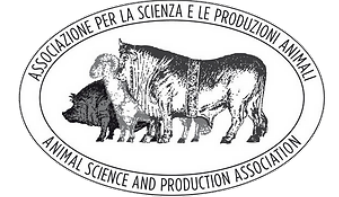




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Feeding dairy ewes with a diet containing agri-food co-products: Implications on rumen metabolism

Foggi G.¹, M. Daghio³, B. Valenti², S. Tinagli¹, A. Silvi¹, L. Turini¹, F. Scicutella, V. Bolletta², C. Viti³, A. Buccioni³, M. Pauselli², M. Mele¹

¹Dipartimento di Scienze Agrarie Alimentari Agro-ambientali, University of Pisa, Pisa, Italy,

²Dipartimento di Scienze Agrarie Alimentari Ambientali, University of Perugia, Perugia, Italy,

³Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali, University of Florence, Florence, Italy

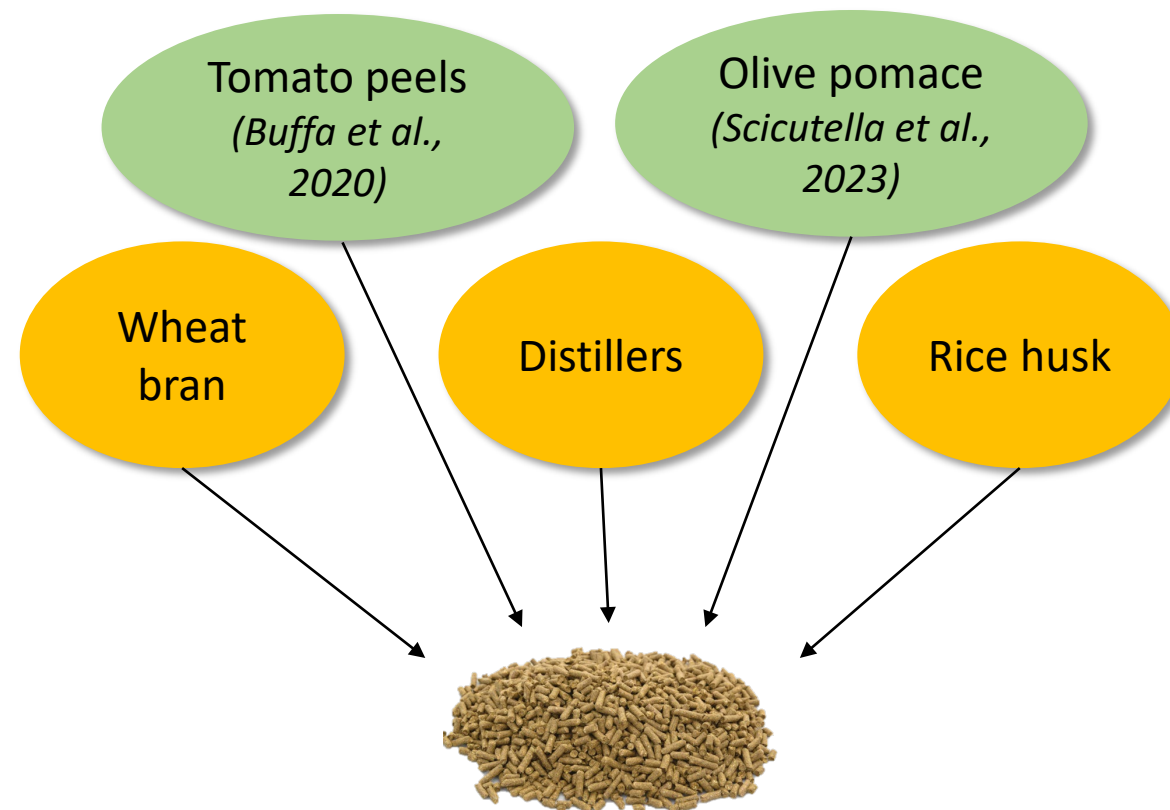
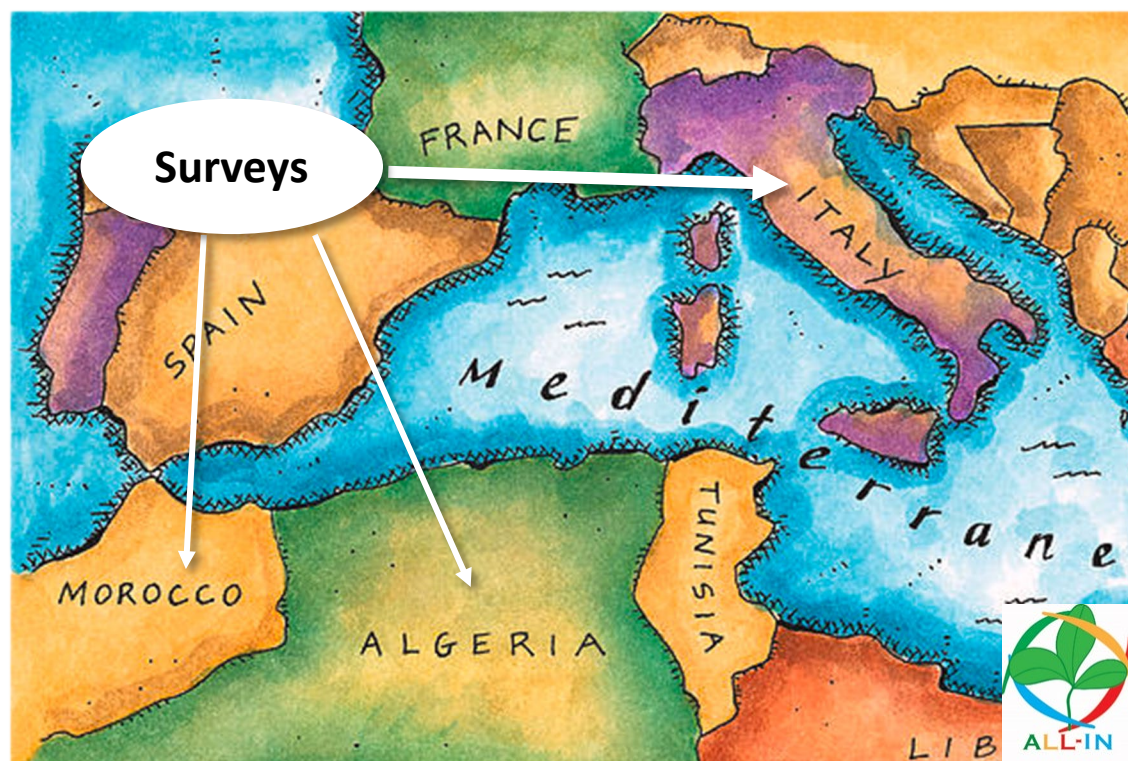


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Pisa Livestock farming, Animal food quality, and Sustainability



Introduction

Can selected by- and co-products replace conventional feedstuffs?

