Relationship between feed efficiency and rumen microbiota in feedlot bulls fed contrasting diets

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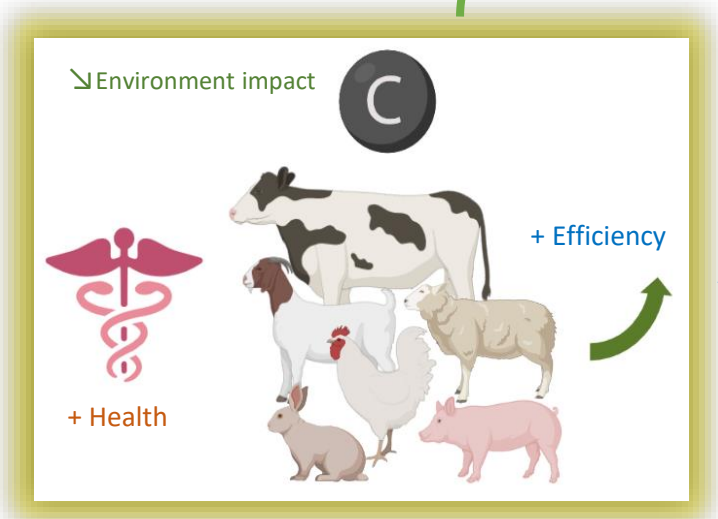Global demand of milk and meat by 2031, ↗~15%

(OECD-FAO, 2022)

= Expansion of cattle herds 🚫 ➕ Animal productivity

Cattle global heads:
2020: 1.53 billons
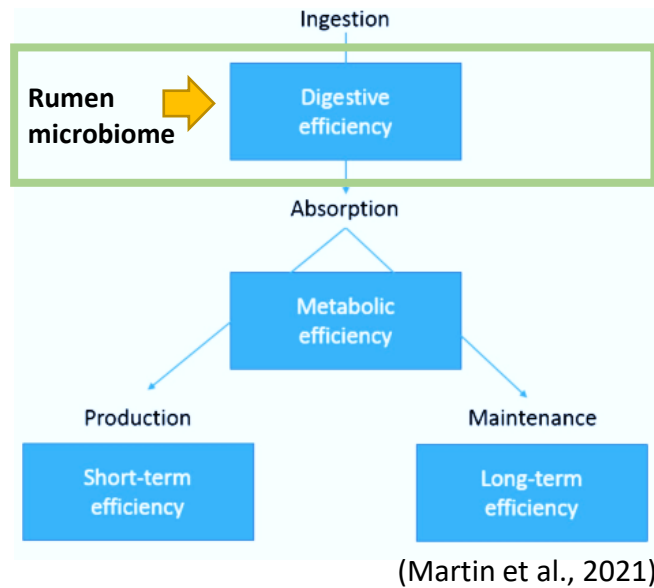By 2029: 1.8 billons (OECD, 2021)

Adverse environmental consequences

↘Environment impact C

+ Efficiency

+ Health

Animal production challenges

Feed efficiency

Residual Feed Intake (RFI) is one of the main ways to improve the **sustainability** and **profitability** of the livestock sector (FAO, 2018)

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• OECD-FAO Agricultural Outlook 2022-2031.
• OECD. Making Better Policies for Food Systems. Paris: OECD; 2021.

Feed efficiency variability

1. Digestive efficiency:

feed → nutrients

2. Metabolic efficiency:

nutrients → products

(Martin et al., 2021)

Why does the rumen microbiota matter?

