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

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Introduction – Material and methods

Intensive milk production systems

Separated from their dams after birth

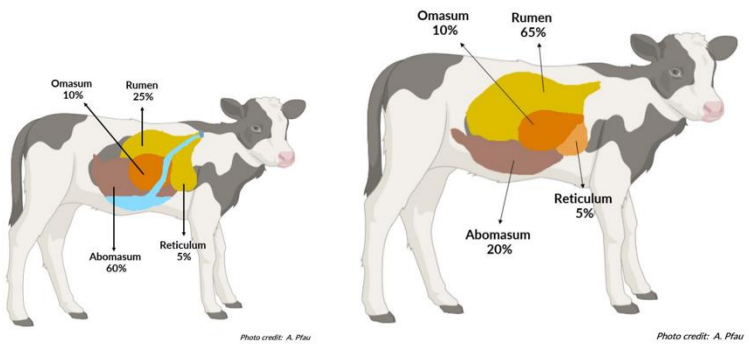
Transported to a rearing farm (21 do)

Transported to a final growing farm

Consequences:

Higher risk of respiratory diseases, diarrhoea, disbiosis

Compromised microbial colonization, rumen development, calf performance



112 male suckling Montbeliarde calves

CTL | SYN | EO | MIX

Day 0 – 45 post-arrival

Day 45 - **MIX**

CTL - SYN– EO – MIX

Day 35	Day 105
preweaning sampling	**postweaning sampling**

Feed additives supplementation

CTL (no additive), **EO** (blend of essential oils), **SYN** (synbiotic yeast probiotic) & **MIX** (essential oils, yeast probiotics, & butyrate) applied until weaning.

Results

Previously Romera-Recio et al. (2025)…

No effects in performance (Body weight gain).
Health status not compromised.

Rumen fermentation:

Beta-hydroxybutyrate (mg/dL)

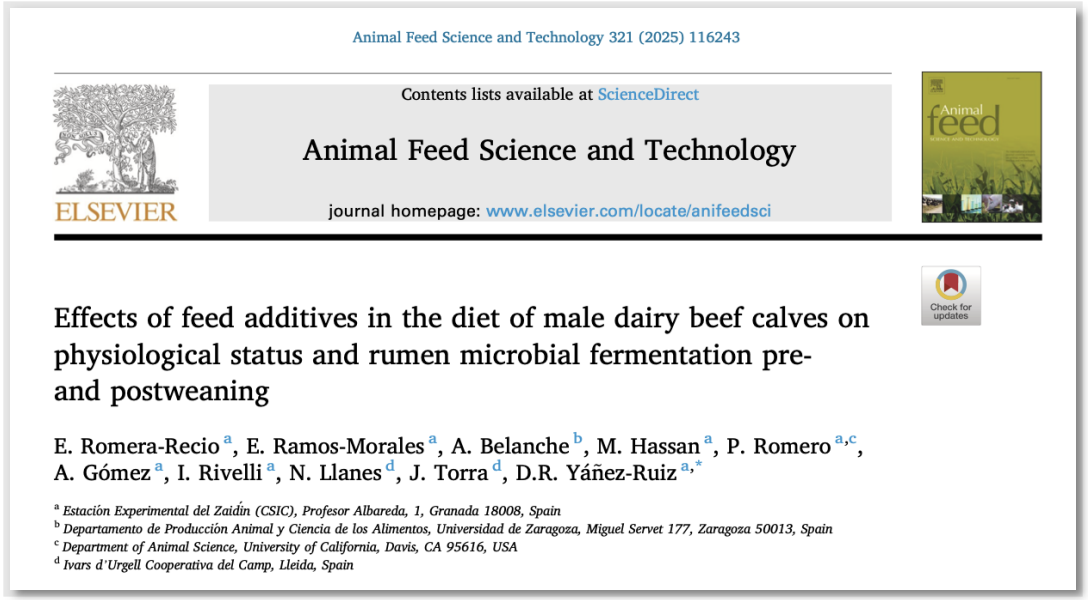
- Preweaning: EO and MIX ≠ CTL
- Postweaning: SYN and MIX ≠ CTL

Total VFA (mM)

- Postweaning: All treatments ≠ CTL

Butyrate (%)

- Postweaning: EO ≠ CTL



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Effects of feed additives in the diet of male dairy beef calves on physiological status and rumen microbial fermentation pre- and postweaning

