

RNA based amplicon sequencing: an emerging approach to study diet related shifts in rumen microbiota

Session 58: Non-invasive biomarkers in nutritional studies

N. Amin, B. Cardazzo, L. Carraro and L. Bailoni

**Department of Comparative Biomedicine and Food Science (BCA),
University of Padua, Italy**

Email: nida.amin@phd.unipd.it

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- Among livestock, ruminants are major methane producers
- Several factors influence methane production by ruminants
 - **Diet & Breed**
- Understanding **rumen microbiota** composition under **different dietary treatments** is essential to
 - **reduce CH₄ emissions**
 - **improve animal health & production**
- Majority of rumen microbes are uncultivable in laboratory
- Next generation sequencing (NGS) allows quick & efficient analysis of microbial communities



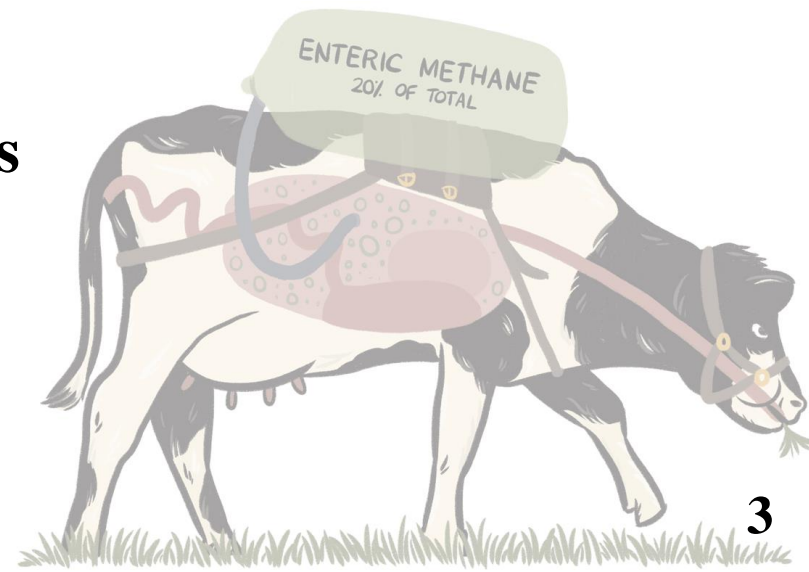
- Majority of published data on rumen microbiota are DNA-based
- DNA-based methods are unable to distinguish between genes stem from live cells or dead cells
- RNA-based methods provide the opportunity to study **potentially active microbiota**

These methods can be applied to invasive samples

➤ **rumen fluid collected by esophageal probe or cannula**

And non-invasive samples

➤ **buccal fluid, chewed bolus or faeces**



1. Monitoring **6 lactating Italian Simmental cows** during **2 different physiological stages & dietary treatments**

➤ **Late lactation**

➤ **Dry Period**

2. Rumen fluid collection during each stage and extraction of RNA

3. Next generation sequencing (NGS) to analyze **diet dependent shifts** in the **metabolically active rumen microbiota**



Italian Simmental cow

Experimental setup

6 Italian Simmental cows (148 to 203 days in gestation)

1st rumen fluid
collection



Late lactation (248 to 332 DIM)

Forage: Concentrate (54:46)



DIET	as fed (kg)	% of DM
Corn silage	28	47.4
Alfalfa hay	4.0	17.4
Meadow hay	3.0	13.1
Protein mix	2.5	10.7
Vit-min mix	0.3	1.4
Energy mix	2.1	8.9
Extruded linseed	0.25	1.1
Total	40.2	100.0

2nd rumen fluid
collection

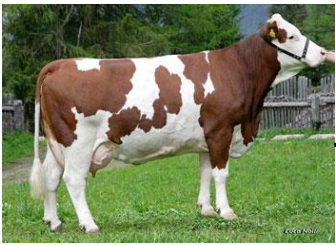


Dry period (2-52 days before calving)

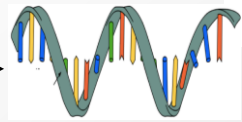
Forage: Concentrate (73:26)



DIET	as fed (kg)	% of DM
Corn silage	7.0	20.3
Wheat straw	5.0	37.8
Meadow hay	3.3	24.6
Protein mix	2.2	16.1
Vit-min mix	0.15	1.2
Total	17.7	100.0