➢ Rumen microbiome play a pivotal role in feed digestion

❖ However, digestive efficiency may differ due to nature of diet

❖ Changing from a forage-rich to a concentrate-rich diet can lead to changes in how animals are classified in terms of RFI (Lahart et al., 2020)

Hypothesis and objective

Hypothesis:

The divergent phenotypes of RFI **would exhibit distinct taxonomic and functional profiles** of the rumen microbiome, but these profiles **would differ depending on the diet**
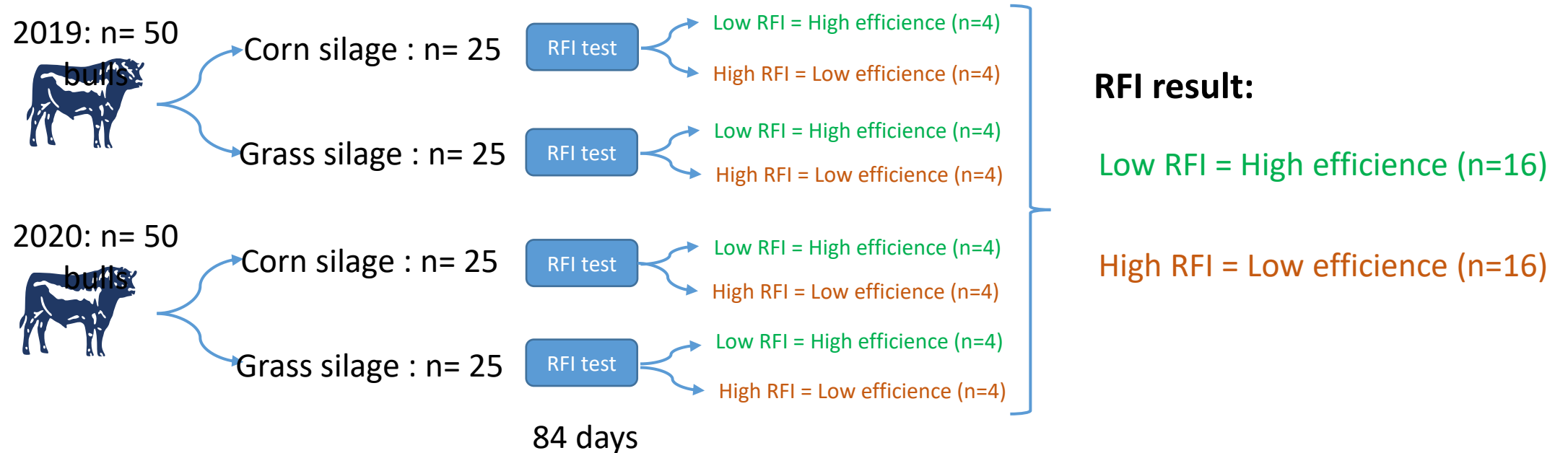
Objective:

To examine the **relationship** between divergent **RFI phenotypes** and the **taxonomic and functional profiles** of the rumen microbiome in fattening Charolais bulls fed two contrasting diets

• Pauline Martin, Vincent Ducrocq, Philippe Faverdin, Nicolas C. Friggens. 2021. Invited review: Disentangling residual feed intake—Insights and approaches to make it more fit for purpose in the modern context, Journal of Dairy Science. https://doi.org/10.3168/jds.2020-19844.
• Lahart, B., Prendiville, R., Buckley, F., Kennedy, E., Conroy, S. B., Boland, T. M., & McGee, M. 2020. The repeatability of feed intake and feed efficiency in beef cattle offered high-concentrate, grass silage and pasture-based diets. *Animal*. https://doi.org/10.1017/S1751731120000853

In brief:

❖ 100 Charolais bulls were evaluated for 2 consecutive years and with two contrasting diets

2019: n= 50 bulls

Corn silage : n= 25 — RFI test
- Low RFI = High efficiency (n=4)
- High RFI = Low efficiency (n=4)

Grass silage : n= 25 — RFI test
- Low RFI = High efficiency (n=4)
- High RFI = Low efficiency (n=4)

2020: n= 50 bulls

Corn silage : n= 25 — RFI test
- Low RFI = High efficiency (n=4)
- High RFI = Low efficiency (n=4)

Grass silage : n= 25 — RFI test
- Low RFI = High efficiency (n=4)
- High RFI = Low efficiency (n=4)

84 days

RFI result:

Low RFI = High efficiency (n=16)

