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Effects of feed additives supplementation on rumen bacterial diversity and composition in calves during pre- and postweaning

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Introduction

In intensive dairy production systems, male crossbred beef calves are separated from their dams shortly after birth, relocated to rearing farms, and group-transported to their final growing facilities. This early-life disruption may have long-term consequences on calf performance, potentially increasing the risk of respiratory diseases, diarrhoea, and impaired microbial colonization (Abecia et al., 2017). A common strategy to support early rumen development is the inclusion of feed additives in starter diets. The rumen, sterile at birth, is gradually colonized during the first days of life (Yeoman et al., 2018), and proper microbial establishment is essential for developing efficient fermentation processes. The **objective** of this study was to assess the impact of different feed additives on rumen microbial colonization during this critical developmental stage in calves.

Materials and methods

- **112 male suckling Montbeliarde calves** were classified into **4 diet groups**: **CTL** (no additives), **EO** (essential oils from plants), **SYN** (yeast probiotics) and **MIX** (mixture of probiotics and essential oils).
- Calves received their respective diet with additives in the concentrate feed **for 45 days (until weaning).** After weaning, all calves **were switched to the MIX diet.**
- Rumen content were collected at **8 weeks old** ("preweaning") **and 18 wo** ("postweaning").
- 16S rRNA amplicon sequencing was performed using Illumina technology, and sequences were processed with QIIME 2 (DADA2 pipeline). Taxonomic assignment was carried out using the Greengenes2 database (version 2024.09). Statistical analyses and data visualization were conducted in R.

Results

Microbial diversity and structure

Alpha-diversity showed limited differences across treatments, although postweaning **SYN** calves tended to have lower ASV richness than controls. Beta-diversity analyses revealed significant treatment-associated shifts at both stages, without clear clustering.

Core and treatment-responsive microbiota

A robust core microbiome was identified, comprising **361 bacterial ASVs,** with **80.1%** (289 ASVs) shared across pre- and postweaning phases.

sPLS-DA revealed distinct preweaning microbial profiles by treatment, with **MIX** and **SYN** groups clearly separated from **CTL** and **EO**. Postweaning, **SYN** and **CTL** remained distinct despite a common diet, suggesting a carryover effect.

Taxonomic shifts with age

Acidaminococcaceae were more abundant preweaning (~7.2%). Postweaning, *Selenomonadaceae* significantly increased (2.5% → 8.9%; p = 0.003), and *Succinivibrionaceae* emerged (~4.3%), reflecting adaptation to solid feed.

Study time	Treatment	N	Bacteria		
			Observed ASVs	*Shannon*	*Simpson*
Preweaning	*CTL*	26	200 ± 22.8	3.90 ± 0.168	0.960 ± 0.011
	EO	27	204 ± 21.8	3.92 ± 0.164	0.961 ± 0.009
	SYN	24	201 ± 15.5	3.89 ± 0.198	0.958 ± 0.013
	MIX	27	197 ± 27.5	3.93 ± 0.229	0.961 ± 0.013
p-value Trt			0.737	0.843	0.742
Postweaning	*CTL*	26	215 ± 38.9	3.94 ± 0.276	0.959^{a} ± 0.015
	EO	27	212 ± 53.8	4.06 ± 0.180	0.967^{b} ± 0.006
	SYN	24	194 ± 49.0	3.93 ± 0.194	0.961^{ab} ± 0.011
	MIX	27	201 ± 50.7	4.00 ± 0.199	0.965^{ab} ± 0.009
p-value Trt			0.099	0.119	0.042
Overall p-value	*Time*		< 0.001	0.004	0.033
	Treatment		0.137	0.163	0.087
	Trt x Time		< 0.001	0.033	0.050

Table 1. Alpha diversity analysis in suckling calves fed commercial additives. Statistical comparisons performed using Kruskal-Wallis rank sum test.