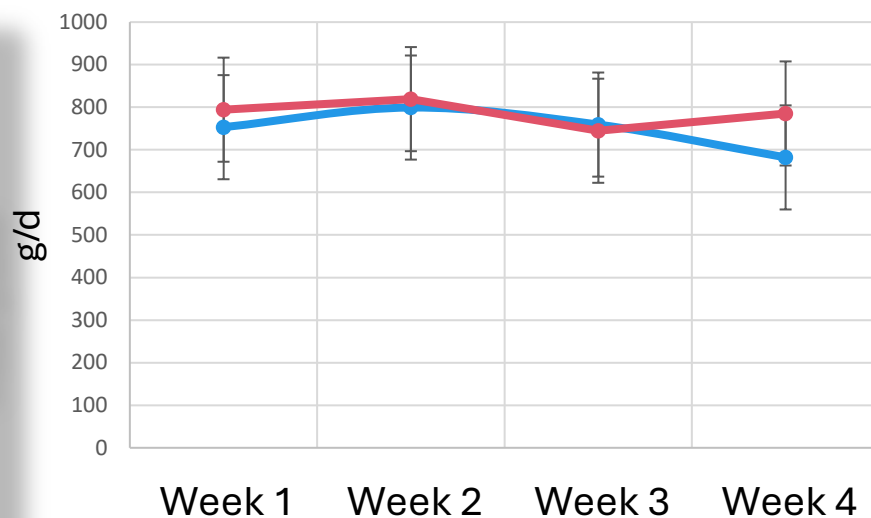


Background

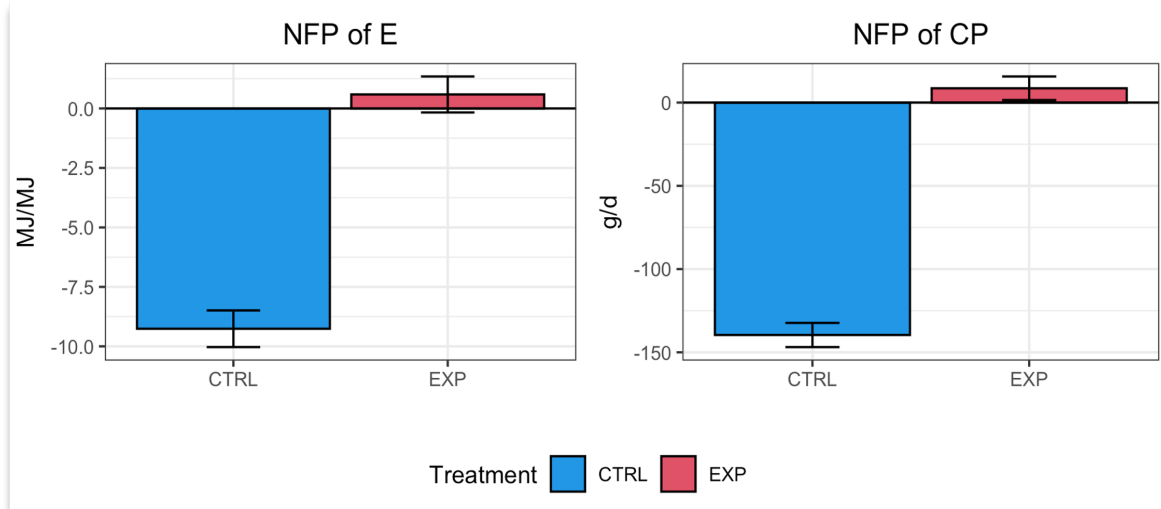
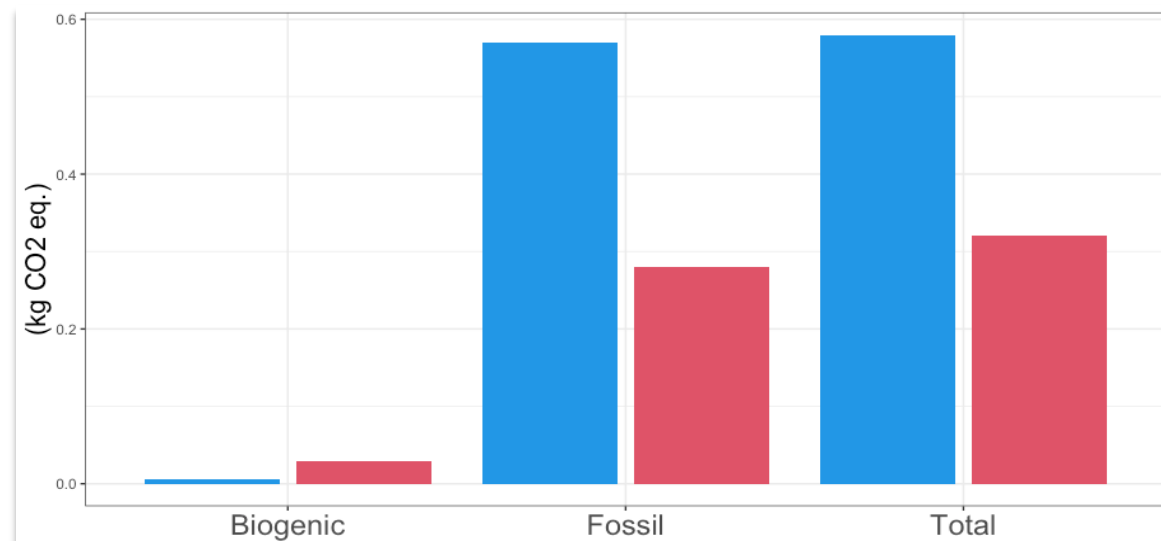


LCA

Milk yield

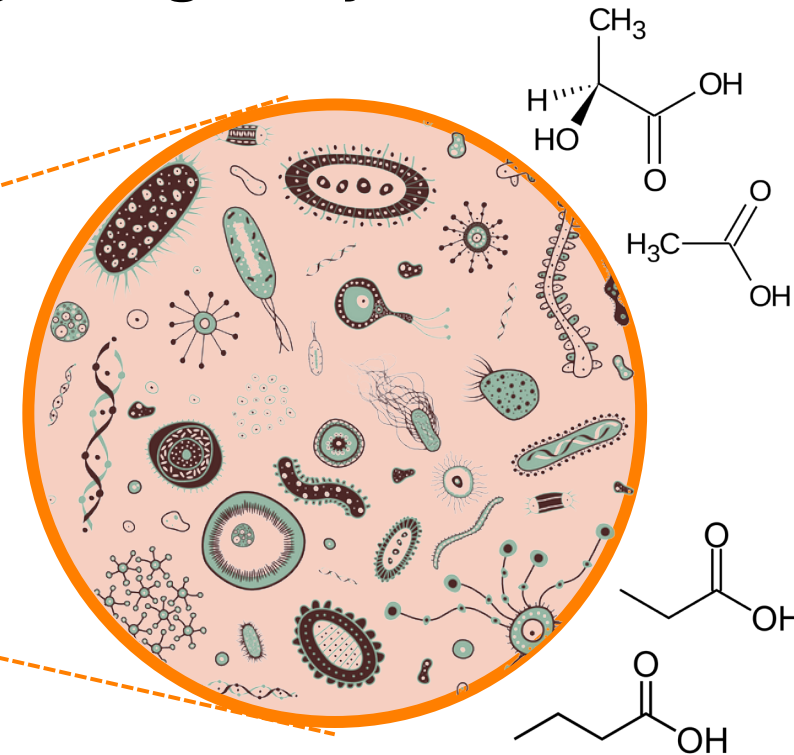
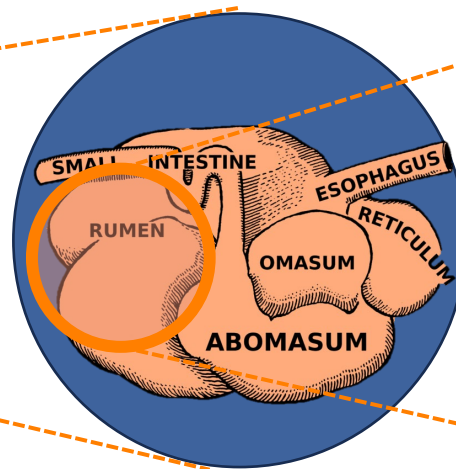
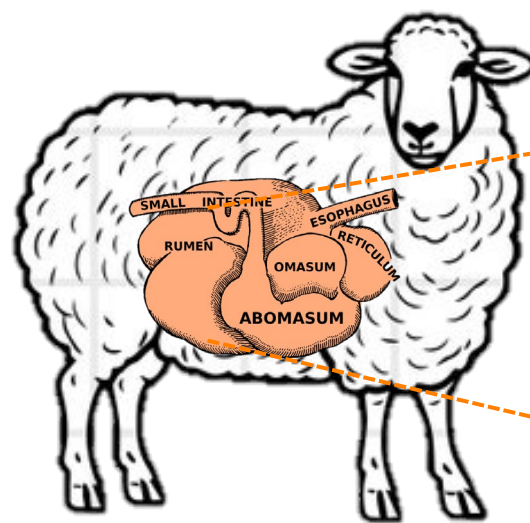


