



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA



Controlling mIcRobiomes
CircuLations for bETter food Systems
H2020-SFS-2018-1



75th EAAP Annual Meeting
1/5 September 2024 - Florence, Italy

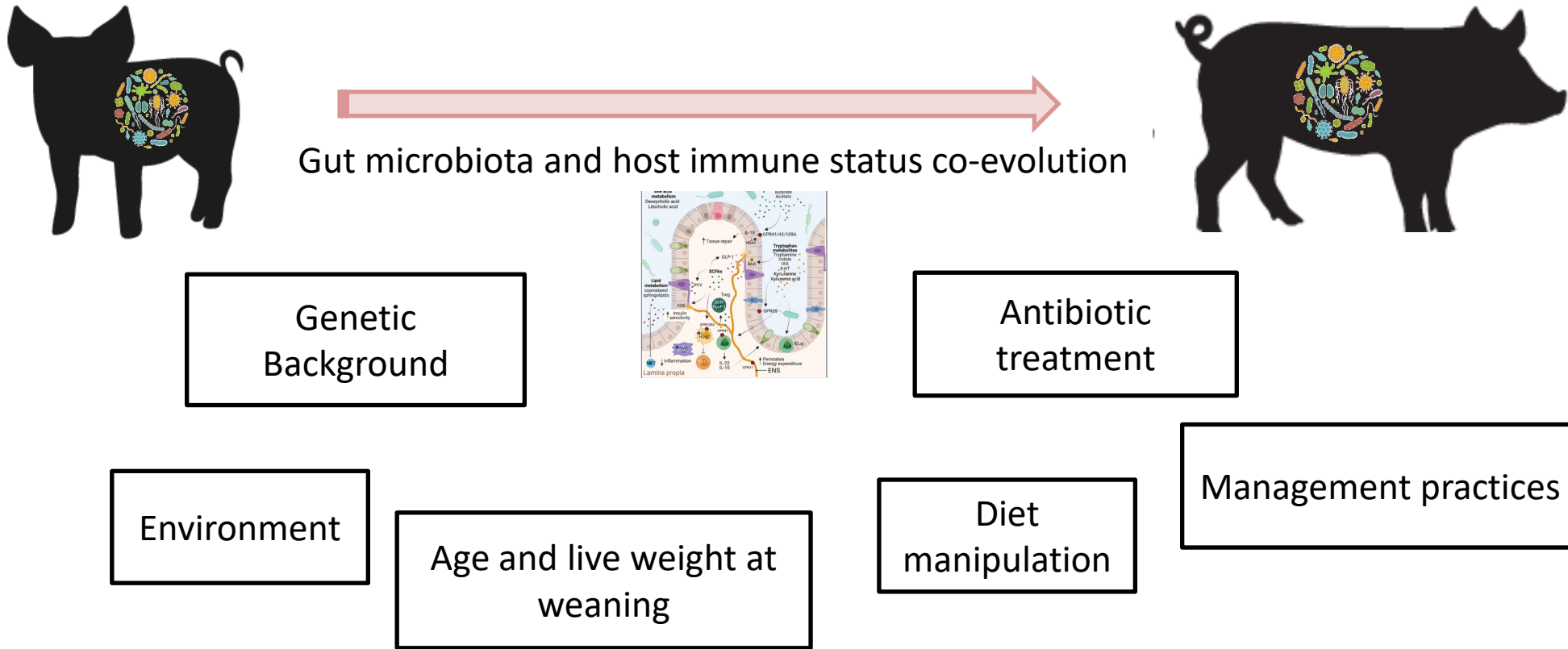


Housing systems and dietary interventions can affect health, performance and microbiome of pigs from weaning to the growing

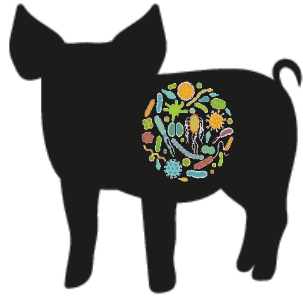
D. Luise¹, G. Palladino², F. Correa¹, F. Palumbo¹, G. Orazzo¹, M. Graziosi¹, E. Perez-Calvo³, G. Litta³, G. Scicchitano², G. Babbi², A. Castagnetti⁴, S. Rampelli², P. Martelli², P. Trevisi¹

