EFFICIENT & ECOLOGICALLY-FRIENDLY PIG AND POULTRY PRODUCTION.

ECOFCE

A WHOLE-SYSTEMS APPROACH TO OPTIMISING FEED EFFICIENCY AND REDUCING THE ECOLOGICAL FOOTPRINT OF MONOGASTRICS.

BASIC DATA

Funding:
EU-FP7
(€ 6 million)

Start date:
1 February 2013

Duration:
48 months
(2013 to 2016)

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FEED EFFICIENCY RELATED GUT MICROBIOTA PROFILES VARY IN CHICKENS RAISED AT TWO LOCATIONS

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Intestinal microbiota

Links between gut microbiota and
- obesity in mice & humans (*Ley et al. 2006*; *Turnbaugh et al. 2006*)
- energy harvest in chickens (*Singh et al. 2012, 2014; Stanley et al. 2012, 2013, 2016*)

Gut microbiota influences
- digestion of food
- colonization resistance
- intestinal function
- development of immune system

Gut microbiota
- competes for nutrients
- produces toxic amino acid catabolites
- decreases fat digestibility
- alters intestinal morphology & function

Genetic background
Sex
Age

Diet composition

Environment

Objectives

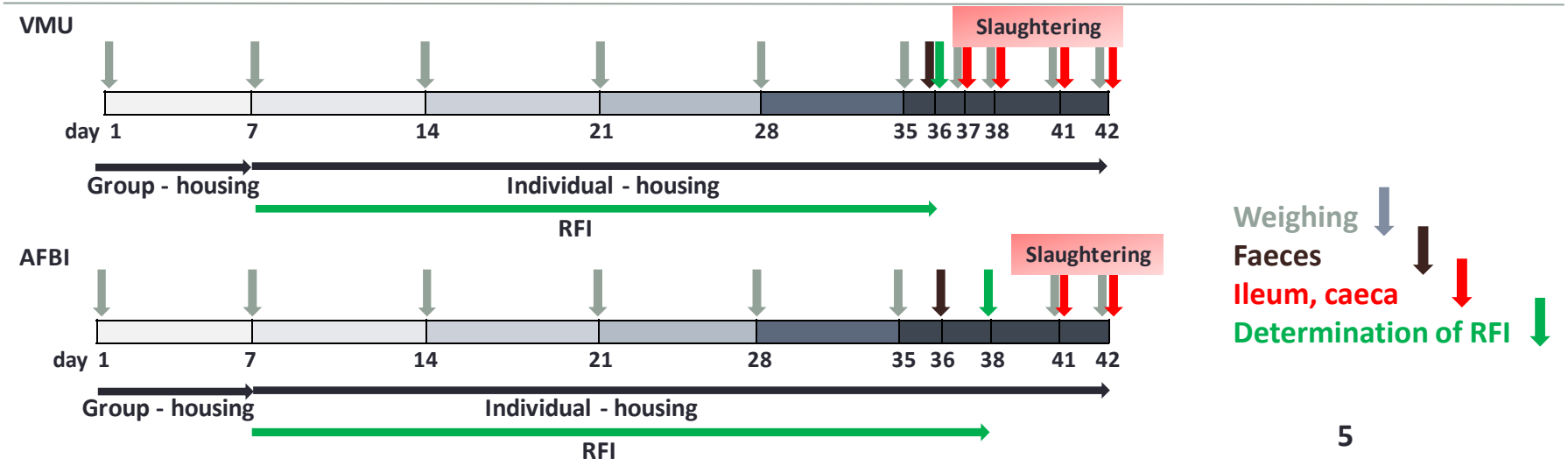
- To characterise and to better understand the gut microbiota of broiler chickens of diverging feed efficiency
- To better understand effects of the environment on gut microbiota and feed efficiency

Hypothesis

- The gut microbiota of broiler chickens of diverging residual feed intake (RFI) – as metric for feed efficiency – differs in key members
- Differences in gut microbiota are persistent between two different rearing environments

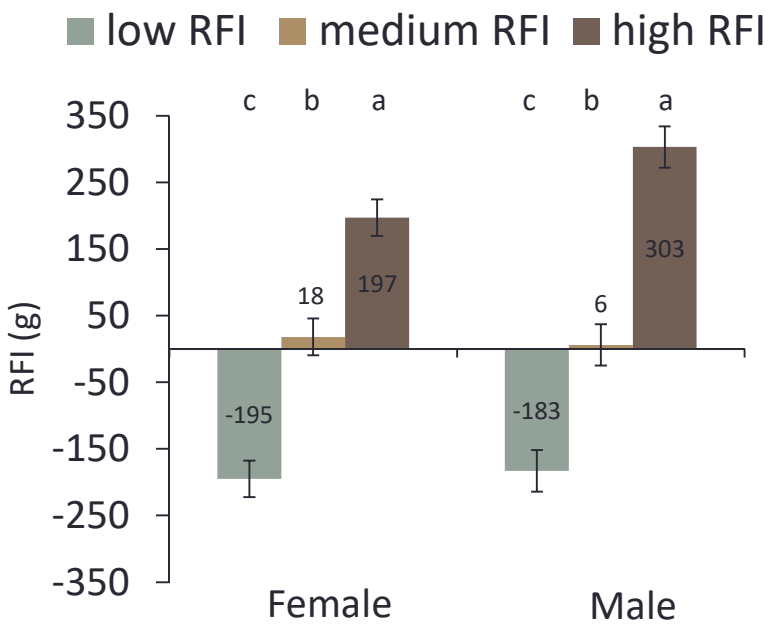
Experimental Design

- 2 identical trials at 2 different locations
  - Austria (University of Veterinary Medicine Vienna, VMU) & Northern Ireland (Agri-Food and Biosciences Institute, AFBI)
  - All conditions kept as constant as possible
- 3 batches with 52 / 64 (♀ & ♂) Cobb 500FF broiler chickens each
- Weighing monitored weekly, feed intake daily
- Selection of chickens on d36 (VMU) / d38 (AFBI) using RFI
- Top and bottom 10% considered as low and high RFI
  - ➢ 15 low RFI / 17 high RFI ♀
  - ➢ 19 low RFI / 18 high RFI ♂