Food / feed competition

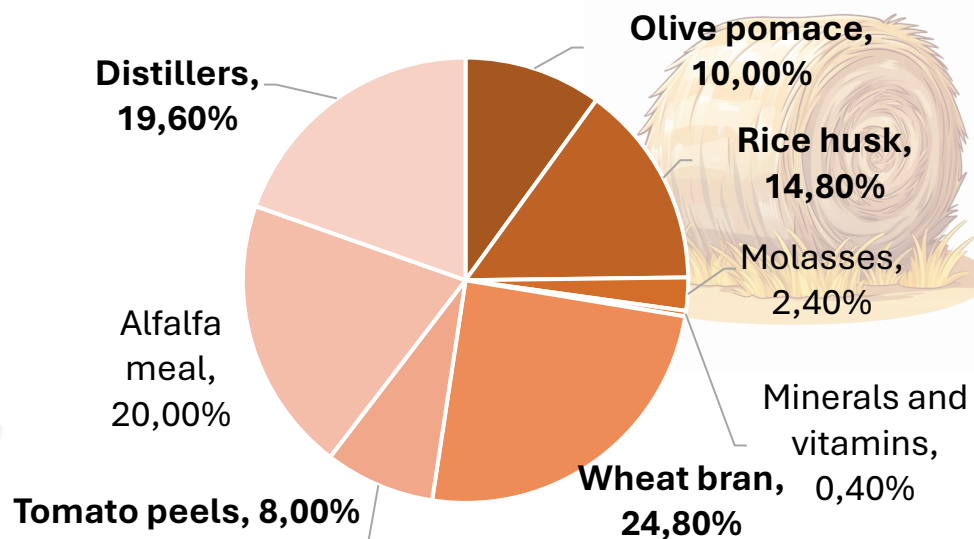
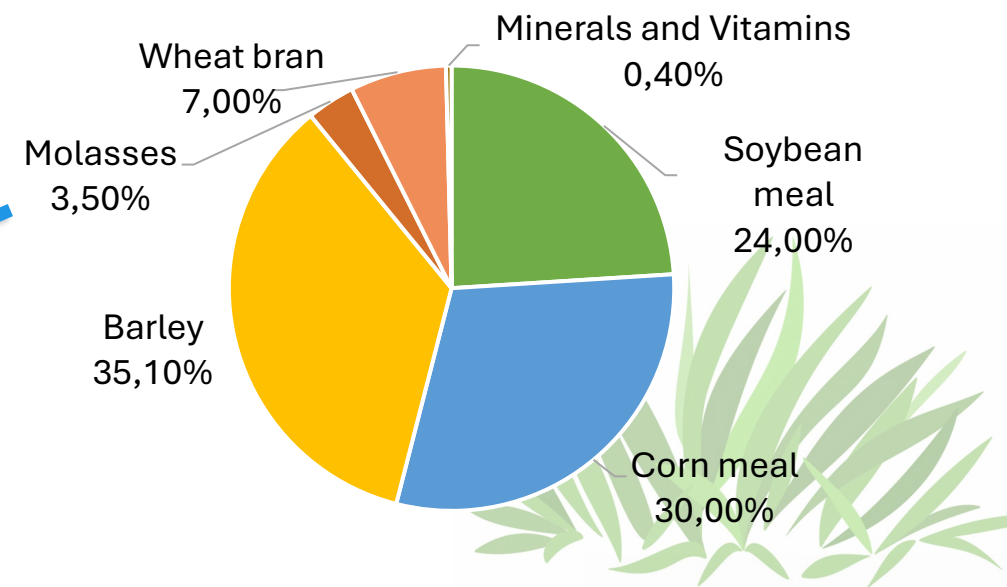
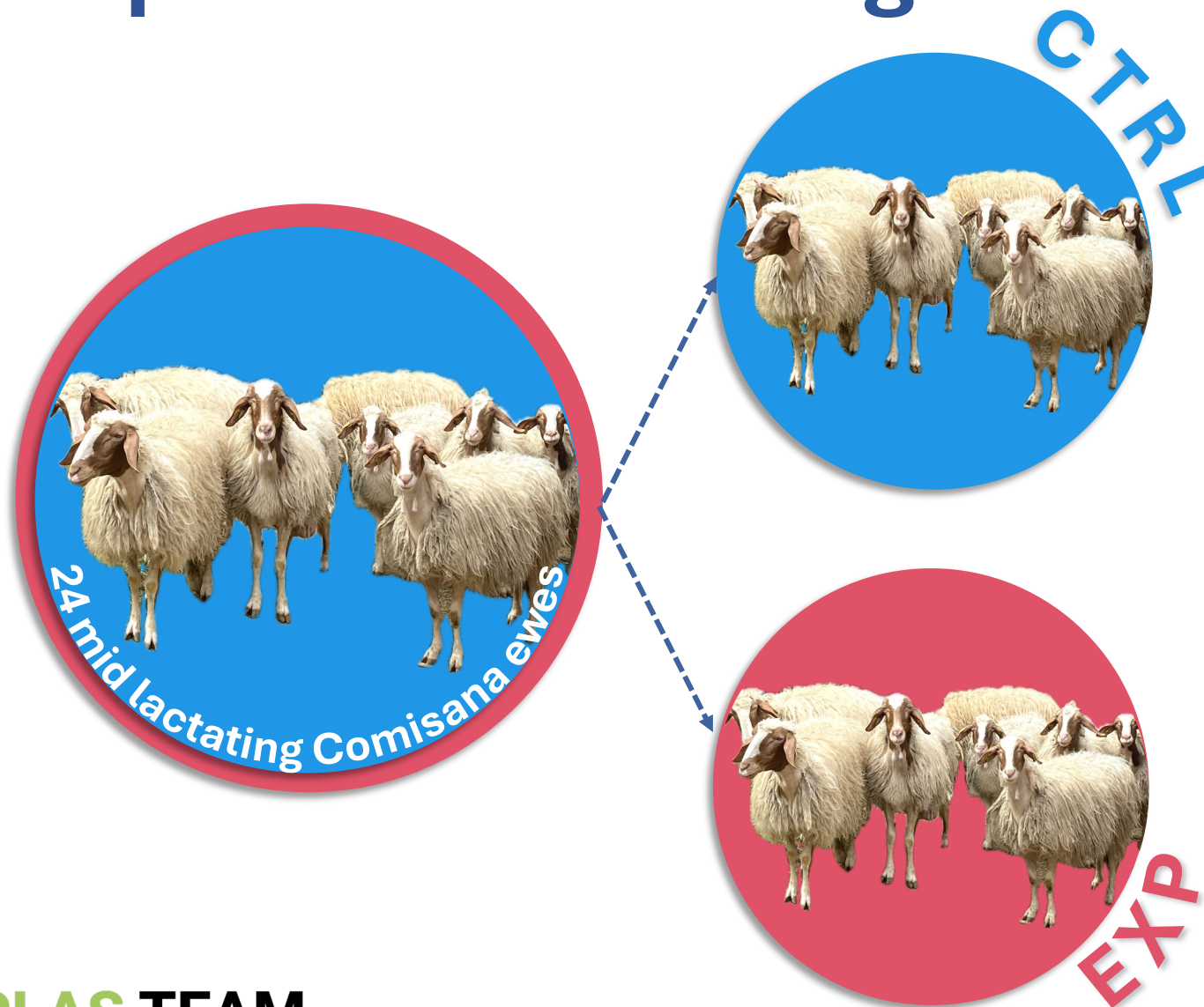


Aim of the study

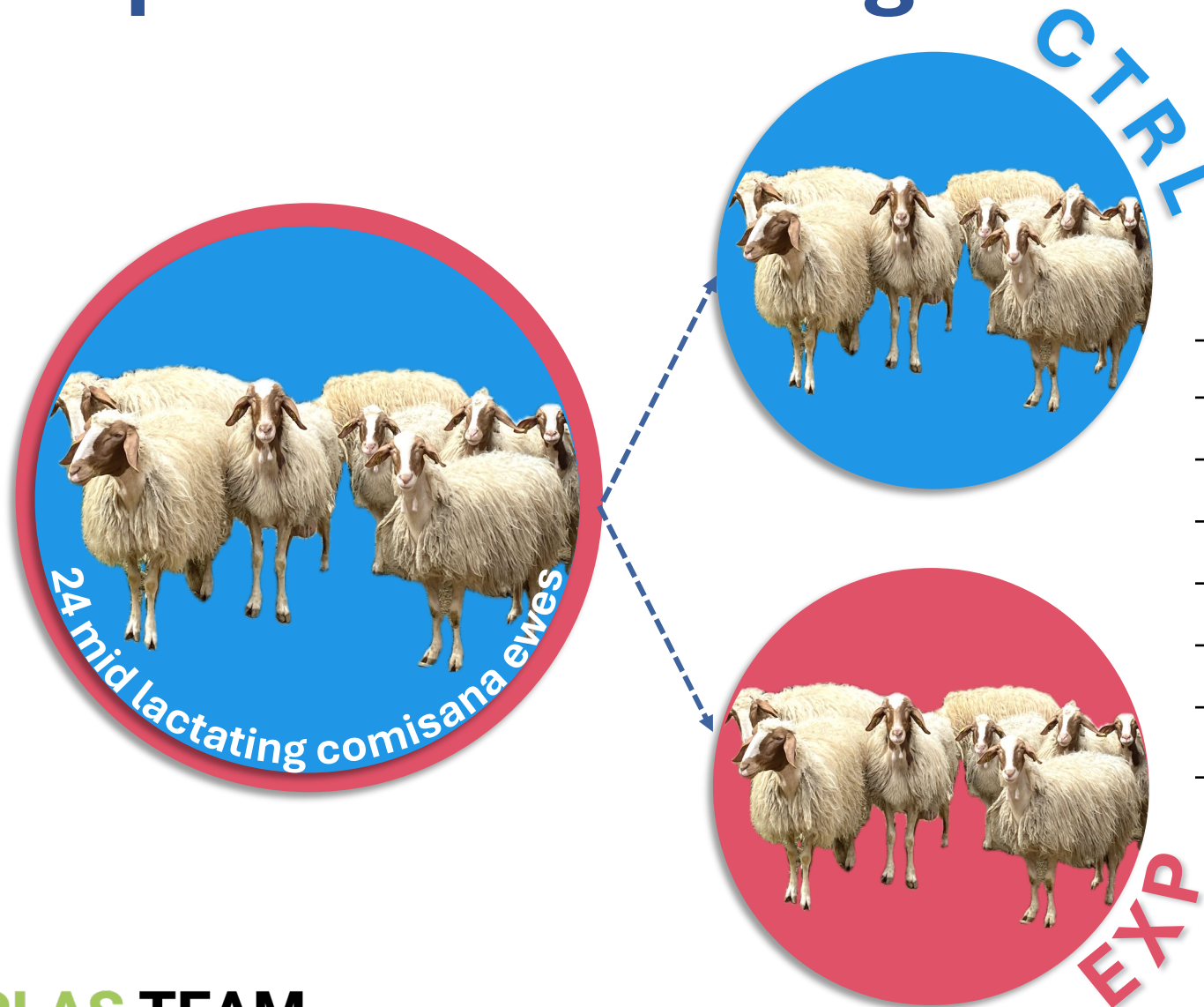
Assessing the effect of the diet based on **by- and co-products** on volatile fatty acids (VFA), fatty acids (FA) concentrations, microbiota composition, and CH_4 from the rumen fluid of grazing dairy ewes