High RFI = Low efficiency (n=16)

❖ Rumen sampling was 3 h post-feeding → Fermentation profile and microbiome analysis

❖ gDNA and RNA extraction → qPCR, 16S rRNA and RNA sequencing

❖ 16S rRNA: QIIME2 pipeline → vegan, phyloseq and MaAsLin2 in R software

❖ RNA seq: MetaTrans pipeline → DeSeq2, MicrobiomeAnalysis and MTX model

Fermentative profiles and qPCR analysis

❖ Total VFA and fermentative profiles did not differ between divergent of RFI phenotypes, and no Diet × RFI interaction was found

❖ Total bacteria and methanogens populations did not differ between RFI groups.

❖ Methanogens expressed per liquid rumen digesta weight tended to decrease in high efficient compared to less efficient bulls (Table 1)

Table 1. Rumen digesta and population of bacteria and methanogens of extreme residual feed intake (RFI) Charolais young bulls fed either a corn silage or grass silage diet.

| Parameters	Grass silage		Corn silage			P-values			Diet×RFI
	Low RFI	High RFI	Low RFI	High RFI	SEM	Diet	Year	RFI	
Absolute quantification, copies Log$_{10}$									
Total bacteria, 1 mL	10.30	10.27	10.31	10.35	0.090	0.569	0.001	0.955	0.609
Total bacteria, LRDW	14.83	14.83	14.82	14.89	0.085	0.796	0.001	0.576	0.582
Methanogens, 1 mL	9.07	9.13	8.86	8.96	0.092	0.011	0.004	0.310	0.500
Methanogens, LRDW	13.59	13.69	13.36	13.53	0.096	0.011	0.001	0.101	0.842

LRDW = Liquid rumen digesta weight.

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Rumen microbiota profiling

Ordination plot and permanova analysis

❖ Diet was the main driver of the rumen microbiota variation, with no overall differences found between the RFI groups (Fig. 1)

❖ Rumen microbiota differed between RFI groups within each diet (Fig. 2A and B)