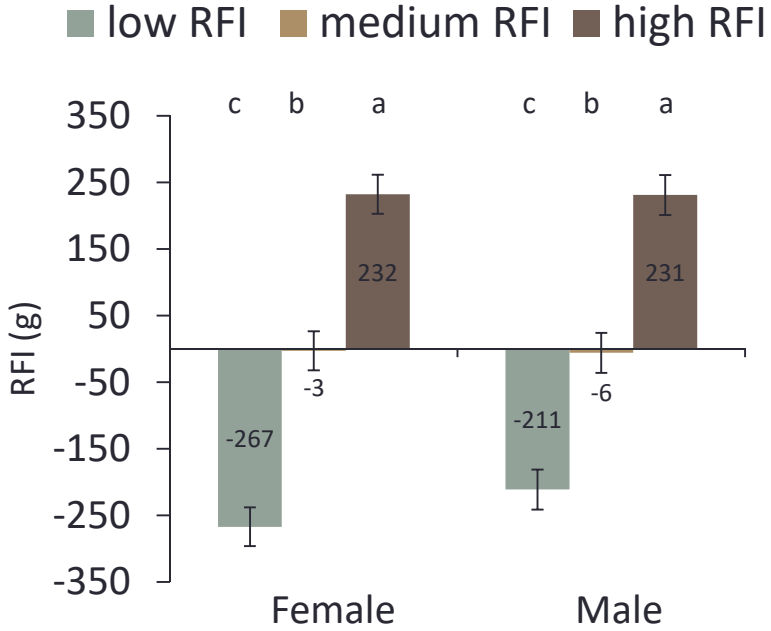


Feed efficiency data

VMU



AFBI



RFI values ⇨ similar in chickens of both locations

Within sex: a, b, c: $P<0.05$

Gut microbiota analysis

Faeces
(*n*=69)

Ileal digesta
(*n*=68)

Caecal digesta
(*n*=68)

Gut microbiota analysis

Gut microbiota analysis

Sample

↓

DNA extraction

↓

16S rRNA **Illumina MiSeq** sequencing

↓

Bioinformatical analysis using **Qiime v 1.9.1**

- Classification in Operational Taxonomic Units (OTUs) at 97% similarity
- Threshold of 10 sequences per OTU
  - 6,404 OTUs

↓

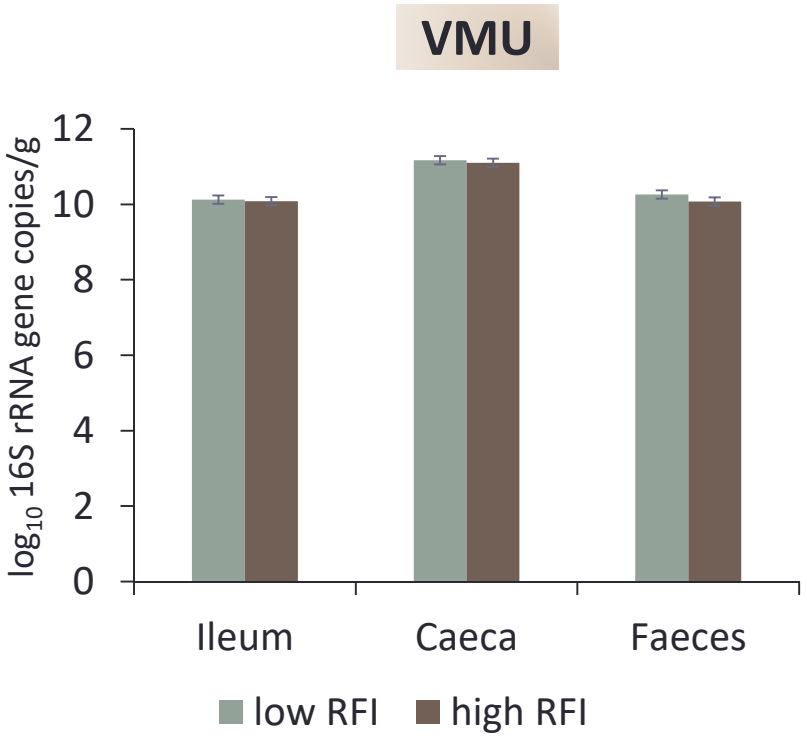
Predictive functional profiling of microbial communities (KEGG* pathways) using **PICRUSt**

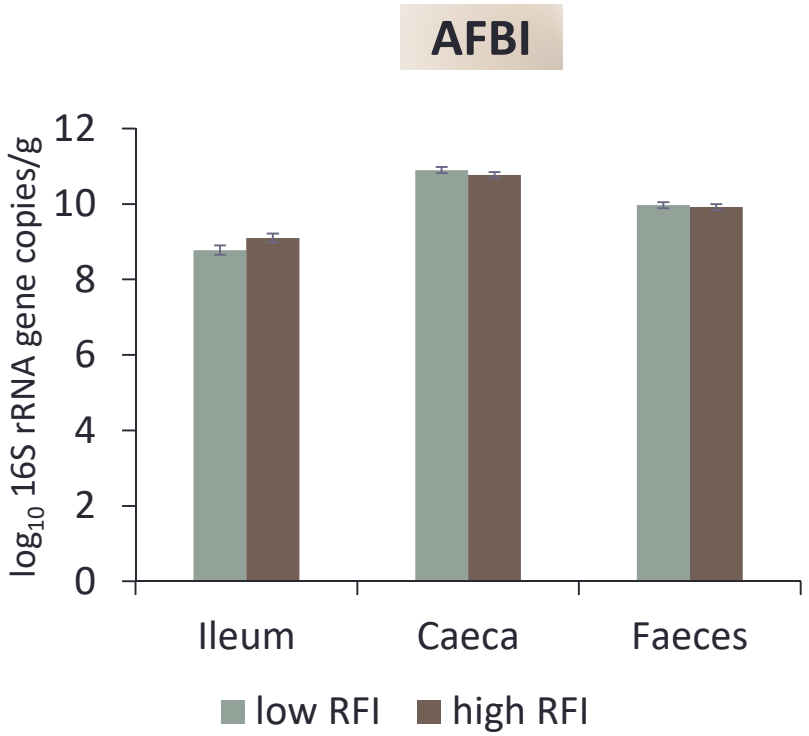
↓

ANOVA using PROC MIXED (**SAS v 9.3**)