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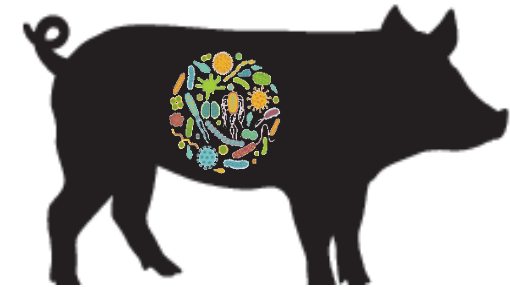
Introduction



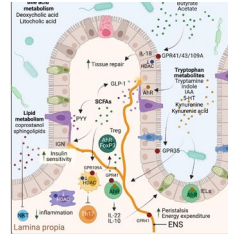
Introduction



Gut microbiota and host immune status co-evolution



Genetic
Background



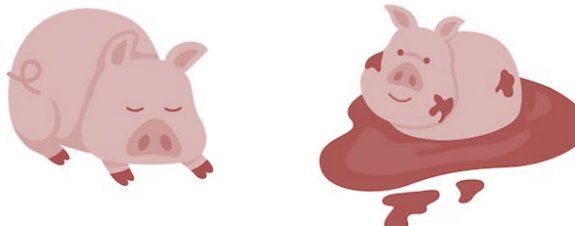
Antibiotic
treatment

Environment

Age and live weight at
weaning

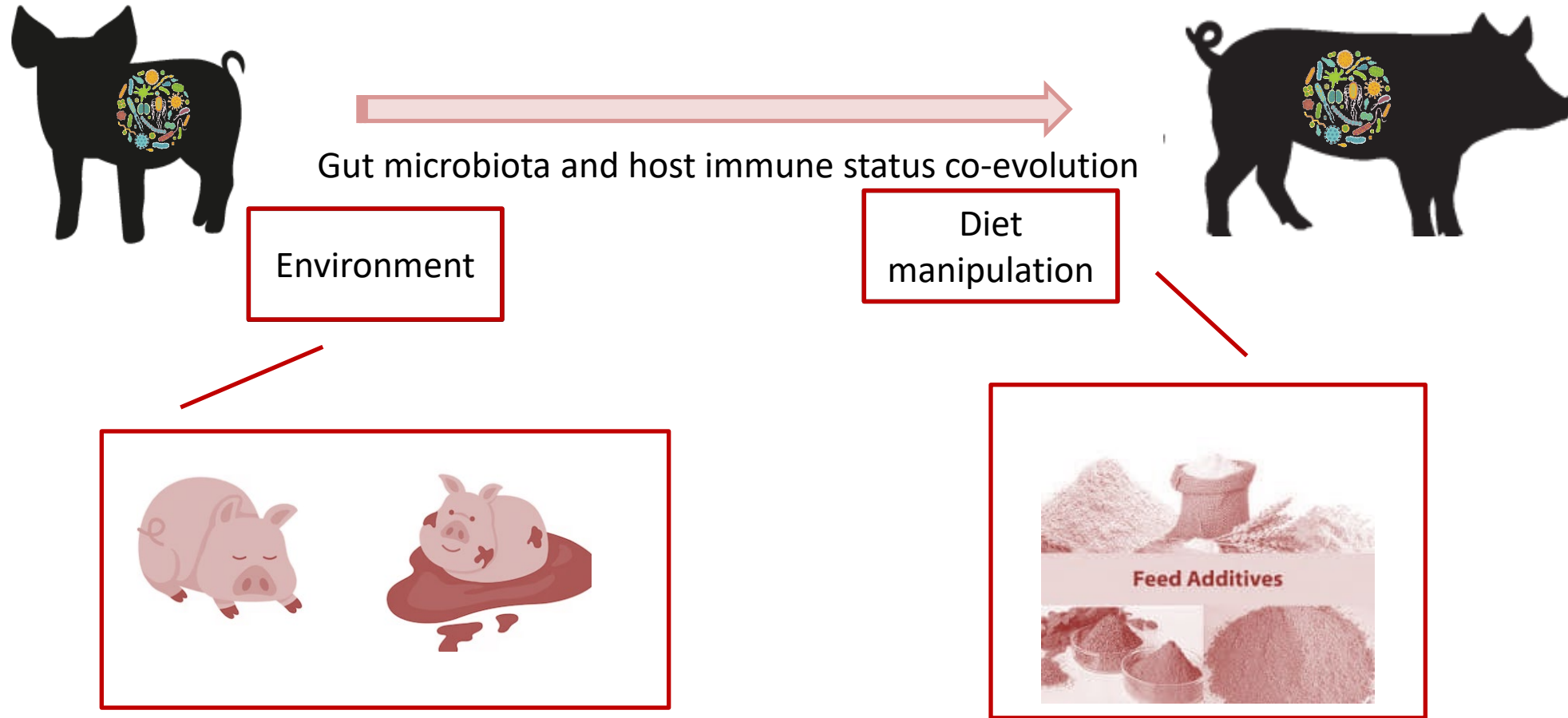
Diet
manipulation

Management practices



SESSION 55: Host physiology/genetics and microbiome interactions – The holobiont concept
EU-CIRCLES project: Evolution of pig microbiota and health under different farming conditions
F. Correa, G. Palladino, D. Luise, D. Schicchitano, B. Giulia, S. Rampelli, P. L. Martelli, P. Trevisi

