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# Effects of Synbiotic Supplementation on Fecal Characteristics and Microbiota in Dogs Submitted to an Abrupt Dietary Change

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**Abstract**

Stressful situations can negatively affect the intestinal microbiota and contribute to gastrointestinal disorders. This study investigated the effects of a synbiotic (SYN) on fecal characteristics and ammonia concentrations, selected fecal bacterial groups, and dysbiosis index (DI) of healthy adult Beagles undergoing an abrupt dietary change. Sixteen adult dogs were divided into two groups: a placebo (PLA, no supplementation, $n = 8$) and a SYN supplementation group ($n = 8$). Dogs from both groups were fed a highly digestible diet for 20 days. On day 21, all dogs underwent an abrupt transition to a less digestible diet, which they continued to receive for an additional 20 days. Fecal samples were collected on days 20, 22, and 40 to analyze fecal dry matter, score, ammonia, pH, microbiota, and DI. The SYN supplementation did not affect fecal characteristics or DI of dogs ($p > 0.05$). After dietary change, an increase in fecal *Bifidobacterium* and universal bacteria was observed only in the PLA group ($p < 0.05$). In addition, dietary transition increased DI and fecal *Escherichia coli* regardless of the SYN supplementation ($p < 0.05$). Dogs fed the highly digestible diet presented greater fecal *Peptacetobacter hiranonis* and lower *Streptococcus*, *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* ($p < 0.05$). Dogs supplemented with SYN presented lower fecal *Clostridium perfringens* than the PLA group when fed the less digestible diet ($p < 0.05$). These results suggest that abrupt dietary change may promote alterations associated with intestinal dysbiosis in dogs. Moreover, SYN supplementation was associated with lower fecal *C. perfringens* abundance in dogs fed the less digestible diet.

**Keywords:** digestibility; dysbiosis; probiotic



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## 1. Introduction

Dogs are frequently exposed to potentially stressful situations, such as weaning, abrupt changes in diet, illness, and medication use. Among these, abrupt dietary changes are particularly relevant in clinical and practical contexts, as they can rapidly alter the substrate reaching the colon for microbial fermentation. These changes may affect the composition and diversity of the intestinal microbiota [1], negatively impacting the gastrointestinal environment and potentially leading to dysbiosis, an imbalance in the dogs' intestinal microbiota.

Recently, functional additives studied in the nutrition of companion animals have demonstrated the potential to positively modulate the intestinal microbiota and its

metabolites [2]. Among these additives, probiotics, prebiotics, and synbiotics are particularly relevant [1].

Probiotics are live microorganisms that can confer health benefits to the host when administered in adequate amounts [3]. Their use may contribute to intestinal functionality through several mechanisms, including the suppression of intestinal pathogens [4], production of antimicrobial substances [5], increase in the host's immune response [6], and regulation of metabolites in the intestine [7]. In dogs, among the most studied probiotic strains, including live bacteria and yeast, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Bacillus subtilis* and *Saccharomyces cerevisiae* have demonstrated notable effects on intestinal functionality. These effects include reductions in potentially pathogenic bacteria and nitrogenous compounds in the intestine, as well as increased fecal IgA concentrations [2,8–10]. In addition, prebiotics are selectively fermentable ingredients that promote beneficial changes in the composition and activity of the gastrointestinal microbiota [3]. The most studied prebiotics for dogs include fructooligosaccharides (FOS), mannanoligosaccharides (MOS), galactooligosaccharides, and inulin, which have been associated with immune modulation (innate and adaptive responses), increased fecal IgA concentrations, and changes in intestinal microbiota composition and its metabolic activity in dogs [11–14]. Therefore, synbiotics are preparations that combine these probiotic microorganisms and prebiotic substrates to promote synergistic effects on intestinal microbiota and gastrointestinal functionality in dogs [3].