*KEGG = Kyoto Encyclopedia of Genes and Genomes

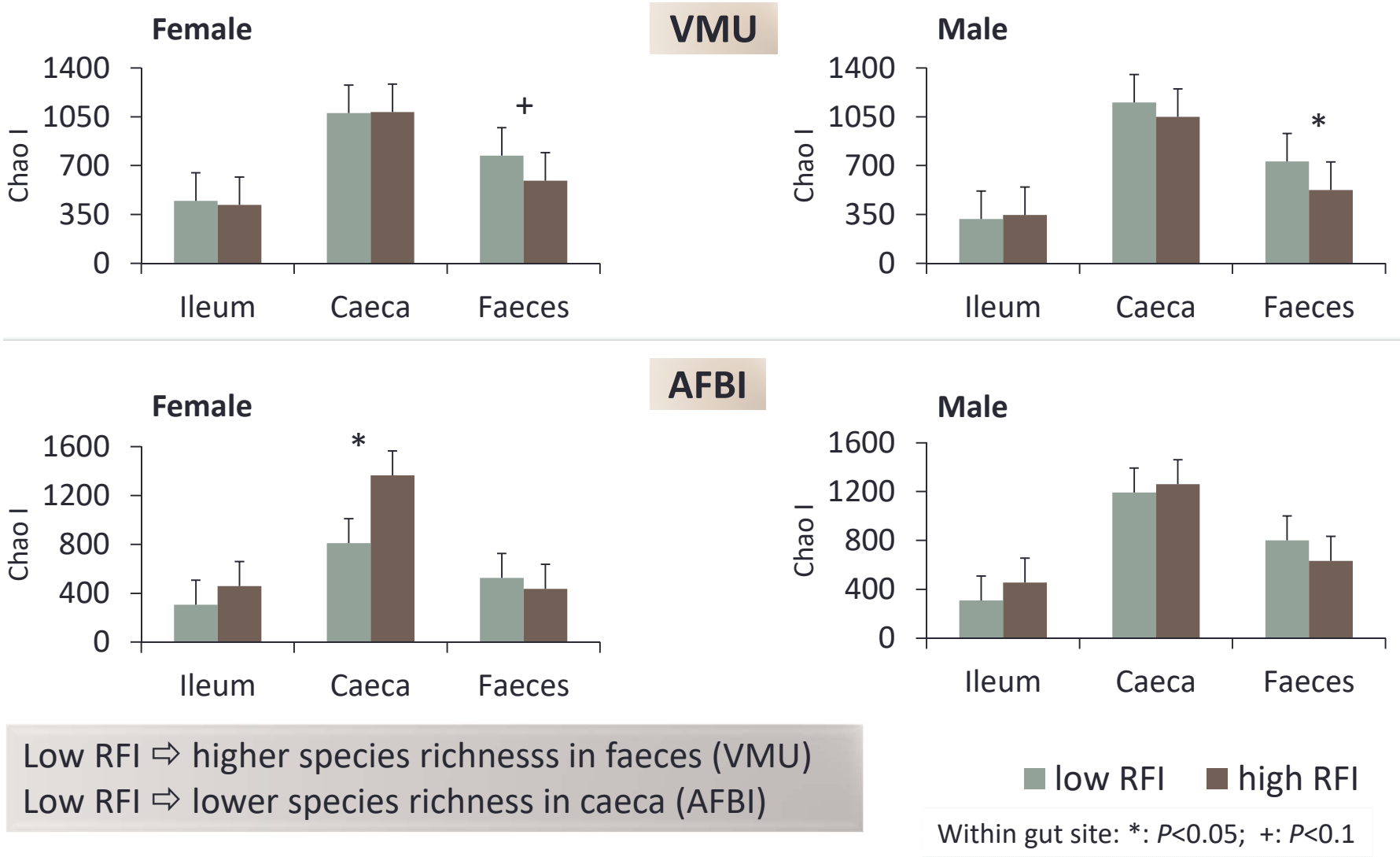
Total bacterial 16S rRNA gene copies in low and high RFI chickens





Log_{10} 16S rRNA gene copies/g ⇨ no RFI effect
⇨ no sex effect
⇨ no location effect

Alpha diversity – Chao I index



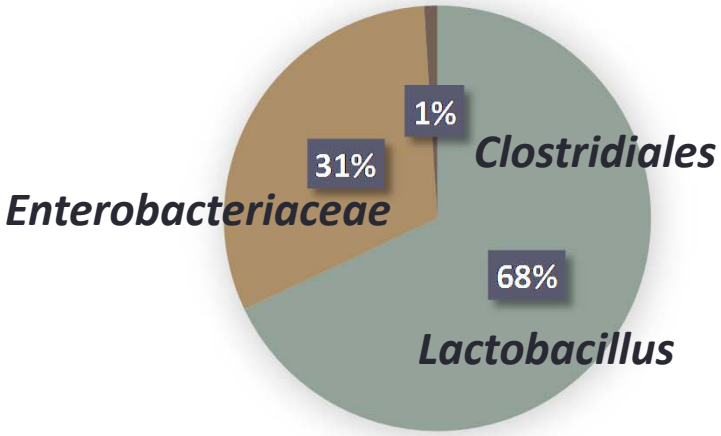
Low RFI ⇨ higher species richnesss in faeces (VMU)
Low RFI ⇨ lower species richness in caeca (AFBI)

Within gut site: *: $P<0.05$; +: $P<0.1$

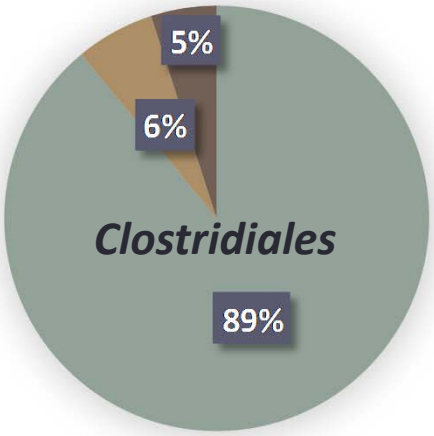
Phylogenetic distribution in the chickens´ gut

