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E FORESTALI



Characterization of buffalo faecal microbiome depending on ration concentrate level

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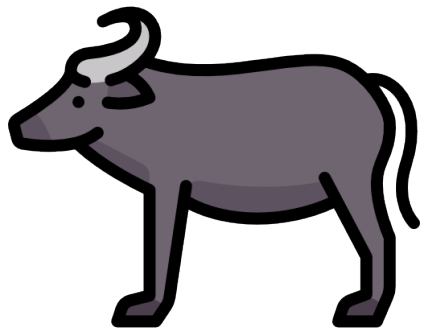
The 75th EAAP Annual Meeting 1/5 September 2024 - Florence, Italy
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INTRODUCTION

The **gastrointestinal tract (GIT) microbiome** of ruminants represents a complex and dynamic ecosystem **crucial to animal health and productivity** (Sharma et al., 2021).

Co-occurring microorganisms were identified within the rumen and fecal microbiomes, revealing a strong association and interdependency between bacterial communities (Andrade et al., 2020). Ruminal sampling being invasive and difficult, obtained only through oral intubation, rumenocentesis, fistulation or after slaughter (Andrade et al., 2020), unlike feces sampling which is rapid and non-invasive.

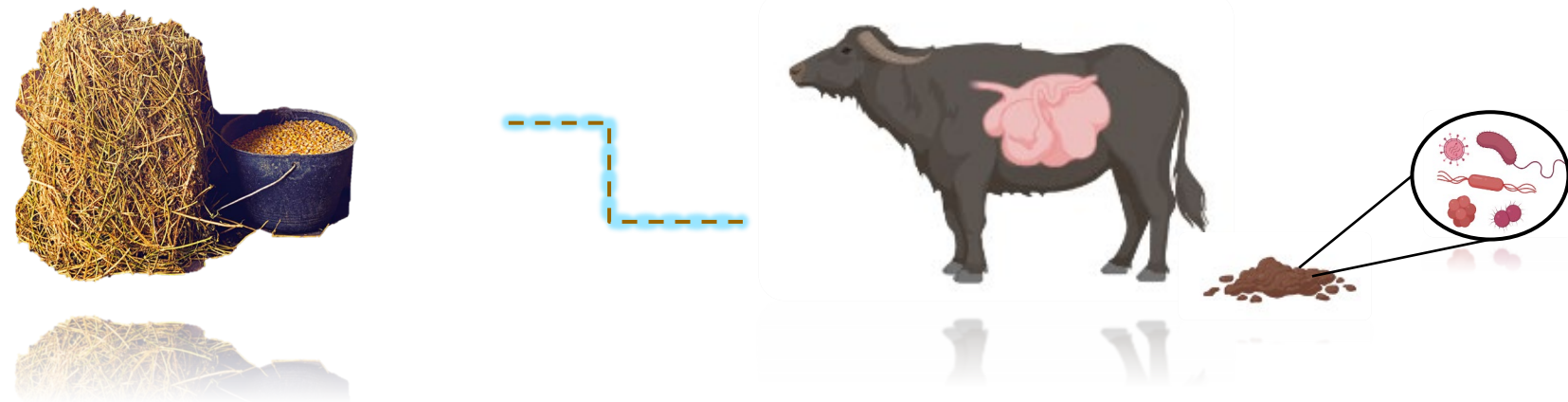
Compared to the rumen, the hindgut microbiota, represented by the fecal microbiome, is poorly characterized, particularly through next-generation sequencing (Noel et al., 2019).



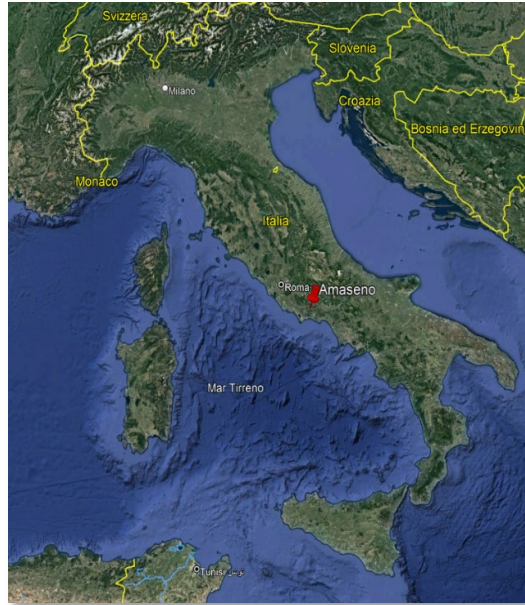
Microbial communities within this tract **are influenced** by a range of **internal and external factors**, including species, breed, age, environment, rearing practices, stocking density, stress, and antibiotic use (Zhao et al., 2024) and oestrus (Sharma et al., 2021). However, the primary factor influencing fecal microbiome composition is the **animal's diet** (Cendron et al., 2020; Couch et al., 2021). In particular, the **forage-to-concentrate ratio** in the diet is the major factor affecting fecal microbiome composition in cattle (Kim et al., 2014).

