75th EAAP 2024

Firenze, Italy, 1-5, September 2024

Polyphenols from Sulla (*Hedysarum coronarium*) affects rumen fluid microbiota of dairy sheep ewes

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PLAS TEAM

Pisa Livestock farming, Animal food quality, and Sustainability

Disolasulla

ministero delle politiche agricole alimentari e forestali

«Polyphenols from sulla forage for improving the welfare of dairy animals and the quality of cheese»

Ruminal fluid

Milk and cheese quality

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Introduction

Hedysarum coronarium

Fresh sulla

It is known for its beneficial effects on animals due to presence of plant secondary metabolites such as polyphenols -> high content of condensed tannins;

Sulla grows in a limited period of the year, primarily in the spring season.

This creates the need to find a preservation method for animal feed, and it is very important to investigate the effects on animals, as the effects of fresh sulla are well known, unlike those of sulla hay and dehydrated sulla.

Sulla hay (SH)

Dehydrated Sulla (DS)

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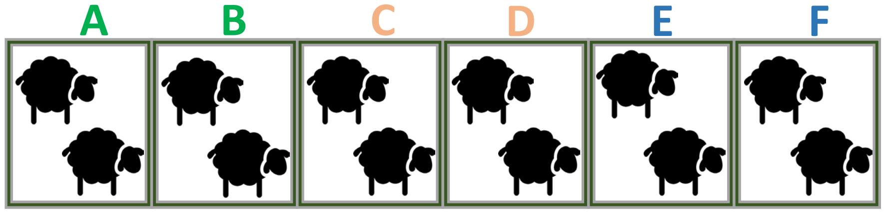
Aim of the study

The aim of this study was to evaluate the effect of two different preservation systems, hay (SH) and dehydrated (DS) on:

- ✓Rumen microbiota
- ✓Rumen metabolism

Materials and method



12 ewes
Same parity
60 days of lactation

Diets composition (Kg)	C	DS	SH
Mixed hay	1	1	1
Alfalfa hay	1.1	-	-
Dehydrated sulla	-	1.1	-
Sulla hay	-	-	1.1
Cracked corn	0.1	0.1	0.1
Oat	0.6	0.6	0.6
Soybean meal	0.2	0.2	0.2

C = control diet;

DH = dehydrated sulla diet;

SH = sulla hay diet

Diets proximate composition (g/100g of DM)	C	DS	SH
Dry matter	90.75	89.83	89.39
Ether extract	2.41	2.69	2.51
NDF	49.99	48.79	47.70
ADF	36.92	33.36	34.06
ADL	7.61	6.54	7.04
Crude protein	12.58	12.03	12.38
Ashes	7.70	8.85	7.29

Phenols content in different diets

Diet	Total phenols mg GAE/g DM	Total tannin phenols mg GAE/g DM	Non-tannin phenols mg GAE/g DM	Condensed tannins mg CAE/g DM	Hydrolysable tannins mg CAE/g DM
C	4.31	1.95	2.36	0.34	1.61
DS	4.94	2.41	2.53	0.40	2.01
SH	5.33	2.48	2.85	0.50	1.99

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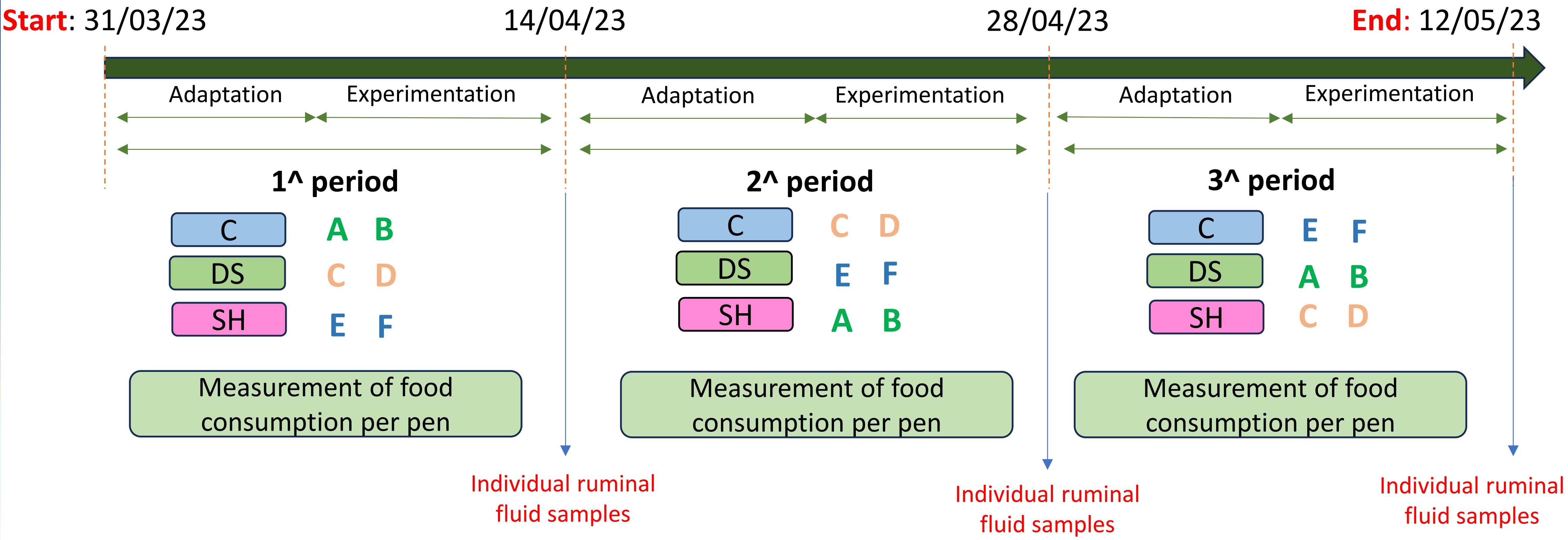
Materials and methods

