

**Gut microbiome and incidence of foodborne pathogens are affected by diet in pasture-raised chickens**J.M. Lourenco<sup>1</sup>, M.J. Rothrock Jr.<sup>2</sup>, Y.M. Sanad<sup>3</sup>, F.L. Fluharty<sup>1</sup> and T.R. Callaway<sup>1</sup><sup>1</sup>University of Georgia, Athens, GA, 30602, USA, <sup>2</sup>USDA-ARS, Athens, GA, 30605, USA, <sup>3</sup>University of Arkansas, Pine Bluff, AR, 71601, USA; jefao@uga.edu

Using a farm-to-fork approach, this study aimed to examine the effects of feeding two distinct diets – one containing soy (SB), and one soy-free (SF) – on the gut microbiome of pasture-raised chickens. Microbial DNA was extracted, and 16r RNA gene sequencing was performed on caecal contents of 1-day-old birds, faeces collected from their pastures (at 4, 7 and 12-weeks-old), caecal contents obtained at the slaughterhouse, whole carcass rinses (WCR) obtained at the slaughterhouse, and WCR obtained from final products (after carcasses were frozen for 1 month). Results showed that the number of observed OTUs significantly increased ( $P < 0.001$ ) as birds fed the SF diet aged (from 1-day-old to 12-weeks old); however, no significant differences ( $P = 0.11$ ) in the number of OTUs were observed as SB-fed birds aged. Relative abundance of the genera *Oscillospira*, *Faecalibacterium* and *Ruminococcus* increased as birds aged ( $P \leq 0.001$ ), whereas the abundance of *Lactobacillus* decreased ( $P \leq 0.02$ ) in both SB and SF diets. For bacterial groups regarded as foodborne pathogens, there was no difference ( $P \geq 0.15$ ) in *Salmonella* abundance between SB and SF in any sample type; however, SF broilers had a lower abundance of *Campylobacter* during their entire lifecycle. This effect was particularly pronounced in the faecal material collected when birds were 12-weeks-old ( $P = 0.003$ ) as well as in the WCR obtained from the final product ( $P = 0.04$ ). *Acinetobacter* abundance was also lower ( $P = 0.05$ ) in the WCR from birds consuming SF. These findings demonstrate that the feed offered to pasture-raised broilers can significantly affect their gut microbial populations. Moreover, they suggest that the use of soy-free diets may represent a viable strategy to reduce contamination of carcasses in pasture-raised chicken production systems since the incidence of foodborne pathogens such as *Campylobacter* and *Acinetobacter* were reduced in broilers fed SF.

## Session 26

## Theatre 9

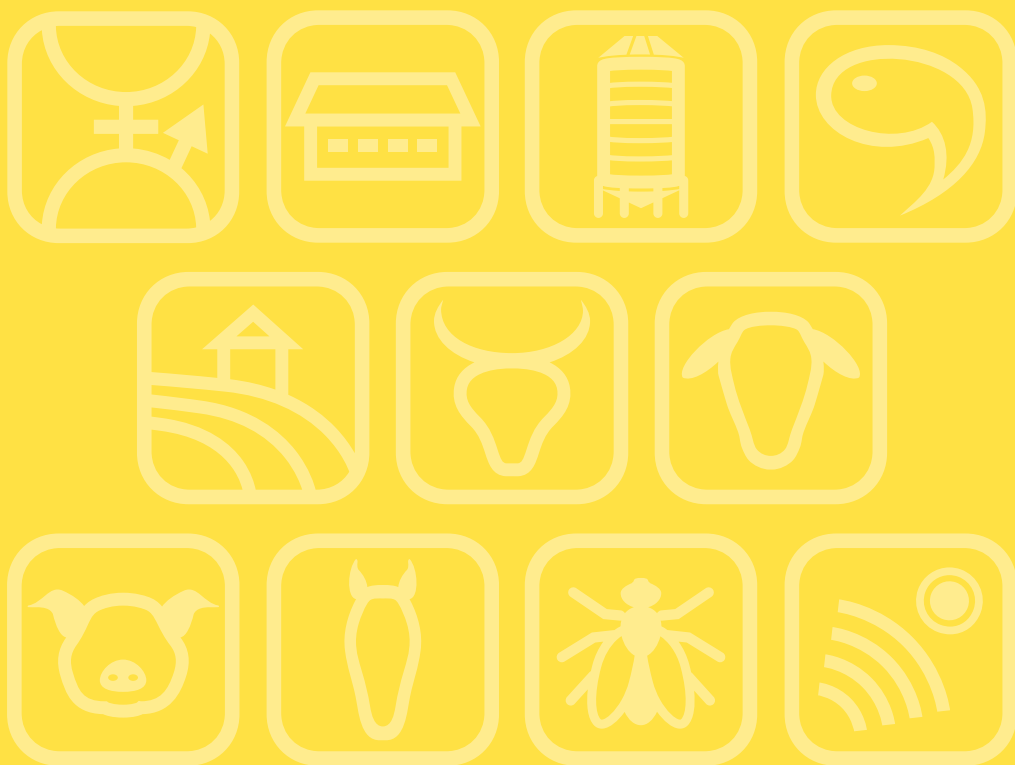
**Selection for intramuscular fat modifies microbial genome for energy metabolic routes in rabbit gut**

