Evaluating Microbial Community Changes in Rumen Samples Using Multiple Distance Metrics

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***Abstract*— This study explored how different dietary treatments affect the microbial communities in the rumen of cattle. We used 16S rRNA gene sequencing to identify and compare microbes across various treatment groups and time periods. To analyze the data, we applied several statistical methods, including Principal Coordinates Analysis (PCoA), Principal Component Analysis (PCA), UniFrac distances, and PERMANOVA. While we did not observe major differences in the overall microbial structure between treatments, we did find noticeable changes in the abundance of specific microbes. These results suggest that certain bacteria respond differently to diet, highlighting the need for further research using functional or gene-level methods like metagenomics or metabolomics to better understand their roles in rumen function and animal performance.**

I. INTRODUCTION

The types and activities of microbes in the rumen can vary significantly depending on the animal's diet. Different diets can impact the abundance and diversity of microbes, as well as their functions. These changes influence not only digestion and nutrient utilization but also the amount of methane (a greenhouse gas) produced by the animal [1]. The rumen is a unique stomach compartment in ruminant animals such as cattle, consisting of a complex and diverse community of microorganisms that include bacteria, archaea, protozoa, and fungi [2]. These microbes help break down fibrous plants into tiny molecules called volatile fatty acids. These acids provide the animal with most of the energy it needs [3]. The types and activities of microbes in the rumen could change a lot depending on what the animal eats. Different diets can impcat how many microbes are there, how varied they are, and what they do. These shifts influne not only digestion and practical utilization, but also how much methane (a greenhouse gas) they produce.

In this study, effect of five dietary treatments called as Control, Tallow, Corn Oil, Tl, and COL on the were evaluted on the structure of the rumen microbbiomee across five different time periods. The goal was to whether dietary lipid supplementation, alone or in combination changed the types of microbes in the rumen over time. The 16S rRNA gene-based high thoughput sequncing was applied to profile bacterial communites. And also, which was used a combiantion of cordination methods (PCA and PCoA) and statistical test to assess treatment and time effcect in microboial community.

II. METHODOLOGY

A. Dataset

Rumen microbial samples were collected from five dairy cows over a 12-week. Rumen microbial samples were collected from five dairy cows over a 12-week period. Each cow was assigned to one of five dietary treatments: Control, Corn Oil, Tallow, COL, or TL. A total of 25 experimental samples were collected, with each cow providing one sample per treatment period. More information about the data set is depicted in Table I. In addition to the experimental samples, the plate also included the following controls, In addition to the experimental samples, the plate also included the following controls Extraction Negative Control, PCR Negative Control and PCR Positive Control.

TABLE I
ANIMAL DISTRIBUTION ACROSS TREATMENT GROUPS AND TIME POINTS

Treatment	Animals Involved	Periods
Control	7075, 7063, 7076, 7101, 7084	Periods 1–5
Corn Oil	7075, 7063, 7076, 7101, 7084	Periods 1–5
Tallow	7075, 7101, 7063, 7076, 7084	Periods 1–5
COL	7075, 7063, 7076, 7101, 7084	Periods 1–5
TL	7075, 7063, 7076, 7101, 7084	Periods 1–5

A total of 28 samples were used for downstream analysis, which included 25 true samples and 3 control samples. The sample IDs were matched with metadata that included animal ID, collection date, treatment group, time period, and sample type. The treatment groups are detailed in Table II.

TABLE II
DESCRIPTION OF DIETARY TREATMENT GROUPS

Treatment	Description
Control	No added fat
Corn Oil	3.5% corn oil
Tallow	3.5% tallow
COL	3.5% corn oil + lysoforte (a digestive aid)
TL	3.5% tallow + lysoforte (a digestive aid)

B. Ordination Analysis Using PCoA and PCA

Two ordination methods were used to explore patterns in microbial community structure across different diets and time points: Principal Component Analysis (PCA) and Principal Coordinates Analysis (PCoA).

PCoA was used to explore differences in microbial communities across samples. UniFrac distances, which consider both the phylogenetic relationships between microbes and their abundance or presence/absence in the samples, were used to perform PCoA [4].

$$D' = -\frac{1}{2} H D^2 H \tag{1}$$

Where D' is the transformed distance matrix and H is a centering matrix used to remove the mean. PCA was applied to normalized microbial abundance data, specifically relative abundance. It identifies new axes known as principal components (PCs) that reveal the greatest variation in the data [5].

$$Z = XW \tag{2}$$

Where, Z is the matrix of principal component scores, X is the input data matrix and W is the matrix of eigenvectors.