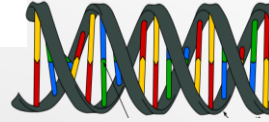
Italian Simmental cow



RNA

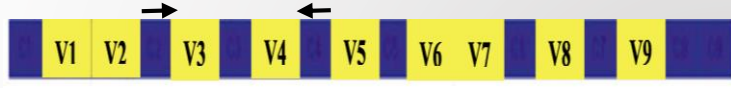
(extracted from rumen fluid)

Reverse transcription



cDNA

Gene amplification by PCR



16S rRNA genes (Bacteria & Archaea)



18S rRNA genes (Protozoa)

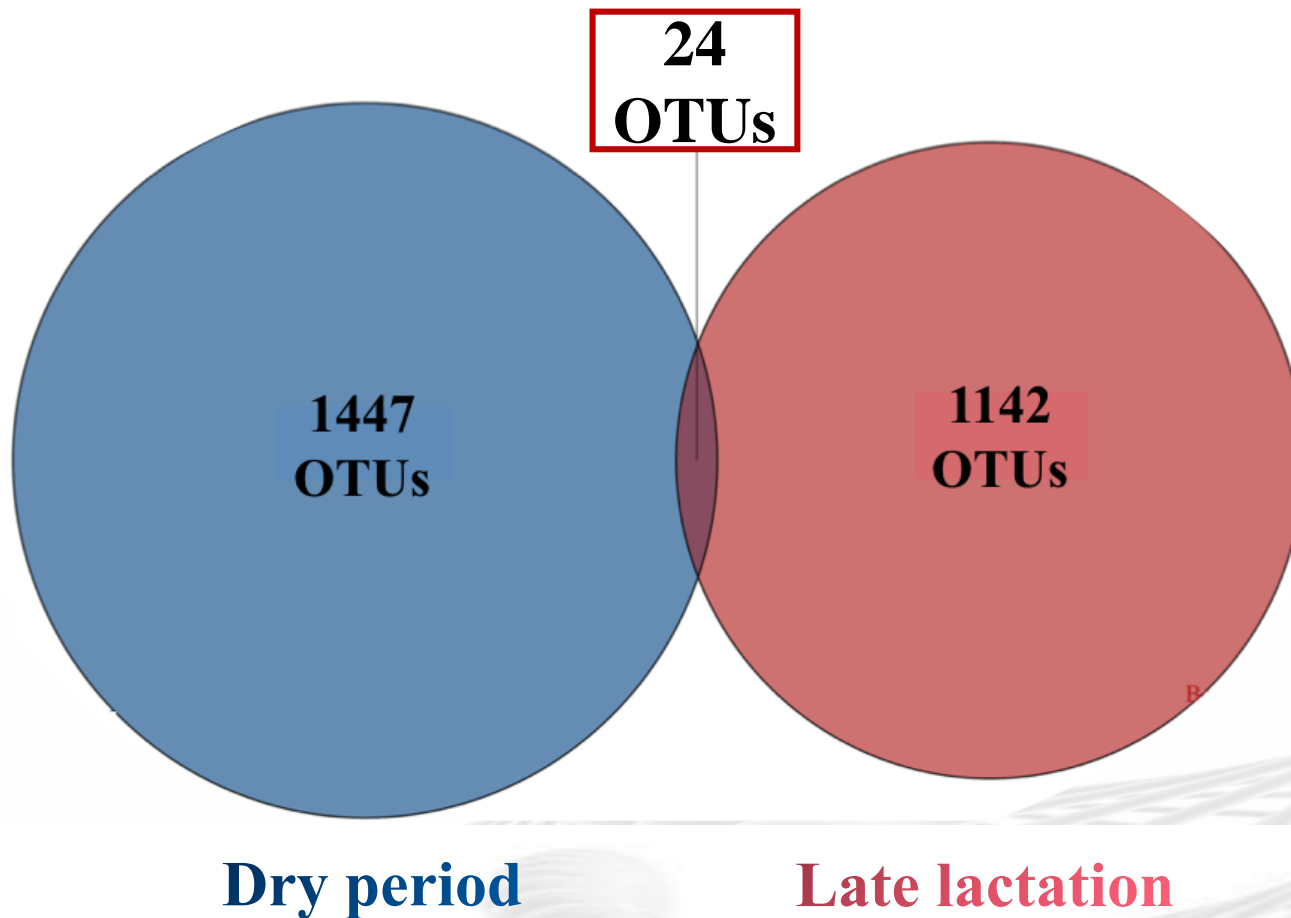
Next generation sequencing
Illumina Miseq platform