AIM OF THE STUDY

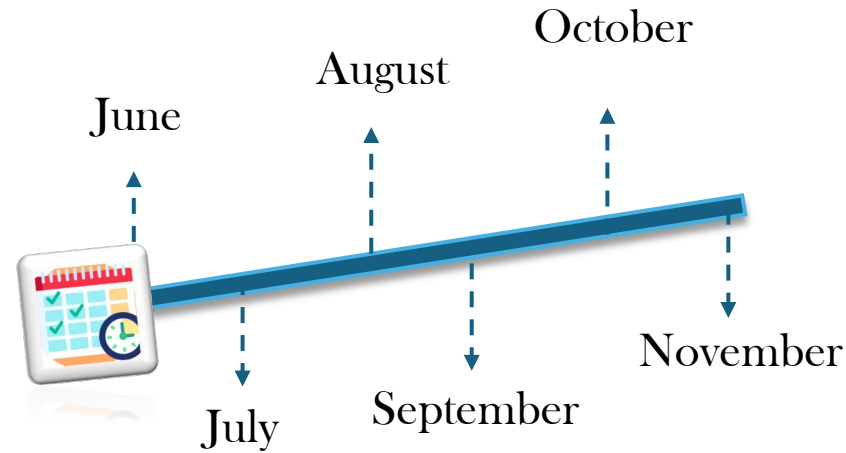
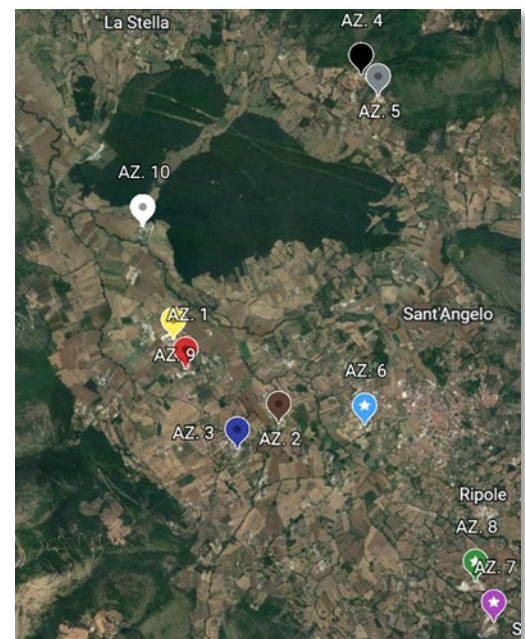
The aim of this study was to evaluate the effect of different forage:concentrate ratio in buffalo's diets on the microbioma composition of faeces. Considering two different **forage-concentrate ratio (F:C)** from **High ($\geq 80:20$)** to **Low ($\leq 70:30$)**



MATERIALS AND METHODS



Survey on 10 dairy buffaloes' farms from June to November 2022



Samples collected

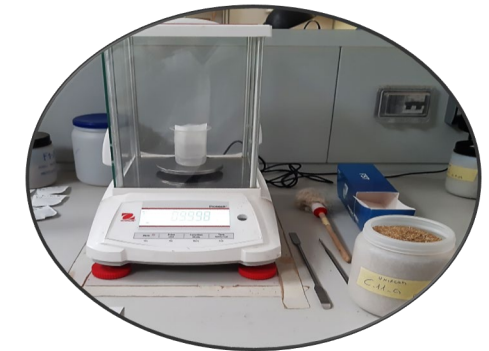
Total mixed ration



Diet's information



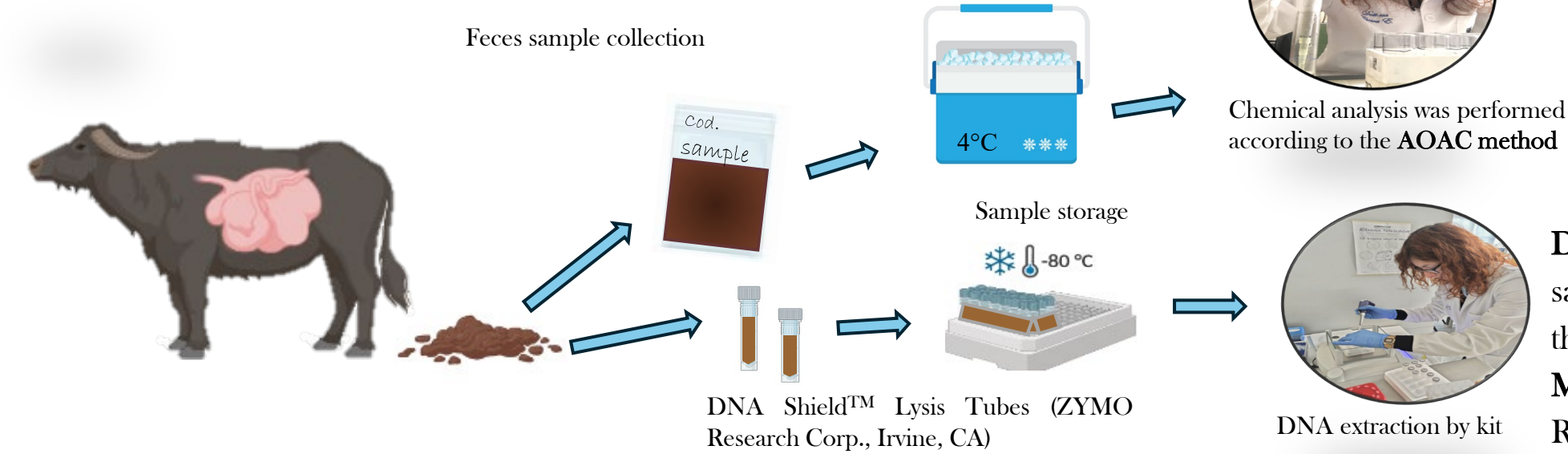
Feces



TMR was analysed for physical characteristics on farm using the 3-screen Penn State Particle Separator (PSPS; Heinrichs, 2013); peNDF was estimated using formula by Mertens (1997); TMR and feces were analysed with wet chemistry (AOAC methods: Dry matter, ash, crude protein, starch, ether extract, and aNDF, ADF, ADL by Van Soest et al. (1991) method).



