UNIVERSITY OF HOHENHEIM

Gut microbiota and efficiency traits in poultry and pig breeding

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Animal Genetics to address Food Security & Sustainability

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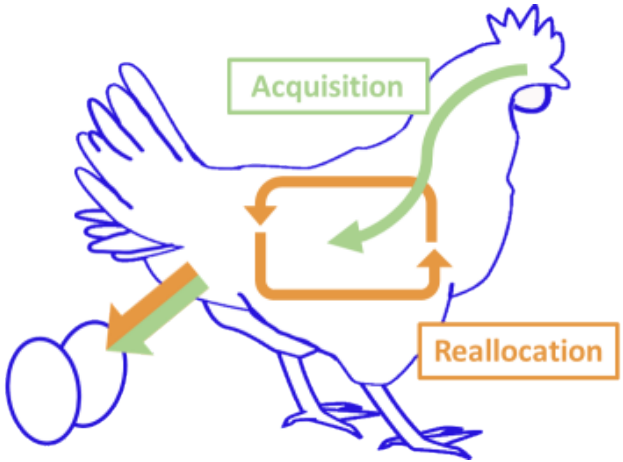
Importance of the Microbiome in Animal Breeding

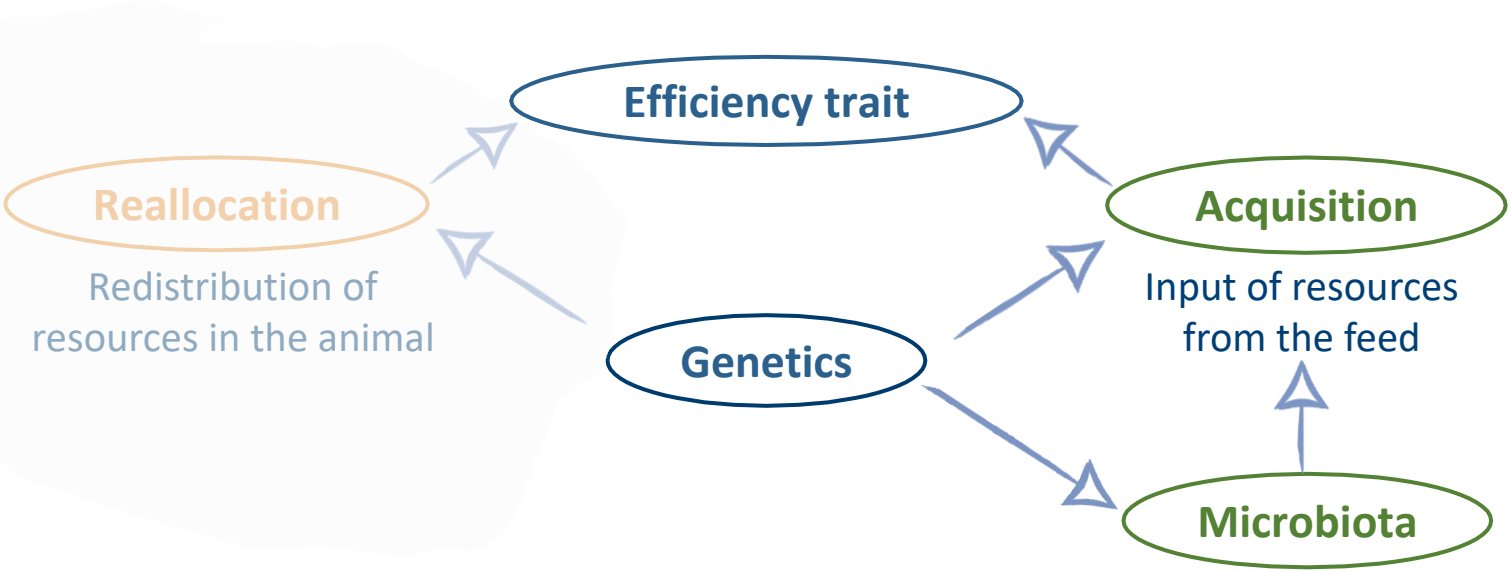
- Efficiency traits consist of two general components: **digestive** and **metabolic** efficiency *(Puilett et al. 2016, Martin et al. 2021)*
- Increasing efficiency: acquisition and reallocation effects *(Puilett et al. 2016)*.



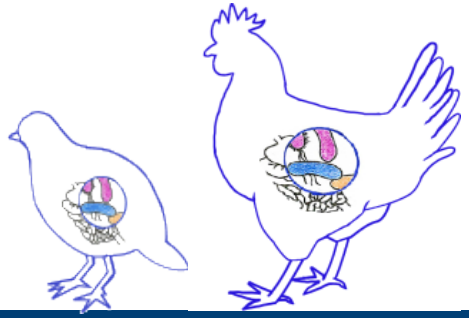
Redistribution of resources in the animal, may compromise animal health *(Huber 2018)*

Importance of the Microbiome in Animal Breeding

Acquisition effects: Influenced by the composition of the microbiota in the gut.



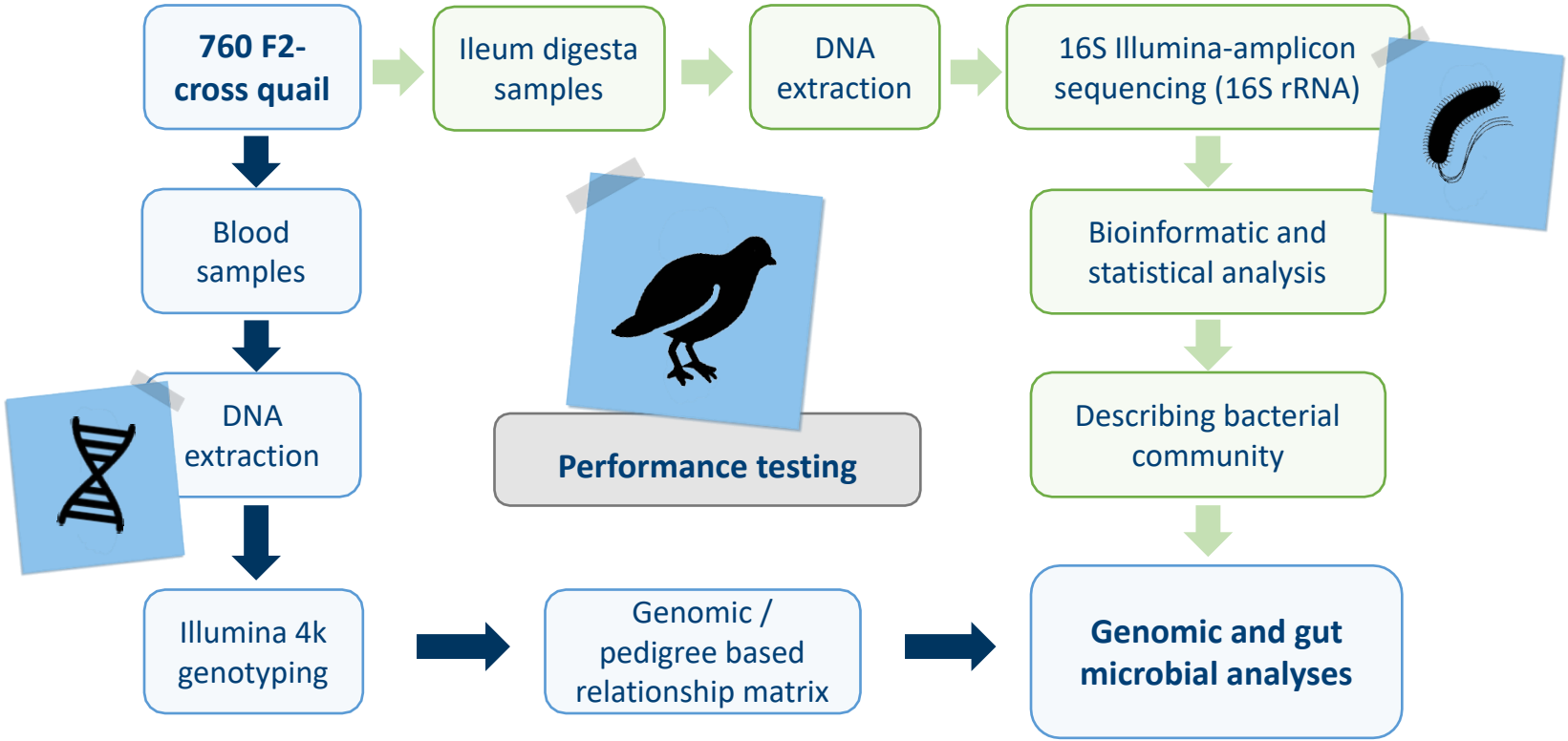
POFOWL
Genetics Physiology Microbiome Nutrition



