The impact of probiotic live yeast in a barley grain-based diet on rumen microbiome and histology of artificially reared lambs

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ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
AGRICULTURAL UNIVERSITY OF ATHENS

LALLEMAND
LALLEMAND ANIMAL NUTRITION

Presenter: Alexandros Mavrommatis

Session 76. Advances in ruminant nutrition, Part 2
Room: Maremmana - Palazzo Affari 2nd Floor
Wednesday 4 September

EAAP
European Federation of Animal Science

Introduction

1

In the intensive dairy sheep systems, lambs are separated from dams and weaned artificially with milk replacers to maximize farmer revenue from raw milk.

2

These practices can expose animals to digestive disorders such as infections or re-configure their gastrointestinal tract microbiome into a dysbiosis model.

3

The transition of lambs from milk to solid feed is based on concentrate feeds where the proportion of starch is high. The high starch content in the rumen is rapidly fermented and increases the risk of metabolic disorders.

Introduction

Live yeasts (LY) such as *Saccharomyces cerevisiae* can be an ally of ruminants' digestive homeostasis in early life by promoting rumen microbial inhabitation and stimulates fibrolytic bacteria.

LY act as oxygen scavengers within the rumen improving its anaerobiosis and stimulating bacterial degradative activities.

LY are capable of either competing with lactic acid bacteria such as *S. bovis* and *Lactobacillus*, for fermentable carbohydrates or encouraging the growth of lactate-utilizing bacteria which results in the low accumulation of lactate.

Probiotic Yeast: How Does It Work?

- Luminal effect
- Trophic action
- Improve rumen fermentation

01

The present study aimed to investigate the effect of a probiotic live yeast supplement under the impact of two factors that regularly stress lambs in early life: weaning through artificially rearing and a high fermentable starch source, on rumen microbiome structure, enzymatic activity, and morphology.

02

To slightly simulate this stressor signal, the concentrate mix in our study was formulated based on barley grains whose starch is more fermentable than that of corn with a faster rate of in situ degradation.

Stage of Conceptualization

Experimental design

- 42 Chios lambs were allocated into two homogenous groups: a) Control (CON) and b) Probiotic LY supplementation (PROB) and were artificially reared until 45th day of lambs age (weaning age).
- Throughout the rearing, the consumption of milk replacer was daily recorded on an individual basis. From the 30th until the 106th day of lambs' age, both groups (CON and PROB) were fed *ad libitum* alfalfa hay and the CON-concentrate mix. Moreover, 100 g of PROB-concentrate which included 0.1 g of *Saccharomyces cerevisiae* CNCM I-1077 live yeast (10^{10} CFU/g of commercial product) was offered to PROB lambs.
- The latter sub-meal was provided before the feeding only in the PROB group to ensure that lambs would receive a constant amount of the live yeast.

Concentrate ingredients and chemical composition of the feeds.

Item	CON-concentrate	PROB-concentrate		
Ingredients (g/kg, as fed)				
Crushed maize	184.0	183.0		
Soybean meal (44 % CP)	251.3	251.3		
Barley	549.2	549.2		
Mineral and vitamin premix	15.5	15.5		
Probiotic yeast product	-	1		
Chemical composition (g/kg dry matter)	**CON-concentrate**	**PROB-concentrate**	**Alfalfa hay**	**Milk powder**
Dry matter (as fed)	908 ± 1.2	905 ± 2.6	913± 0.8	953 ± 2.9
Ash	54 ± 1.5	58 ± 2.4	107 ± 0.7	72 ± 1.1
Crude Protein	194 ± 4.5	192 ± 4.6	244 ± 4.0	230 ± 3.5
Ether extract	24 ± 1.2	27 ± 0.8	14 ± 0.8	230 ± 0.7
Non-fibre carbohydrates	737 ± 3.9	750 ± 6.1	637 ± 4.6	-
aNDF	212 ± 9.0	201 ± 18.6	442 ± 31.9	-
ADF	71 ± 4.7	71 ± 9.6	303 ± 24.2	-
ADL	20 ± 7.6	26 ± 6.8	72 ± 3.7	-
Starch	432 ± 21.9	444 ± 10.6	-	-

Sample collection

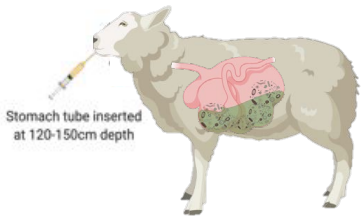
- On the 45th and 100th day of lambs ages, ten (10) out of twenty-one lambs/group were randomly selected for rumen digesta collection 4 hours after the morning feeding using an electric vacuum pump.

- At 106th day of lambs age, rumen wall portions dorsal to the cranial groove (from serosa to mucosa) were collected at the slaughterhouse and were snap-frozen in liquid nitrogen and stored at -80 ^{o}C.

Analyses pipeline

1

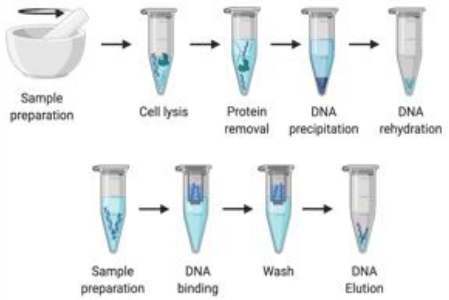
Rumen fluid collection



Rumen fluid collection

2

DNA extraction



1g of frozen rumen digesta was grinded using liquid nitrogen

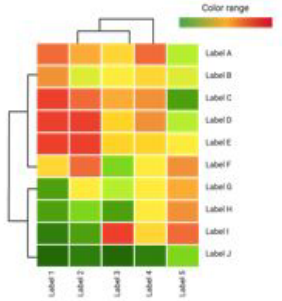
3

Illumina NGS 16s Metagenomics analysis

Hyper-variable regions V3-V4 of the ribosomal gene 16s

4

Bioinformatics and interpretation of data



Data were evaluated with holistic statistical tools

5

RT-qPCR relative abundance

Targeting species of interest

6

Protease and amylase were measured spectrophotometrically

Azo-casein method for protease and 3,5-dinitrosalicylic acid method for amylase

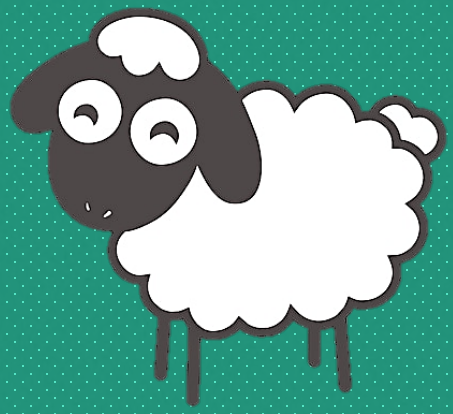
7

Cellulase and xylanase were evaluated using petri dishes

Xylanase and Cellulase were determined with petri dishes method

Analyses of rumen morphology

- Four µ thick cryotome sections were cut on poly-L-lysine coated slides using a LEICA Cryostat CM 1500.
- The sections were subsequently processed for histochemistry treated with the following 4 histochemical stains, to assess at a preliminary level basic characteristic of the rumen histology:
- a) Haematoxylin and eosin to display tissue morphology,
- b) Mallory's trichrome according to McFarlane to display collagen, elastic fibers, and smooth muscle,
- c) Modified reticular fibers stain to display reticular fibers, and
- d) Dane's stain to display prekeratins, keratins and mucins.

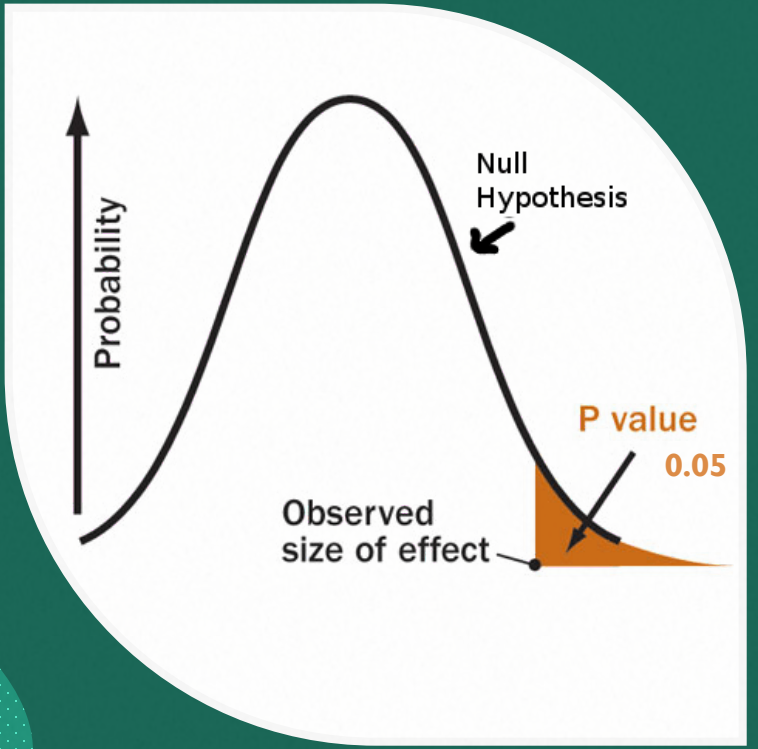


Statistical analysis

- The statistical experimental analysis was performed using the SPSS statistical software (v 26.0 IBM Corp., Armonk, NY, USA) and the experimental data are demonstrated as mean ± standard error of means (SEM).