Group Feces sampling



DNA extractions from fecal samples were performed by using the **Quick-DNA™ Fecal/Soil Microbe Miniprep Kit** (ZYMO Research Corp., Irvine, CA) using 300 mg of each sample, as stated by the standard protocol. Libraries were prepared on V3-V5 region of 16S gene and sequenced on Miseq with PE 300bp output

Apparent digestibility was estimated for aNDF (dNDF), starch (dStarch), crude protein (dProtein) and lipid (dLipid) following equation (1) by Fredin et al. (2014) using ADL as a marker and expressed as % on DM; apparent digestibility of DM (dDM) was estimated by equation (2).

$$(1) \text{ Apparent digestibility (\%)} = \left\{ 1 - \left[\frac{ADL_{TMR}}{ADL_{faeces}} \times \frac{Analyte_{faeces}}{Analyte_{TMR}} \right] \right\} \times 100$$

$$(2) \text{ Apparent digestibility DM (\%)} = \left\{ \left[\frac{ADL_{faeces} - ADL_{TMR}}{ADL_{faeces}} \right] \right\} \times 100$$

MATERIALS AND METHODS_statistical analysis



Bioinformatic analysis was performed using QIIME2 pipeline. Sequences were clustered into Amplicon Sequence Variant (ASVs) using DEBLUR, and the **taxonomic assignment** was conducted using the **GreenGenes2** database. Statistical analysis (i.e. α - and β -diversity metrics and identification of differentially abundant species) was done with DESeq2.

To evaluate the differences between High and Low, a factorial ANOVA (Analysis of variance) was carried out considering the season and the F:C ratio.

* * The farms are not included in the model because they match High and Low, (i.e. the farms that were H were always H as well as those that were low).

To highlight relationships between genus statistically significant and the chemical-physical composition of TMR and feces, a Pearson correlation was made.

The program used was R (R core team).

Significance differences was declared at $p < 0.05$



RESULTS

Table 1. Effect of forage to concentrate level and season on **phisiochemical diet's composition** (Lsmean $\pm$ SD).

	F:C_H	F:C_L	F:C ratio	Season	Interaction
Forage to concentrate ratio	85:15	65:35	0.001	ns	ns
Dry Matter (%)	54.57$\pm$5.26	46.82$\pm$5.17	0.001	ns	ns
Ashes (% DM ⁻¹)	6.93 $\pm$ 0.73	6.65 $\pm$ 0.72	ns	ns	ns
Crude Protein (% DM⁻¹)	11.12$\pm$1.66	12.46$\pm$1.16	0.029	ns	ns
Ethereal Extract (% DM ⁻¹)	2.28 $\pm$ 0.32	2.42 $\pm$ 0.38	ns	ns	ns
Crude fiber (% DM ⁻¹)	28.84 $\pm$ 6.76	29.60 $\pm$ 6.91	ns	0.0001	ns
aNDF (% DM ⁻¹)	48.28 $\pm$ 4.36	47.97 $\pm$ 4.05	ns	ns	ns
ADF (% DM ⁻¹)	32.68 $\pm$ 3.02	31.17 $\pm$ 2.61	ns	ns	ns
ADL (% DM ⁻¹)	6.31 $\pm$ 1.06	6.14 $\pm$ 0.66	ns	ns	ns
Starch (% DM ⁻¹)	13.11 $\pm$ 2.46	12.76 $\pm$ 2.51	ns	ns	ns
Upper Sieve (%)	25.22 $\pm$ 20.46	22.93 $\pm$ 14.24	ns	ns	ns
Middle Sieve (%)	27.88 $\pm$ 12.50	20.79 $\pm$ 7.29	ns	0.047	ns
Lower Sieve (%)	26.04 $\pm$ 9.34	29.40 $\pm$ 6.36	ns	0.000	ns
Bottom (%)	20.84$\pm$5.19	27.29$\pm$6.61	0.007	ns	ns
peNDF (%)	38.36 $\pm$ 5.59	35.19 $\pm$ 56.19	ns	ns	ns
MFU (kg DM⁻¹)	0.82$\pm$0.06	0.87$\pm$0.03	0.033	ns	ns

aNDF= neutral detergent fiber; ADF=acid detergent fiber; ADL=lignin acid deterse, MFU=milk forage unit.

Chemical composition of the rations were similar to those reported in recent studies on dairy buffaloes, except for the fiber content which was higher in this study (Evangelista et al., 2022; Masucci et al., 2024).



