UNDERSTANDING THE IMPORTANCE OF THE RUMEN MICROBIOME TO EXPLAIN VARIATION IN MILK FAT CONTENT IN DAIRY CATTLE USING A COMBINATION OF OMICS APPROACHES

Dr Marc D. AUFFRET

EAAP 2019, Ghent, Belgium

Leading the way in Agriculture and Rural Research, Education and Consulting

Background and Hypothesis

Milk products are an important component of human diets, with beneficial effects for human health, but also one of the major sources of saturated fatty acids (SFA) increasing the risk of cardiovascular diseases.

DIET
63% forage-
37% concentrate

Low SFA milk
(24) → (8 extremes)

Rumen → Rumen microbiome

High SFA milk
(24) → (8 extremes)

Importance of the rumen microbiome composition and activities controlling milk FA composition

1) Methodologies

Milk

Milk composition

Fatty acids

Rumen Microbiome

Metagenomics

Microorganisms
Their genes

Metabolomics

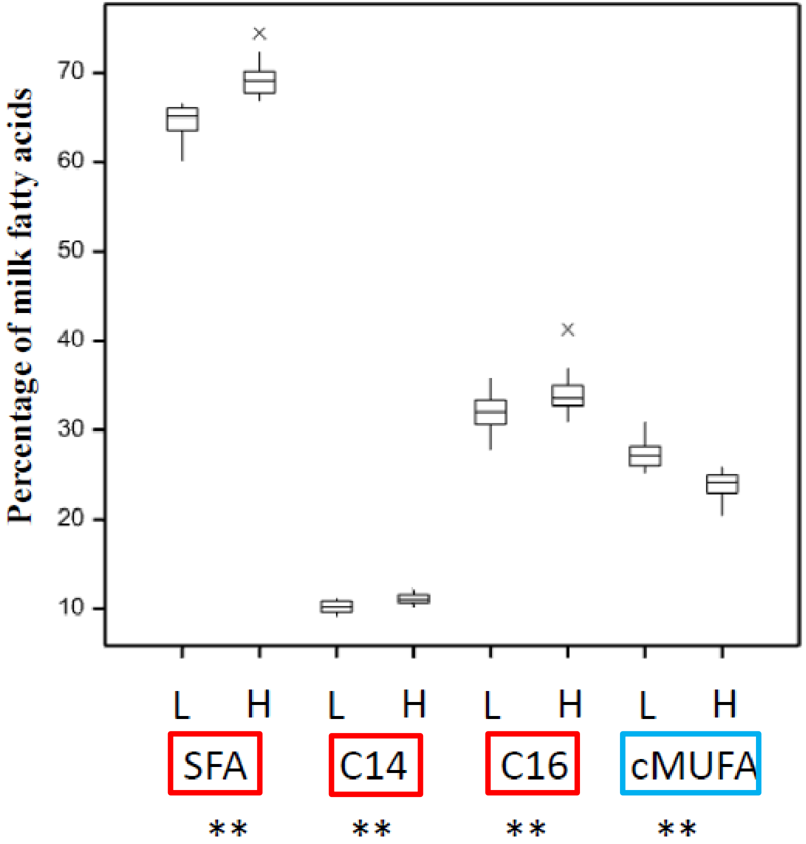
Metabolites
and compounds

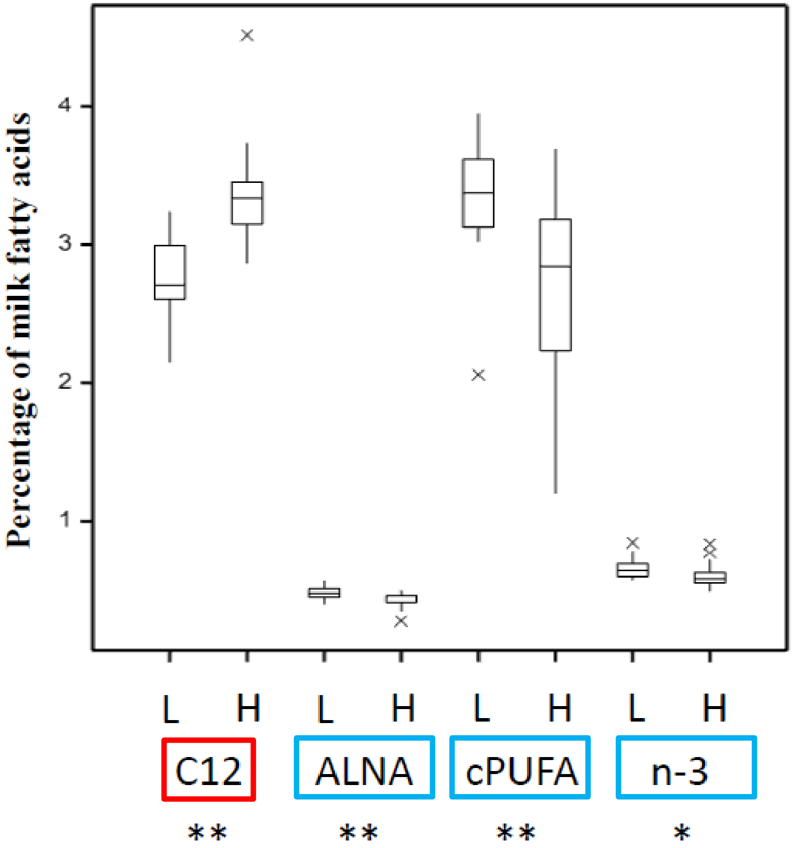
Statistics and Modelling

Partial least square (PLS) and Orthogonal Projection to Latent Structures Discriminant Analysis (O-PLS DA) analysis