C. UniFrac Distances

UniFrac is a distance measure that indicates how much of the microbial DNA in each sample comes from different branches of the phylogenetic tree. This not only shows which microbes are present but also provides insight into how closely related they are [6], [7]. Two types of Unifrac methods were used. Equation 3 calculates the distance between two samples.

$$d_{\text{UniFrac}}(A, B) = \frac{\sum w_e \cdot |p_{e,A} - p_{e,B}|}{\sum w_e} \tag{3}$$

Where: w_e is the length of each branch in the phylogenetic tree, $p_{e,A}, p_{e,B}$ are the amounts of microbes in A and B that come from that branch.

- Unweighted UniFrac shows only at whether or not a microbe is present. It is good at detecting rare microbes.
- Weighted UniFrac considers how abundant each microbe is. This gives more importance to the dominant microbes.

III. Results

Figure 1 shows rarefaction curves for microbial samples, which helps to assess the depth of the sequencing and the richness of the species. Each curve represents a sample, and the number of observed species increases with more sequencing reads until it level. The red dashed line indicates the minimum sequencing depth across all samples.

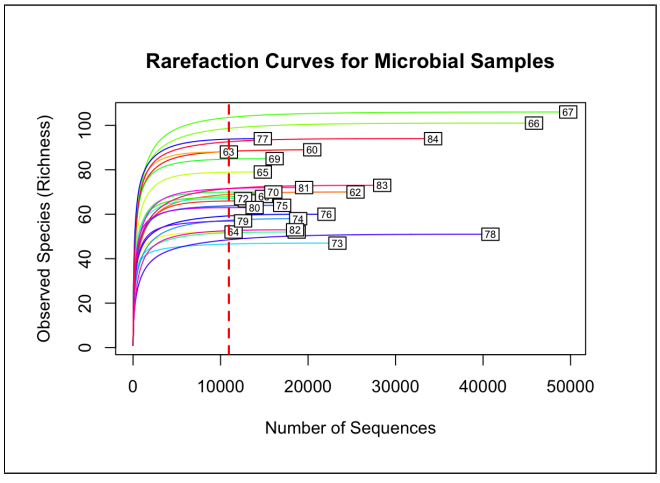

Fig. 1. Rarefaction curves for microbial samples.

This Figure 2 depicts the alpha diversity of the microbial communities in five treatment groups COL, Control, Corn Oil, Tallow, and TL. To reduce noise and improve statistical power, we applied a prevalence-abundance filter using the `filterfun()` function. Only ASVs that appeared in at least one samples and had a relative abundance greater than 0.15% in those samples were retained for downstream analysis. This approach helps to focus on more biologically meaningful features by removing rare and low-abundance taxa.

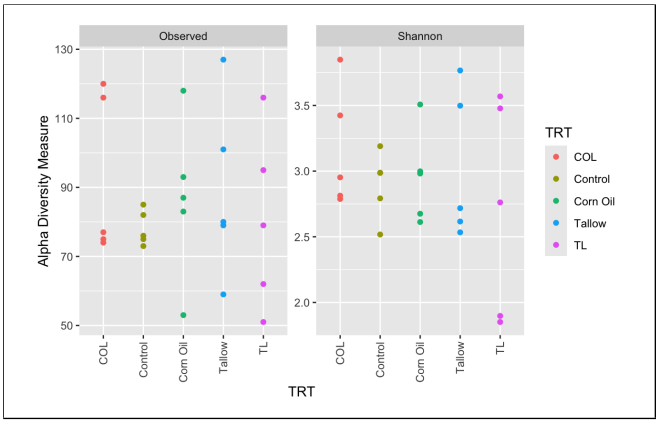

Fig. 2. Alpha diversity of microbial communities.

The observed panel shows how many different types of microbes were found in each sample. The Shannon panel displays for both the number of types and how evenly they are distributed. Tallow samples show a lot of variation and include some of the highest diversity levels. TL samples also have some high diversity values, but some samples are much lower. COL and Control samples generally have lower diversity in both panels. Corn Oil samples show a moderate level of diversity with less variation between samples.