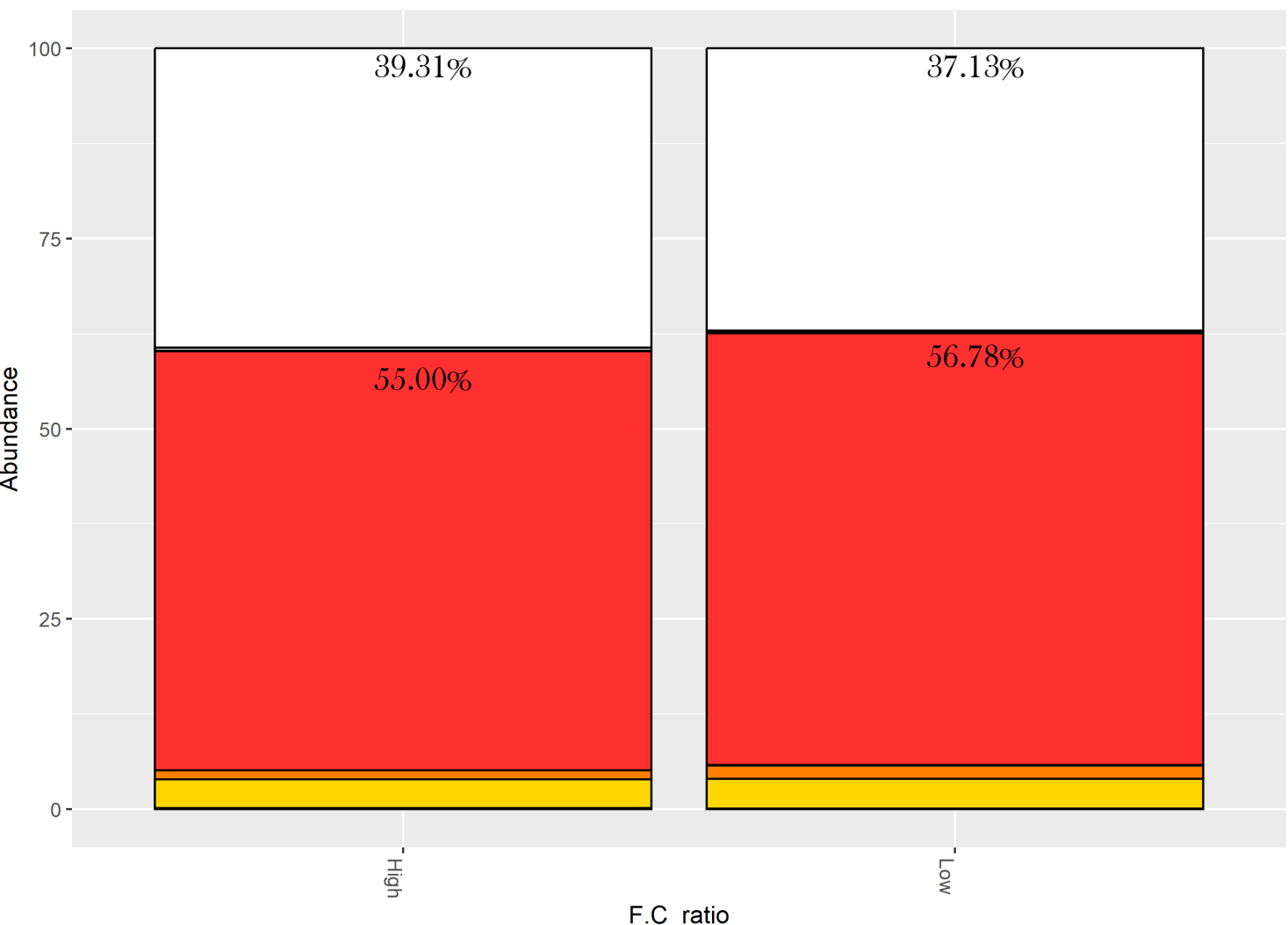
Table 2. Effect of forage to concentrate level and season on **chemical feces's composition** and **apparent digestibility** (Lsmean $\pm$ SD).

	F:C_High	F:C_Low	F:C ratio	Season	Interaction
DM (%)	13.80 $\pm$ 1.10	13.91 $\pm$ 1.80	ns	0.01	ns
Ashes (% DM ⁻¹)	12.01 $\pm$ 1.35	11.73 $\pm$ 1.39	ns	ns	ns
Crude Protein (% DM ⁻¹)	14.07 $\pm$ 1.60	14.02 $\pm$ 1.12	ns	ns	ns
Ethereal extract (% DM ⁻¹)	1.12 $\pm$ 0.15	1.51 $\pm$ 0.46	0.003	ns	ns
aNDF (% DM ⁻¹)	56.97 $\pm$ 3.74	55.28 $\pm$ 4.85	ns	0.01	ns
ADF (% DM ⁻¹)	45.91 $\pm$ 2.60	41.09 $\pm$ 2.91	0.0001	ns	0.02
ADL (% DM ⁻¹)	15.88 $\pm$ 2.38	13.12 $\pm$ 1.63	0.0001	ns	0.03
Starch (% DM ⁻¹)	1.30 $\pm$ 0.69	1.84 $\pm$ 1.40	0.02	0.01	0.01
pH	6.46 $\pm$ 0.23	6.17 $\pm$ 0.25	0.008	ns	ns
dDM	59.87 $\pm$ 6.43	52.90 $\pm$ 5.22	0.002	ns	0.04
dNDF	52.19 $\pm$ 10.01	45.78 $\pm$ 5.36	0.047	ns	ns
dStarch	96.17 $\pm$ 1.37	93.15 $\pm$ 5.35	0.001	0.005	0.003
dProtein	48.62 $\pm$ 9.94	46.64 $\pm$ 8.39	ns	ns	ns
dLipid	79.99 $\pm$ 4.48	69.84 $\pm$ 11.71	0.001	ns	0.049

aNDF= neutral detergent fiber; ADF=acid detergent fiber; ADL=lignin acid deterse; dDM=apparent digestibility of DM; dNDF=apparent digestibility of NDF; dStarch=apparent digestibility of starch; dProtein=apparent digestibility of Protein; dLipid=apparent digestibility of Lipid.

The feces chemical composition are consistent with values reported by Bovera et al. (2007) and Guerra et al. (2024). Also, the apparent digestibility (Guerra et al., 2024).

Figure 1. Relative abundance at **Phylum level** between High vs. Low F:C.



