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Enrichment of Beneficial Genera and Suppression of Pathobionts in the Cat Oral Cavity Following Bifidobacterium-Lactobacillus Supplementation

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ABSTRACT

Probiotics have been shown to alter oral microbial communities and enhance oral health in both humans and laboratory animals. Despite this, their potential effects on the oral microbiome of felines are not yet well defined. In the present 42-day experiment, twelve healthy cats were evenly divided into two treatments: a control (CON) and a composite probiotic group (CPG). The CPG diet was fortified with Bifidobacterium animalis subsp. lactis HN019, Lactobacillus acidophilus NCFM, and Lactobacillus casei LC-11, each supplied at roughly 1×10^{10} CFU per kg of feed. Oral swabs from the gingiva, tooth surface, and tongue were collected on days 0 and 42 for 16S rRNA sequencing. Across all oral regions, Bacteroidetes, Firmicutes, and Proteobacteria represented the most prevalent phyla. In the gingiva of CPG cats, seven bacterial genera—including Moraxella, Actinomyces, and Frederiksenia—were elevated. On tooth surfaces, Bergeyella and Streptococcus increased, while on the tongue, Bergeyella, Flavobacterium, and Luteimonas were enriched. Conversely, eight genera, such as Bacteroides, Desulfovibrio, and Filifactor, declined in the gingiva, Helcococcus, Lentimicrobium, and Campylobacter decreased on teeth, and Porphyromonas, Treponema, and Fusibacter reduced on the tongue. These data indicate that this probiotic blend reshapes feline oral microbiota by favoring commensal species while limiting potential pathogens, suggesting benefits for oral hygiene in cats.

Keywords: Probiotics, Feline oral microbiota, Gingiva; Dental surfaces, Tongue

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Introduction

Oral health is fundamental to general animal health, functioning as a primary interface for both immune and digestive activity. In domestic settings, cats commonly experience oral conditions including halitosis, tartar formation, and periodontal disorders [1]. Even minor issues like bad breath may hinder human–cat interaction, whereas severe inflammation, such as gingivitis or periodontitis, can cause pain, feeding difficulty, and systemic complications [2]. The oral microbial ecosystem—second in size only to the gut microbiota—has a strong association with oral disease onset and progression in cats [3, 4]. Microbes are critical to plaque development, a precursor to gingivitis and periodontitis [5]. Typically, an imbalance in oral flora supports excessive microbial accumulation within biofilms, encouraging persistent plaque and tartar buildup [3, 6]. Once these deposits form above or below the gum line, bacterial by-products can trigger mucosal irritation and inflammation, ultimately causing periodontal tissue damage [7].

Professional dental procedures, such as scaling, are often needed once plaque hardens into calculus [8]. Consequently, prevention through daily oral management is vital. However, consistent brushing or use of antiseptic rinses can be difficult for cat owners to perform regularly. Therefore, modifying the oral microbiome through diet-based approaches may represent a more practical long-term preventive option. Probiotics can influence oral microbial balance by competitive exclusion, secretion of antimicrobial compounds, and modulation of immune functions [9, 10]. Such actions have been linked to reduced periodontal damage in both humans and rodents [11, 12]. Certain strains, including Bifidobacterium animalis subsp. lactis HN019 [11], Lactobacillus acidophilus NCFM [13], and Lactobacillus casei LC-11 [14] have demonstrated the ability to limit pathogen proliferation, produce acidic environments, and disrupt biofilm maturation. Incorporating such probiotics into feline diets may therefore support oral health. Nonetheless, most existing feline studies have concentrated on gastrointestinal outcomes, leaving limited information about their oral effects.

The cat's oral cavity presents a complex environment, where microbial communities differ by surface type and oxygen exposure [15]. Aerobic microbes usually colonize areas like the tongue, mucosal lining, and teeth, whereas anaerobic species thrive in gingival crevices and subgingival pockets [16]. Human studies have shown that seven oral regions contain three distinct microbial clusters, with subgingival and supragingival communities differing markedly from those of the palate, gingiva, mucosa, tongue, and saliva [17]. Comparable findings have been reported in dogs, where oral microbial diversity varies according to habitat characteristics, pH, and oxygen tension [18, 19]. Bacterial groups associated with supragingival and subgingival zones are especially linked to canine dental disease [20]. Yet, investigations into feline oral microbiota often rely on pooled or single-site samples, rarely exploring site-specific variation within the same analysis.

Accordingly, this study incorporated three probiotic strains—previously confirmed to improve oral conditions in humans and rodents—into feline diets. Using high-throughput sequencing, we examined changes in microbial communities across three oral niches (gingiva, tooth surface, and tongue) to assess whether dietary probiotic supplementation can alter microbial composition and enhance the oral environment. The outcomes contribute to understanding how probiotic nutrition supports balanced oral microbiota in cats and may inform future dietary approaches for feline dental care.

Materials and Methods

Animals

The animal trial received ethical clearance from the Institutional Animal Care and Use Committee of China Agricultural University (approval number AW50503202-2-6). Twelve sterilized adult cats were included—six Chinese domestic and six British Shorthair—balanced for sex (three males and three females per breed). All animals had no prior oral hygiene care but were confirmed healthy and free of oral disease. Before enrollment, each underwent a complete clinical assessment, including hematological and serum profiles, nutritional condition, appetite, fecal quality, and parasite examination. All findings indicated a normal health status. None of the cats had received antibiotics, medications, or any functional diets during the three months preceding the study. The median body weight was 4.35 kg (range: 3.62–5.15 kg), and the median age was 3 years (range: 2–4 years). According to the 9-point body condition scale, the mean score was 5.50 ± 0.50. Housing and feeding were conducted at the Pet Feeding Center, Feed Industry Centre, Ministry of Agriculture and Rural Affairs, China Agricultural University. Animal management followed the standards outlined by the National Research Council.

Study design

The experiment employed a randomized design with twelve cats assigned evenly into two dietary treatments (n = 6 per group) while maintaining equal sex and breed distribution. The control group (CON) was provided with a basal diet alone, whereas the composite probiotic group (CPG) received the same diet supplemented post-processing with a mixed probiotic powder. The CPG feed incorporated Bifidobacterium animalis subsp. lactis HN019, Lactobacillus acidophilus NCFM, and Lactobacillus casei LC-11, each added at roughly 1×10^{10} CFU per kilogram of diet. The composite probiotic product, purchased from Aikoyou Health Technology Co., Ltd. (Suzhou, China), contained maltodextrin as a carrier and each bacterial strain at around 2×10^{10} CFU/g. The experimental diets for both groups were produced by Hangzhou Wangmiao Biotechnology Co., Ltd. (Hangzhou, China). The nutritional ingredients and chemical profile of the basal feed are shown in **Table 1**.

Feed components were examined in duplicate for moisture, crude fat, protein, fiber, and ash contents according to the AOAC International Official Methods [21]. The trial lasted for 42 days, with unrestricted access to food and water. Prior to the main phase, cats were acclimated to the basal diet for 30 days. All feeding practices complied with the 2006 NRC nutrient recommendations for adult cats.

Table 1. Composition and nutrient level of the basal diet.