Although previous studies in dogs have demonstrated the beneficial effects of synbiotics on fecal microbiota diversity, modulation of microbial populations, and a reduction in the incidence of diarrhea, most of these investigations were conducted under stable dietary conditions or using formulations with highly variable compositions, including different probiotic strains and prebiotic combinations [15–17]. In addition, differences in study design, prebiotic or probiotic sources, supplementation periods, and evaluated outcomes make comparisons across studies difficult. Consequently, evidence regarding the efficacy of synbiotic supplementation specifically during abrupt dietary changes remains limited and inconsistent, despite this being a common situation in canine management and clinical practice. Furthermore, few studies have simultaneously evaluated fecal characteristics, microbiota modulation, and the dysbiosis index (DI) under these conditions. These parameters are relevant because fecal score and fecal dry matter are commonly used indicators of stool quality and intestinal tolerance to the diet, while fecal ammonia concentrations and pH may reflect changes in colonic fermentation patterns and substrate utilization. In addition, the DI and selected bacterial taxa have been proposed as biomarkers of gastrointestinal health in dogs [18,19].

Therefore, our hypothesis is that the synbiotic supplementation would mitigate the negative effects of an abrupt dietary change by improving fecal characteristics, reducing DI, and favorably modulating the composition of the gut microbiota compared to dogs in the placebo group. Based on this hypothesis, the aim of this study was to evaluate the effects of a synbiotic on fecal characteristics and ammonia concentrations, selected fecal bacterial groups and DI in healthy adult dogs undergoing an abrupt dietary change using a placebo-controlled, double-blinded experimental design.

## 2. Materials and Methods

### *2.1. Ethical Statements*

The use of animals for this study was approved by the Ethics Committee on Animal Use of the Agrarian Sciences Sector of the Federal University of Paraná, Curitiba, PR, Brazil, under protocol no. 066/2023. The study was conducted at the Research Laboratory on Canine Nutrition—LENUCAN in Curitiba, Paraná, Brazil (25°25′40″ S, 49°16′23″ W).

### 2.2. Animals and Facilities

Sixteen neutered/spayed adult Beagle dogs (8 males and 8 females) of approximately 1.5 ± 0.03 years old, with an average body weight of 11.22 ± 1.10 kg and a body condition score of 4.8 ± 0.6 on a scale of 1 to 9, were used [20]. All animals underwent prior clinical evaluation and were considered healthy. During most of the experimental period, dogs had free access to a 1137 $m^2$ outdoor area for 4 h/day for voluntary exercise and socialization. During fecal collection, the dogs were individually housed in brickwork kennels (5 m long × 2 m wide) containing a bed and free access to fresh water. The facilities had side wall bars that allowed limited visual interaction with neighboring dogs. The ambient temperature varied from 16 °C to 28 °C, with a 12 h light-dark cycle (light from 6:00 a.m. to 6:00 p.m.).

### 2.3. Experimental Groups

Two experimental treatments were evaluated: a placebo (PLA) and a synbiotic (SYN). The SYN used was a commercially available product. Each of the 8 dogs received two tablets of either PLA or SYN, administered orally twice daily in a placebo-controlled, double-blind trial for 40 consecutive days. The dogs were divided based on sex and body weight, with each group consisting of 4 males and 4 females and an approximately equivalent average body weight. Each tablet of the SYN treatment consisted of 500 mg of a blend containing $1.8 \times 10^8$ CFU of *Lactobacillus lactis*, $1.2 \times 10^8$ CFU of *Bifidobacterium bifidum*, $2.4 \times 10^8$ CFU of *Enterococcus faecium*, $3.0 \times 10^8$ CFU of *Lactobacillus acidophilus*, $2.4 \times 10^8$ CFU of *Lacticaseibacillus casei*, $9 \times 10^7$ CFU of *Saccharomyces cerevisiae*, $1.2 \times 10^8$ CFU of *Bacillus subtilis*, 1700 mg/kg of MOS, 880 mg/kg of inulin, 9800 mg/kg of glutamine, 290 ug/kg of cyanocobalamin, silicon dioxide, magnesium stearate, maltodextrin and flavoring (LactobacDog® Plus Tabs, Organnact, Curitiba, Brazil). The PLA tablet contained silicon dioxide (1.5 mg), magnesium stearate (2.5 mg), maltodextrin (211 mg), and flavoring (2.5 mg). The SYN and PLA products were prepared in identical tablets and coded to ensure blinding both the researchers responsible for animal care and sample collection and the evaluators in charge of laboratory analyses and data.

During the first 20 days of the experiment, all dogs received a highly digestible (HD) complete dry extruded diet for adult dogs that did not contain any functional additives that may impact intestinal functionality, such as prebiotics or probiotics. On the 21st day, the dogs underwent an abrupt dietary change to a less digestible (LD) complete dry extruded diet for adult dogs, also free of functional additives. This dietary change was intended to promote differences in fermentable substrate available to the intestinal microbiota of dogs. The dogs consumed the LD diet for another 20 days, totalizing 40 experimental days. The animals were fed twice a day according to their metabolizable energy requirements for maintenance [21]. Feed intake (g/dog/day) was recorded daily for each animal.