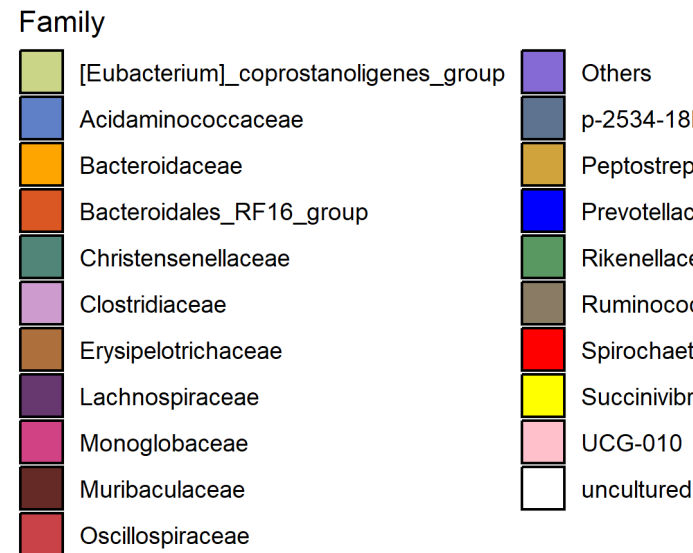
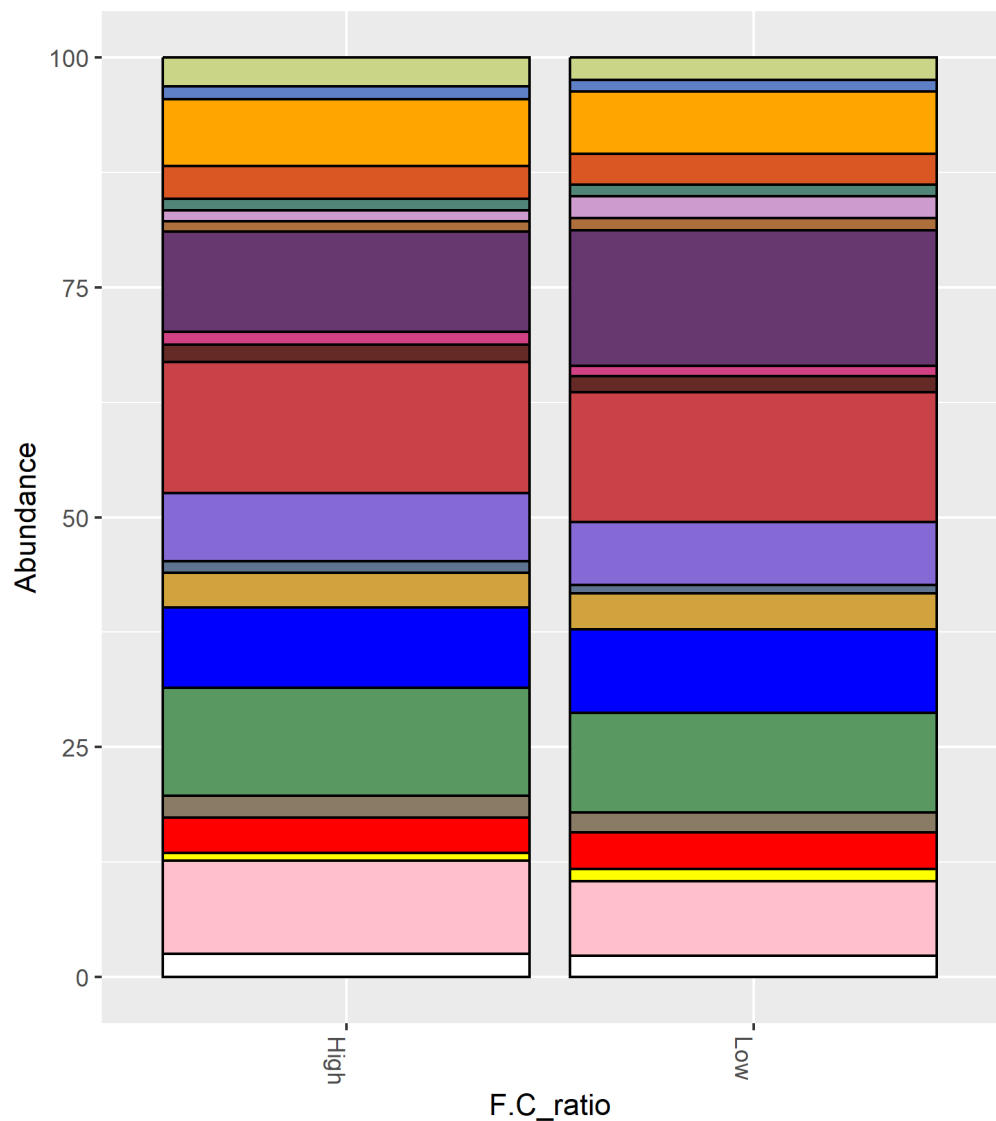
The study identified 10 phyla, with the most predominant being **Firmicutes** (55-56%) and **Bacteroidota** (37-39%), together accounting for **over 94%** of the total abundance. These results agree with several other studies, including those by Zhao et al. (2024) in Murrah and Nili-Ravi buffalo feces, as well as Plaizier et al. (2017) and Liu et al. (2020) in dairy cow feces.

Phylum	Phylum	F:C_high	F:C_low
	Bacteroidota	39.31	37.13
	Cyanobacteria	0.46	0.26
	Desulfobacterota	0.02	0.01
	Elusimicrobiota	0.02	0.01
	Fibrobacterota	0.05	0.03
	Firmicutes	55.00	56.78
	Patescibacteria	0.02	0.03
	Proteobacteria	1.23	1.74
	Spirochaetota	3.72	3.91
	Verrucomicrobiota	0.17	0.10
	F/B ratio	1.40	1.53

Bacteroidota, **Fibrobacterota** and **Cyanobacteria** populations are **positively associated** with **NDF** and **ADF** levels in diet (Martinez-Fernandez et al., 2020).