Ingredient	%
Chicken meal	54.50
Chicken fat	8.00
Fish oil	2.00
Tapioca	3.00
Potato starch	19.00
Rice	4.00
Chicken liver powder	5.00
Alfalfa meal	3.00
Choline chloride	0.30
Salt	0.50
Taurine	0.20
Mineral complexes and vitamins[1]	0.50
Nutrient Content	%
Moisture	7.12
Crude protein	41.65
Crude fat	20.28
Crude fiber	1.82
Ash	7.87

[1] The vitamin–mineral premix supplied per kilogram of feed: vitamin A (14, 500 IU), vitamin D_3 (1, 000 IU), vitamin E (156 IU), vitamin B_1 (32.0 mg), vitamin B_2 (30.0 mg), vitamin B_3 (120 mg), vitamin B_5 (88.0 mg), vitamin B_6 (13.0 mg), vitamin B_{12} (0.20 mg); Fe ($FeSO_4$) 100 mg, Cu ($CuSO_4$) 7.00 mg, Co ($CoSO_4$) 1.00 mg, I (CaI_2) 20.0 mg, Mn ($MnSO_4$) 20.0 mg, Zn ($ZnSO_4$) 68.0 mg, Se (Na_2SeO_3) 0.50 mg.

Sample collection

Microbial specimens were collected from all cats on days 0 and 42. Feed was withdrawn eight hours prior to sampling to limit food residues, while water remained available. All operations were performed aseptically with sterile gloves. Sterile cotton swabs were used to gently wipe both the upper and lower gingiva as well as the dorsal and ventral tongue surfaces, avoiding tooth contact. Each surface was swabbed three times, lasting about 5 seconds per stroke. To sample tooth-associated microbes, sterile swabs were rubbed across teeth numbered 104, 204, 108, and 208 in the upper jaw and 304, 404, 309, and 409 in the lower jaw, as described by the modified Triadan system [22]. Three replicate swabs were taken from each region per animal. The samples were transferred into cryogenic tubes and promptly frozen at −80 °C until further use.

Oral microbiota analysis

DNA was isolated from the swab samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). The V3–V4 region of the bacterial 16S rRNA gene was amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Purified amplicons were pooled in equimolar concentrations and sequenced on an Illumina MiSeq platform (San Diego, CA, USA). Raw reads were filtered for quality with fastp (v0.19.6) and assembled with Flash (v1.2.11), following the procedures outlined in a previous study [23].

Operational taxonomic units (OTUs) were generated in UPARSE (v11) at 97% sequence similarity. Taxonomic identification employed the RDP Bayesian classifier (v2.13) with a confidence threshold of 0.7 using the Silva138/16S_bacteria database. Microbial composition was characterized across multiple taxonomic ranks. Alpha diversity was calculated in Mothur (v1.30.2) using Faith's phylogenetic diversity (Faith's PD), Ace, Chao,

Simpson, Shannon, and Sobs indices. Beta diversity was determined using weighted UniFrac distances, followed by ANOSIM statistical testing and visualization by principal coordinate analysis (PCoA). Differential taxa between treatments were identified using linear discriminant analysis effect size (LEfSe), applying an LDA score cutoff > 3 (or 4) and $p < 0.05$ for significance.

Statistical analysis

All datasets were processed in IBM SPSS Statistics 26.0 (Chicago, IL, USA). Graphs illustrating α-diversity parameters were generated with GraphPad Prism 9.0 (San Diego, CA, USA). Analyses and visual summaries of sequencing outputs were completed using R software. ggplot2 was used to construct bar graphs of microbial profiles, while vegan produced heatmaps of community structures.

Group comparisons for both diversity indices and microbial relative abundance were evaluated using the Wilcoxon rank-sum test. Adjustments for multiple hypothesis testing were handled by the false discovery rate (FDR) method. Bootstrap resampling was applied to estimate 95% confidence intervals. Statistical differences were considered meaningful when $p < 0.05$, and all results are expressed as means ± SEM.

Results and Discussion

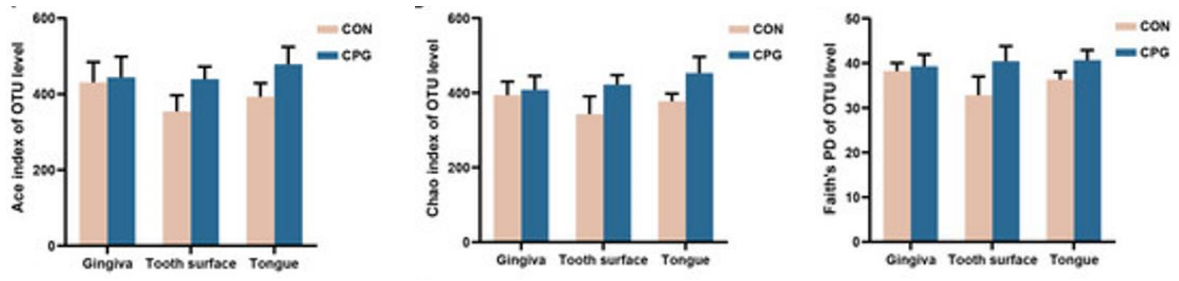
Initial oral microbial landscape of cats

After filtering and quality control, the Illumina sequencing produced an average of 58, 846 effective 16S rRNA sequences per specimen. Subsequent analyses were standardized to a sequencing depth of 18, 583 reads per sample.

Alpha diversity was characterized using Ace, Chao, Faith's PD, Simpson, Shannon, and Sobs indices. Prior to the intervention, no significant distinctions in any of these indices were noted between the control (CON) and composite probiotic (CPG) groups within the gingiva, tooth surface, or tongue ($p \geq 0.05$; (**Figures 1a–1f)**). Similarly, principal coordinate analysis (PCoA) derived from weighted-UniFrac distances revealed no clear clustering among the two groups across any oral region ($p \geq 0.05$; (**Figures 1g–1i)**).

The taxonomic comparison of bacterial abundance at the phylum, family, and genus levels is detailed in **Table 2**.

- In gingival samples, Desulfomicrobiaceae, Caulobacteraceae, Desulfomicrobium, and norank_f_Propionibacteriaceae showed elevated levels in the CPG group ($p < 0.05$). Meanwhile, Parabacteroides, Granulicatella, and unclassified_f_Anaerovoracaceae were less represented than in CON cats ($p < 0.05$).
- On tooth surfaces, Campilobacterota, unclassified_c_Gammaproteobacteria, Frederiksenia, and norank_f_Pasteurellaceae exhibited a higher abundance in the CPG cats relative to CON ($p < 0.05$).
- Within the tongue microbiota, Synergistota, Synergistaceae, Fretibacterium, and Prevotellaceae_UCG-003 were depleted, while unclassified_f_Lachnospiraceae was increased in the probiotic group ($p < 0.05$).



a) b) c)