Table 1 describes the diets' chemical composition, apparent digestibility coefficients (ADC), and metabolizable energy (ME). The ADC and ME of the diets were determined in a previous internal validation study (not published) using the same experimental diets and the same 16 Beagle dogs enrolled in the present study (8 males and 8 females; approximately 1 year of age; mean body weight of 10.3 ± 0.86 kg). Nutrient digestibility and ME were determined using the total fecal collection method according to the Association of American Feed Control Officials [22]. Total fecal output and food intake were recorded, and nutrient analyses were performed according to the Association of Official Analytical Chemists (AOAC) procedures [23].

**Table 1.** Chemical composition (%, dry matter), apparent digestibility coefficients (ADC, %), and metabolizable energy (ME, kcal/kg) of highly digestible (HD) and less digestible (LD) diets.

| Item | HD [1] | LD [2] |
|---|---|---|
| Chemical composition | | |
| Dry matter | 93.89 | 93.64 |
| Crude protein | 24.76 | 24.18 |
| Crude fiber | 1.78 | 3.81 |
| Ether extract in acid hydrolysis | 13.09 | 10.40 |
| Ash | 7.51 | 9.19 |
| Calcium | 1.65 | 2.74 |
| Phosphorus | 0.81 | 1.14 |
| ADC | | |
| Dry matter | 82.6 | 70.7 |
| Organic matter | 85.7 | 75.2 |
| Crude protein | 86.1 | 78.2 |
| Ether extract in acid hydrolysis | 91.2 | 89.0 |
| Metabolizable energy | 3925.2 | 3265.8 |

[1] Main ingredients of the HD diet: corn, poultry by-product meal, rice bran, defatted rice bran, hydrolyzed poultry liver, poultry fat, vitamins, and minerals. [2] Main ingredients of the LD diet: corn, wheat bran, meat and bone meal, hydrolyzed feather meal, hydrolyzed poultry liver, poultry fat, vitamins, and minerals.

### *2.4. Chemical Composition, Fecal Characteristics, and Microbiota-Related Analyses*

For the determination of the chemical composition of the diets, each of them (HD and LD) was ground to 1 mm in a hammer mill (Arthur H. 149 Thomas Co., Philadelphia, PA, USA) and analyzed for dry matter (DM) at 105 °C for 12 h, nitrogen (N, method 954.01), crude protein (CP), which was calculated as N × 6.25, crude fiber (method 994.13), ash (method 942.05), and acid-hydrolyzed ether extract (AEE) (method 954.02). All analyses followed the recommendations of the AOAC [23]. Gross energy (GE) was determined in a calorimeter pump (IKA C2000 Basic, IKA-Werke, Staufen, Germany). The organic matter was calculated by: 100%–% ash.

Fresh feces were collected with up to 15 min of defecation on days 20, 22, and 40 for analyses of fecal DM (fDM), score, pH, ammonia, and microbiota. Fecal scores were always assessed by the same researcher, assigning points from 1 to 5, being 1 = soft and unformed feces; 2 = soft and poorly formed feces; 3 = soft, formed, and moist feces; 4 = well-formed and consistent feces; and 5 = well-formed, hard, and dry feces, according to [24]. Fecal pH was measured using a digital pH meter (331, Politeste Instrumentos de Teste Ltd.a., São Paulo, Brazil) in 3.0 g of fresh feces diluted in 30 mL of distilled water. Fecal ammonia concentrations were determined according to [25].

For fecal microbiota analyses, DNA extraction from 100 mg of each fecal sample was performed using the MoBio PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions and as previously described by [18]. Quantitative PCR (qPCR) assays were performed according to the validated protocol described by [18].