A. PCoA using Bray-Curtis

Figure 3 was performed PCoA using Bray-Curtis dissimilarity and two axes explaining 21. 1% and 11. 6% of the variation, respectively. The samples showed some clustering according to treatment and sampling period, although no clear separation was observed.

We used PERMANOVA with Bray-Curtis dissimilarity to investigate how microbial communities were affected by the animal, treatment and sampling period (Table III). The analysis showed that the animal had a strong and significant effect on the microbial composition (p = 0.001), explaining about 29% of the total variation. The sampling period explained around 16% of the variation but was not statistically significant (p = 0.133). Similarly, treatment did not have a significant effect (p = 0.584), accounting for about 13% of the variation. These results indicate that individual animal differences were the main factor that influenced the variation of the microbial community in this study.

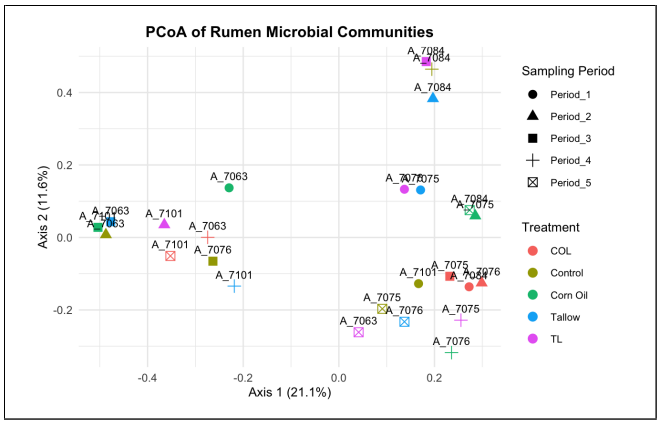

Fig. 3. PCoA using Bray-Curtis dissimilarity.

TABLE III
PERMANOVA RESULTS BASED ON BRAY-CURTIS DISSIMILARITY

Source	Df	Sum of Squares	R^2	F-value	Pr(>F)
Animal	4	2.6996	0.29499	2.1580	0.001
TRT	4	1.1902	0.13006	0.9515	0.584
Period	4	1.5086	0.16485	1.2059	0.133
Residual	12	3.7529	0.41009		
Total	24	9.1512	1.00000		

B. Unweighted UniFrac

Figure 4 explains that PCoA-based unweighted UniFrac distances revealed clustering patterns in microbial communities according to treatment and sampling periods. The axes explained 58.5% of the total variation (33.7% by Axis 1 and 14.8% by Axis 2). Each point represents a sample from one animal, and the label (e.g, A_7075) shows the animal ID. Some animals in the same treatment group are close to each other, which suggests that the type of diet may have influenced the kinds of microbes found in their samples.

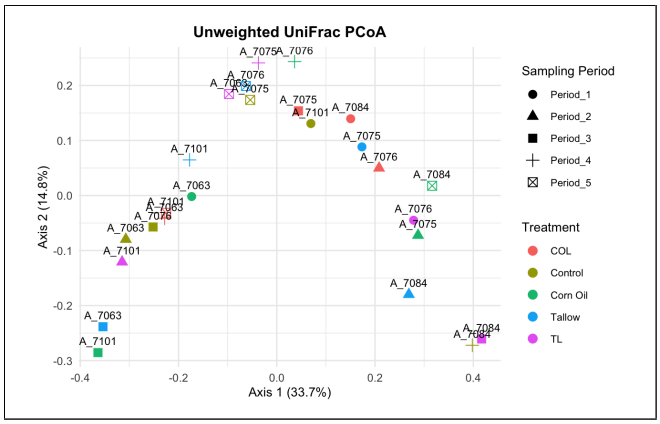

Fig. 4. PCoA using Unweighted UniFrac.

We performed a PERMANOVA analysis using unweighted UniFrac distances to evaluate the effects of the animal, treatment, and sampling period on the composition of the microbial community (Table IV). The results showed that individual animals had a significant influence on microbial structure (p = 0.001), which explained approximately 35% of the total variation. The sampling period had a marginal effect (p = 0.097), which accounts for 17% of the variation. In contrast, treatment did not have a significant impact on microbial composition (p = 0.458). These results suggest that host-specific differences were the strongest drivers of microbial variation in this study.