M. Martínez-Álvarez, A. Zubiri-Gaitán, C. Casto-Rebollo, A. Blasco and P. Hernández

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High intramuscular fat content (IMF) of meat has an economic interest, as it improves meat quality. An exceptional experimental material to study the genetic basis of IMF deposition was developed in rabbits, consisting of two lines from a common genetic origin and divergently selected for IMF. In this study we investigate the changes on the cecum microbes genome, identifying the metabolic routes modified by the selection. A correlated response on the microbiome would imply a link between host genome and its metagenome. Cecum content samples were collected from 16 and 17 rabbits from each of the two selected lines for high (H) and low (L) IMF, in the 10<sup>th</sup> generation of selection. Samples were sequenced with an Illumina NextSeq instrument. Metagenomic data were pre-processed using the centred log ratio transformation due to its compositional nature. Distinct microbial genes between the two lines were identified using Projection to Latent Structures Discriminant Analysis (PLS-DA), selecting variables with a variable importance for projection (VIP)  $> 1$ . The final model included 105 microbial genes, showing a classification ability after cross-validation ( $Q^2$ ) of 91.6%. These 105 microbial genes coded for proteins involved in several metabolic pathways, being one of the most represented the energy metabolism pathway (18 genes). These genes showed different relative abundance in H and L lines. For instance, two genes involved in methane metabolism, and two involved in the metabolism of mannose and fructose specific carbohydrates were more abundant in the H line, whereas genes related lipopolysaccharides biosynthesis were more abundant in L line. Besides, when analysing only these 18 genes in PLS-DA model, its explanatory power was high ( $Q^2 = 55.7\%$ ). This preliminary analysis highlights the importance of the gut microbiome in the muscular lipid deposition in rabbits and shows that selection for IMF led to a correlated response in their metagenomics profile, particularly in the energy metabolic routes. These results imply a link between the genes of the individual and the genes of its gut microbes.

# Book of Abstracts of the 70<sup>th</sup> Annual Meeting of the European Federation of Animal Science



**Book of abstracts No. 25 (2019)**  
**Ghent, Belgium,**  
**26-30 August 2019**



**Correlated responses to selection for IMF on microbial genomes in rabbits (using compositional data analysis techniques)**

Martínez-Álvaro M., Zubiri-Gaitán A., Hernández P. and Blasco A.

