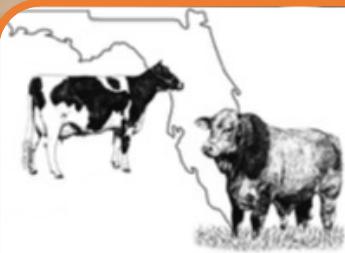


THE RUMEN MICROBIOME AND LINKS WITH THE GENOME AND PRODUCTION IN DAIRY COWS



35th Annual Meeting

UF|IFAS UNIVERSITY of FLORIDA UF | ANIMAL SCIENCES

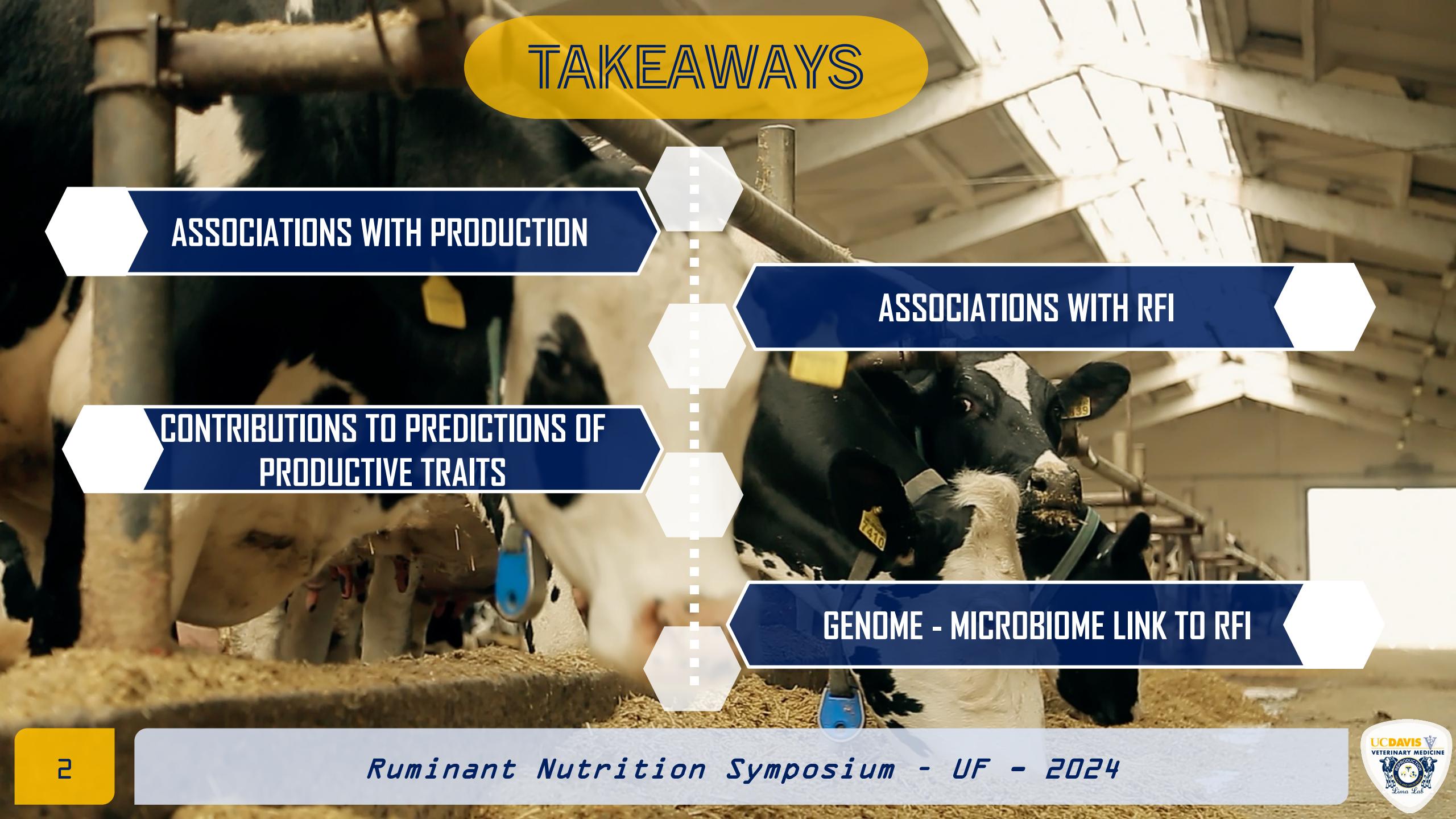
02/28/24



Fabio Lima, DVM, MS, PhD, Diplomate ACT

Assist Prof of Livestock & Theriogenology
Department of Population Health & Reproduction





TAKEAWAYS

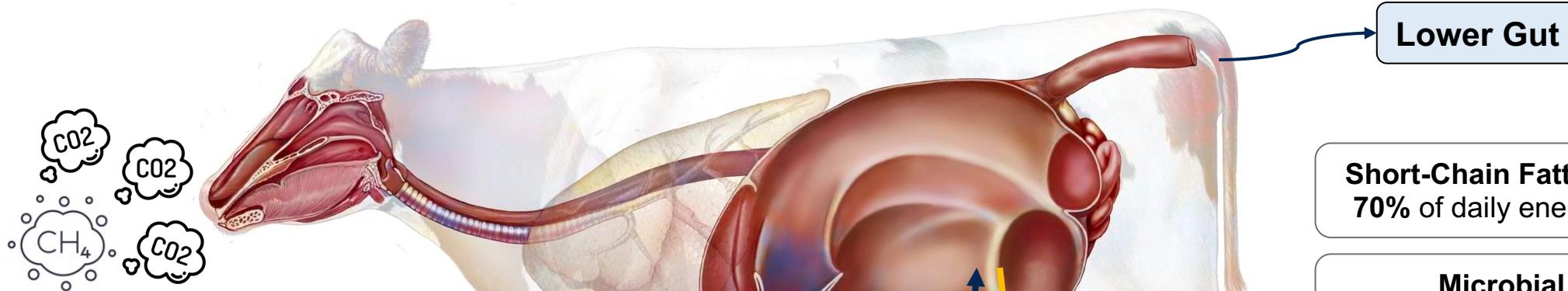
ASSOCIATIONS WITH PRODUCTION

CONTRIBUTIONS TO PREDICTIONS OF
PRODUCTIVE TRAITS

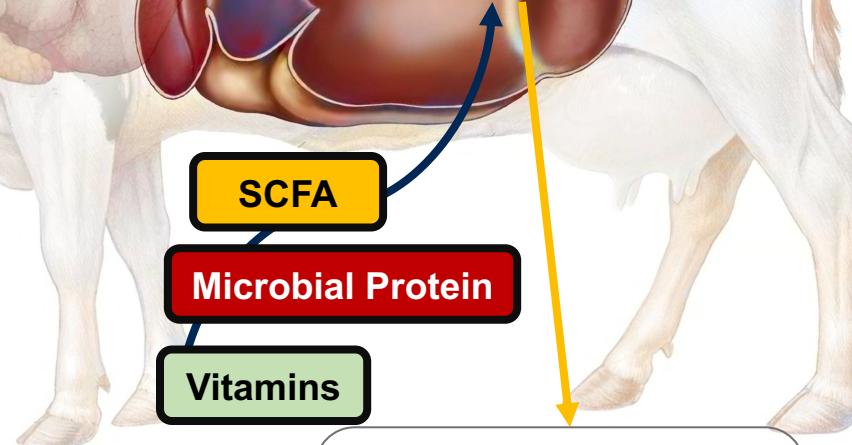
ASSOCIATIONS WITH RFI

GENOME - MICROBIOME LINK TO RFI

ENTERIC FERMENTATION IN RUMINANTS



CATTLE RELY ON FERMENTATION TO BREAK DOWN COMPLEX NUTRIENTS AND UTILIZE THAT ENERGY FOR MILK AND MILK COMPONENTS

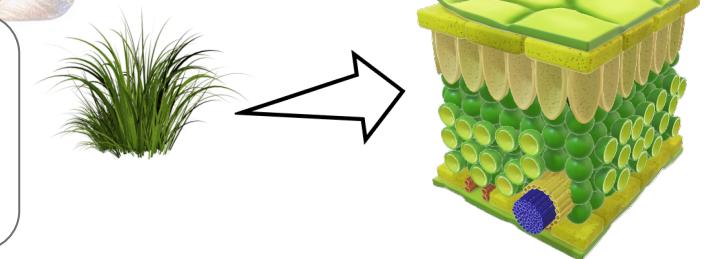


Bacteria (1×10^{11} cells/mL)
Archaea (1×10^9)
Protozoa (1×10^6)
Fungi (1×10^5)
Viruses (1×10^9)

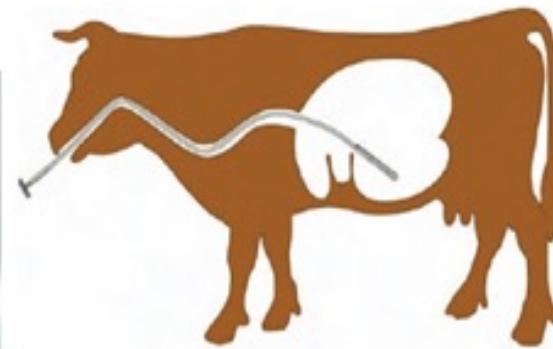
Van Soest (1994)

Short-Chain Fatty Acids (SCFA)
70% of daily energy requirement

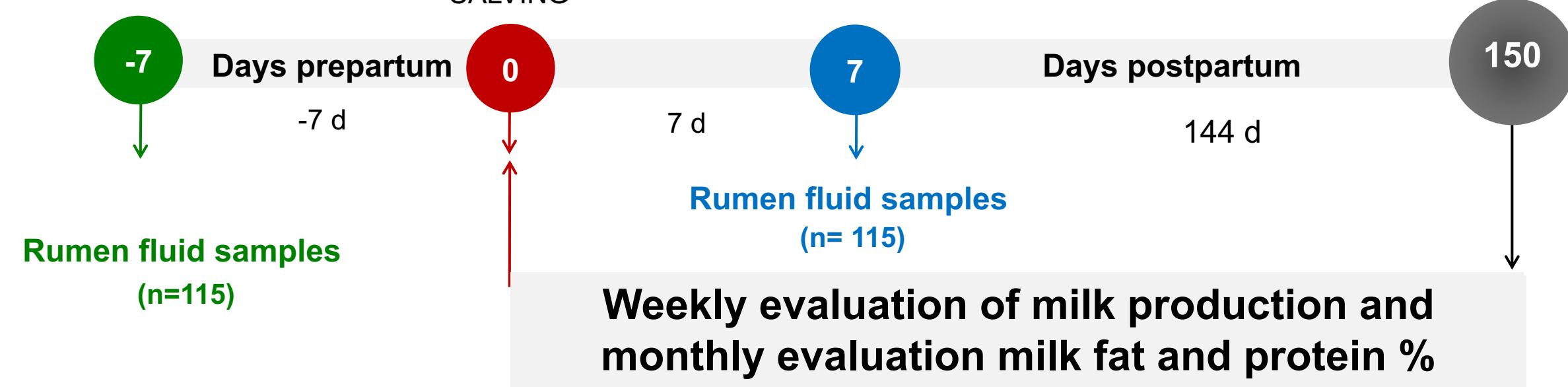
Microbial Protein
60% of daily protein requirement



EXPERIMENTAL DESIGN

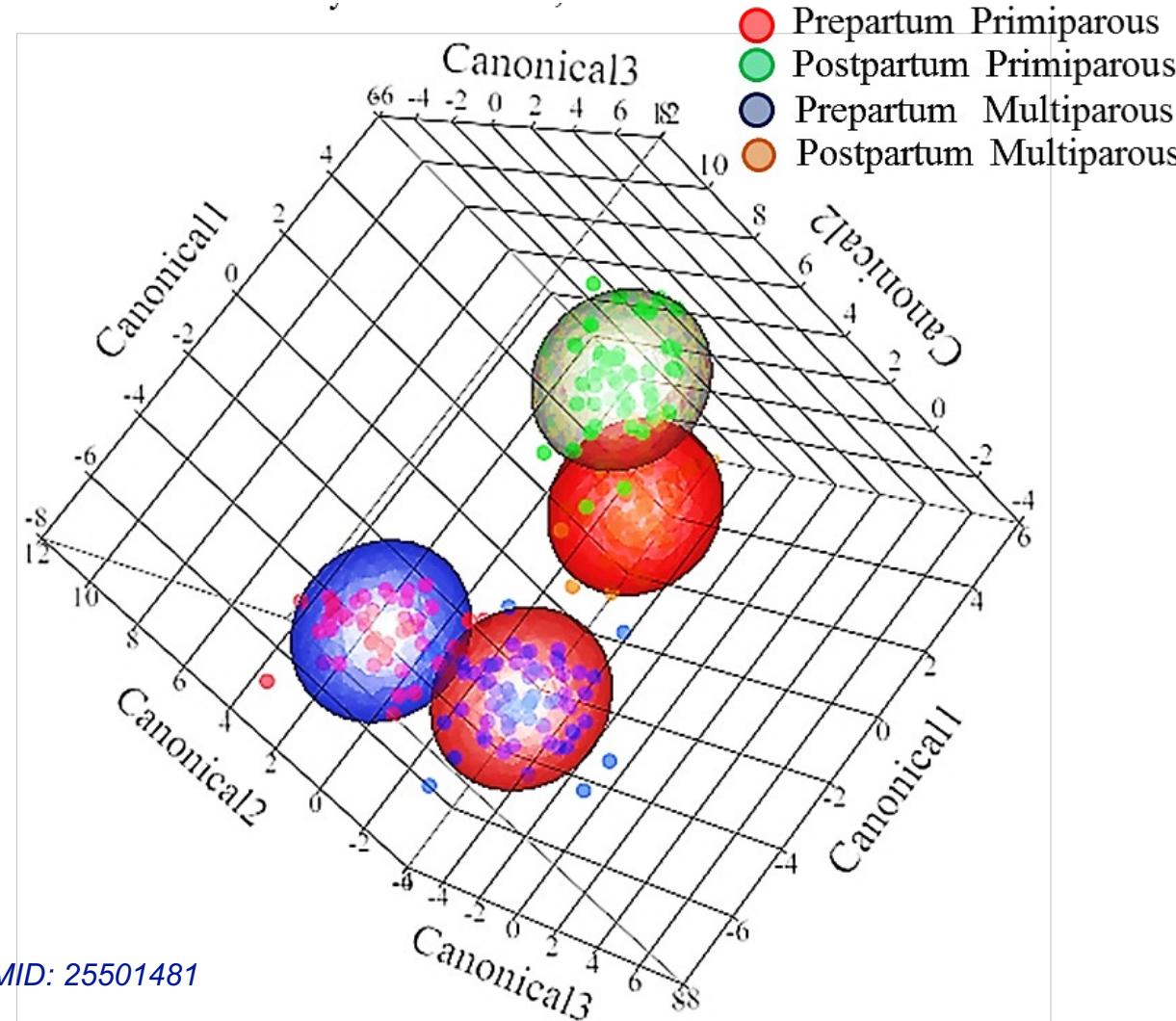


CALVING



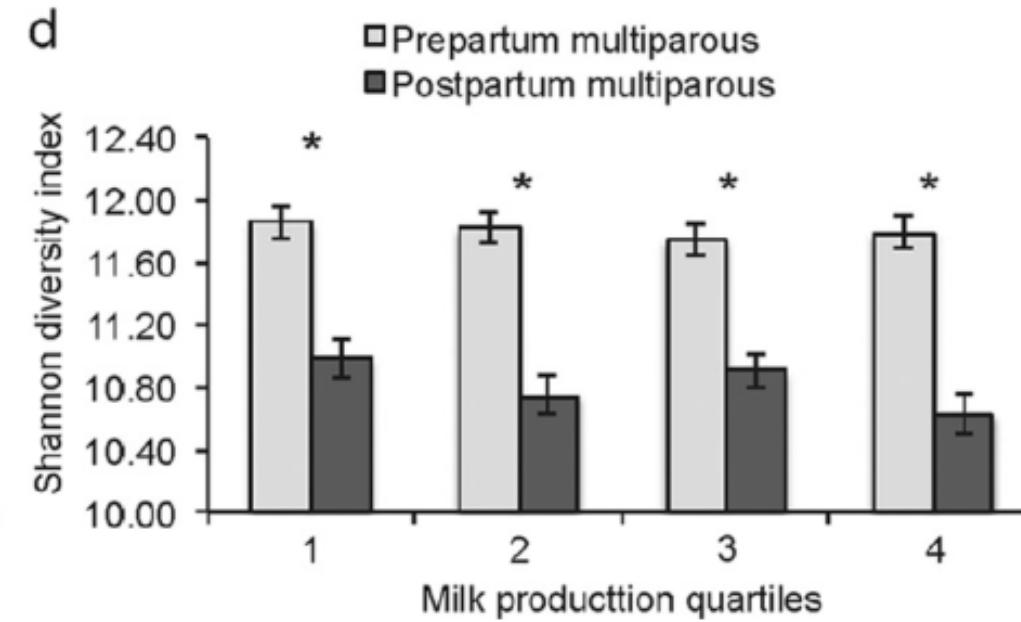
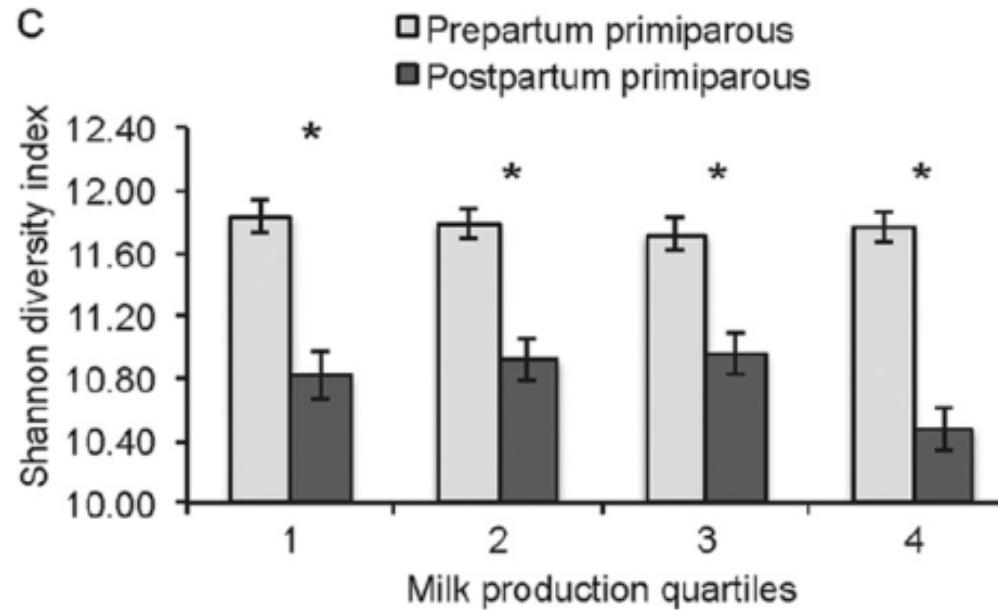
Lima et al., 2015. *Appl Environ Microbiol.* PMID: 25501481