Results from Japanese Quail Projects

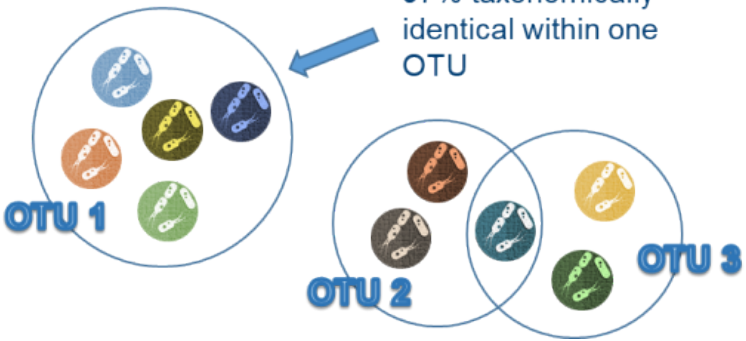
for details see Vollmar et al. 2020 and Haas et al. 2022

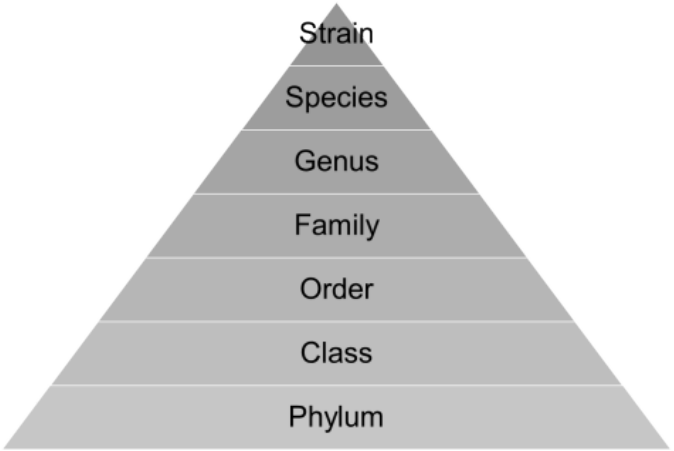
Study Design *(Vollmar et al. 2021, Borda-Molina et al. 2020)*



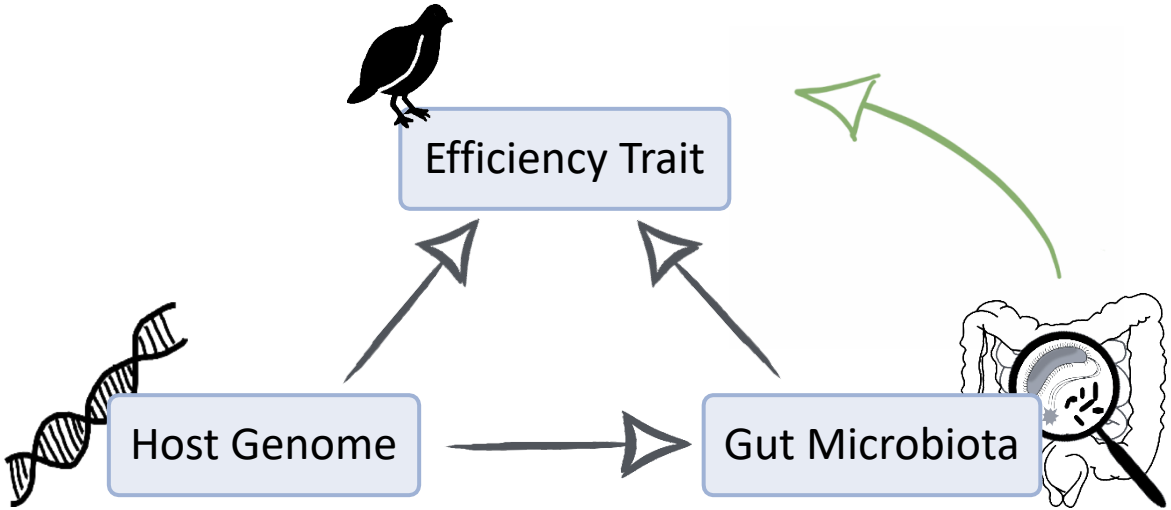
Microbial Community

- Characterization of the microbiota in the ileum samples by amplicon sequencing of the 16S rRNA → **Genus and OTU data set**
- Reads were clustered at 97% identity into operative taxonomic units (OTU)





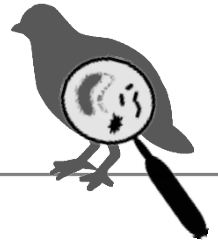
Microbial Influence on Efficiency Traits?



Microbiability of Quantitative Traits *(Difford et al. 2018)*

Microbial linear mixed models:

$$\mathbf{y} = \boldsymbol{\mu}\,\mathbf{1} + \mathbf{Z_{td}td} + \mathbf{m} + \mathbf{e}$$



efficiency traits **y**

- P utilization (PU)
- Feed intake (FI)
- Body weight gain (BWG)
- Feed per gain (F:G)

$\mathbf{y}$	= observation vector (efficiency traits)
$\boldsymbol{\mu}\mathbf{1}$	= the overall mean and the vector of ones
$\mathbf{td}$	= vector of random test day effects $\mathbf{td} \sim N(0, \mathbf{I}\sigma^2_{td})$
$\mathbf{Z_{td}}$	= design matrix of random effect
$\mathbf{m}$	= vector with random microbiota effects $\mathbf{m} \sim \mathrm{N}(0, \mathbf{M}\sigma^2_m)$ and σ^2_m microbial variance
$\mathbf{M}$	= microbial relationship matrix
$\mathbf{e}$	= vector of residuals

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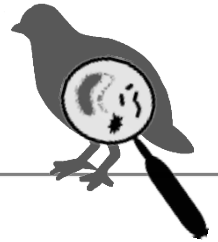

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OTU information of the quail

Microbiability of Quantitative Traits *(Difford et al. 2018)*

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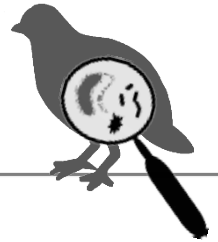

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OTU information of the quail

efficiency traits **y**

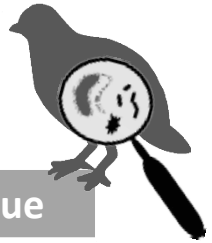
- P utilization (PU)
- Feed intake (FI)
- Body weight gain (BWG)
- Feed per gain (F:G)

$$m^2 = \frac{\sigma^2_m}{\sigma^2_p}$$

with

$$\sigma^2_p = \sigma^2_m + \sigma^2_{td} + \sigma^2_e$$

Estimated Microbiability *(Vollmar et al. 2020)*



Trait	m^2	SE	p value
P utilization	0.15	0.05	< 0.001
Feed intake	0.09	0.04	< 0.001
Body weight gain	0.18	0.05	< 0.001
Feed conversion ratio	0.27	0.05	< 0.001

→ Moderate microbiabilities show that microbiota play a substantial role in efficiency traits.