The qPCR panel used for DI calculation included total bacteria (universal 16S rRNA gene), *Faecalibacterium* spp., *Turicibacter* spp., *Escherichia coli*, *Streptococcus* spp., *Blautia* spp., *Fusobacterium* spp., and *Peptacetobacter hiranonis*. In addition, *Bacteroides*, *Clostridium perfringens*, *Lactobacillus*, *Ruminococcus*, *Prevotella*, *Collinsella*, and *Bifidobacterium* were also quantified by qPCR as described before [26]. Briefly, the conditions for qPCR were as follows: initial denaturation at 98 °C for 2 min, then 40 cycles with denaturation at 98 °C for 3 s, and annealing for 3 s. Melt curve analysis was performed to validate the specific generation of the qPCR product using these conditions: 95 °C for 1 min, 55 °C for 1 min, and increasing incremental steps of 0.5 °C for 80 cycles for 5 s each. Each reaction was

run in duplicate. Data were expressed as log DNA abundance (fg) per 10 ng of total isolated DNA.

The abundance of the bacterial groups included in the validated panel was used to calculate the DI according to the mathematical algorithm previously described by [18]. Dysbiosis was classified as significant (DI > 2), mild to moderate (DI 0–2), minor changes (DI < 2 with individual bacterial groups outside the reference interval), and normal (DI < 2 without overall shifts in intestinal microbial balance).

### *2.5. Statistical Analysis*

Data were analyzed for normality by the Shapiro–Wilk test. Data with normal distribution were subjected to mixed effects analysis of variance (ANOVA), considering the effects of diet (HD on day 20, LD on day 22, and LD on day 40), supplementation (PLA and SYN), and the diet x supplementation interaction as fixed effects. Dogs were considered random effects and accounted for repeated measures obtained from the same animal over time. In addition, each dog was considered an experimental unit, totaling 8 repetitions per treatment. When the ANOVA F-test indicated a statistical difference for diet or diet x supplementation interaction ($p < 0.05$), the means were compared using the Tukey test ($p < 0.05$). The analyses were performed using MiniTab®18 software.

## 3. Results

There were no episodes of food refusal, vomiting, or diarrhea during the experiment. All dogs maintained constant weight and body condition scores throughout the study and fully consumed the tablets and diets, with no differences in food intake among groups ($p > 0.05$). Table 2 presents the DI and fecal bacteria abundances.

**Table 2.** Means of the dysbiosis index (DI) and fecal bacteria (logDNA) of dogs fed the highly digestible (HD) and less digestible (LD) diets and receiving placebo (PLA) or synbiotic (SYN) supplementation (SU).

| Diet (D) | HD | | CHLD [1] | | LD | | SEM [2] | *p*-Value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SU | SYN | PLA | SYN | PLA | SYN | PLA | | D | SU | D × SU |
| DI | −1.93 [b] | −2.76 [b] | −0.64 [a] | −1.05 [a] | −0.66 [a] | −0.32 [a] | 0.169 | <0.001 | 0.222 | 0.143 |
| Total bacteria | 10.79 [ab] | 10.76 [ab] | 10.60 [b] | 10.94 [a] | 10.69 [b] | 10.78 [ab] | 0.027 | 0.758 | 0.008 | 0.008 |
| *Faecalibacterium* | 4.46 [b] | 4.48 [b] | 4.41 [b] | 4.65 [b] | 5.27 [a] | 5.04 [a] | 0.091 | 0.002 | 0.939 | 0.511 |
| *Turicibacter* | 8.35 [a] | 8.36 [a] | 7.88 [b] | 8.12 [b] | 8.24 [ab] | 7.98 [ab] | 0.053 | 0.018 | 0.984 | 0.133 |
| *Streptococcus* | 6.34 [b] | 5.64 [b] | 6.52 [b] | 6.38 [b] | 7.38 [a] | 7.59 [a] | 0.133 | <0.001 | 0.285 | 0.172 |
| *Escherichia coli* | 5.11 [b] | 4.92 [b] | 5.94 [a] | 6.00 [a] | 5.35 [b] | 5.52 [b] | 0.092 | <0.001 | 0.920 | 0.601 |
| *Blautia* | 10.46 [a] | 10.37 [a] | 10.29 [b] | 10.18 [b] | 10.23 [ab] | 10.29 [ab] | 0.031 | 0.036 | 0.426 | 0.449 |
| *Fusobacterium* | 7.98 [b] | 7.85 [b] | 7.95 [b] | 7.98 [b] | 8.50 [a] | 8.45 [a] | 0.088 | 0.017 | 0.761 | 0.918 |
| *Peptacetobacter hiranonis* | 6.86 [a] | 6.81 [a] | 6.31 [b] | 6.57 [b] | 6.36 [b] | 6.50 [b] | 0.039 | <0.001 | 0.040 | 0.065 |
| *Bifidobacterium* | 4.37 [b] | 5.37 [b] | 4.38 [b] | 6.82 [a] | 6.90 [a] | 6.94 [a] | 0.201 | <0.001 | <0.001 | 0.001 |
| *Bacteroides* | 5.04 [b] | 4.98 [b] | 4.96 [b] | 5.04 [b] | 5.54 [a] | 5.75 [a] | 0.089 | 0.003 | 0.648 | 0.785 |
| *Clostridium perfringens* | 4.20 [a] | 4.00 [a] | 2.80 [b] | 2.76 [b] | 1.68 [c] | 2.64 [b] | 0.157 | <0.001 | 0.233 | 0.049 |
| *Lactobacillus* | 5.94 [b] | 6.71 [b] | 7.37 [a] | 7.49 [a] | 6.61 [a] | 7.44 [a] | 0.115 | <0.001 | 0.001 | 0.200 |
| *Ruminococcus* | 9.99 | 10.01 | 10.15 | 10.12 | 10.38 | 10.19 | 0.070 | 0.308 | 0.648 | 0.821 |
| *Prevotella* | 12.12 [b] | 12.52 [b] | 13.04 [b] | 12.53 [b] | 13.75 [a] | 13.73 [a] | 0.125 | <0.001 | 0.818 | 0.139 |
| *Collinsella* | 13.51 [a] | 13.59 [a] | 13.13 [b] | 13.30 [b] | 13.33 [b] | 13.25 [b] | 0.032 | <0.001 | 0.267 | 0.149 |