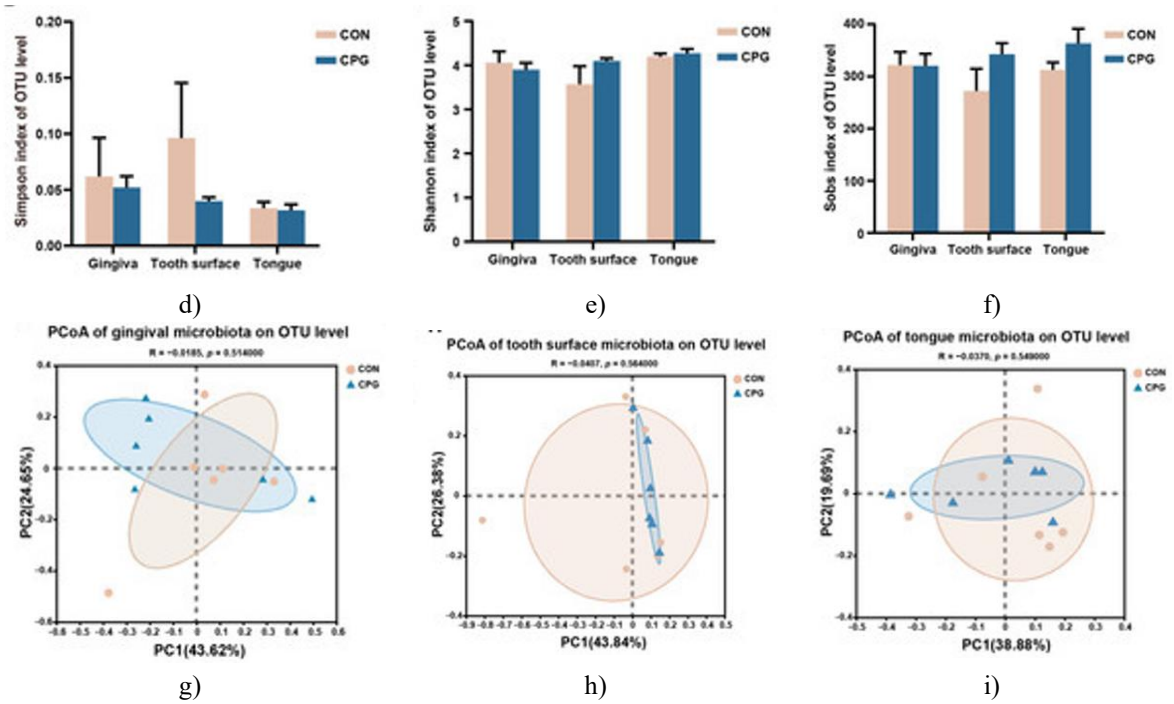


Figure 1. Baseline α- and β-diversity metrics of feline oral microbiota.
Panels (a–f) depict the Ace, Chao, Faith's PD, Simpson, Shannon, and Sobs indices; panels (g–i) show PCoA plots based on weighted-UniFrac distances for gingiva, teeth, and tongue samples. CON = basal diet only; CPG = basal diet plus probiotic blend. Data shown as mean ± SEM (n = 6).

Table 2. Comparative baseline bacterial composition of feline oral samples.

Oral Site	Taxon	CON (Mean ± SE)	CPG (Mean ± SE)	p-Value
Gingiva	Desulfomicrobiaceae	0.011 ± 0.004	0.096 ± 0.046	0.03
	Caulobacteraceae	0	0.022 ± 0.013	0.03
	Parabacteroides	1.743 ± 1.295	0.044 ± 0.028	0.03
	Granulicatella	0.201 ± 0.156	0	0.01
	Desulfomicrobium	0.011 ± 0.004	0.096 ± 0.046	0.03
	unclassified_f_Anaerovoracaceae	0.071 ± 0.046	0	0.03
	norank_f_Propionibacteriaceae	0.004 ± 0.004	0.031 ± 0.011	0.03
Tooth surface	Campilobacterota	0.326 ± 0.284	0.839 ± 0.276	0.04
	unclassified_c_Gammaproteobacteria	0.030 ± 0.015	0.209 ± 0.083	0.008
	Frederiksenia	1.423 ± 0.644	4.214 ± 0.797	0.03
	unclassified_c_Gammaproteobacteria	0.030 ± 0.015	0.209 ± 0.083	0.008
	norank_f_Pasteurellaceae	0.004 ± 0.002	0.018 ± 0.005	0.03
Tongue	Synergistota	0.770 ± 0.424	0.177 ± 0.058	0.04
	Synergistaceae	0.770 ± 0.424	0.177 ± 0.058	0.04
	Fretibacterium	0.767 ± 0.423	0.176 ± 0.059	0.04
	unclassified_f_Lachnospiraceae	0.042 ± 0.018	0.145 ± 0.026	0.03
	Prevotellaceae_UCG-003	0.079 ± 0.038	0.003 ± 0.003	0.004

CON: cats fed basal diet; CPG: cats fed basal diet supplemented with probiotics. Results expressed as mean ± SEM (n = 6); significant differences at $p < 0.05$.

Influence of composite probiotics on oral microbial diversity

The variations in oral microbial indices after treatment are shown in **Figure 2**.

At day 42, there were no significant differences in overall microbial richness (Ace, Chao, Sobs) or diversity (Faith's PD, Simpson, Shannon) between CON and CPG cats across gingiva, teeth, and tongue sites ($p \geq 0.05$; (**Figures 2a–2f)**).

Nonetheless, weighted-UniFrac PCoA demonstrated a distinct shift in clustering of microbial communities between the two treatments in both the gingival and tongue samples ($p < 0.05$; (**Figures 2g and 2i**)). Conversely, microbial populations on the tooth surfaces displayed no notable group separation ($p \geq 0.05$; (**Figure 2h)**).