In total: **7 Phyla + 1 unassigned kingdom**

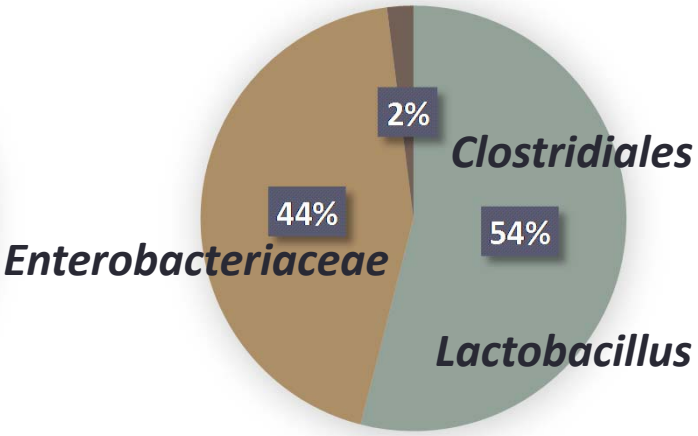
Ileal digesta*



Caecal digesta*



Faeces*



*VMU + AFBI

Tenericutes, Bacteroidetes, Actinobacteria, ?, Cyanobacteria, Verrucomicrobia

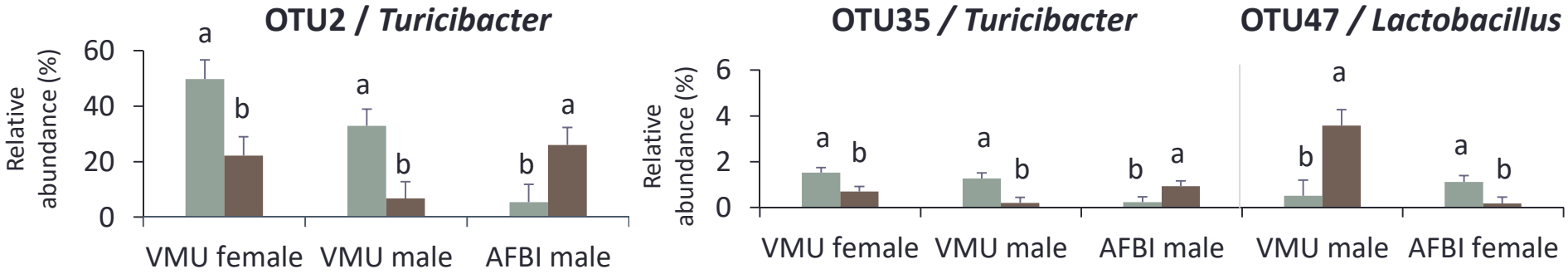
Firmicutes Proteobacteria Others

OTUs differing in low and high RFI chickens

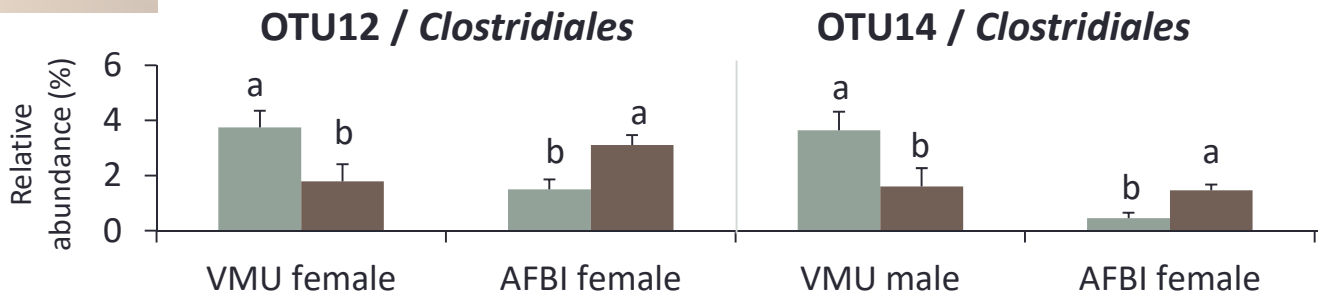
VMU	AFBI
30 OTUs differing	36 OTUs differing
18 in ♀	24 in ♀
7 ↑in low RFI	7 ↑in low RFI
11 ↑in high RFI	17 ↑in high RFI
17 in ♂	16 in ♂
6 ↑in low RFI	8 ↑in low RFI
11 ↑in high RFI	8 ↑in high RFI