TABLE IV
PERMANOVA RESULTS BASED ON UNWEIGHTED UNIFRAC DISTANCE

Source	Df	Sum of Squares	R^2	F-value	Pr(>F)
Animal	4	1.5395	0.35379	2.9743	0.001***
TRT	4	0.5187	0.11920	1.0022	0.458
Period	4	0.7405	0.17017	1.4306	0.097.
Residual	12	1.5527	0.35684		
Total	24	4.3513	1.00000		

C. Weighted UniFrac

The Weighted UniFrac PCoA Figure 5 shows the differences in microbial communities between treatments and time points. The first axis (Axis 1) explains 32.1% of the variation between samples, and the second axis (Axis 2) explains 18.9%.

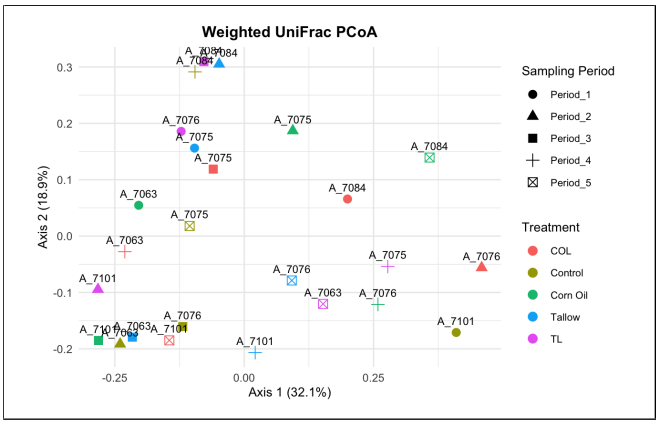

Fig. 5. PCoA using using Weighted UniFrac.

We used PERMANOVA to assess the effects of individual animal, treatment, and sampling period on the structure of the microbial community based on weighted UniFrac distances. The results showed that the individual animal had a significant effect on the microbiom composition (p = 0.022), which explains approximately 28% of the total variation. However, treatment and sampling period did not significantly influence community structure (p = 0.924 and p = 0.729, respectively). Almost 48% of the variation remained unexplained, indicating that other unmeasured factors may contribute to microbial differences.

D. Aitchison PCA (CLR-transformed)

In the Figure 6 PC1 and PC2 explain 17.3% and 14% of the variation. Describes a significant part of the differences between samples. Together, these two axes account for 31.3% of the differences observed between the samples. Samples

TABLE V

PERMANOVA RESULTS BASED ON WEIGHTED UNIFRAC

Source	Df	Sum of Squares	R^2	F-value	Pr(>F)
Animal	4	1.0398	0.28272	1.7697	0.022
TRT	4	0.3889	0.10576	0.6620	0.924
Period	4	0.4864	0.13227	0.8279	0.729
Residual	12	1.7626	0.47926		
Total	24	3.6778	1.00000		

from different treatment groups appear to form loose clusters in some areas of the plot, suggesting that the type of treatment may have influenced the microbial composition in the rumen.

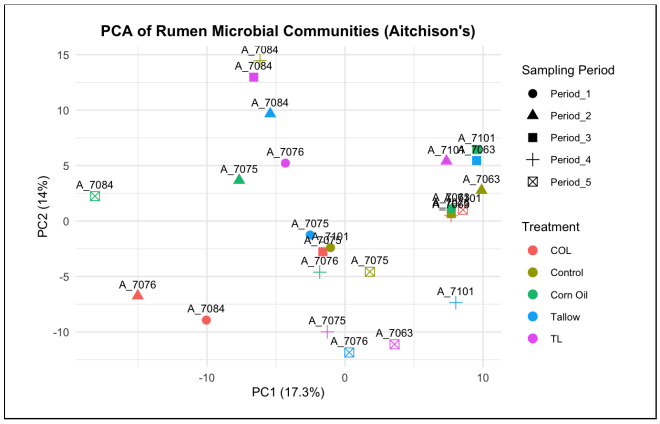

Fig. 6. PCA using using Atichoson's.

We performed a PERMANOVA analysis using Aitchison's distance to examine how microbial communities varied by animal, treatment, and sampling period (Table VI). The results showed that the animal had a strong and significant effect on the microbial composition (p = 0.001), which explained about 32% of the total variation. The sampling period had a moderate effect (p = 0.081), accounting for 17% of the variation, though it was only marginally significant. In contrast, treatment had no significant impact (p = 0.487) and explained about 13% of the variation. These findings suggest that individual differences between animals had the greatest influence on the structure of the microbial community.