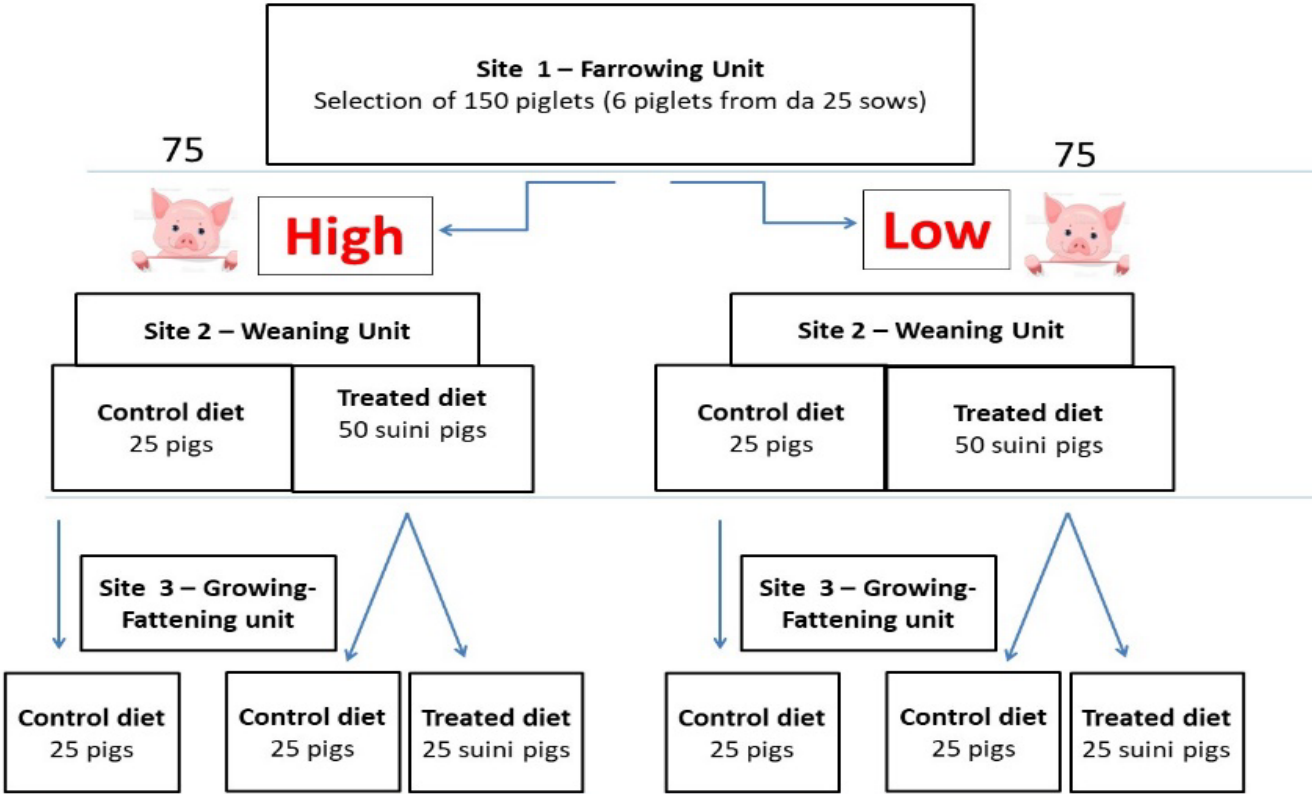




Aim of the study

The study aimed to evaluate the effect of two housing systems and a mixture of feed additives from weaning to growing phase on the health, performance and microbiome of pigs

Material and methods



Sampling

Time	Age	Site	BW	Faeces	Blood
T1 ¹	d 28	Site 1	150	150	
T2 ²	d 42	Site 2	150	150	150
T3 ²	d 80	Site 2	150	150	150
T4 ²	d 98	Site 3	150	150	150



Shot-gun metagenomics



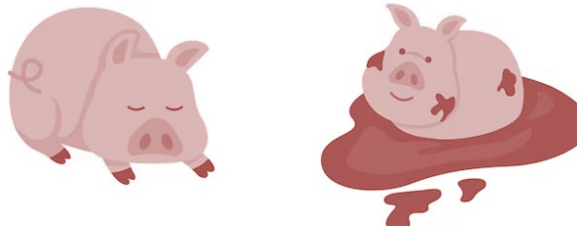
Blood Formula
Immunoglobulins
Antioxidant status

Material and methods

SESSION 55: Host physiology/genetics and microbiome interactions – The holobiont concept

EU-CIRCLES project: Evolution of pig microbiota and health under different farming conditions

F. Correa, G. Palladino, D. Luise, D. Schicchitano, B. Giulia, S. Rampelli, P. L. Martelli, P. Trevisi



- *Prevotella* 9
- Prevotellaceae NK3B31
- Rikenellaceae RC9
- *Lactobacillus amylovorus*
- *Butyricicoccus porcorum*
- *Escherichia Shigella*
- *Megashaera*

The dietary intervention was designed to stimulate **Smart Microbiome Modulators** (SMMs) which were defined in the Observation phase of the project