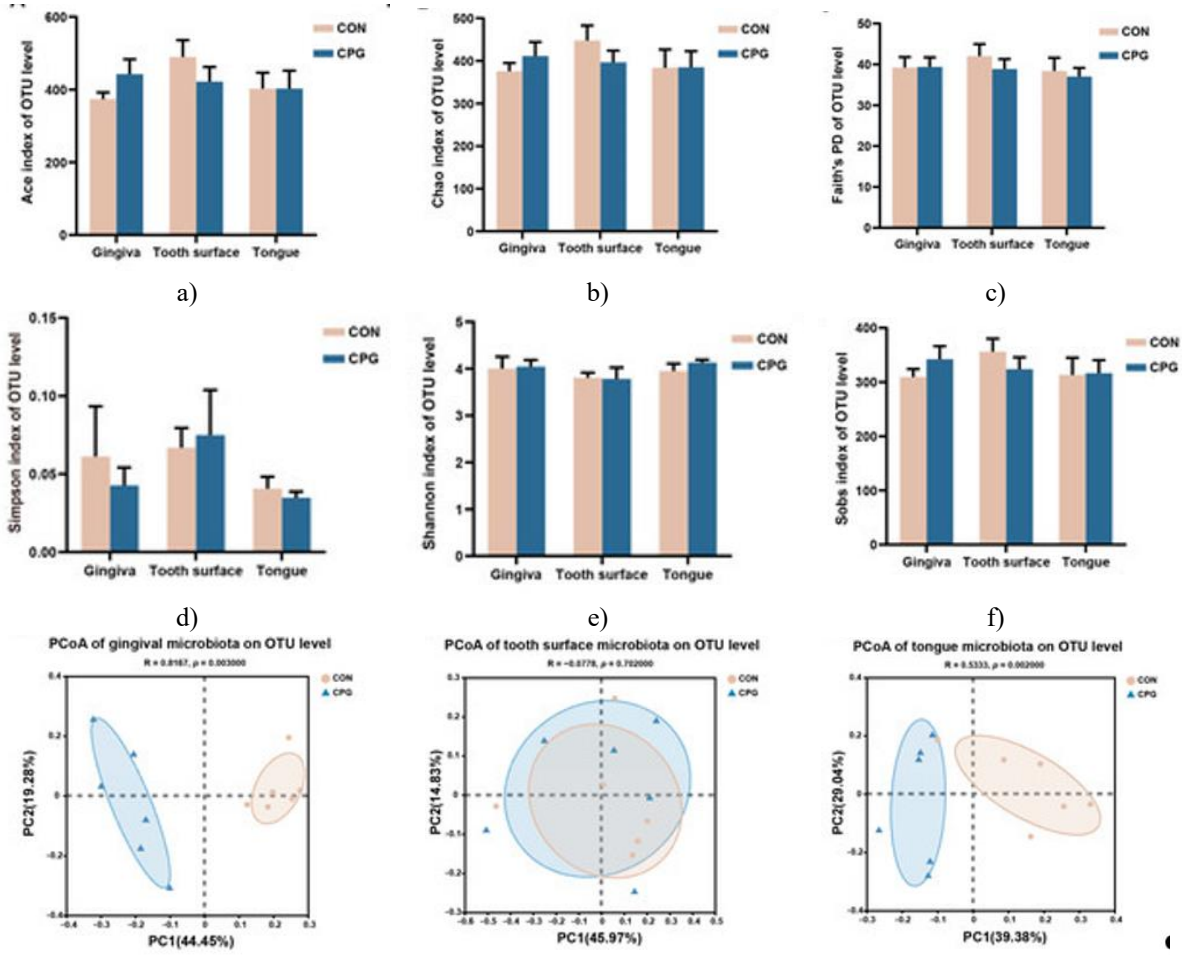


Figure 2. α- and β-diversity of feline oral microbiota after 42 days.
Panels (a–f) illustrate the Ace, Chao, Faith's PD, Simpson, Shannon, and Sobs indices; (g–i) present PCoA analyses based on weighted-UniFrac distances of gingival, dental, and lingual samples. CON = basal diet; CPG = basal diet plus composite probiotic. Values are mean ± SEM (n = 6).

Influence of the composite probiotic on gingival bacterial communities in cats

Analysis of the gingival samples revealed 407 operational taxonomic units (OTUs) that were shared by both groups, while 173 OTUs were unique to the CON cats and 427 OTUs occurred exclusively in the CPG group. Across all samples, sequences were distributed among 17 phyla, 34 classes, 83 orders, 143 families, 255 genera, and 480 species.

At the phylum level, the three most represented lineages in both groups were Bacteroidota (CON: 26.59%; CPG: 30.27%), Firmicutes (CON: 39.07%; CPG: 15.50%), and Proteobacteria (CON: 7.18%; CPG: 32.97%), collectively accounting for more than 70% of total bacterial reads **(Figure 3a)**. Compared with the control cats, those receiving the probiotic supplement displayed reduced relative abundances of Firmicutes, Patescibacteria, Desulfobacterota, and Spirochaetota, whereas Proteobacteria and Actinobacteriota were proportionally higher in the CPG group ($p < 0.05$, (**Table 3)**).

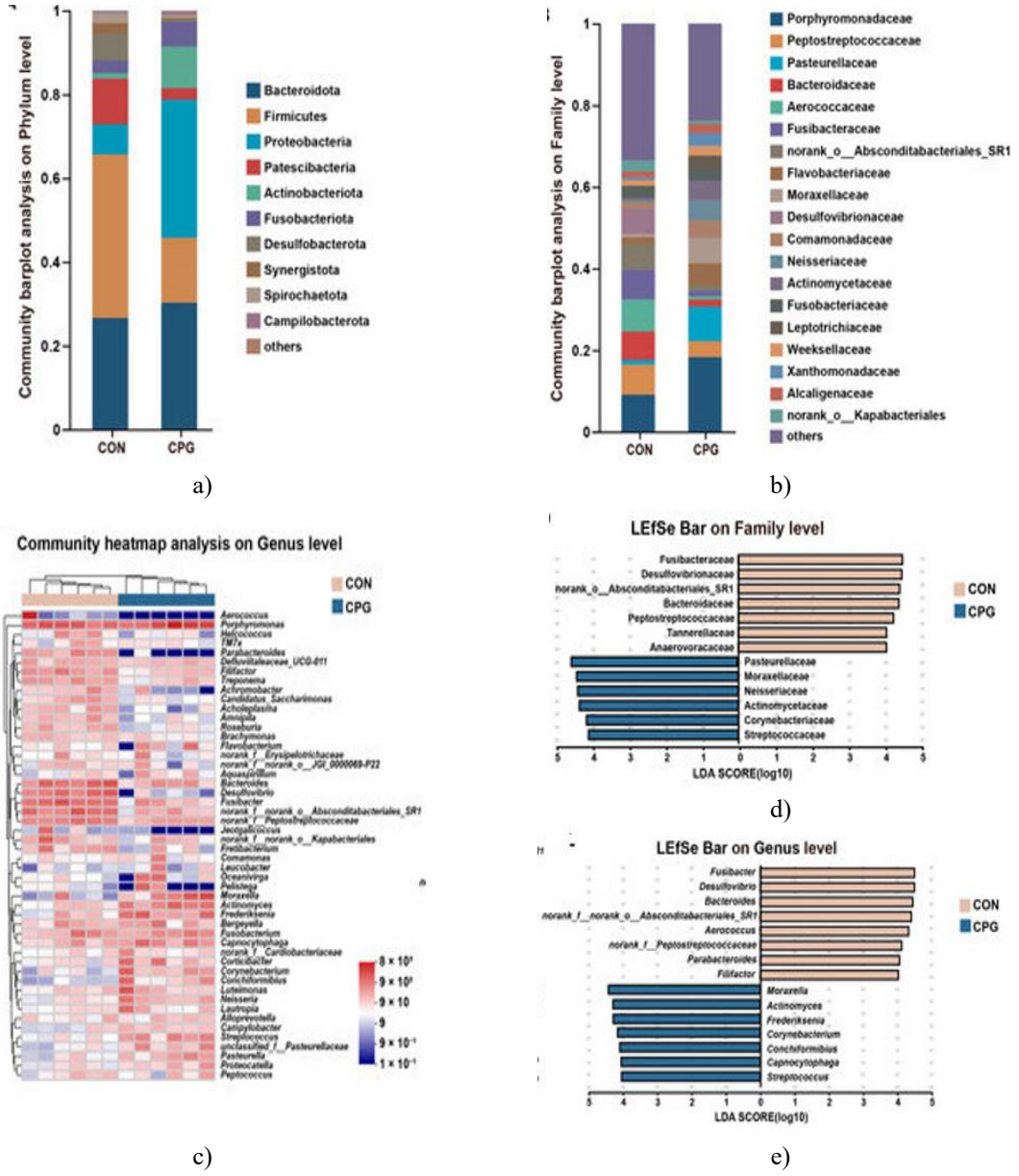


Figure 3. Gingival microbial community structure after 42 days of dietary intervention.
(a, b) Distribution at the phylum and family levels.
(c) Heatmap showing genus-level abundance.
(d, e) LEfSe outputs for discriminant taxa.
CON = basal diet only; CPG = basal diet plus post-sprayed composite probiotic.

Table 3. Differences in gingival bacterial composition on day 42.