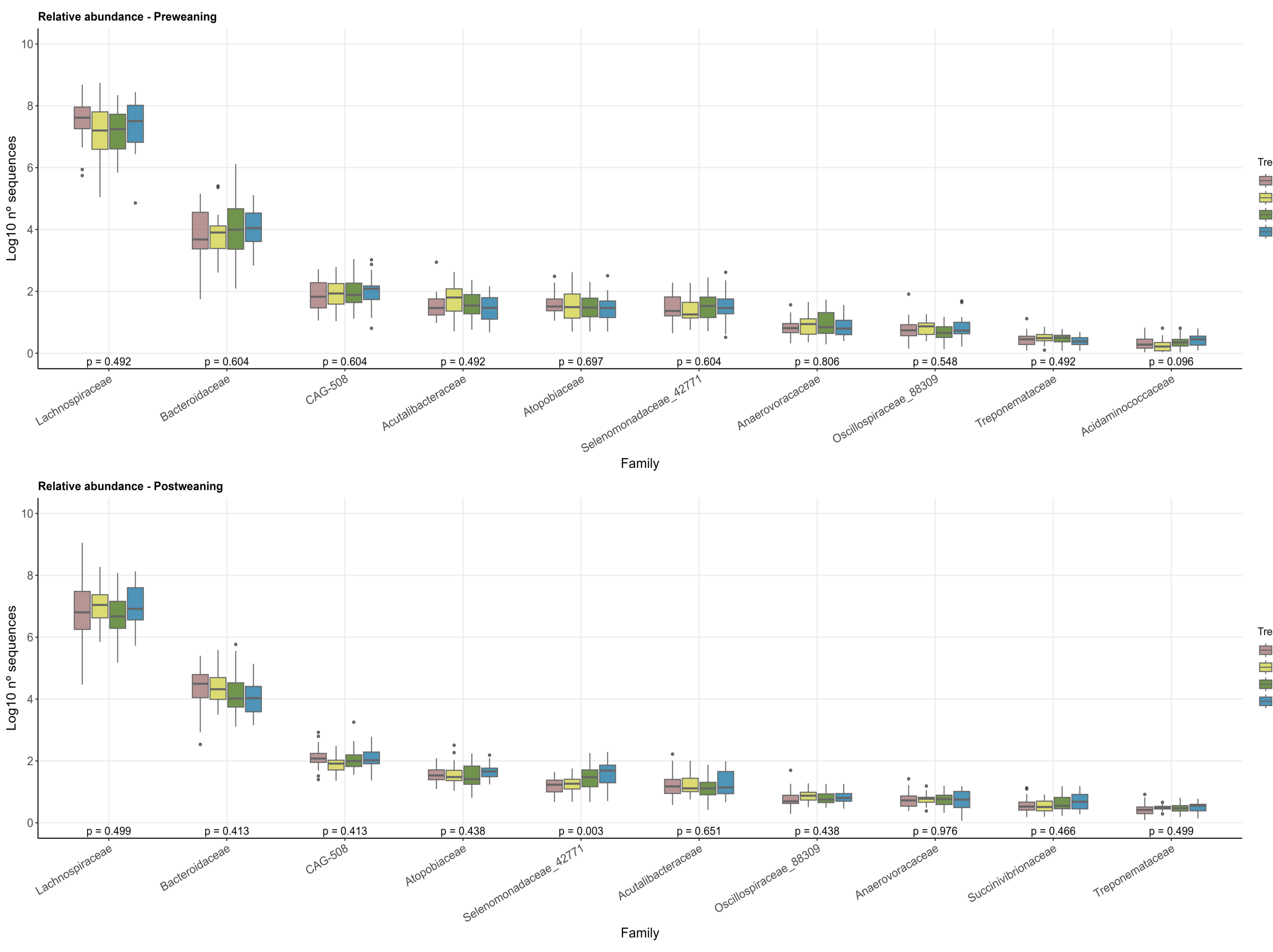

Figure 1. Box plot of the relative abundance (Log10 number of sequences) of the 10 most abundant bacterial families pre- and postweaning, by treatment.