PARITY AND TIME RELATIVE TO CALVING: ≠ RUMEN MICROBIOME



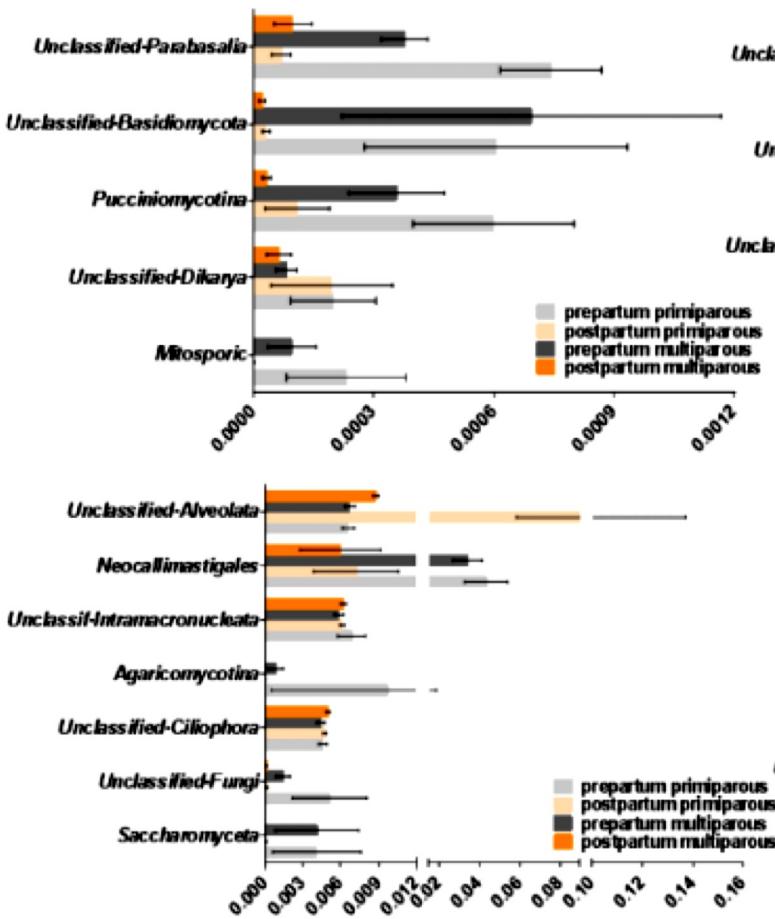
Lima et al., 2015. *Appl Environ Microbiol.* PMID: 25501481

PREPARTUM MICROBIOME – HIGHER DIVERSITY

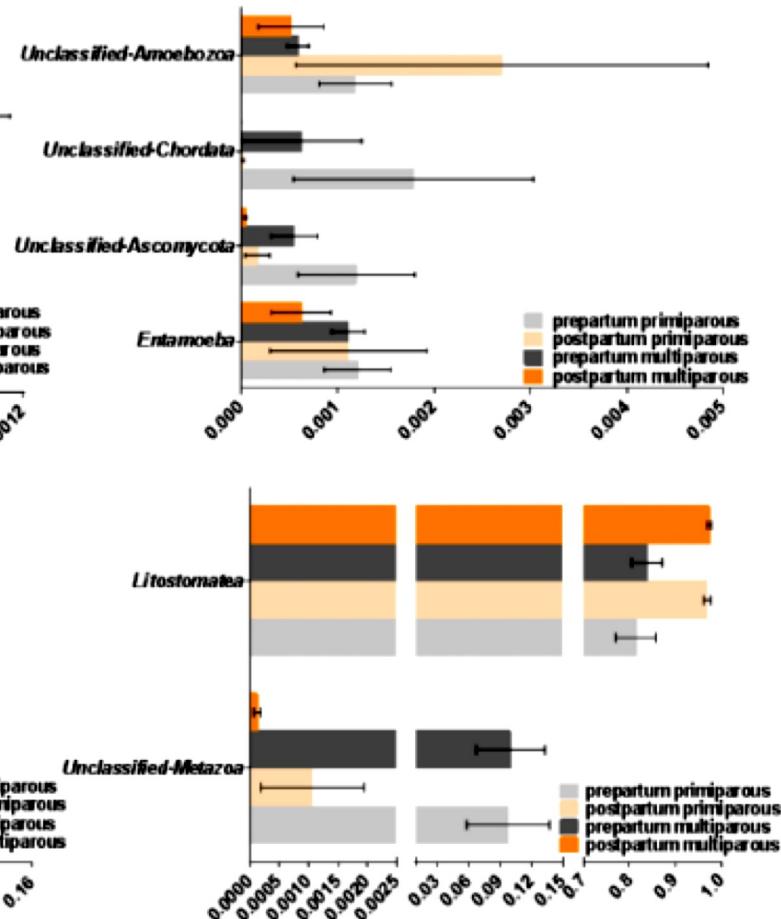


Lima et al., 2015. *Appl Environ Microbiol.* PMID: 25501481

FUNGI: ↑ PREPARTUM



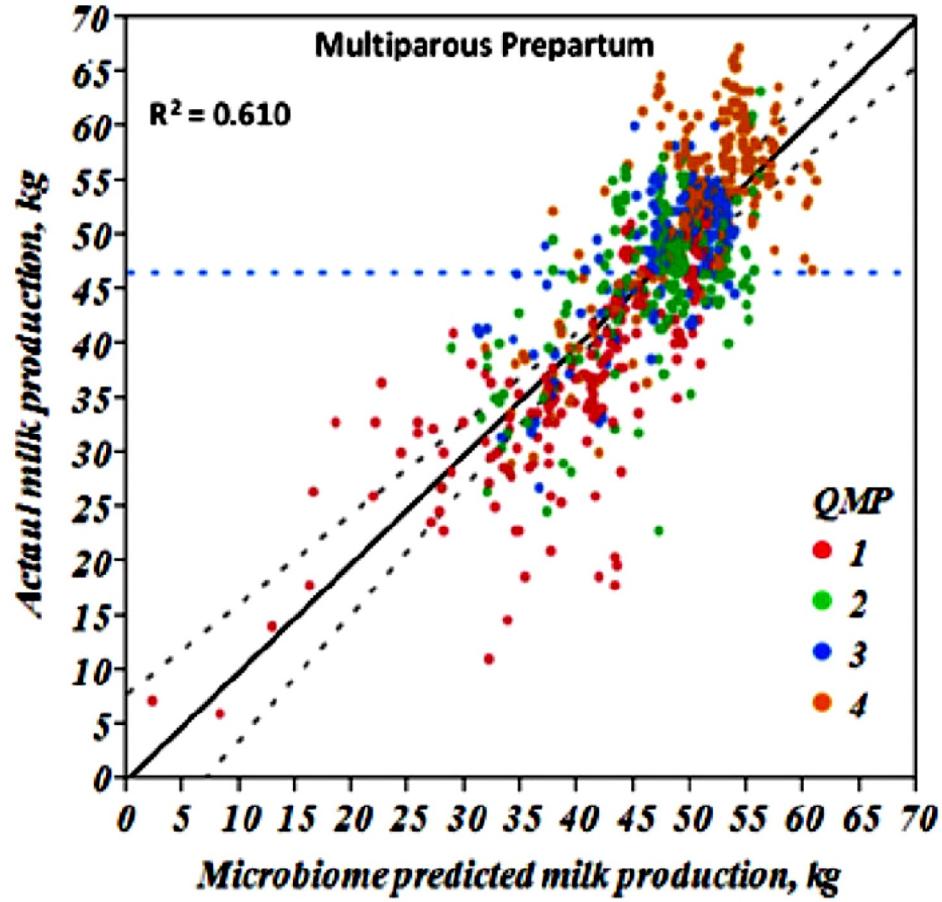
PROTOZOA: ↑ POSTPARTUM



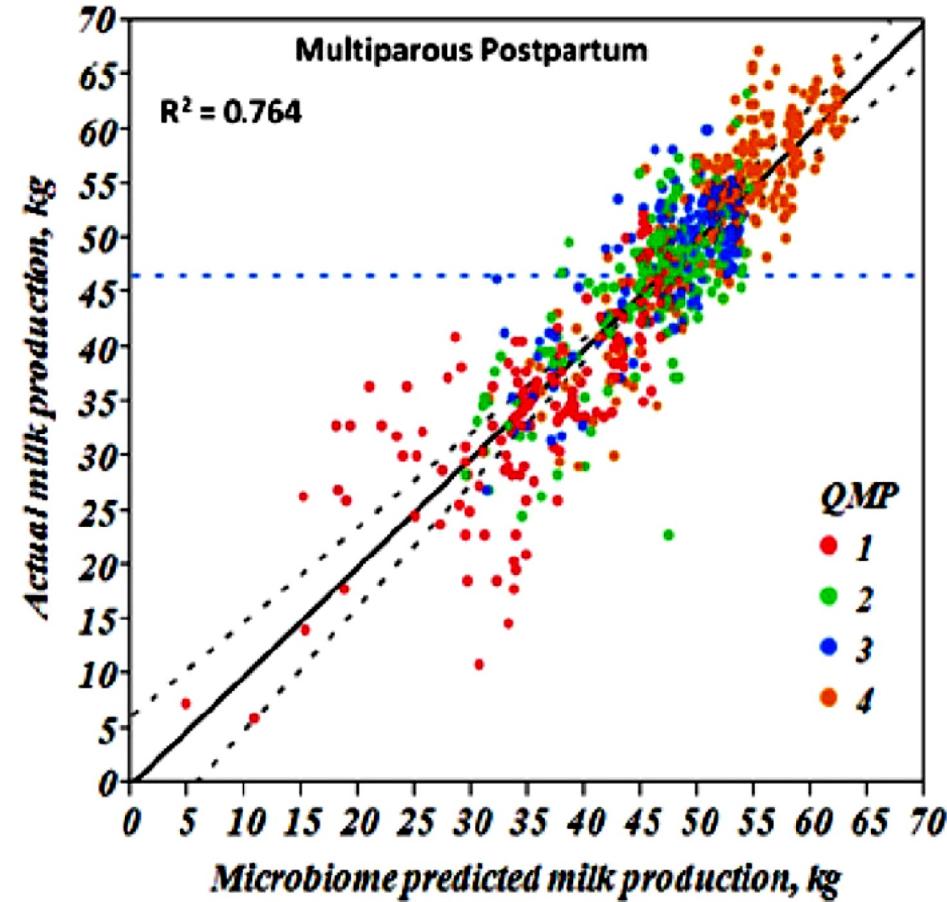
Lima et al., 2015. Appl Environ Microbiol. PMID: 25501481

MICROBIOME PREDICTED AND ACTUAL MILK PRODUCTION

C



d



Lima et al., 2015. Appl Environ Microbiol. PMID: 25501481

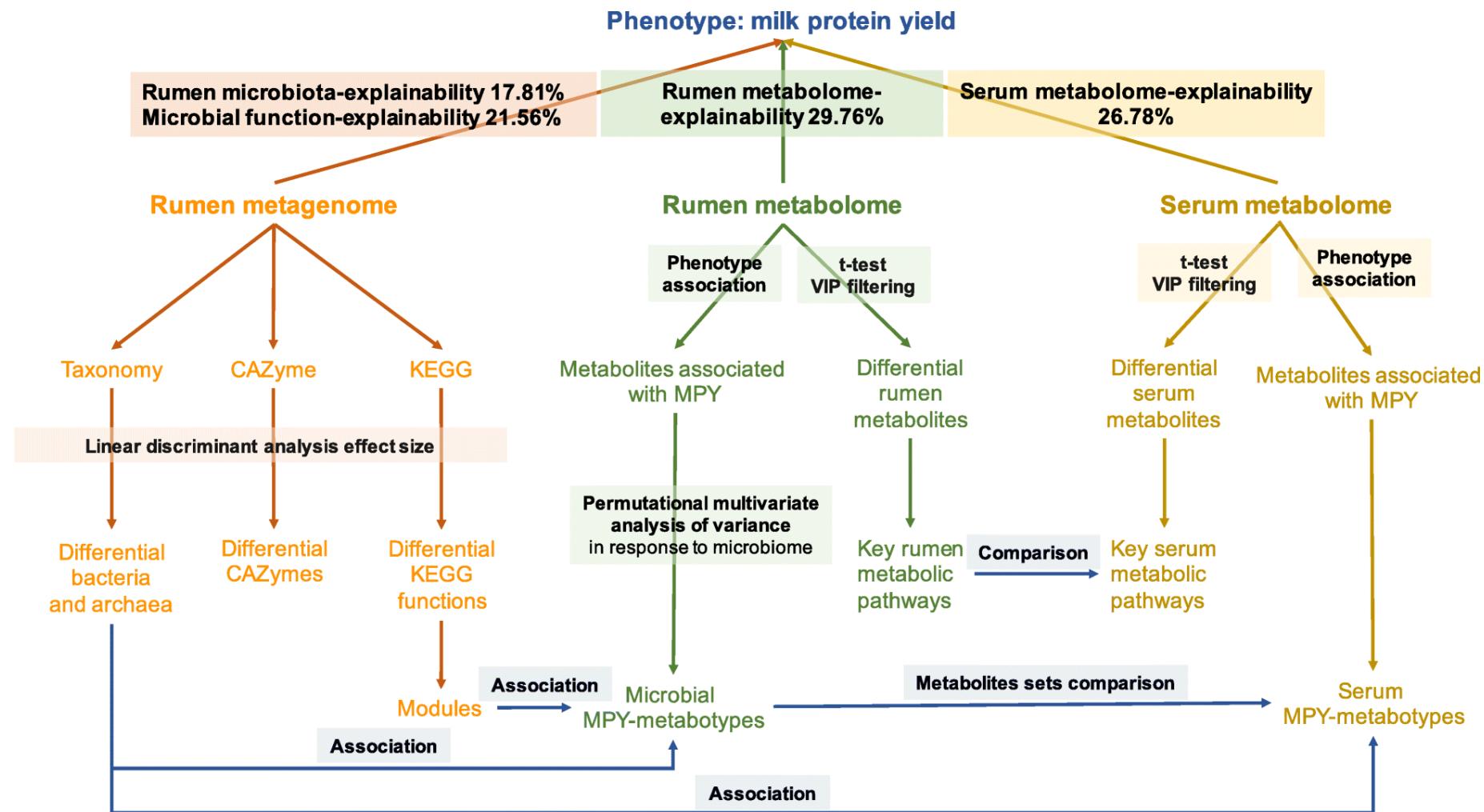
Deltaproteobacteria, *Faecalibacterium* and *Virgibacillus* Prevotellaceae, Micrococcaceae and Butyrivibrio

CONCLUDING REMARKS

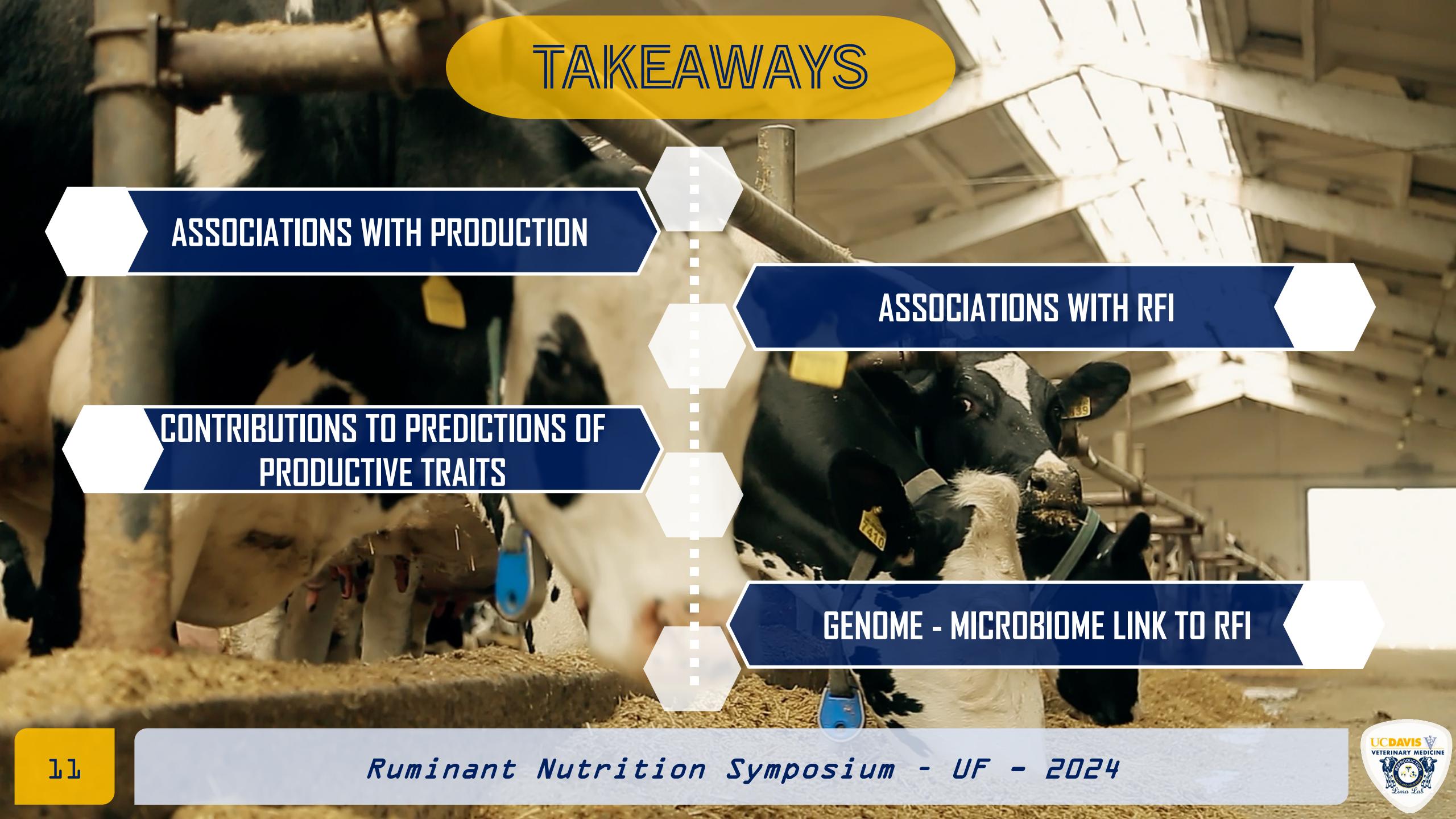
- ▶ Pre and postpartum microbiome: different prevalence of classic cellulolytic and amylolytic bacteria
- ▶ Prepartum = increased prevalence of fungi associated with cellulose digestion
- ▶ Postpartum = increased prevalence of protozoa associated with starch digestion
- ▶ Rumen microbiome model had a high goodness of fit of the regression models for milk production

Lima et al., 2015. Appl Environ Microbiol. PMID: 25501481

HOW DOES THE MICROBIOME CONTRIBUTE TO MILK PRODUCTION EFFICIENCY?