Some studies (El Kaoutari et al., 2013; Plaizier et al., 2017) explained that Bacteroidota are more efficient degrader of structural carbohydrates than Firmicutes. This may explain the lower apparent digestibility previously observed in low rations.

Figure 2. Relative abundance at **Family level** between High vs. Low F:C.

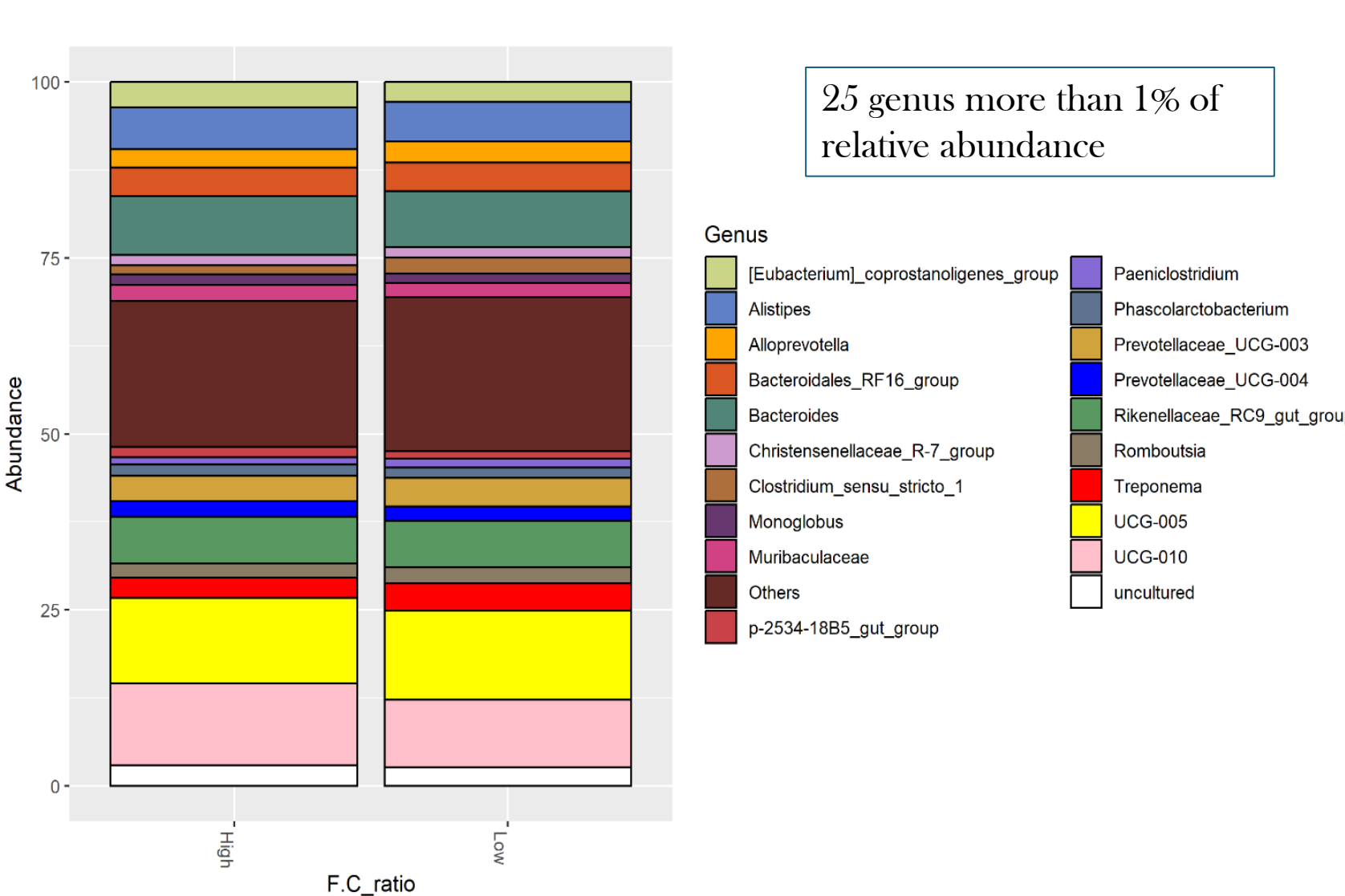


Family	F:C_high	F:C_low
Oscillospiraceae	14.23	14.18
Rikenellaceae	11.72	10.86
Lachnospiraceae	10.92	14.71
UCG-010	10.13	8.12
Prevotellaceae	8.76	9.09
Bacteroidaceae	7.28	6.78
Spirochaetaceae	3.82	4.00
Peptostreptococcaceae	3.80	3.90
Bacteroidales_RF16_group	3.55	3.38
[Eubacterium]_coprostanoligenes_group	3.17	2.46
uncultured	2.55	2.28
Ruminococcaceae	2.38	2.14
Muribaculaceae	1.91	1.75
Monoglobaceae	1.38	1.08
Acidaminococcaceae	1.36	1.26
Christensenellaceae	1.30	1.25
p-2534-18B5_gut_group	1.22	0.87
Clostridiaceae	1.16	2.32
Erysipelotrichaceae	1.09	1.39
Butyrificoccaceae	1.07	0.87
Succinivibrionaceae	0.82	1.34

Lachnospiraceae and Succiniovibrionaceae family is more present in environments with more soluble carbohydrates and in feces derived from cows fed with diet with higher fine particles (Castillo Lopez et al., 2019).