- The effects of the dietary treatment among the two groups in microbiomes were evaluated using a non-parametric permutation-based t-test (equivalent to Mann-Whitney U-test) with 999 random permutations while other parameters following normal distribution were evaluated using independent t-test.

- For all the statistical tests, significance level was set at 0.05.



Lambs' performances

Probiotic diet resulted in:

- Improved carcass traits
- Cleaner dung score after weaning
- Lower IgA after weaning
- Lower expression of *IL1B* and *IL10* in neutrophils and monocytes

Contents lists available at ScienceDirect

Animal Feed Science and Technology

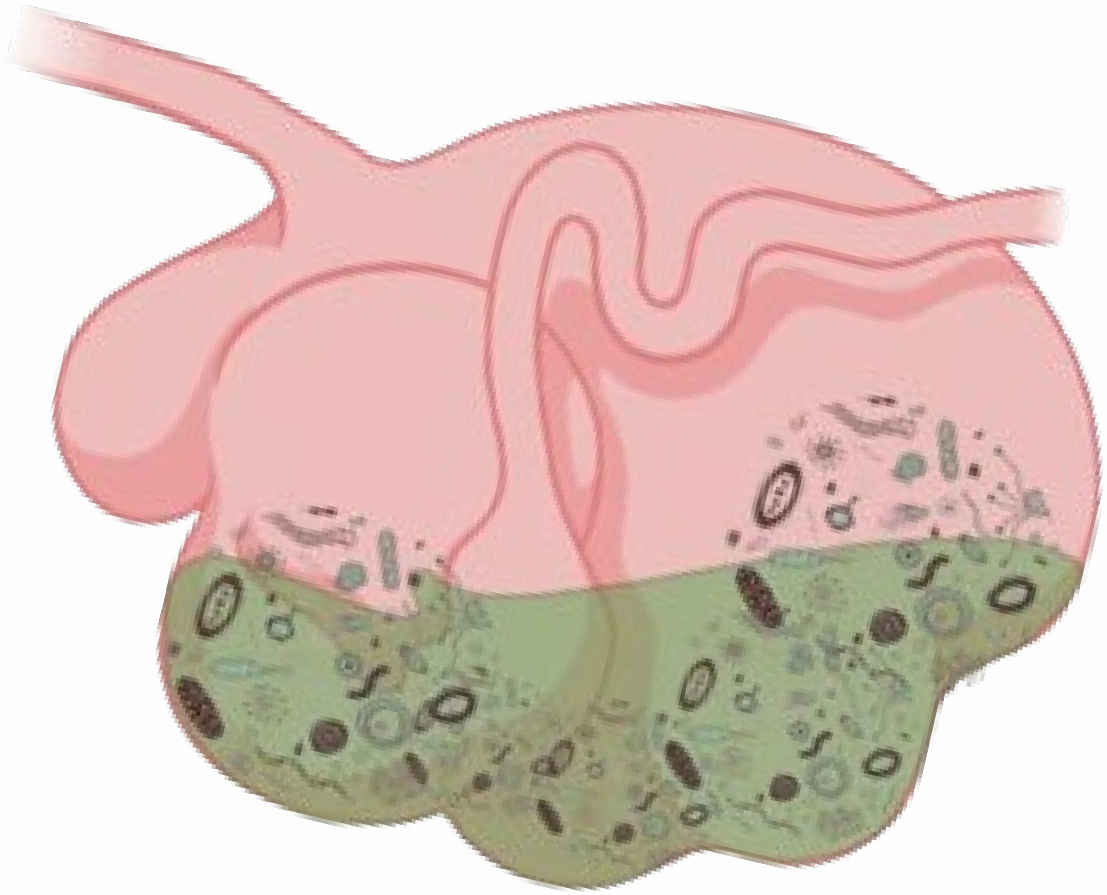
journal homepage: www.elsevier.com/locate/anifeedsci

The impact of probiotic live yeast in a barley grain-based diet on feed efficiency, carcass traits, and immune-oxidative status of artificially reared lambs

Alexandros Mavrommatis [a], Christos Christodoulou [a], Panagiota Kyriakaki [a], Alexis Skourtis [a], Basiliki Kotsampasi [b], Vladimiros Christodoulou [b], George Symeon [b], Maria Karatzia [b], Despoina Karatosidi [b], Soumela Savvidou [b], Federico Righi [c], Georgios Arsenos [d], Eric Chevaux [e], Eleni Tsiplakou [a,*]

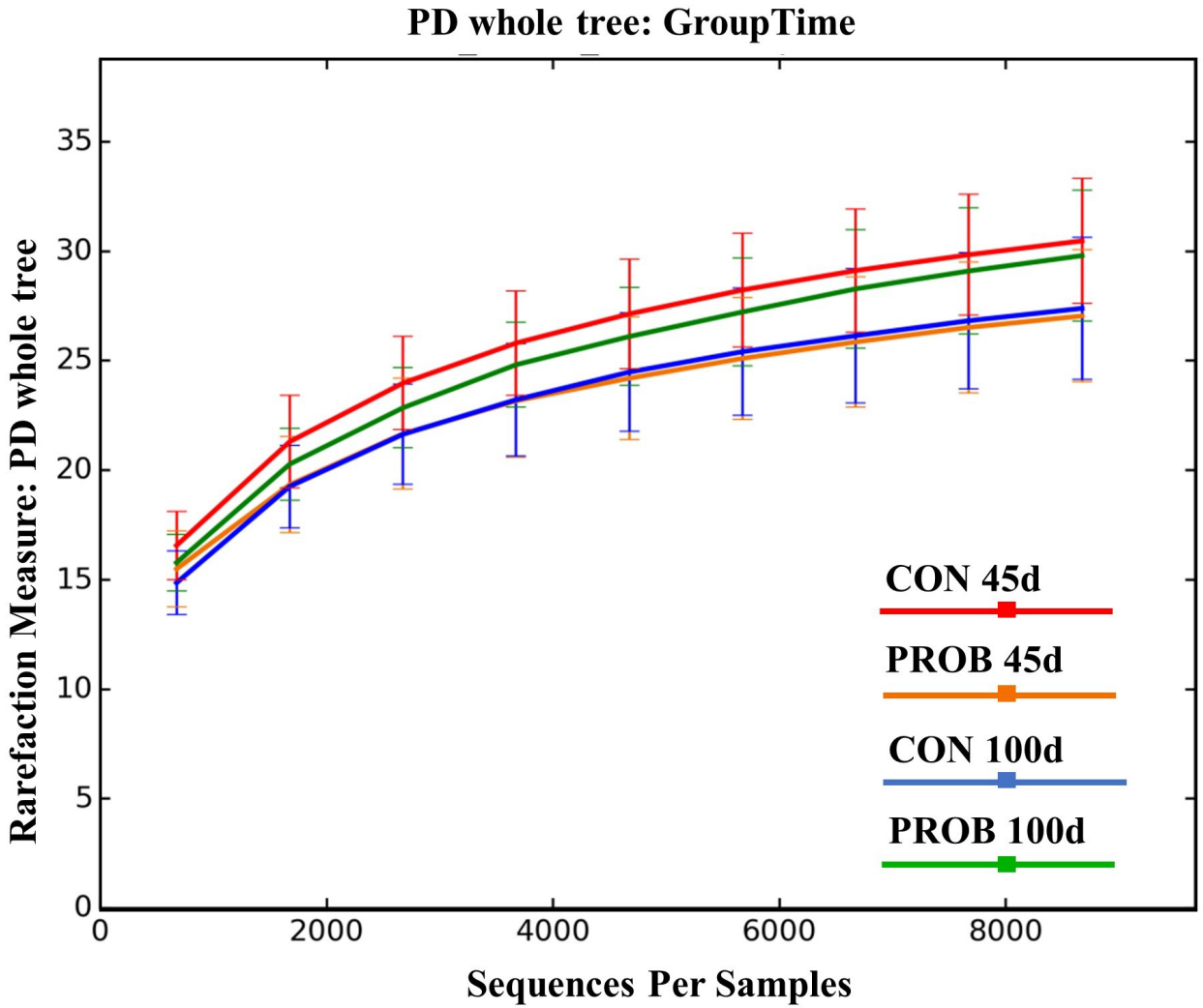
	CON		PROB		Significance
	Mean	SE[a]	Mean	SE[a]	P-value
DMI milk as skimmed (g/day)					
Birth to 30-days-old	323.14	7.25	321.17	6.26	0.838
30- to weaning (45d)	261.29	4.20	258.21	6.08	0.680
DMI concentrates (g/day)					
30- to weaning (45d)	58.71	5.15	44.53	3.50	0.028
Weaning (45d) to slaughter (106d)	757.2	28.07	720.2	26.54	0.339
DMI alfalfa hay (g/day)					
30- to weaning (45d)	52.69	3.59	50.0	2.96	0.566
Weaning (45d) to slaughter (106d)	160.9	4.88	154.2	5.97	0.383
Transformed date of birth	4.00	0.28	4.00	0.23	1.000
Body weight (kg)					
Birth BW	3.45	0.12	3.43	0.09	0.927
30-days-old	8.84	0.34	8.72	0.29	0.786
45-days-old (weaning)	12.17	0.43	11.78	0.32	0.479
106-days-old (slaughter)	**31.78**	0.79	**31.26**	0.62	0.680
Average Daily Gain (ADG; g/day)					
30- to weaning (45d)	0.238	0.014	0.215	0.009	0.193
Weaning (45d) to slaughter (106d)	0.318	0.039	0.319	0.035	0.943
Feed Conversion Ratio (FCR; kg feed/kg BW gain)					
30- to weaning (45d)	1.64	0.08	1.63	0.05	0.905
Weaning (45d) to slaughter (106d)	**2.81**	0.22	**2.72**	0.18	0.182