ICTA, Universitat Politècnica de València

70<sup>th</sup> Annual Meeting of EAAP  
27<sup>th</sup> August 2019, Ghent



**WHY INTRAMUSCULAR FAT?**

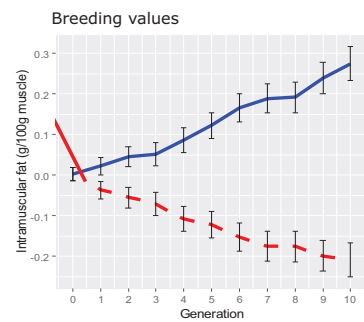


**WHY IN RABBITS?**



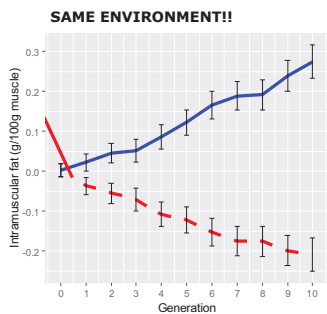
**Divergent selection for IMF**

**Response to selection for IMF**



0.5 SD per gen

**Divergent selection for IMF**



CORRELATED RESPONSES TO SELECTION FOR IMF IN OTHER TRAITS

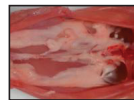


PHENOTYPIC DIFFERENCES HIGH LINE - LOW LINE

**Divergent selection for IMF**

**CORRELATED RESPONSES IN MEAT QUALITY TRAITS**

Carcass fat



**Divergent selection for IMF**

**CORRELATED RESPONSES IN MEAT QUALITY TRAITS**

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**Divergent selection for IMF**

**CORRELATED RESPONSES IN MEAT QUALITY TRAITS**

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IMF in other muscles



**Divergent selection for IMF**

**CORRELATED RESPONSES IN MEAT QUALITY TRAITS**

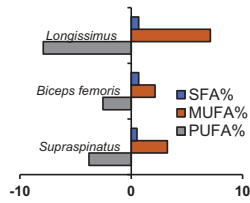
Carcass fat



IMF in other muscles



Meat Fatty Acid profile



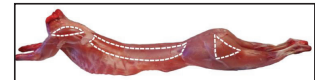
**Divergent selection for IMF**

**CORRELATED RESPONSES IN MEAT QUALITY TRAITS**

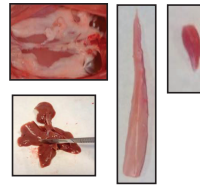
Carcass fat



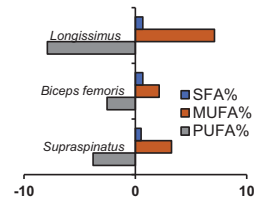
IMF in other muscles



Lipogenic activity



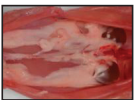
Meat Fatty Acid profile



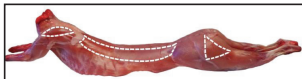
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**CORRELATED RESPONSES IN MEAT QUALITY TRAITS**

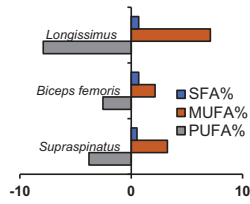
Carcass fat



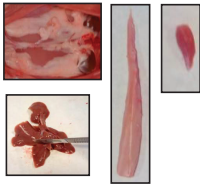
IMF in other muscles



Meat Fatty Acid profile



Lipogenic activity



Adipocytes size

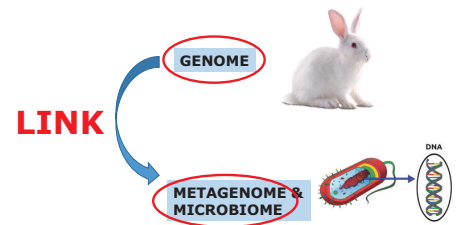


Liver size



**OBJECTIVE**

**CORRELATED RESPONSES IN MICROBIALS GENOME AND COMMUNITIES**



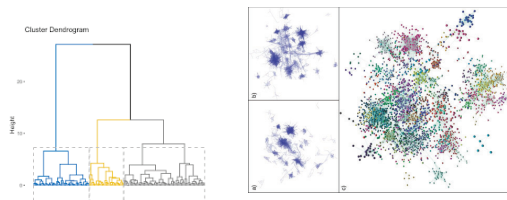
## SOME IDEAS ABOUT COMPOSITIONAL DATA ANALYSIS...