Xue et al., 2020. *Microbiome*. PMID: 32398126



TAKEAWAYS

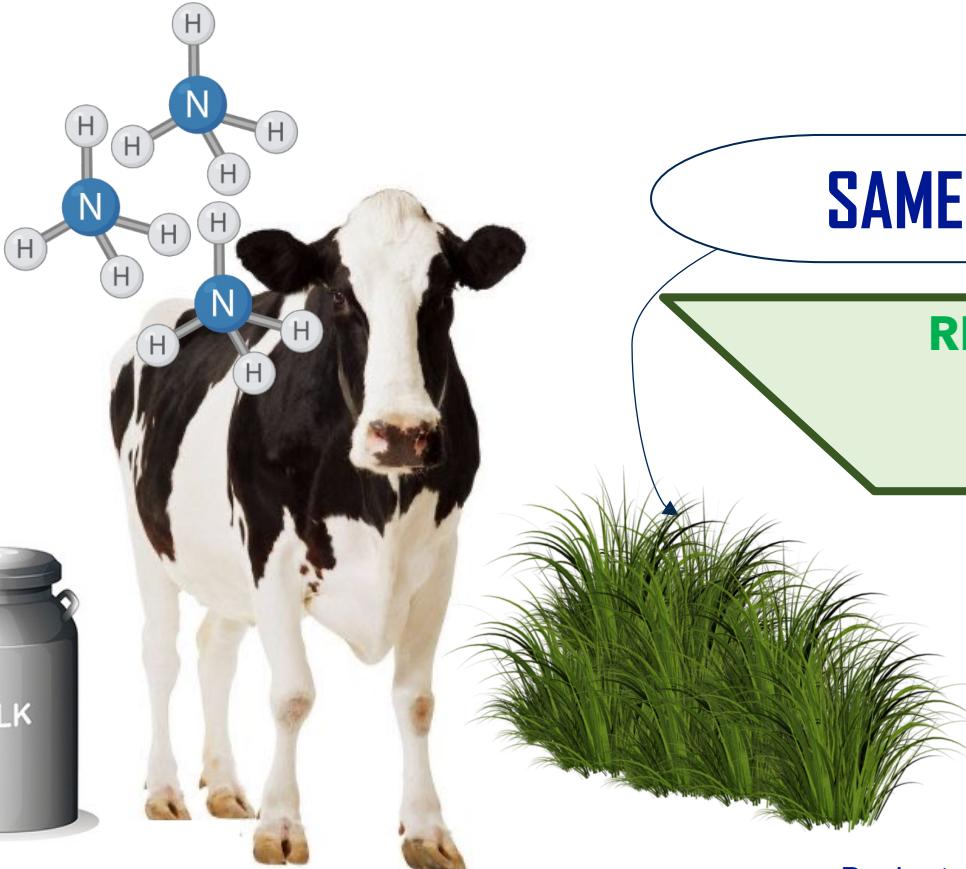
ASSOCIATIONS WITH PRODUCTION

CONTRIBUTIONS TO PREDICTIONS OF
PRODUCTIVE TRAITS

ASSOCIATIONS WITH RFI

GENOME - MICROBIOME LINK TO RFI

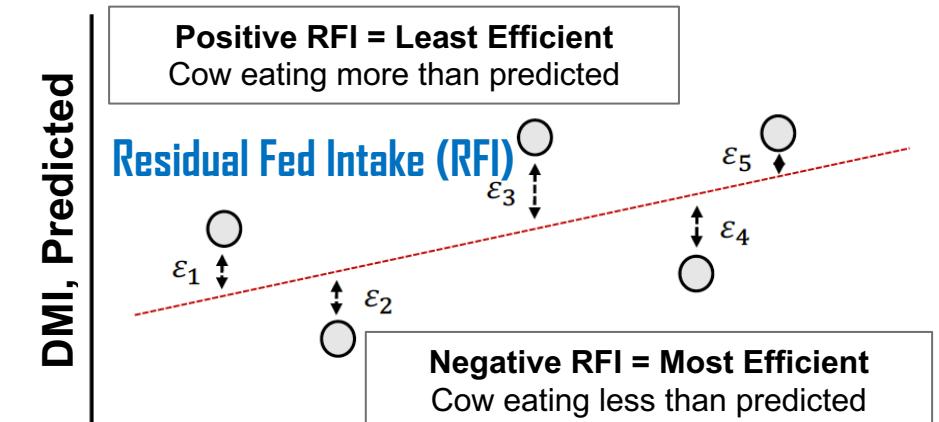
RUMEN MICROBIOME RESILIENCE AND ASSOCIATION WITH FEED EFFICIENCY



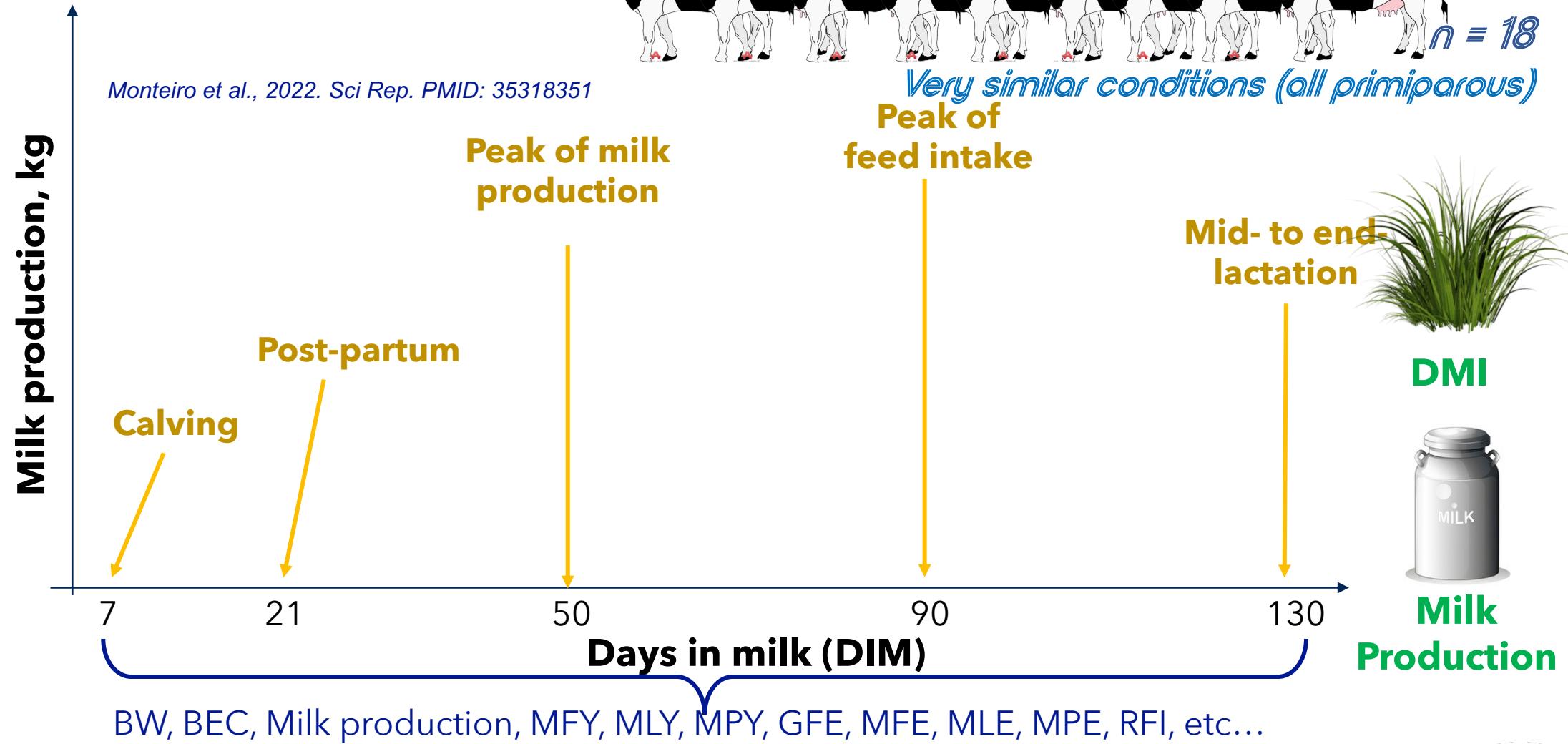
SAME LEVEL OF PRODUCTION

RFI in the U.S. Holstein
Heritability = 0.14
Reliability = 0.24

Bach et al. (2020); Connor et al. (2013); Freetly et al. (2020)

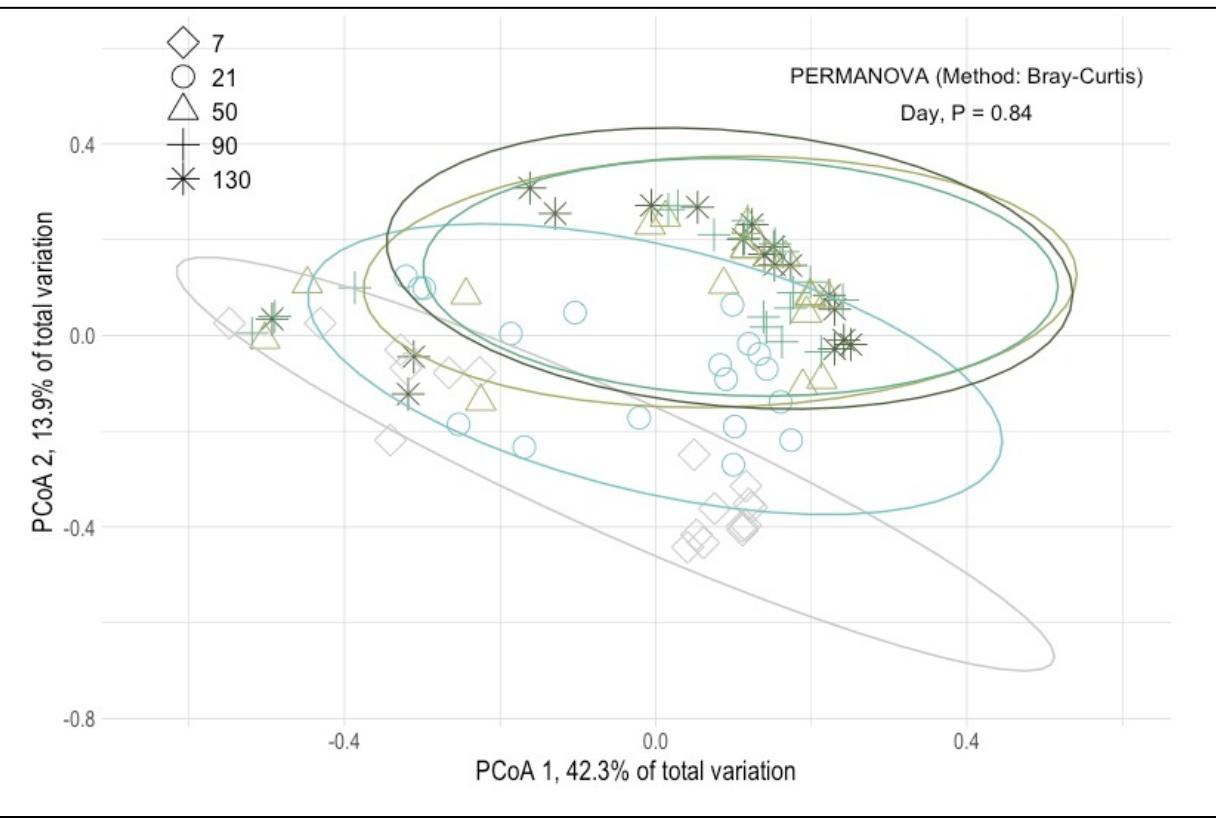


EXPERIMENTAL DESIGN

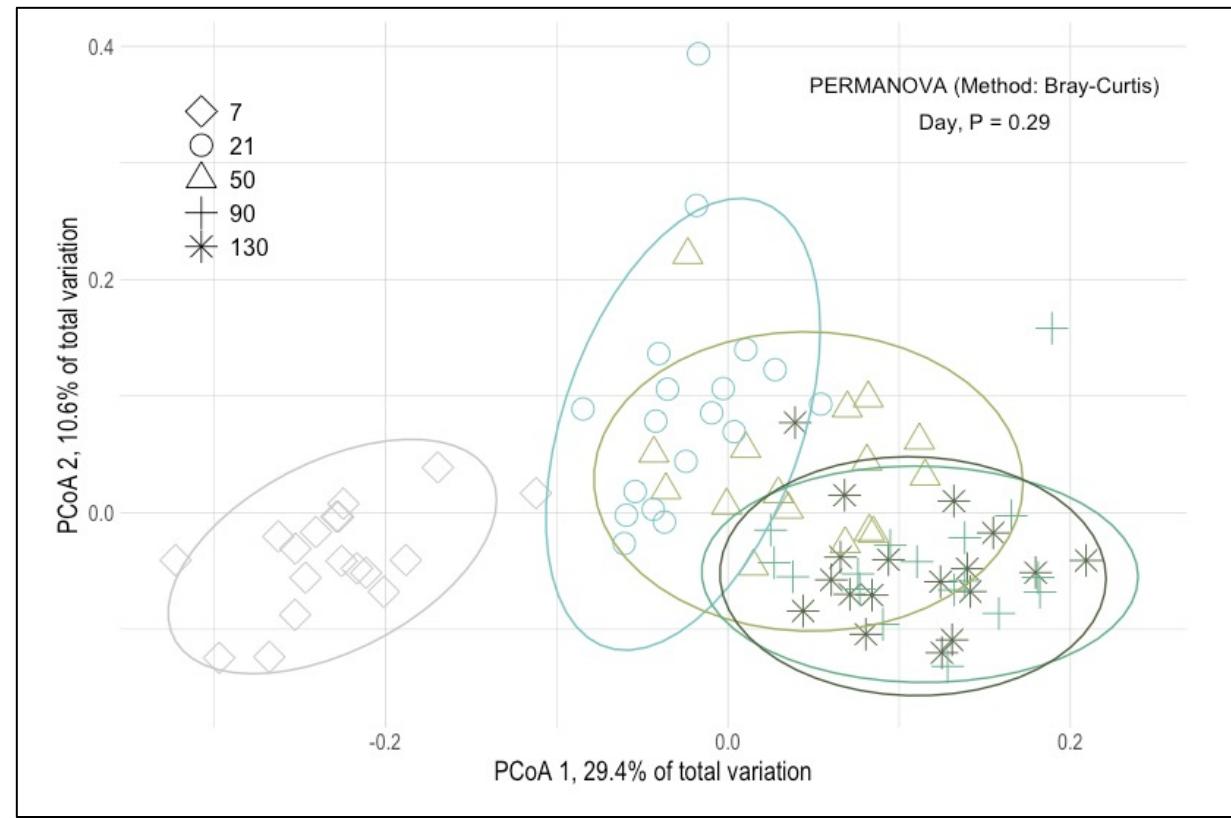


SAMPLING EFFECT

RUMEN MICROBIOME



LOWER GUT MICROBIOME



*No interaction with **DAY** was detected for the remaining variables

Monteiro et al., 2022. Sci Rep. PMID: 35318351

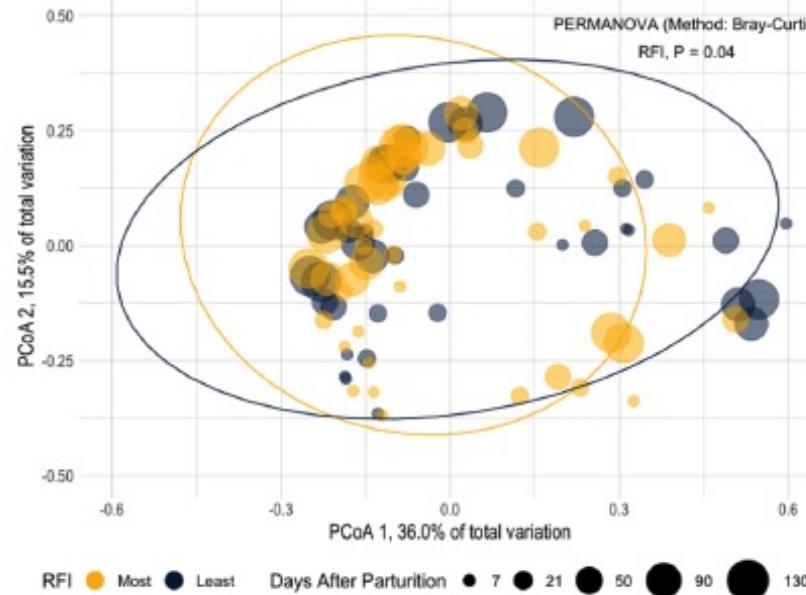
RESULTS – PERMANOVA CORRECTED FOR DMI

Item	P-Values	
	Rumen	Lower Gut
Day	0.84	0.29
DMI, kg/d	< 0.01	< 0.001
Milk production, kg/day		
ECM	< 0.01	< 0.001
Milk fat	< 0.001	< 0.01
Milk lactose	< 0.001	< 0.01
Milk protein	< 0.001	< 0.001
Feed efficiency		
Residual feed intake, RFI	0.04	0.04
<i>RFI variables</i>		
MBW, kg	< 0.001	< 0.001
BEC, Mcal/d	< 0.001	0.26
NESec, Mcal/d	< 0.01	< 0.001
<i>RFI variables, unit/kg DMI</i>		
MBW	0.01	0.19
BEC	0.03	0.01
NESec	< 0.001	< 0.001
Production efficiency, kg/kg DMI		
Energy-corrected milk, a.k.a. GFE	0.18	< 0.01
Milk fat	0.12	0.03
Milk lactose	0.30	0.19
Milk protein	0.49	< 0.01