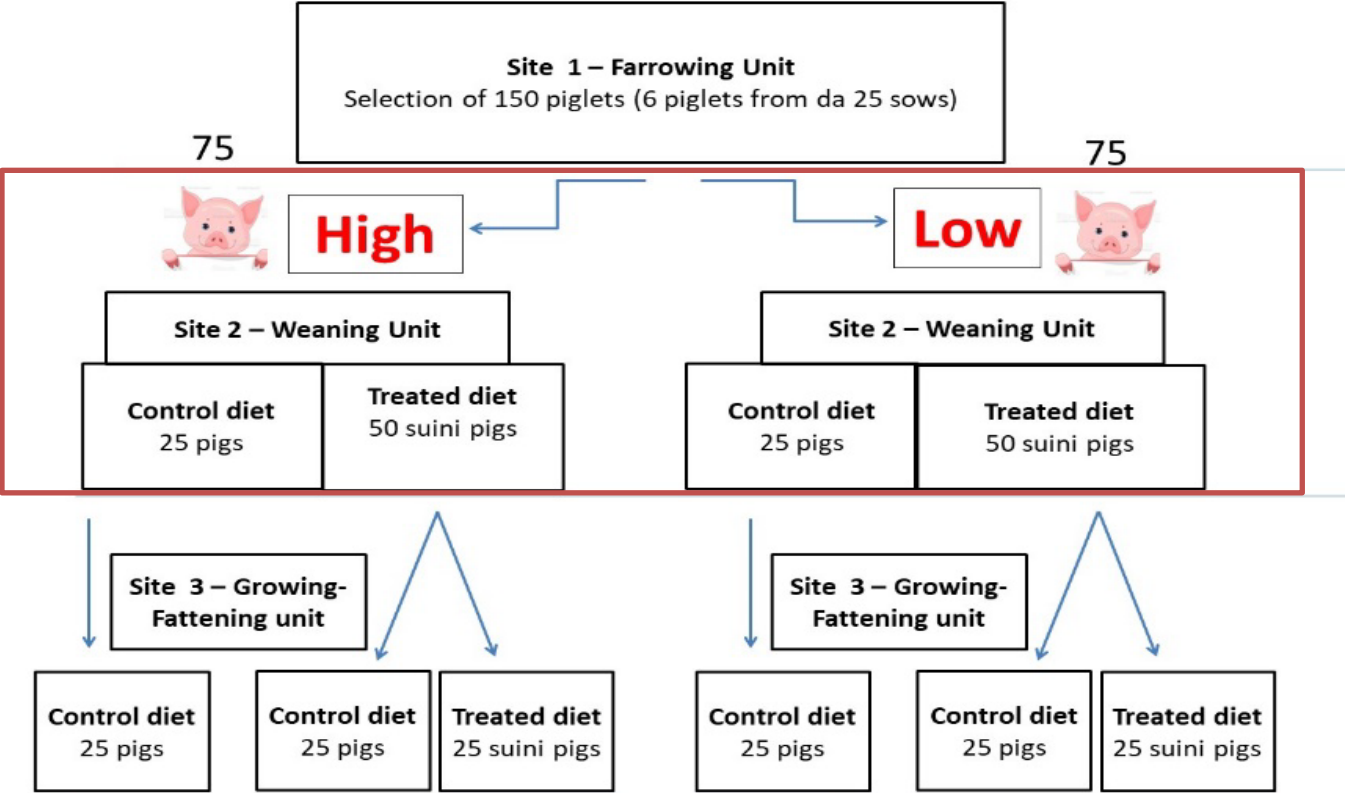
Dietary intervention

Additive	Weaning (T2 and T3)	Growing (T4)
	From weaning to 30 kg	From 25 to 50 kg
Benzoic acid (mg/kg)	5000	5000
<i>E. faecium</i> (mg/kg)	35	-
Muramidase (mg/kg)	500	-

- Benzoic acid is able to select *Prevotella* genus (Correa et al., 2021);
- *E. faecium* can directly inhibit potential pathogens such as *Escherichia coli* (Pajarillo et al., 2015). It is also known to promoting immune system development (Bednorz et al., 2013)