Experimental design

3x3 latin square model with two replicates



The last 3 days before the end of each period, we measured the individual methane concentration 5h after the last feeding

Authorised by OPBA of Pisa University 22/2022

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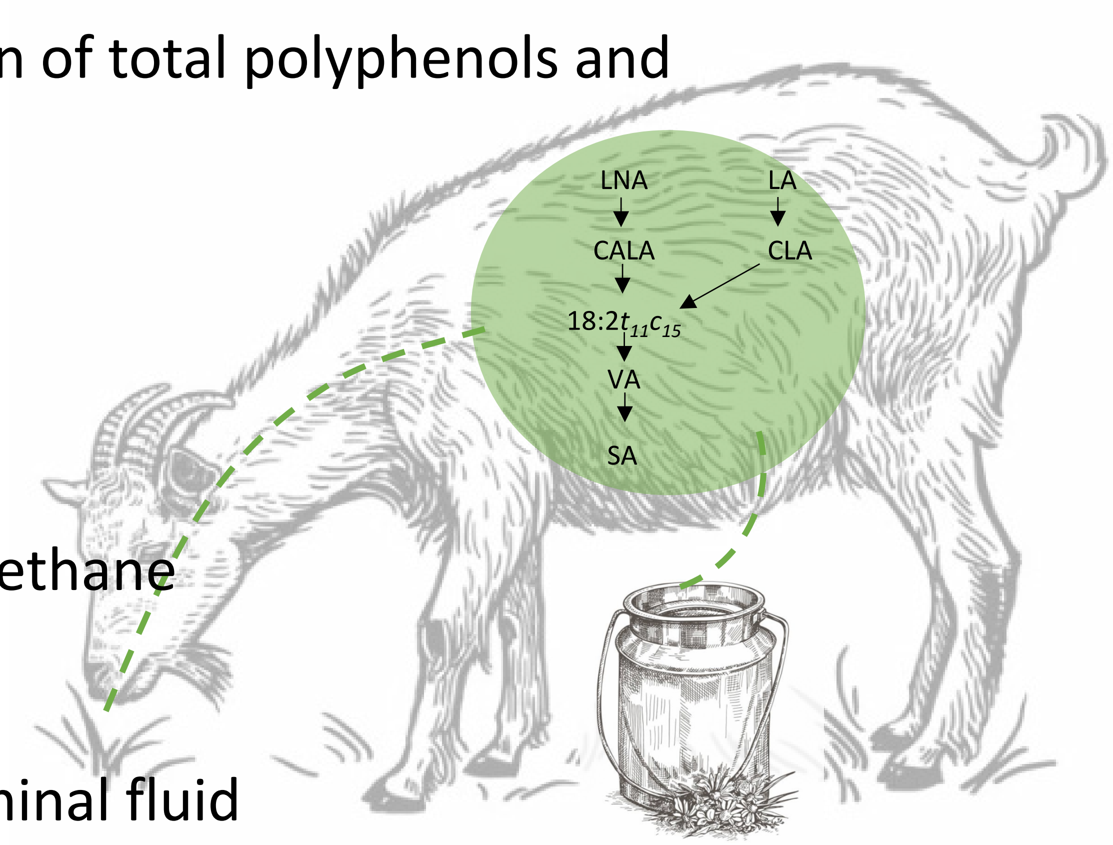
Materials and methods

Analysis

The analytical methods used are:

- Proximate composition and fiber fractions (A.O.A.C., Van Soest)
- Spectrophotometric method → Quantification of total polyphenols and their different fractions
- HPLC UV-DAD → Determination of VFAs
- GC-FID → Determination of FAs
- Handheld laser methane detector (LMD) → methane concentration
- V3-V4 of 16s rRNA region → microbiota in ruminal fluid



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Statistical analysis

Mixed linear model

$$Y_{ijk} = \mu + D_i + P_j + D_i x P_j + A_k + \varepsilon_{ijk}$$

FA, VFA, CH_4, poliphenols

Where:

Y_{ijk} = variable;

μ = common mean to all observations;

D_i = fixed effect of i^{th} diet (C, DS, SH);

P_j = fixed effect of j^{th} period (1, 2, 3);

D_i x P_j = interaction of fixed effect;

A_k = random effect of K^{th} ewes (from 1 to 12);