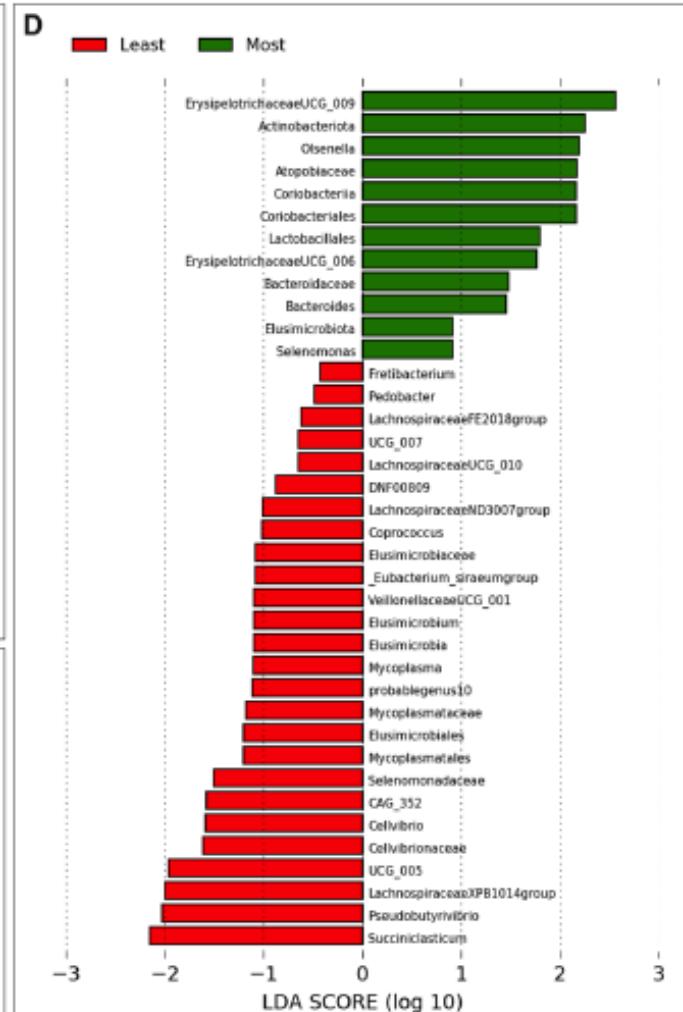
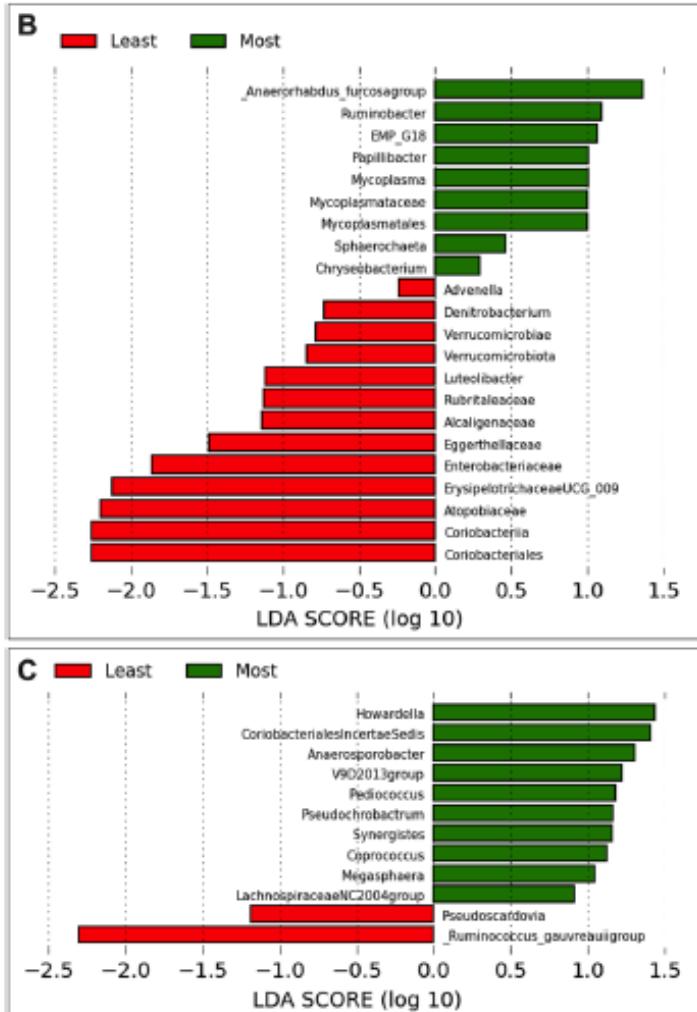
PCOA, PERMANOVA & LEFSE FOR MBW, BEC, & NESEC

Residual Feed Intake (RFI) - Rumen Microbiome

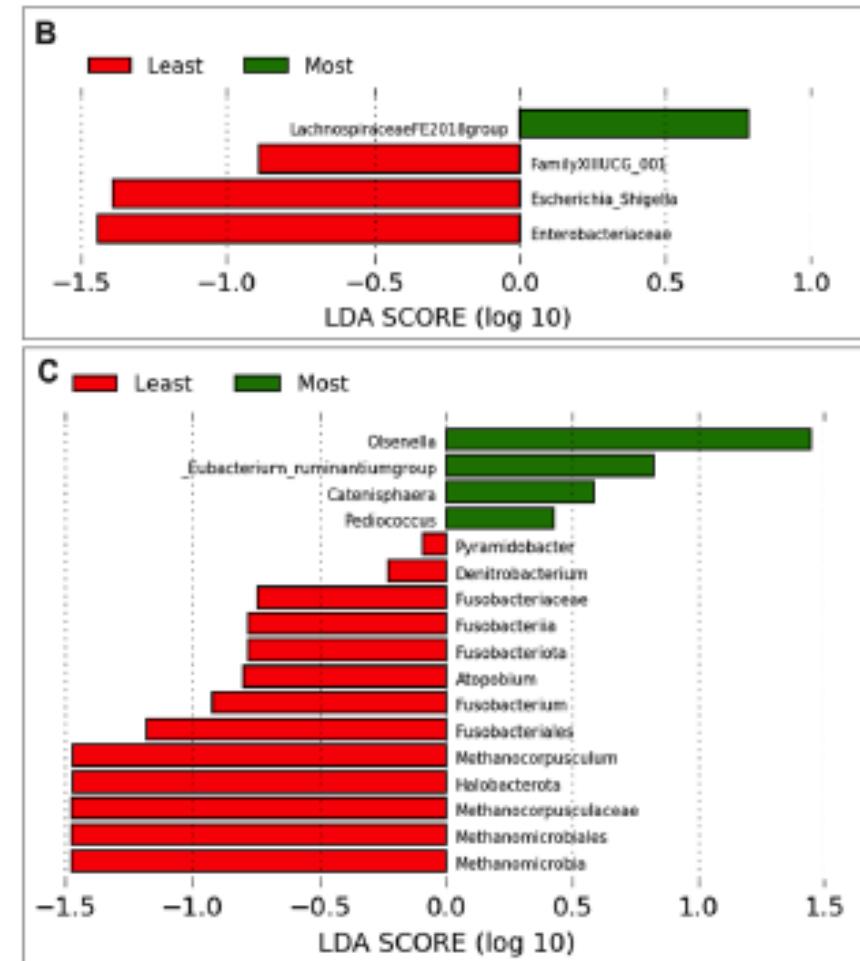
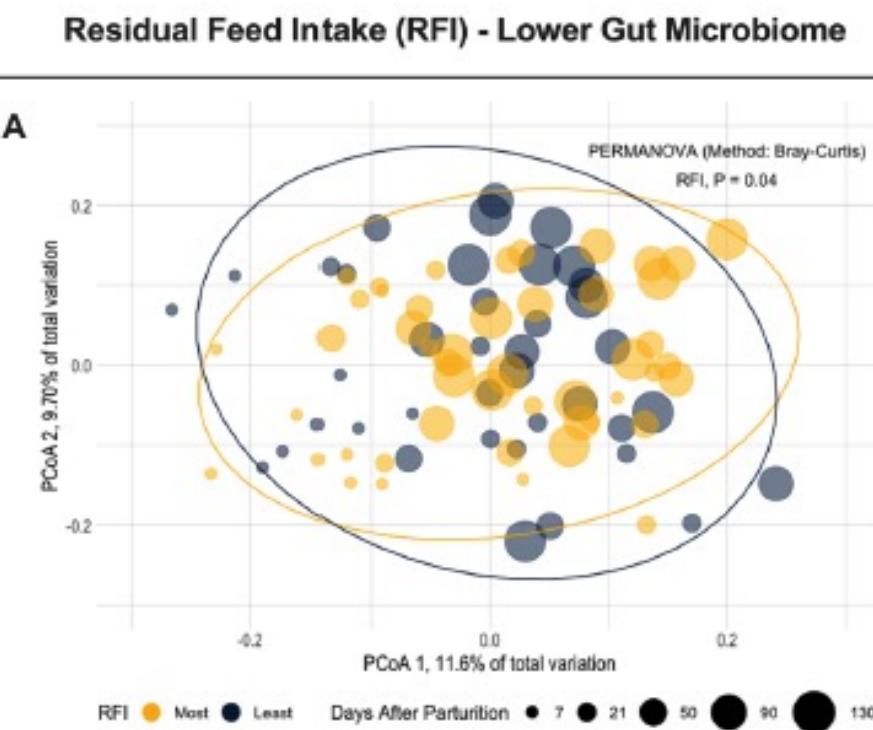
A



