

Article

# The Canine Gut Health: Impact of a New Feed Supplement on Microbiota Composition

## Supplementary Materials

Supplemental Table 1: Guaranteed Analysis of commercially available diet provided to both groups.

New Formulation Ingredients (mg/g)	
Crude Protein (min.)	22.0%
Crude Fat (min.)	12.0%
Crude Fiber (max.)	4.1%
Moisture (max.)	10.0%
Eicosapentaenoic Acid (EPA) (min.)	0.17%
Docosahexaenoic Acid (DHA) (min.)	0.07%
Glucosamine* (min.)	818 mg/kg
Chondroitin sulfate* (min.)	8 mg/kg

\*Not recognized as an essential nutrient by the AAFCO Dog Food Nutrient Profiles

Supplemental Table 2: Composition of the tested supplement and the placebo

New Formulation Ingredients (mg/g)	
<i>Lentinula edodes</i>	10.0
Quercetin	13.5
Bromelain	13.5
Maltodextrin	583.4
Appetite stimulants	379.6
Total	1000.0
Placebo ingredients (mg/g)	
Maltodextrin	1000.0

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Supplemental Table 3: Differentially abundant ASVs by time within dogs in the control group as determined by ANCOM-BC (T0 vs T28).

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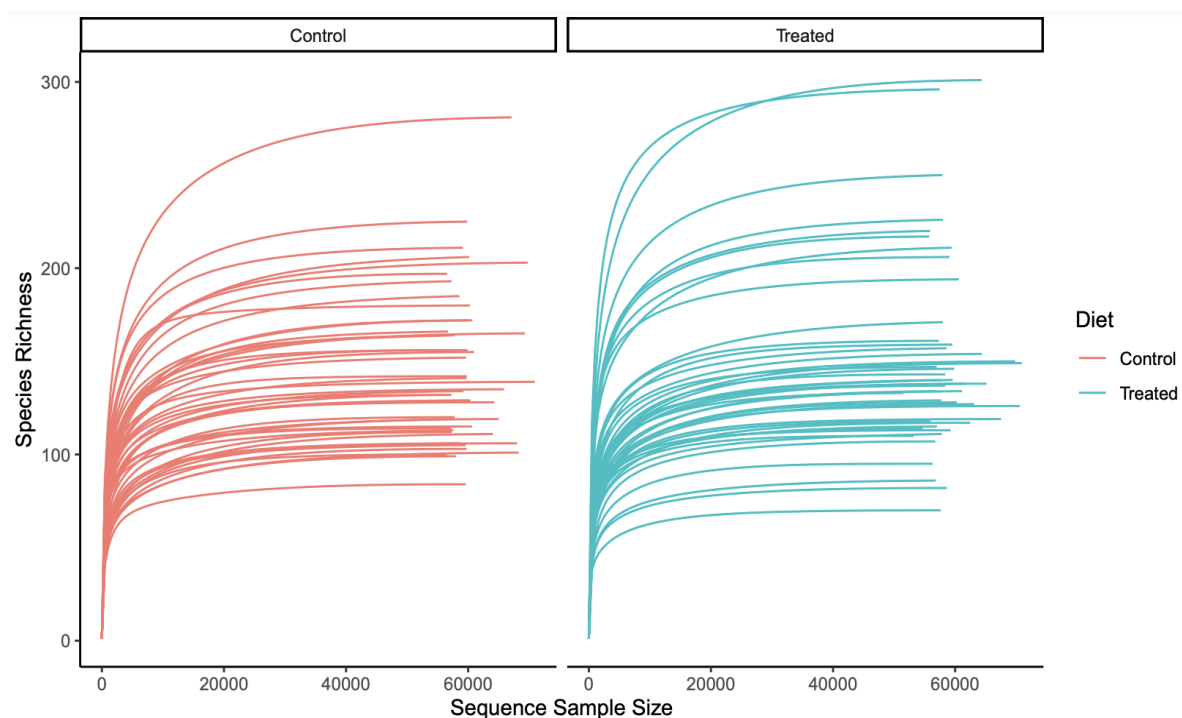
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Taxonomic classification	Higher Abundance Timepoint	Log2-Fold Change	Standard deviation	W statistic	p-value	Corrected p-value	ASV_code
Lachnospiraceae_unclassified	T0	-0.040	0.014	-2.862	0.004	0.081	ASV002
Clostridiales_unclassified	T0	-0.036	0.013	-2.767	0.006	0.081	ASV349
Lachnospiraceae_unclassified	T0	-0.054	0.013	-4.228	0.000	0.006	ASV781
<i>Ruminococcus lactaris</i>	T28	0.037	0.012	3.114	0.002	0.058	ASV969
Fusobacteria_unclassified	T0	-0.058	0.021	-2.752	0.006	0.081	ASV029
<i>Collinsella stercoris</i>	T28	0.042	0.015	2.808	0.005	0.081	ASV259
<i>Paraeggerthella hongkongensis</i>	T28	0.045	0.017	2.670	0.008	0.089	ASV527
Coriobacteriaceae_unclassified	T28	0.021	0.008	2.697	0.007	0.088	ASV345
<i>Bifidobacterium</i> sp	T28	0.019	0.007	2.786	0.005	0.081	ASV018
<i>Bifidobacterium animalis</i>	T28	0.054	0.020	2.743	0.006	0.081	ASV117
<i>Bifidobacterium</i> sp	T28	0.047	0.016	3.035	0.002	0.058	ASV772
Lactobacillaceae_unclassified	T28	0.084	0.027	3.132	0.002	0.058	ASV256
Lactobacillaceae_unclassified	T28	0.057	0.016	3.565	0.000	0.029	ASV585
Lactobacillaceae_unclassified	T28	0.100	0.029	3.418	0.001	0.038	ASV098
<i>Lactobacillus hamsteri</i>	T28	0.081	0.025	3.211	0.001	0.058	ASV440
<i>Streptococcus equi</i>	T28	0.080	0.026	3.042	0.002	0.058	ASV222
<i>Anaerorhabdus furcosa</i>	T0	-0.034	0.012	-2.818	0.005	0.081	ASV086
<i>Eubacterium</i> sp	T0	-0.048	0.018	-2.660	0.008	0.089	ASV825
Erysipelotrichaceae_unclassified	T28	0.056	0.021	2.644	0.008	0.089	ASV255
<i>Enterococcus cecorum</i>	T28	0.035	0.012	2.836	0.005	0.081	ASV036
<i>Pediococcus</i> sp	T28	0.050	0.016	3.081	0.002	0.058	ASV844
<i>Lactobacillus</i> sp	T28	0.102	0.026	3.880	0.000	0.013	ASV046