Experimental design

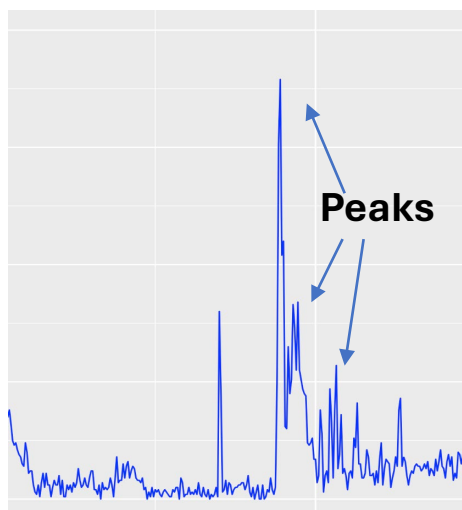


Experimental design

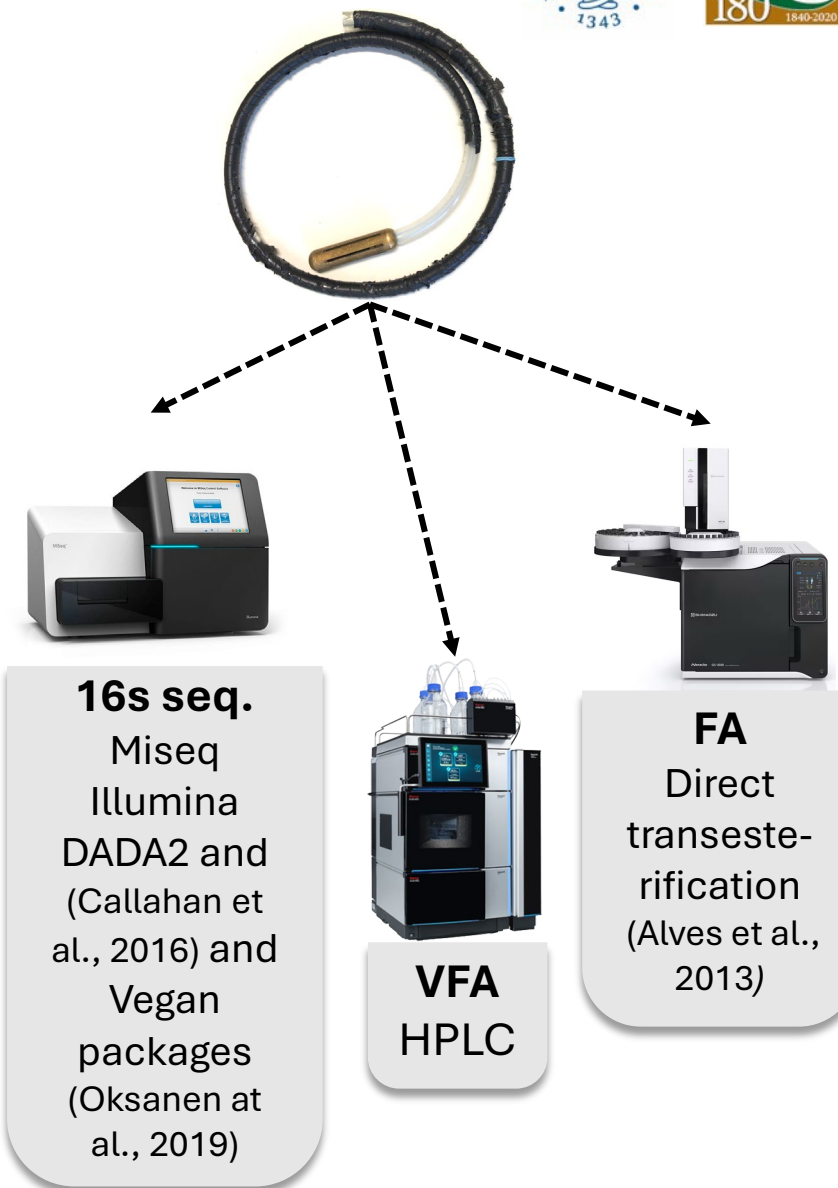
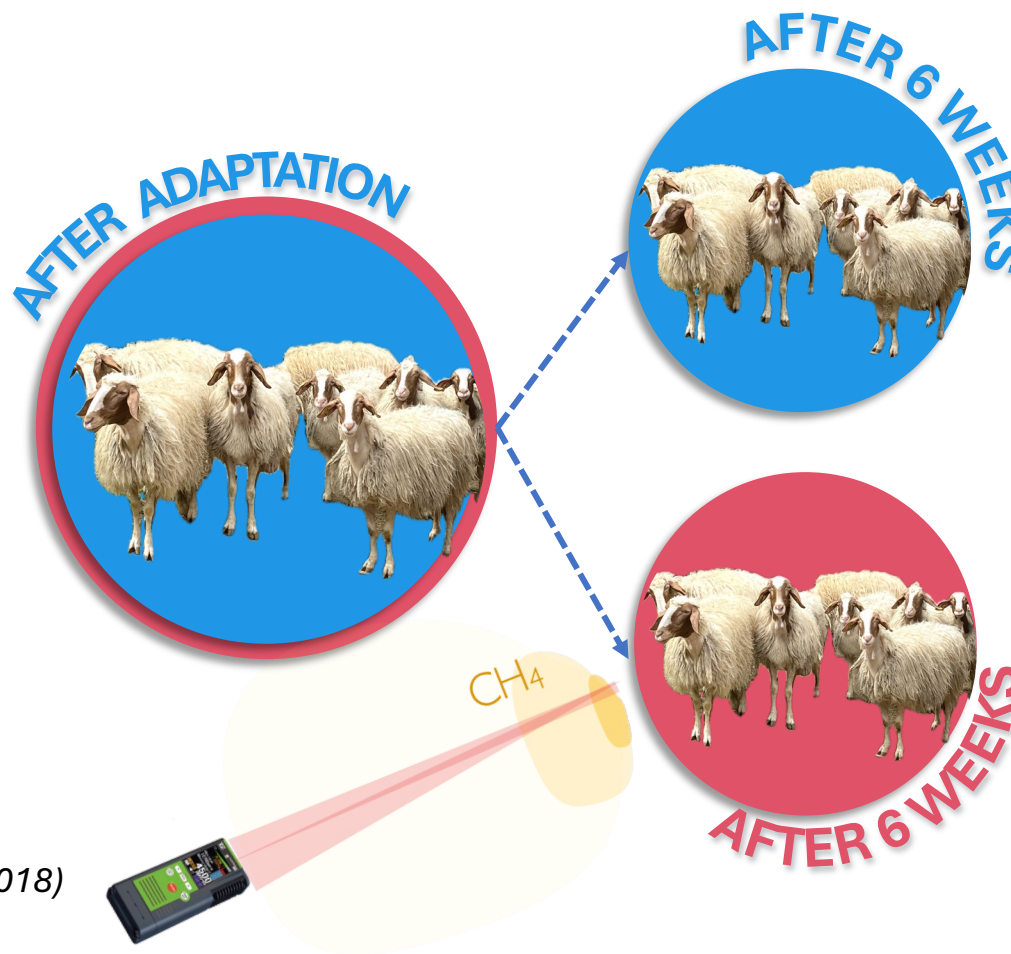


	EXP pellet	CTRL pellet	Pasture	Hay
g/kg DM				
CP	21.80	21.10	13.84	16.71
EE	1.36	0.62	2.43	1.24
NDFom	44.83	6.94	48.08	65.5
ADFom	25.33	4.02	33.99	39.39
ADL	8.61	-	7.56	5.12
NFC	23.74	66.89	9.61	24.51
Ashes	8.27	4.45	11.14	6.93