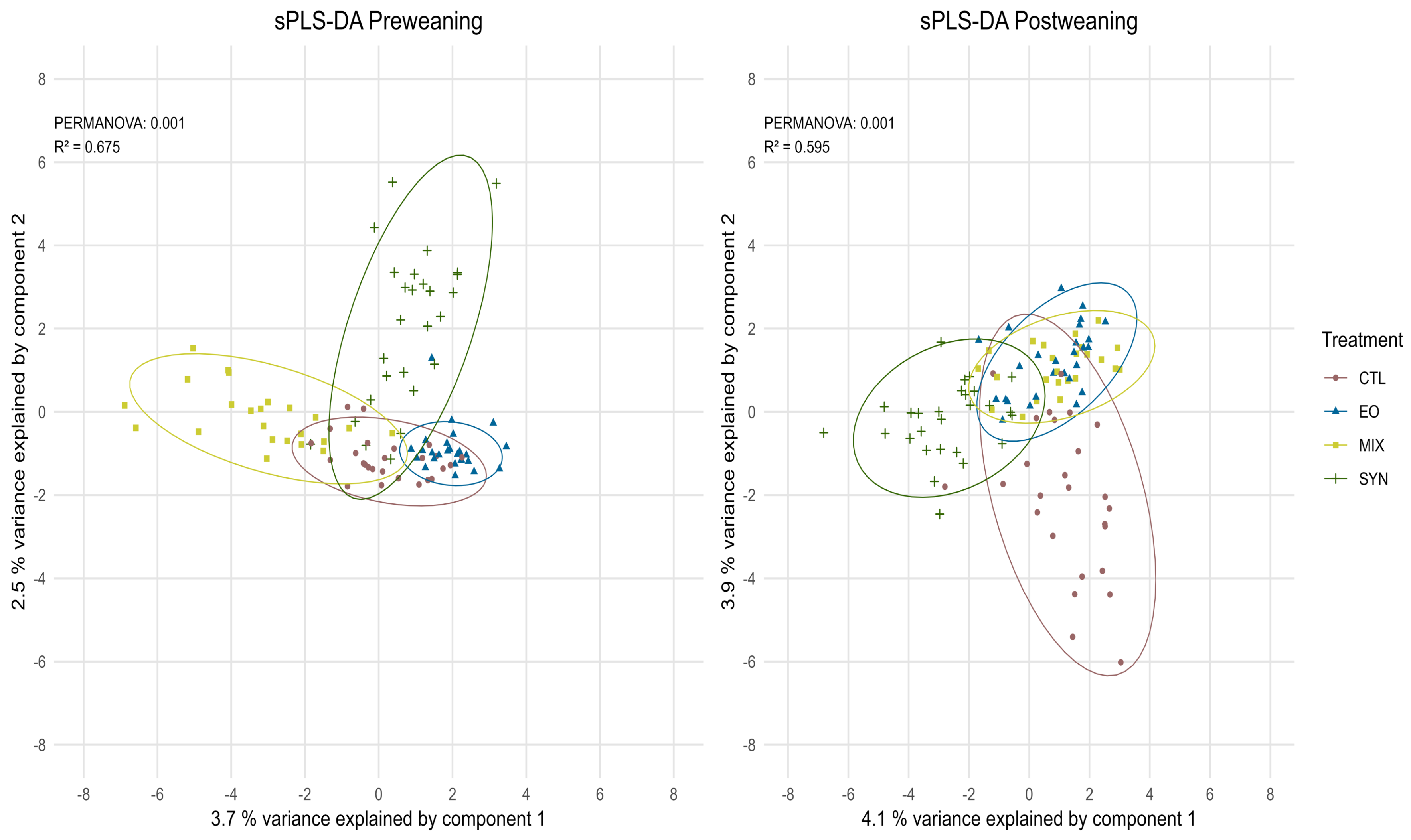

Figure 2. Sparse Partial Least Squares Discriminant Analysis (sPLS-DA) of rumen bacterial communities by treatment and time period.

Conclusions

Early dietary treatments shaped distinct rumen bacterial profiles during the preweaning period, with lasting effects postweaning despite a common diet, suggesting microbial imprinting.

While a stable core microbiome was identified, and specific taxa shifted with age, overall alpha diversity remained similar across treatments. The high unexplained variance (83.6%) in β-diversity points to additional influences on microbiota development, highlighting the complexity of rumen microbial assembly and the potential for early-life modulation to influence long-term microbial development.

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