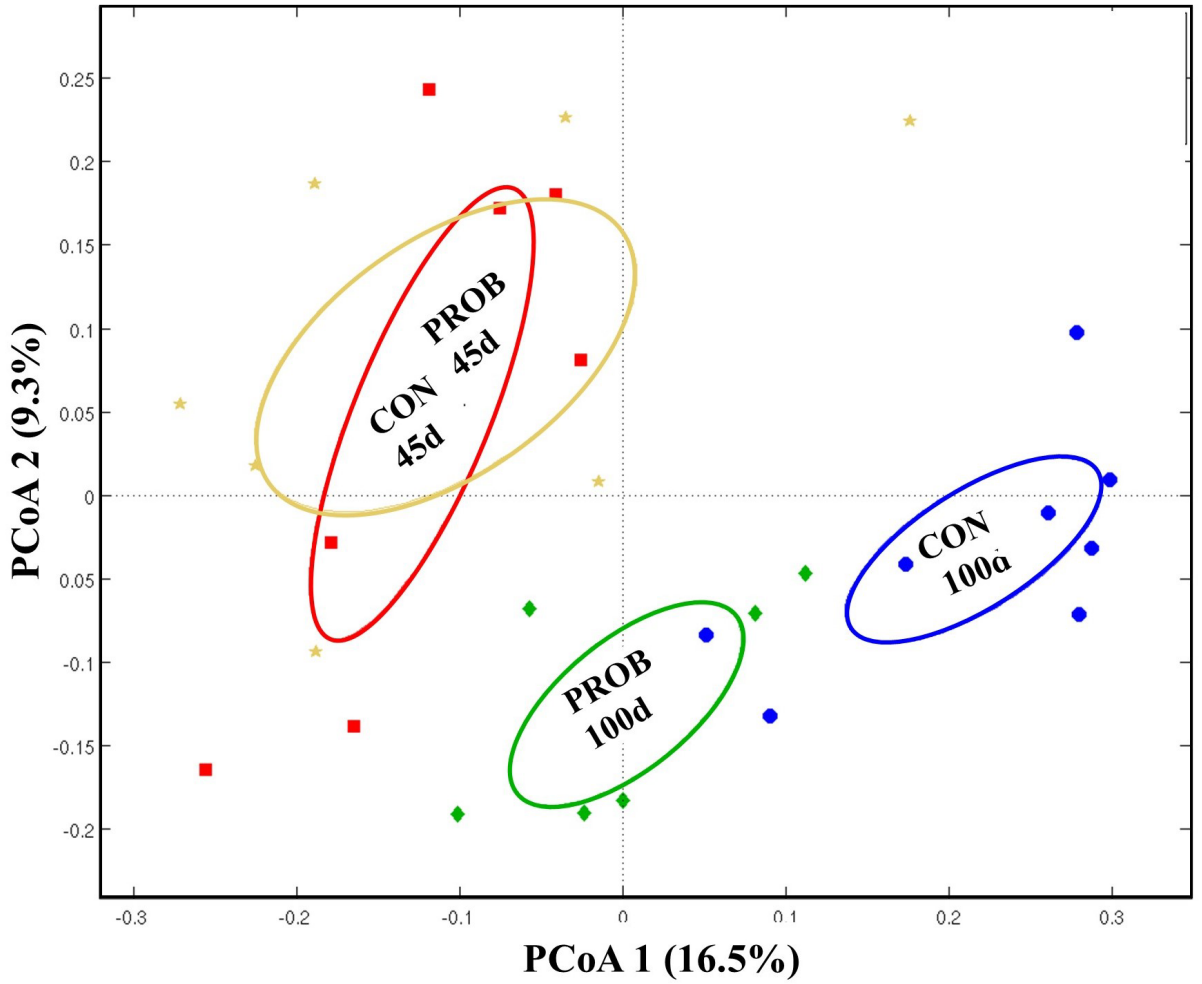
Rumen microbiome



The qualitative performance of the sequencing was very efficient, with 80%-90% of the reads retained after the qualitative filtering. After zOTUs clustering, there were still retained 45%-60% of the initial reads of the dataset. The analysis of the rarefaction curves for both the chao1 and the observed species metrics determined that all the samples show a tendency toward reaching a plateau at around 10.000 reads. All analyses were performed in QIIME 1.9.0 suite while taxonomic assignment was performed by RDP classifier against SILVA 138 database using 0.8 as confidence threshold.

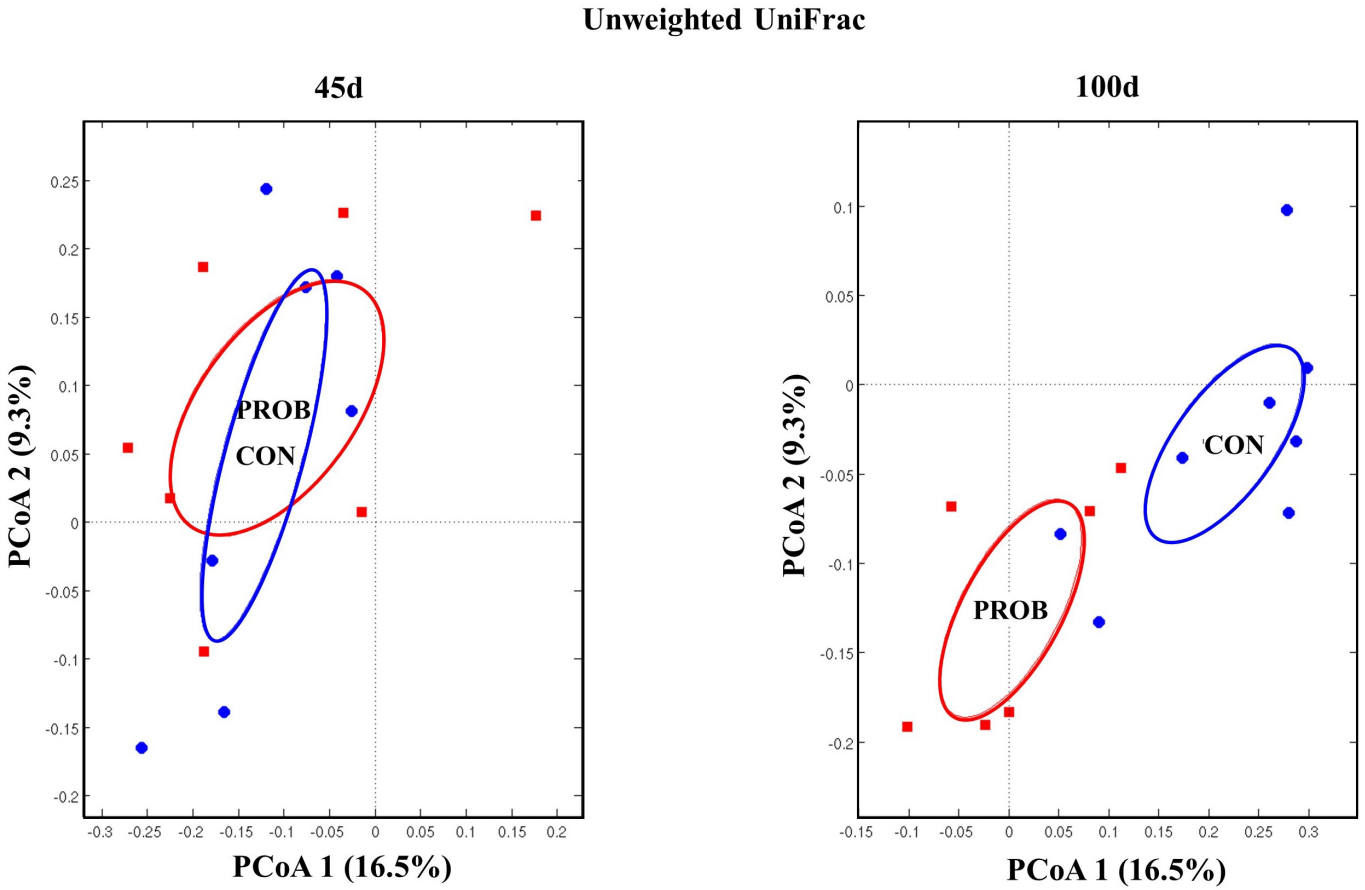
Alpha diversity as PD whole tree and beta-diversity as Principal Coordinate Analysis