RFI effects differ between locations

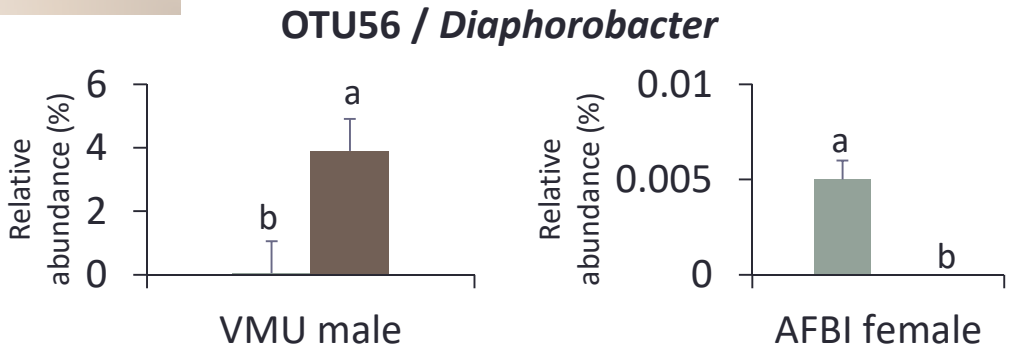
Ileal digesta



Caecal digesta



Faeces

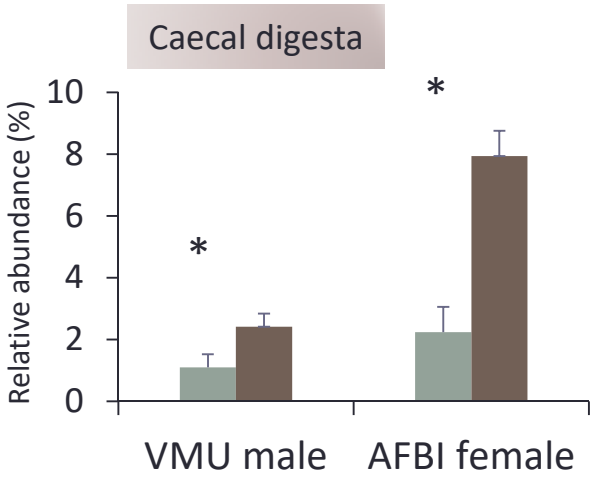


low RFI high RFI

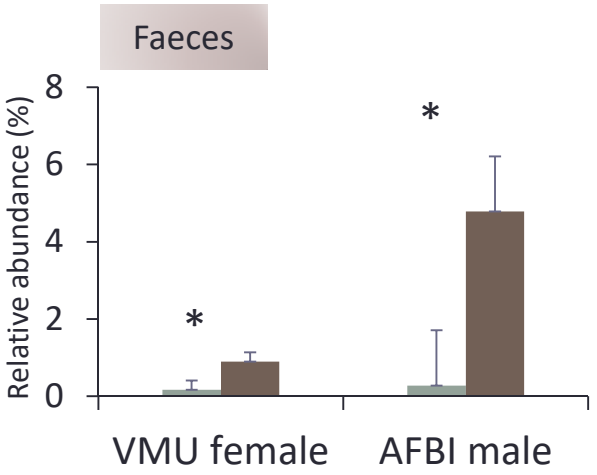
Within sex & location: a, b: $P<0.05$

Taxa showing same RFI effects at both locations

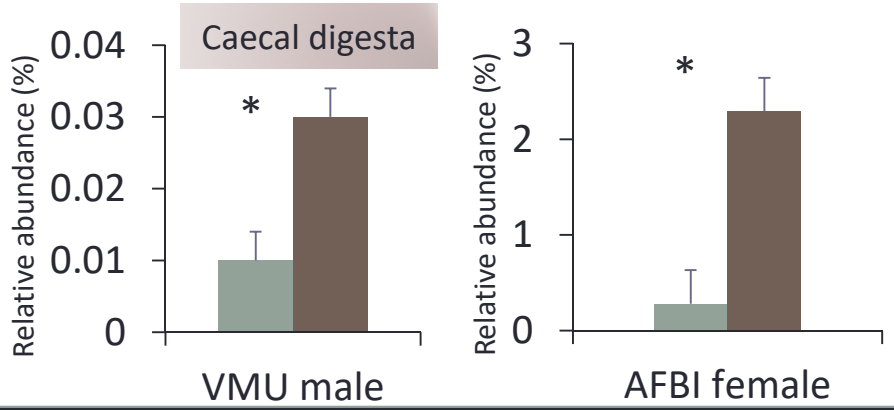
_RF39_ unclassified family



Clostridiaceae



OTU32 / *Clostridiales*



No taxa associated with low RFI at both locations

low RFI high RFI

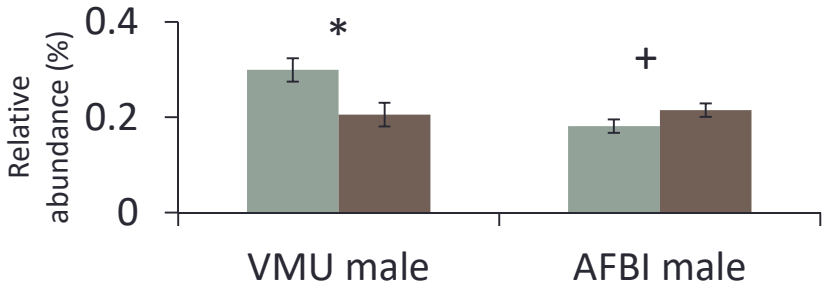
Within sex & location: *: $P<0.05$

Predictive functional profiling of microbial communities – KEGG pathways

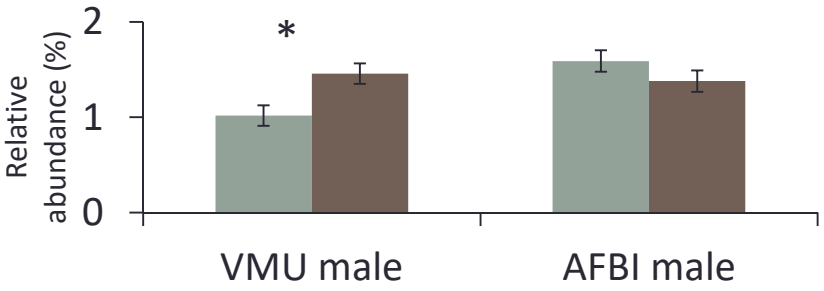
Within sex & location: *: *P*<0.05; +: *P*<0.1

Ileal digesta

Amino acid metabolism

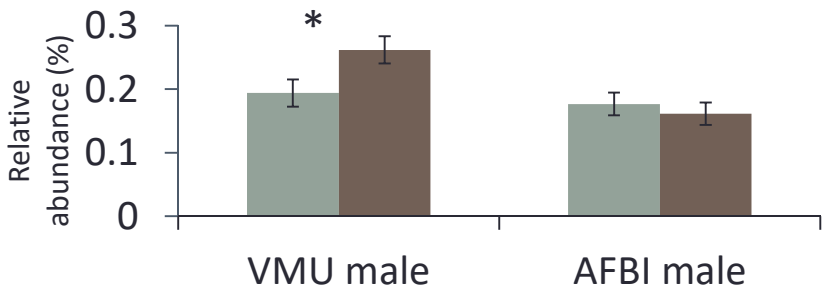


Aminoacyl-tRNA biosynthesis

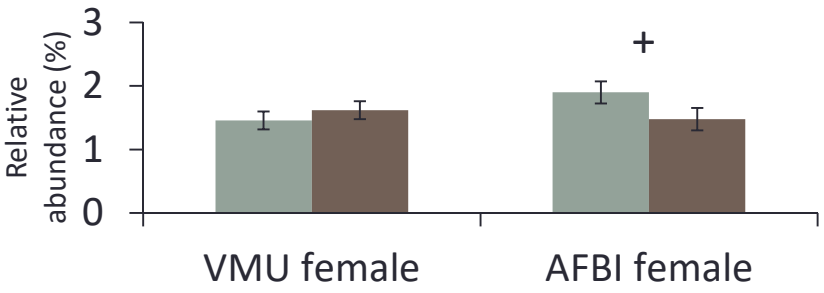


Faeces

Aminobenzoate degradation



Bacterial motility proteins

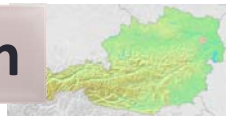


Predicted metagenomics ⇨ differences between locations

low RFI high RFI

Conclusion

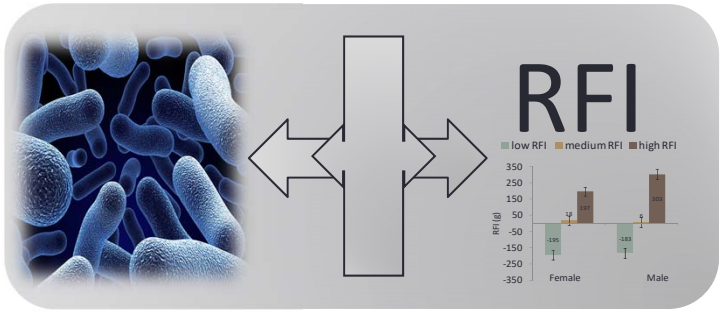
Location



Presence / absence of specific taxa, interaction of gut microbiota

Handling of chickens and environmental microbes

Sex



Difficult to clearly assign specific bacterial taxa and metabolic functions to chicken's RFI

Acknowledgements

- Lab team of the Institute of Animal Nutrition and Functional Plant Compounds, Vetmeduni and AFBI Hillsborough
- Cobb-Vantress
- Devenish Animal Nutrition, Garant-Tiernahrung GmbH
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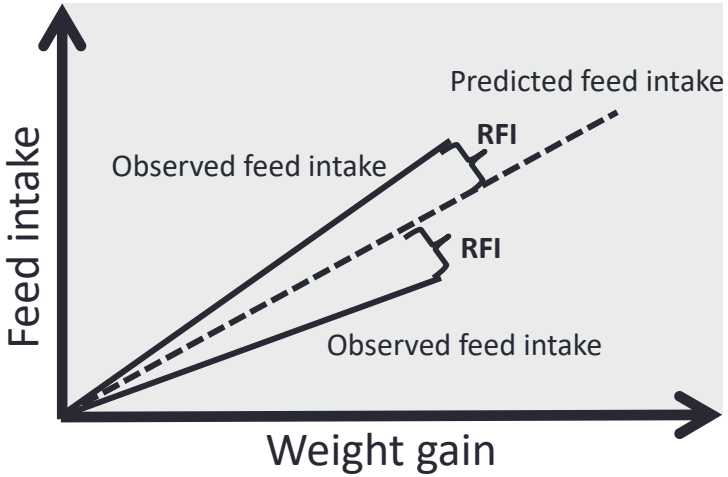
Calculation of RFI

RFI = difference between observed and predicted feed intake, with lower RFI values indicating greater energy efficiency

$$\textbf{RFI} = TFI\,[a + b_1 \times BW^{0.75} + b_2 \times TBWG],$$

where a is the intercept and b_1 and b_2 are partial regression coefficients of total feed intake (TFI), $BW^{0.75}$ and total body weight gain (TBWG), respectively