Fig. 2

Fig. 1
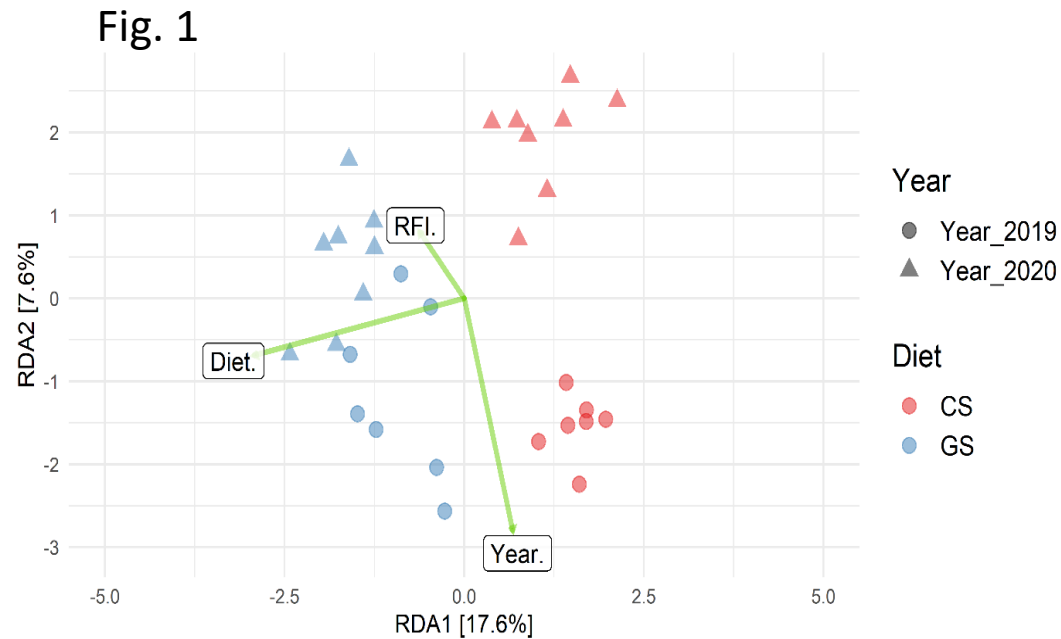
Redundancy analysis of the rumen microbiota. Adonis2 Test: Diet effect, $R^2 = 0.18$, P < 0.001, Year effect, $R^2 = 0.09$, P < 0.001, RFI effect ($R^2 = 0.03$, P = 0.168)
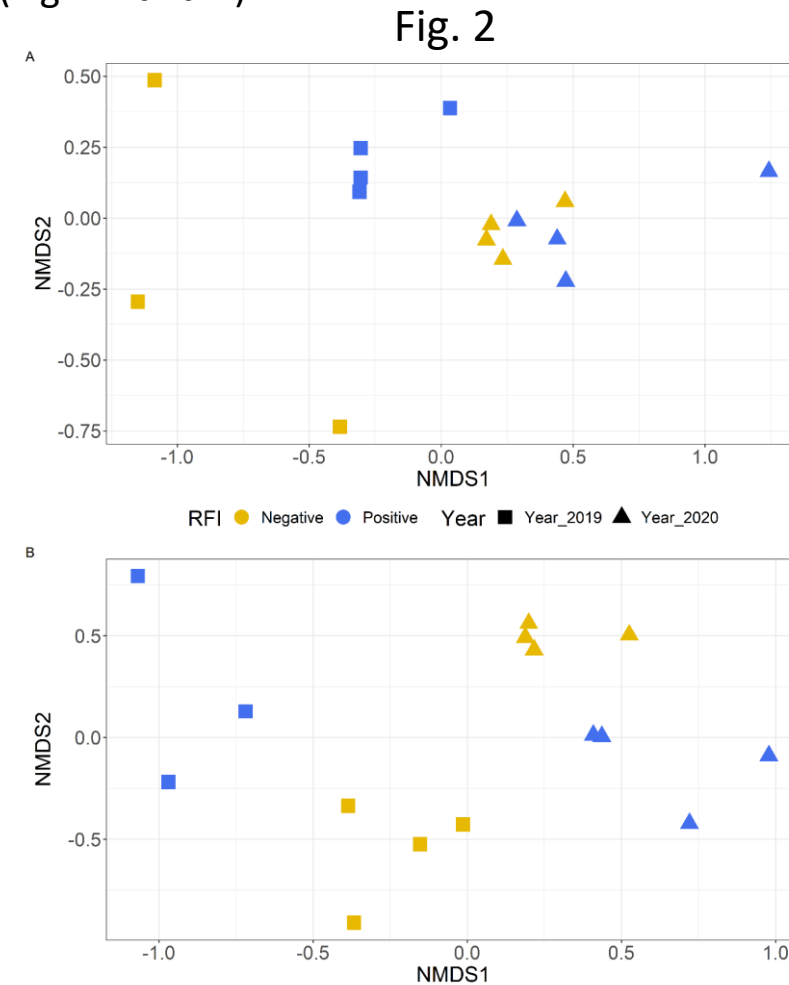
Grass silage diet

Adonis2 Test:
RFI: R2 = 0.09, P = 0.04
Year: R2 = 0.20, P < 0.001

Corn silage diet

Adonis2 Test:
RFI: R2 = 0.07, P = 0.10
Year: R2 = 0.24, P < 0.001

Rumen microbiota profiling

Differential abundances analysis

❖ Different profiles of rumen microbiota abundance were observed for each diet

Grass silage

Parameters	Taxon	Low RFI (%)	High RFI (%)	q-value
Family level	*RF16*	0.80 ± 0.146	1.02 ± 0.171	0.082
Family level	*Desulfovibrionaceae*	0.19 ± 0.022	0.24 ± 0.030	0.046
Family level	*Peptostreptococcaceae*	0.11 ± 0.009	0.16 ± 0.017	0.099
Family level	*Acidobacteriaceae*	0.04 ± 0.028	0.00 ± 0.000	0.003
Family level	*Unclassified Bacilli*	0.01 ± 0.006	0.00 ± 0.000	0.099
Family level	*Rhodocyclaceae*	0.00 ± 0.000	0.04 ± 0.023	0.082
Genus level	Unclassified *Elusimicrobiaceae*	0.07 ± 0.044	0.00 ± 0.000	0.045