Items	CON	CPG	p-Value
Firmicutes	39.074 ± 5.236	15.495 ± 2.310	0.005
Proteobacteria	7.181 ± 1.415	32.968 ± 6.508	0.008
Patescibacteria	10.917 ± 1.415	2.783 ± 0.804	0.005
Actinobacteriota	1.307 ± 0.252	9.877 ± 2.152	0.005
Desulfobacterota	6.355 ± 1.197	0.322 ± 0.112	0.005
Spirochaetota	2.344 ± 0.282	0.644 ± 0.230	0.008
Peptostreptococcaceae	7.414 ± 0.652	3.926 ± 0.886	0.02
Pasteurellaceae	1.107 ± 0.411	8.409 ± 1.961	0.005
Bacteroidaceae	6.970 ± 1.566	1.727 ± 0.511	0.02
Fusibacteraceae	7.240 ± 1.177	1.369 ± 0.549	0.005
norank_o_Absconditabacteriales_SR1	6.380 ± 1.326	1.222 ± 0.418	0.008

Moraxellaceae	0.822 ± 0.788	6.179 ± 2.563	0.03
Desulfovibrionaceae	6.192 ± 1.214	0.111 ± 0.081	0.005
Neisseriaceae	0.430 ± 0.148	5.002 ± 2.589	0.005
Actinomycetaceae	0.565 ± 0.156	4.832 ± 1.068	0.005
Corynebacteriaceae	0.218 ± 0.108	2.822 ± 1.379	0.008
Streptococcaceae	0.257 ± 0.090	2.764 ± 0.652	0.01
Anaerovoracaceae	2.690 ± 0.624	0.300 ± 0.101	0.005
Tannerellaceae	2.641 ± 0.496	0.330 ± 0.137	0.005
Bacteroides	6.970 ± 1.566	1.727 ± 0.511	0.02
Fusibacter	7.240 ± 1.177	1.369 ± 0.549	0.005
Aerococcus	7.636 ± 7.616	0	0.002
norank_f_norank_o_Absconditabacteriales_SR1	6.380 ± 1.326	1.222 ± 0.418	0.008
Moraxella	0.818 ± 0.788	6.086 ± 2.582	0.03
Desulfovibrio	6.187 ± 1.214	0.111 ± 0.081	0.005
norank_f_Peptostreptococcaceae	4.206 ± 0.521	1.558 ± 0.267	0.005
Actinomyces	0.565 ± 0.156	4.832 ± 1.068	0.005
Frederiksenia	0.688 ± 0.319	4.675 ± 1.473	0.02
Capnocytophaga	1.212 ± 0.406	3.532 ± 0.967	0.04
Filifactor	2.722 ± 0.540	0.719 ± 0.161	0.005
Corynebacterium	0.218 ± 0.108	2.822 ± 1.379	0.008
Streptococcus	0.257 ± 0.090	2.764 ± 0.652	0.01
Parabacteroides	2.362 ± 0.468	0.038 ± 0.038	0.004
Conchiformibius	0.057 ± 0.024	2.266 ± 1.236	0.005

Values expressed as mean ± SEM (n = 6); $p < 0.05$ considered significant.

At the family level **(Figure 3b)**, the dominant groups were Porphyromonadaceae (CON: 9.08%; CPG: 18.32%), Peptostreptococcaceae (CON: 7.41%; CPG: 3.93%), Pasteurellaceae (CON: 1.11%; CPG: 8.41%), and Bacteroidaceae (CON: 6.97%; CPG: 1.73%).

When classified by genus **(Figure 3c)**, the most abundant taxa were Porphyromonas (CON: 9.08%; CPG: 18.32%), Bacteroides (CON: 6.97%; CPG: 1.73%), and Fusibacter (CON: 7.24%; CPG: 1.37%).

Differential analysis combining LEfSe (LDA > 4) and the Wilcoxon rank-sum test identified that in the CON group, the following families and genera were significantly enriched: Fusibacteraceae, Desulfovibrionaceae, norank_o_Absconditabacteriales_SR1, Bacteroidaceae, Peptostreptococcaceae, Anaerovoracaceae, Tannerellaceae, Fusibacter, Desulfovibrio, Bacteroides, norank_f_norank_o_Absconditabacteriales_SR1, Aerococcus, norank_f_Peptostreptococcaceae, Parabacteroides, and Filifactor ($p < 0.05$).

By contrast, the CPG cats showed increased representation of Pasteurellaceae, Moraxellaceae, Neisseriaceae, Actinomycetaceae, Corynebacteriaceae, Streptococcaceae, Moraxella, Actinomyces, Frederiksenia, Capnocytophaga, Corynebacterium, Conchiformibius, and Streptococcus ($p < 0.05$) **(Figures 3d and 3e; Table 3)**.

Influence of the probiotic blend on tooth-surface microbial profiles in cats

For the microbial communities on tooth surfaces, 524 OTUs were shared by both groups, while 360 were unique to CON and 308 were exclusive to CPG. Taxonomic classification identified 17 phyla, 32 classes, 74 orders, 129 families, 233 genera, and 445 species.

As shown in **Figure 4a**, the most represented phyla were Bacteroidota (CON: 32.90%; CPG: 30.43%), Firmicutes (CON: 26.23%; CPG: 25.52%), and Proteobacteria (CON: 21.06%; CPG: 24.67%), accounting for roughly 80% of total bacterial sequences. At the family level **(Figure 4b)**, Porphyromonadaceae (CON: 20.97%; CPG: 12.54%), Staphylococcaceae (CON: 8.52%; CPG: 11.41%), Pasteurellaceae (CON: 6.55%; CPG: 8.69%), and Moraxellaceae (CON: 7.56%; CPG: 7.19%) predominated.

The main genera **(Figure 4c)** were Porphyromonas (CON: 20.97%; CPG: 12.54%), Moraxella (CON: 7.56%; CPG: 7.19%), and Staphylococcus (CON: 2.94%; CPG: 9.23%). Although principal coordinate analysis indicated no overall statistical separation of tooth-surface bacterial communities between groups, LEfSe (LDA > 3) revealed several discriminatory taxa.

Specifically, CPG cats showed higher abundances of Weeksellaceae, Streptococcaceae, Bergeyella, and Streptococcus, while Lachnospiraceae, Campylobacteraceae, Lentimicrobiaceae, Catonella, Helcococcus, Campylobacter, and Lentimicrobium were significantly depleted compared with CON ($p < 0.05$; (**Figures 4d–4e; Table 4**)).