Bioinformatics analysis
QIIME 2™ platform

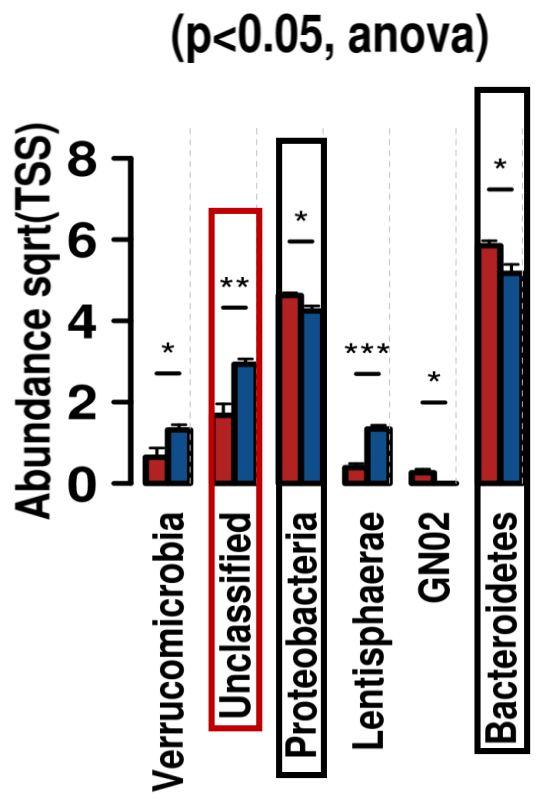
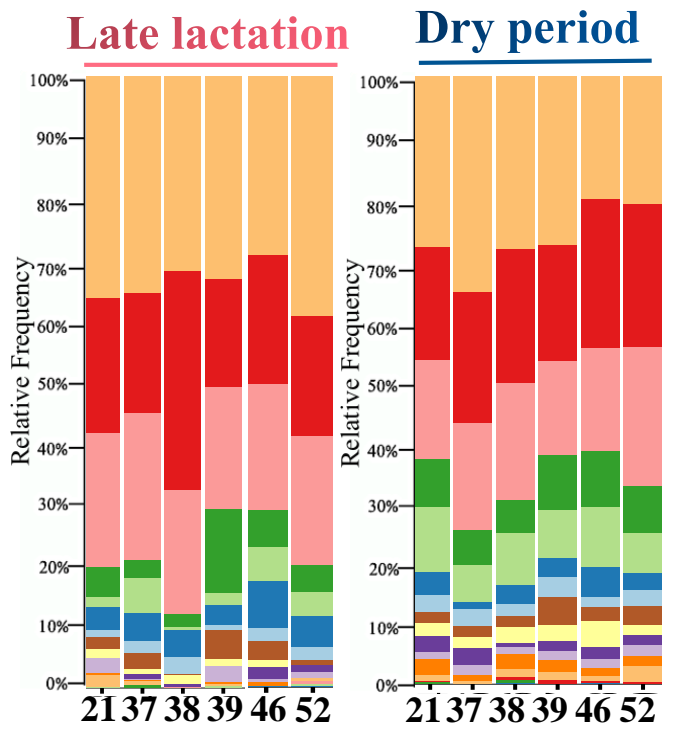
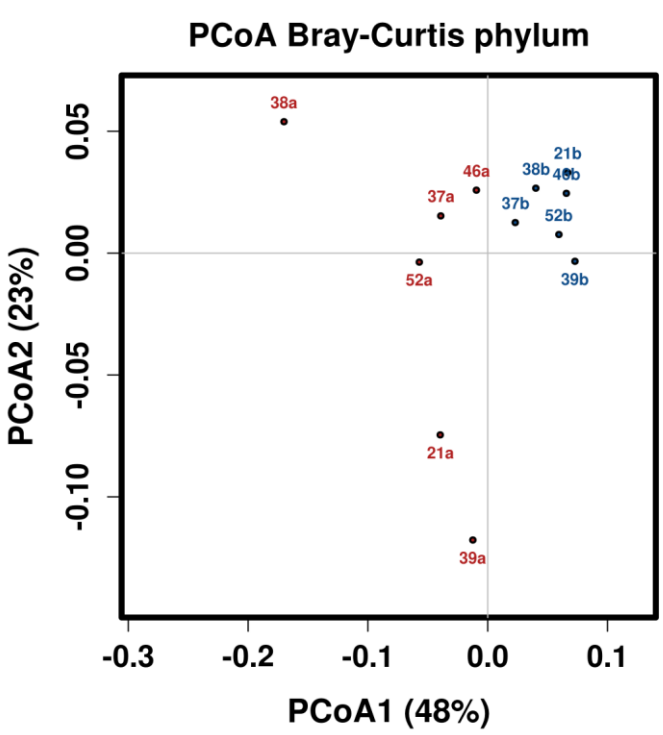
Sequences clustered into
Operational Taxonomic
Units' (OTUs)