- Muramidase can improve the feed efficiency and increase the peptidoglycans turnovers affecting the microbiota. It increased the *Lactobacillus* and *Butyricicoccus* genera in broilers (Wang et al., 2021; Brugaletta et al., 2022)



Results: Weaning unit



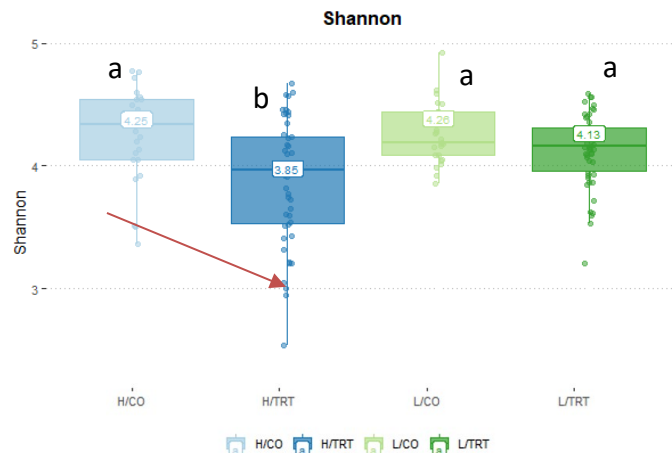
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Results: metagenomics of faecal samples, T2 and T3