[1] CHLD: Change to the less digestible diet. [2] SEM: standard error of the mean. [a,b,c] Means followed by different letters differ by Tukey's test ($p < 0.05$).

Before the dietary transition (day 20), no significant differences were observed between treatment groups for DI or fecal bacteria abundances ($p > 0.05$).

After transitioning to the LD diet (day 22), the DI increased in all experimental groups ($p < 0.05$), demonstrating that the challenge altered the fecal microbiota composition. However, all DI values remained below 0, which is expected for healthy dogs without gastrointestinal disorders [18]. SYN supplementation did not affect the DI throughout the study ($p > 0.05$).

Overall, the dietary transition presented a greater effect on fecal microbiota composition than the SYN supplementation. In both treatments, consumption of the LD diet increased the fecal abundance of *Faecalibacterium*, *Streptococcus*, *Fusobacterium*, *Bacteroides*, *Prevotella*, and *Escherichia coli* (day 22 only) compared with the HD diet ($p < 0.05$). Additionally, dogs fed the HD diet presented greater fecal abundance of *P. hiranonis* and *Collinsella*, while *Lactobacillus* was lower in dogs fed the HD diet ($p < 0.05$), regardless of supplementation.

Total bacteria and *Bifidobacterium* were greater in the PLA group compared with the SYN group after the dietary transition on day 22 ($p < 0.05$). Dogs in the PLA group also presented a higher fecal abundance of *P. hiranonis* and *Lactobacillus*, regardless of diet ($p < 0.05$). In contrast, SYN supplementation was associated with lower fecal abundance of *Clostridium perfringens* in dogs fed the LD diet ($p < 0.05$).

Dogs fed the HD diet presented higher fDM, fecal score, ammonia, and pH compared with dogs fed the LD diet ($p < 0.05$), indicating diet-related changes in fecal characteristics and fermentation products. The SYN supplementation did not influence dogs' fecal characteristics and fecal ammonia concentrations ($p > 0.05$, Table 3).

**Table 3.** Means of fecal characteristics of dogs fed the highly digestible (HD) and less digestible (LD) diets receiving placebo (PLA) or synbiotic (SYN) supplementation (SU).