## STATISTICAL ANALYSIS OF METAGENOMIC DATA



### MULTIVARIATE ANALYSIS DATA BASED ON COV OR CORRELATIONS

PLS, Clusters, Correlation networks, Multiple regression, etc



## STATISTICAL ANALYSIS OF METAGENOMIC DATA INTERPRETATION PROBLEM



### ABSOLUTE VALUES

IMF (g/100 g)	1	2	5
Microbial gene A	1	10	40
Microbial gene B	4	6	8

↑ IMF ↑ Microbial Genes A & B

## STATISTICAL ANALYSIS OF METAGENOMIC DATA INTERPRETATION PROBLEM

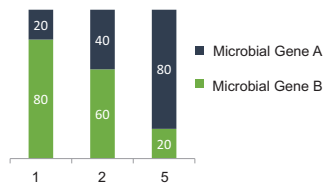


### ABSOLUTE VALUES

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### RELATIVE VALUES



## STATISTICAL ANALYSIS OF METAGENOMIC DATA INTERPRETATION PROBLEM

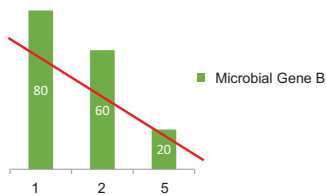


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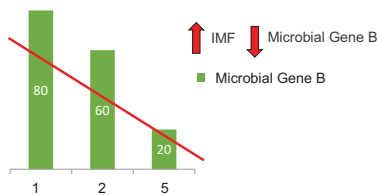


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### RELATIVE VALUES



## CONFUSION



**STATISTICAL ANALYSIS OF METAGENOMIC DATA**



**COMPOSITIONAL DATA**

SIMPLEX  
restricted space

Aitchison geometry  
(not real)

**STATISTICAL ANALYSIS OF METAGENOMIC DATA**



**COMPOSITIONAL DATA**

SIMPLEX  
restricted space

Aitchison geometry  
(not real)

Transformations  
Log ratio  
Clr  
Ailr  
Ilr

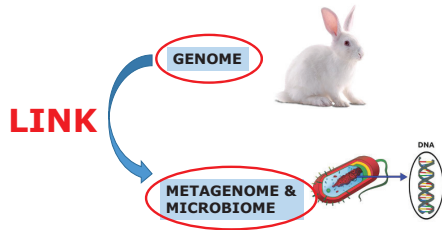


From Simplex to Real Space

Metrics in Euclidean  
geometry of real space

**OBJECTIVE**

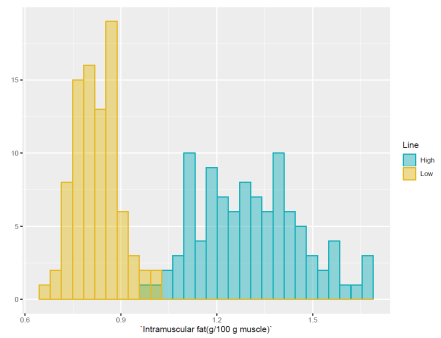
**CORRELATED RESPONSES IN  
MICROBIALS GENOME AND  
COMMUNITIES**



**M & M**

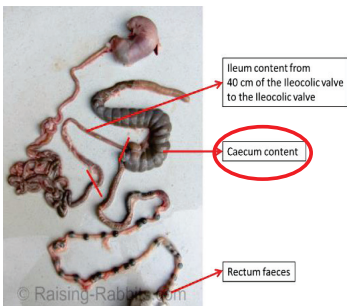
**ANIMALS**

Distribution of IMF fat data in the 10<sup>th</sup> generation



**M & M**

**METAGENOMIC MEASUREMENTS**



N = 33 ( 16 H & 17 L)

- Illumina NextSeq
- Reads (2 x 150 bp)
- KEGG database

**M & M**

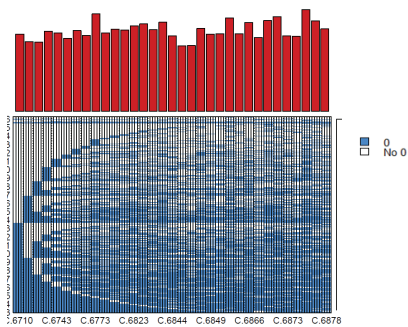
**STATISTICAL PIPELINE**

1. "0s" study and replacement (zCompositions R)