Diet composition

Item	Starter day 1-10 of life	Grower day 11-21 of life	Finisher day 22-end of experiment
Ingredient, g/kg (as-fed basis)			
Corn	612	660	679
Soybean meal	331	282	260
Soybean oil	17.5	20.6	27.7
Limestone flour	11.0	9.8	7.0
Salt	2.0	2.0	2.3
Dicalcium phosphate	16.1	15.0	13.4
Vitamin-Mineral-Premix	11.0	11.0	10.0
Analyzed composition (g/kg DM)			
DM	926	923	914
ME (MJ/kg), calculated	13.7	14.3	14.6
Crude protein	243	223	216

Qiime workflow

1. split_libraries_fastq.py

- Max number of consecutive low quality base calls allowed before truncating a read [default: 3]
- Min number of consecutive high quality base calls to include a read (per single end read) as a fraction of the input read length [default: 0.75]
- Maximum number of N characters (ambiguous base) allowed in a sequence to retain it – this is applied after quality trimming, and is total over combined paired end reads if applicable [default: 0]
- The maximum unacceptable Phred quality score [default: 3]
- Maximum number of errors in barcode [default: 1.5]

→ 8543871 sequences
→ sequence lengths (mean +/- std): 556.9701 +/- 16.1065)

2. identify_chimeric_seqs.py (usearch61)

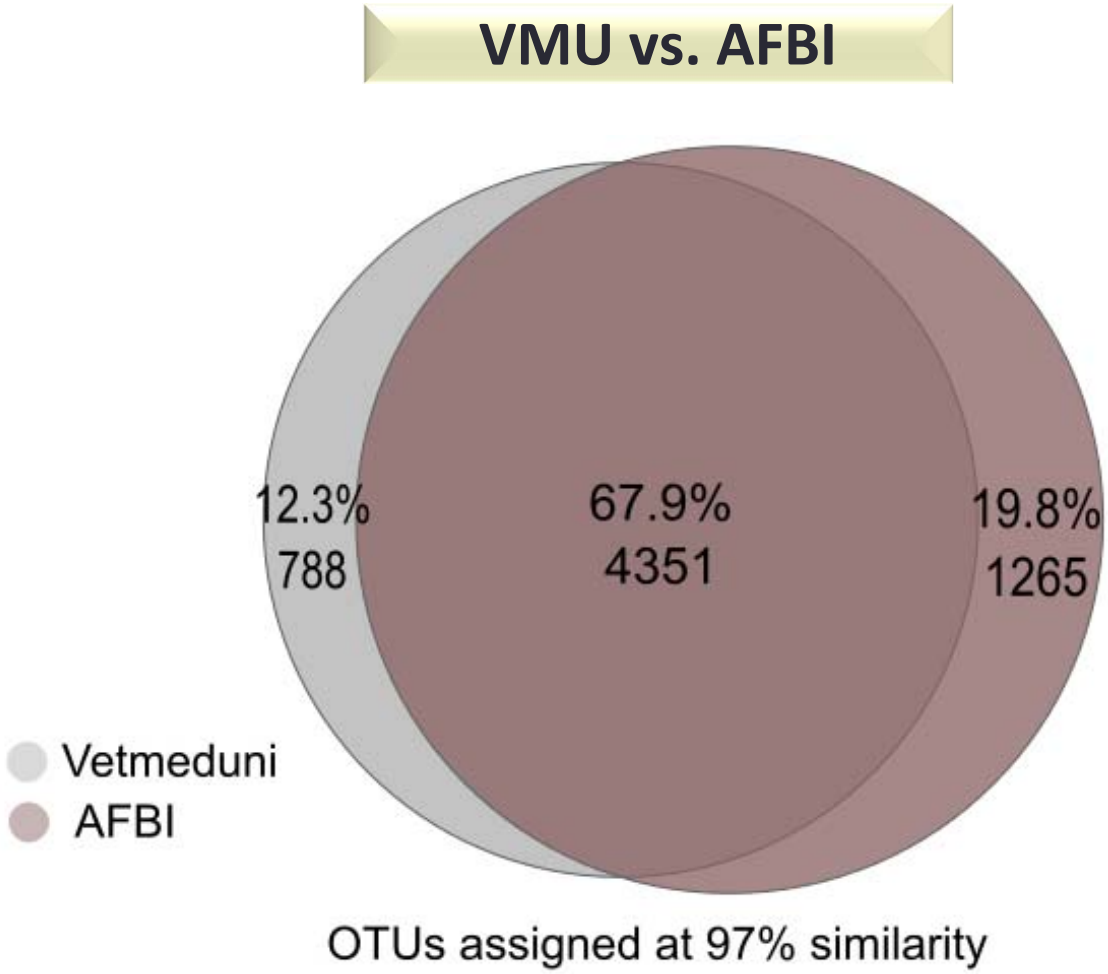
- Minimum length of sequence allowed [default: 64]
- Word length value [default: 8]
- Max_accepts value [default: 1]
- Max_rejects value [default: 8]

→ 8518063 sequences

3. pick_open_reference_otus.py (uclust)