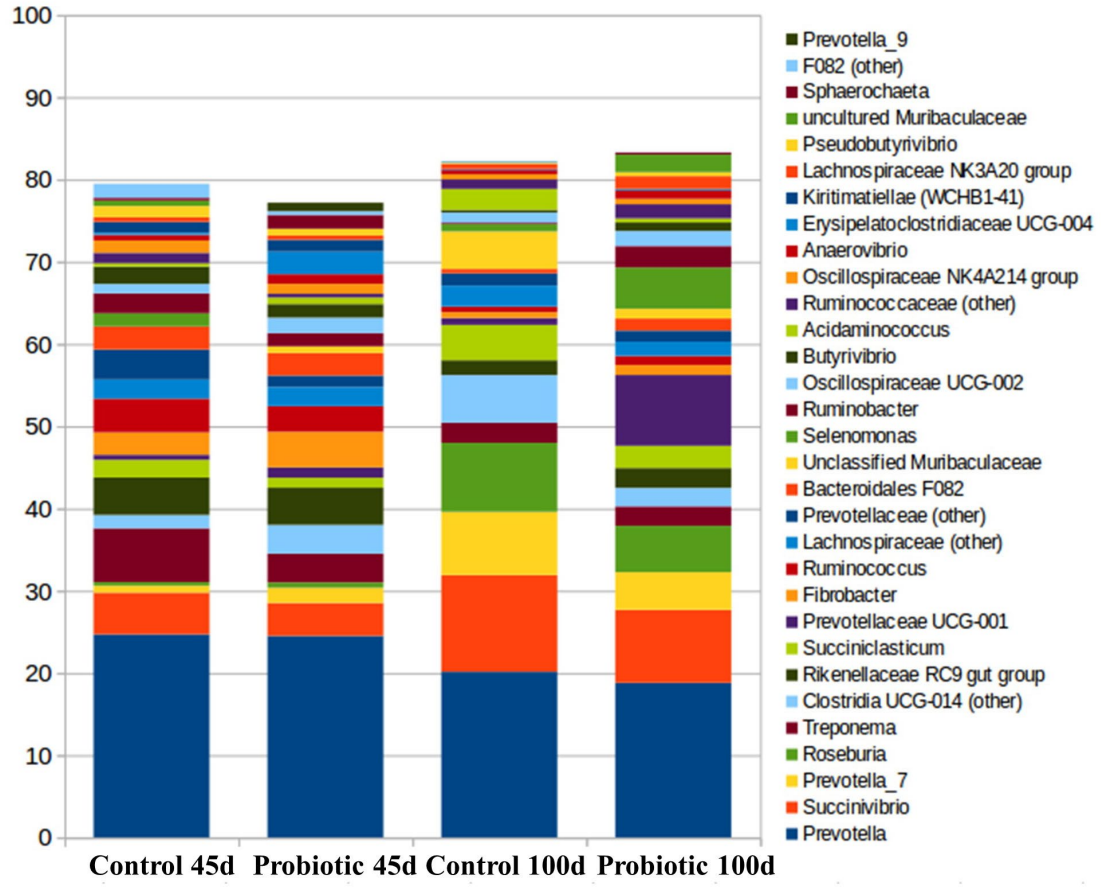




Considering together the effects of diet and time points on the rumen microbiome highlighted that, although no difference was evident for the alpha-diversity, several interesting features were evident for the beta-diversity.

Rumen bacteriome





Analyzing the microbial composition and diversity over the time (100d vs 45d) regardless of the diet revealed that, although biodiversity (alpha-diversity) was similar, the microbial composition was significantly different (p=0.001 for both the unweighted and weighted UniFrac distances).

Rumen bacteriome-metagenomic analysis

	Treatments				SEM	P value			
	Weaning (W)		End (E)			W	E	C	P
	C	P	C	P		C vs P	C vs P	W vs E	W vs E
Phylum									
Bacteroidetes	44.20	44.04	40.03	43.11	1.034	0.955	0.282	0.121	1.000
Firmicutes	32.98	34.12	38.66	36.30	1.279	0.779	0.282	0.281	0.662
Proteobacteria	8.10	6.36	13.42	12.60	1.559	0.867	0.852	0.443	0.142
Spirochaetota	6.90	5.18	2.46	2.61	0.693	0.463	0.755	0.121	0.142
Fibrobacterota	2.72	4.34	0.70	1.22	0.514	0.416	0.228	0.152	0.282
Verrucomicrobiota	**1.50**	1.55	**0.10**	0.17	0.331	0.281	0.755	0.004	0.228
Cyanobacteria	0.13	0.17	**1.99**	**0.93**	0.199	0.536	0.081	<0.001	0.059
Euryarchaeota	0.72	0.68	0.81	0.93	0.081	0.536	0.491	0.336	0.228

Rumen bacteriome-metagenomic analysis

	Treatments				SEM	P value			
	Weaning (W)		End (E)			W	E	C	P
	C	P	C	P		C vs P	C vs P	W vs E	W vs E
Family									
Prevotellaceae	31.96	33.40	31.28	34.43	1.093	0.681	0.491	0.779	0.852
Lachnospiraceae	10.93	9.87	14.20	14.51	0.894	0.918	1.000	0.232	0.142
Succinivibrionaceae	7.83	5.89	12.17	11.86	1.557	0.681	0.950	0.536	0.142
Spirochaetaceae	6.90	5.18	2.46	2.61	0.693	0.351	0.755	0.121	0.142
Ruminococcaceae	**5.54**	4.75	**2.39**	3.50	0.565	0.606	0.345	**0.072**	0.414
Acidaminococcaceae	2.59	2.00	**6.86**	**3.19**	0.530	0.142	**0.059**	**<0.001**	0.573
Oscillospiraceae	4.18	4.70	2.24	2.63	0.539	1.000	0.662	0.152	0.142
Clostridia UCG-014	1.61	3.49	**5.78**	**2.25**	0.429	0.114	**0.008**	**0.004**	0.142
Rikenellaceae	4.71	4.55	1.82	2.42	0.584	0.681	0.282	**0.094**	0.282
Selenomonadaceae	2.92	2.28	1.62	6.87	0.767	0.758	0.142	1.000	0.228
Muribaculaceae	1.55	1.08	5.26	3.95	0.667	0.299	0.755	**0.072**	**0.013**
Bacteroidales F082	4.56	3.26	**0.77**	**1.47**	0.401	0.142	**0.081**	**0.001**	**0.020**
Fibrobacteraceae	2.72	4.34	0.70	1.22	0.514	0.606	0.228	0.152	0.282
Erysipelatoclostridiaceae	1.22	3.15	0.92	0.62	0.665	0.470	0.345	0.613	0.414
Kiritimatiellae (WCHB1-41)	**1.32**	1.45	**0.10**	0.16	0.310	0.252	0.755	**0.004**	0.414
Methanobacteriaceae	0.72	0.68	0.81	0.93	0.081	0.470	0.491	0.336	0.228
Bacteroidales RF16 group	0.91	1.08	0.49	0.31	0.230	1.000	0.414	0.955	0.491
Erysipelotrichaceae	0.55	0.71	0.89	0.44	0.106	0.408	0.414	0.397	0.662
Christensenellaceae	0.73	0.60	0.41	0.33	0.095	0.252	0.414	0.029	0.755

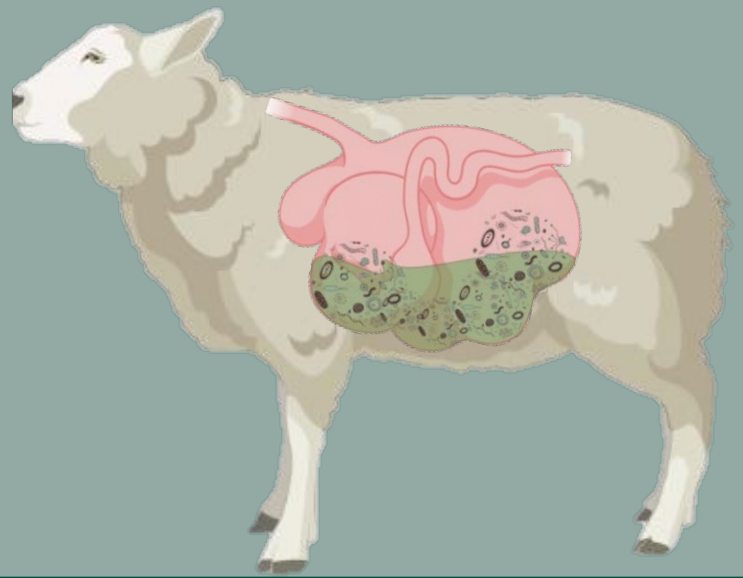
Rumen bacteriome-metagenomic analysis

	Treatments				SEM	P value			
	Weaning (W)		End (E)			W	E	C	P
	C	P	C	P		C vs P	C vs P	W vs E	W vs E
Genus									
Prevotella	24.70	**24.51**	20.14	**18.81**	1.246	1.000	0.755	0.536	**0.081**
Succinivibrio	5.06	4.01	11.74	8.87	1.573	0.779	0.662	0.336	0.181
Treponema	6.56	3.49	2.45	2.37	0.642	0.336	0.852	0.152	0.491
Clostridia UCG-014 (other)	**1.61**	**3.49**	**5.78**	**2.25**	0.429	**0.054**	**0.008**	**0.004**	0.142
Succiniclasticum	**2.17**	**1.20**	4.30	2.69	0.303	**0.021**	0.181	**0.001**	0.282
Prevotellaceae UCG-001	**0.62**	**1.26**	**0.85**	**8.61**	0.939	**0.094**	**0.059**	0.613	0.228
Fibrobacter	2.72	4.34	0.70	1.22	0.514	0.463	0.228	0.152	0.282
Ruminococcus	4.10	3.10	0.69	1.11	0.433	0.613	0.108	**0.006**	**0.043**
Selenomonas	1.62	0.00	0.89	5.03	0.676	0.694	0.142	0.121	**<0.001**
Ruminobacter	2.41	1.59	0.18	2.60	0.601	0.463	0.142	0.397	0.345
Butyrivibrio	2.11	1.64	**0.31**	**1.03**	0.320	0.463	**0.029**	**0.006**	0.414
Acidaminococcus	0.42	0.79	**2.56**	**0.50**	0.301	0.867	**0.029**	**0.014**	0.755
Anaerovibrio	0.68	1.19	**0.53**	**0.97**	0.159	0.613	**0.043**	0.463	0.852
Lachnospiraceae NK3A20 g	0.57	0.51	0.58	1.53	0.147	0.189	0.142	0.779	**0.081**
Pseudobutyrivibrio	1.37	0.80	**0.12**	**0.47**	0.180	0.189	**0.029**	**0.002**	0.662
Sphaerochaeta	0.33	1.68	**0.01**	**0.25**	0.202	0.336	**0.008**	**<0.001**	0.142
Veillonellaceae UCG-001	0.37	0.46	**0.09**	**0.79**	0.126	0.779	**0.013**	0.336	0.228
Lachnospiraceae NK4A136 g	0.73	0.35	**0.10**	**0.41**	0.105	0.397	**0.059**	**0.072**	0.414

!