TABLE VI

PERMANOVA RESULTS BASED ON ATICHOSON'S DISTANCE

Source	Df	Sum of Squares	R^2	F-value	Pr(>F)
Animal	4	10495	0.32241	2.5361	0.001
TRT	4	4138	0.12713	1.0000	0.487
Period	4	5504	0.16909	1.3301	0.081·
Residual	12	12415	0.38137		
Total	24	32552	1.00000		

E. Differentially Abundant

Corn Oil vs Control

Figure 7 displays the top 20 ASVs that were significantly different between the Corn Oil and Control groups. ASVs from *Ruminococcus*, *Selenomonadaceae*, and *Succinivibrio* were enriched in the Corn Oil group. Meanwhile, several taxa, including *Treponema, Muribaculaceae*, and *Tractidigestivibacter*, were reduced. This suggests that corn oil supplementation altered microbial composition, promoting certain fiber-degrading and fermentative bacteria.

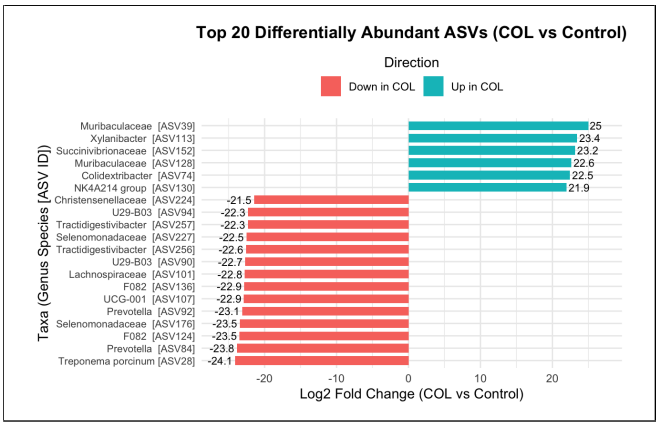

Fig. 7. Corn Oil vs Control.

Tallow vs Control

As shown in Figure 8, Tallow supplementation significantly increased the abundance of ASVs from *CAG-196*, *Ruminococcaceae*, and *Acidaminococcus*. In contrast, ASVs such as *Bifidobacterium*, *Prevotella*, and *Muribaculaceae* were notably decreased. These findings highlight specific microbial shifts associated with dietary tallow, which may influence gut fermentation and fat metabolism.

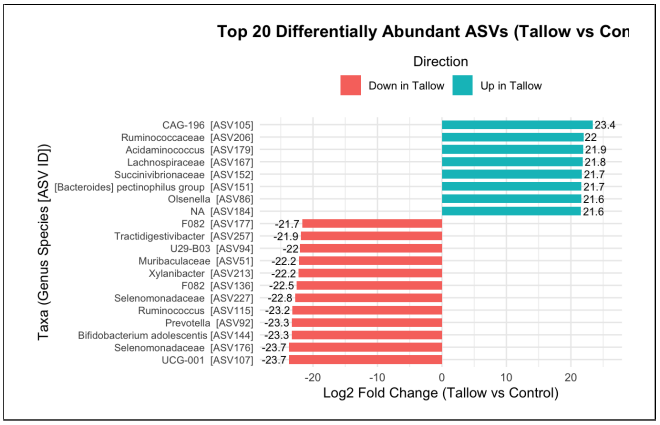

Fig. 8. Tallow vs Control.

TL vs Control

Figure 10 illustrates the differential abundance of ASV between the TL and Control groups. Taxa including *Xylanibacter, Prevotellaceae UCG-001*, and *Treponema* were significantly more abundant in the TL group. Conversely, *Segatella, Selenomonadaceae*, and *Tractidigestivibacter* were markedly reduced. These changes reflect how the TL treatment reshaped microbial populations, possibly impacting carbohydrate utilization pathways.