Monteiro et al., 2022. Sci Rep. PMID: 35318351

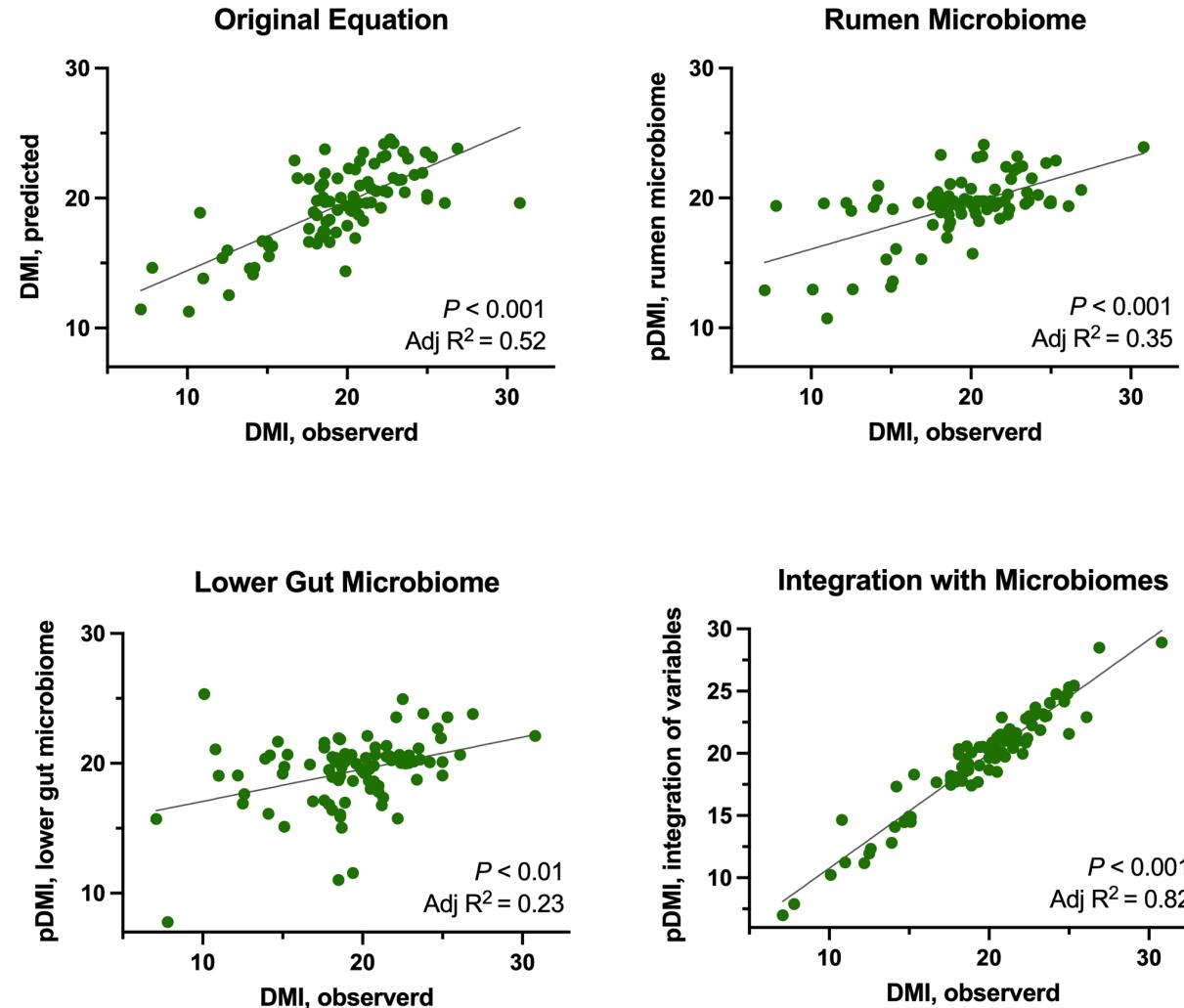


PCOA, PERMANOVA & LEFSE FOR BEC, & NESEC

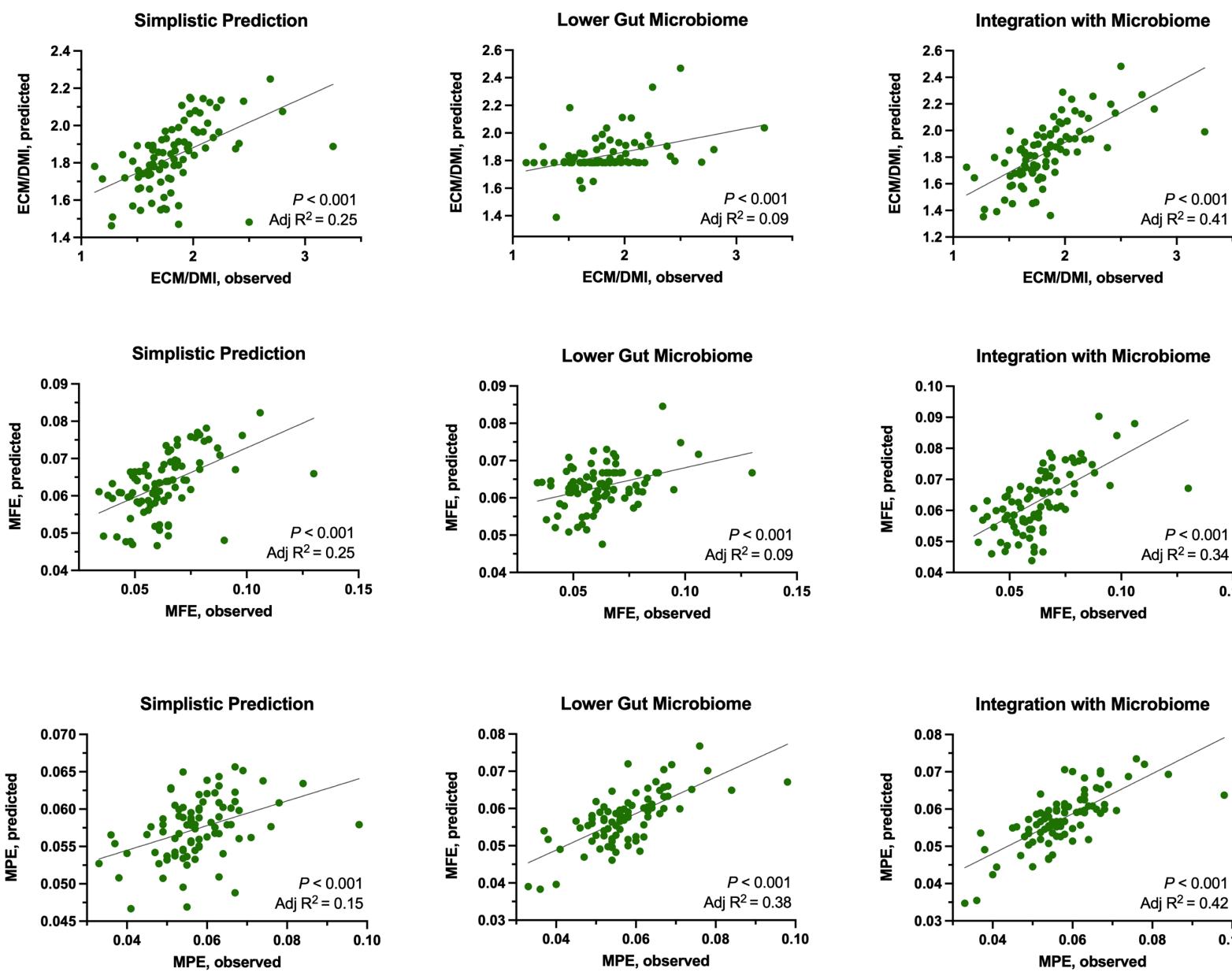


Monteiro et al., 2022. Sci Rep. PMID: 35318351

CORRELATION OF RUMEN & LOWER GUT MICROBIOME WITH DMI



Monteiro et al., 2022. Sci Rep. PMID: 35318351



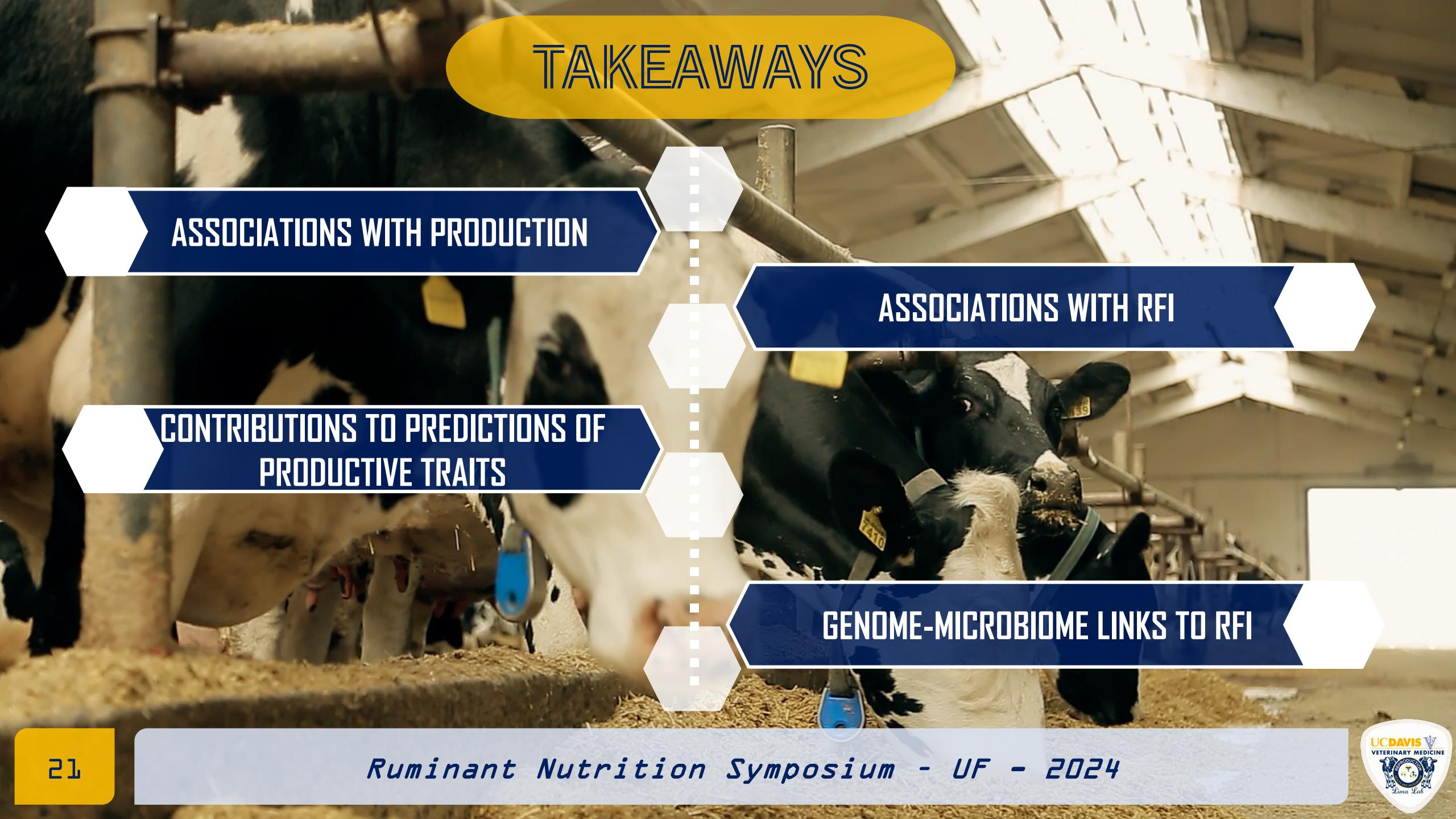
CORRELATION OF RUMEN & LOWER GUT MICROBIOME WITH DMI

Monteiro et al., 2022. Sci Rep. PMID: 35318351

CONCLUDING REMARKS

- ▶ The microbiome from both locations has temporal stability throughout lactation.
- ▶ Yet factors such as feed intake levels significantly shape microbiome diversity.
- ▶ The composition of the rumen microbiome was dependent on feed intake.
- ▶ In contrast, the lower gut microbiome was less dependent on feed intake and associated with a potentially enhanced ability to digest dietary nutrients.
- ▶ Therefore, milk production traits may correlate more with microorganisms in the lower gut than previously expected.

Monteiro et al., 2022. *Sci Rep.* PMID: 35318351



TAKEAWAYS

ASSOCIATIONS WITH PRODUCTION

CONTRIBUTIONS TO PREDICTIONS OF
PRODUCTIVE TRAITS

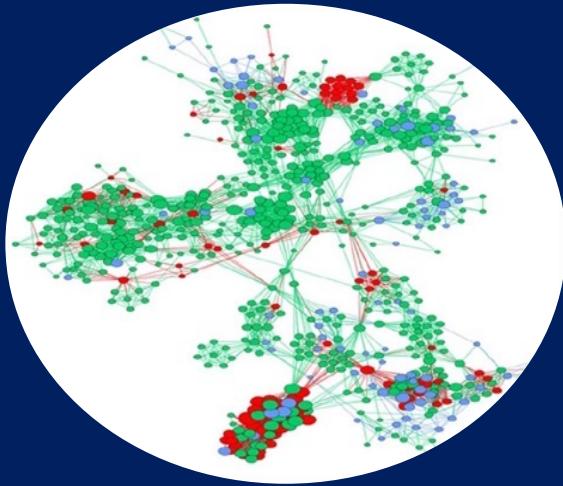
ASSOCIATIONS WITH RFI

GENOME-MICROBIOME LINKS TO RFI

SOURCES OF VARIATION FOR FEED AND MILK PRODUCTION EFFICIENCY



Animal Genetics



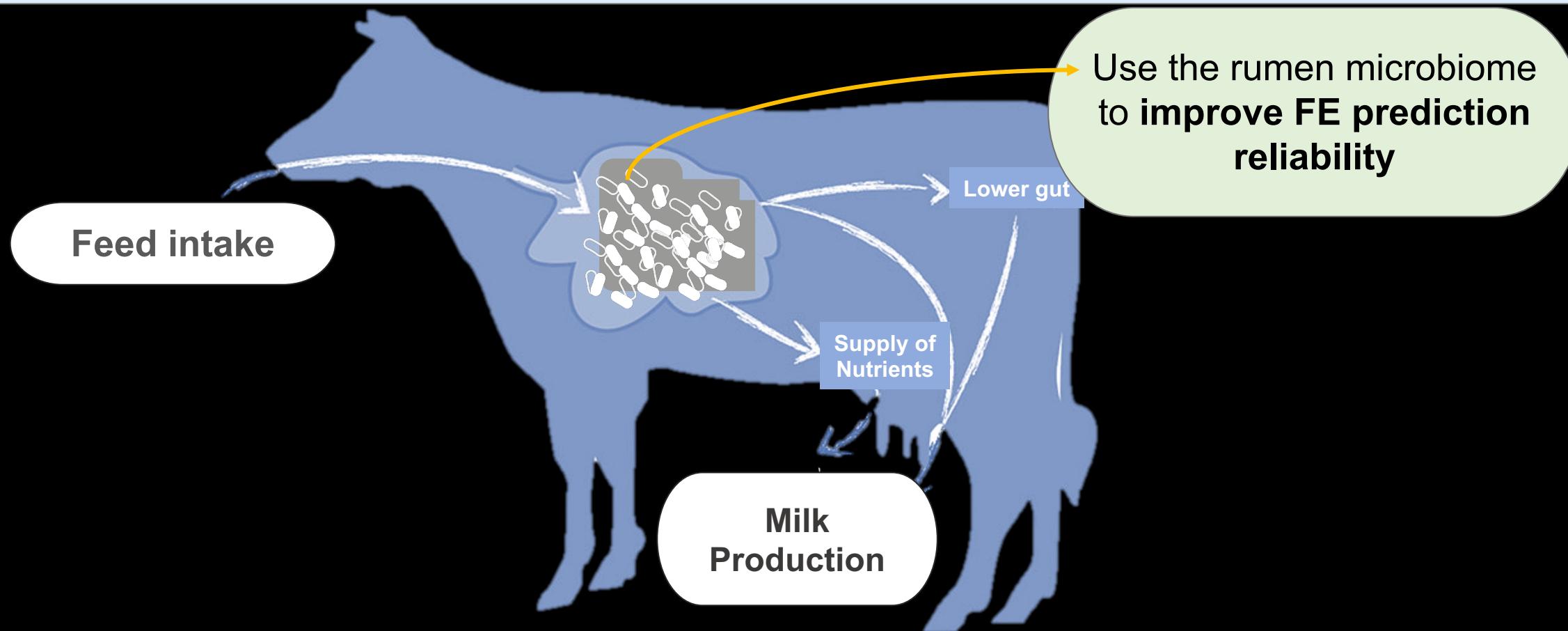
**Gastrointestinal
Fermentation**



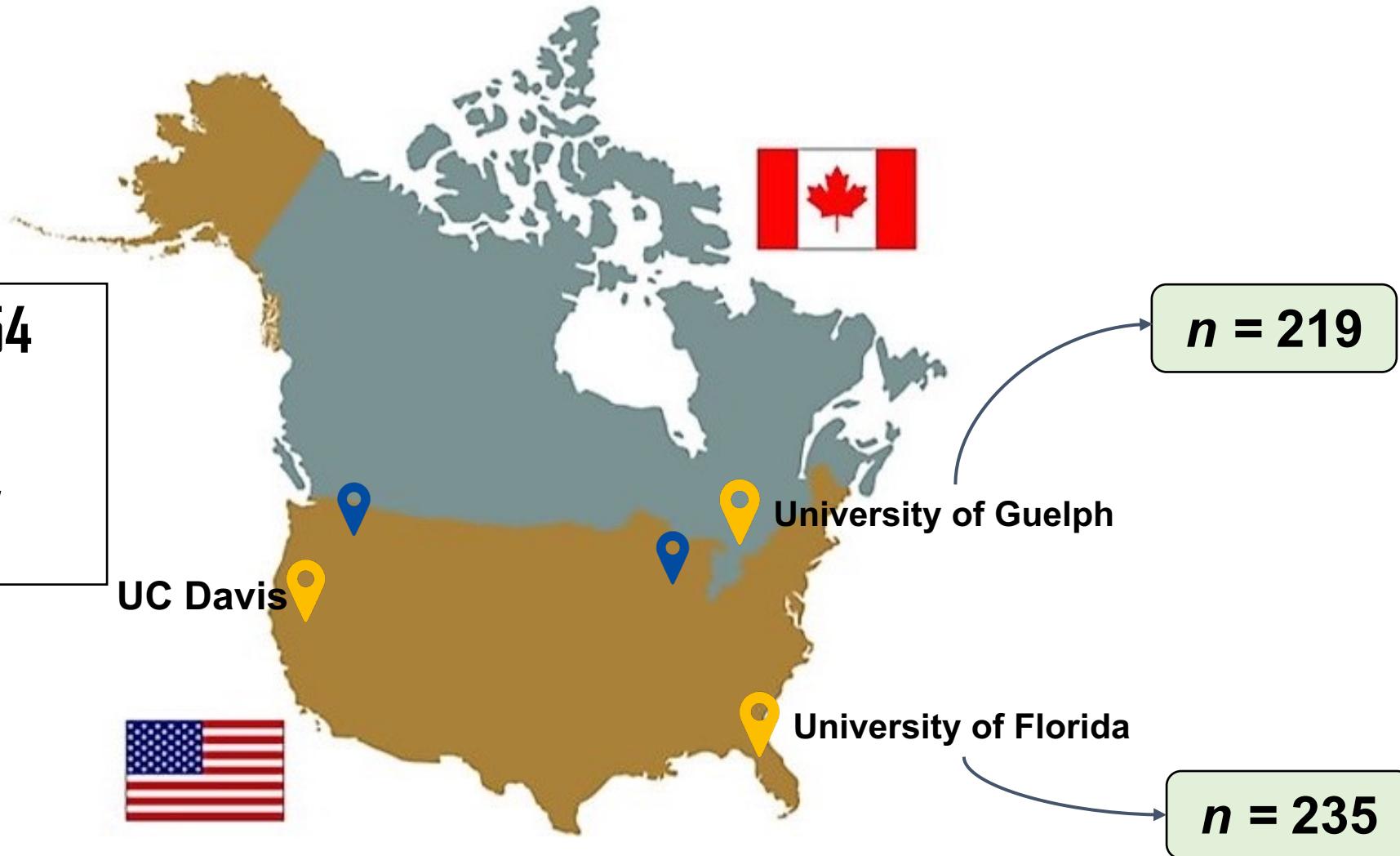
Management

HYPOTHESIS

The rumen microbiome plays a major role in feed efficiency variation and can be a path to identify highly feed-efficient dairy cows.



COLLABORATION US - CANADA



Artificial intelligence opportunities

MULTICOLLINEARITY

Monteiro et al. *Animal Microbiome* (2024) 6:5
<https://doi.org/10.1186/s42523-024-00289-5>

Animal Microbiome

RESEARCH

Open Access

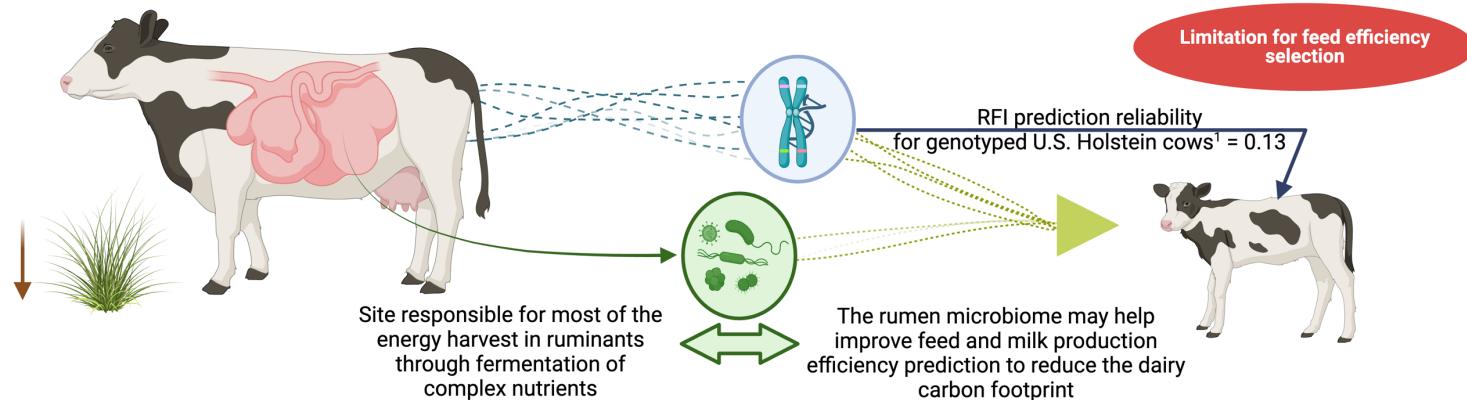


An artificial intelligence approach of feature engineering and ensemble methods depicts the rumen microbiome contribution to feed efficiency in dairy cows

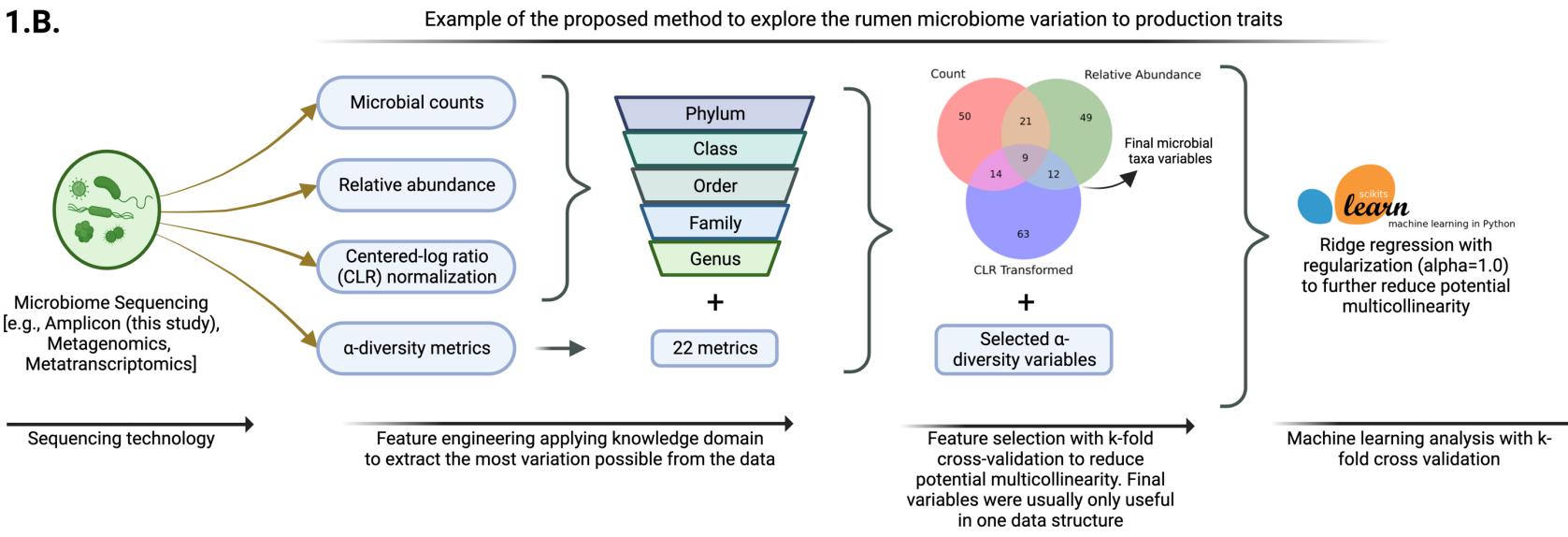
Hugo F. Monteiro¹, Caio C. Figueiredo^{2,3}, Bruna Mion⁴, José Eduardo P. Santos⁵, Rafael S. Bisinotto³, Francisco Peñagaricano⁶, Eduardo S. Ribeiro⁴, Mariana N. Marinho⁵, Roney Zimpel⁵, Ana Carolina da Silva⁵, Adeoye Oyebade⁵, Richard R. Lobo⁵, Wilson M. Coelho Jr¹, Phillip M. G. Peixoto³, Maria B. Ugarte Marin³, Sebastian G. Umaña-Sedó³, Tomás D. G. Rojas³, Modesto Elvir-Hernandez³, Flávio S. Schenkel⁴, Bart C. Weimer¹, C. Titus Brown¹, Ermias Kebreab⁷ and Fábio S. Lima^{1*}

HIDDEN PATTERNS IN COMPOSITIONAL DATA

1.A.