ε_{ijk} = residual error

α and β diversity of microbiota in rumen fluid

α– diversity

- Chao1
- ACE
- Shannon
- Simpson

Multivariate discriminant analysis

$$Z = c_1X_1 + c_2X_2 + \ldots + c_nX_n$$

Where:

$c_1, c_2, \ldots c_n$ = discriminant coefficients;

$X_1, X_2 \ldots X_n$ indipendent variables

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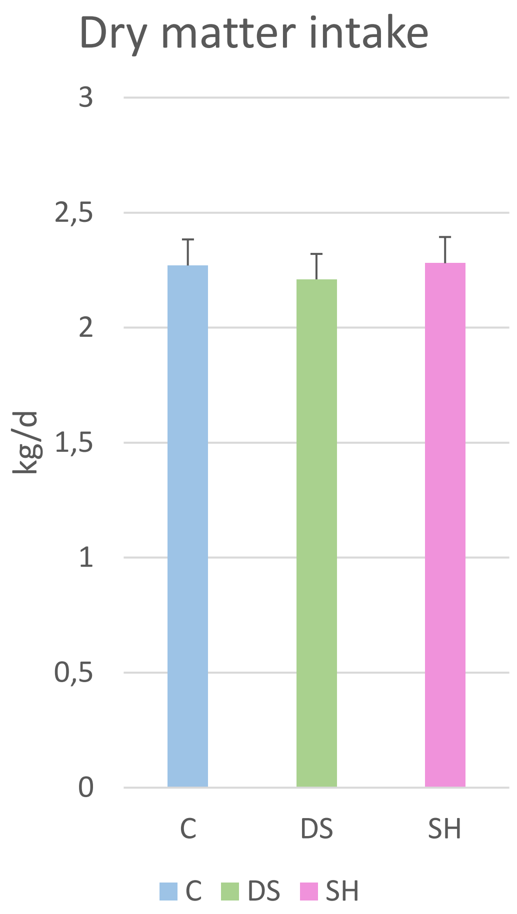
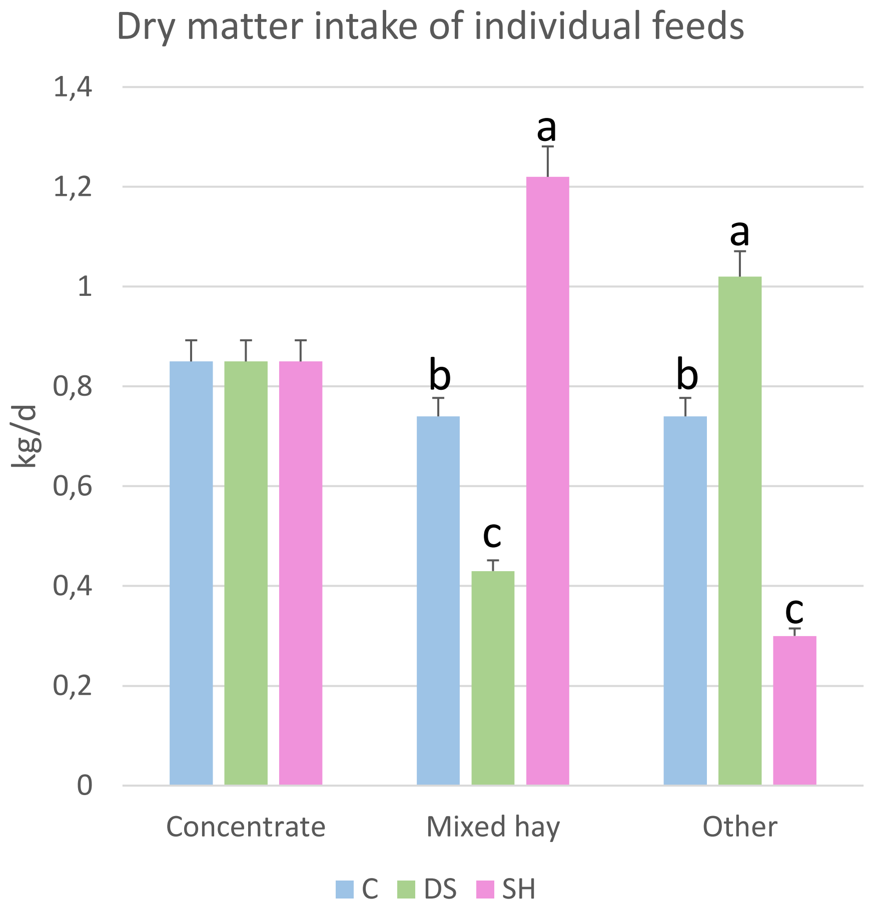
Results