Taxonomic classification

Bacterial and Archaeal microbial community profile



i. Phylum level comparison of active bacterial communities

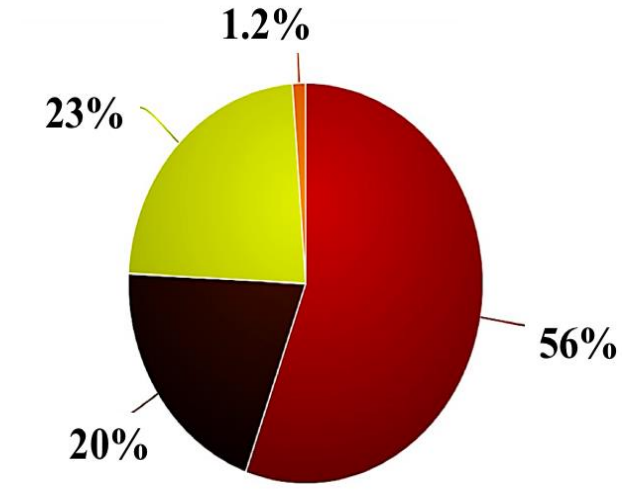
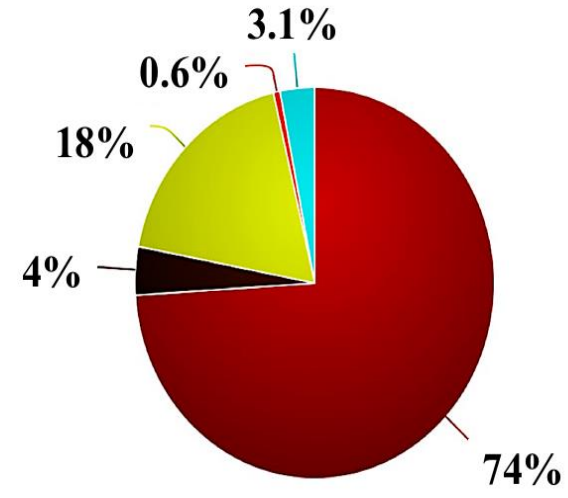
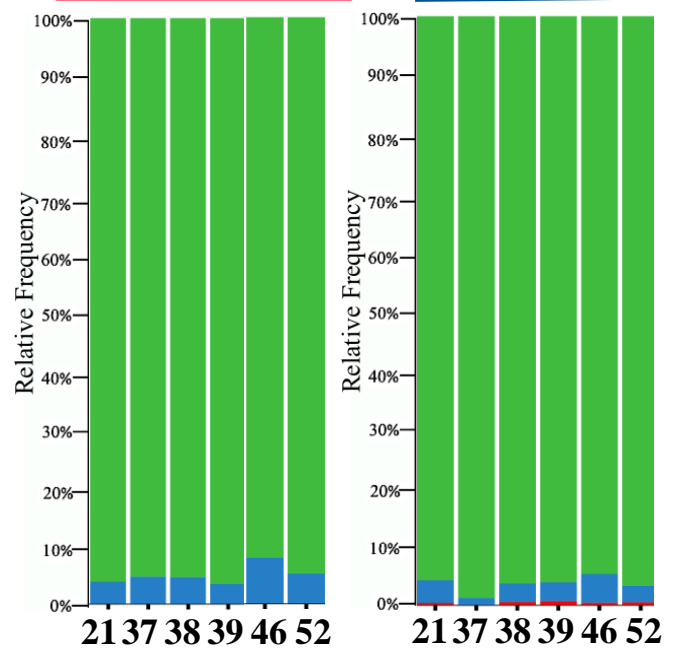