1.B.



Monteiro et al., 2024. *Animal Microbiome*. 6:5 PMID:38321581

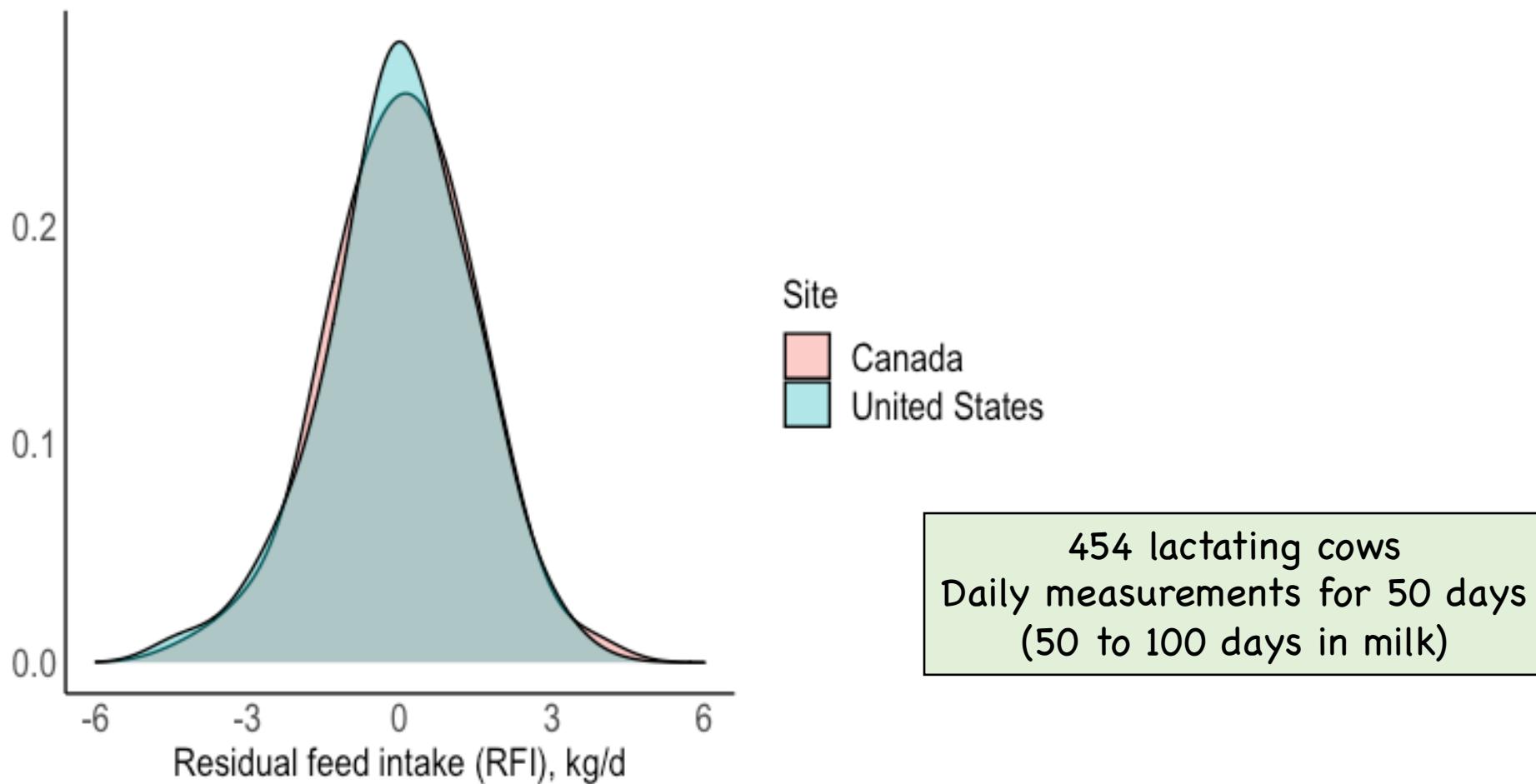
PREDICTING DRY MATTER INTAKE (DMI)

Table 1. Results from a mixed model based on Type 3 sum of squares for dry matter intake and gross milk production efficiency traits in 454 lactating Holstein cows in the US and Canada

Item	R ²	Estimate	SE ¹	P-value
Dry matter intake, kg/d				
<i>Parity</i> ²	0.02	0.87	0.22	< 0.001
<i>MBW, kg</i>	0.12	0.09	0.01	< 0.001
<i>BEC, Mcal/d</i>	0.05	0.17	0.02	< 0.001
<i>NESec, Mcal/d</i>	0.39	0.37	0.02	< 0.001
<i>Treatment (random effect)</i>	0.07			
<i>Residual (residual feed intake; RFI)</i>	0.36			

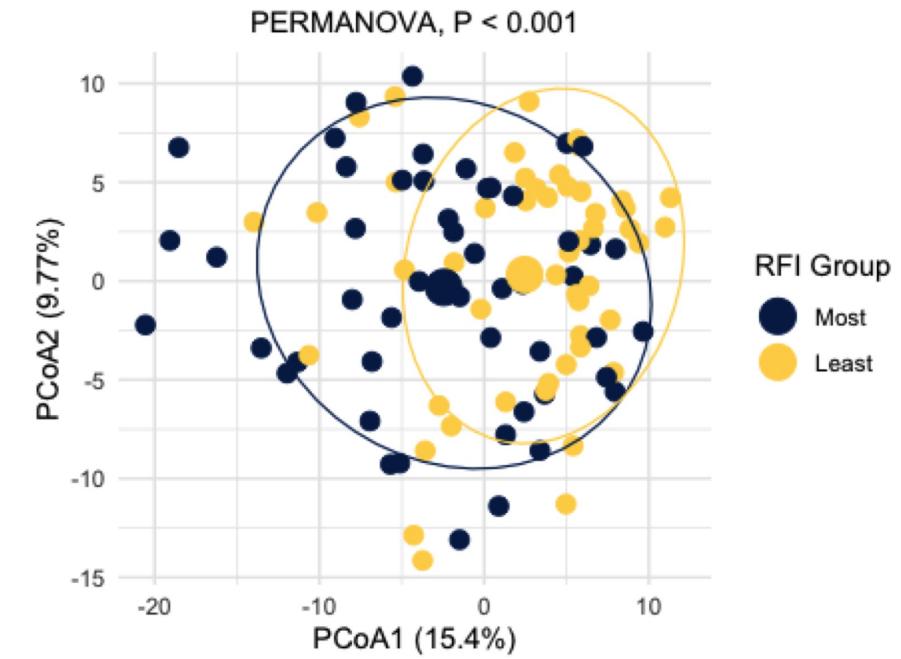
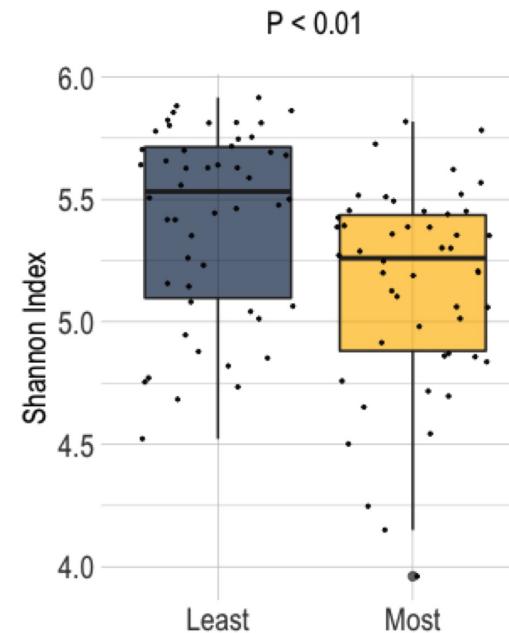
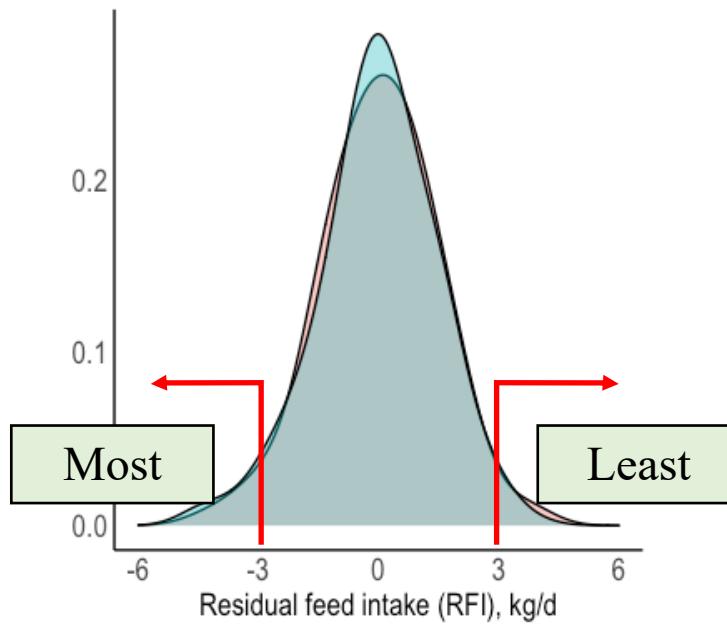
Monteiro et al., 2024. *Animal Microbiome*. 6:5 PMID:38321581

DISTRIBUTION OF RFI IN THE STUDIED POPULATION



Monteiro et al., 2024. *Animal Microbiome*. 6:5 PMID:38321581

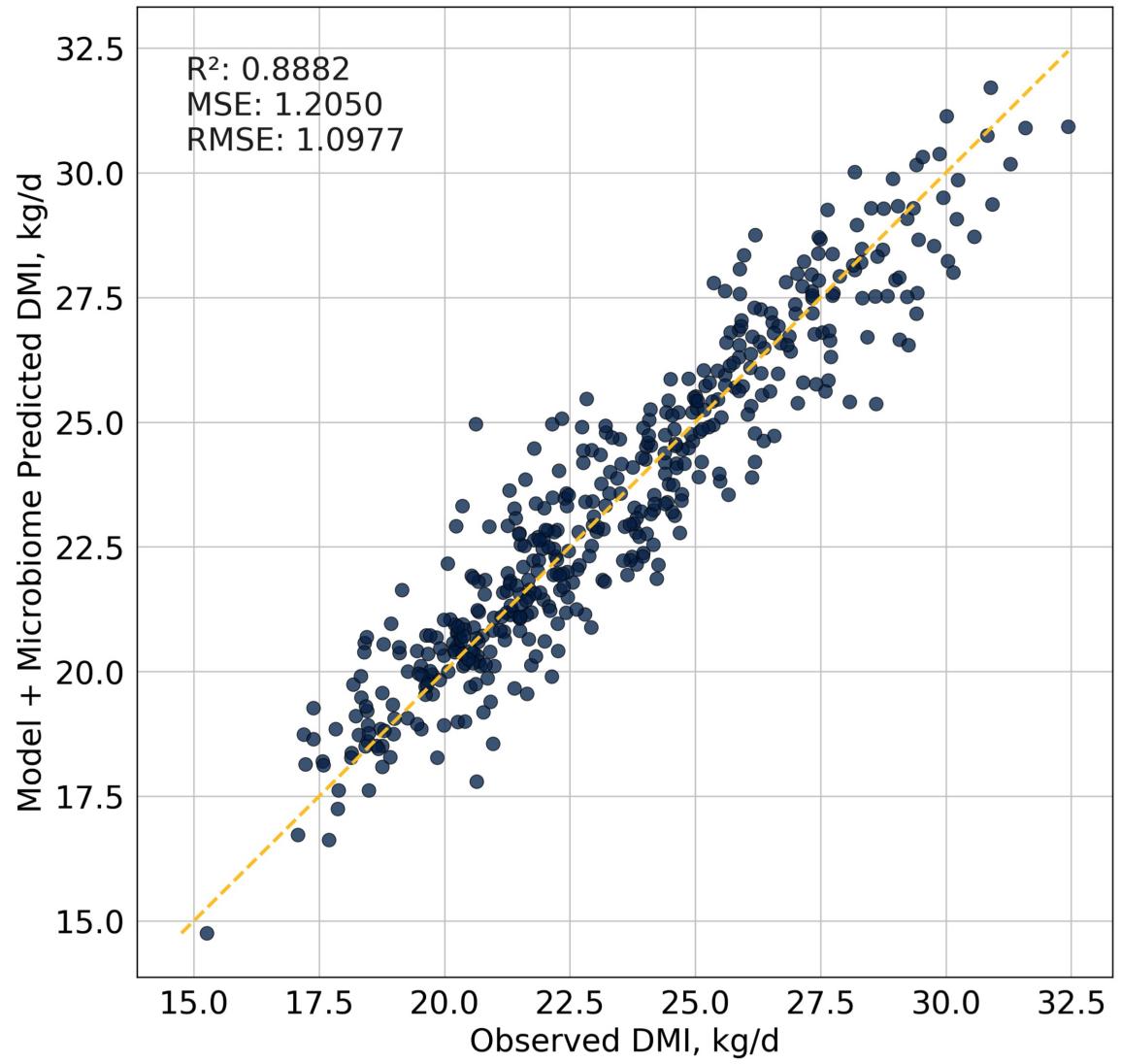
RUMEN MICROBIOME DIFFERENCES ($N = 454$)



Monteiro et al., 2022. *J Dairy Sci.* 106(1):141-142

INCLUDING THE MICROBIOME ON DMI PREDICTION

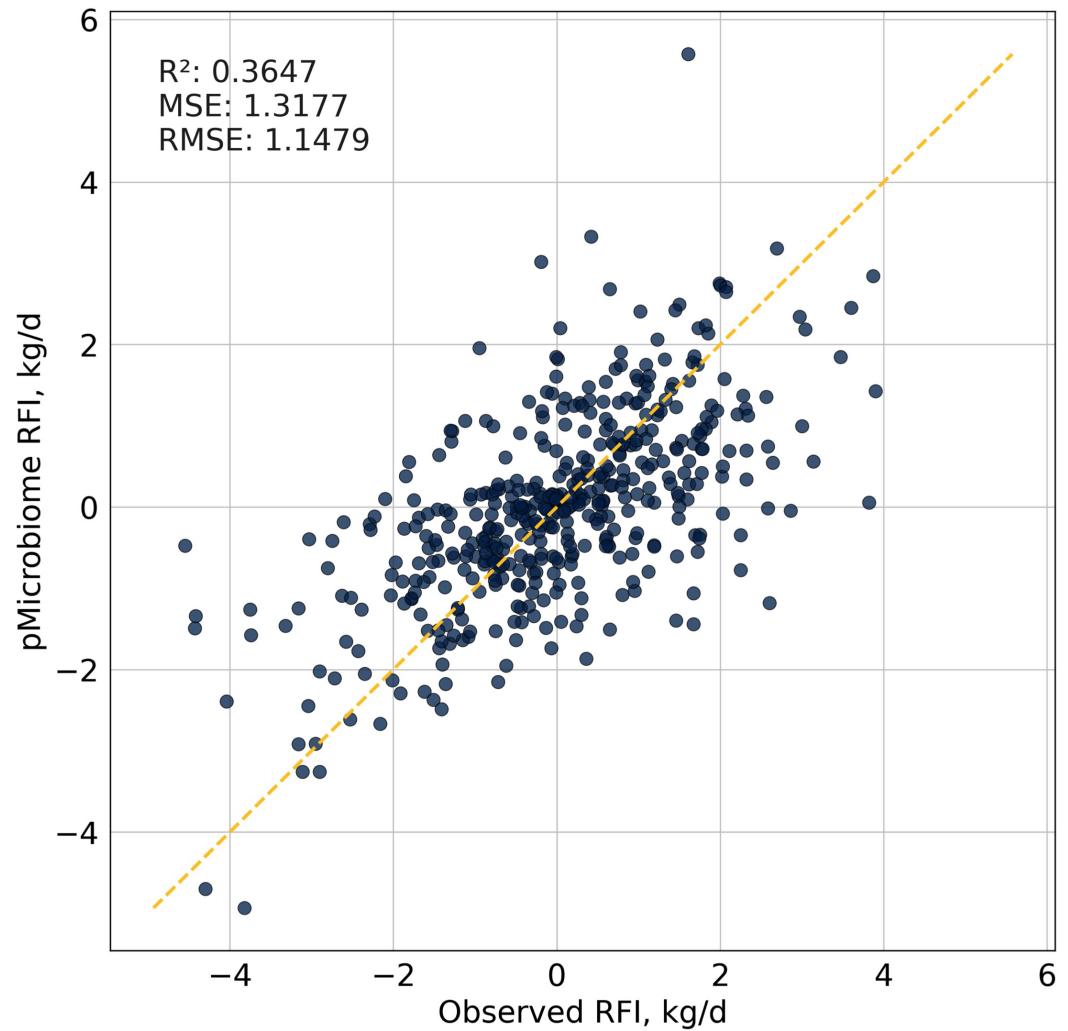
Monteiro et al., 2024. *Animal Microbiome*. 6:5 PMID:38321581



$$\begin{aligned} \text{DMI, kg/d} = & \text{Parity} + \text{NESec} + \text{BW}^{0.75} + \text{BEC} + \text{Treatment} \\ & + \text{Microbiome} + \epsilon \end{aligned}$$