All changes observed in the rumen bacteriome are correlated with dysbiosis conditions, like SARA, in previous experimental trials

Isaac Newton wrote in a 1675 letter to fellow scientist Robert Hooke, "it is by standing on the shoulders of giants."



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ORIGINAL RESEARCH
published: 07 August 2020
doi: 10.3389/fmicb.2020.01813

PacBio and Illumina MiSeq Amplicon Sequencing Confirm Full Recovery of the Bacterial Community After Subacute Ruminal Acidosis Challenge in the RUSITEC System

*Melanie Brede[1], Theresa Orton[1], Beate Pinior[2], Franz-Ferdinand Roch[2], Monika Dzieciol[2], Benjamin Zwirzitz[3], Martin Wagner[2,3], Gerhard Breves[1] and Stefanie U. Wetzels[2,3]**

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Anaerobes in animal disease

Rumen microbial abundance and fermentation profile during severe subacute ruminal acidosis and its modulation by plant derived alkaloids *in vitro*

Elsayed Mickdam [a, b, d], Ratchaneewan Khiaosa-ard [a, d], Barbara U. Metzler-Zebeli [c, d], Fenja Klevenhusen [a, d], Remigius Chizzola [a], Qendrim Zebeli [a, d, *]

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PLOS ONE

Characterization of the Core Rumen Microbiome in Cattle during Transition from Forage to Concentrate as Well as during and after an Acidotic Challenge

Renee M. Petri[1,2], Tyler Schwaiger[1,2], Greg B. Penner[2], Karen A. Beauchemin[1], Robert J. Forster[1], John J. McKinnon[2], Tim A. McAllister[1]*

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J. Dairy Sci. 92:4456–4466
doi:10.3168/jds.2008-1722

Effect of pH and level of concentrate in the diet on the production of biohydrogenation intermediates in a dual-flow continuous culture

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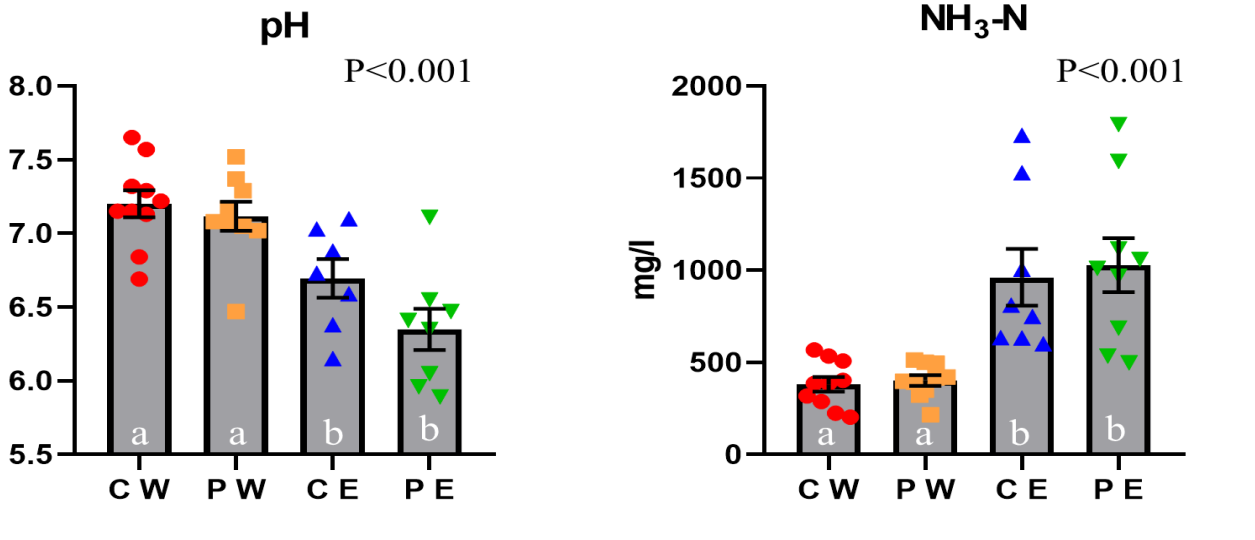
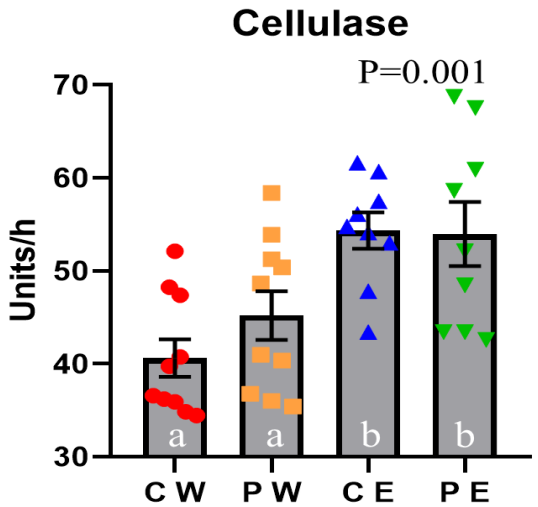
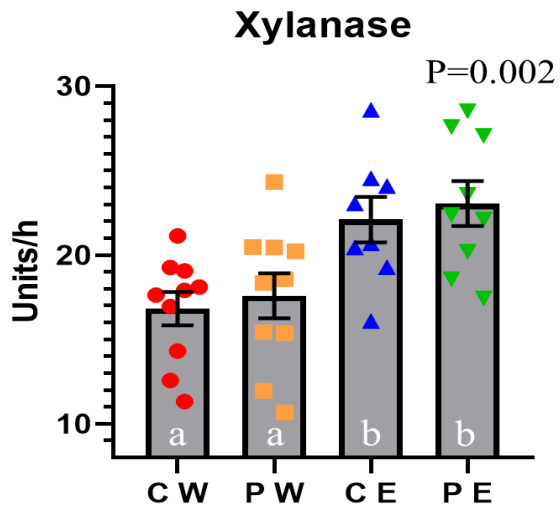
RT-qPCR targeted analysis

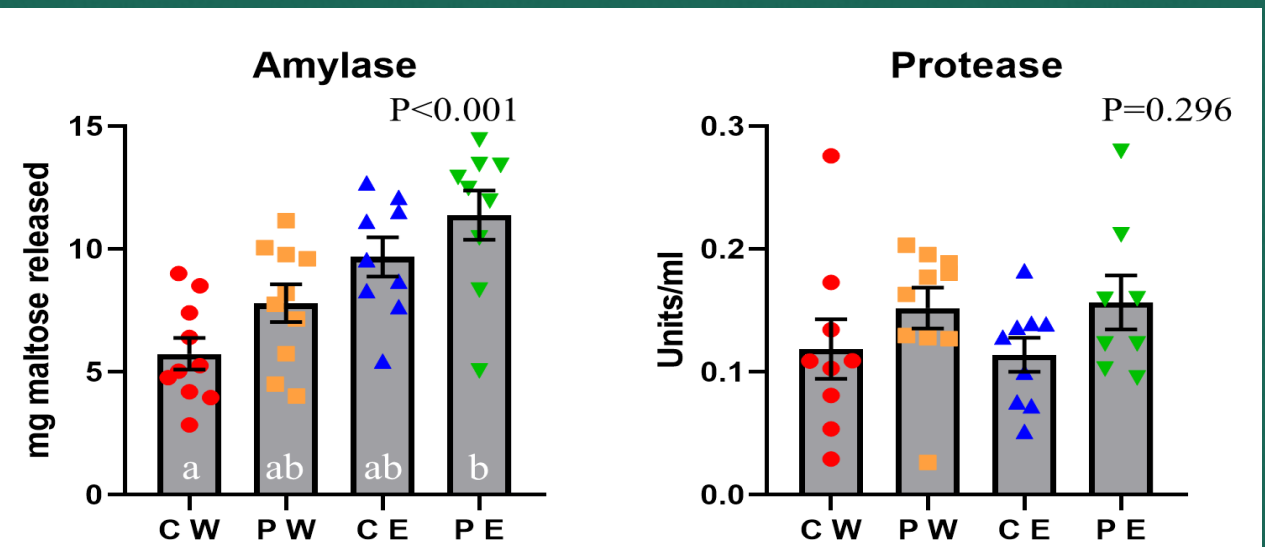
	Treatments				SEM	P value			
	W		E			W	E	C	P
	C	P	C	P		C vs P	C vs P	W vs E	W vs E
Bacteroidetes	39.22	35.44	41.68	40.83	2.350	0.236	0.888	0.721	0.340
Firmicutes	35.14	32.49	35.5	33.36	3.001	0.963	0.673	0.743	0.888
F/B	0.91	1.01	1.07	1.09	0.146	0.673	0.370	0.442	0.489
a-Proteobacteria	0.159	0.103	0.069	0.069	0.018	0.779	0.259	0.383	0.152
Total fungi	0.001	0.009	0.000	0.000	<0.001	0.481	1.000	0.673	0.258
Neocallimastigales	0.036	0.046	0.000	0.000	0.007	0.963	0.796	**0.021**	**0.040**
Saccharomyces cerevisiae	**0.0000**	**0.0009**	**0.0000**	**0.0007**	<0.001	**<0.001**	**<0.001**	0.541	0.222
Protozoa	0.258	0.546	0.387	0.929	0.190	1.000	0.918	0.743	0.758
Entodinium sp.	0.059	0.000	0.002	0.034	0.014	0.277	0.297	1.000	0.031
Prevotella sp.	20.28	19.84	34.39	27.51	1.704	1.000	0.423	**<0.001**	0.258
Butyrivibrio fibrisolvens	1.11	1.05	1.15	1.48	0.134	1.000	0.863	0.743	0.666
Ruminococcus flavefaciens	0.012	0.007	0.000	0.004	0.003	0.888	0.113	**0.096**	0.931
Ruminococcus albus	0.141	0.153	0.016	0.054	0.027	0.370	0.136	**0.011**	0.340
Streptococcus bovis	0.0002	0.0005	0.0001	0.0001	<0.001	0.798	0.863	0.139	0.673
Fibrobacter succinogenes	**0.002**	0.012	**0.0003**	0.0015	0.001	0.613	0.666	**0.046**	**0.091**
Bifidobacterium	0.373	0.025	**0.120**	**0.015**	0.056	0.236	**0.063**	0.606	0.666
Lactobacillus	**0.0000**	0.0000	**0.0023**	0.0001	<0.001	0.445	0.130	**0.043**	0.281