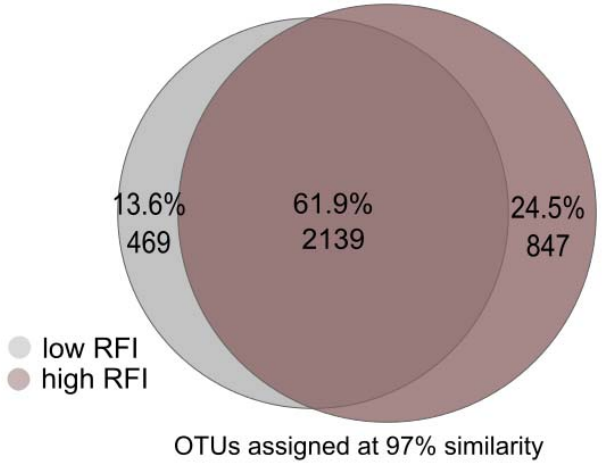
→ 7726361 sequences

Similarity between microbial communities

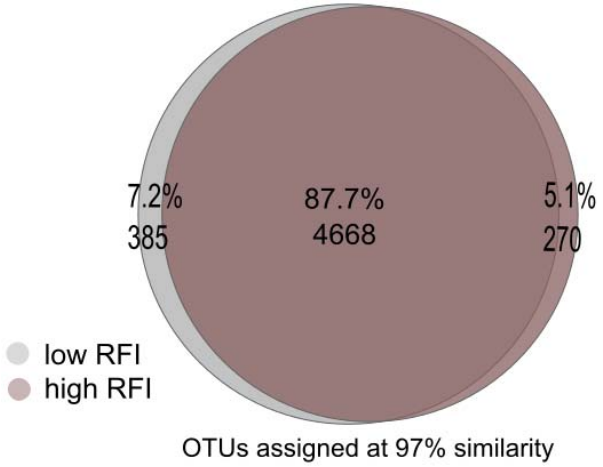


Similarity between microbial communities

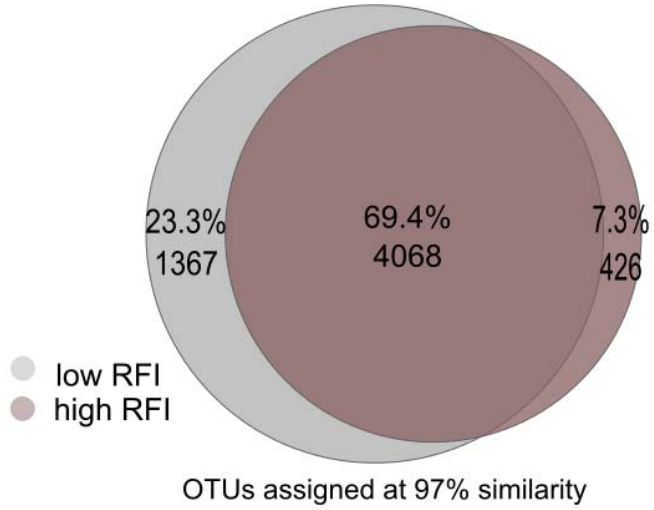
Ileum: low RFI vs. high RFI



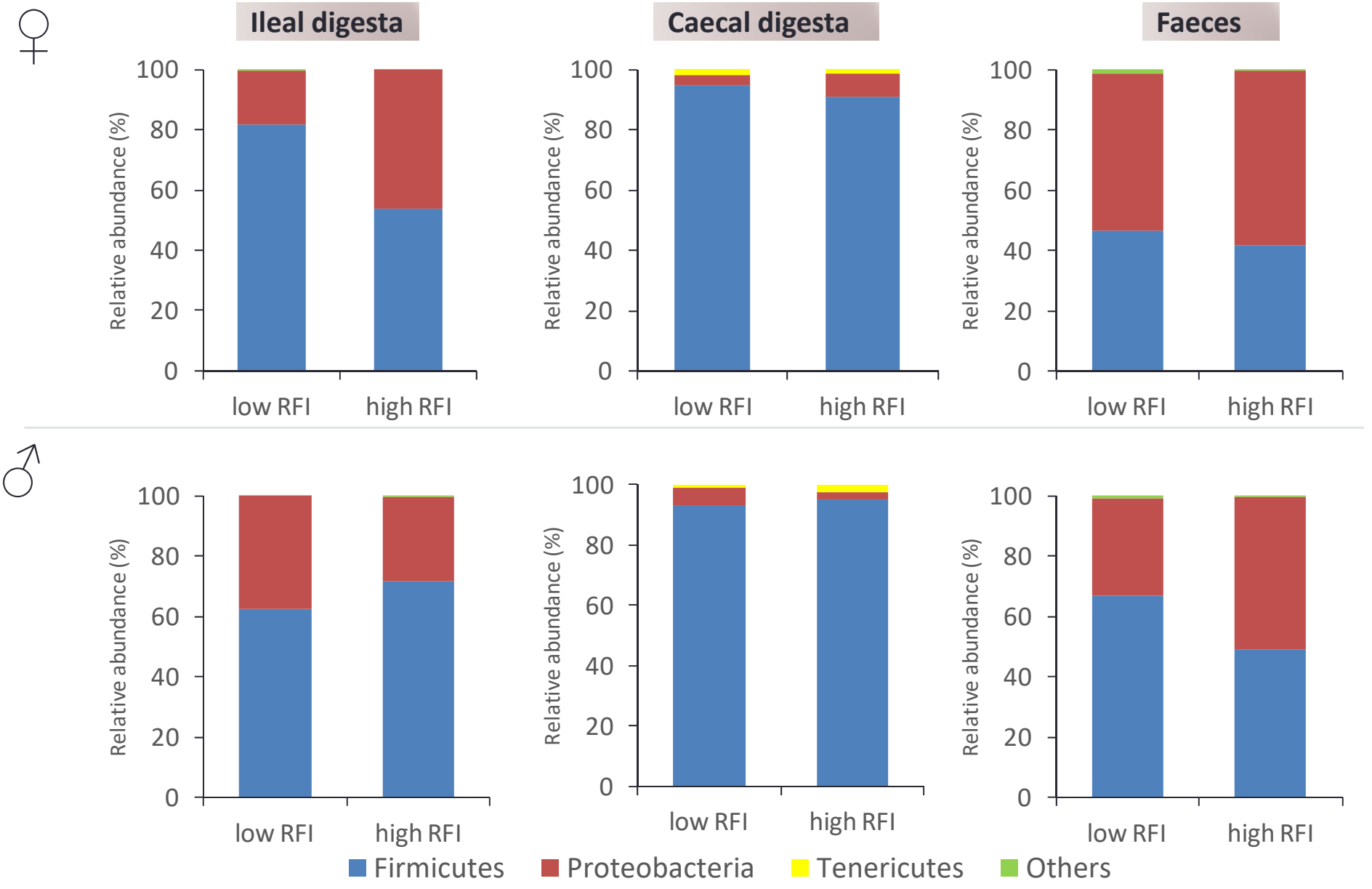
Caecum: low RFI vs. high RFI



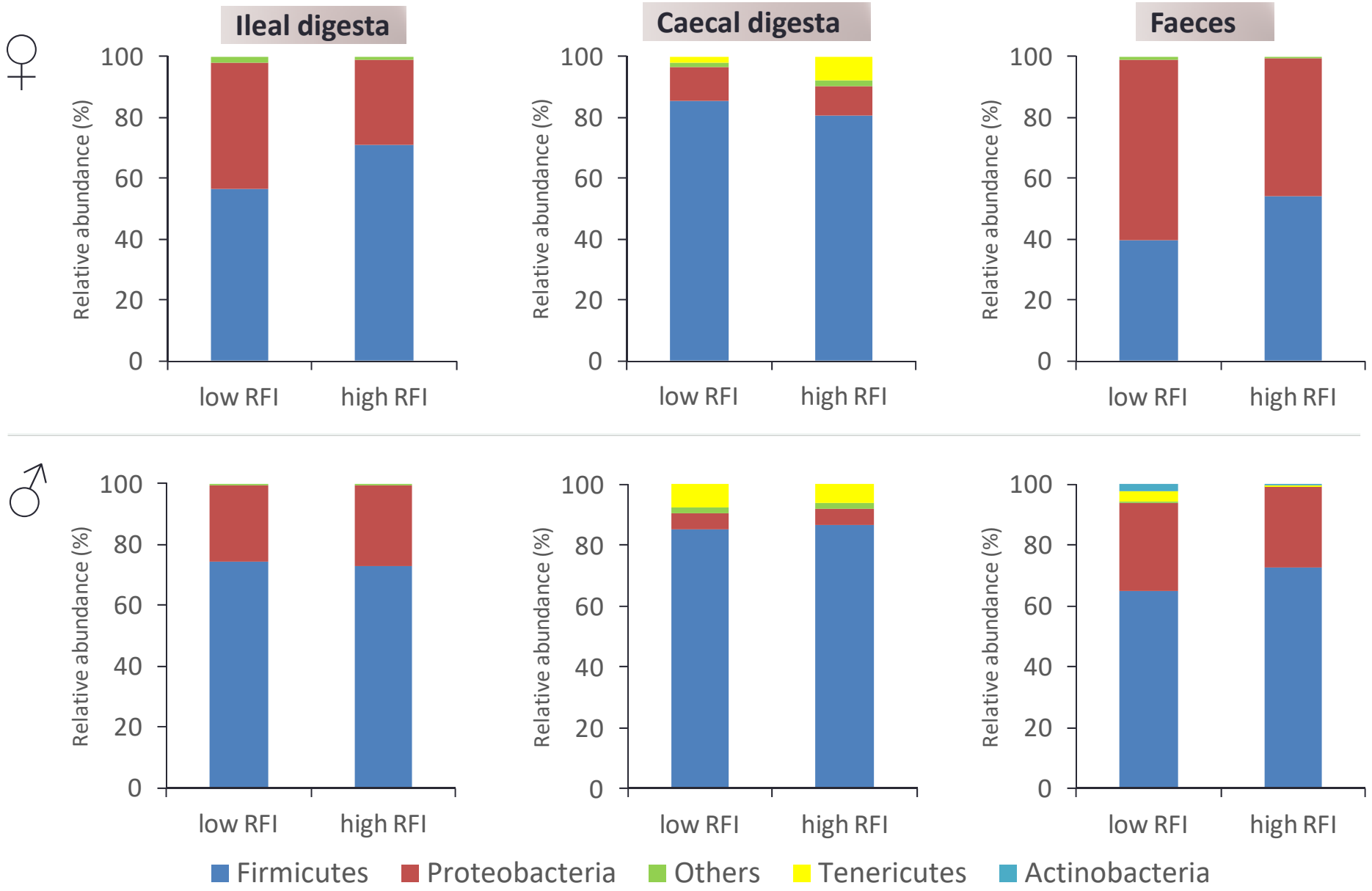
Faeces: low RFI vs. high RFI



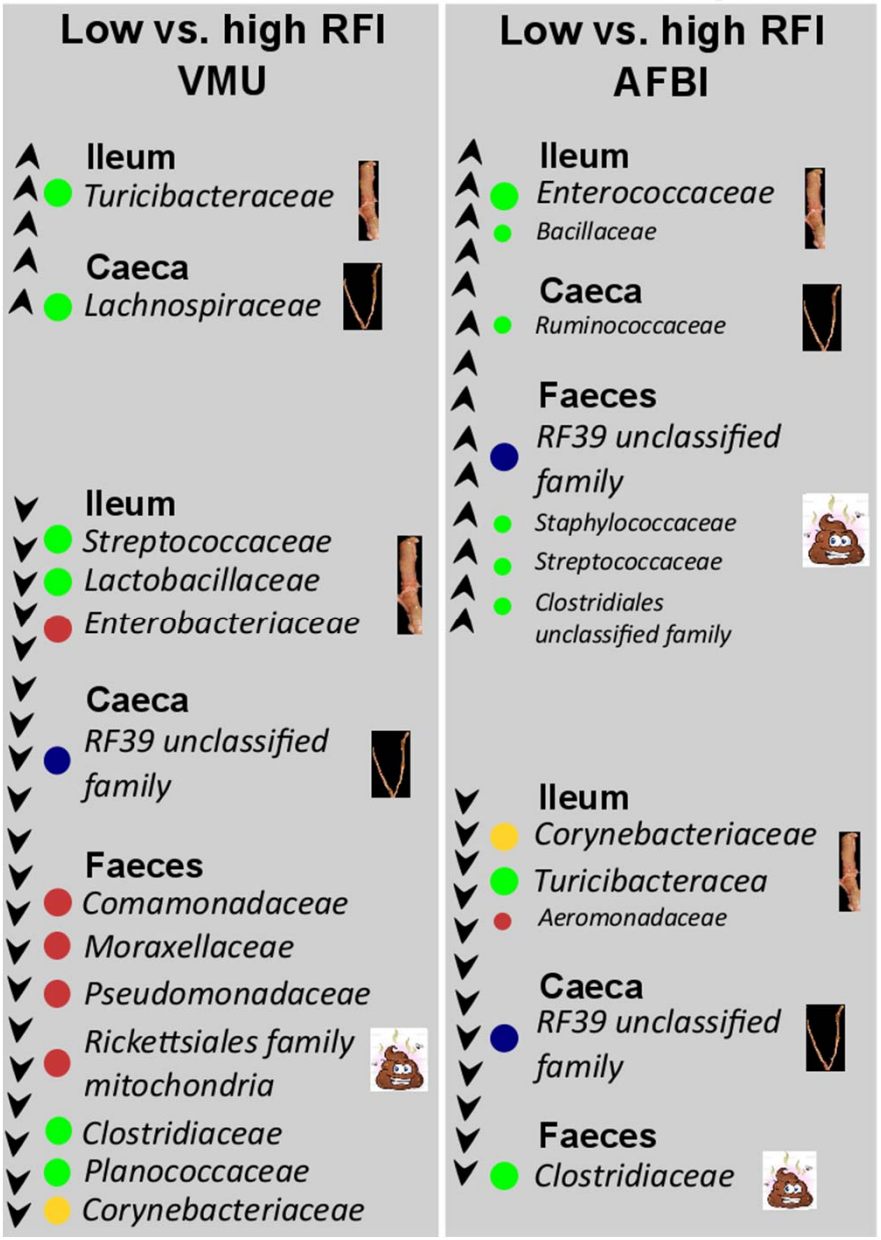
Phylogenetic distribution in the chicken´s gut – VMU



Phylogenetic distribution in the chicken´s gut – AFBI



Bacterial families differing in low and high RFI chickens



RFI ⇨ differences between locations

- *P*<0.05
- 0.05<*P*<0.1

- Phylum *Firmicutes*
- Phylum *Proteobacteria*
- Phylum *Tenericutes*
- Phylum *Actinobacteria*

Microbial composition – OTU-level

Ileal digesta dominated by members of

- *Lactobacillus*
- *Enterobacteriaceae*
- *Clostridiales*


poultrydisease.ir

- OTU2 (*Turicibacter*) highest abundant OTU in VMU chickens, 2nd highest abundant OTU in AFBI chickens

Caecal digesta almost exclusively occupied by members of *Clostridiales*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Faeces dominated by members of

- *Clostridiales*
- *Lactobacillus*
- *Enterobacteriaceae*


depositphotos.com

- OTU2 (*Turicibacter*) 2nd highest abundant OTU in VMU and AFBI chickens