USING THE MICROBIOME TO PREDICT RFI

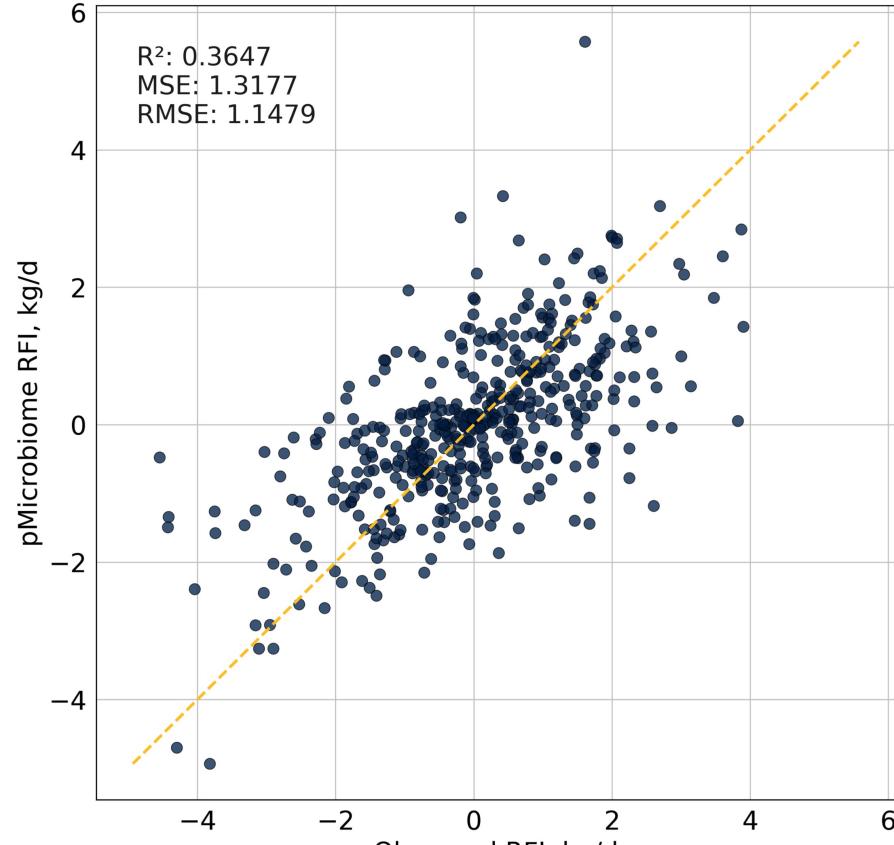
Monteiro et al., 2024. *Animal Microbiome*. 6:5 PMID:38321581



$$\text{RFI, kg/d} = \text{Microbiome} + \epsilon$$

USING THE MICROBIOME TO PREDICT RFI

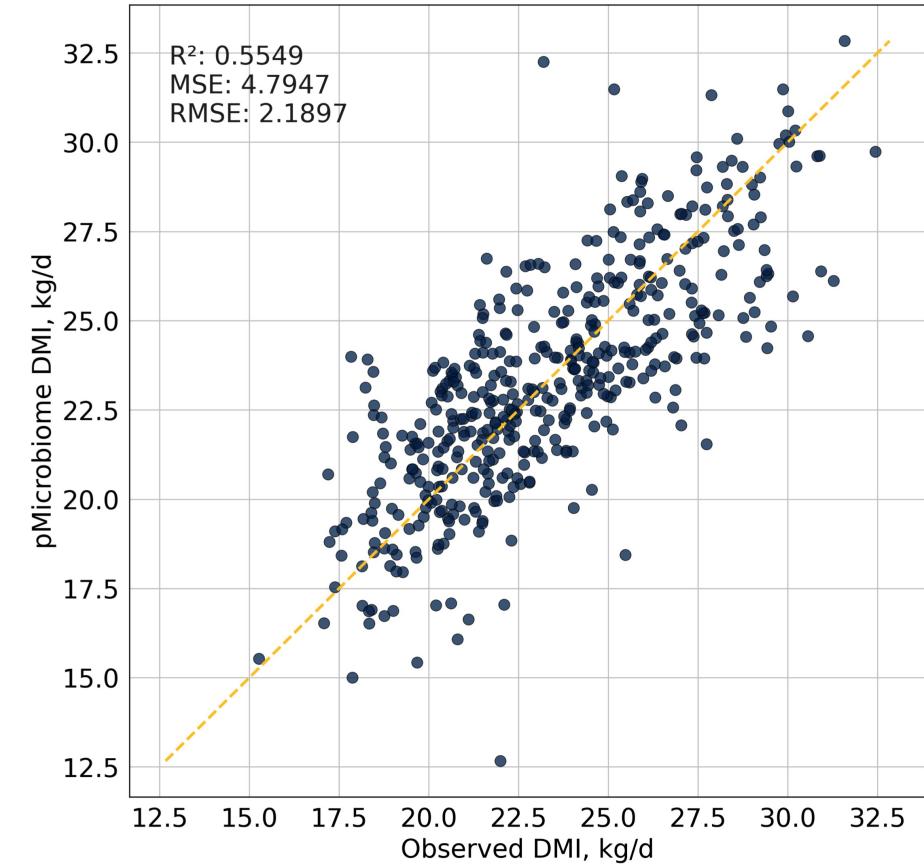
RFI



$$RFI, \text{kg/d} = \text{Microbiome} + \epsilon$$

Monteiro et al., 2024. *Animal Microbiome*. 6:5 PMID:38321581

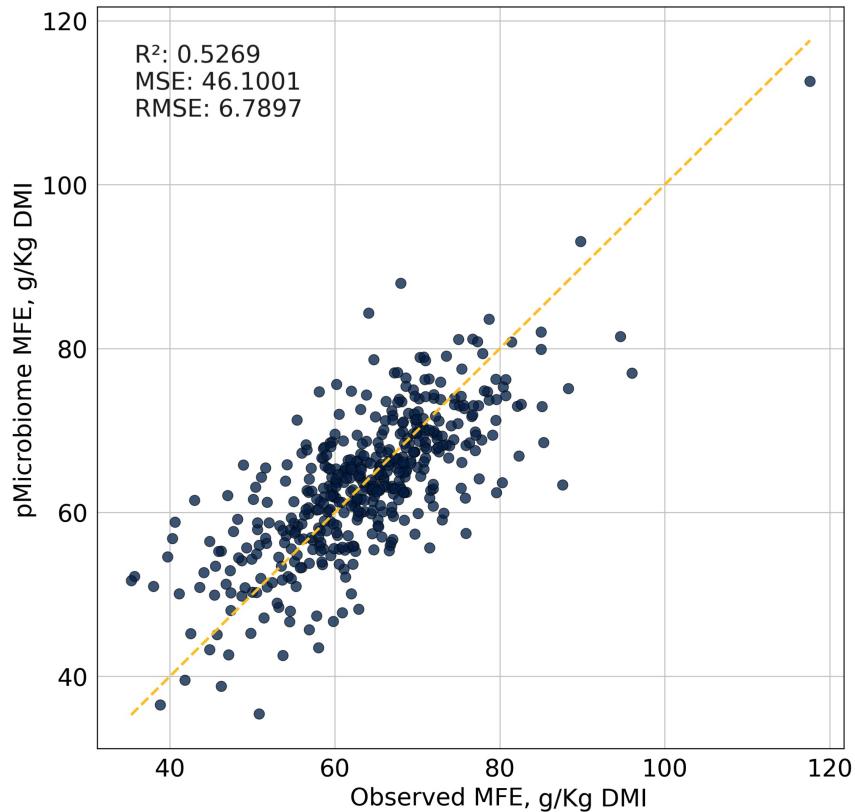
DMI



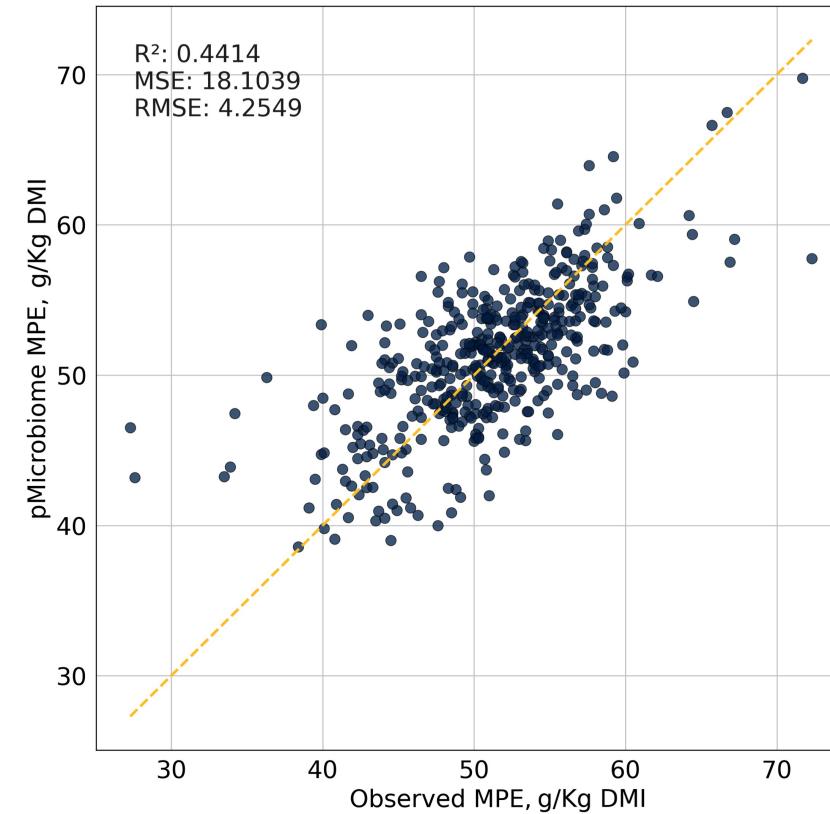
$$DMI, \text{kg/d} = \text{Microbiome} + \epsilon$$

THE MICROBIOME AND MILK PRODUCTION EFFICIENCY

MILK FAT EFFICIENCY



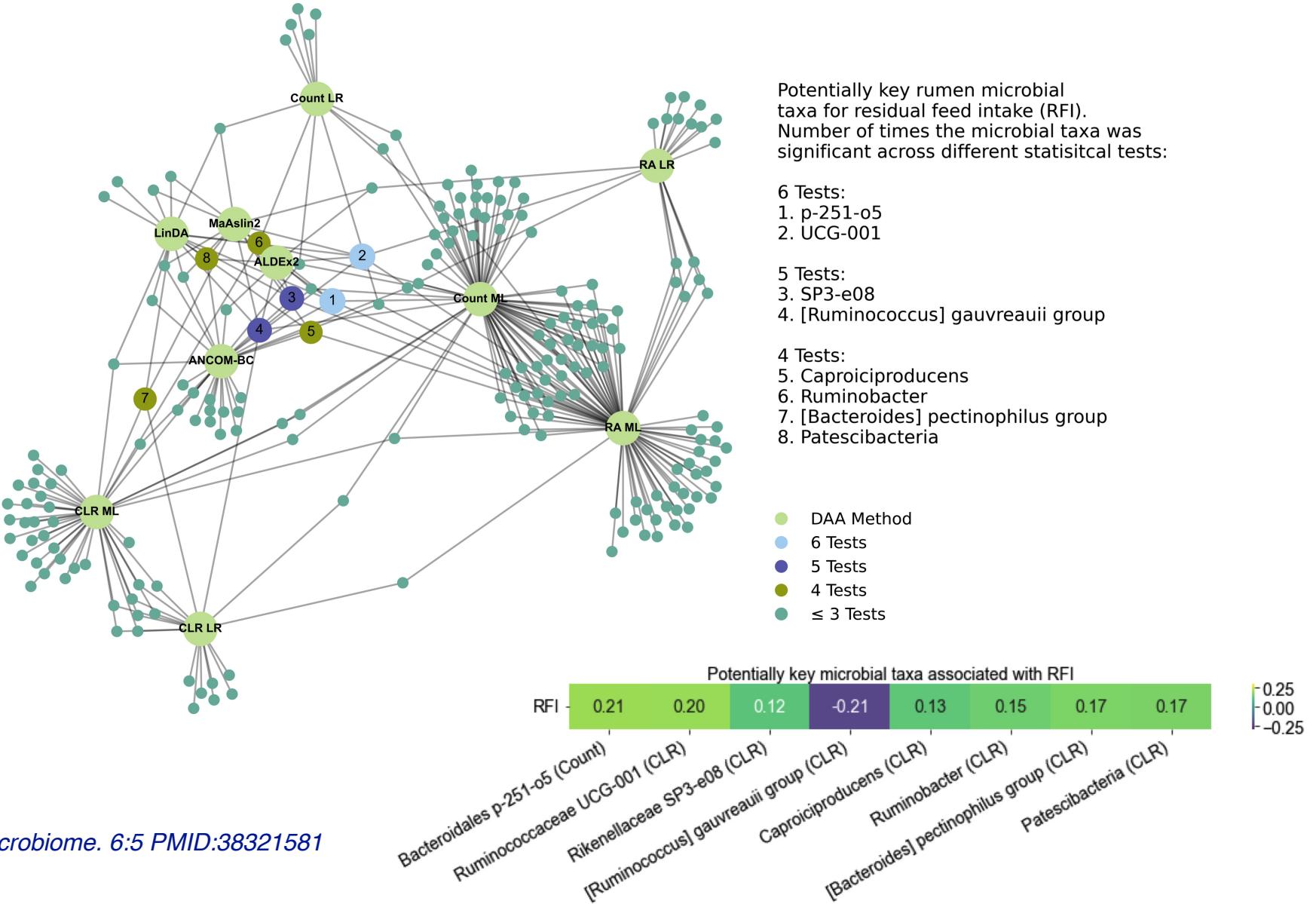
MILK PROTEIN EFFICIENCY



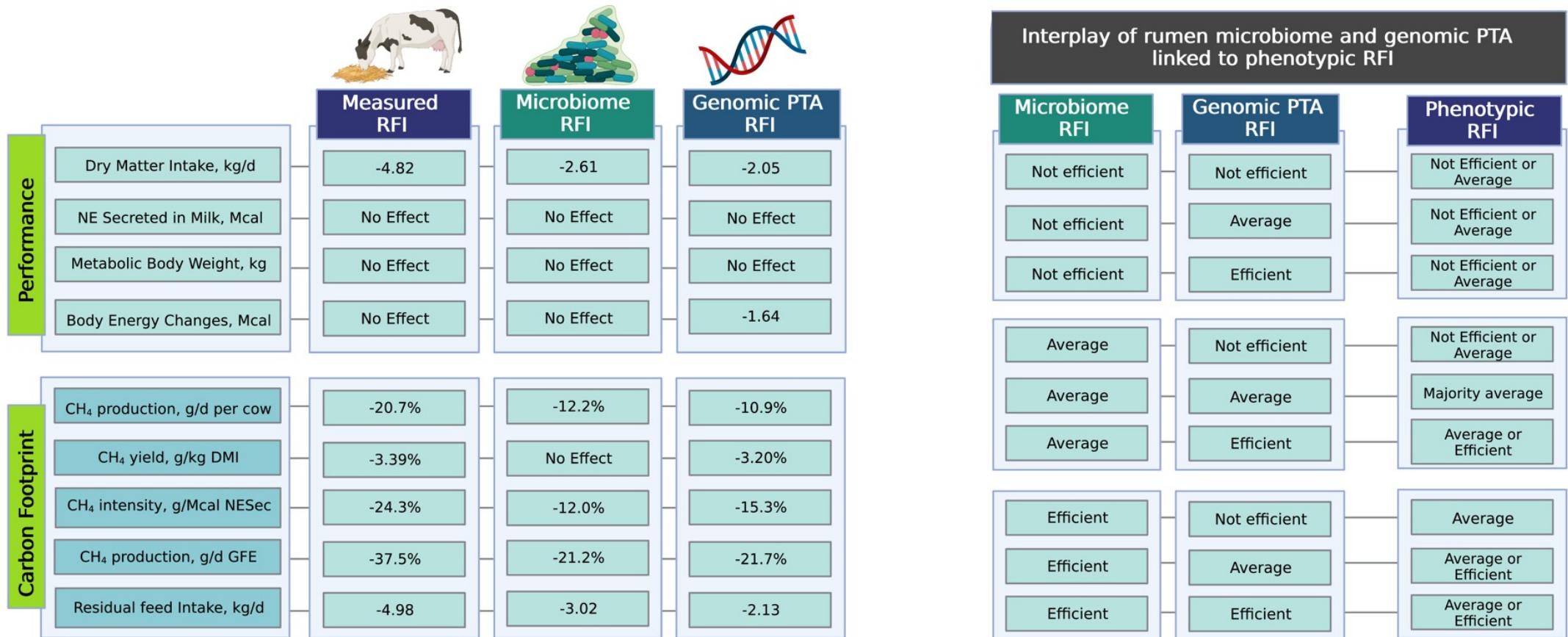
Monteiro et al., 2022. *J Dairy Sci.* 106(1):141-142

BACTERIA ARE ASSOCIATED WITH RFI

Monteiro et al., 2024. *Animal Microbiome*. 6:5 PMID:38321581



HYPOTHETICAL SELECTION FOR RFI, AND THE RUMEN MICROBIOME INTERPLAY WITH GENOMIC PTA, AND PHENOTYPIC RFI



Monteiro et al., 2024. *Animal Microbiome*. 6:5 PMID:38321581

CONCLUDING REMARKS

- ▶ Rumen microbiome composition explains a significant portion of the variation in RFI, presenting a promising site of exploration for future improvements in predictive models to decrease the dairy sector's carbon footprint.
- ▶ The associations of RFI, as well as MFE, MPE, and their residuals with the rumen microbiome, unraveled through an ensemble method, further indicate key microbial players that could be targeted further to evaluate their effect on the efficiency of dairy cows.
- ▶ Additionally, the predictability of heritable traits by the rumen microbiome underscores the need for future research to dissect host-microbiome interactions in shaping feed and milk production efficiency.