Microbiome-wide Association Analyses (MWAS)

From the microbial model solutions, the effect of the single OTUs on the phenotypic traits were approximated by back-solving the model *(Camarinha-Silva et al. 2017)*

$$\mathbf{y} = \boldsymbol{\mu}\,\mathbf{1} + \mathbf{Z}_{\mathbf{td}}\mathbf{td} + \mathbf{m} + \mathbf{e}$$

$$\hat{\boldsymbol{u}} = \frac{\boldsymbol{X'M^{-1}\hat{m}}}{\boldsymbol{N}}$$

$\hat{u}$ = Estimated OTU effect

X' = $N * n$ Matrix, with N OTUs and n animals

M^{-1} = Inverted microbial relationship matrix

$\hat{m}$ = Estimated random effect of the animal

efficiency traits $\mathbf{y}$

- P utilization (PU)
- Feed intake (FI)
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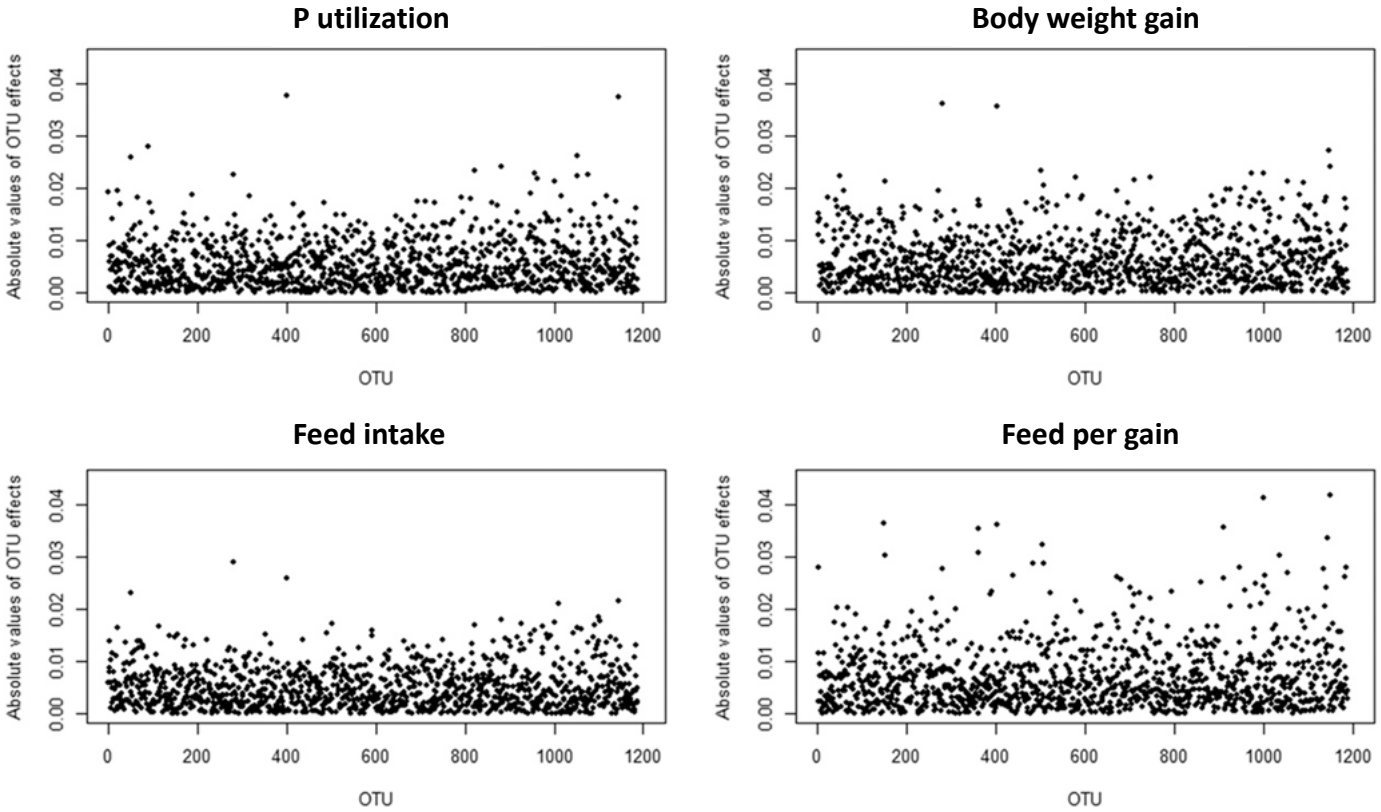
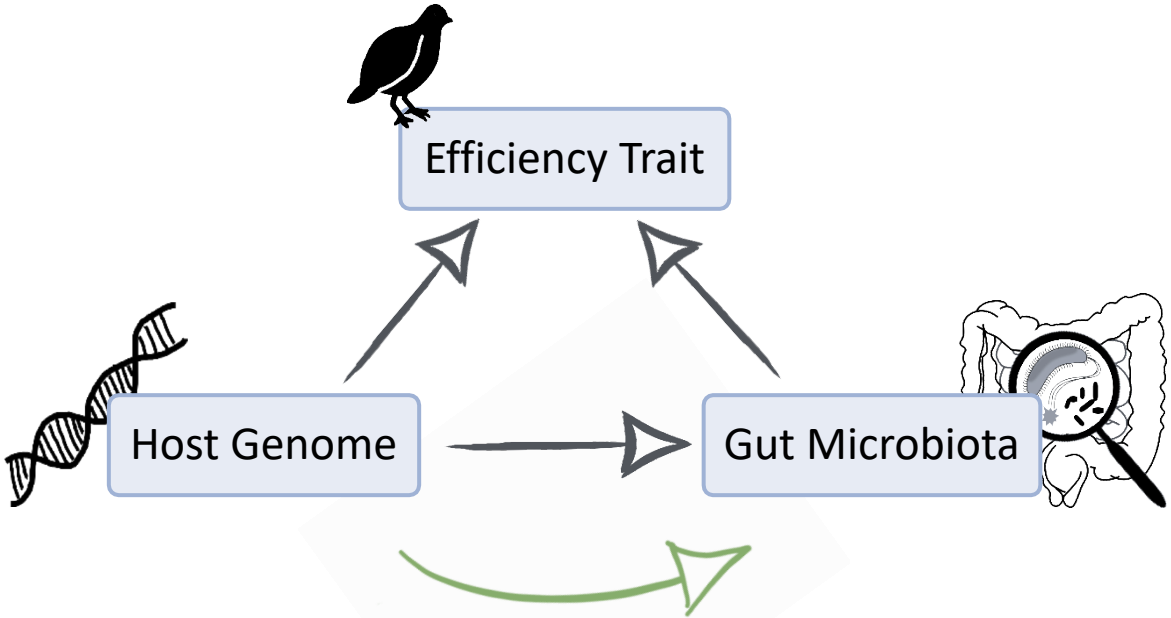
- Feed per gain (F:G)

Microbiome-wide Association Analyses (MWAS)



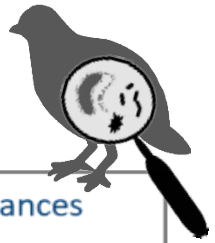
Host Genome Influence on Gut Microbiota?