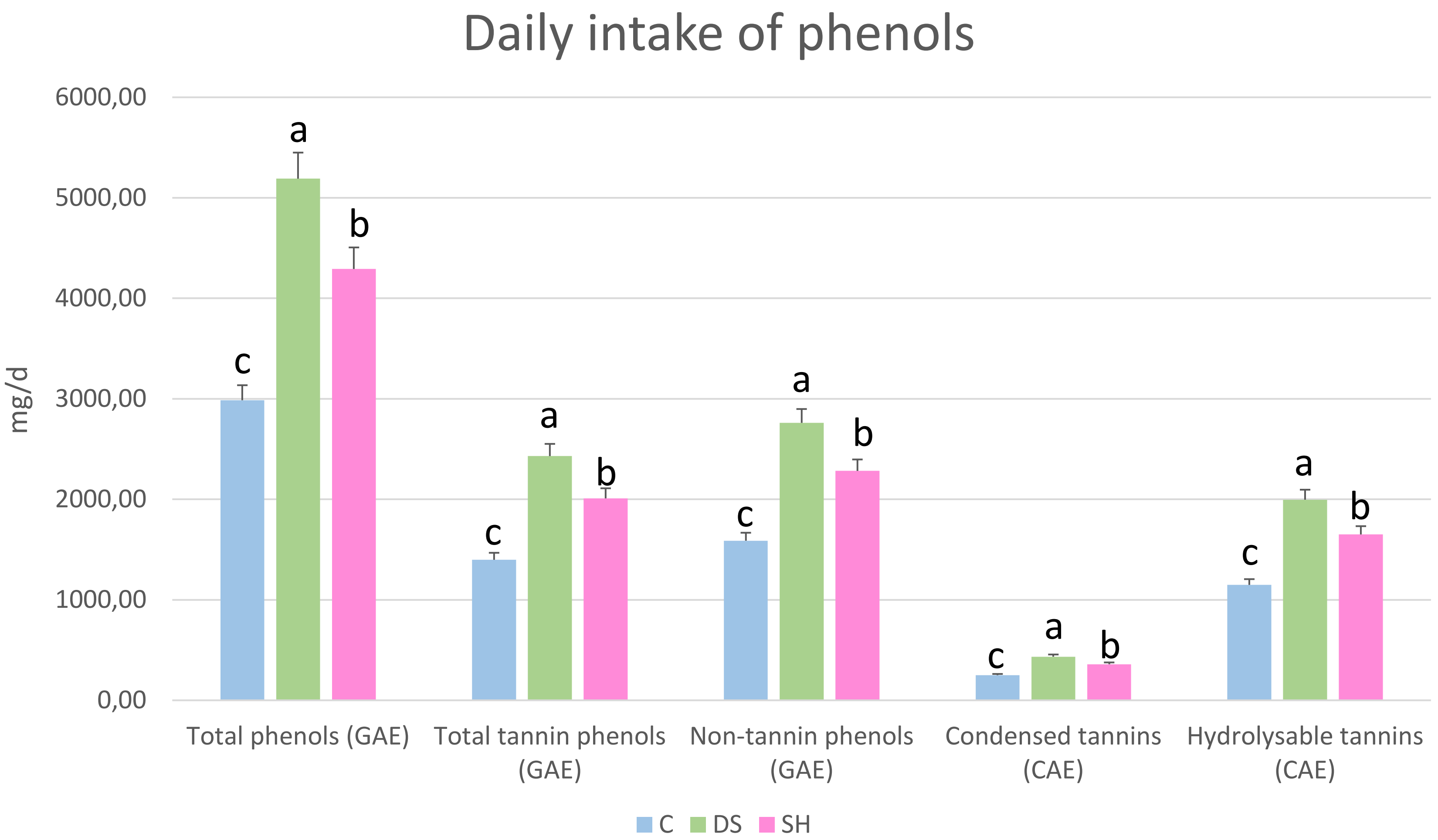




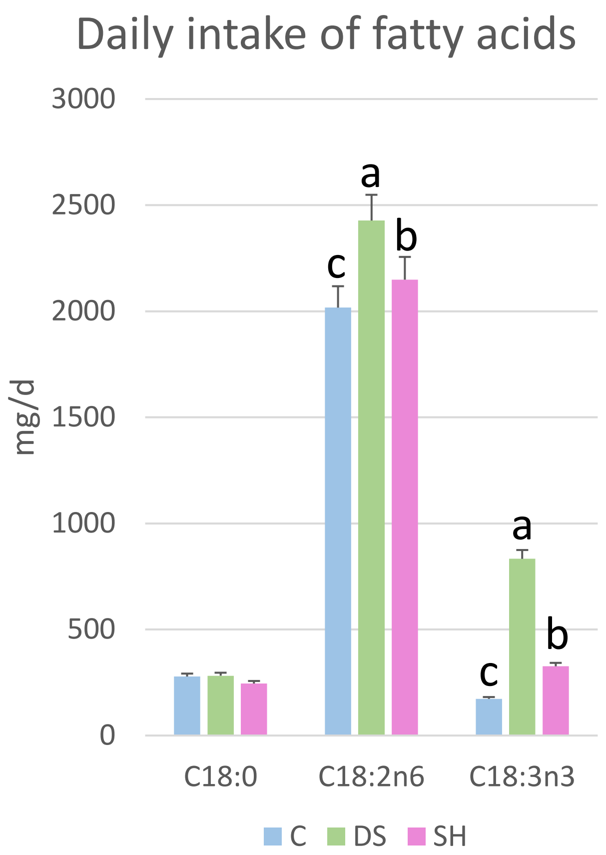
Where other:

- C = alfalfa hay;
- DH = dehydrated sulla;
- SH = sulla hay

Results



P = 0.03



P = 0.02

❌ α diversity ✅ β diversity

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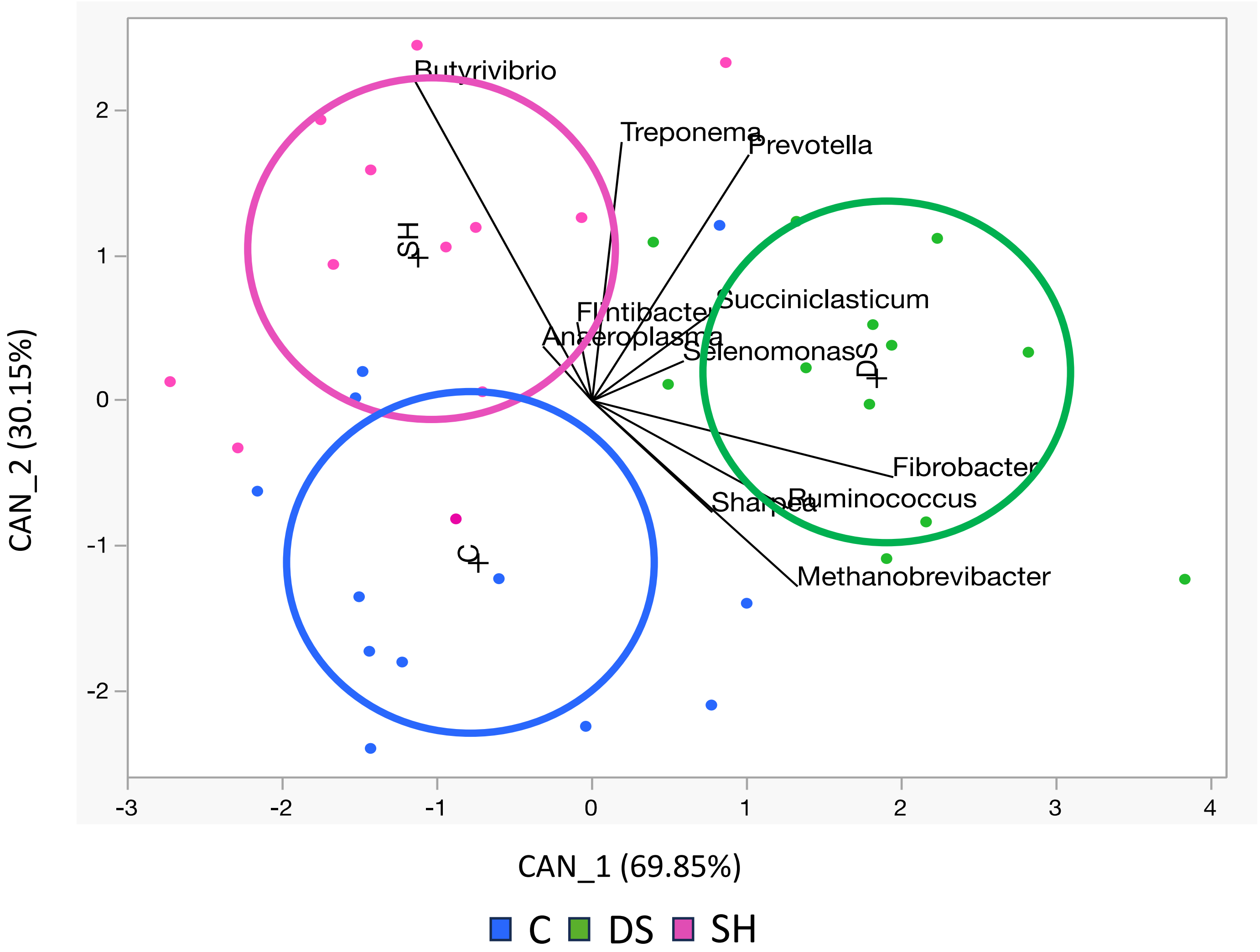
Results



	CAN_1	CAN_2
Anaeroplasma	-0.21	0.25
Butyrivibrio	-0.76	**1.47**
Fibrobacter	**1.30**	-0.35
Flintibacter	-0.06	0.36
Methanobrevibacter	**0.89**	**-0.85**
Prevotella	0.67	**1.13**
Ruminococcus	**0.85**	-0.49
Selenomonas	0.40	0.18
Sharpea	0.52	-0.51
Succiniclasticum	0.54	0.42
Treponema	0.13	**1.19**

Hotteling-Lawley test p = 0.004

Results

Polyphenols affect:

1.Carbohydrates

2. Proteins

3. Lipids

Degradation and fermentation

Biohydrogenation

Effect?