M & M

STATISTICAL PIPELINE

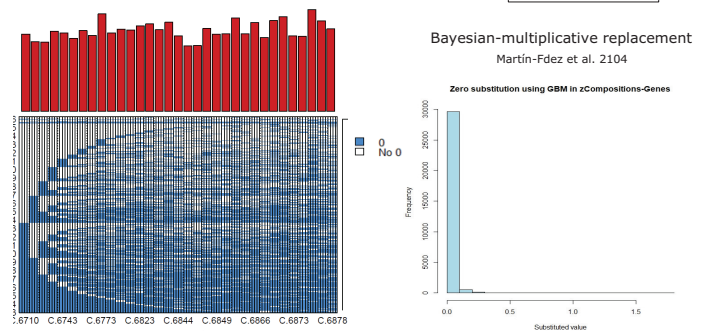
1. "0s" study and replacement (zCompositions R) 16 % of 0s



M & M

STATISTICAL PIPELINE

1. "0s" study and replacement (zCompositions R) 16 % of 0s



M & M

STATISTICAL PIPELINE

1. "0s" study and replacement (zCompositions R)
2. Estimation of relative abundances

M & M

STATISTICAL PIPELINE

1. "0s" study and replacement (zCompositions R)
2. Estimation of relative abundances
3. **Weighed** centered log ratio transformation

$$\text{clr}(x_i) = \text{bg} \frac{x_i}{G} = \text{bg} x_i - \frac{1}{n} \sum_1^n \text{bg}(x_i)$$

M & M

STATISTICAL PIPELINE

1. "0s" study and replacement (zCompositions R)
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$$\text{clr}(x_i) = \text{bg} \frac{x_i}{G} = \text{bg} x_i - \frac{1}{n} \sum_1^n \text{bg}(x_i)$$

**Weighed mean**

1/n → Mean relative abundance

M & M

STATISTICAL PIPELINE

1. "0s" study and replacement (zCompositions R)
2. Estimation of relative abundances
3. **Weighed** centered log ratio transformation
4. Selection of discriminant microbial genes with DA - PLS
  - VIP
  - Regression coefficients

**RESULTS**

**DISCRIMINATING MICROBIAL GENES DA-PLS**

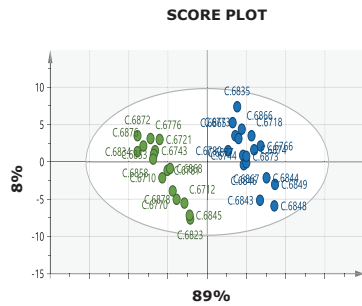
PC	R <sup>2</sup>	Q <sup>2</sup>
1	0.89	0.84
2	0.97	0.91
3	0.99	0.92

251 GENES INCLUDED IN THE MODEL

SELECTION FOR IMF  
MODIFIED THE MICROBIAL  
GENOME OF THE LINES!!!

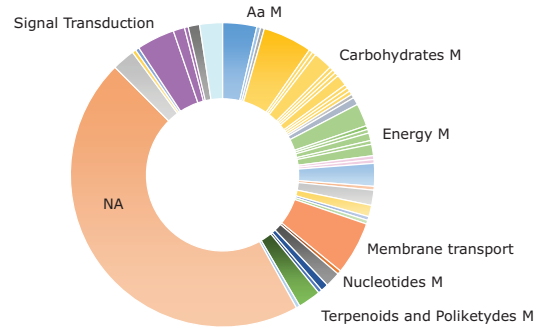


LINK  
HOST GENOME-METAGENOME



**RESULTS**

**WHICH 251 MICROBIAL GENES?**



**RESULTS**

**LOG TRANSFORMATION EFFECTS IN PRACTISE  
ANALYSIS WITHOUT wclr TRANSFORMATION**

**RESULTS**

**LOG TRANSFORMATION EFFECTS IN PRACTISE  
ANALYSIS WITHOUT wclr TRANSFORMATION**

**PREDICTION ABILITY OF DA-PLS MODEL**

	PC	R <sup>2</sup> Y	Q <sup>2</sup>	Number of genes
Rel. abundances	3	0.987	0.917	227