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Hypothesis:

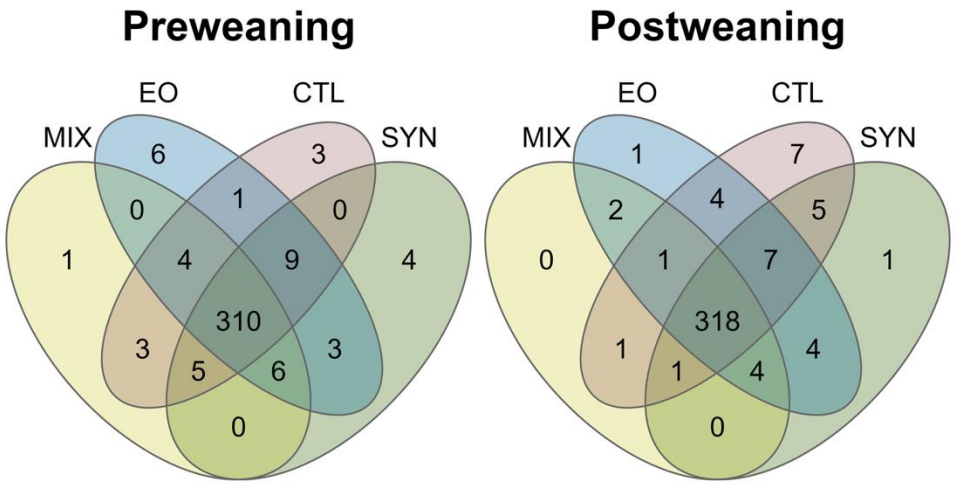
To assess whether different **feed additives** influence **rumen microbial colonization** during the critical early developmental stage in calves.

Results - MetaT

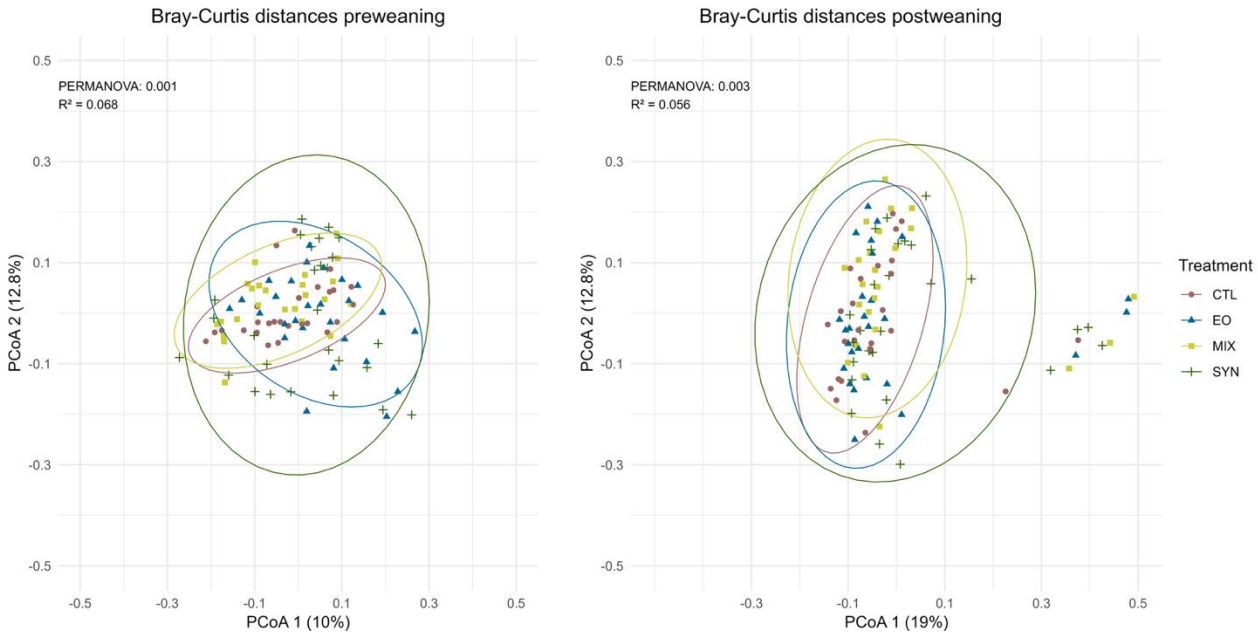
Alpha diversity

Study time	Treatment	N	Bacteria		
			Observed ASVs	*Shannon*	*Simpson*
Preweaning	*CTL*	26	200	3.90	0.960
	EO	27	204	3.92	0.961
	SYN	24	201	3.89	0.958
	MIX	27	197	3.93	0.961
p-value			0.737	0.843	0.742
Postweaning	*CTL*	26	215	3.94	0.959[a]
	EO	27	212	4.06	0.967[b]
	SYN	24	194	3.93	0.961[ab]
	MIX	27	201	4.00	0.965[ab]
p-value			0.099	0.119	0.042