AGV

CH_4

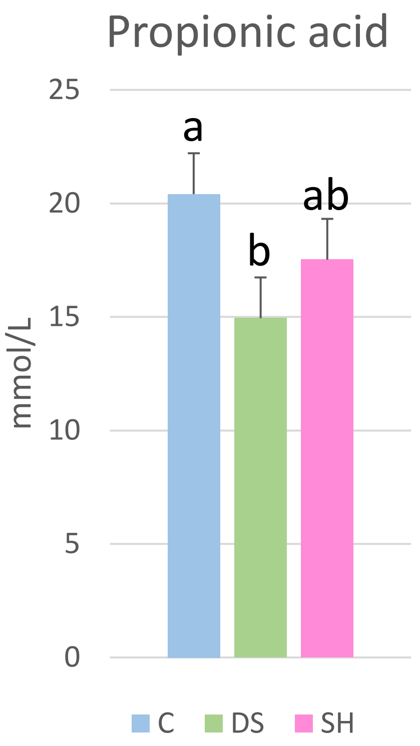
Nutraceutical properties

Results

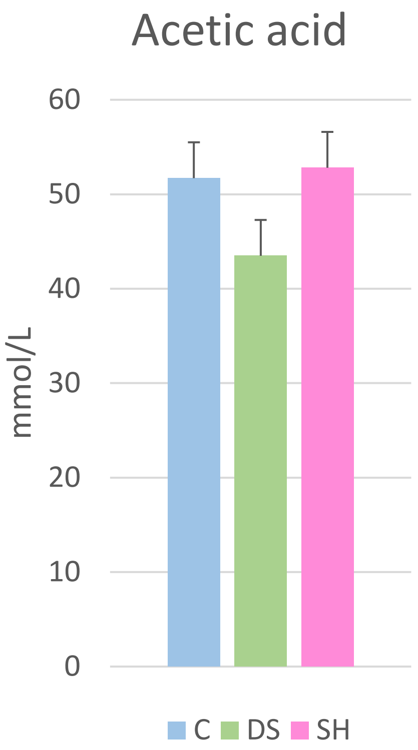
1. Effect on carbohydrates fermentation

Starch → Propionic acid

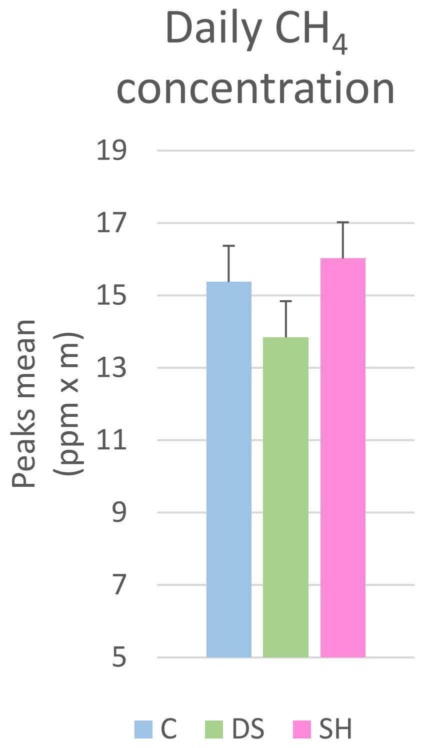
Structural carbohydrates → Acetic acid, CH_4



P =0.03



P = 0.07



P = 0.1

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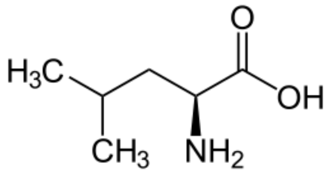
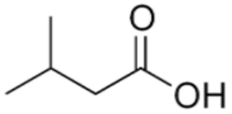
Results

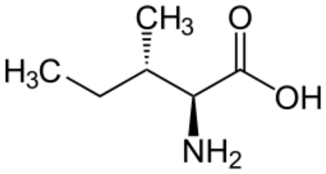
2. Effect on proteins



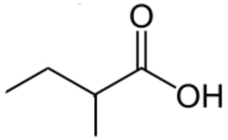
Leucine



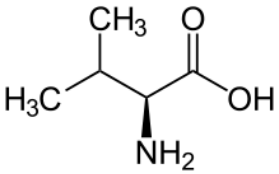
Isovaleric



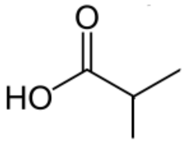
Isoleucine



2-methylbutyric



Valine

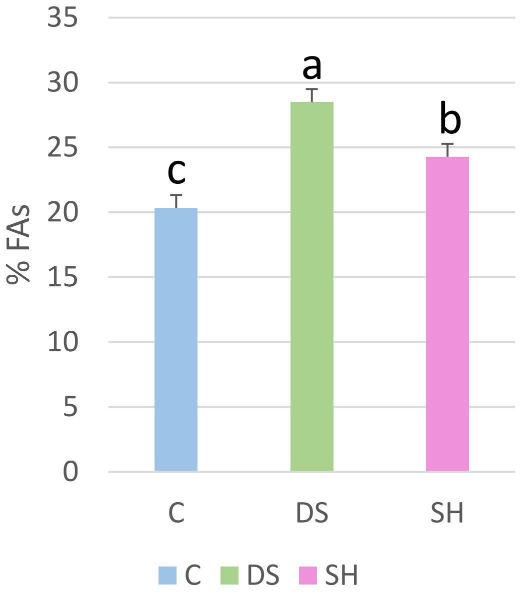


Isobutyric

Elongation

ISO-C15:0 ISO-C17:0	ANTEISO-C15:0 ANTEISO-C17:0	ISO-C14:0 ISO-C16:0
Zhan T. et al 2023	*Kupczynsky et al. 2024*	*Salazar N. et al 2022*