Monteiro et al., 2024. *Animal Microbiome*. 6:5 PMID:38321581

TAKEAWAYS

ASSOCIATIONS WITH PRODUCTION

CONTRIBUTIONS TO PREDICTIONS OF
PRODUCTIVE TRAITS

ASSOCIATIONS WITH RFI

GENOME-MICROBIOME LINKS TO RFI

Host and rumen microbiome contributions to feed efficiency traits in Holstein cows

Guillermo Martinez Boggio,^{1*} Hugo F. Monteiro,² Fabio S. Lima,² Caio C. Figueiredo,³ Rafael S. Bisinotto,⁴ José E. P. Santos,⁵ Bruna Mion,⁶ Flávio S. Schenkel,⁶ Eduardo S. Ribeiro,⁶ Kent A. Weigel,¹ and Francisco Peñagaricano¹

¹Department of Animal and Dairy Sciences, University of Wisconsin, Madison 53706

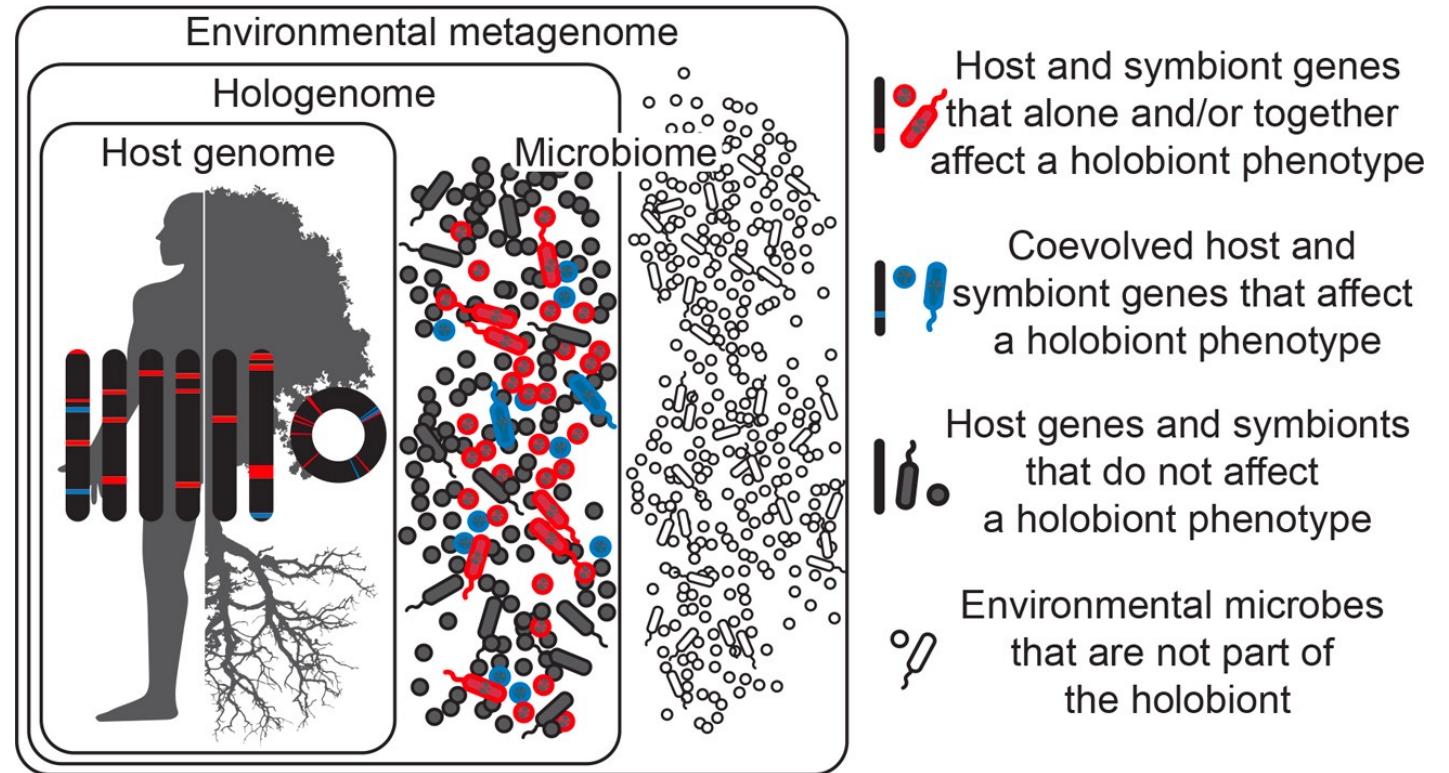
²Department of Population Health and Reproduction, University of California, Davis 95616

³Department of Veterinary Clinical Sciences, Washington State University, Pullman 99163

⁴Department of Large Animal Clinical Sciences, University of Florida, Gainesville 32610

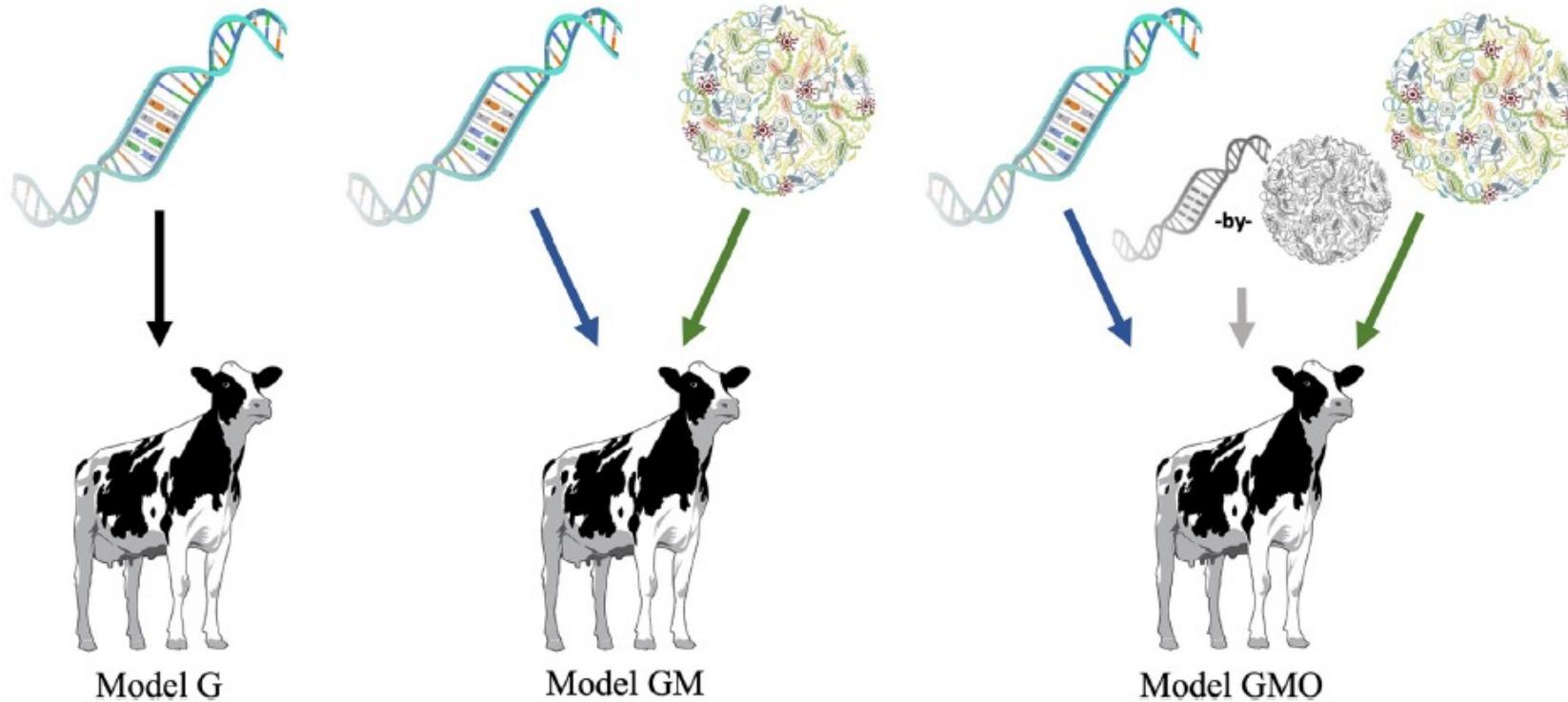
⁵Department of Animal Sciences, University of Florida, Gainesville 32611

⁶Department of Animal Biosciences, University of Guelph, Guelph N1G 2W1



Boggio et al., 2024. J Dairy Sci. TBC. PMID: 38135048

MODELS INCLUDING GENOME, MICROBIOME, AND GENOME-BY-MICROBIOME INTERACTION EFFECTS TO EXPLAIN THE PHENOTYPE



Boggio et al., 2024. J Dairy Sci. TBC. PMID: 38135048

VARIANCE, HERITABILITY, DIRECT HERITABILITY, MICROBIALITY, AND HOLOBIABILITY

Table 2. Estimates of variance components, and heritability, direct heritability, microbiability, genome-by-microbiome interaction, and holobiability for dry matter intake, milk energy, and residual feed intake using three different models

Model	Trait	DIC	σ_g^2	$\sigma_{g_d}^2$	σ_m^2	$\sigma_{g\times m}^2$	σ_{ho}^2	σ_e^2	h^2	h_d^2	m^2	$g\times m^2$	ho^2
G	DMI	1968.6	2.37 ± 0.58					3.33 ± 0.50	0.41 ± 0.09				
GM	DMI	1909.1		1.56 ± 0.42	1.37 ± 0.36		2.92 ± 0.51	2.68 ± 0.43		0.28 ± 0.07	0.24 ± 0.06		0.52 ± 0.07
GMO	DMI	1882.3		1.31 ± 0.39	1.18 ± 0.33	0.84 ± 0.26	3.33 ± 0.50	2.37 ± 0.41		0.23 ± 0.06	0.21 ± 0.05	0.15 ± 0.04	0.58 ± 0.07
G	NESec	2478.5	8.16 ± 2.00					10.1 ± 1.72	0.45 ± 0.09				
GM	NESec	2415.6		5.56 ± 1.55	4.88 ± 1.33		10.4 ± 1.87	8.04 ± 1.49		0.30 ± 0.08	0.26 ± 0.06		0.56 ± 0.08
GMO	NESec	2384.7		4.50 ± 1.39	4.20 ± 1.23	2.77 ± 0.95	11.6 ± 1.77	7.07 ± 1.40		0.24 ± 0.07	0.22 ± 0.06	0.15 ± 0.05	0.62 ± 0.08
G	RFI	1556.9	0.61 ± 0.17					1.46 ± 0.17	0.29 ± 0.08				
GM	RFI	1529.3		0.40 ± 0.13	0.38 ± 0.11		0.78 ± 0.18	1.30 ± 0.17		0.19 ± 0.06	0.18 ± 0.05		0.37 ± 0.08
GMO	RFI	1513.9		0.33 ± 0.13	0.31 ± 0.10	0.28 ± 0.11	0.91 ± 0.19	1.18 ± 0.18		0.16 ± 0.06	0.15 ± 0.05	0.13 ± 0.05	0.43 ± 0.08

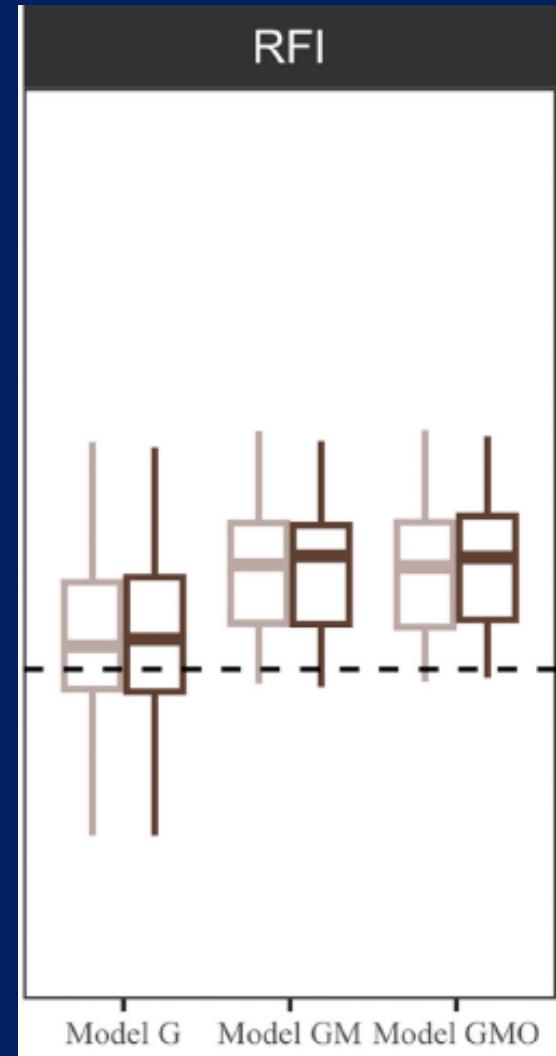
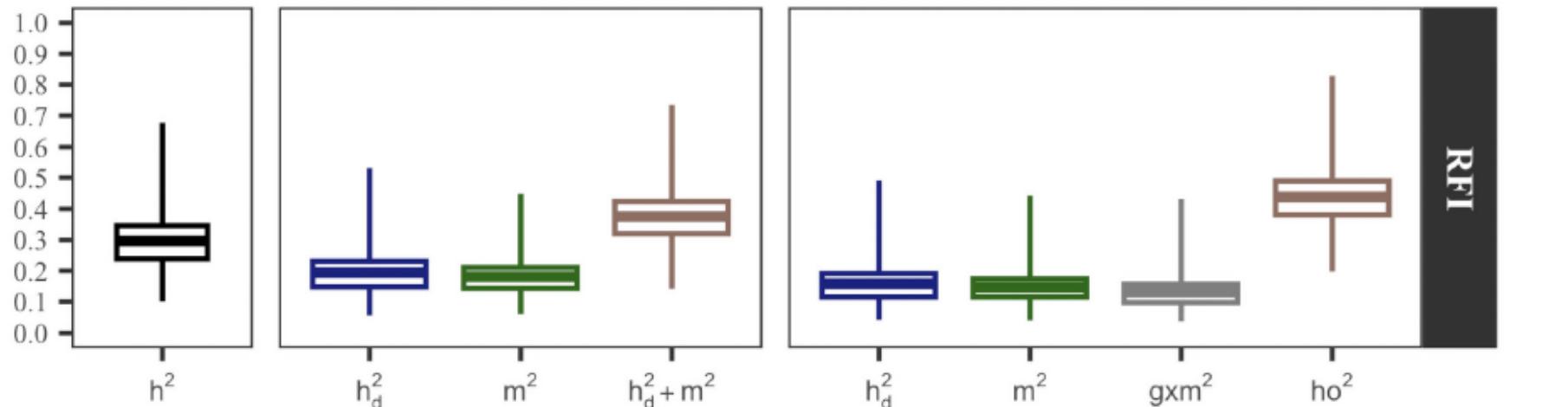
Models: G = models including only the cow genome; GM = models including cow genome and rumen microbiome; GMO = models including cow genome, rumen microbiome, and genome-by-microbiome interaction.

Variance components and parameters: σ_g^2 = additive genetic variance; $\sigma_{g_d}^2$ = direct additive genetic variance; σ_m^2 = microbiome variance; $\sigma_{g\times m}^2$ = genome-by-microbiome interaction variance; σ_{ho}^2 = for model GM is the total variance explained by genome and microbiome, and for model GMO is the variance explained by the holobiont; σ_e^2 = residual variance; h^2 = heritability; h_d^2 = direct heritability; m^2 = microbiability; $g\times m^2$ = genome-by-microbiome interaction; ho^2 = for model GM is the sum of h_d^2 and m^2 , and for model GMO is holobiability.

Traits: DMI = dry matter intake; NESec = net energy secreted in milk; RFI = residual feed intake.

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PROPORTION OF VARIANCE EXPLAINED BY MODELS



PREDICTIVE ABILITY OF
KERNEL-BASED MODELS

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CONCLUDING REMARKS

- ▶ Incorporating the rumen microbiome information in addition to genomic data allows for revealing the relative effects of the host genome and the microbiome on feed efficiency traits in dairy cattle.
- ▶ Rumen microbiome data can be used to estimate host direct and indirect genetic effects on feed efficiency.
- ▶ Indeed, the differences obtained between the heritability and the direct heritability strongly suggest that the microbiome mediates part of the host genetic effect.
- ▶ The holobiont model, which incorporates the host genome-by-microbiome interaction, provides further insights into the biological mechanisms underlying dairy cow feed efficiency.

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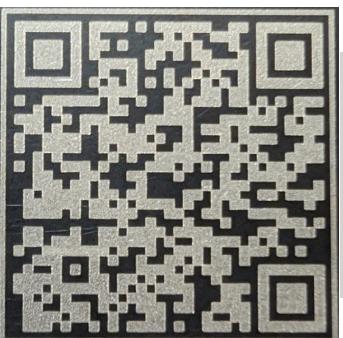
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QUESTIONS??



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