Core microbiome



Bray-Curtis



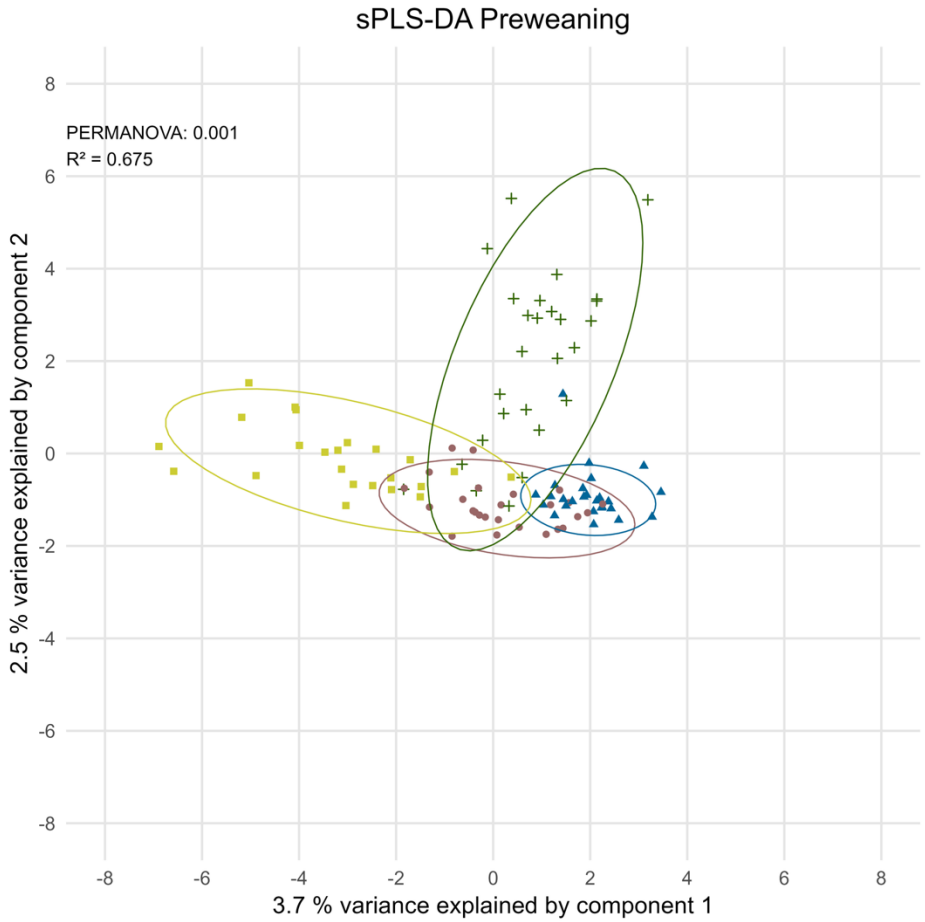
Significant differences between dietary treatments.