● Late lactation
● Dry period

■ Bacteroidetes
■ Firmicutes
■ Proteobacteria
■ Spirochaetes
■ Unclassified

ii. Genus level comparison of active archaeal communities

Late lactation Dry period



Late lactation

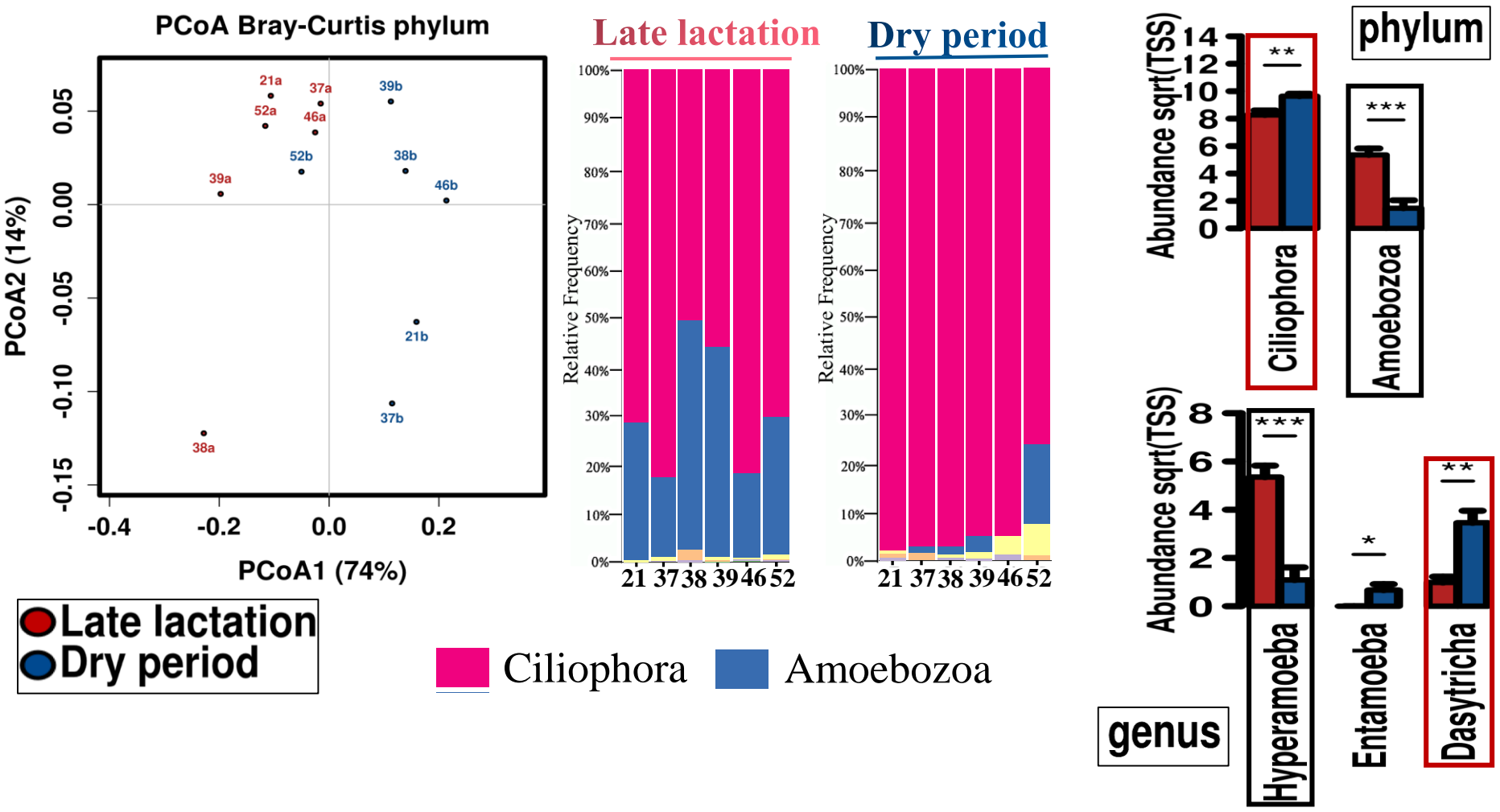
Dry period

- Bacteria
- Archaea
- Unassigned