**RESULTS**

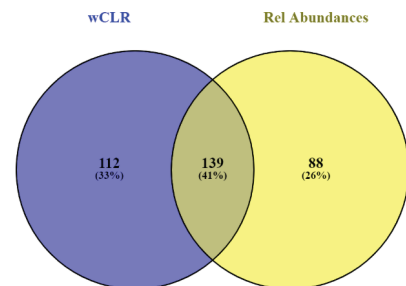
**LOG TRANSFORMATION EFFECTS IN PRACTISE  
ANALYSIS WITHOUT wclr TRANSFORMATION**

**PREDICTION ABILITY OF DA-PLS MODEL**

	PC	R <sup>2</sup> Y	Q <sup>2</sup>	Number of genes
Rel. abundances	3	0.987	0.917	227
wClr	3	0.987	0.922	251

**RESULTS**

**LOG TRANSFORMATION EFFECTS IN PRACTISE  
ANALYSIS WITHOUT wclr TRANSFORMATION  
OVERLAPPING BETWEEN MICROBIAL GENES SELECTED BY DA-PLS**



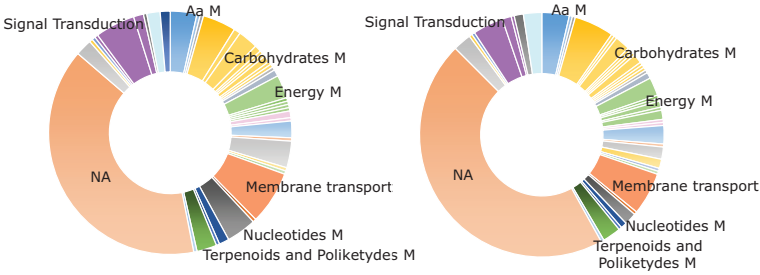


**RESULTS**  
**LOG TRANSFORMATION EFFECTS IN PRACTISE**  
**ANALYSIS WITHOUT wCLR TRANSFORMATION**

**OVERLAPPING BETWEEN MICROBIAL GENES SELECTED BY DA-PLS**

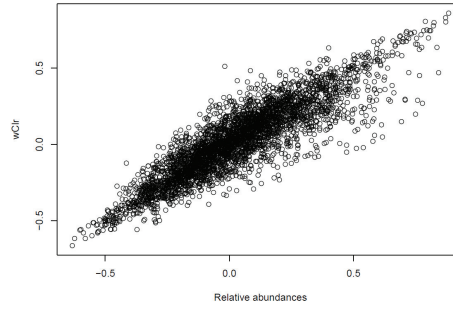
**RELATIVE ABUNDANCES**

**wCLR**



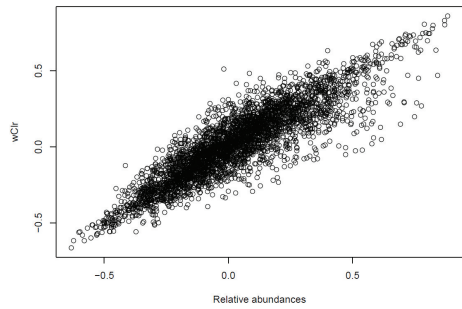
**RESULTS**  
**LOG TRANSFORMATION EFFECTS IN PRACTISE**

**CORRELATIONS BETWEEN MICROBIAL GENES IN wCLR VS. REL. ABUNDANCES**



**RESULTS**  
**LOG TRANSFORMATION EFFECTS IN PRACTISE**

**CORRELATIONS BETWEEN MICROBIAL GENES IN wCLR VS. REL. ABUNDANCES**



**Rank of relative abundances**  
 $[1 \times 10^{-6}, 1 \times 10^{-1}]$

**CONCLUSIONS**



**Selection for IMF modifies the microbials genome**

**Link genome - metagenome**

**Correlated responses in genes involved in several metabolic pathways, as carbohydrates and energy metabolism**

**In our study, log transformations did not change results**



**THANKS!**