Correlation taxa/genes with metabolites between low SFA and high SFA animals

1) Results: METADATA

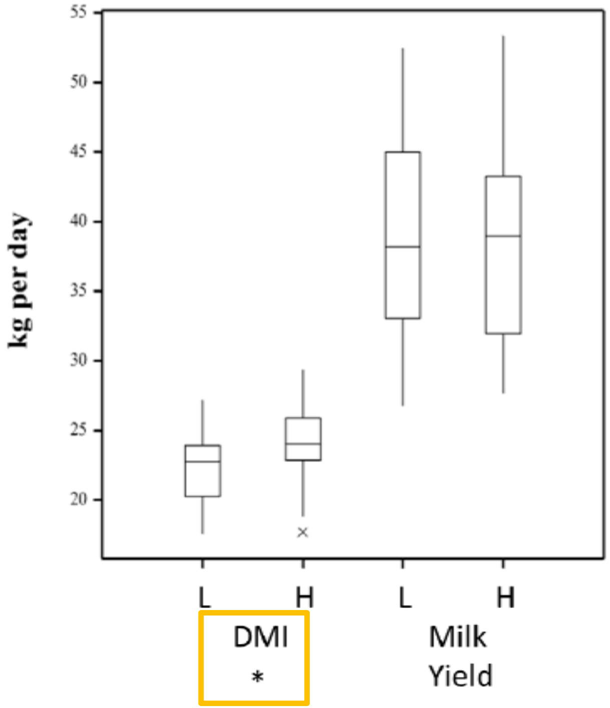




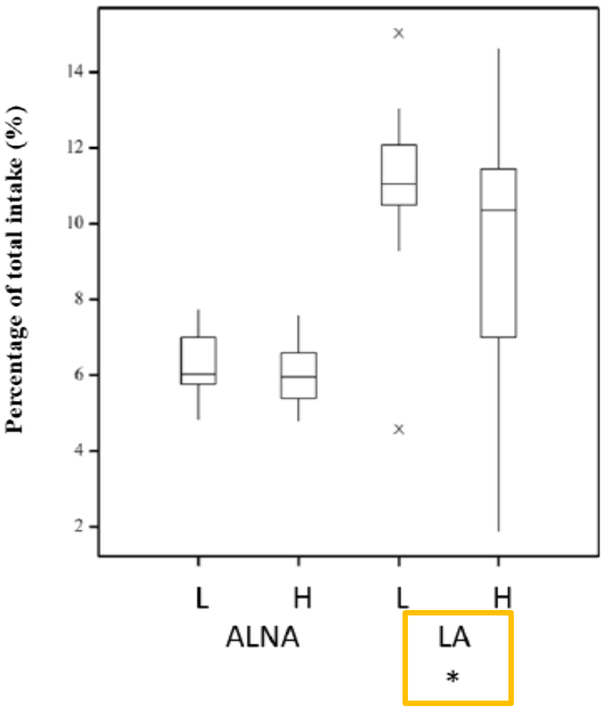
* $P<0.05$
** $P<0.01$

1) Results: METADATA

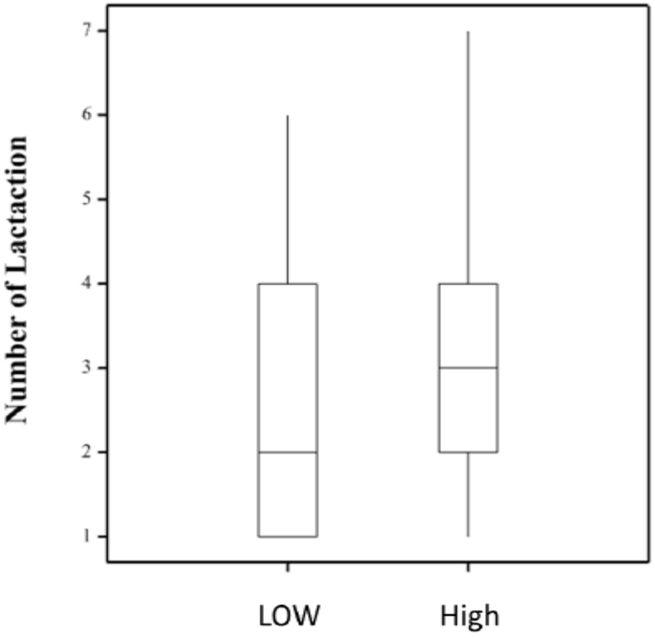
S1A.



S1B. Transfer



S1C. Number of lactation



2) Results: MICROBIOME

39 genes explained on **average 85% of the variability** observed in the different milk FA

Genes involved in:

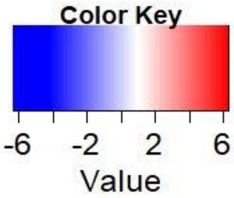
- Oxidative stress resistance
- Cell wall and transporters = adaptation to new environment
- Lactate metabolism

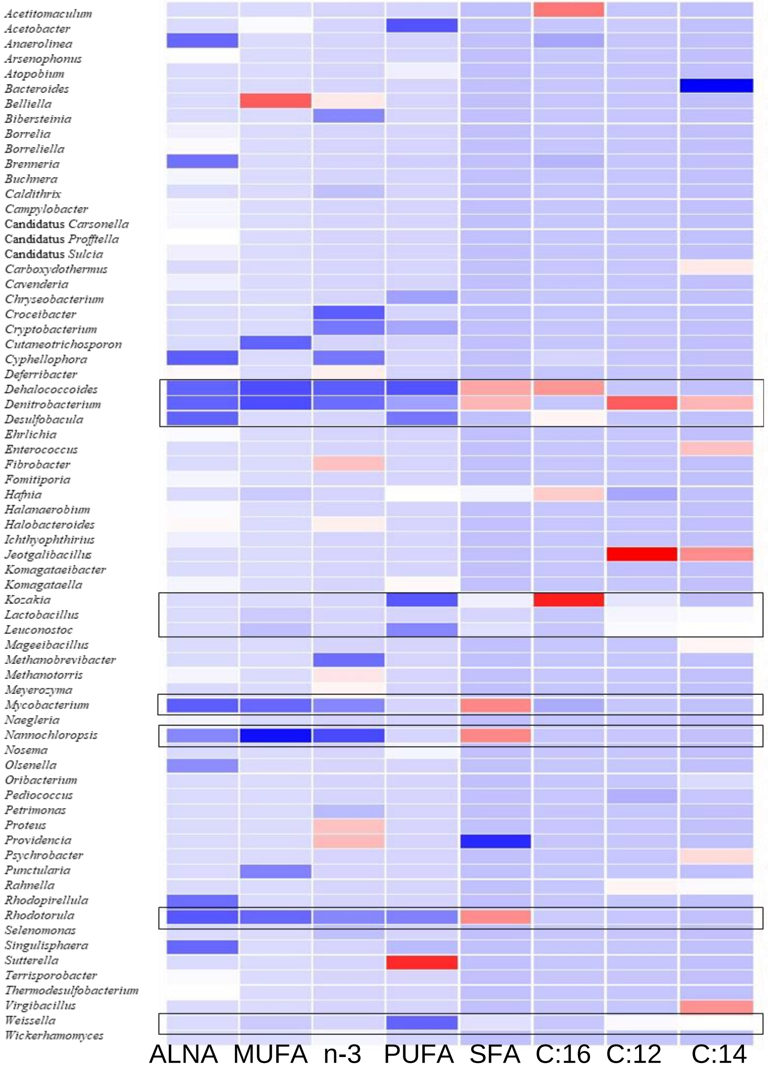
Taxa carrying these genes and explaining the variability observed in the different milk FA were mostly:

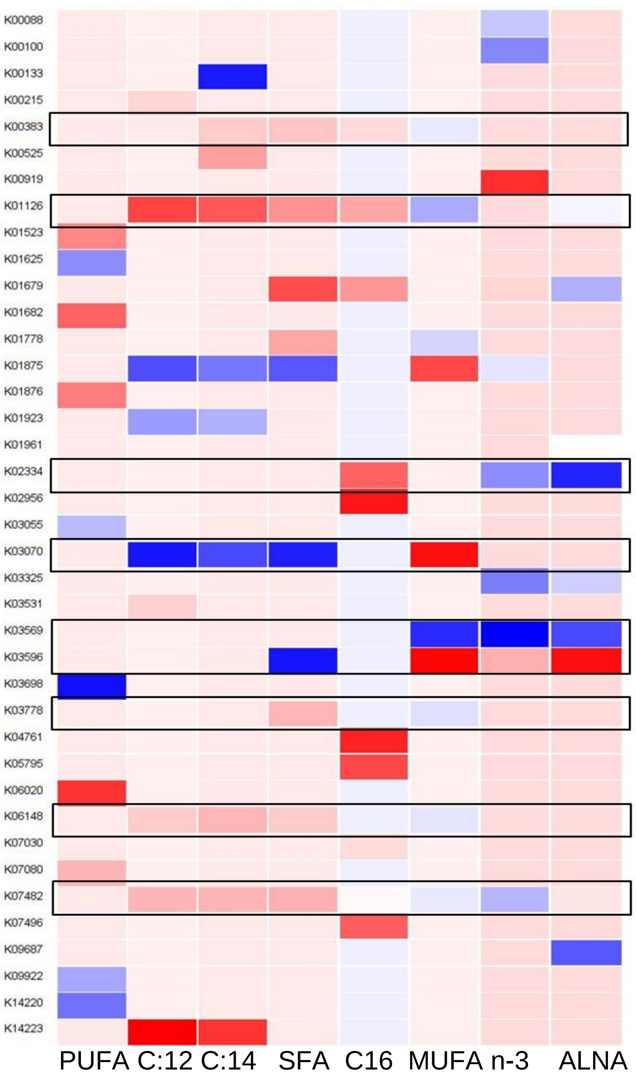
- **Lactic acid bacteria** = *Lactobacillus*, *Leuconostoc* and *Weissella*
- **Acetogenic bacteria** = *Acetobacter* and *Kozakia*
- **Phytate degrader** = *Hafnia*

Lactate degraders = *Megasphaera* and *Selenomas* were not significantly different