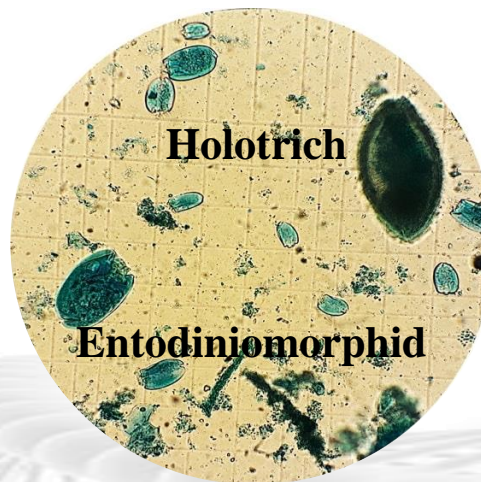
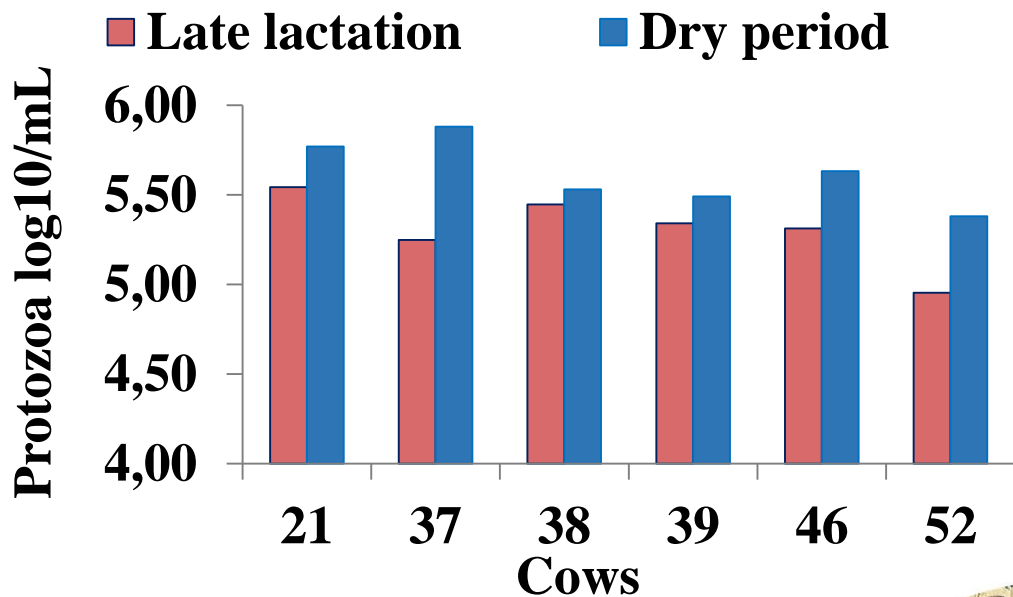
- vadinCA11
- Methanimicrococcus
- Methanosphaera

- Methanobrevibacter
- Unclassified.Methano-massiliicoccaceae
- Methanoplanus

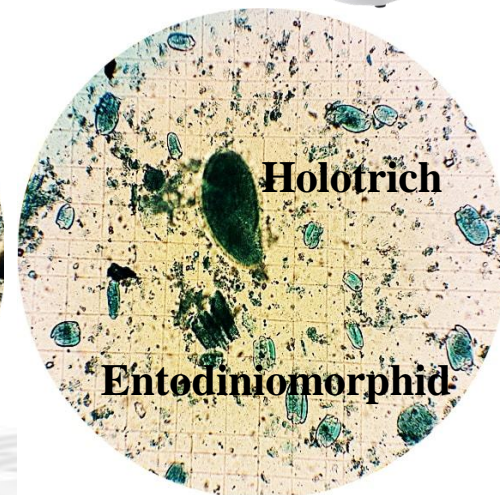
iii. Phylum & genus level comparison of active protozoal communities