Sampling and analyses



CH₄ profile of dairy ewes,
adapted from *Sorg et al. (2018)*

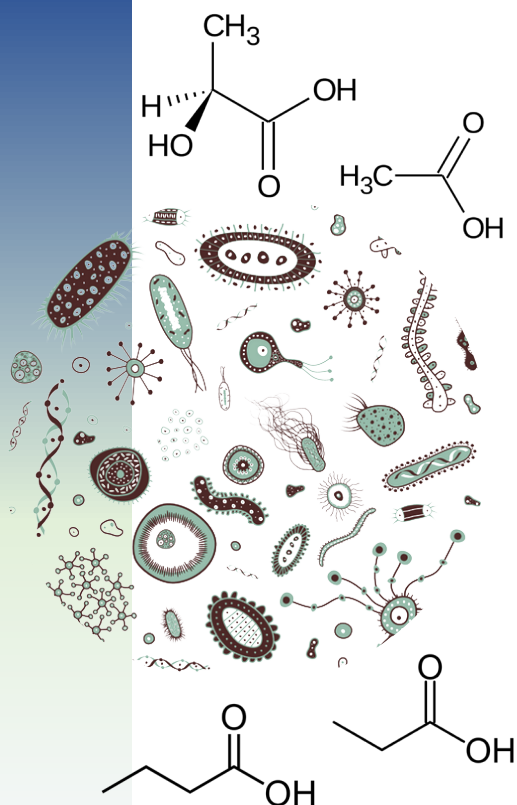


Data Handling and Analysis

- Methanogenic profiles were elaborated as proposed by Sorg et al. (2018). The mean of methane peaks (ppm_{xm}) was considered estimating the absolute CH₄.
- The Chao1 index, the ACE index, the Shannon diversity index, and the Simpson index were calculated to estimate the α -diversity. A non-metric multidimensional scaling (NMDS) and a permutational multivariate analysis of variance (PERMANOVA) were used to detect differences in β -diversity.
- All the ruminal data were analyzed using a linear mixed-effect model (R version 4.2.0) considering the fixed effects of treatment and the relative covariate for each response variable.
- For the microbiota data, a Kruskal-Wallis test was performed.



Results – VFA

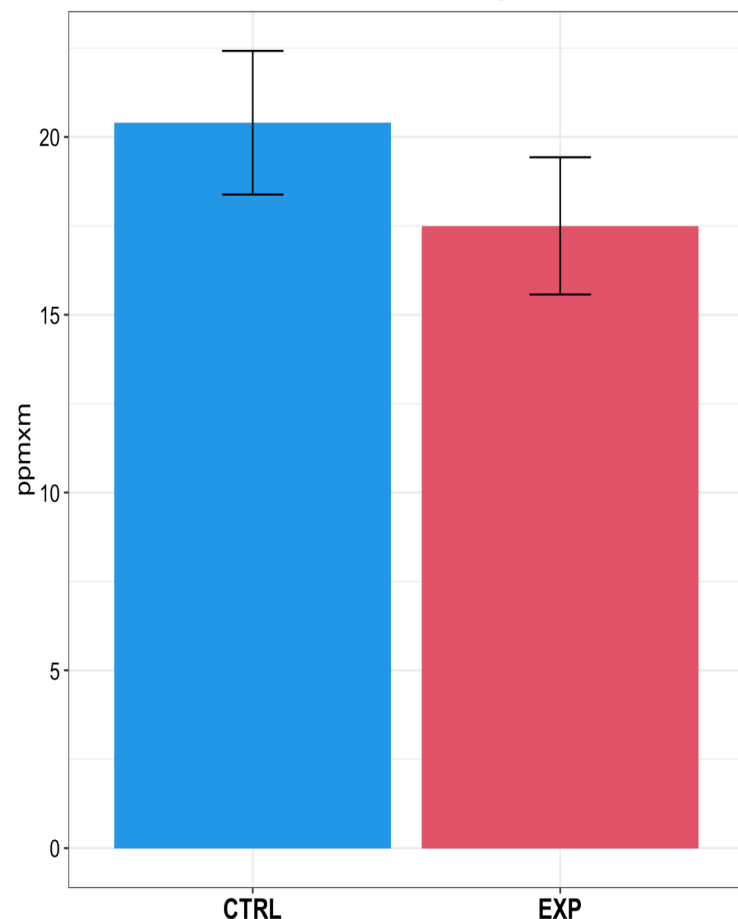


	CTRL	EXP	SEM	<i>P-value</i>
pH	7.44	7.47	0.07	0.706
Lactate (mM)	9.09	4.83	1.48	0.055
Tot VFA (mM)	87.41	76.38	7.33	0.306
Acetate (%)	64.98	66.73	1.70	0.626
Propionate (%)	22.23	22.49	1.70	0.504
iso-butyrate (%)	1.85	1.71	0.14	0.518
Butyrate (%)	8.78	7.11	0.35	<0.001
iso-valerate (%)	2.27	1.60	0.18	0.014
Valerate (%)	0.43	0.35	0.06	0.351
Acetate:propionate	3.14	3.33	0.31	0.593

→ Both related to reactions incorporating H₂ (Ungerfeld, 2020); however, no significant reductions of **acetate produced** (lower NDFom diet) or **methanogenesis** were detected.