| Diet (D) | HD | | CHLD [1] | | LD | | SEM [2] | *p*-Value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SU | SYN | PLA | SYN | PLA | SYN | PLA | | D | SU | D × SU |
| fDM (%) | 36.70 [a] | 37.94 [a] | 29.00 [b] | 29.66 [b] | 30.19 [b] | 30.98 [b] | 0.60 | <0.001 | 0.200 | 0.922 |
| Score | 3.5 [a] | 3.6 [a] | 3.1 [b] | 3.0 [b] | 3.0 [b] | 3.2 [b] | 0.09 | 0.049 | 0.462 | 0.483 |
| Ammonia (g/kg) | 1.71 [a] | 1.84 [a] | 1.59 [a] | 1.62 [a] | 0.99 [b] | 1.28 [b] | 0.06 | <0.001 | 0.135 | 0.570 |
| pH | 7.08 [a] | 6.87 [a] | 6.71[b] | 6.31 [b] | 6.81 [ab] | 6.93 [ab] | 0.07 | 0.023 | 0.346 | 0.166 |

[1] CHLD: Change to the less digestible diet. [2] SEM: standard error of the mean. Score: 1 = liquid stool to 5 = dry stool. fDM: fecal dry matter (%). [a,b] Means followed by different letters differ by Tukey's test ($p < 0.05$).

## 4. Discussion

The present study used an abrupt transition from an HD to an LD diet as a challenge model to evaluate the effects of a SYN in healthy dogs. Abrupt dietary changes are known to rapidly modulate the intestinal microbiota and may influence the DI depending on diet composition and digestibility [2,27]. Gut microbiome imbalance is commonly associated with gastrointestinal disorders, demonstrating the importance of maintaining a stable microbiome [28].

In this context, dogs consuming the HD diet presented higher fecal *P. hiranonis* abundance. This bacterium is present in healthy animals and plays an important role in converting primary bile acids into secondary ones in the intestine [29]. As *P. hiranonis* is also a key taxon included in the canine DI, and its reduction has been consistently associated with chronic enteropathies and dysbiosis [18,30], the higher abundance observed in dogs fed the HD diet may reflect a more favorable intestinal microbial environment associated with highly digestible diets.

This interpretation is consistent with the fecal characteristics observed in dogs fed the HD diet, which also exhibited higher fecal consistency and higher fDM content than animals fed the LD diet. Since fecal characteristics are influenced by both nutrient digestibility and dietary fiber content, these results probably reflect differences in the amount and composition of substrates reaching the large intestine, affecting water retention and microbial fermentation patterns [31]. The HD diet also exhibited higher CP ADC (ADC = 86.1 vs. 78.2%) than the LD diet, which may have contributed to these responses.

Interestingly, dogs fed the HD diet also exhibited higher fecal ammonia concentrations. Given the higher CP digestibility of this diet and the similar CP contents between the diets, lower fecal ammonia concentrations would be expected [32]. Therefore, the observed increase in fecal ammonia cannot be explained only by the amount of undigested protein reaching the large intestine and may reflect differences in microbial nitrogen metabolism and ammonia utilization. However, since other fermentation metabolites were not evaluated, the mechanisms responsible for this response remain unclear.

In contrast, the greater availability of protein and peptides in the large intestine associated with the LD diet may favor an environment favorable to the growth of potentially pathogenic bacteria such as *E. coli* and *C. perfringens* [32,33]. However, dogs receiving SYN showed lower fecal *C. perfringens* abundance than dogs fed PLA, suggesting that the SYN supplementation may have partially attenuated this response. This effect may be explained by multiple synergistic mechanisms, including competitive exclusion by probiotic strains, modulation of the intestinal environment, and the effects of fermentable substrates such as inulin and yeast-derived cell wall components. Inulin may contribute to shifts in the composition of the gut microbiota [34], and MOS may reduce pathogen colonization by acting as ligand receptors for bacterial adhesion [35]. In addition, probiotic strains such as *Lactobacillus* spp. may inhibit *C. perfringens* through the production of antimicrobial compounds, including bacteriocins, as well as through modulation of host immune responses and intestinal barrier function [33]. However, these mechanisms were not investigated, nor were toxin production or virulence factors evaluated. Therefore, although this finding may suggest a modulatory effect of SYN on this bacterial population, its biological and clinical significance should be interpreted with caution.