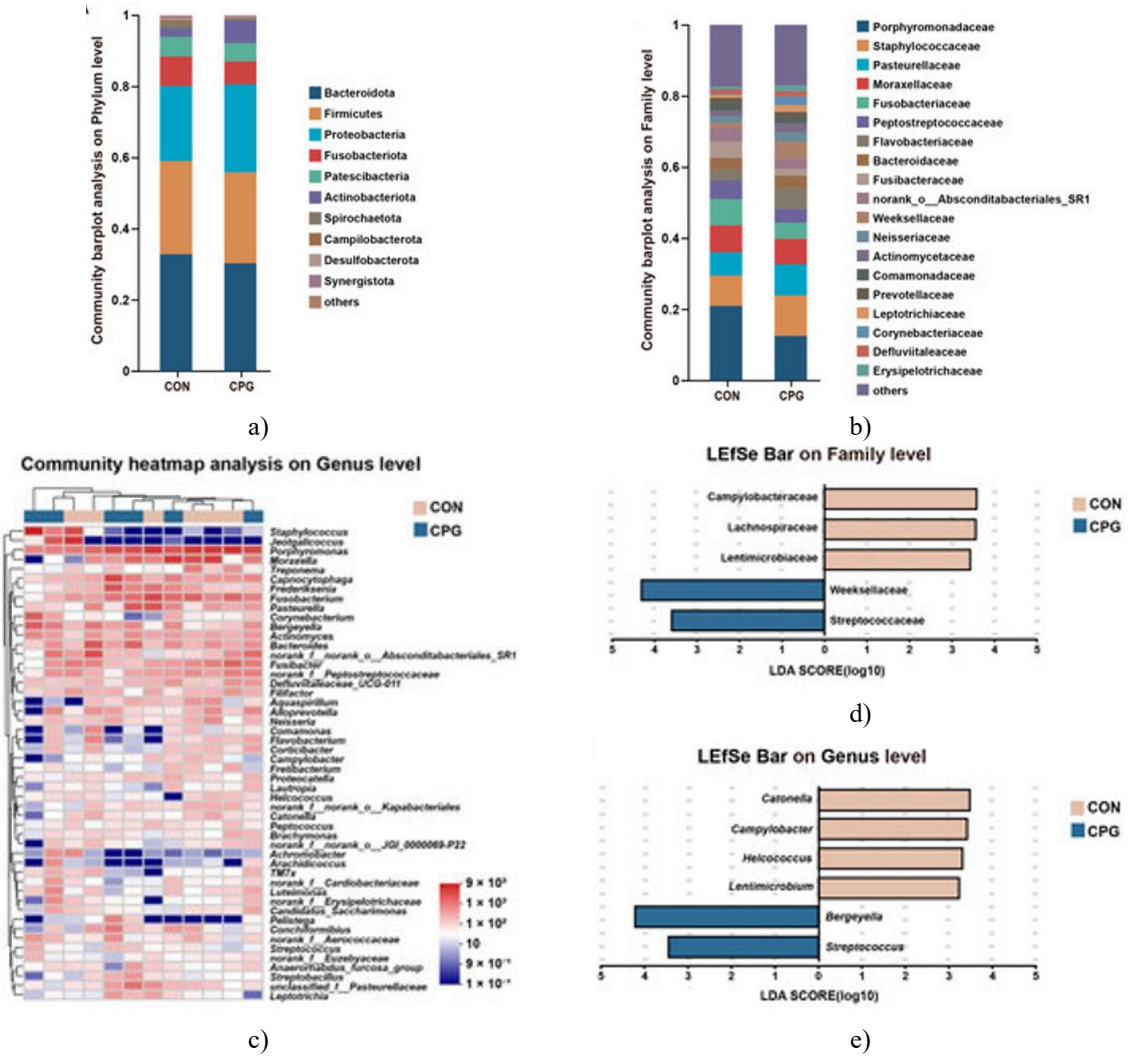


Figure 4. Influence of dietary supplementation on microbial populations attached to feline tooth surfaces at day 42.

(a, b) Relative microbial abundance displayed at phylum and family classification levels.

(c) Genera distribution visualized via heatmap.

(d, e) Identification of discriminant taxa through LEfSe assessment at family and genus ranks.

CON = cats consuming a basal diet; CPG = cats offered the same diet enhanced with a surface-applied probiotic mixture.

Table 4. Microbial shifts on the tooth surfaces of cats were recorded on day 42.

Items	CON	CPG	p-Value
Weeksellaceae	1.499 ± 0.822	4.919 ± 1.207	0.04
Lachnospiraceae	1.626 ± 0.182	0.904 ± 0.184	0.04
Streptococcaceae	0.170 ± 0.053	0.675 ± 0.220	0.04
Campylobacteraceae	0.600 ± 0.183	0.152 ± 0.090	0.04
Lentimicrobiaceae	0.037 ± 0.015	0.001 ± 0.001	0.02
Bergeyella	1.379 ± 0.837	4.612 ± 1.282	0.04
Catonella	0.887 ± 0.227	0.227 ± 0.057	0.02

Helcococcus	0.632 ± 0.138	0.259 ± 0.081	0.04
Streptococcus	0.167 ± 0.052	0.673 ± 0.221	0.04
Campylobacter	0.600 ± 0.183	0.152 ± 0.090	0.04
Lentimicrobium	0.037 ± 0.015	0.001 ± 0.001	0.02

CON = basal diet group; CPG = group fed basal diet with post-coated composite probiotics. Data are presented as mean ± SEM (n = 6). Significance threshold: $p < 0.05$.

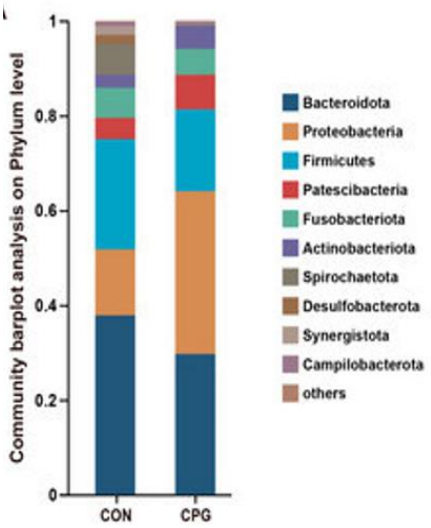
Impact of composite probiotics on tongue microbiota of cats

Sequencing results revealed 463 operational taxonomic units (OTUs) shared by both groups, while 342 were unique to the CON group and 259 were exclusive to CPG. Across the entire dataset, microbes were classified into 18 phyla, 35 classes, 81 orders, 139 families, 246 genera, and 449 species.

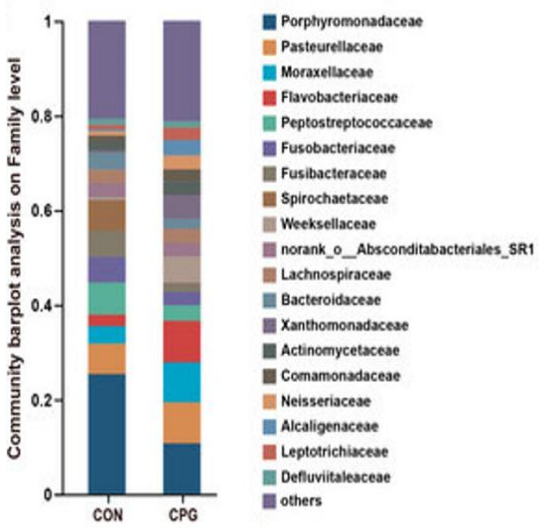
At the phylum level **(Figure 5a)**, microbial communities were primarily dominated by Bacteroidota (CON: 37.87%; CPG: 29.72%), Proteobacteria (CON: 13.90%; CPG: 34.36%), and Firmicutes (CON: 23.28%; CPG: 17.26%). Statistical testing using the Wilcoxon rank-sum method showed that Proteobacteria abundance increased significantly ($p < 0.05$), while Spirochaetota decreased ($p < 0.05$) in CPG relative to CON **(Table 5)**.