Corn silage

| Parameters	Taxon	Low RFI (%)	High RFI (%)	q-value
Family level	*Unclassified Bacteroidales*	19.6 ± 0.922	20.4 ± 1.238	0.083
Family level	*Lachnospiraceae*	8.2 ± 0.498	7.9 ± 0.498	0.049
Family level	*Methanobacteriaceae*	3.1 ± 0.303	2.4 ± 0.275	0.083
Family level	*Spirochaetaceae*	4.0 ± 0.849	3.0 ± 0.432	0.083
Family level	*Veillonellaceae*	2.4 ± 0.181	2.1 ± 0.180	0.012
Family level	Unclassified *Firmicutes*	1.5 ± 0.094	1.2 ± 0.111	0.001
Family level	*S24_7*	0.00 ± 0.000	0.09 ± 0.043	0.001
Family level	Unclassified *HA64*	0.00 ± 0.000	0.04 ± 0.021	0.001
Family level	Unclassified *Flavobacteriales*	0.00 ± 0.000	0.01 ± 0.007	0.001
Genus level	*Methanobrevibacter*	3.1 ± 0.302	2.4 ± 0.274	0.077
Genus level	*Succinispira*	1.31 ± 0.134	1.16 ± 0.169	0.068
Genus level	*Clostridium*	1.02 ± 0.109	0.87 ± 0.113	0.041
Genus level	*Anaerobiospirillum*	0.02 ± 0.011	0.00 ± 0.000	0.042

q-value was obtained after false discovery rate (FDR) correction using the Benjamini-Hochberg procedure.

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Functional microbiota profiling

PCA and functional categories

❖ Diet was the main driver of the functional profiles of the rumen microbiota (Fig. 3A), with no overall differences found between the RFI groups (Fig. 3B)

Fig. 3
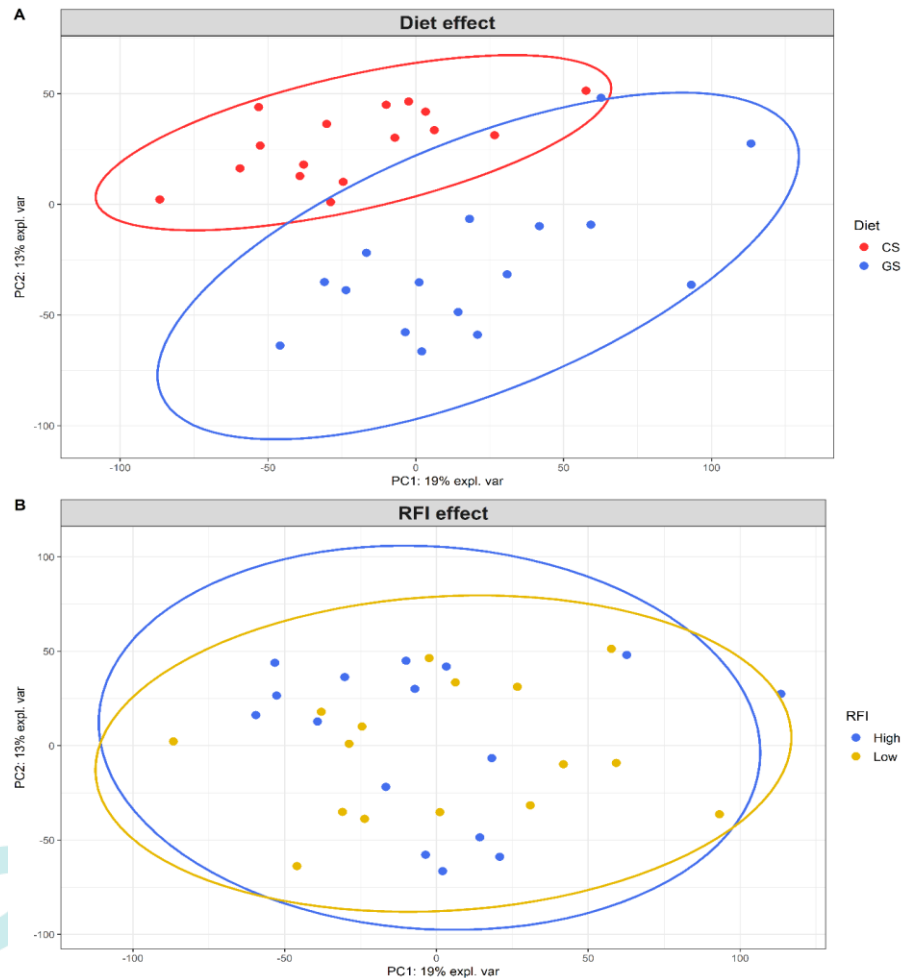
Table 2. Microbial functional categories abundances (cpm, mean ± SEM) of Charolais young bulls fed either a grass silage or corn silage diet.