Each FA has a specific genera and gene profile



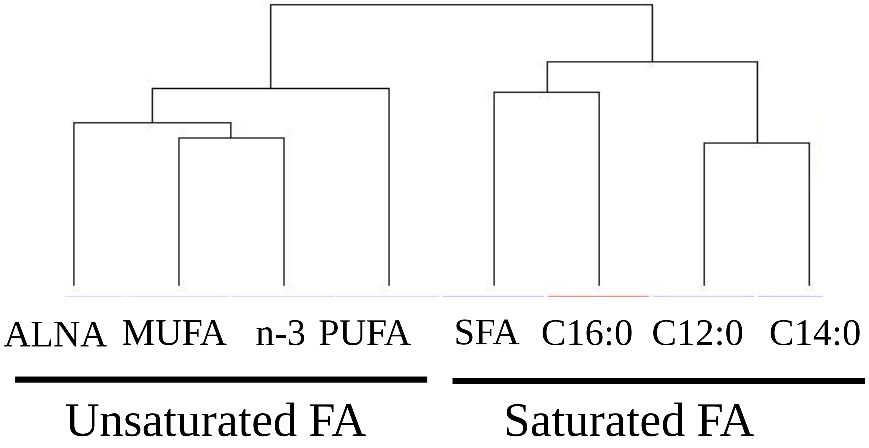




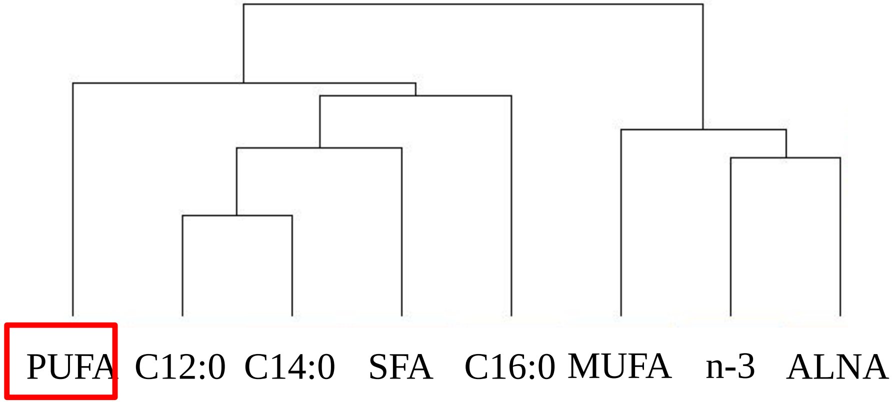
2) Results: MICROBIOME

Each FA has a specific genera and gene profile

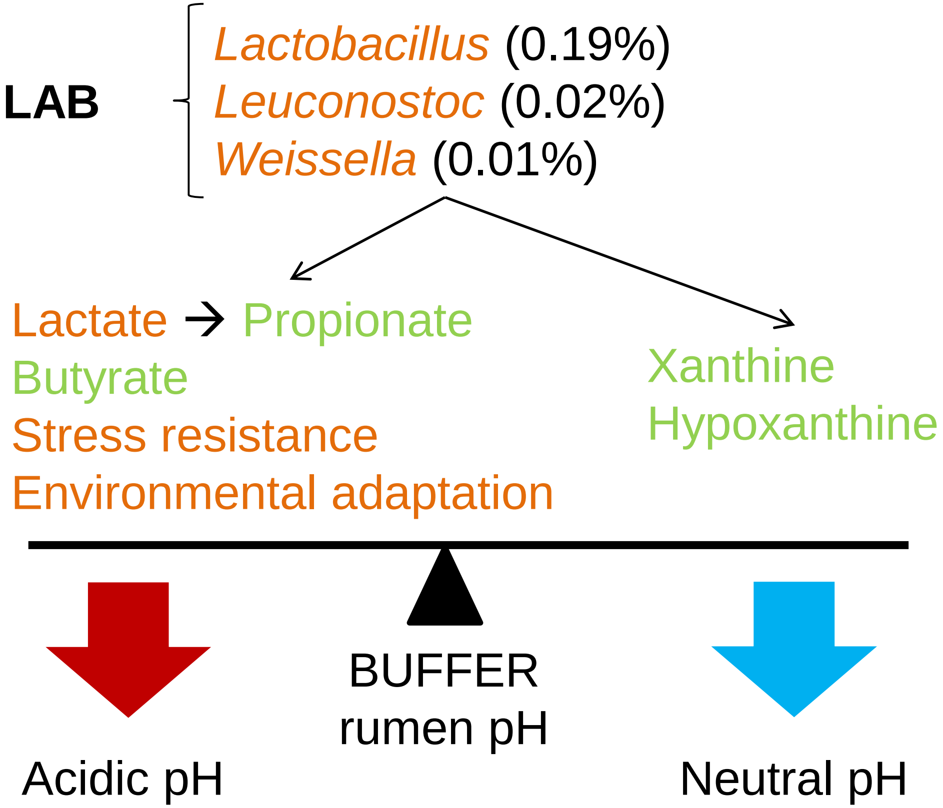
Genera



Genes



3) Results: MECHANISMS



LAB

Lactobacillus (0.19%)
Leuconostoc (0.02%)
Weissella (0.01%)

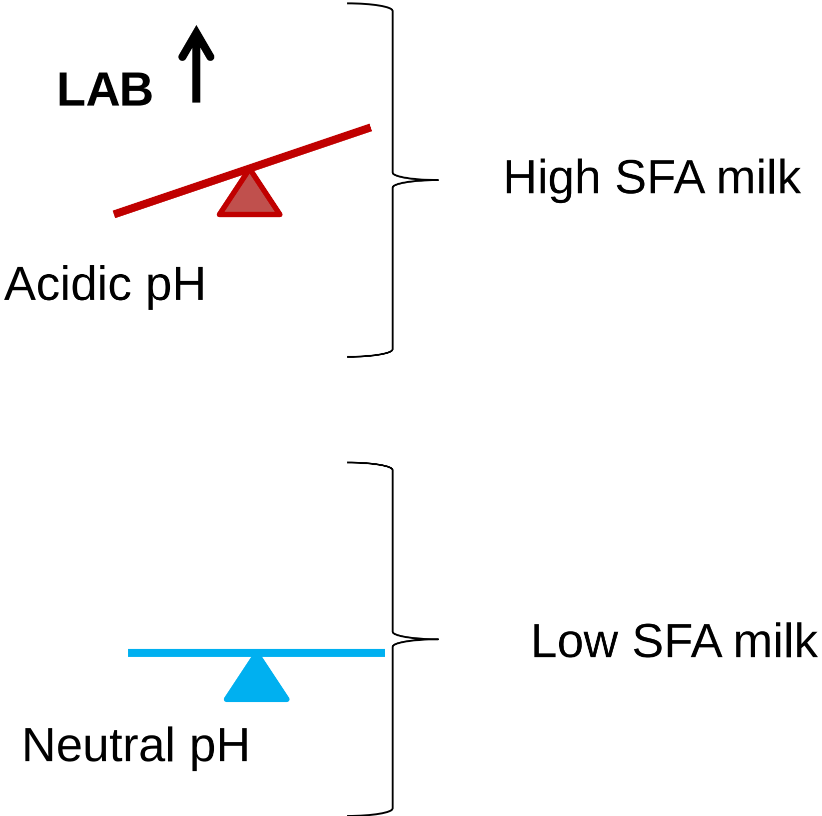
Lactate → Propionate
Butyrate
Stress resistance
Environmental adaptation