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Supplementary Figure 1: Rarefaction curves for all samples for treated and control groups

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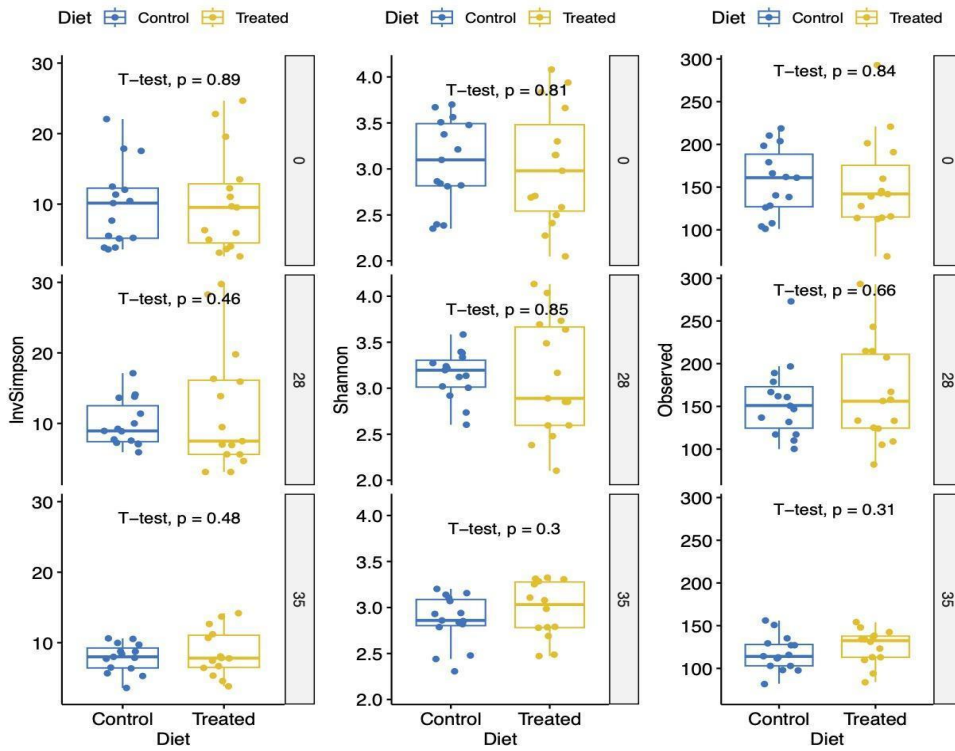