 = p < 0.05

Figure 3. Relative abundance at **Genus level** between High vs. Low F:C

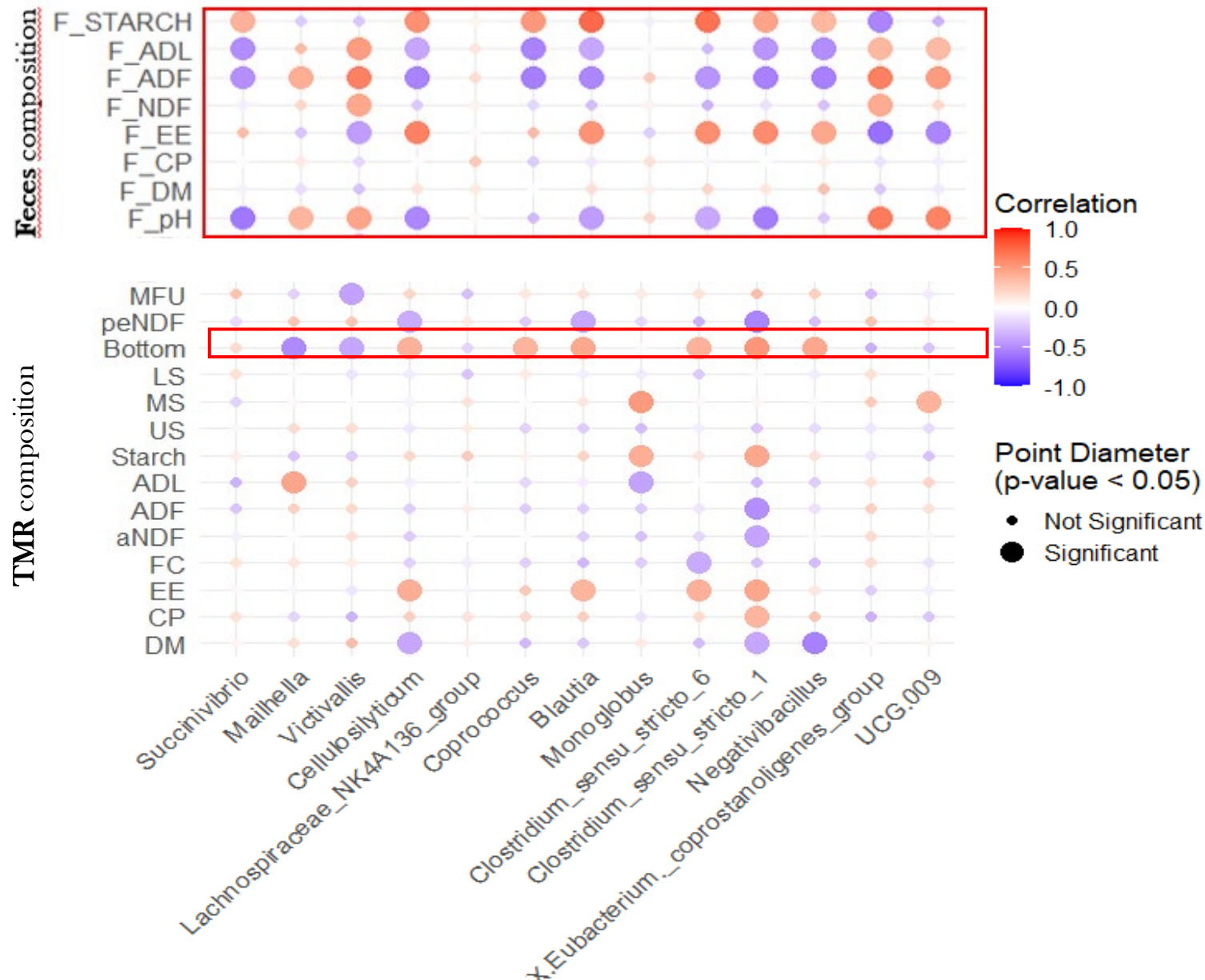


Genus	F:C_high	F:C_low
UCG-005 (Oscillospiraceae)	12.18	12.66
UCG-010	11.62	9.56
Bacteroides	8.35	8.02
Rikenellaceae_RC9_gut_group	6.64	6.65
Alistipes	5.94	5.53
Bacteroidales_RF16_group	4.07	3.99
[Eubacterium]_coprostanoligenes_group	3.64	2.89
Prevotellaceae_UCG-003	3.60	4.04
uncultured	2.92	2.69
Treponema	2.86	3.88
Alloprevotella	2.57	3.07
Muribaculaceae	2.20	2.07
Prevotellaceae_UCG-004	2.18	2.00
Romboutsia	2.03	2.26
Monoglobus	1.59	1.28
Phascolarctobacterium	1.56	1.50
GWE2-31-10	1.48	0.83
Christensenellaceae_R-7_group	1.48	1.45
p-2534-18B5_gut_group	1.40	1.02
Clostridium_sensu_stricto_1	1.26	2.32
uncultured	1.25	1.02
UCG-009	1.19	0.98
Paeniclostridium	1.11	1.23
Ruminobacter	0.87	1.37
Turicibacter	0.86	1.15

The most abundant genera found in this study are consistent with other studies on farmed (Zhao et al., 2024) and wild buffalo (Couch et al., 2021).