Xanthine
Hypoxanthine

BUFFER
rumen pH

Acidic pH

Neutral pH



LAB ↑

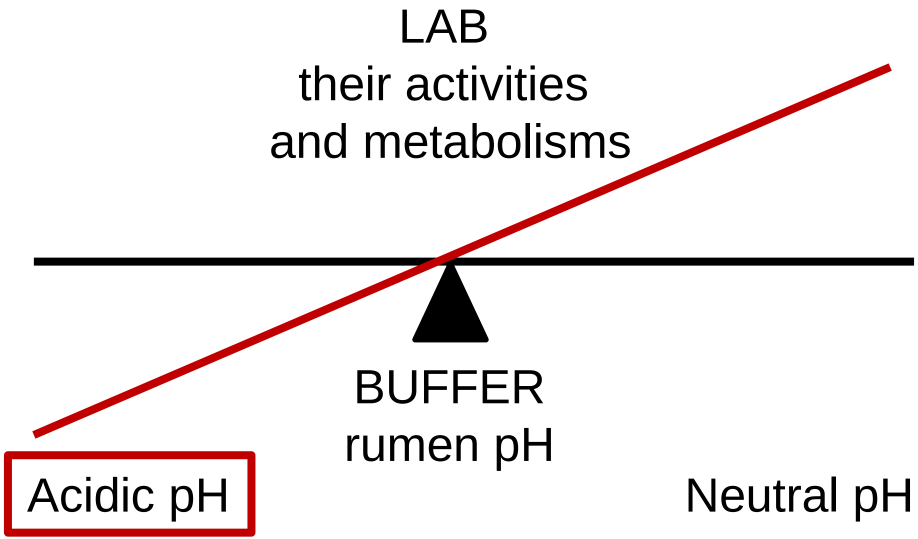
Acidic pH

High SFA milk

Neutral pH

Low SFA milk

3) Results: MECHANISMS



LAB
their activities
and metabolisms

BUFFER
rumen pH

Acidic pH

Neutral pH

- Fibre degradation by fungi (*Punctularia; P<0.05*) →Acetate production → SFA production in the mammary gland
- Phytate degradation (*Hafnia; P<0.05*) →fermentation and VFA released like butyrate and propionate
- Pathogens (*P<0.05*): *Babesia*, *Mycobacterium*

Conclusions-Suggestions

MECHANISM: Lactic acid bacteria → adaptation to low pH and stress environment: Lactate-VFA/Xanthine and Hypoxanthine→ milk FA

Event → LAB → Disturbance Rumen pH → **Lactate-VFA/** Xanthine Hypoxanthine → Adaptation Acidic pH → High SFA milk

RUMEN FERMENTATION: Potentially more efficient

HEALTH: More pathogens in the rumen microbiome when acidic pH and high milk SFA

SUGGESTIONS: Such knowledge can be used to develop farming strategies to maintain a fine balance between high milk productivity, good herd health and an improved environmental footprint of dairy production.

THANK YOU FOR YOUR ATTENTION

SRUC

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ROSLIN

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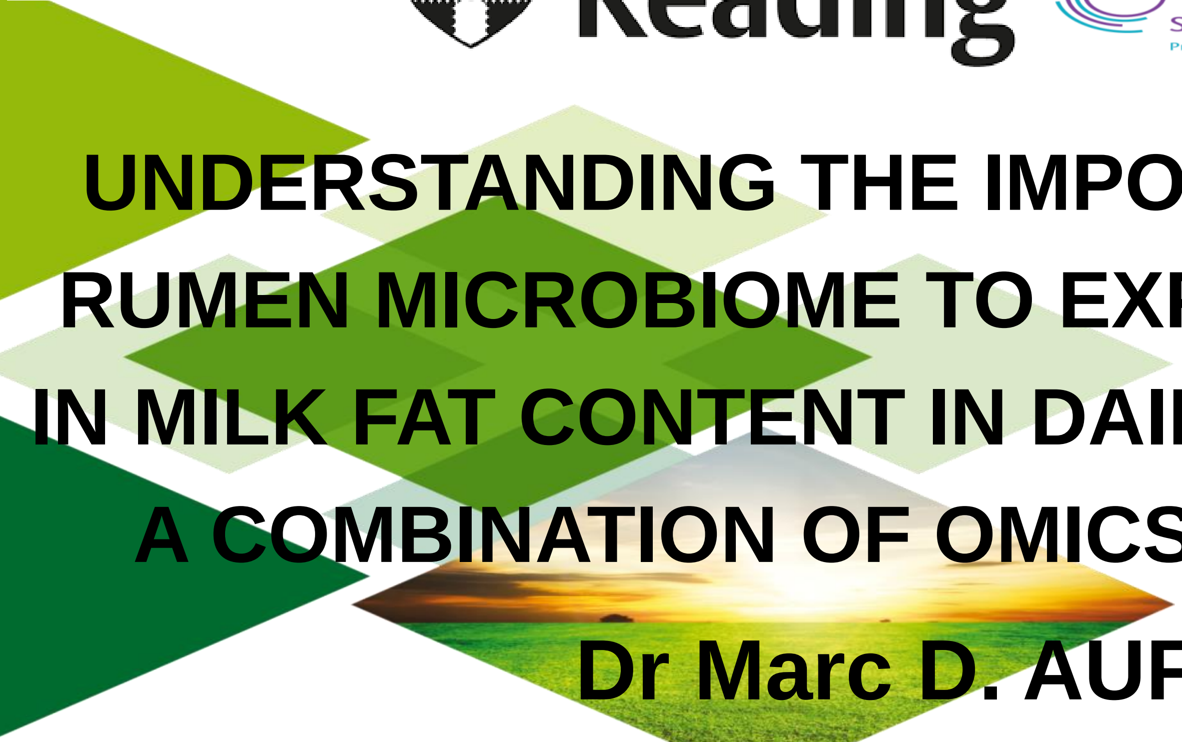

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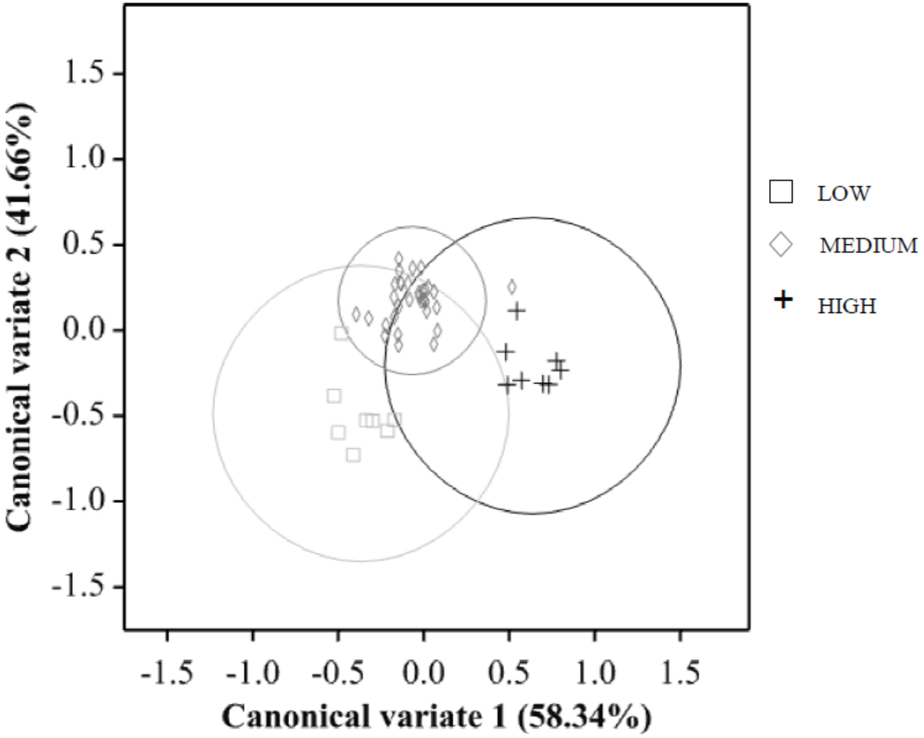
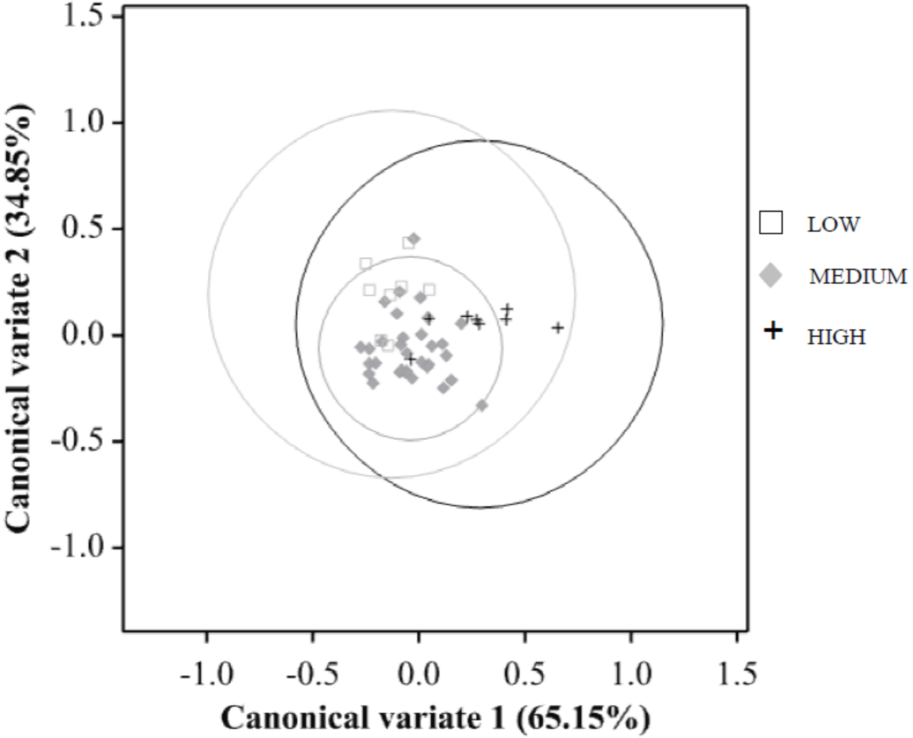
2) Results: MICROBIOME