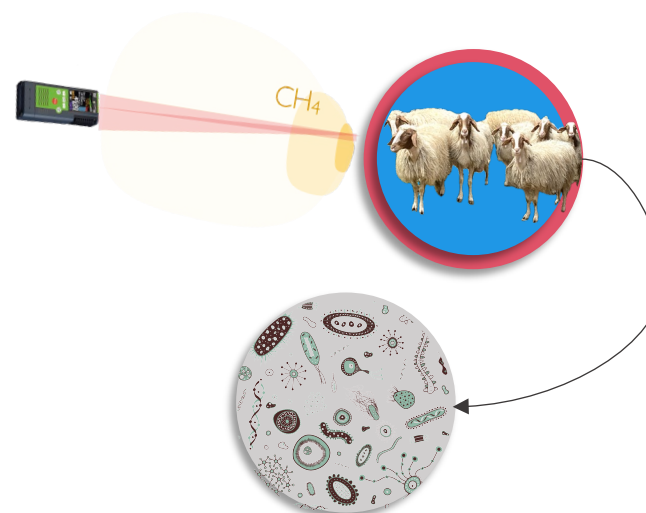
Results – enteric CH₄ emission

CH₄ peaks average *



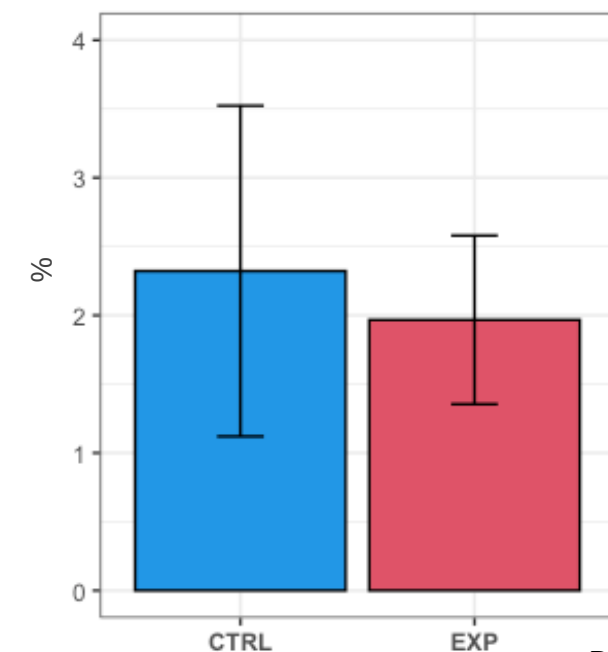
P=0.32

No differences despite NDFom content was EXP>>CTRL



No differences of Archaea at Domain level

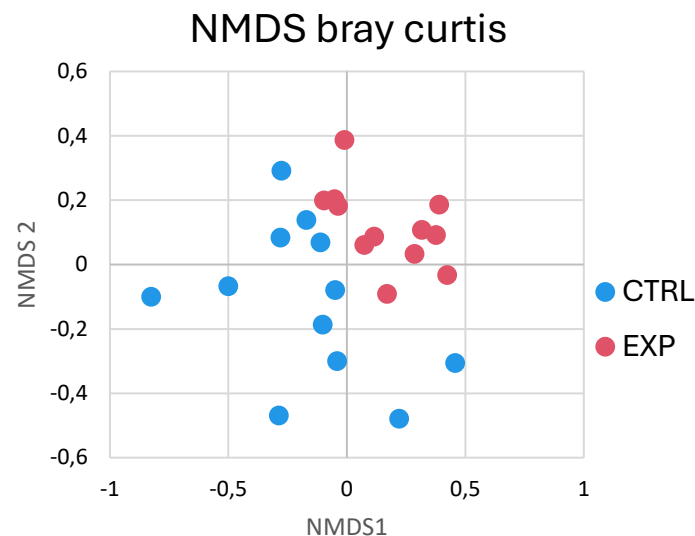
Archaea relative abundance



P=0.49

Results – bacterial community structure

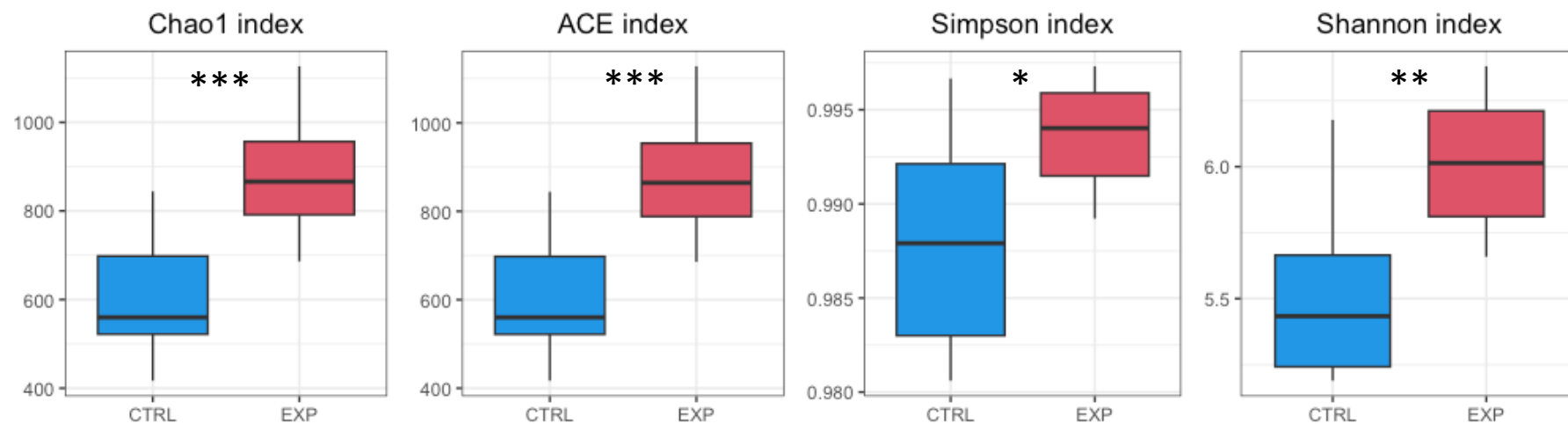
β -diversity



Permanova:

- CTRL vs. EXP – $p = 0.002$

Significant higher
 α -diversity in EXP

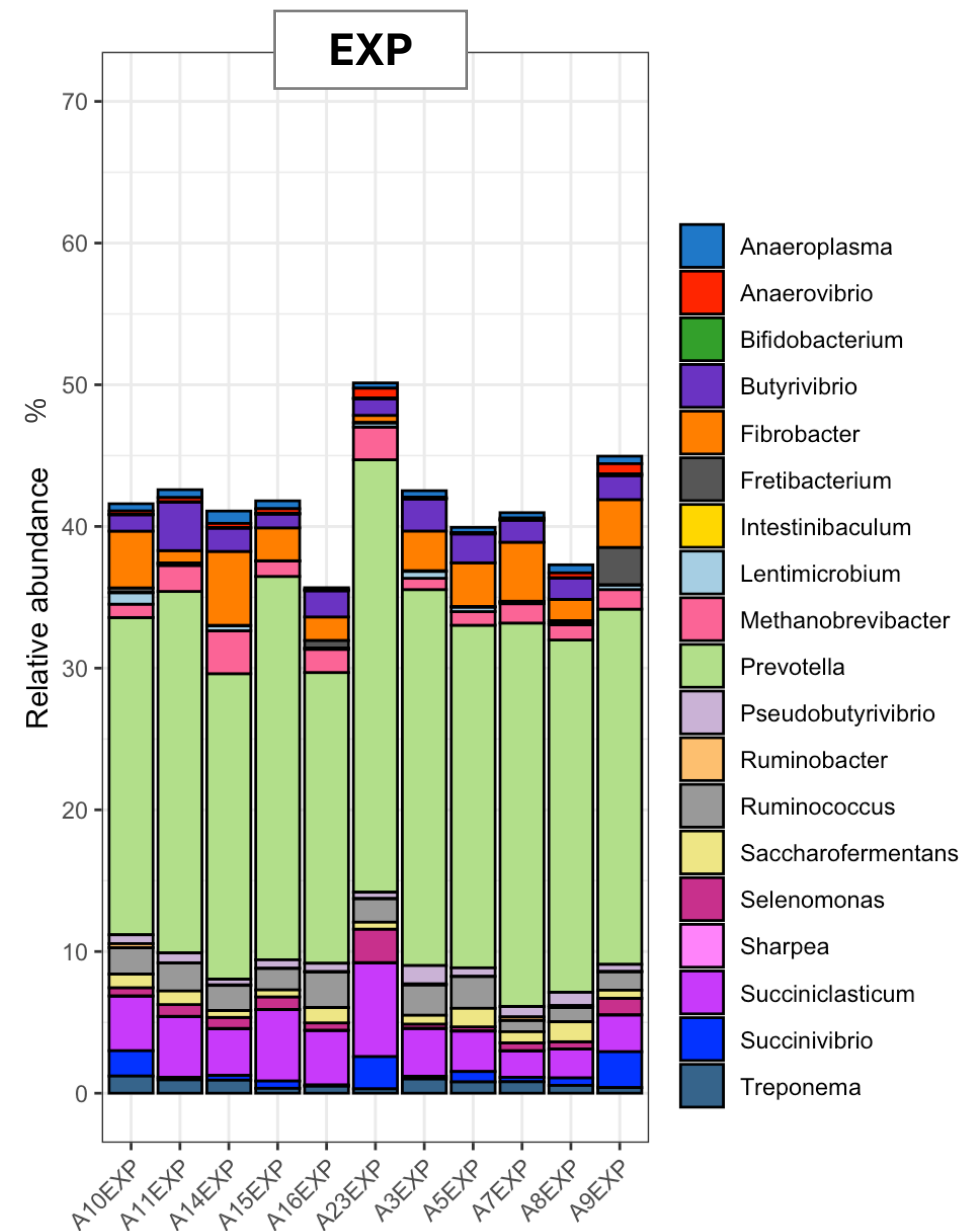
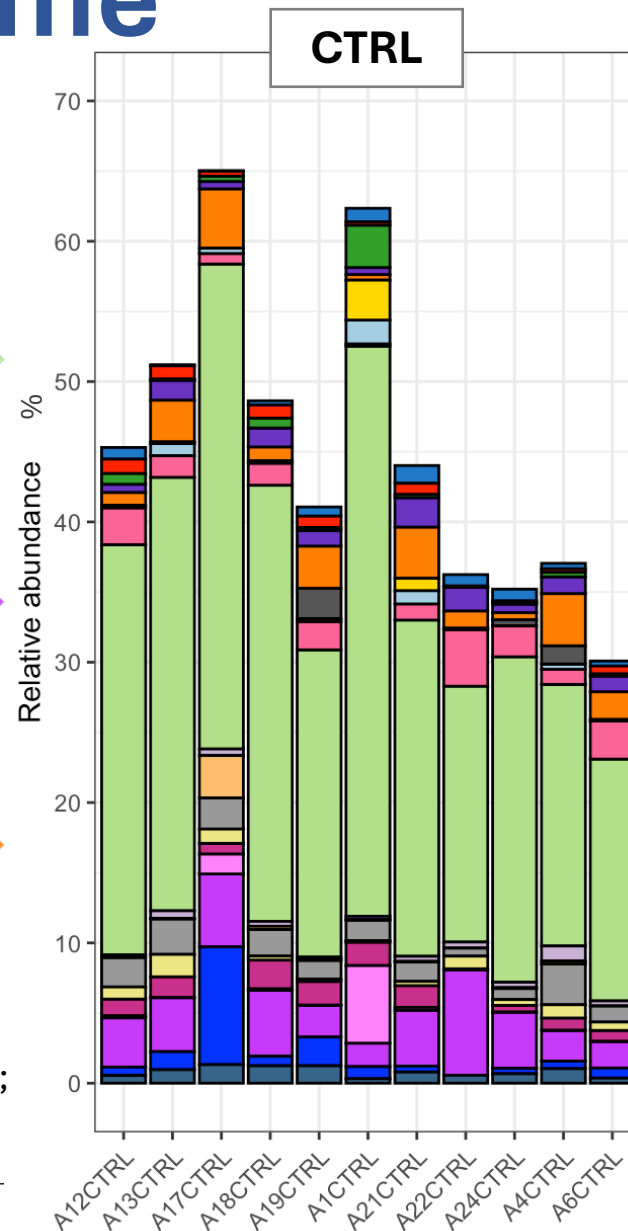


Core bacteriome

Prevotella spp.

Succinoclasticum spp.

Fibrobacter spp.