 = $p < 0.05$

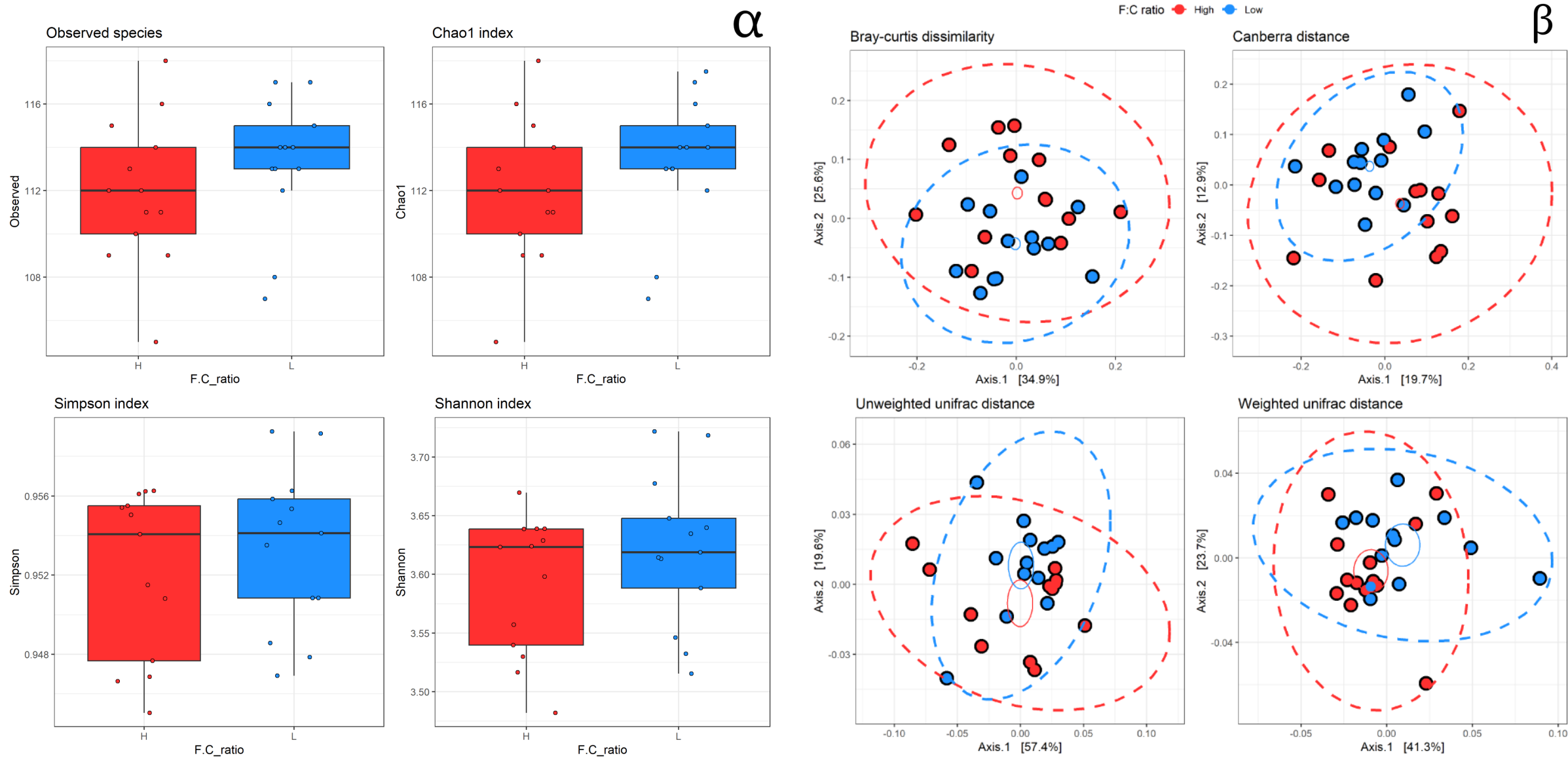
Figure 4. Correlation among genus and TMR and feces composition



Most of the correlations are with the chemical composition of the feces only a few correlations are with TMR

Only genus that were statistically significant between H and L were taken for correlation

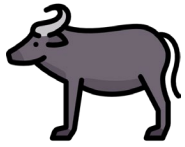
Figure 5. alpha and beta diversity



	Observed	Chao1	Shannon	Simpson
F:C ratio	0.2161	0.2162	0.3622	0.5446

 = $p < 0.05$

	Bray-curtis dissimilarity	Canberra distance	Unweighted unifracs	Weighted unifracs
F:C ratio	0.0292	0.0026	0.3346	0.0375



CONCLUSIONS AND FUTURE PROSPECTS



Our preliminary study revealed that different F:C ratio in diets significantly influenced the distribution of fecal bacterial communities, although the specific species remained unchanged;



The chemical composition of feces has a significant impact on the development of specific bacterial taxa in the feces of dairy buffaloes;

Also, the physical characteristic of ration seems to be influent on fecal microbiome



Further research is needed to understand how physical and chemical composition of diet can influences the fecal microbioma.

Characterization of buffalo faecal microbiome

depending on ration concentrate level

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Amaseno, June 2022



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Composition of 2 diets (average ± SD).

Mean of kg/head/day	F:C_H	F:C_L
Corn silage	14.64±1.94	12.33±2.05
Wheat silage	11.80±8.15	13.50±1.50
Mixed hay	14.10±2.52	14.49±1.90
Green forage		4.60±1.50
Corn mash	3.00±0.71	---
Threshers	---	6.38±1.48
Pellet feed	4.30±0.40	---
Corn meal	2.05±0.87	2.66±0.68
Soy meal	1.52±0.90	0.83±0.54
Barley meal	--	---
Feed bran	1.52±0.27	---
Barley rootlets	1.33±0.24	1.00±---
Broad bean	0.87±0.09	1.50±0.41
Linomix	0.50±---	0.50±---
Supplements	0.51±0.29	0.39±0.19
F:C ratio	85:15	65:35