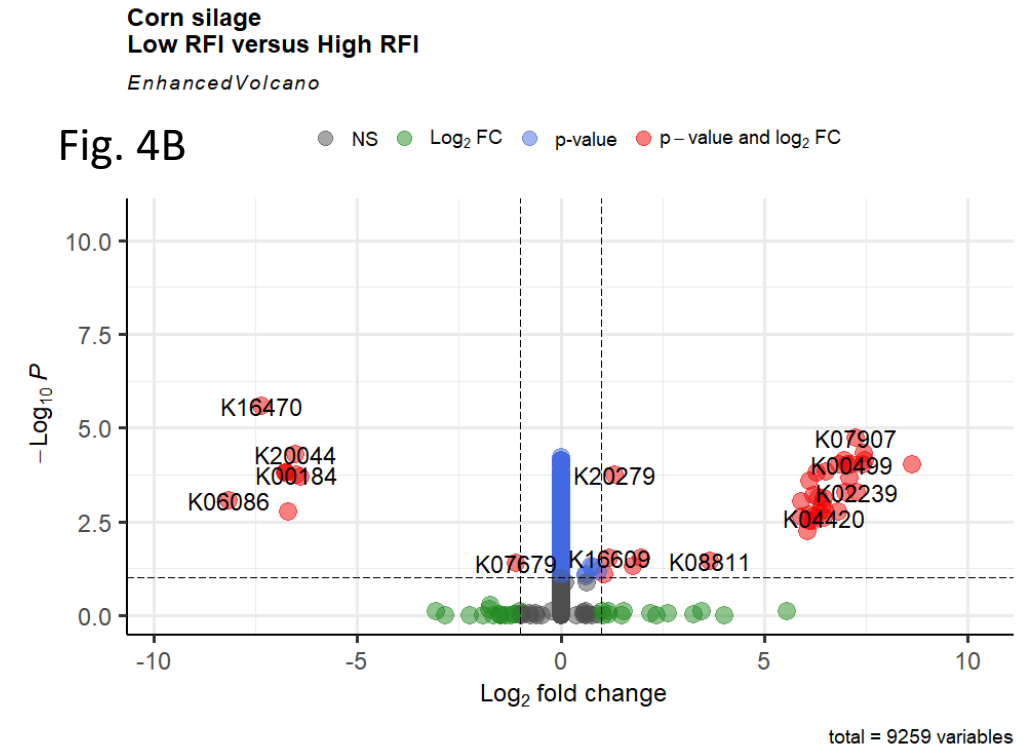| Parameters	Grass silage	Corn silage	q-value
KEGG functional category			
Glycan biosynthesis and metabolism	23,234 ± 872	20,025 ± 676	0.043
Nucleotide metabolism	87,311 ± 1,874	78,642 ± 2,006	0.043
Biosynthesis of other secondary metabolites	22,274 ± 761	19,593 ± 737	0.098
Energy metabolism	234,000 ± 4,984	247,000 ± 3,417	0.250
Lipid metabolism	39,141 ± 835	37,105 ± 1,201	0.651
Carbohydrate metabolism	297,000 ± 5,485	305,000 ± 5,464	0.704
Metabolism of other amino acids	32,685 ± 842	31,332 ± 695	0.704
Metabolism of cofactors and vitamins	53,979 ± 2,963	55,576 ± 1,264	0.739
Xenobiotics biodegradation and metabolism	23,008 ± 2,517	21,467 ± 539	0.739
Metabolism of terpenoids and polyketides	16,996 ± 465	16,755 ± 619	0.788
Amino acid metabolism	170,000 ± 5,556	168,000 ± 6,884	0.839