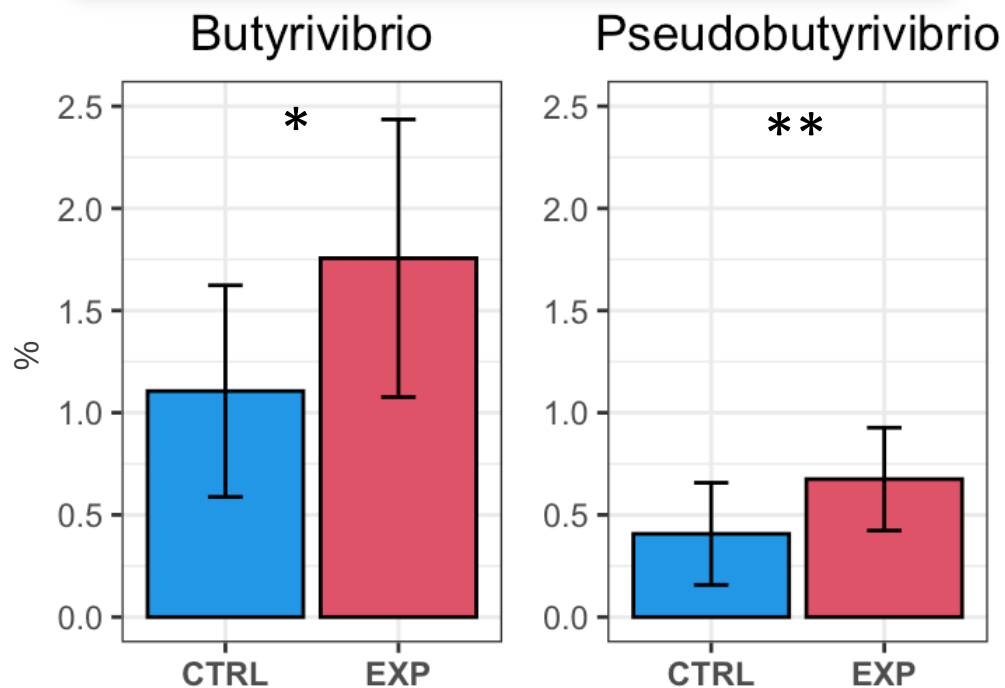


(Henderson et al., 2015;
Manneli et al., 2018)



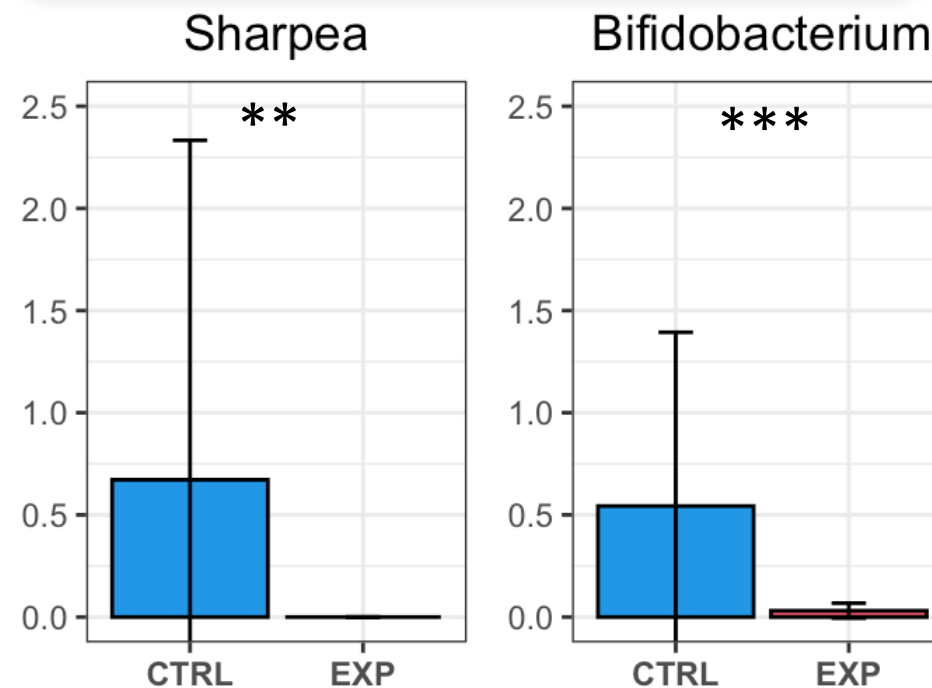
Differences at genus level

Fibrolytic activity



They occupy similar niches but apply different degradation strategies (*Palevich et al., 2020*).

Lactic acid producers



More active when simple sugars are available (*Pokusaeva et al., 2011, Kumar et al., 2018*). Higher abundances supported by the higher lactic acid conc.

Results – Diets FAs

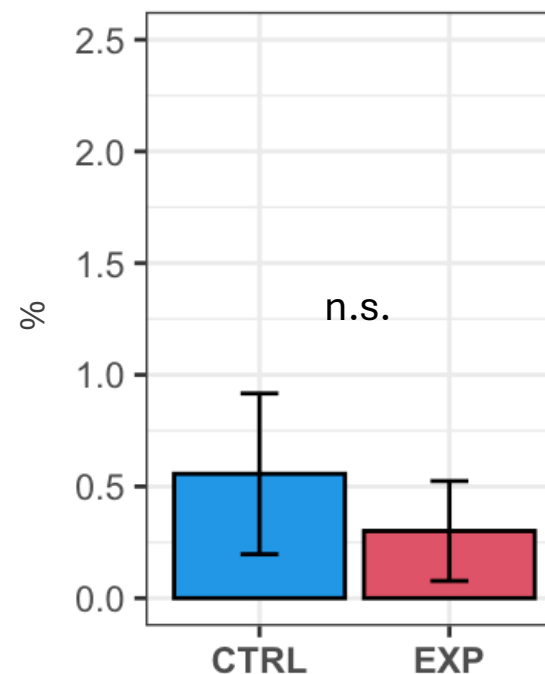
DIETS

	CTRL pellet	EXP pellet	Pasture
FA (g/100 g FAs)			
C14:0	0.19	0.29	0.71
C16:0	14.97	15.47	17.34
C16:1 cis-9	0.13	0.22	
C17:0	0.25	0.48	0.41
C18:0	2.89	2.59	2.01
C18:1 n-9	26.01	36.36	4.38
C18:1 cis-11	0.76	0.95	0.23
C18:2 n-6	48.43	34.61	20.72
C18:3 n-3	3.32	3.08	46.57
C20:0	0.39	0.59	1.03
C20:1 cis-11	0.37	0.47	
C22:0	0.47	0.59	0.73
C24:0	0.51	0.79	0.23
C22:5 n-3	0.29	0.28	
Others	1.02	3.21	5.65

X

Lipolysis

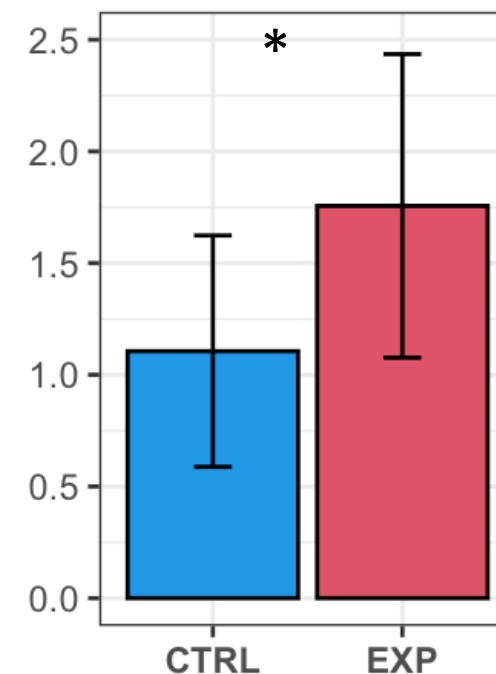
Anaerovibrio



✓

Biohydrogenation

Butyrvibrio



In contrast with previous results obtained feeding olive pomace (*Mannelli et al., 2018*)