At the family level **(Figure 5b)**, the prevalent groups were Porphyromonadaceae (CON: 25.33%; CPG: 10.67%), Pasteurellaceae (CON: 6.42%; CPG: 8.70%), Moraxellaceae (CON: 3.67%; CPG: 8.41%), and Flavobacteriaceae (CON: 2.46%; CPG: 8.79%). The most common genera **(Figure 5c)** included Porphyromonas (CON: 25.33%; CPG: 10.67%), Moraxella (CON: 3.66%; CPG: 8.40%), and Fusobacterium (CON: 5.46%; CPG: 2.82%).

The LEfSe evaluation (LDA > 4) revealed clear distinctions between groups, identifying eight families and six genera that varied significantly. Four families—Porphyromonadaceae, Spirochaetaceae, Fusibacteraceae, and Peptostreptococcaceae—were enriched in CON ($p < 0.05$), whereas Flavobacteriaceae, Xanthomonadaceae, Weeksellaceae, and Neisseriaceae were more abundant in CPG ($p < 0.05$) **(Figure 5d; Table 5)**. On the genus scale, Porphyromonas, Treponema, and Fusibacter were characteristic of CON samples ($p < 0.05$), while Luteimonas, Bergeyella, and Flavobacterium were predominant in CPG ($p < 0.05$) **(Figure 5e; Table 5)**.



a)



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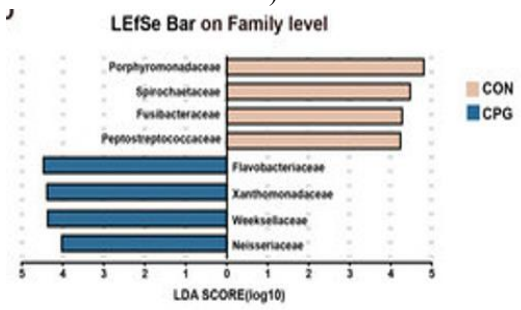
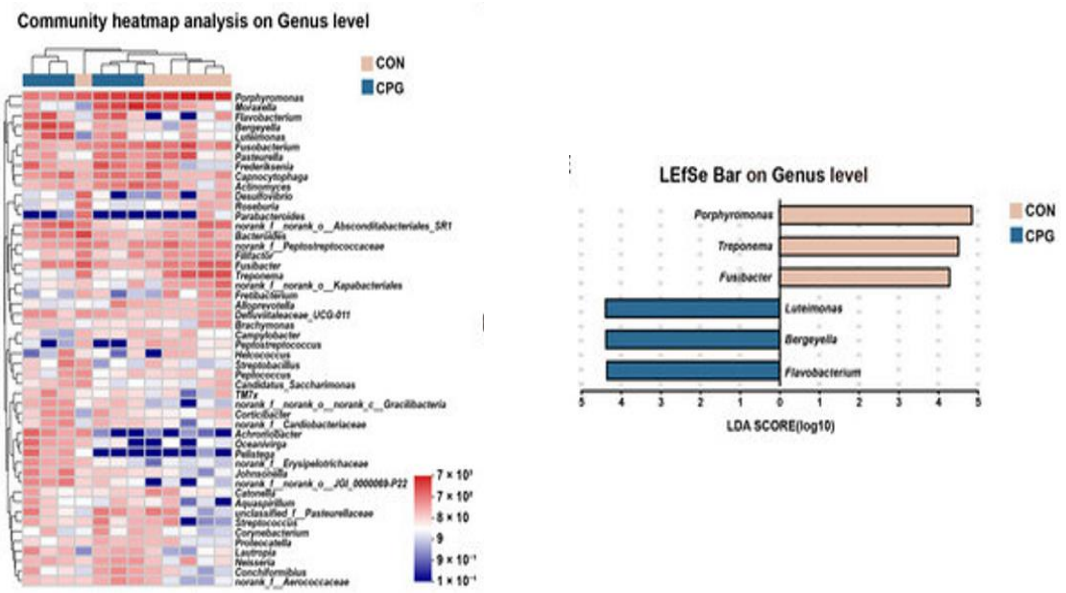


d)



c e

Figure 5. Comparative patterns of tongue microbial composition in cats at day 42 under distinct dietary conditions.

(a, b) Relative bacterial proportions are shown for phylum and family ranks.

(c) Genus-level distribution visualized through heatmap clustering.

(d, e) LEfSe results showing significantly enriched taxa for each treatment.

CON = control diet; CPG = diet supplemented with surface-applied composite probiotics.

Table 5. Microbial differences in tongue samples collected on day 42.

Items	CON	CPG	p-Value
Proteobacteria	13.900 ± 4.604	34.358 ± 2.901	0.01
Spirochaetota	6.544 ± 1.704	0.186 ± 0.036	0.005
Porphyromonadaceae	25.328 ± 4.148	10.671 ± 2.892	0.02
Flavobacteriaceae	2.465 ± 0.984	8.788 ± 1.762	0.01
Peptostreptococcaceae	6.767 ± 0.824	3.280 ± 0.797	0.03
Fusibacteraceae	5.448 ± 1.175	1.739 ± 0.477	0.03
Spirochaetaceae	6.536 ± 1.704	0.185 ± 0.036	0.005
Weeksellaceae	0.595 ± 0.261	5.599 ± 2.121	0.01
Xanthomonadaceae	0.439 ± 0.293	5.057 ± 1.765	0.01
Neisseriaceae	0.916 ± 0.324	2.909 ± 0.545	0.02
Porphyromonas	25.328 ± 4.148	10.671 ± 2.892	0.02
Fusibacter	5.448 ± 1.175	1.739 ± 0.477	0.03
Treponema	6.504 ± 1.696	0.176 ± 0.036	0.005
Bergeyella	0.413 ± 0.229	5.222 ± 2.128	0.03
Flavobacterium	0.422 ± 0.367	5.058 ± 1.697	0.01
Luteimonas	0.419 ± 0.294	4.718 ± 1.790	0.02

All data are given as mean ± SEM (n = 6); significance is defined at $p < 0.05$.

The oral cavity of mammals hosts a complex microbial community that strongly influences the onset and course of dental and gingival disorders [24]. Adjusting the diet to influence this ecosystem provides an opportunity to improve oral conditions. Research in humans and rodent models has demonstrated that adding probiotics to daily nutrition can rebalance the oral microbiome and reduce disease severity [11, 12]. However, little evidence exists for their role in feline species. To address this gap, the present experiment evaluated how a blended probiotic supplement affects microbial organization across oral sites in cats.

The findings suggest that probiotic administration can remodel microbial populations differently in each oral microenvironment. Increased abundance of beneficial or commensal organisms was observed, including Moraxella, Actinomyces, and Frederiksenia within gingival tissues; Bergeyella and Streptococcus on dental surfaces; and Bergeyella, Flavobacterium, and Luteimonas within the tongue community. Meanwhile, bacteria linked with oral pathology—such as Bacteroides, Desulfovibrio, and Filifactor in gingival samples; Helcococcus and Campylobacter on teeth; and Porphyromonas and Treponema on the tongue—showed marked reductions in prevalence.

These alterations indicate that dietary inclusion of the composite probiotic may promote the proliferation of favorable taxa while suppressing potentially harmful ones, contributing to a healthier oral microbial equilibrium in cats.