Heritabilities of Microbial Genera

Heritabilities (h^2) of each microbial genus, with:

$$\mathbf{y} = \boldsymbol{\mu}\,\mathbf{1} + \mathbf{Z_{td}td} + \mathbf{Z_a a} + \mathbf{e}$$



$\mathbf{y}$	= Vector of Box-Cox transformed relative abundances
$\boldsymbol{\mu}\,\mathbf{1}$	= Trait mean and vector of ones
$\mathbf{td}$	= Vector of random test day effects $\mathbf{td} \sim N(0, \mathbf{I}\sigma^2_{td})$
$\mathbf{a}$	= Vector of random animal effects $\mathbf{a} \sim N(0, \mathbf{A}\sigma^2_a)$
$\mathbf{A}$	= Pedigree-based relationship matrix
$\mathbf{Z_{td}}$, $\mathbf{Z_a}$	= Design matrices of random effects
$\mathbf{e}$	= Vector of the residuals

Genera filtering of 0.01% relative abundances: 59 genera for analysis

$$h^2 = \frac{\sigma^2_a}{\sigma^2_p}$$

with

$$\sigma^2_p = \sigma^2_a + \sigma^2_{td} + \sigma^2_e$$

Heritabilities of Ileal Microbial Genera

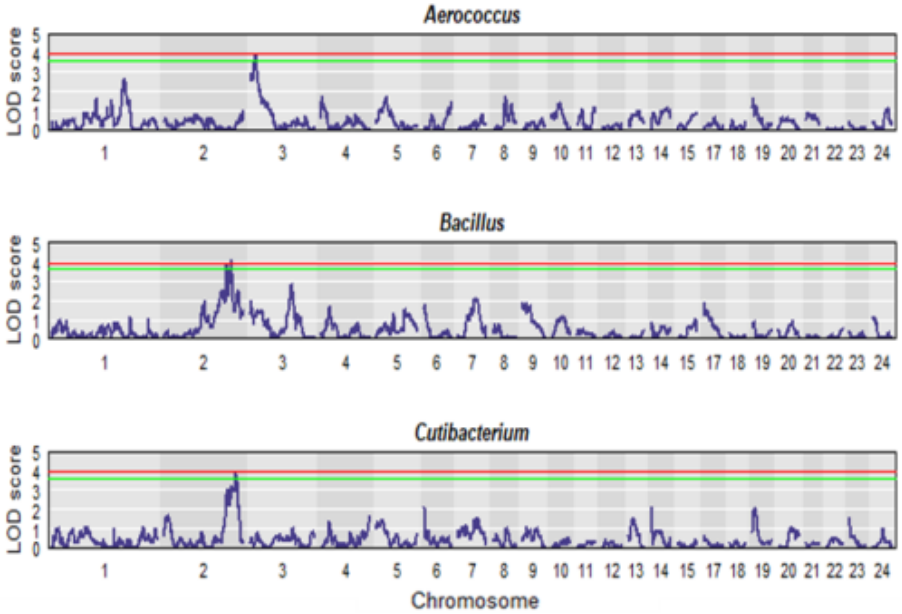
Genus	Averaged relative abundance (%)	Heritability	SE	p value
Clostridium sensu stricto	14.11	0.17	0.07	< 0.001
Lactobacillus	24.33	0.12	0.05	< 0.001
Bifidobacterium	0.48	0.10	0.05	< 0.001
Escherichia/Shigella	14.17	0.09	0.05	0.001
Cutibacterium	0.06	0.08	0.04	0.002
Aerococcus	0.47	0.08	0.04	0.003
Streptococcus	8.25	0.08	0.05	0.022
Tyzzerella	0.08	0.07	0.04	0.007
Enterococcus	3.75	0.06	0.04	0.011
Bacillus	0.08	0.06	0.03	0.006

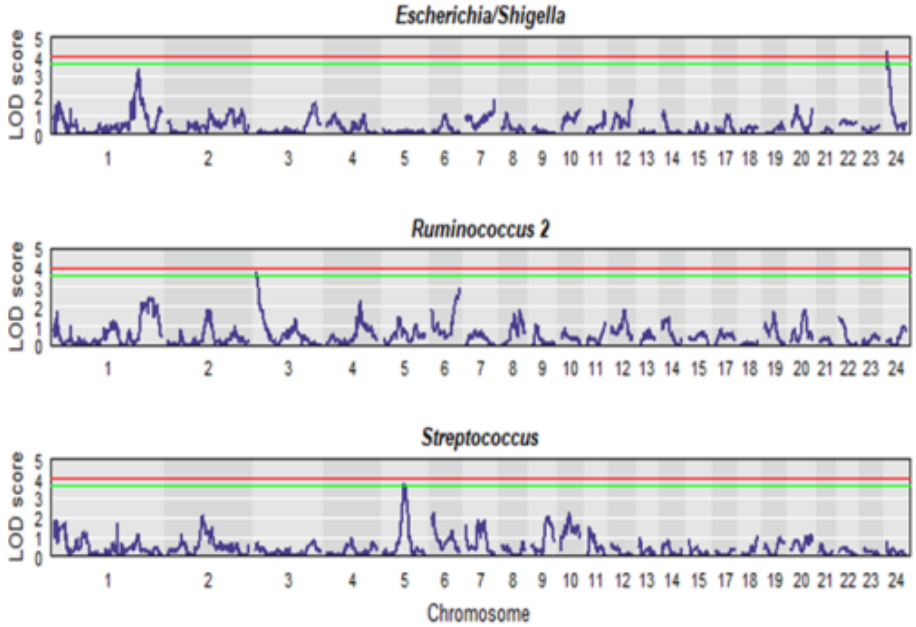
→ Of 59 genera, 24 showed significant heritability.

Host Genome Scans of Microbial Genera

QTL linkage mapping

- using R package MAPfastR and R/qlt2 *(Nelson et al. 2013; Broman et al. 2019)*





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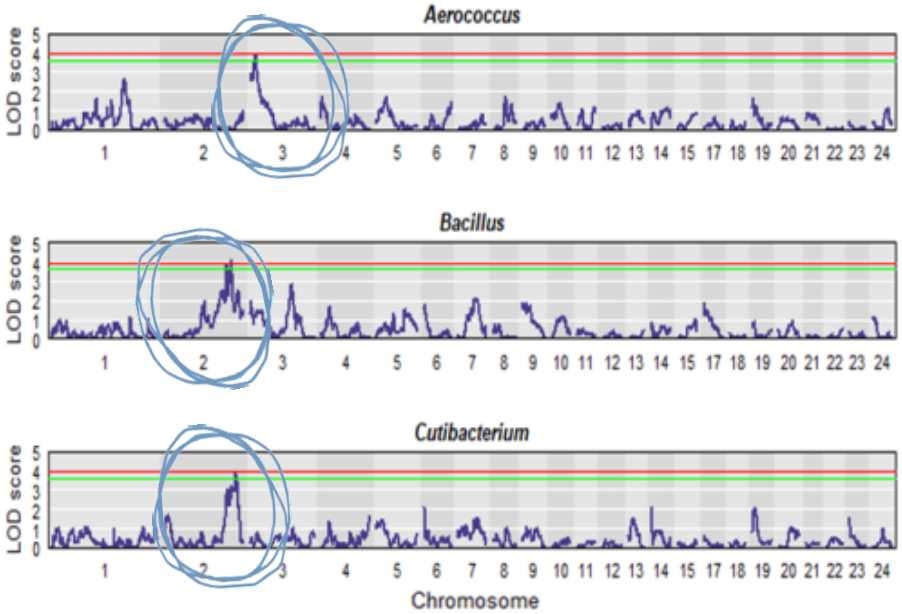
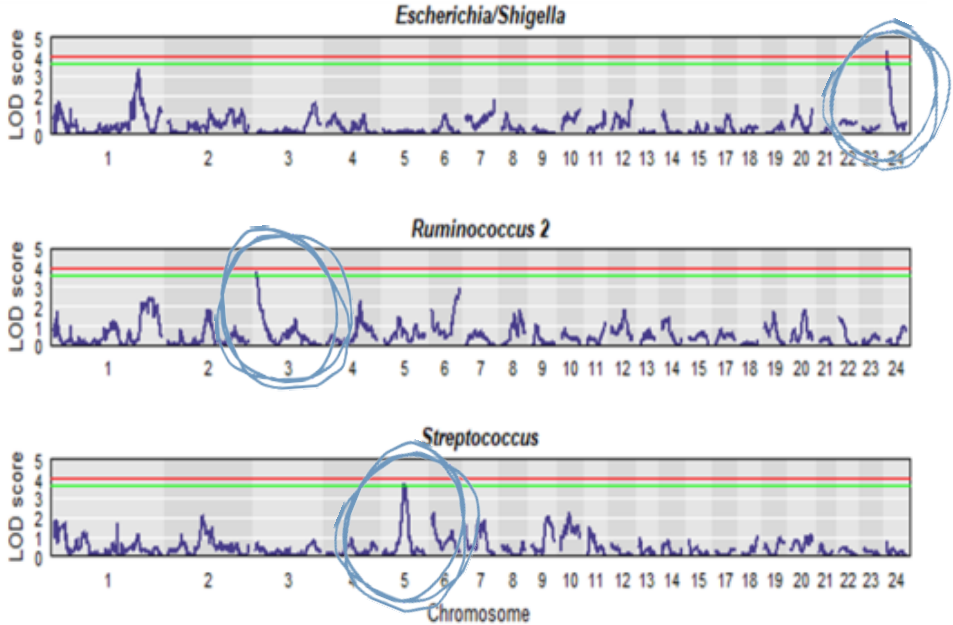