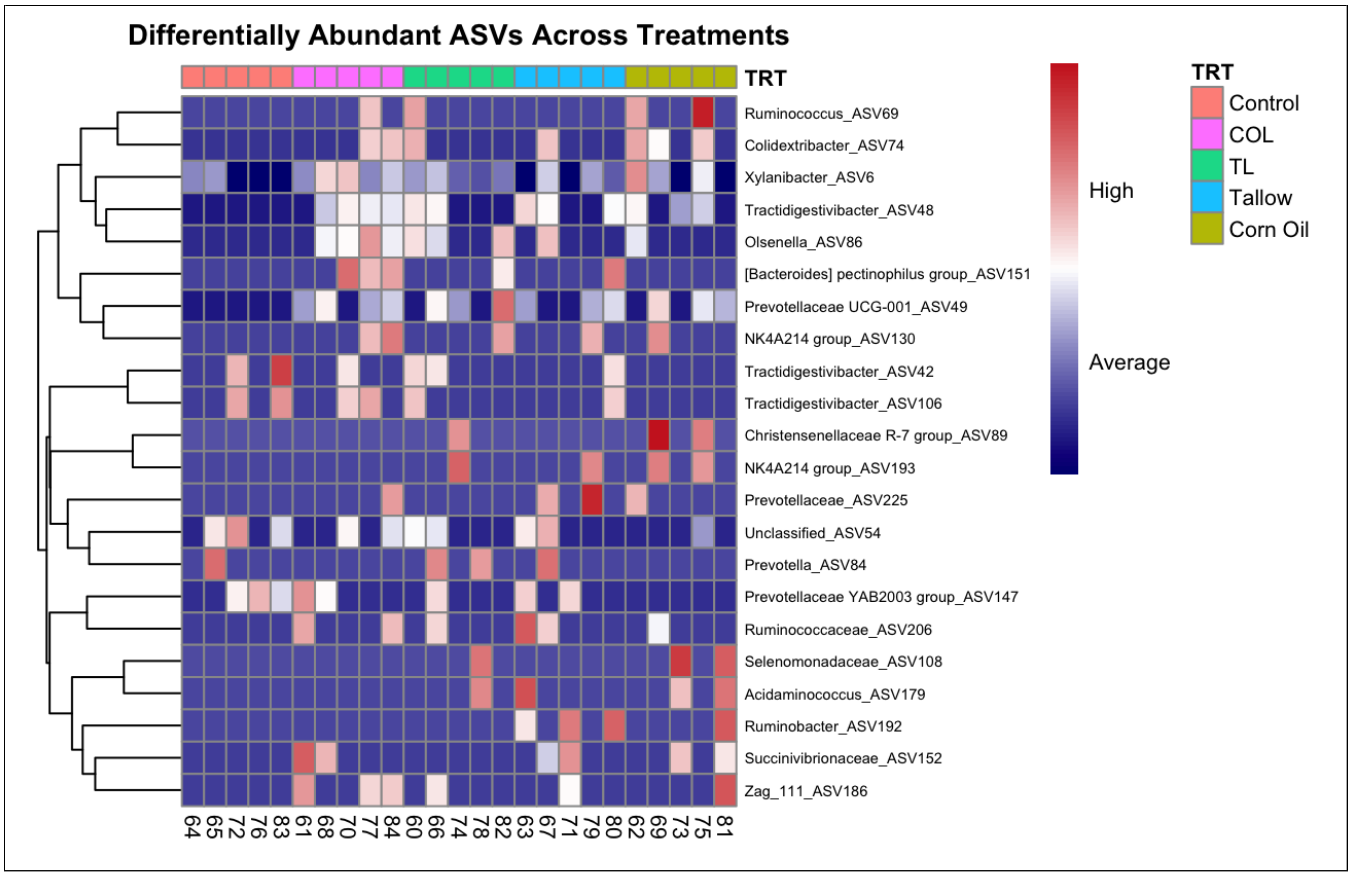

Fig. 9. Differentially abundant ASVs between treatments. The heatmap illustrates normalized and standardized abundance patterns across samples from all treatment groups.

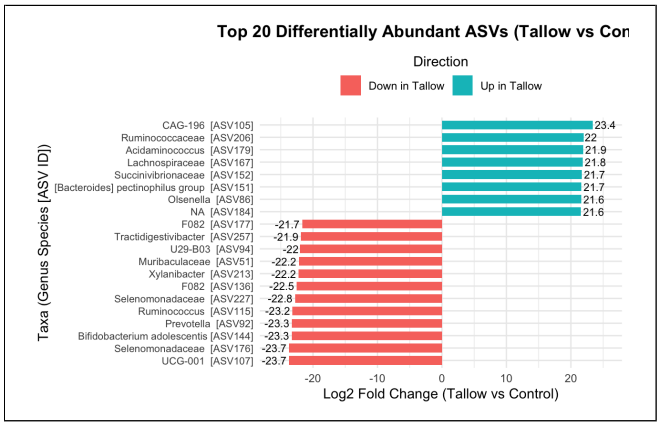

Fig. 10. TL vs Control.