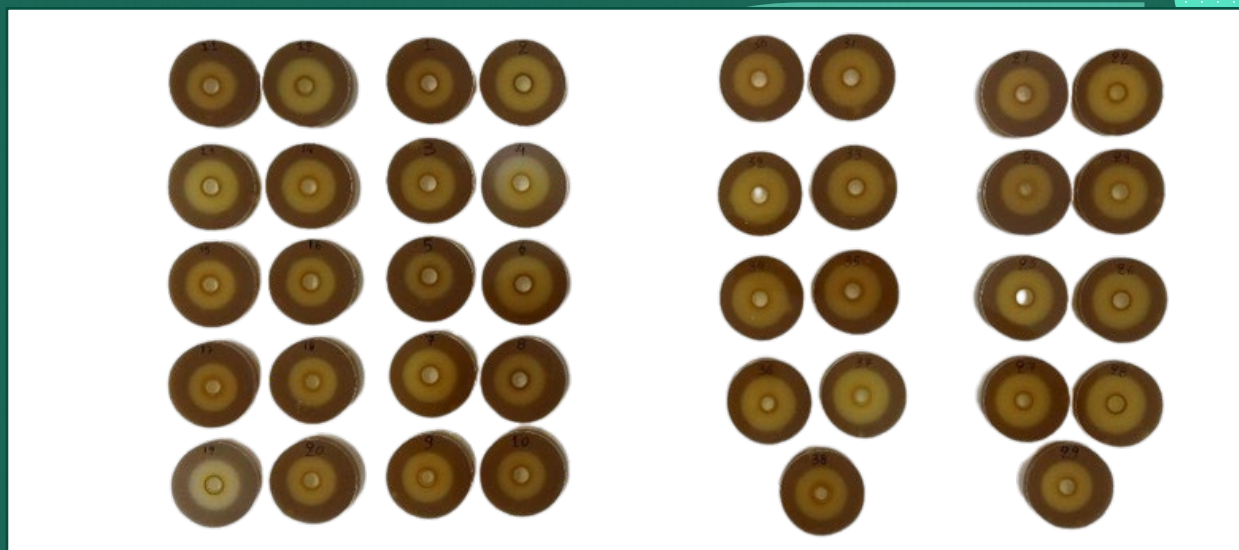
Effect of probiotic yeast supplementation on pH, ammonia concentration, alpha-amylase, protease, cellulase, and xylanase activity in the rumen of lambs at weaning (W) and the end (E) of the experiment.