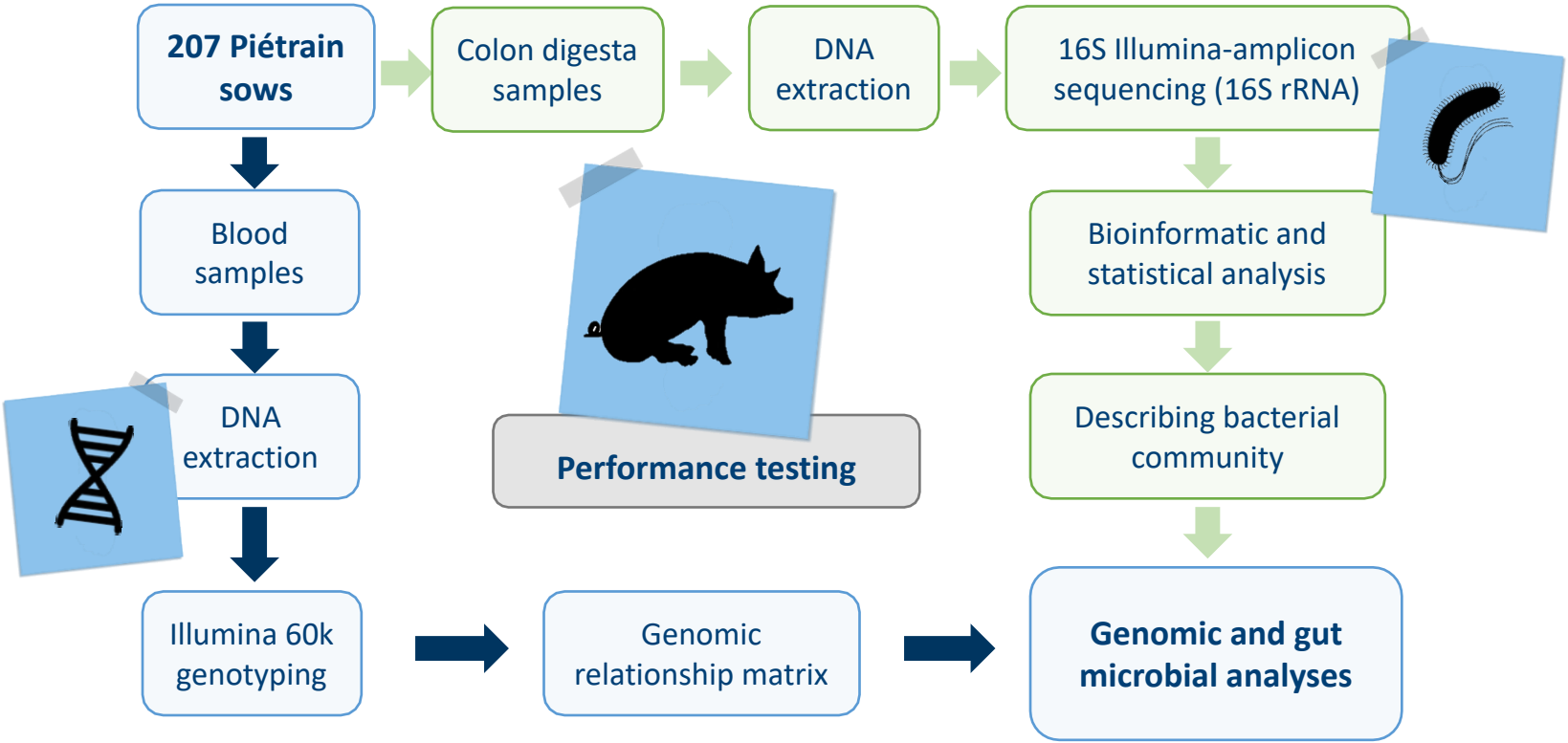


HoLMiR
Hohenheim Center for Livestock Microbiome Research

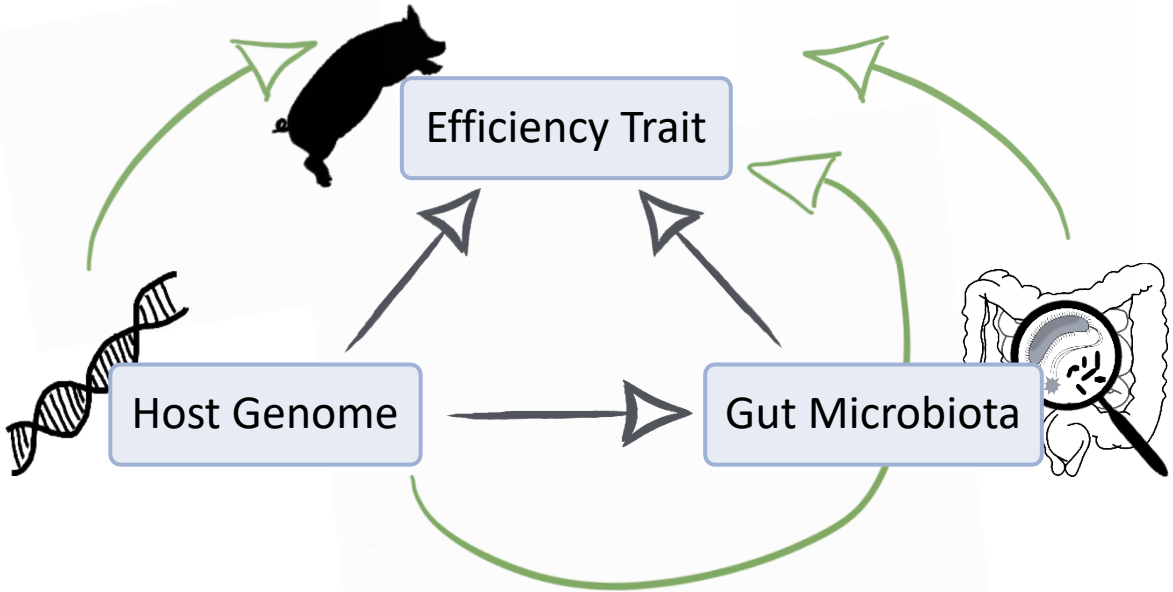
Results from Piétrain Pigs

for details see Weishaar et al. 2020

Study Design *(Camarinha-Silva et al. 2017)*



Trait Predictions? *(Weishaar et al. 2020)*



Methods Trait Predictions

In GBLUP, with the genomic relationship matrix **G** *(VanRaden et al. 2008)*:

(1) $$\mathbf{y} = \mathbf{X_b b} + \mathbf{Z_{sd} sd} + \mathbf{Z_{pen} pen} + \mathbf{g} + \mathbf{e}$$

In MBLUP, with the microbial relationship matrix **M** *(Camarinha-Silva et al. 2017)*:

(2) $$\mathbf{y} = \mathbf{X_b b} + \mathbf{Z_{sd} sd} + \mathbf{Z_{pen} pen} + \mathbf{k} + \mathbf{e}$$

efficiency traits **y**

- Feed conversion rate (FC)
- Residual feed intake (RFI)
- Daily gain (DG)

Methods Trait Predictions

GBLUP of the microbiota-mediated part of the trait *(Weishaar et al. 2020)*:

(2) $$\mathbf{y} = \mathbf{X_b b} + \mathbf{Z_{sd} sd} + \mathbf{Z_{pen} pen} + \mathbf{k} + \mathbf{e}$$

(3) $$\hat{\mathbf{k}} = \boldsymbol{\mu}\,\mathbf{1} + \mathbf{Im} + \mathbf{e}$$

+

efficiency traits **y**
- Feed conversion rate (FC)
- Residual feed intake (RFI)
- Daily gain (DG)

$\hat{\mathbf{k}}$ = estimated microbiota effects for each animal and trait (FC, RFI, or DG)

$\mathbf{m}$ = vector with random animal effects $\mathbf{m} \sim N(0, \mathbf{G}\sigma^2_m)$, with the SNP variance σ^2_m of the microbiota effects and the genomic relationship matrix $\mathbf{G}$ *(VanRaden et al. 2008)*

Accuracies of Predictions

Trait	GBLUP	
	Accuracy[1]	95% CI
FCR	0.23	-0.04:0.48
RFI	0.31	0.14:0.56
DG	0.37	0.11:0.59

[1] Correlation of $\boldsymbol{y}$ and genomic predictions of $\boldsymbol{y}$

Accuracies of Predictions

Trait	GBLUP		MBLUP	
	Accuracy[1]	95% CI	Accuracy[2]	95% CI
FCR	0.23	-0.04:0.48	0.33	0.06:0.55
RFI	0.31	0.14:0.56	0.36	0.09:0.60
DG	0.37	0.11:0.59	0.43	0.20:0.63

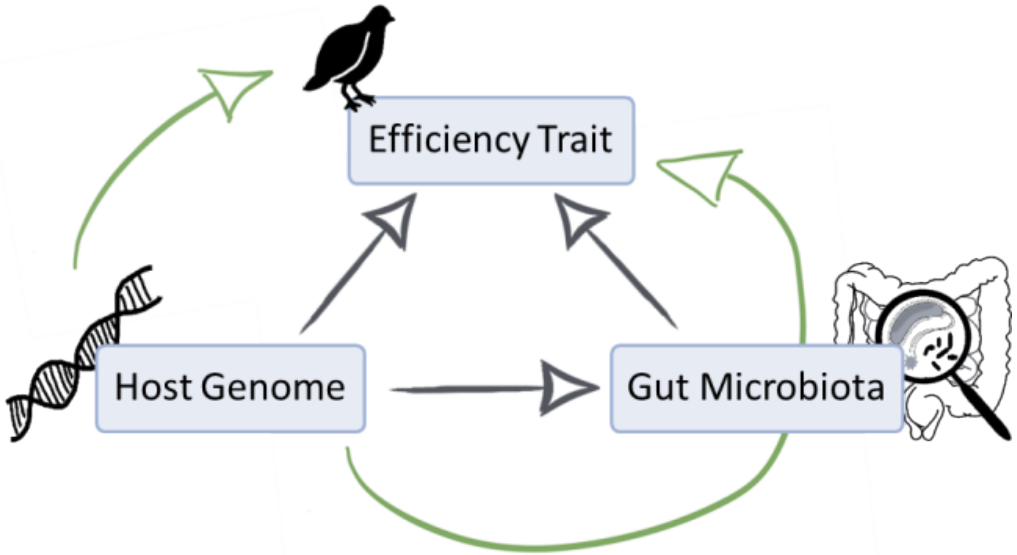
[1] Correlation of $\boldsymbol{y}$ and genomic predictions of $\boldsymbol{y}$
[2] Correlation of $\boldsymbol{y}$ and microbial predictions of $\boldsymbol{y}$

Accuracies of Predictions

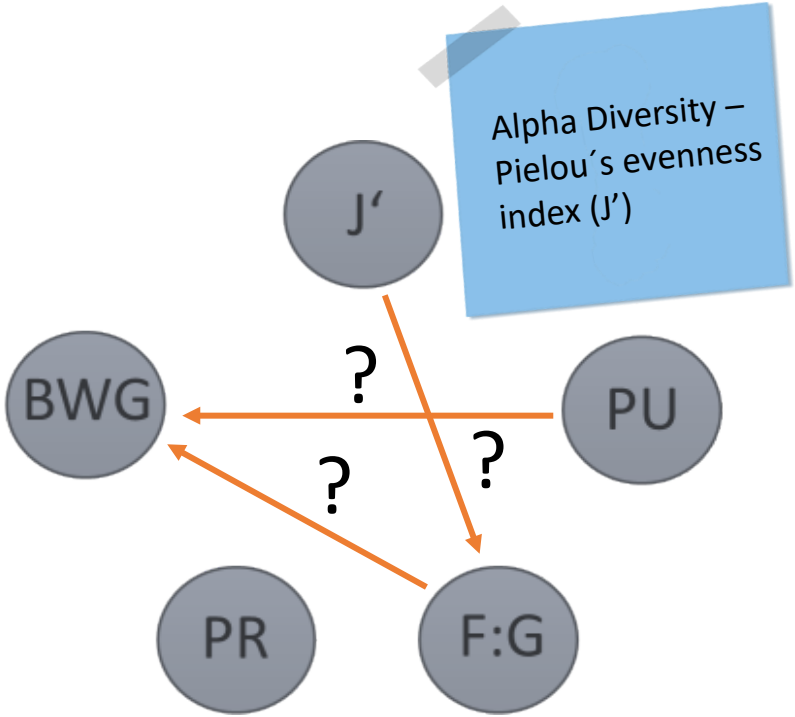
Trait	GBLUP		MBLUP		Microbiota-mediated GBLUP	
	Accuracy[1]	95% CI	Accuracy[2]	95% CI	Accuracy[3]	95% CI
FCR	0.23	-0.04:0.48	0.33	0.06:0.55	0.14	-0.19:0.42
RFI	0.31	0.14:0.56	0.36	0.09:0.60	0.06	-0.25:0.36
DG	0.37	0.11:0.59	0.43	0.20:0.63	0.32	0.07:0.56

[1] Correlation of $\boldsymbol{y}$ and genomic predictions of $\boldsymbol{y}$
[2] Correlation of $\boldsymbol{y}$ and microbial predictions of $\boldsymbol{y}$
[3] Correlation of $\boldsymbol{y}$ and genomic predictions of microbiota-driven $\boldsymbol{y}$