iv. Total protozoa number in late lactation and dry period



Late lactation



Dry period

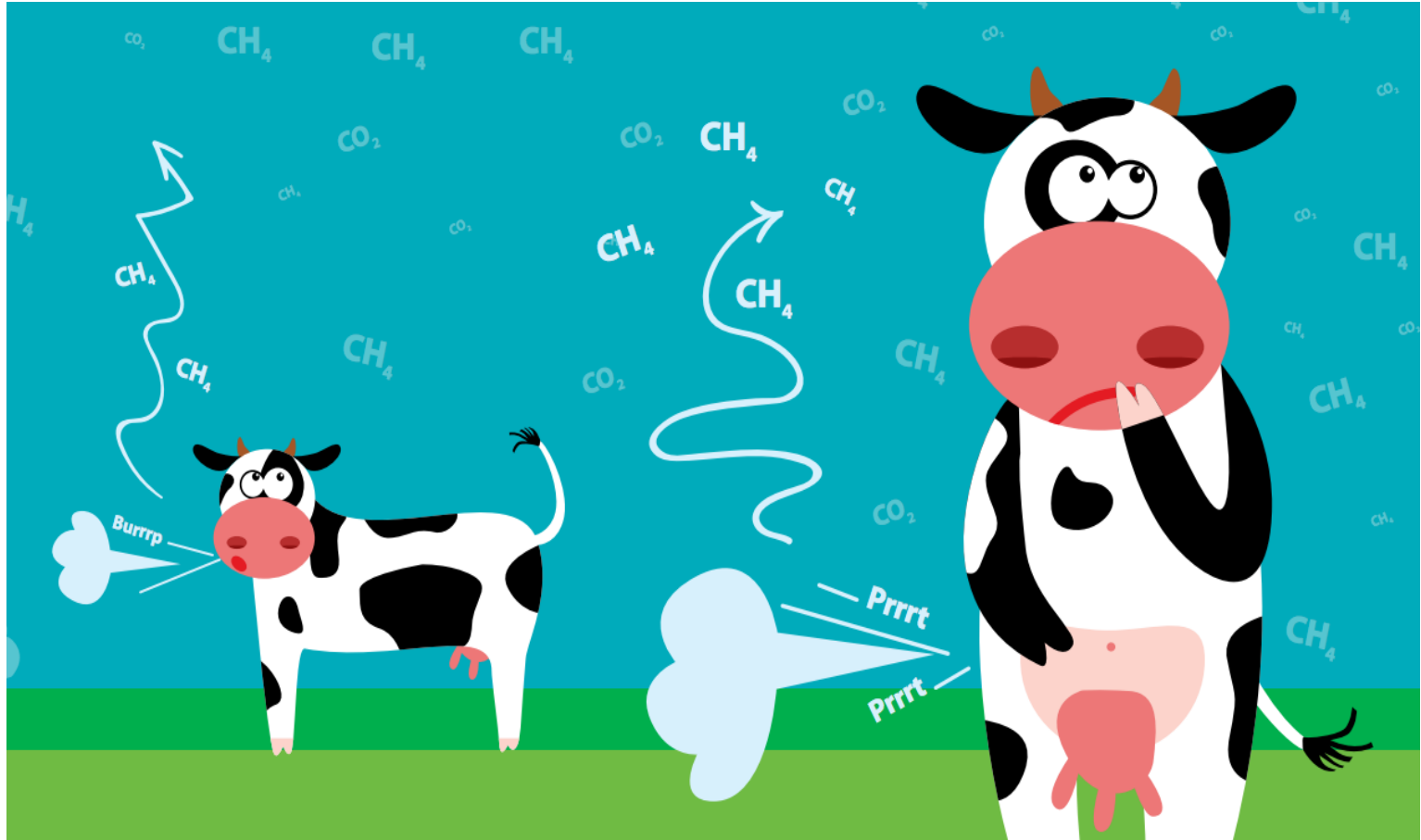
The results of this study have shown

- i. A clear effect of different diets on rumen microbiota composition**
- ii. Dry period diet resulted in higher abundance of methanogenic microorganisms**

Future perspective:

- **Microbial programming** by controlling feeding managements in early life that persisted in later life
- Therefore, more research is needed to gain insight into the rumen microbiota composition under different dietary treatments

THANK YOU FOR YOUR ATTENTION



Late lactation Cow

Dry Cow