GENERA



GENES



Conclusions-Suggestions

MECHANISM: Lactic acid bacteria → adaptation to low pH and stress environment: Lactate-VFA/Xanthine and Hypoxanthine→ milk FA

MILK PRODUCTION: tyrosine

Higher abundance of protist and bacteria, known to be rich in polyunsaturated fat, were associated with higher production of milk saturated fat, in contrast with currently suggested probiotic strategies to increase milk content of polyunsaturated fatty acids.

METHANE: methanogenesis also tend to explain part of the variation in milk saturated fat content.

HEALTH: More pathogens in the rumen microbiome when acidic pH and high milk SFA.

SUGGESTIONS: such knowledge can be used to develop farming strategies to maintain

Hypotheses

Microbial mechanisms explaining Dysbiosis:

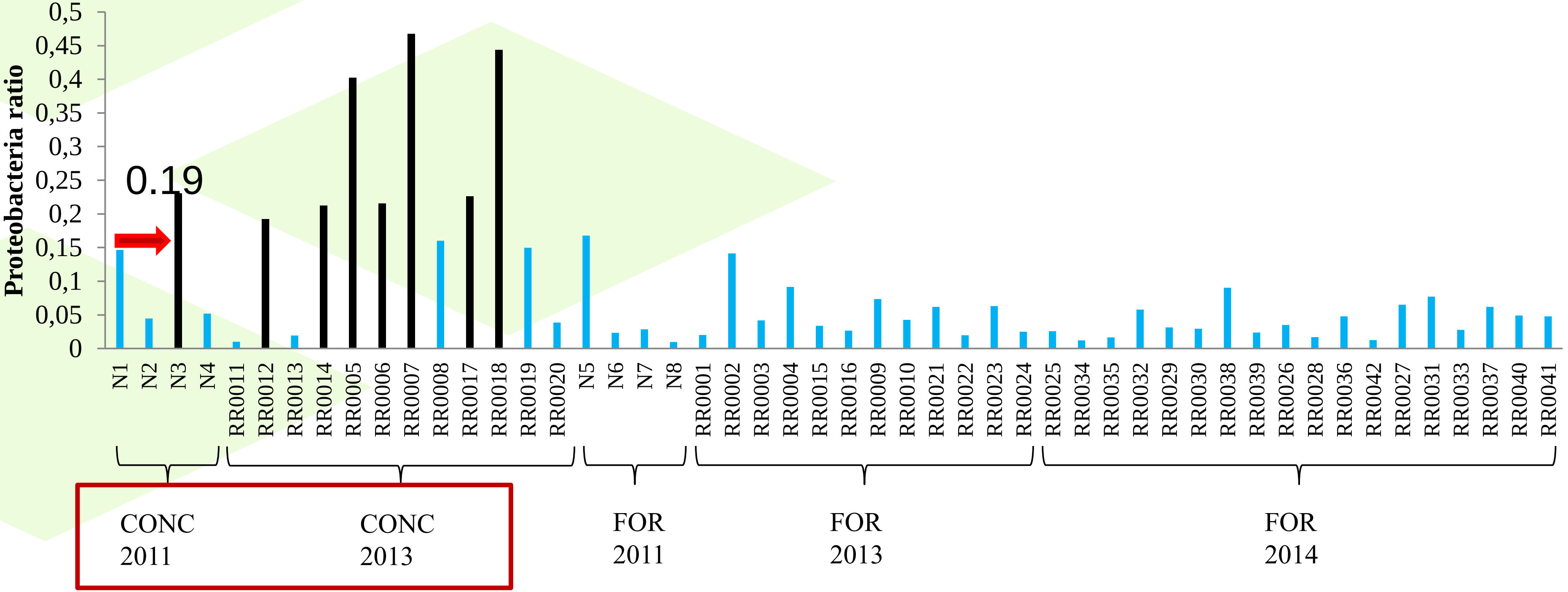
1) Increase in Proteobacteria abundance

2) Increase in abundance of AMR and pathogenicity-related genes