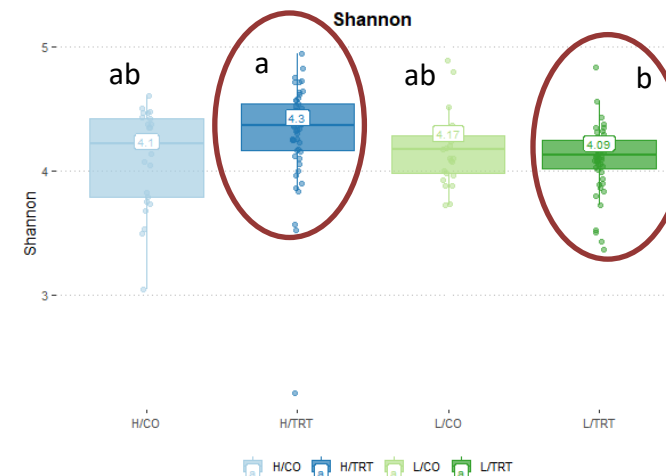
T2

Farm, P = N.S. | Diet, P <0.001 | Diet x Farm, P<0.05



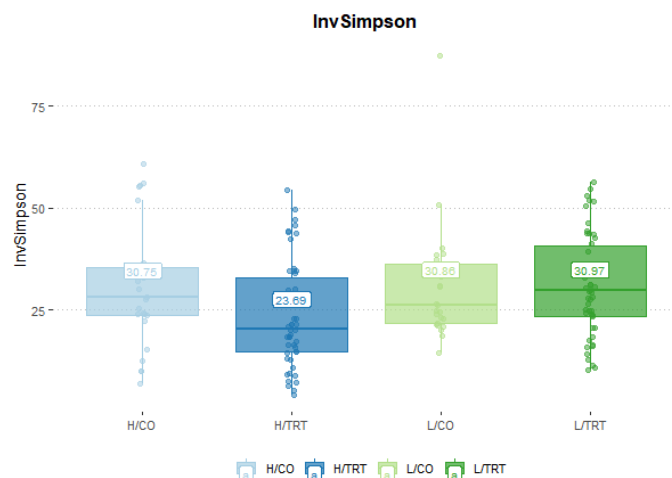
T3

Farm, P = N.S. | Diet, P <0.05. | Diet x Farm, P<0.05



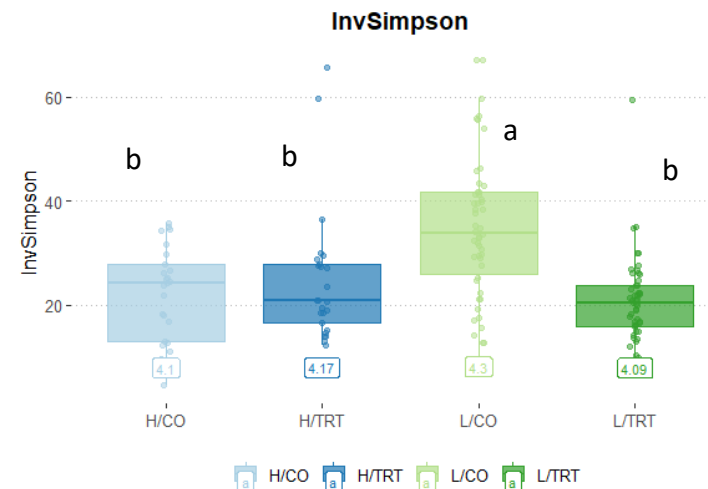
T2

Farm, P = N.S. | Diet, P<0.05 | Diet x Farm, P= N.S.