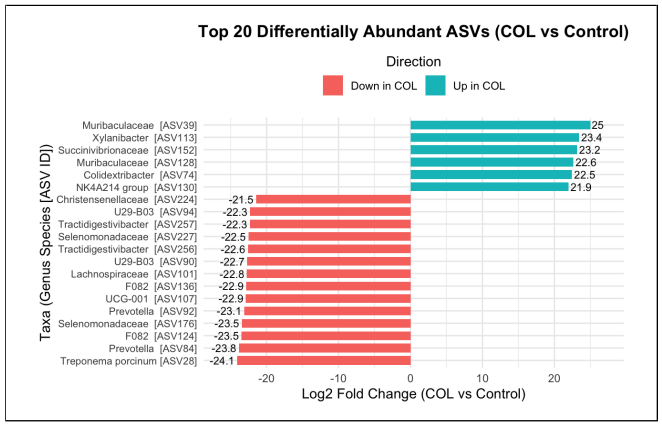

Fig. 11. COL vs Control.

COL vs Control

The Figure 11 shows the top 20 ASVs that were significantly different between the COL and Control groups. Several ASVs, such as *Muribaculaceae, Xylanibacter*, and *Succinivibrionaceae*, were more abundant in the COL group. However, ASVs like *Prevotella, Tractidigestivibacter*, and were lower in abundance compared to the Control treatment.

Heatmap

The heatmap in Figure 9 shows the variationin then the abundances of specific amplicon sequence vari(ASV) in different dieteatments. Each row represents an ASV, and each column represents an individual sample. *Ruminococcus_ASV69* and *Christensenellaceae R-7 group_ASV89* show relatively higher abundance in some treatment groups compared to others, suggesting a treatment-specific response.

Likewise, certain ASVs such as *Prevotella_ASV84* and *Succinivibrionaceae_ASV152* appear more abundant in specific treatment groups, indicating that dietary fat type and composition influence the structure of the rumen microbiome.

IV. Discussion

This study explained how different dietary fat supplements (Corn Oil, Tallow, TL, and COL) affected the rumen microbial community in dairy cows. The differentially abundant ASVs and explored microbial shifts across treatments were analysised using 16S rRNA gene sequencing. In the Figure 9, ASVs like *Ruminococcus, Christensenellaceae*, and *Succinivibrionaceae* were more abundant in samples from cows fed Corn Oil and Tallow, while ASVs such as *Tractidigestivibacter* and *Prevotella* were less abundant in those same treatments. These bacteria play key roles in fiber degradation and Short-Chain Fatty Acids (SCFA) production, which are essential for energy supply in cows. However, *Tractidigestivibacter, Prevotella* and *Selenomonadaceae* were reduced in several fat-supplemented groups. These bacteria are also involved in carbohydrate and protein fermentation. So their reduction may suggest that certain fats inhibit their growth or activity. Interestingly, some ASVs increased only in one treatment group, while others decreased across multiple treatments. This suggests that different fat sources can selectively promote or suppress certain bacterial groups. These microbial changes could impact rumen fermentation patterns, nutrient digestion, and possibly animal performance. Although the PERMANOVA results did not show a statistically significant difference in overall community structure between treatments. And, the heatmap clearly highlights that individual microbial populations were strongly influenced by diet. These microbial shifts may impact rumen fermentation, nutrient breakdown, and ultimately, animal performance.

V. Conclusion

This study shows that different types of dietary fats can change the rumen microbial community in dairy cows. Although the overall microbial structure was not significantly different between treatments, several specific ASVs showed distinct changes in abundance in response to the type of dietary fat. Some fat sources increased the abundance of microbes involved in fiber breakdown and SCFA production, while others reduced certain microbial groups. These changes suggest that the type of dietary fat can influence rumen fermentation and possibly affect feed efficiency and animal performance. Understading how different diets affect the rumen microbiome is important for improving cow health and milk production. Future studies using technologies like metagenomics or metabolomics can help us learn how changes in microbes affect digestion and how well cows grow and produce milk.

Data and Code Availability

All the following codes used for data preprocessing, data analysis, and visualization in this study is publicly available in the following GitHub repository: https://github.com/sachinkavindaa/KobzaMicrobial

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