After 20 days on the LD diet, fecal *E. coli* decreased, while *Faecalibacterium*, *Streptococcus*, *Fusobacterium*, *Bifidobacterium*, *Bacteroides*, and *Prevotella* increased in both groups. Except for *Streptococcus*, these taxa are associated with eubiosis in healthy dogs [18,29]. This pattern may reflect the composition of the LD diet, which has a higher crude fiber content (3.81%), possibly altering the availability of substrate for intestinal fermentation and promoting the proliferation of specific bacterial groups. This may help explain the increased abundance of genera such as *Prevotella* and *Bacteroides* in this period, since these taxa are associated with fiber fermentation [36]. In contrast, *Collinsella* abundance was reduced after the dietary transition, corroborating previous studies reporting increased *Collinsella* abundance in dogs fed lower-fiber diets [2]. However, the lack of characterization of soluble and insoluble fiber fractions limits more precise interpretation of specific effects of fiber on the gut microbiota.

Collectively, these microbial changes may indicate an adaptive response of the intestinal microbiota following the dietary transition. Similarly, a previous study reported stabilization of the fecal microbiota within two weeks after an abrupt dietary change in dogs [27]. This modulation after 20 days may suggest a degree of microbiota resilience and adaptive capacity following the dietary transition. However, whether these microbial changes represent transient adaptations or persist over longer periods could not be determined within the duration of the present study. It is also important to consider that laboratory dogs may be more accustomed to dietary changes than household pets, potentially influencing the magnitude of observed responses, as already demonstrated in other studies with dietary changes [2].

With regard to the effects of the synbiotic, no influence was observed on the dogs' DI or fecal characteristics. This may be partly explained by the use of healthy dogs, which likely limited the extent of microbiota modulation achievable through supplementation. Although beneficial effects of synbiotic supplementation on intestinal functionality have been reported in dogs [16,37,38], evidence indicates that responses are often modest and highly individualized, even when relatively high doses ($2 \times 10^{10}$ CFU/day) and multi-

strain formulations are used [16]. Furthermore, factors such as supplementation duration and gastrointestinal disorders have been identified as important determinants of treatment response [39,40]. In this regard, one study observed more pronounced effects in healthy dogs presenting higher fecal *Proteobacteria* and lower *Lactobacillus* abundance before supplementation, suggesting that baseline microbiota composition may also influence treatment response [16].

Interestingly, we observed an increase in fecal universal bacteria and *Bifidobacterium* immediately after the abrupt transition to the LD diet, but only in the PLA group (day 22). Although the genus *Bifidobacterium* is not typically associated with dysbiosis in dogs [18], its abundance may also vary according to substrate availability and dietary composition [41]. Supporting this, a study in dogs with exocrine pancreatic insufficiency reported increased fecal abundance of *Bifidobacterium* in affected animals compared to healthy controls [42], probably reflecting altered substrate availability in the colon. Therefore, the increase in fecal *Bifidobacterium* observed in the present study may represent a microbial response to the dietary change rather than an indicator of imbalance.

Although no gastrointestinal signs were observed and the DI remained below 0 throughout the study, together, these results reinforce the importance of considering both dietary composition and microbiota interventions when evaluating strategies to support canine gastrointestinal health.

Some limitations should be considered when interpreting these results: (1) the relatively small number of animals may have reduced the statistical power to detect biological effects; (2) the study did not include a control group maintained on the HD diet throughout the experimental period, which limits the ability to distinguish temporal microbiota adaptations from responses specifically associated with the dietary transition; and (3) it is important to note that microbiota assessment was intentionally based on the canine DI and selected bacterial taxa with established clinical relevance in dogs. Therefore, the objective was not to comprehensively characterize the canine microbiome, but rather to evaluate microbial responses using a validated and clinically applicable approach. Finally, further studies should integrate sequencing approaches and fecal metabolomic analyses, along with inflammatory and immunological biomarkers, in a long-term study to enable a more comprehensive characterization of host–microbiota interactions.

## 5. Conclusions

An abrupt transition to a less digestible diet induced dysbiosis-associated changes in the fecal microbiota of healthy dogs, characterized by increased fecal *E. coli* and *Streptococcus* and reduced *P. hiranonis*, without inducing clinically relevant dysbiosis according to the dysbiosis index. The SYN supplementation was associated with lower fecal abundance of total bacteria, *Bifidobacterium*, and *C. perfringens* during the dietary transition, indicating a modest modulation of the microbiota response to dietary change.

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## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| PLA | Placebo |
| SYN | Synbiotic |
| HD | Highly digestible |
| LD | Less digestible |
| ADC | Apparent digestibility coefficients |
| ME | Metabolizable energy |
| DM | Dry matter |
| fDM | Fecal dry matter |
| SU | Supplementation |
| DI | Dysbiosis index |

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