Even Iso Branched chain fatty acids



$P = 0.04$

Results

3. Effect on lipids biohydrogenation in rumen fluid

C	DS	SH	Fatty acid
b	a	b	18:3 $c_9c_{12}c_{15}$ (LNA)
b	a	a	18:3 $c_9t_{11}c_{15}$ (CALA)
b	a	b	18:2$t_{11}c_{15}$
b	a	b	18:1 t_{11} (VA)
a	b	a	18:0 (SA)

Fatty acid	C	DS	SH
18:2 c_9c_{12} (LA)	b	a	b
18:2 c_9t_{11} (CLA)	b	a	b

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Conclusions

Condensed tannins

Effect on

Metabolic pathways of proteins, carbohydrates and lipids

Part of these effects maybe is due to the different intake of polyphenols.

Preservation system

Dehydrated sulla was preffered by the ewes

Higher intake of polyphenols

Enhancing the effects more clearly

In summary…

- ✓ The dehydrated sulla resulted in greater feed intake, likely due to its better palatability and physical form
- ✓ The dehydration process required to produce dehydrated sulla is energy-intensive. It would be important to find renewable energy sources to make it more sustainable
- ✓ Sulla hay would be an alternative preservation system but we observed that the ewes tended to select certain parts of the plant, especially flower and leaves, likely because the stems were quite thick. Additionally, producing sulla hay remains a challenge, especially in Tuscany.

THANK YOU FOR YOUR ATTENTION

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Special thanks to Matteo Daghio for his support in bioinformatics analysis

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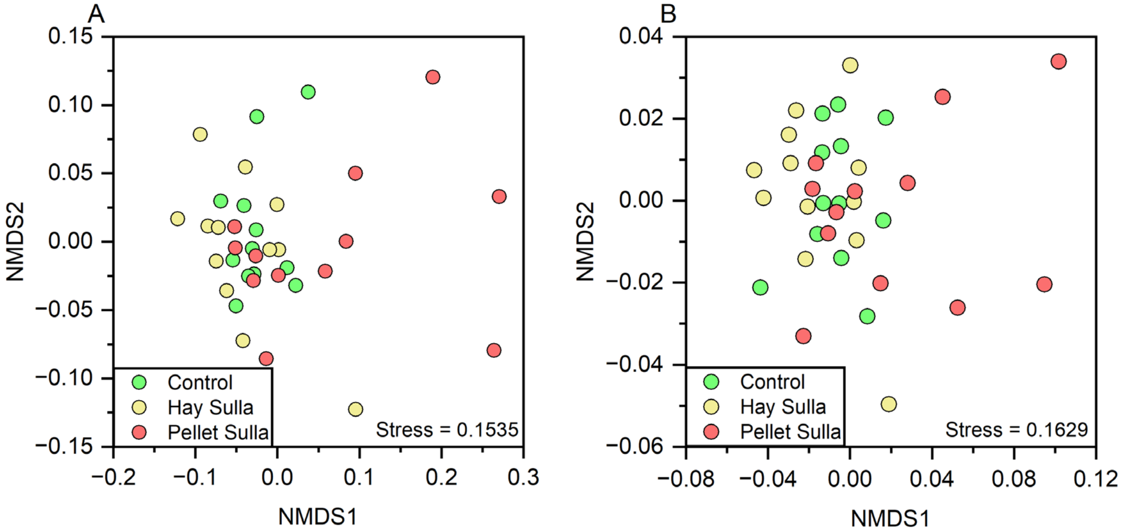
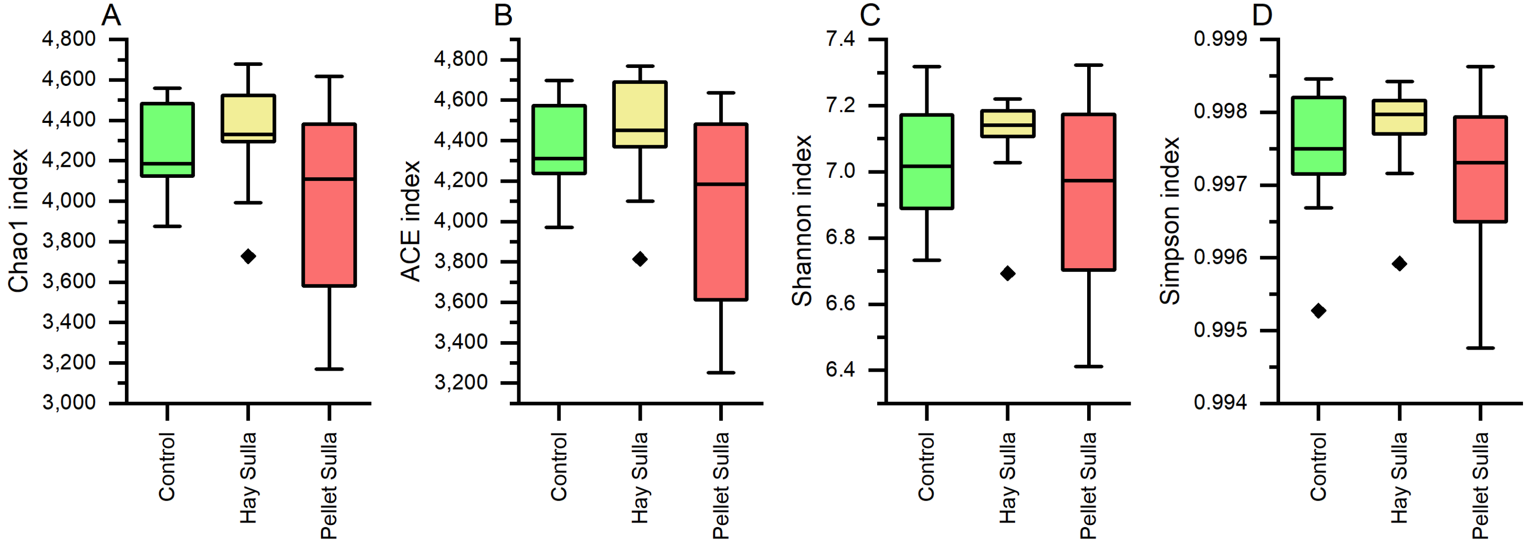
Supplementary