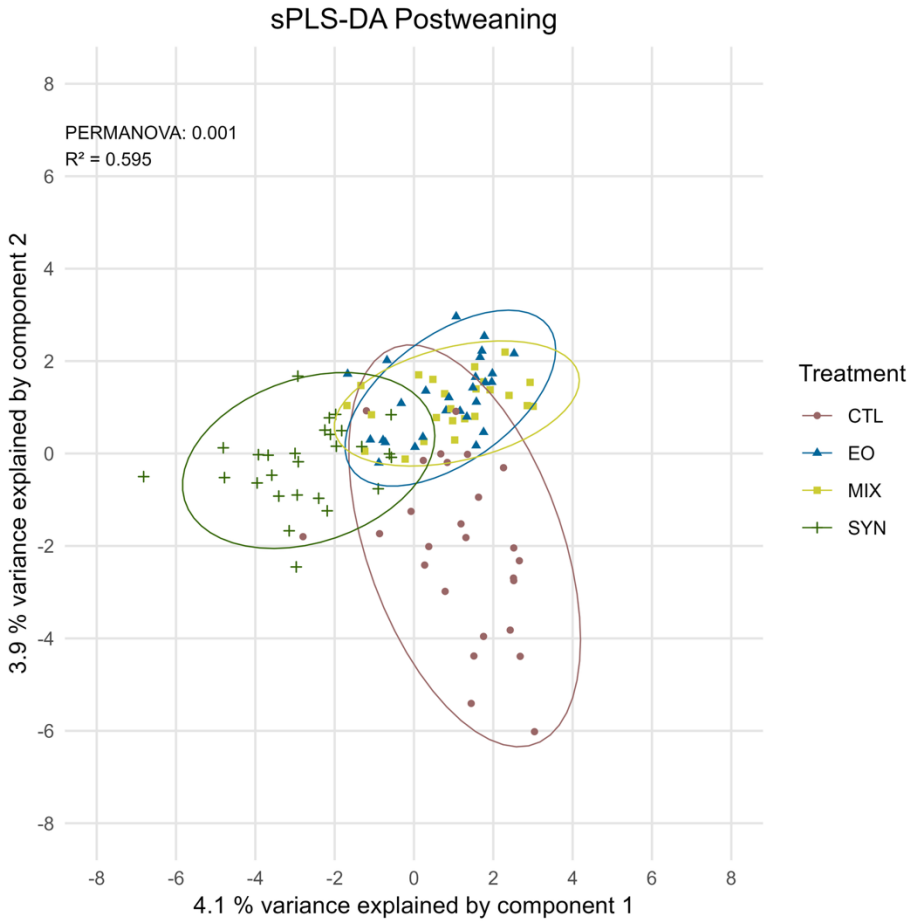
No clear clustering pattern observed between groups.

Explained variation (R^2): Preweaning: 6.80% vs. postweaning: 5.60%

Results - MetaT

Sparse partial least squares discriminant analysis





MIX diet after weaning

MIX and SYN separated from CTL and EO

Microbial profiles of SYN and CTL remained distinct

R^2

- Preweaning: 0.675
- Postweaning: 0.595

Distinct microbial profiles emerged in response to the different dietary treatments.

Summary

- Dietary treatments shaped **distinct rumen bacterial profiles.**
- A **resilient core microbiome** was present across all treatments (80.1% shared across pre- and postweaning phases).
- Taxonomic shifts occurred with age, but **alpha diversity remained similar** among treatment groups.
- **Beta diversity** showed significant but modest group separation, with more pronounced **preweaning** effects.
- **sPLS-DA** revealed strong discrimination, especially preweaning, with lasting effects postweaning despite common diet, supporting a microbial carryover effect.

Early dietary modulation may impact long-term microbial maturation

Acknowledgement

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HoloRuminant
Understanding microbiomes of the ruminant holobiont

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Results - MetaT

Unique ASVs detected in the treatments

Preweaning		
Treatment	**ASVs**	**Taxonomy (Family)**
EO	ASV82, ASV58, ASV169, ASV162, ASV180, ASV17	Bacteroidaceae, Lachnospiraceae, Acidaminococcaceae
SYN	ASV30, ASV78, ASV68, ASV75	Atopobiaceae, Eubacteriaceae, Lachnospiraceae, Acidaminococcaceae
MIX	ASV343	Anaerovoracaceae
CTL	ASV119, ASV44, ASV347	Lachnospiraceae, Bacteroidaceae, Coprobacillaceae
EO + SYN + MIX	ASV276, ASV231, ASV291, ASV170, ASV332, ASV35	Oscillospiraceae, Sphingomonadaceae, Acutalibacteraceae, Coprobacillaceae, Acidaminococcaceae

Postweaning		
Treatment	**ASVs**	**Taxonomy (Family)**
EO	ASV40	Lachnospiraceae
SYN	ASV332	Clostridia (CAG-508)
MIX	-	-
CTL	ASV360, ASV36, ASV138, ASV143, ASV188, ASV368,ASV64	Lachnospiraceae, Coprobacillaceae, Anaerovoracaceae
EO + SYN + MIX	ASV304, ASV209, ASV68, ASV103	Lachnospiraceae, Erysipelotrichaceae, Clostridia (CAG-508)