0.35				
Day 28	0.70	0.60	0.71	0.79				
Others								
Day 0 [a]	1.32	1.01	0.29	0.06	0.10	0.091	0.038	0.612
Day 14 [ab]	0.49	0.40	0.09	0.00				
Day 28 [b]	0.02	0.00	0.01	0.06				
F/B ratio								
Day 0	318	370	199	271	1348	0.420	0.181	0.456
Day 14	2629	2086	17,669	1034				
Day 28	857	993	884	442				
Genus								
Peptoclostridium								
Day 0 [ab]	19.5	19.2	15.7	14.9	0.62	0.545	0.018	0.142
Day 14 [a]	17.6	21.5	20.0	24.7				
Day 28 [b]	16.3	19.3	17.9	15.6				
Romboutsia								
Day 0	7.12	8.54	8.54	10.3	0.44	0.183	0.051	0.806
Day 14	11.2	9.41	9.09	12.9				
Day 28	11.8	9.50	9.89	12.4				
Turicibacter								
Day 0 [b]	7.31	7.70	5.51	7.2	0.47	0.193	<0.001	0.746
Day 14 [a]	12.5	13.3	8.57	10.6				
Day 28 [a]	10.3	11.5	11.3	9.9				

Table A1. *Cont.*

Relative Abundance (%) [1]	Groups				SEM [2]	*p*		
	Control	1% CT	2% CT	2% CP		Group	Time	Group × Time
Blautia								
Day 0 [b]	7.5	7.64	5.96	4.97	0.35	0.407	0.008	0.306
Day 14 [ab]	8.78	6.39	8.05	9.49				
Day 28 [a]	10.3	7.85	9.54	8.81				
Lactobacillus								
Day 0	4.03	4.13	9.98	14.7	0.81	0.085	0.065	0.194
Day 14	3.86	4.06	4.84	3.63				
Day 28	7.86	6.47	4.02	12.3				
Streptococcus								
Day 0	10.3	7.64	5.73	6.62	0.74	0.390	0.109	0.789
Day 14	9.76	6.25	4.88	3.37				
Day 28	4.38	7.09	3.84	2.01				
Clostridium_sensu_stricto_1								
Day 0	4.67	4.68	5.36	7.29	0.28	0.494	0.540	0.227
Day 14	6.95	6.98	4.71	6.07				
Day 28	6.65	5.69	4.95	5.69				
Allobaculum								
Day 0	3.01	3.34	4.42	2.18	0.30	0.245	0.433	0.926
Day 14	3.19	3.99	4.90	3.79				
Day 28	2.62	4.01	5.70	3.07				
Collinsella								
Day 0	3.74	2.50	1.66	1.56	0.20	0.723	0.051	0.067
Day 14	2.38	1.99	2.60	3.66				
Day 28	3.52	3.21	4.21	2.89				
Holdemanella								
Day 0	4.58	2.79	1.22	2.11	0.24	0.715	0.896	0.011
Day 14	2.20	2.93	2.13	2.75				
Day 28	1.89	3.57	3.45	1.87				
Bifidobacterium								
Day 0	0.82	1.11	1.53	2.45	0.27	0.348	0.471	0.266
Day 14	0.64	3.70	2.76	1.09				
Day 28	0.63	3.07	2.40	2.13				
[Ruminococcus]_torques_group								
Day 0 [b]	1.05	1.44	1.15	0.87	0.08	0.864	0.003	0.140
Day 14 [ab]	1.23	1.07	1.10	1.83				
Day 28 [a]	2.11	1.61	1.66	1.68				
Enterococcus								
Day 0 [a]	1.28	2.27	1.66	2.99	0.16	0.355	<0.001	0.578
Day 14 [a]	1.32	1.00	1.52	1.77				
Day 28 [b]	0.87	0.43	0.61	1.01				
Terrisporobacter								
Day 0 [b]	0.99	0.71	0.73	1.46	0.10	0.231	0.007	0.225
Day 14 [a]	2.26	1.59	1.14	1.41				
Day 28 [a]	1.76	1.00	1.38	1.42				
Dubosiella								
Day 0	1.29	0.83	2.89	0.96	0.23	0.458	0.641	0.686
Day 14	0.95	1.26	2.01	0.42				
Day 28	1.54	0.57	1.32	1.01				
Faecalibacterium								
Day 0 [a]	0.80	0.91	0.70	0.45	0.06	0.315	0.015	0.246
Day 14 [a]	0.50	0.37	0.29	0.50				
Day 28 [ab]	1.11	0.63	0.40	0.81				
Escherichia–Shigella								
Day 0 [a]	0.60 [b]	0.63 [b]	0.52 [b]	1.45 [a]	0.07	0.003	0.009	0.917
Day 14 [b]	0.15	0.17	0.19	0.55				
Day 28 [ab]	0.17	0.15	0.19	0.76				

Table A1. *Cont.*

Relative Abundance (%) [1]	Groups				SEM [2]	*p*		
	Control	1% CT	2% CT	2% CP		Group	Time	Group × Time
Prevotella								
Day 0	0.67	0.23	0.23	0.32	0.05	0.283	0.093	0.815
Day 14	0.33	0.01	0.00	0.02				
Day 28	0.08	0.08	0.03	0.24				
Bacteroides								
Day 0 [a]	0.35	0.33	0.35	0.16	0.03	0.999	0.009	0.567
Day 14 [b]	0.03	0.02	0.03	0.03				
Day 28 [a]	0.05	0.03	0.04	0.21				

[1] Different superscript letters between days and groups indicate significant differences ($p < 0.05$). [2] Pooled standard error of means. [a,b] Values within the same row with different superscript letters are significantly different ($p < 0.05$); values sharing a common superscript letter are not significantly different.

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