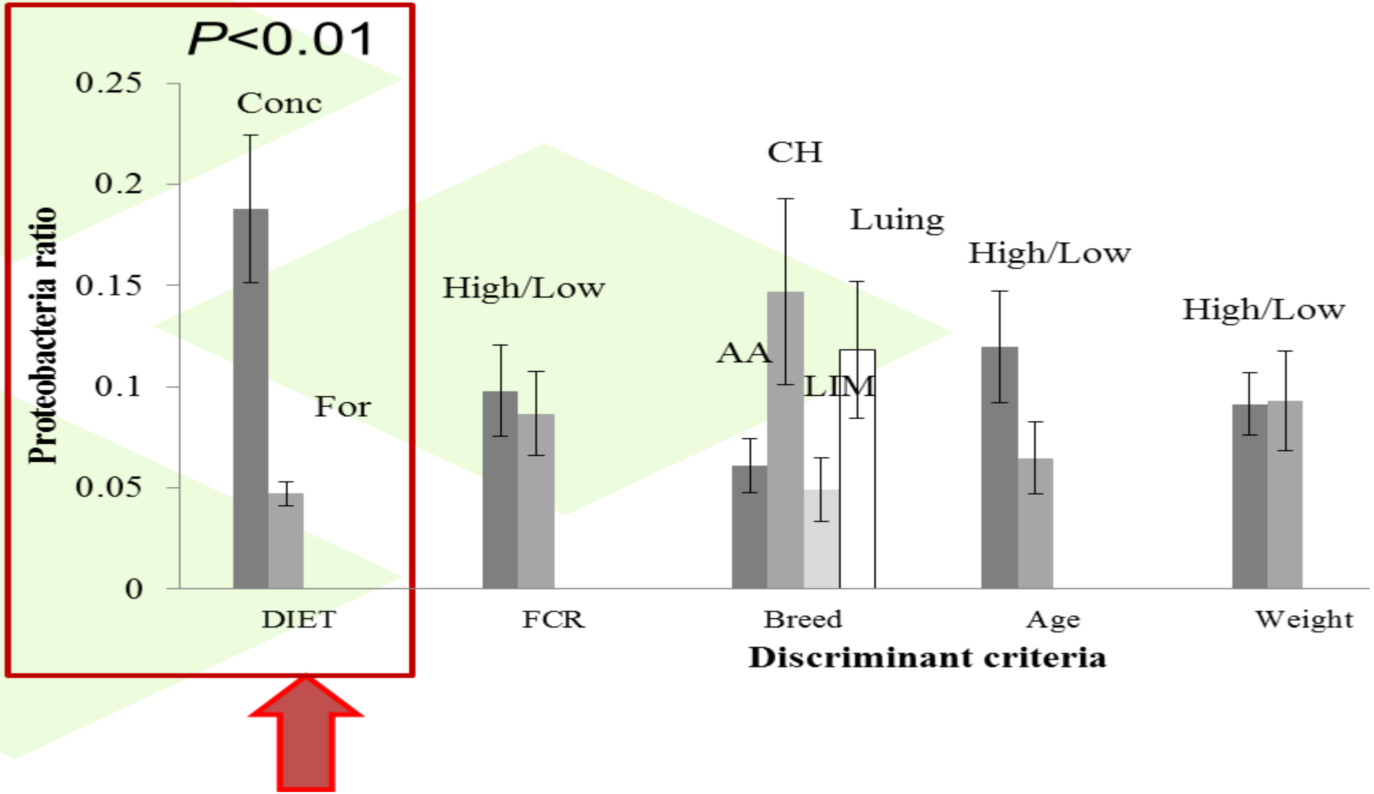
→

Lead to disease
Increase the risk of AMR transfer
Human health threat

1) Results: Proteobacteria



1) Results: Proteobacteria



2) Results: AMR and pathogenicity-related genes

50 animals
Rumen microbiome: 204 selected genes

AMR genes

Resistance to:
- Beta-lactam
- Chloramphenicol
- Macrolide
- Aminoglycoside
- Microcin (peptide)

Communication and Sensing genes

Genes for:
- Fucose sensing
- Quorum sensing
- Two-component signal transduction systems

Stress responses genes

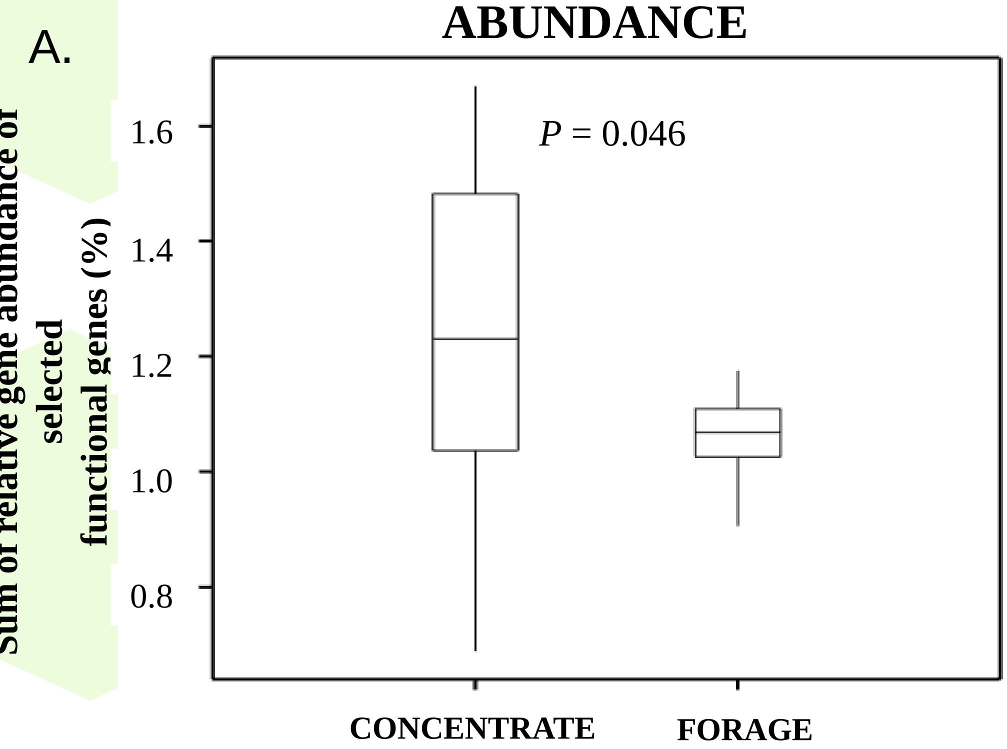
Genes for:
- Oxidative stress
- Heat/cold shock prot.
- Toxic resistance
- Drugs resistance

Pathogenicity Colonization genes

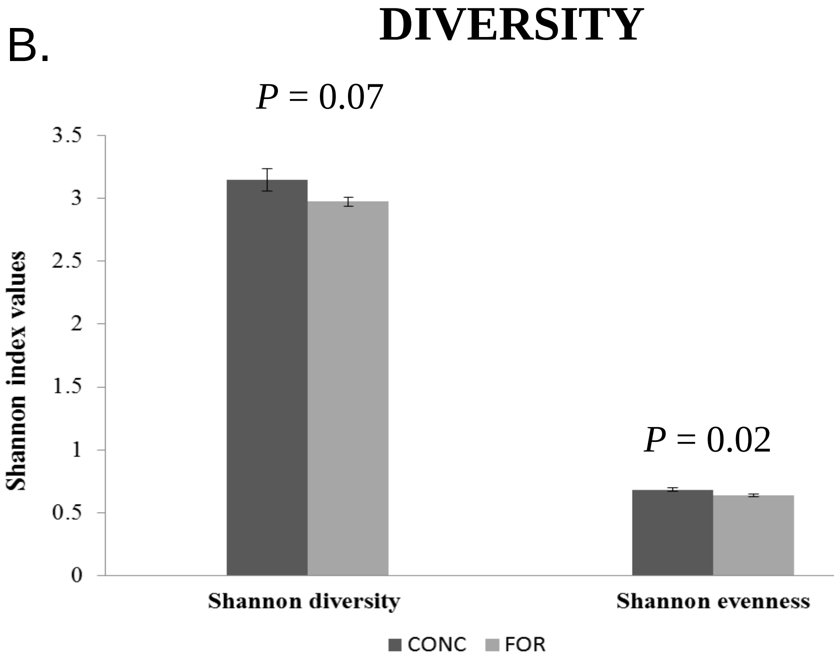
Genes for:
- Motility and hooking to cells
- Adhesion/Type IV pilus
- Secretion systems
- Biofilm

2) Results: AMR and pathogenicity-related genes

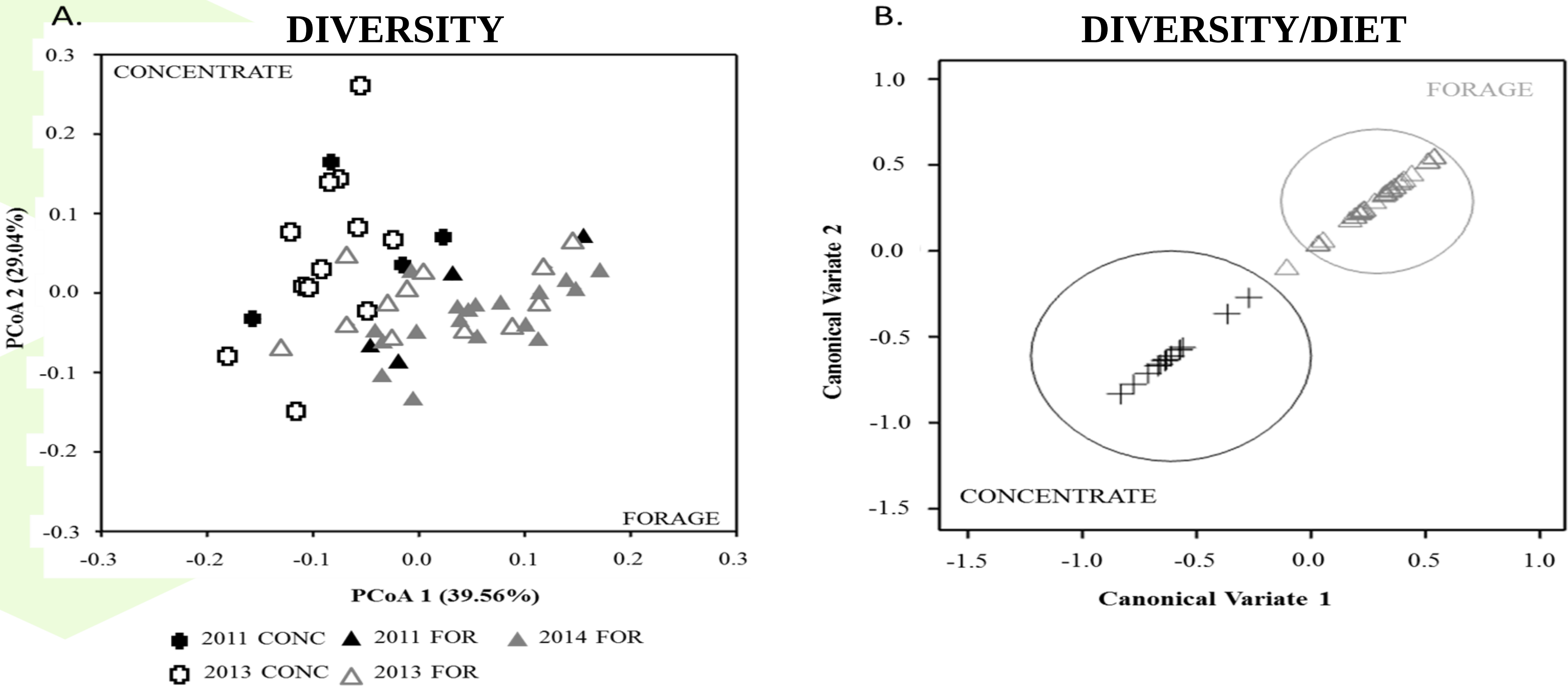
A.