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Supplementary Figure 2: Alpha diversity analysis of treated vs control groups at T0, T28 and T35

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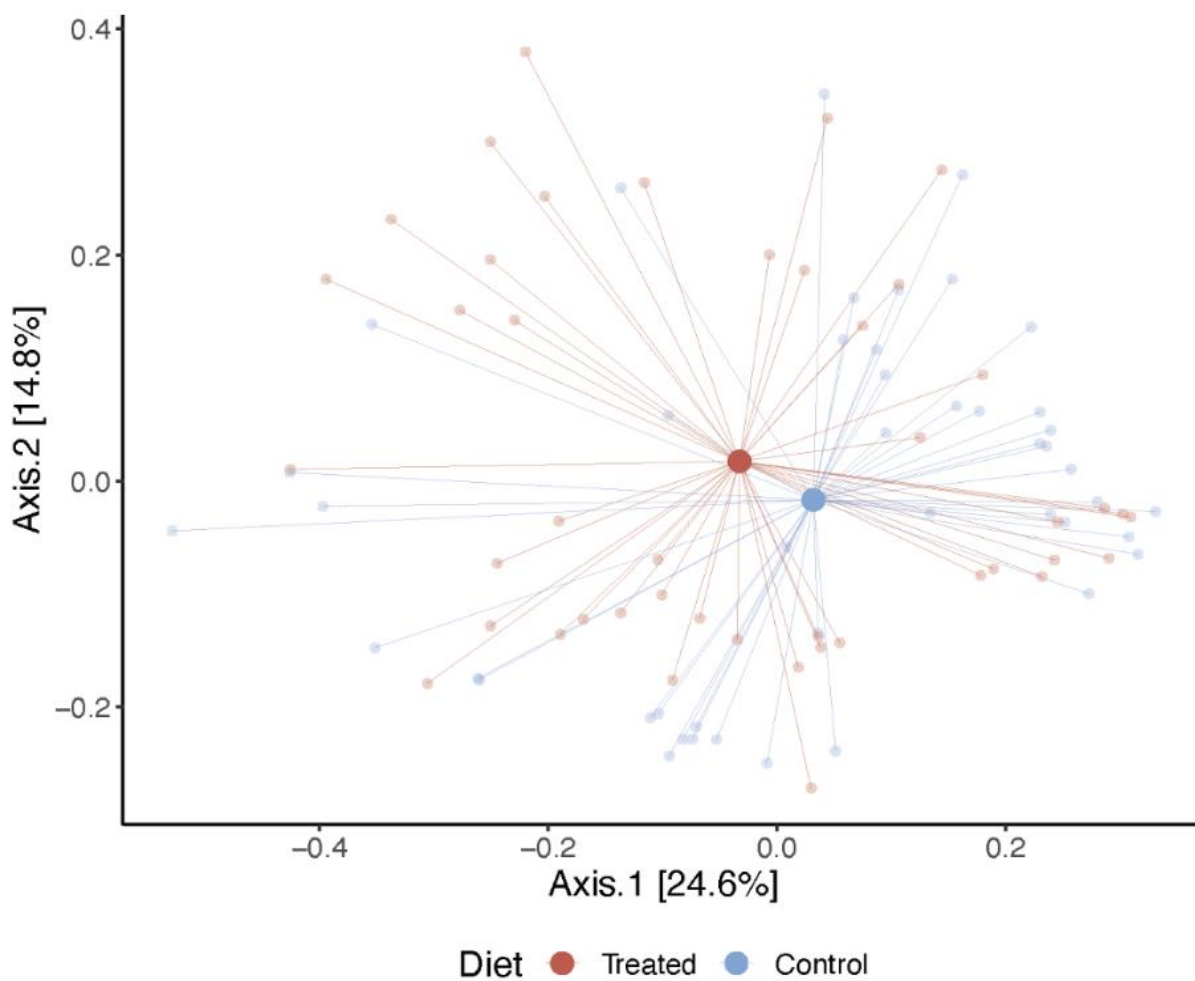
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Supplementary Figure 3: A principal coordinate analysis using Bray-Curtis dissimilarity matrix for  $\beta$  diversity colored by treated and control groups.

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