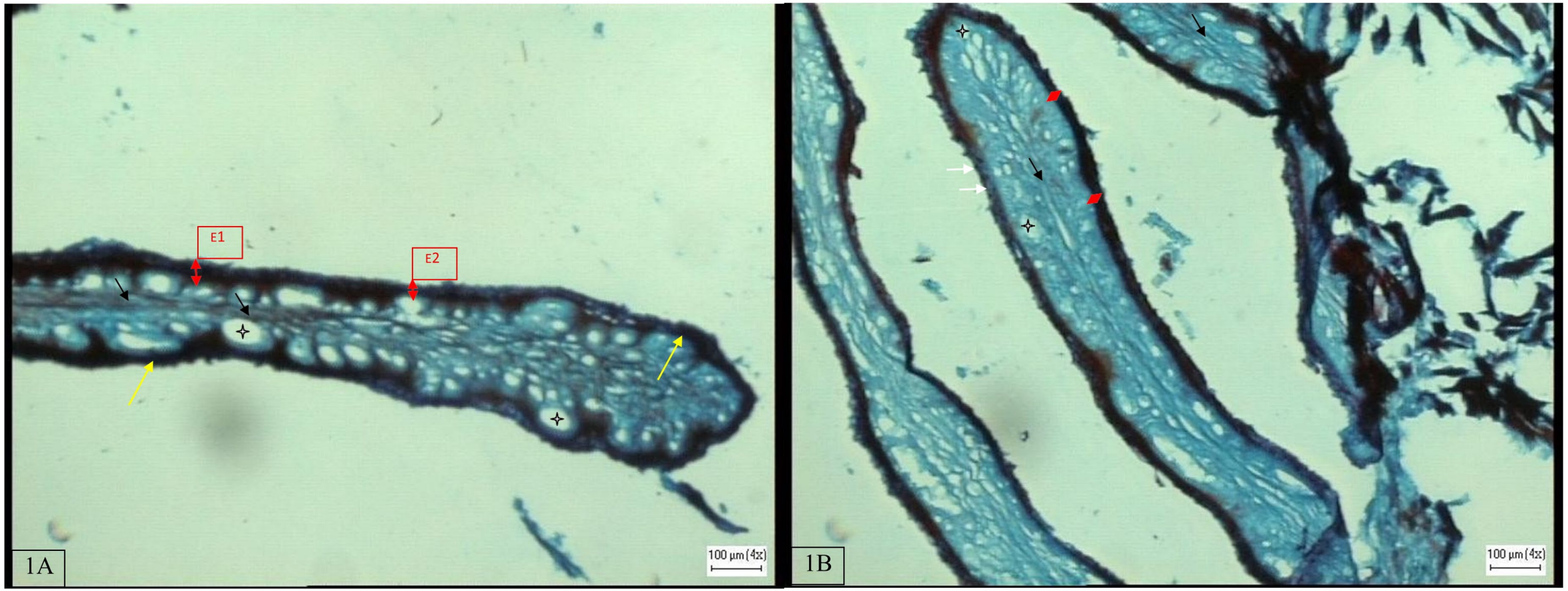






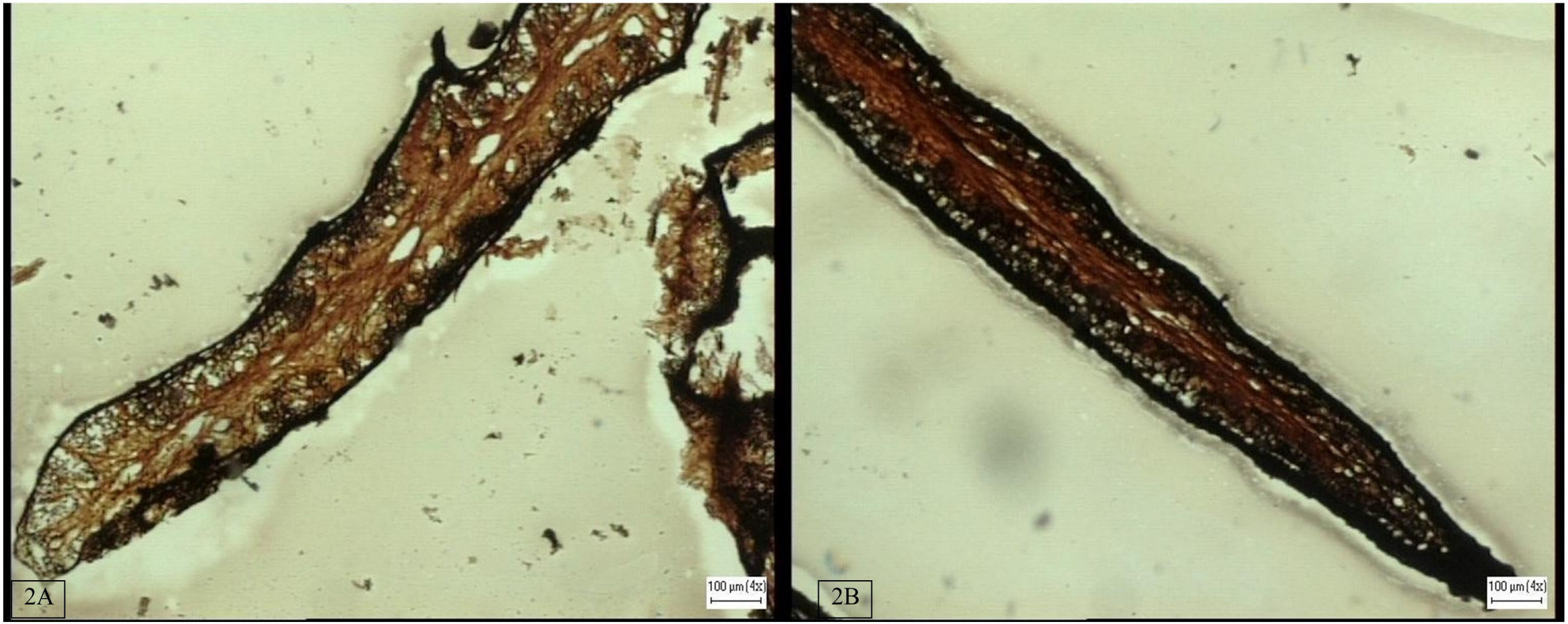


Rumen morphology



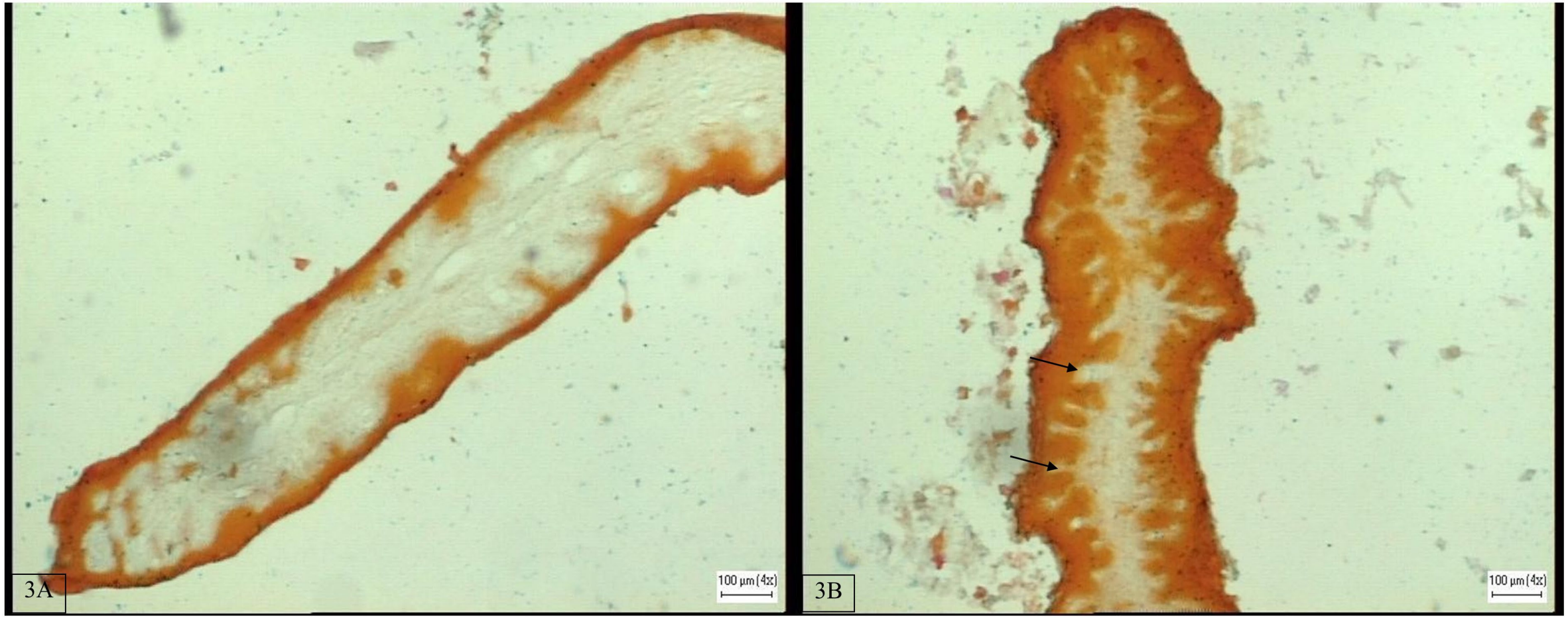
- The Mallory's trichrome stain revealed the extensive presence of collagen in the lamina propria (shades of blue), delineated blood vessels' lumens (stars) and labelled smooth muscle cells (shades of violet, arrows) (Figure 1A&B).
- The epithelium was of variable thickness (red double arrows- compare E1 with E2) and its layers were not uniformly presented in the periphery of the papillae in control lambs, where keratinization (yellow arrows) was very prominent (Figure 1A).
- In contrast, in Probiotic lambs' papillae epithelium was of uniform thickness (Figure 1B) and with well-delineated layers and often visible epithelial cell nuclei (white arrows).

Rumen morphology



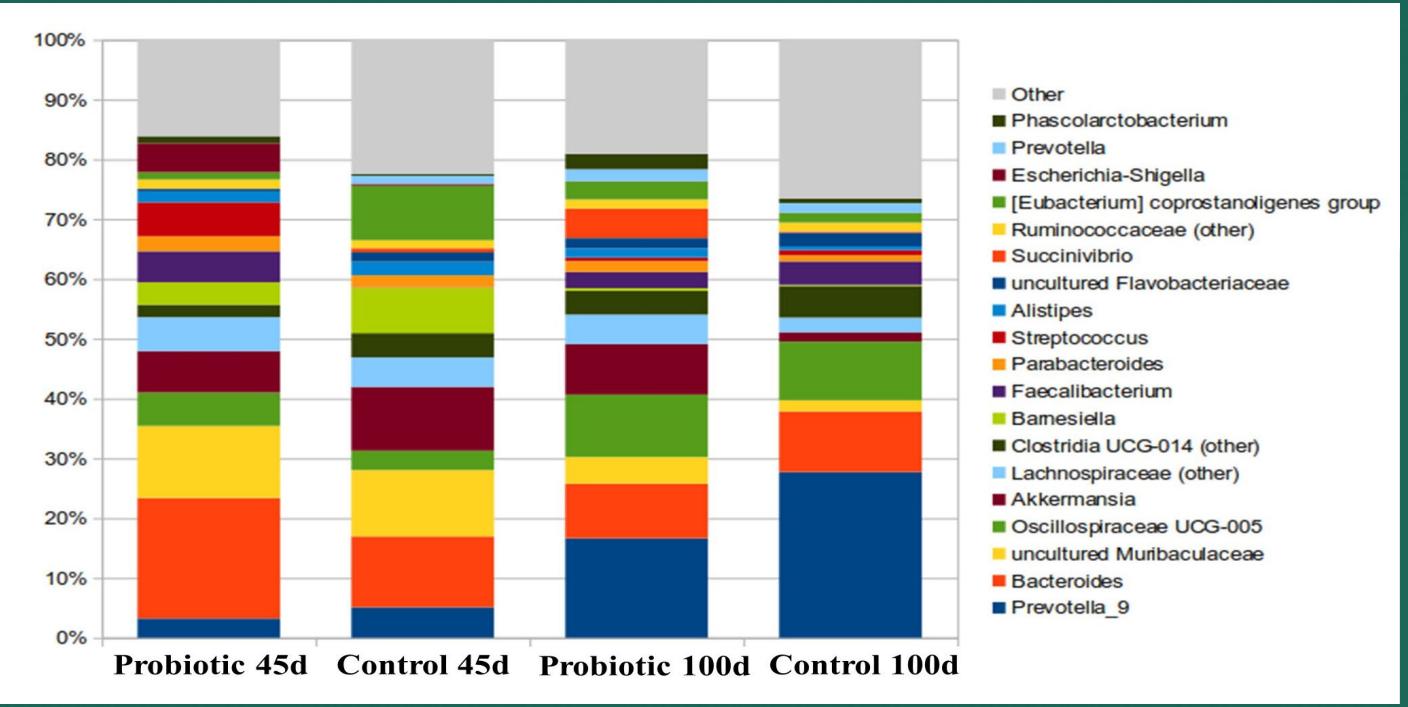
- The modified reticular fiber stain confirms the presence of reticular (black) and collagen fibers (brown) in the lamina propria (Figure 2A&B) but confirms that both (reticular and collagen fibers) were more densely packed and structurally supported, as they should, the mucosa and its constituents (compare Figure 2 A to Figure B).

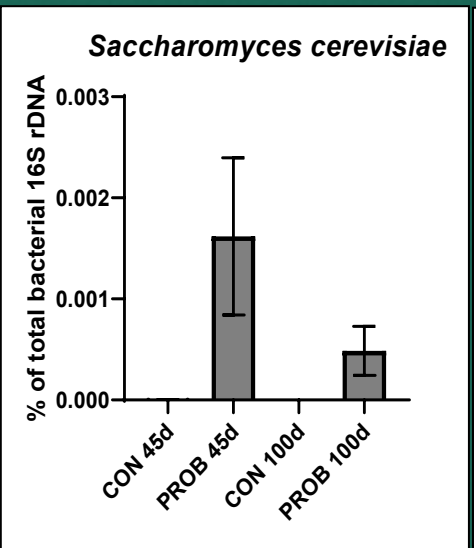
Rumen morphology

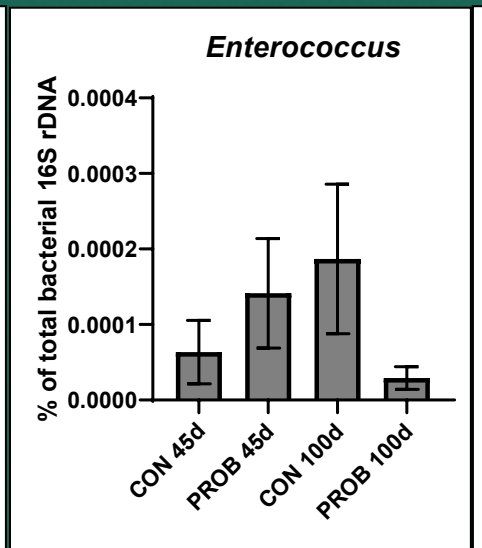


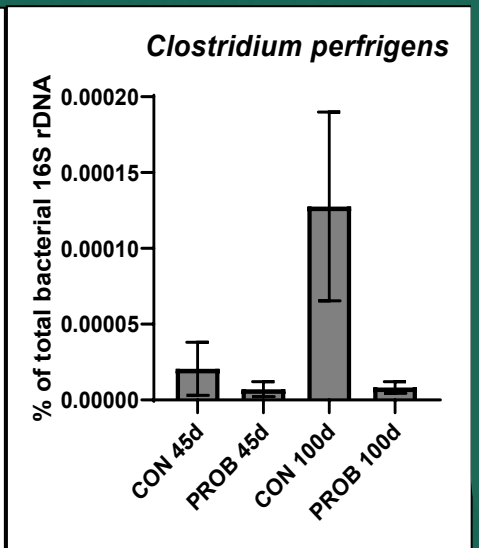
- As regards Dane's stain the presence of prekeratins and keratins (shades of orange) was evident in both control and Probiotic lambs' papillae (Figure 2A&B), but their distribution was prominent in the Probiotic lambs, delineating epithelial pegs (arrows), when compared to controls.