Causal Relationships? *(Haas et al. 2023)*



Bayesian Networks

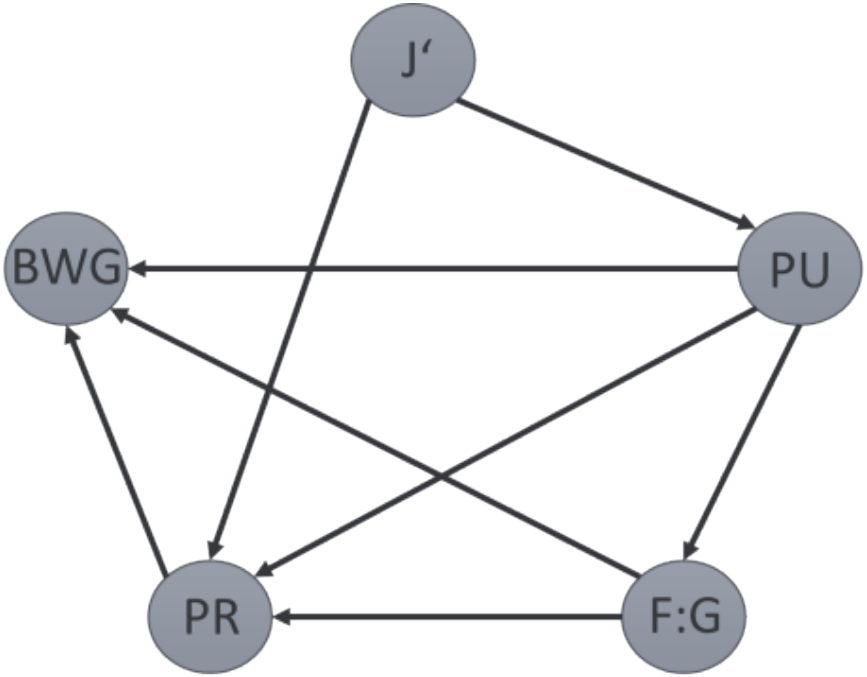


Traits **y**

- Pielou's evenness index (J')
- Phosphorus utilization (PU)
- Feed per gain (F:G)
- Phosphorus retention (PR)
- Body weight gain (BWG)

Bayesian Networks

Hill-Climbing (**HC**) learning algorithm *(Daly and Qiang, 2007)* in the R package bnlearn *(Scutari, 2010)*



Traits **y**

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Structural Equation Model Association Analyses

Calculate the SNP effects in a trivariate network:

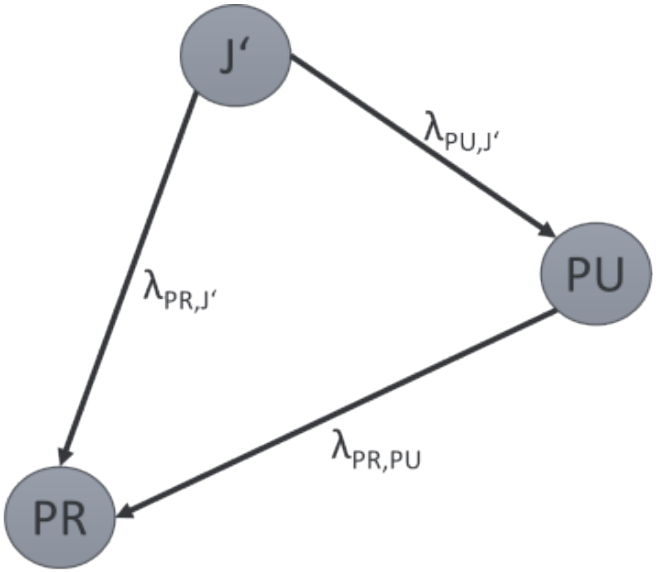
$$\mathbf{y} = (\Lambda \otimes \mathbf{I_n})\mathbf{y} + \mathbf{Ws} + \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

$\mathbf{y}$ = vector of observations (J', PU, and PR)

$\mathbf{I_n}$ = identity matrix

$\mathbf{\Lambda}$ = off-diagonal Matrix (t x t) of structural coefficients $\lambda_{i,j}$

$$\mathbf{\Lambda} = \begin{bmatrix} 0 & 0 & 0 \\ \lambda_{PU,J'} & 0 & 0 \\ \lambda_{PR,J'} & \lambda_{PR,PU} & 0 \end{bmatrix}$$



Structural Equation Model Association Analyses

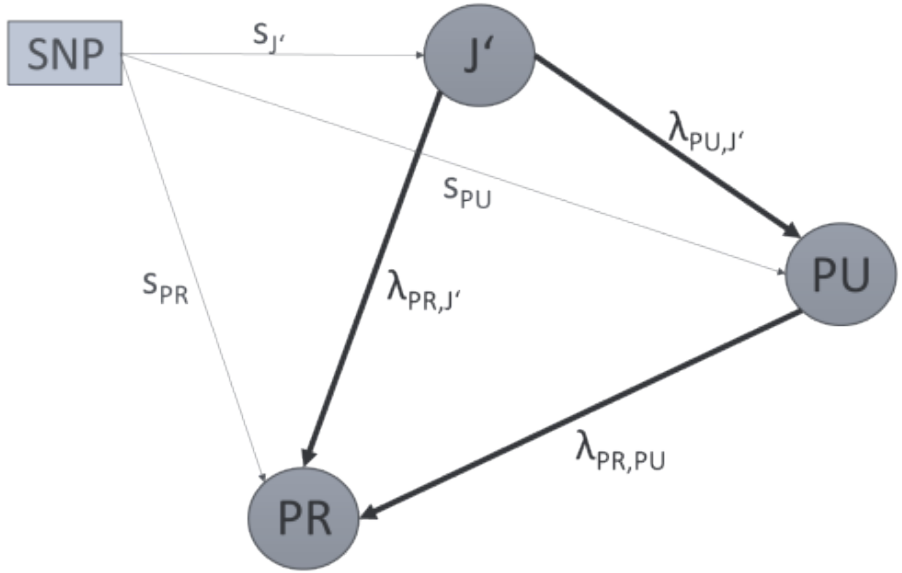
Calculate the SNP effects in a trivariate network:

$$\mathbf{y} = (\Lambda \otimes \mathbf{I_n})\mathbf{y} + \mathbf{Ws} + \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