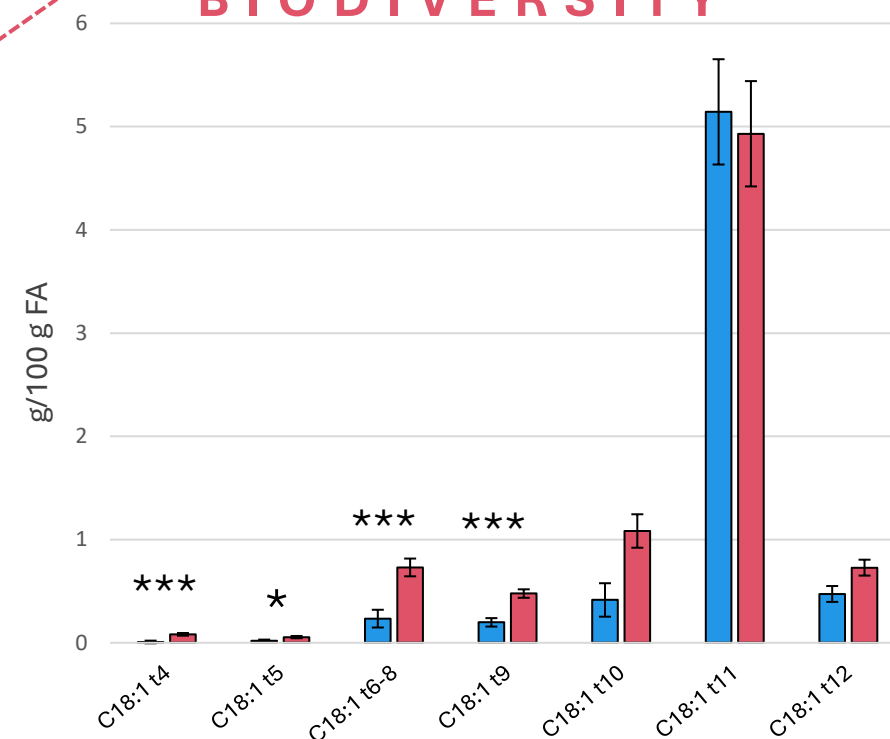
Results – Ruminal FAs

Diet **C18:2 cis-9,12** CTRL > EXP as in rumen liquor (10.5 vs. $6.3 \pm 1.3 \%$, $p=0.04$)



Pasture

BIODIVERSITY



- C18:1 c9 did not statistically differed (8.5 CTRL vs. $7.7 \text{ EXP} \pm 1.1 \%$).
- C18:0 (26.2 CTRL vs. $35.3 \text{ EXP} \pm 5.0 \%$)

Conclusions

- The study provides new evidence on the potential effect of **by-products** on the **ruminal microbiome**, which has been little studied (*Marcos et al., 2022*).
- Relative abundances at genus, despite the fact they cannot reflect the ruminal activities, had a good correspondence with the measured **fermentative traits**.
- Our previous research showed that feeding EXP did not reduce milk yield in ewes; instead, it improved their **environmental efficiency**.
- In the present study, the EXP diet notably enhanced the **α -diversity**, suggesting that when simple sugars are less available, the **rumen environment effectively adapts** by supporting microorganisms capable of exploiting structural carbohydrates.
- Further research should investigate rumen metabolism and microbiota **across different lactation phases** and with varying mixtures of by-products.





Thank you for your kind attention!

giulia.foggi@agr.unipi.it



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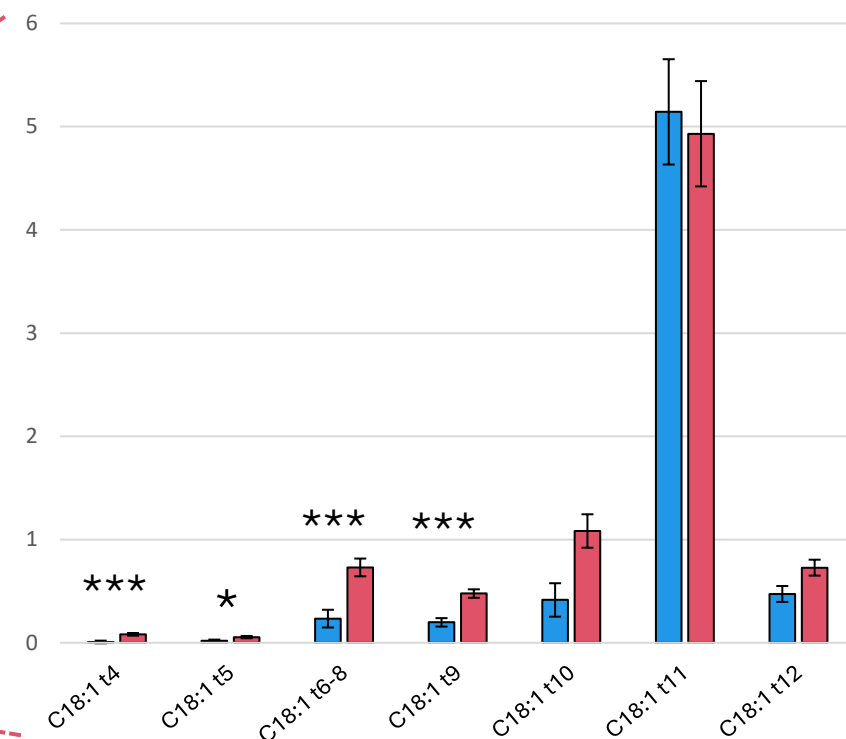
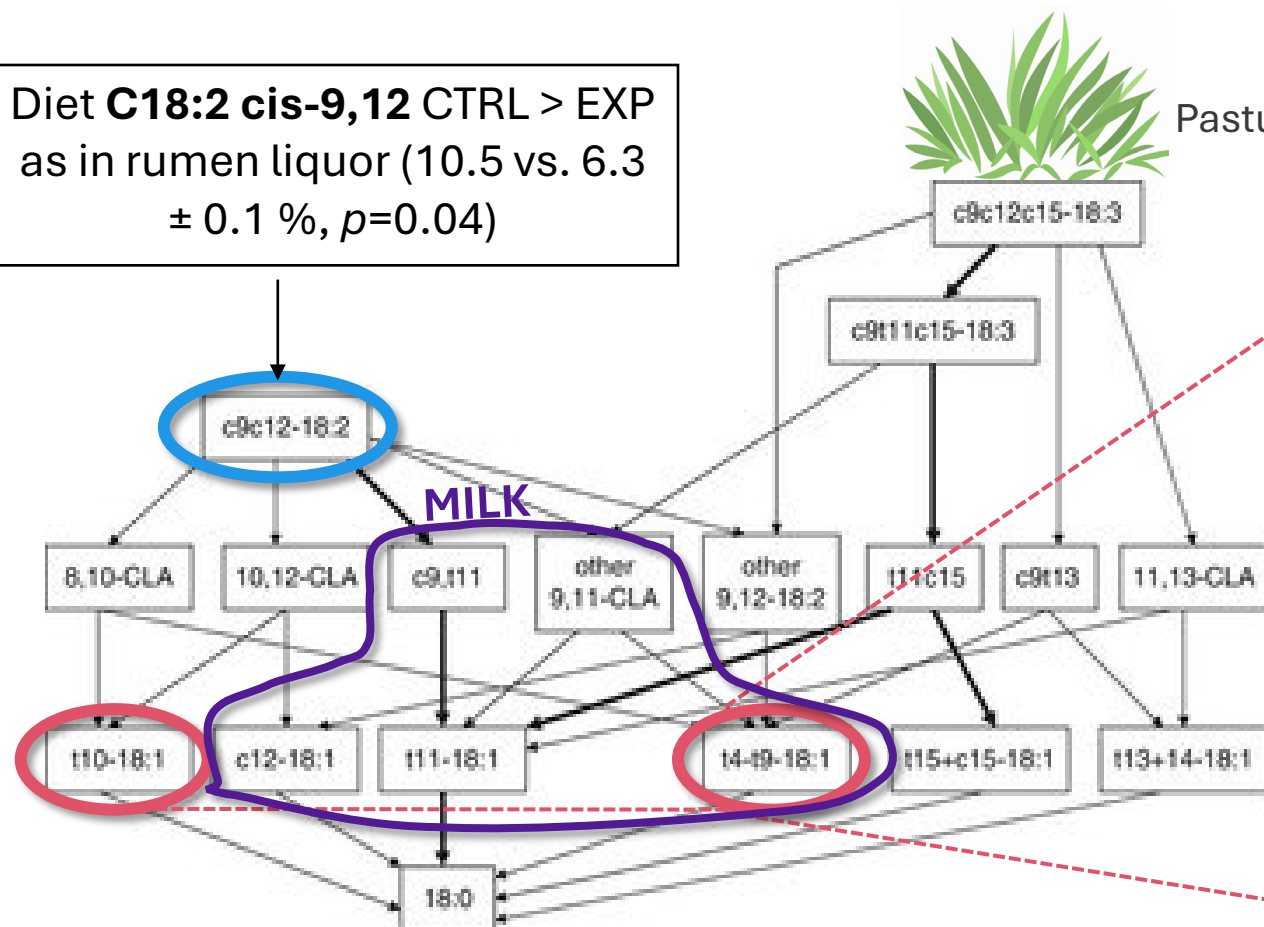
The authors acknowledge the financial support for this project provided by transnational funding bodies, partners of the H2020 ERA-NETs SUSFOOD2 and CORE Organic Cofund, under the Joint SUSFOOD2/CORE Organic Call 2019 (ALL-IN project) and Agritech National Research Center funded from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022), Spoke 5, Task 5.3.3.

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Supplementary – Ruminal FAs

Diet **C18:2 cis-9,12** CTRL > EXP
as in rumen liquor (10.5 vs. 6.3
± 0.1 %, $p=0.04$)



C18:1 c9 did not statistically differed (8.5 CTRL vs. 7.7 EXP ± 1.1 %).

Supplementary material