Functional microbiota profiling

DESeq2 and volcano plot analysis

❖ Minor differences were found in the differential expression between the RFI groups for the grass (Fig. 4A) or corn silage diet (Fig. 4B)

Fig. 4A

Grass silage
Low RFI versus High RFI

EnhancedVolcano
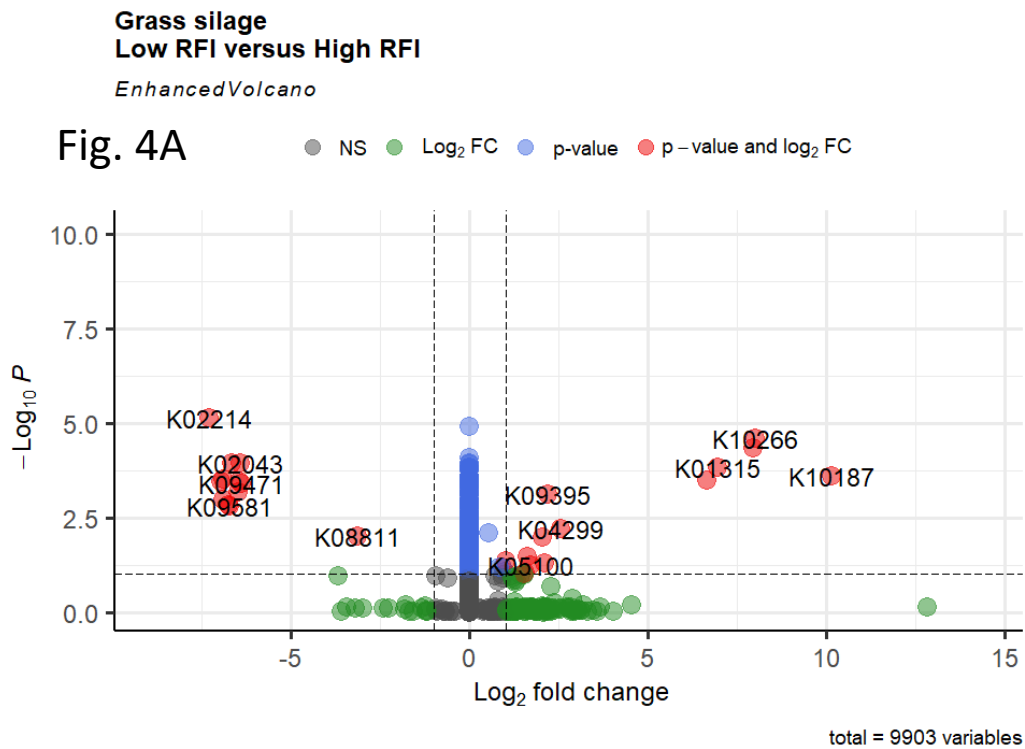
253 deKEGGs = 0.06% of the total read counts

Fig. 4B

Corn silage
Low RFI versus High RFI

EnhancedVolcano

282 deKEGGs = 0.08% of the total read counts

Previous research studies:

➢ No differences in rumen microbial ecosystem between RFI groups (<mark>with only two exceptions</mark>)

➢ No consensus in relative abundances of rumen microbiota members between RFI groups

| N° | Metadata | | | | | | | | | | Results | | | |
	Sp	Age	Stage	Breed	Sex	Diet	Sample	Method	Type of	Sampling	Region	Betadi	DA, list	DA Method	Autors
1	Bos indic	Steers	Growing	Nellore	M	Concentr	Rumen	Illumina	Slaughte	Pre_feed	V4	Not cha	LRFI: >Lachnospiraceae, Ruminococcaceae and Christensenellaceae	LDA LEfSe	Lopes et al. 2022
2	Bos tauru	Steers	Growing	Charolais	M	Concentr	Rumen	Illumina	Tubing	Post-fee	V4	Not cha	LRFI: >Mogibacteriaceae, Methanomassiliicoccaceae, Ruminobacter and Lactobaci	MaAsLin2	McGovern et al 2020
3	Bos tauru	Heifers	Finishing	Angus	F	50/50, F/	Rumen	Illumina	Slaughte	Pre_feed	V3-V4	Differed	LRFI: >Proteobacteria, Rikenellaceae, Ruminococcaceae, and Lachnospiraceae	T-Test	Liu et al 2022
4	Bos indic	Steers	Finishing	Nellore	M	Concentr	Rumen	Illumina	ND	ND	V4	Not cha	LRFI; >Lachnospiraceae, Ruminococcaceae, and Bacteroidales	Ven diagram	Lopes et al. 2019
5	Bos tauru	Steers	Growing	Angus	M	Forage	Rumen	Illumina	Tubing (li	ND	V1-V3	Not cha	LRFI: >Flavobacteriia and Fusobacteriia	RF clasification	Clemmons et al 2019
6	Bos indic	Bull	Finishing	Nellore	M	Concentr	Rumen	Illumina	Slaughte	Pre_feed	V3	Not cha	LRFI: <Christensenellaceae, Succinivibrio and Prevotella. >Rikenellaceae RC9	ANCOM and MaAsLin2	Andrade et al 2022
7	Bos tauru	Steers	Finishing	Crossbre	M	Concentr	Rumen	Illumina	Slaughte	ND	V1-V3	Not cha	HRFI: >Lachnospiraceae, Lactobacillaceae, and Veillonellaceae	LDA LEfSe	Li and Guan 2017
8	Bos tauru	Steers	Finishing	Angus	M	Concentr	Rumen	Illumina	Slaughte	Pre-feed	V4	Not cha	LRFI: >Ruminococcaceae, <Bifidobacteriaceae	ANOVA	Welch et al 2020
9	Bos tauru	Bull	Finishing	Simment	M	Concentr	Rumen	Illumina	Slaughte	Pre-feed	V4	Not cha	LRFI: >Fibrobacteres, Cyanobacteria and Tenericutes	Wilcoxon rank sum test	McGovern et al 2018

nature
COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-022-28034-z   OPEN

Microbiome differential abundance methods produce different results across 38 datasets

Jacob T. Nearing [1,7 ✉], Gavin M. Douglas[1,7], Molly G. Hayes [2], Jocelyn MacDonald[3], Dhwani K. Desai[4], Nicole Allward[5], Casey M. A. Jones[6], Robyn J. Wright[6], Akhilesh S. Dhanani [4], André M. Comeau [4] & Morgan G. I. Langille[4,6]

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Summary

1. Diet-related factors were the main drivers of rumen microbiota and function variations

2. The microbial profile slightly differed between RFI phenotypes but in diet-depend manner, suggesting an interaction between the diet and RFI

3. No differences in fermentative profile and microbiota functional profiling were observed between divergent RFI

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Future research

✓ To perform a meta-analysis-level study, as this would incorporate a larger number of animals,

in particular, a common method for analyzing differential rumen microbiota abundance

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Thanks for your attention!

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