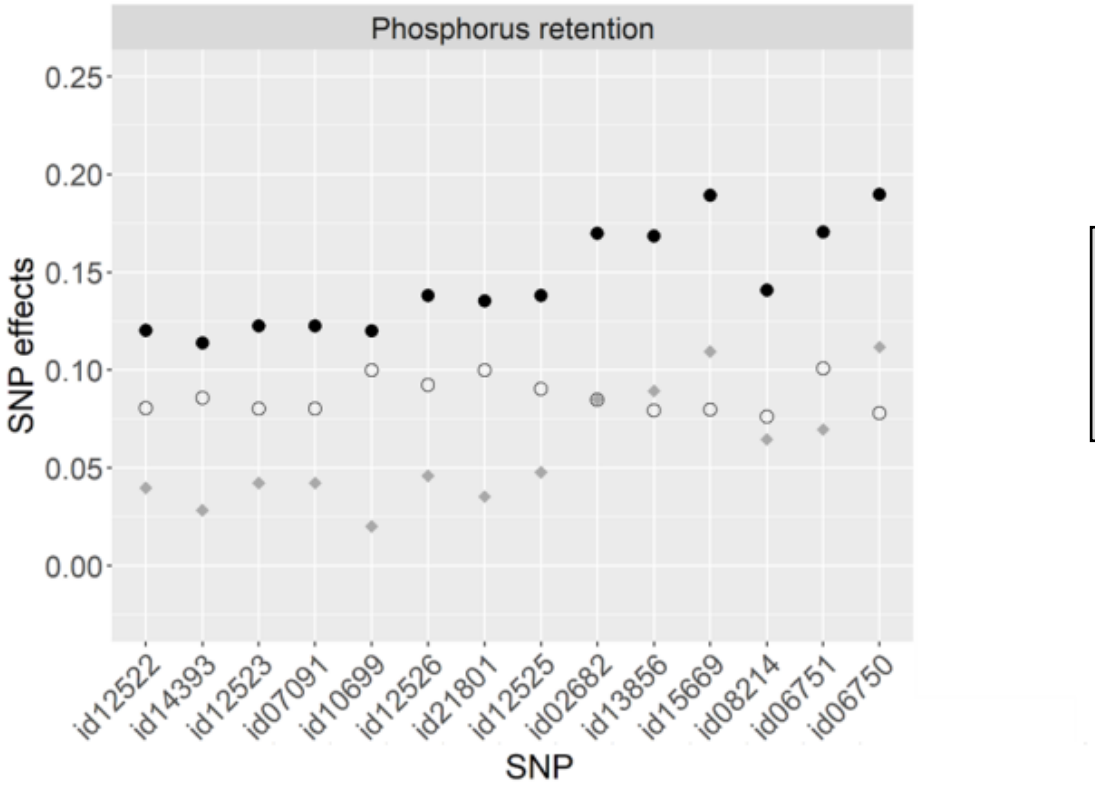
W = individual´s genotype codes for SNP

s = trait additive effects of SNP with

$$\mathbf{s} = [s_{J'}, s_{PU}, s_{PR}]$$

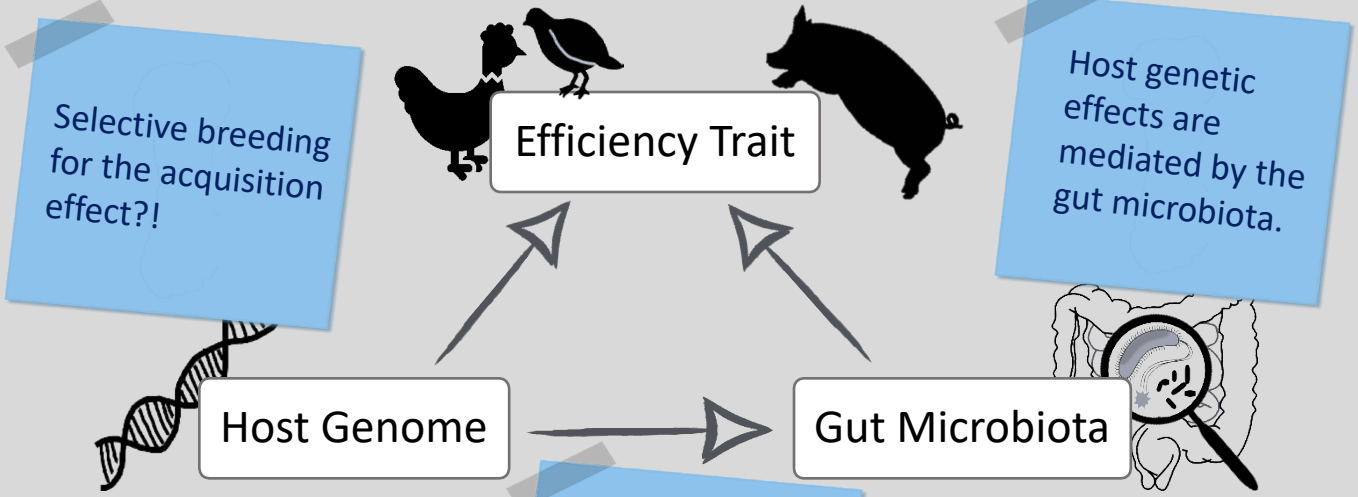


SNP Effects from SEM Association Analyses



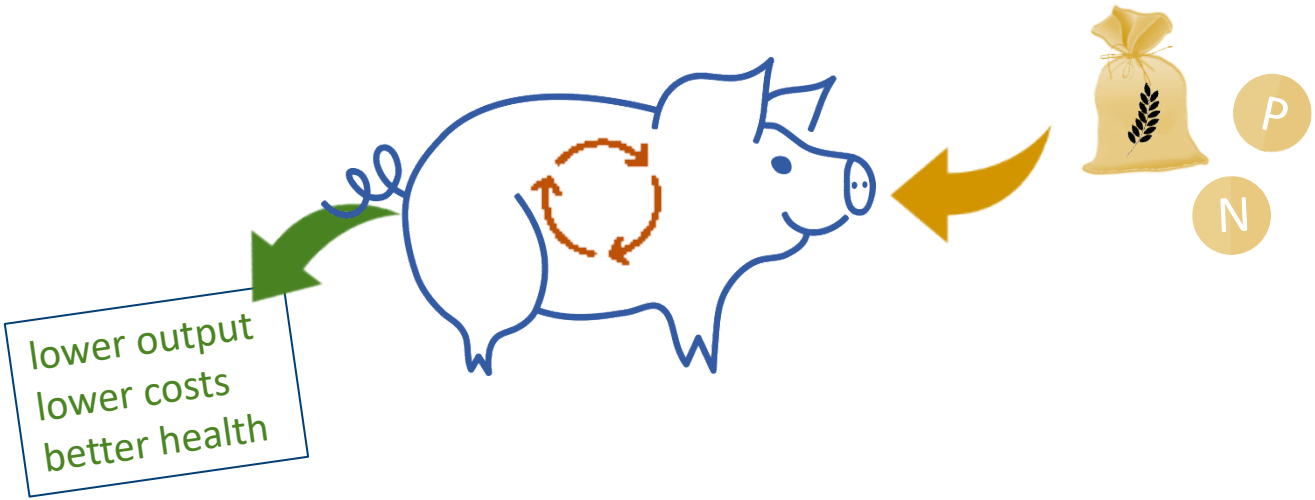
Total SNP effects
Direct SNP effects
∑ Indirect SNP effects

Take Home Message



Take Home Message

Food Security and Sustainability



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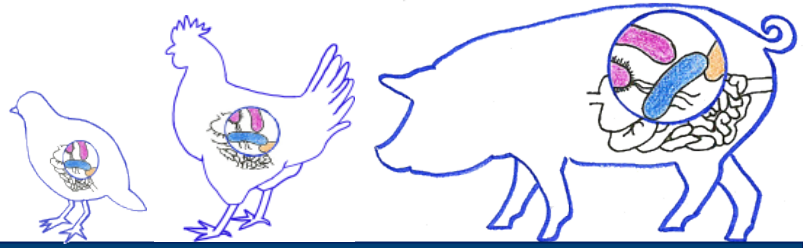

Thank you for your attention!

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Appendix – Additional Slides

Heritabilites and correlations from publication 3

Traits	J’	PU	F:G	PR	BWG
J’	**0.19 (0.08)***	-0.10 (0.04)*	0.06 (0.04)	-0.15 (0.05)*	-0.16 (0.05)*
PU	-0.17 (0.32)	**0.14 (0.07)***	-0.38 (0.04)*	0.79 (0.02)*	0.57 (0.03)*
F:G	-0.06 (0.34)	0.03 (0.40)	**0.11 (0.08)***	-0.26 (0.05)*	-0.62 (0.05)*
PR	-0.46 (0.23)*	0.58 (0.22)*	0.41 (0.29)	**0.26 (0.12)***	0.82 (0.02)*
BWG	-0.62 (0.22)*	0.37 (0.33)	0.23 (0.38)	0.81 (0.11)*	**0.17 (0.11)***

* 95% highest posterior density interval not including 0.

Appendix – Additional Slides

Resiudal feed intake and feed conversion in Piétrain pigs:

Feed conversion rate

$$\mathbf{FC} = \frac{\textbf{Feed intake in kg (FI)}}{\textbf{weight gain in kg (DG)}}$$

Residual feed intake *(Saintilan et al. 2013)*

$$\mathbf{RFI} = \textbf{observed FI} - \textbf{expected FI (of the production level)}$$