B.



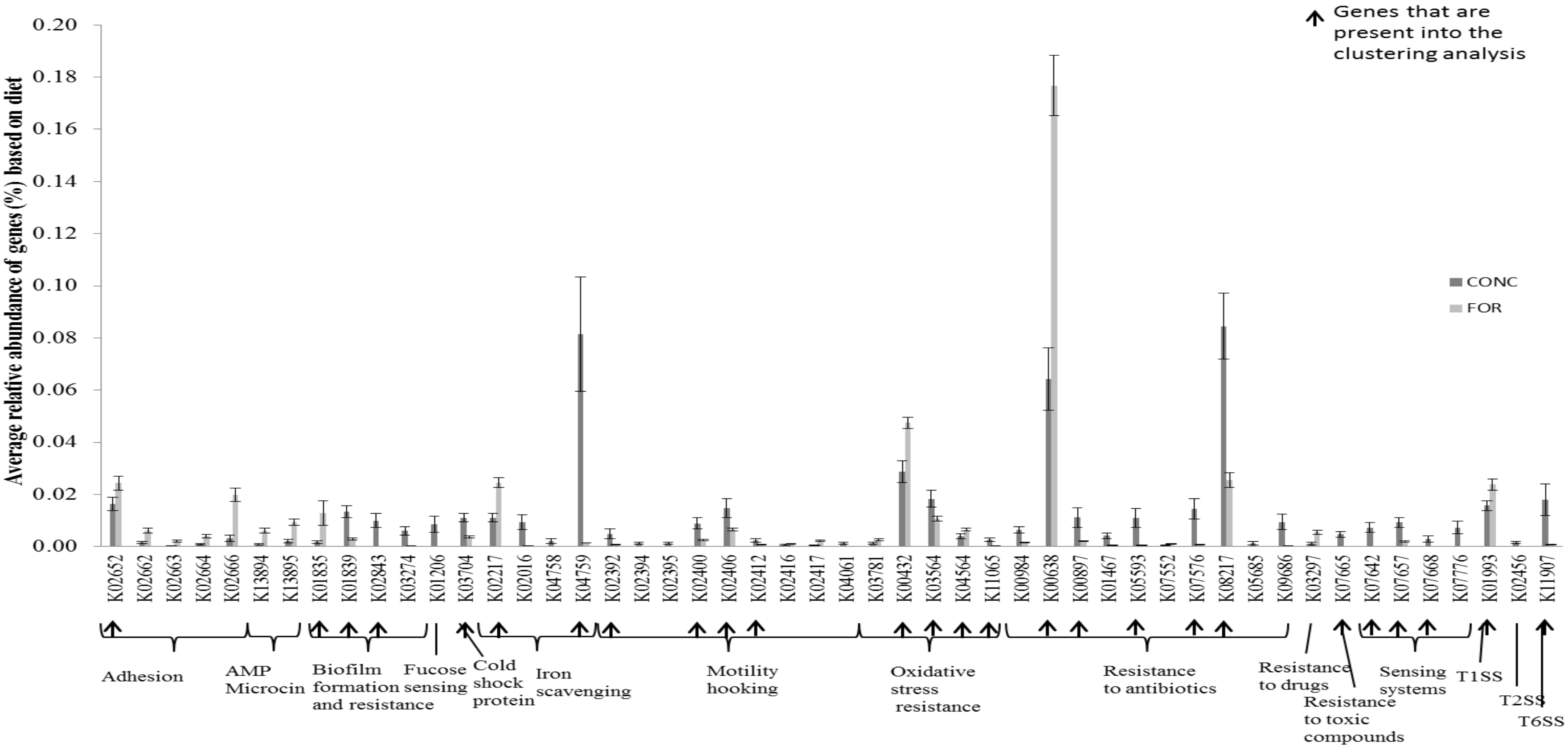
2) Results: AMR and pathogenicity-related genes