β diversity





α diversity

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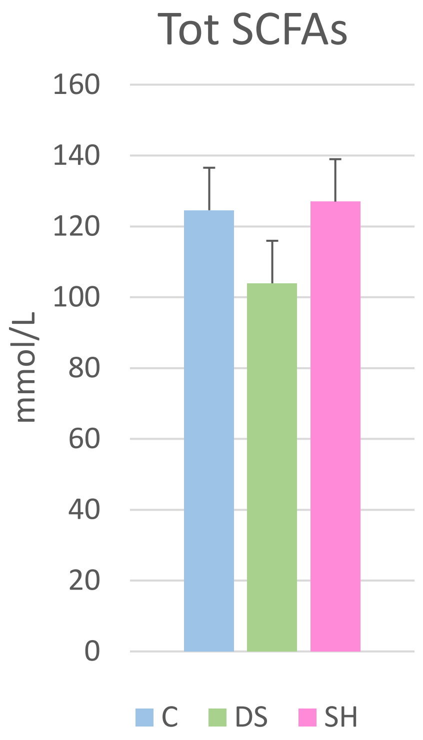
Tot SCFAs
160
140
120
100
80
60
40
20
0
mmol/L
C
DS
SH

Volatile fatty acids in rumen fluid (mmol/L)					
	C	DS	SH	SE	P value
Lactic	41.63	36.59	46.19	8.71	0.6347
Acetic	51.70	43.48	52.80	3.48	0.0709
Proprionic	20.41[a]	14.94[b]	17.52[ab]	1.83	0.03
Isobutirric	0.34	0.36	0.47	0.14	0.7956
Butirric	8.02	7.17	7.86	0.70	0.4887
Isovaleric	1.38[a]	0.67[b]	1.13[a]	0.14	0.0016
Valeric	1.06	0.71	0.98	0.18	0.2184
Tot	124.55	103.93	126.97	12.17	0.1805

Volatile fatty acids in rumen fluid (%)					
	C	**DS**	**SH**	**SE**	**P value**
Lactic	28.34	27.42	35.57	3.49	0.1594
Acetic	44.20	45.79	42.48	2.20	0.3726
Proprionic	18.30	16.77	13.61	1.77	0.2858
Isobutirric	0.29	0.70	0.38	0.26	0.5401
Butirric	6.74[ab]	7.91[a]	6.24[b]	0.53	0.0400
Isovaleric	1.16[a]	0.65[b]	0.88[ab]	0.10	0.0016
Valeric	0.96	0.75	0.83	0.17	0.5748

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BCFA in rumen fluid (%)				
	Tot iso	TOT ante	TOT iso even	TOT iso odd
Control	42.68	57.31	20.34	22.34
DH	49.14	50.85	28.50	20.64
SH	42.61	57.39	24.28	18.32

Fatty acid composition of milk (g/100 g FAs)					
	Control	Hay	Pellet	SE	P value
C14:0	8.9[b]	9.0[b]	9.4[a]	0.15	0.0019
C16:0	22.3[a]	22.3[a]	21.5[b]	0.28	0.0050
C18:0	14.1[a]	14.1[a]	12.0[b]	0.51	0.0003
C18:1 11t	0.59[b]	0.63[b]	0.87[a]	0.03	<0.0001
C18:1c9	23.9[a]	23.5[a]	20.4[b]	0.41	<0.0001
C18:2 n6	2.11	2.09	2.13	0.08	0.90
CALA	0.04[b]	0.04[b]	0.06[a]	0.01	0.0002
C18:3 n3	0.40[b]	0.40[b]	0.87[a]	0.04	<0.0001
CLA9:11ct	0.29[b]	0.30[b]	0.40[a]	0.30	<0.0001

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