This research applied a mixed probiotic formulation consisting of Bifidobacterium animalis subsp. lactis HN019, Lactobacillus acidophilus NCFM, and Lactobacillus casei LC-11. Prior laboratory findings have shown that Bifidobacterium strains can hinder the proliferation of periodontal pathogens such as Porphyromonas gingivalis while sparing beneficial species like Streptococcus [25]. In addition, a clinical investigation in humans reported that supplementation with B. animalis subsp. lactis HN019 eased systemic inflammation in individuals suffering from advanced chronic periodontitis, diminished the presence of pathogenic organisms in periodontal pockets, and possibly colonized the subgingival niche [12].

The probiotic genus Lactobacillus is well recognized for several oral health benefits, including anti-inflammatory capacity [26], reduction of oral malodor [27], inhibition of disease-associated microbes [28], and suppression of biofilm accumulation through the production of metabolites such as hydrogen peroxide, organic acids, and bacteriocins [29, 30]. Furthermore, L. acidophilus and L. casei have been demonstrated to reduce periodontal inflammation and to influence microbial balance within the oral environment [13, 14, 31].

A total of twelve healthy adult cats were included in a 42-day randomized controlled experiment. Before treatment began, differences in microbial diversity between the two dietary groups were assessed to account for natural individual variation. Initial analyses showed no notable differences in α- or β-diversity of the gingival, dental, or lingual microbiota. Reports in the literature have noted that cats with gingivostomatitis or periodontitis typically display greater α-diversity indices (e.g., Shannon and Chao1) than healthy cats [3], though this trend is not universal [32]. Likewise, oral conditions in humans have been associated with expanded microbial richness and variety [33].

During this study, supplementation for 42 days with the composite probiotic did not cause significant changes in microbial diversity or species richness at any oral site, which aligns with what would be expected in healthy individuals. Nevertheless, prior investigations revealed that cats affected by oral disorders experience microbial dysbiosis, as reflected by β-diversity showing clear community separation from that of healthy controls [3]. It is known that dietary adjustments can reshape microbial communities in the feline mouth [34]; notably, by day 42, probiotic treatment produced distinct alterations in microbial composition on the gingiva and tongue.

Microbial profiling further confirmed that the major bacterial phyla—Bacteroidota, Firmicutes, and Proteobacteria—were consistently dominant on the gums, teeth, and tongue, consistent with profiles previously documented in healthy felines [35]. Earlier studies have described higher proportions of Bacteroidota, Firmicutes, Synergistota, Chloroflexi, Fusobacteria, and Spirochaetota in diseased cats, whereas Proteobacteria generally predominate in healthy animals [3, 36]. In the present work, probiotic administration led to a reduction of Spirochaetota populations on both the gingival and lingual surfaces and an increase in Proteobacteria, while Firmicutes levels declined in the gingival samples. A significant drop in Treponema—recognized as a periodontal disease indicator—was also observed on the tongue. Treponema, a member of the phylum Spirochaetota, contributes to host tissue inflammation through metabolic toxins and adhesion-related mechanisms [37].

The phylum Actinobacteriota, which plays an important role as an early colonizer contributing to oral ecological balance and periodontal integrity [36, 38], increased following probiotic intake. Specifically, Actinomyces and Corynebacterium were significantly more abundant in the gingival microbiota of supplemented cats, echoing prior research findings [3, 4]. In contrast, Desulfovibrio—a genus within Desulfobacterota identified as a marker of periodontitis—was markedly reduced in the gingival community after probiotic administration [39].

In this experiment, the bacterial families Porphyromonadaceae and Pasteurellaceae remained among the three most represented taxa across all examined oral niches of cats. These two families have been consistently reported as core constituents of the feline oral ecosystem [35]. The genus Porphyromonas, although normally present in the oral cavity, has been strongly implicated in periodontal lesions [40]. Certain species within this genus can

avoid host immune detection and suppress bactericidal activity, creating inflammatory and dysbiotic oral conditions [41, 42]. Furthermore, Porphyromonas is capable of producing lipopolysaccharides and proteolytic enzymes that deteriorate soft periodontal tissues [42]. Conversely, Frederiksenia, a member of the Pasteurellaceae family, was previously found in greater proportions in healthy felines compared with those affected by gingivitis [4]. In the current trial, cats supplemented with the composite probiotic showed a decline in Porphyromonas on the tongue and an increase in Frederiksenia at gingival sites, suggesting that the formulation might enhance oral microbial balance.

Consistent with previous literature, Moraxella and Fusobacterium were also abundant in the oral microbiome of healthy cats [3], aligning with the patterns observed here. Notably, variations were detected in the dominant bacterial hierarchy across the gingiva, teeth, and tongue, both at the phylum and genus levels. Such variability likely reflects microenvironmental differences—including fluctuations in oxygen availability, surface structure, epithelial composition, and local immune defense [17, 43]. Additionally, the microbial populations at these three sites responded differently to probiotic intake, indicating that the effects of supplementation may depend on the specific oral habitat.

After 42 days of dietary treatment, there was a rise in Streptococcus on the gingival and dental surfaces and an increase in Bergeyella on both the tongue and teeth. Earlier studies noted that these genera function as symbiotic inhabitants and are negatively associated with oral plaque formation and disease [19, 35]. It has been proposed that the anaerobic, nutrient-rich environment of the subgingival zone favors the growth of Gram-negative anaerobes, promoting oral pathology [44, 45]. Supporting this, previous findings revealed elevated Gram-negative populations in cats with gingival and periodontal disorders [3, 32].

In addition to these taxa, the present work recorded notable declines in Bacteroides and Parabacteroides in gingival samples and in Campylobacter and Lentimicrobium from tooth surfaces after probiotic administration. The CPG group also showed reduced presence of genera linked to oral disease, including Filifactor [3], Aerococcus [46], and Helcococcus [47], while exhibiting higher frequencies of bacteria typically associated with oral health, such as Capnocytophaga [48], Conchiformibius [4], Flavobacterium [4], and Luteimonas [49], particularly in the gingival and lingual areas. Taken together, these shifts imply that the probiotic blend may support microbial stability and defense against disease-associated bacteria within the feline oral environment.

Some restrictions apply to this study. The 42-day trial duration might have been too brief to reveal the full effects of probiotic feeding; long-term studies could provide stronger evidence. Moreover, this experiment involved a small sample of healthy cats, aiming to explore preventive potential rather than therapeutic benefit. Future research should include larger cohorts and cats diagnosed with oral disorders to test whether probiotics could aid in treatment. Although this work clarifies how probiotic intake reshapes oral microbial patterns, additional research is required to explain the mechanistic pathways responsible for these microbial shifts.

Conclusion

Administration of the composite probiotic—comprising Bifidobacterium animalis subsp. lactis HN019, Lactobacillus acidophilus NCFM, and Lactobacillus casei LC-11—altered the oral microbial landscape of clinically healthy cats. Distinct oral habitats displayed differential responses, with reductions in pathogenic taxa such as Bacteroides, Desulfovibrio, and Filifactor on the gingiva; Helcococcus and Campylobacter on tooth surfaces; and Porphyromonas and Treponema on tongues. At the same time, beneficial taxa like Moraxella, Actinomyces, and Frederiksenia were enriched on the gingiva; Bergeyella and Streptococcus on teeth; and Bergeyella, Flavobacterium, and Luteimonas on tongues. These microbial adjustments indicate that the tested probiotic complex could contribute to maintaining oral microbial equilibrium and potentially assist in the prevention or management of feline oral diseases, though confirmation through longer-term and disease-model studies is still warranted.

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Ethics Statement: None

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