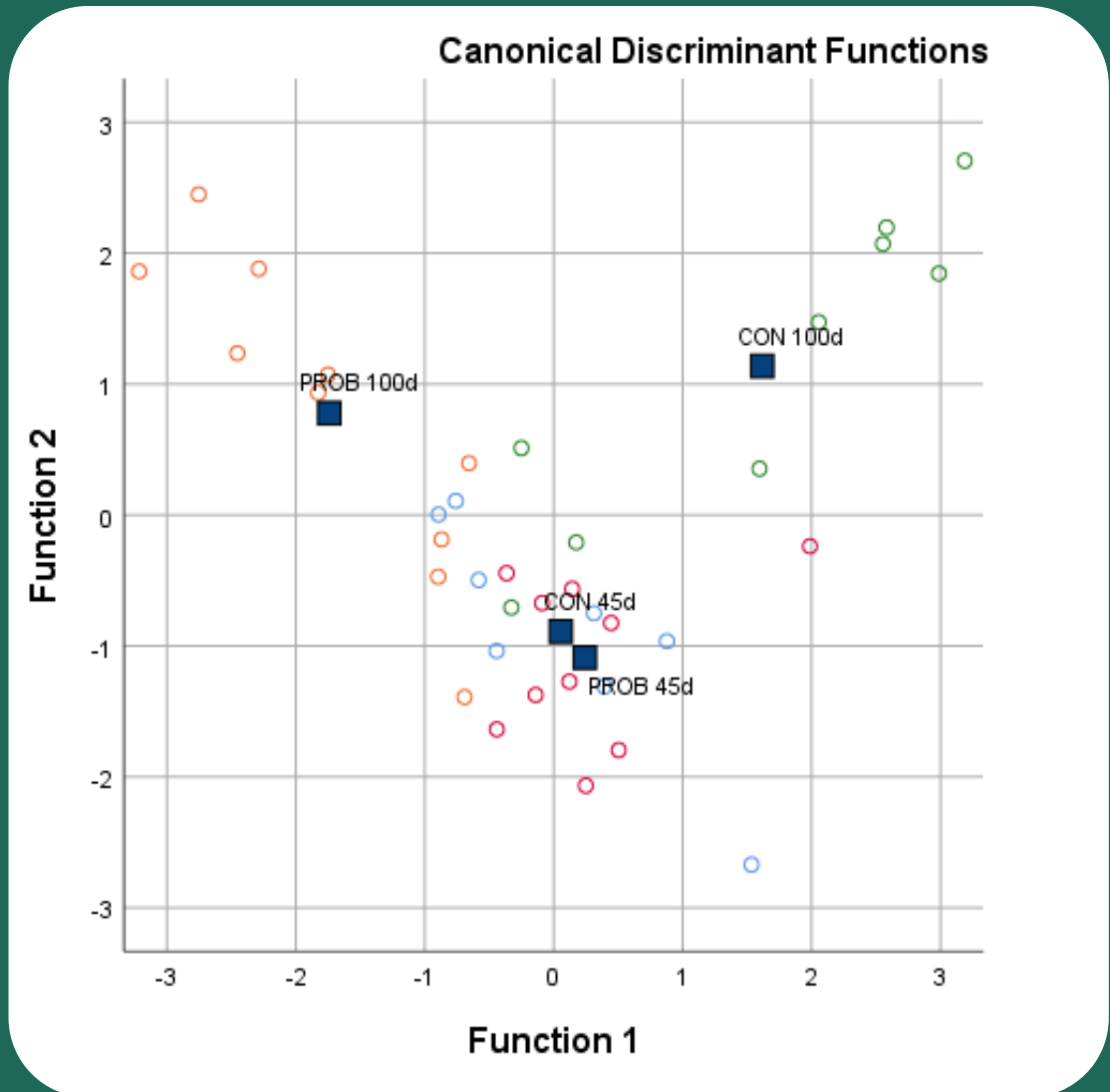
Fecal microbiome











Conclusions

1

The inclusion of probiotic live yeast supported a "better" rumen bacteriome habitat in lambs reared artificially and fed a starter feed with high fermentable carbohydrates (barley).

2

Nevertheless, rumen biochemistry did not follow bacteriome changes between CON and PROB dietary treatments. Similarly, our results on rumen morphology showed no effect in rumen histology due to the treatment.

3

However, our observations on mucosa histology confirm that PROB lambs outweighed that of the CON as regards the appearance and distribution of the extracellular matrix components, the prekeratin and keratin contents and finally the appearance of the epithelium, which was much less keratinized and displayed all five of its constituent layers in uniform thickness.

4

Although the detrimental impact of high fermentable carbohydrate diet was concealed considering the animal performances, our results are arguing for a positive effect of the Probiotic on rumen. In adult life these changes can bring differences also in productivity level.

ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
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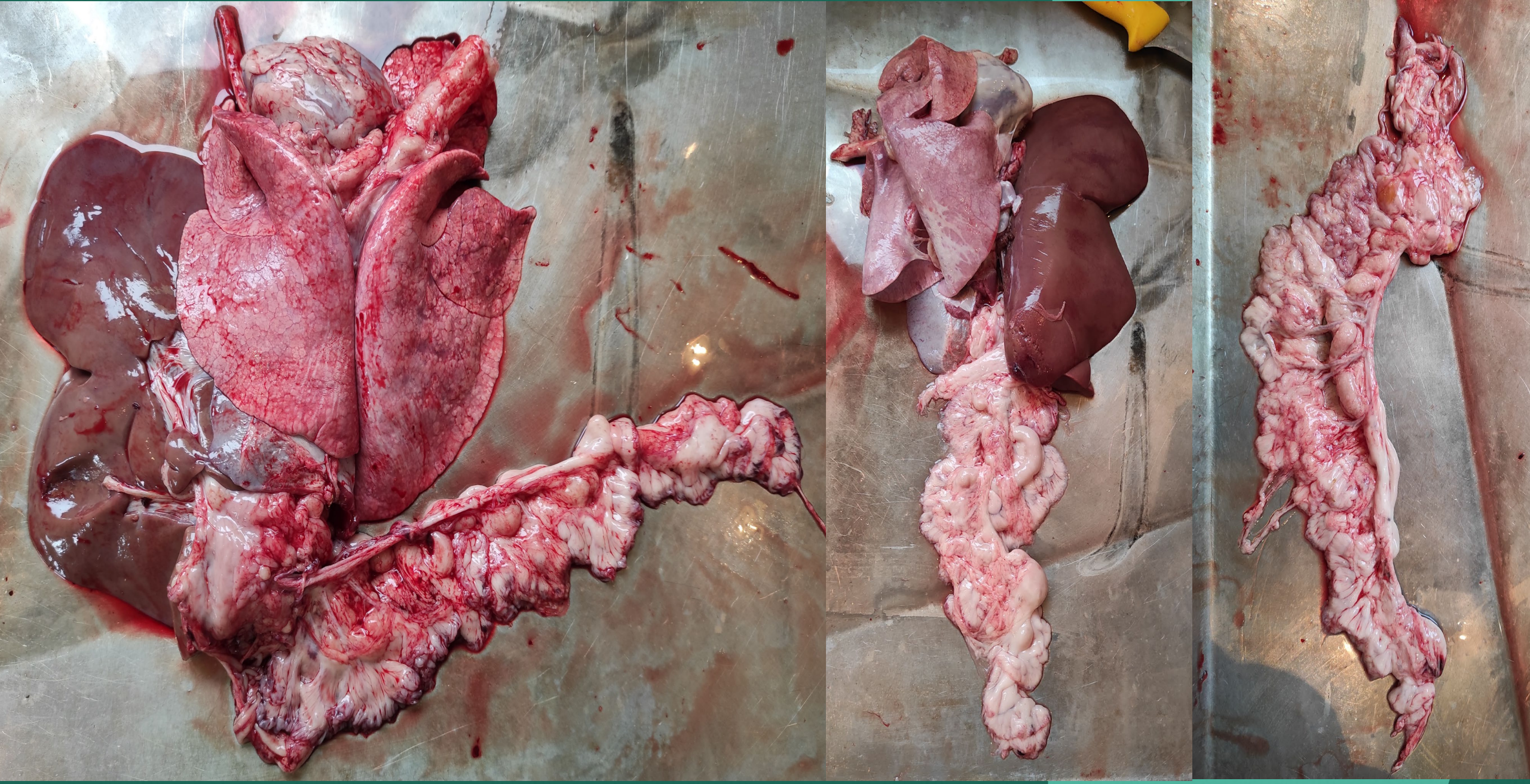
Yeast
Control


YEAST
CONTROL



Rumen microbiome