DIVERSITY

DIVERSITY/DIET

2) Results: AMR and pathogenicity-related genes



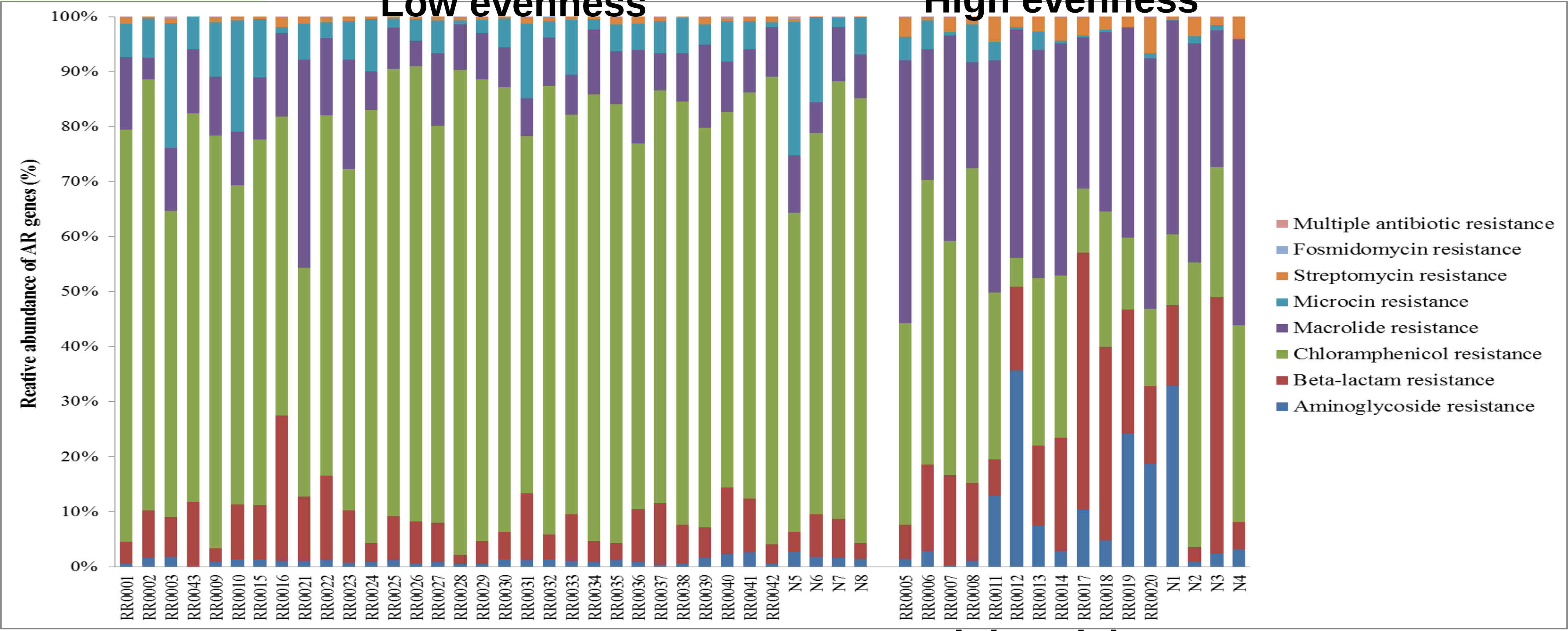
3) Results: AMR genes

FORAGE

Low evenness

CONCENTRATE

High evenness

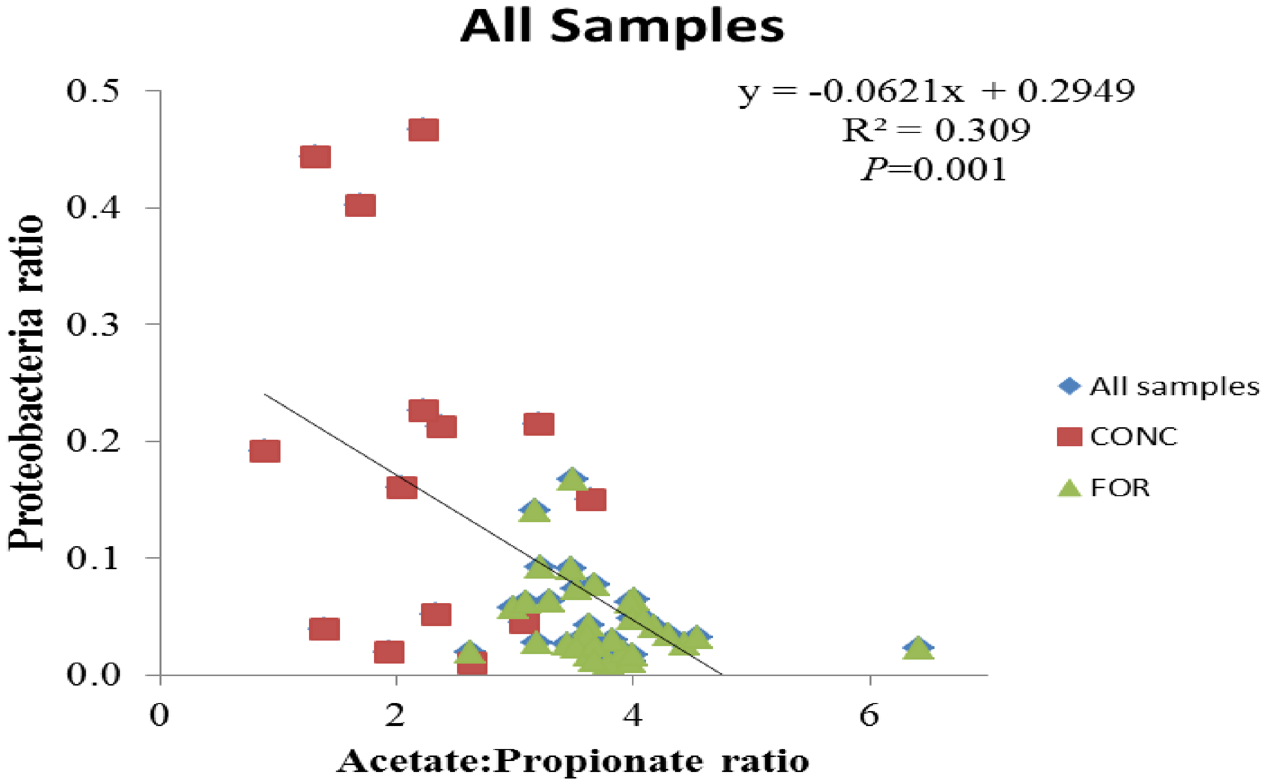


Lower risk

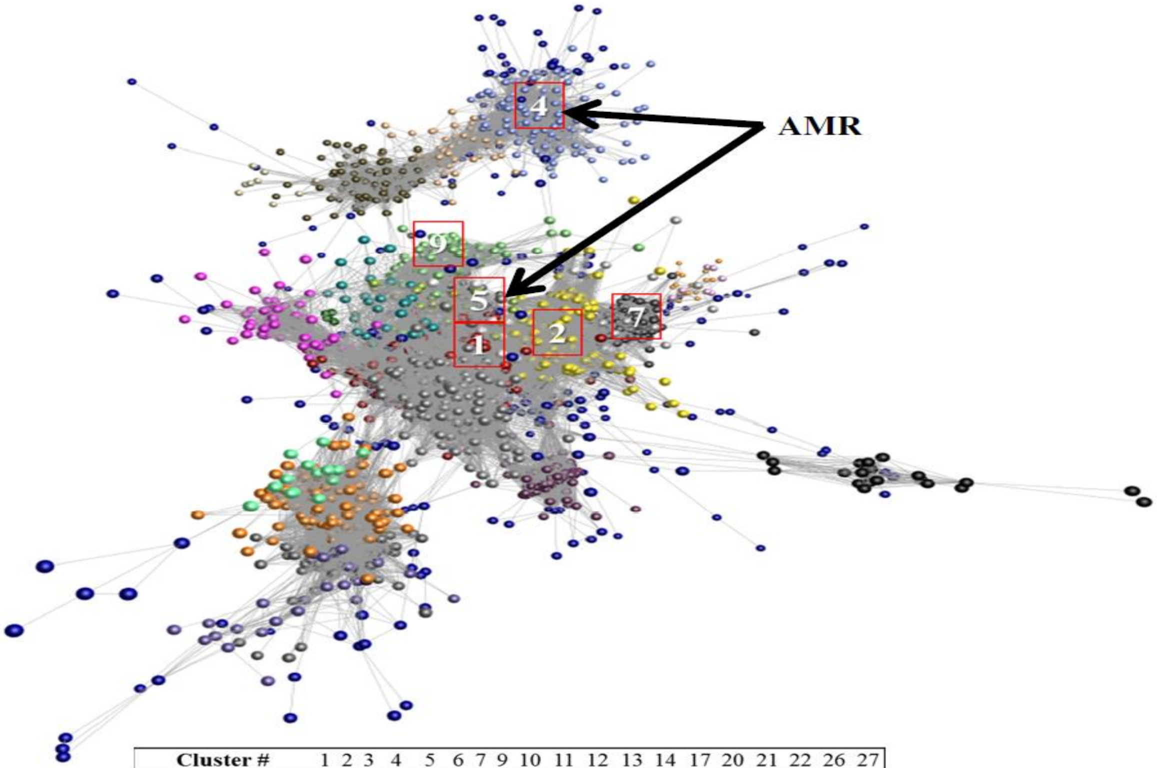
Higher risk

4) Results: Impact of VFA on Proteobacteria ratio

SRUC



5) Results: Network analysis



Cluster #	1	2	3	4	5	6	7	9	10	11	12	13	14	17	20	21	22	26	27
Number of genes	8	8	3	**14**	**22**	1	6	8	3	2	1	3	4	2	1	1	2	1	3

Conclusions-Suggestions

CONCLUSIONS: Concentrate diet significantly increase:

- The relative abundance of **Proteobacteria** including pathogens = **Dysbiosis**
- The number of genes associated with colonization, sensing and **AMR genes**
- The diversity of **microbial mechanisms** to avoid the host immune system
- The **risk of disease**
- Potentially the **risk of AMR gene transfer into human pathogens** and therefore it is a **threat to human heath**.

SUGGESTIONS:

Forage-based diet in cattle should be prioritized over concentrate diet.

Identification of a **baseline for AMR genes** abundance in antibiotic-free animals will help to improve policies about antibiotic treatments.

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