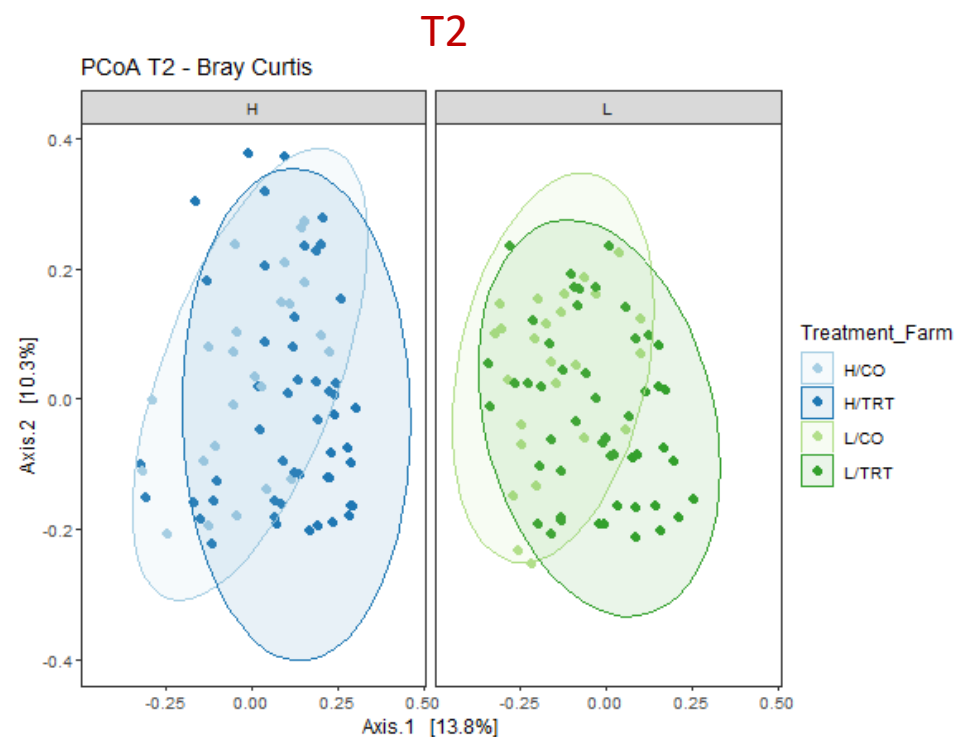


T3

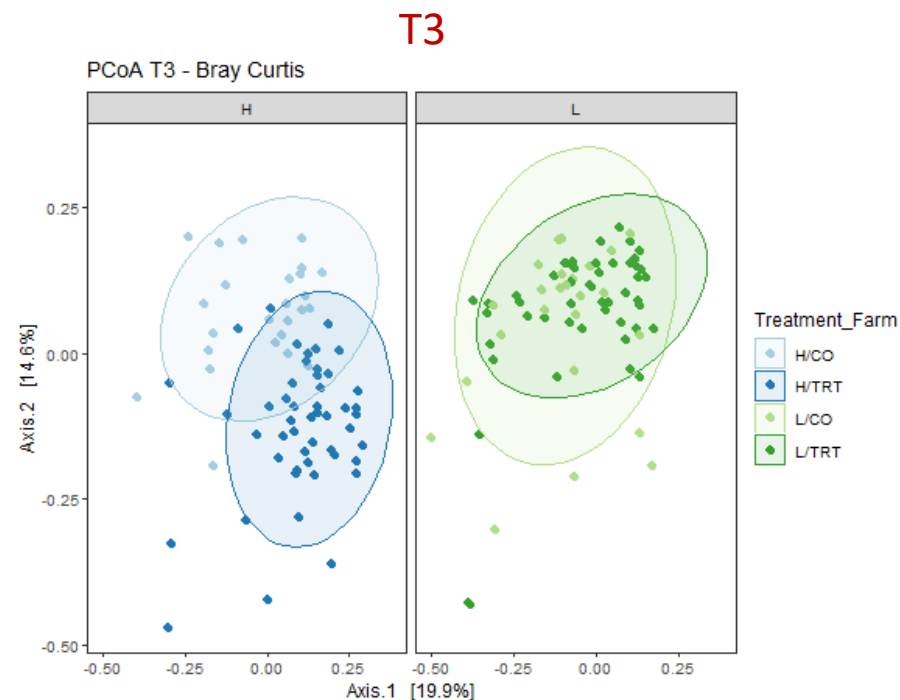
Farm, P = N.S. | Diet, P <0.001. | Diet x Farm, P<0.001



Results: metagenomics of faecal samples, T2 and T3



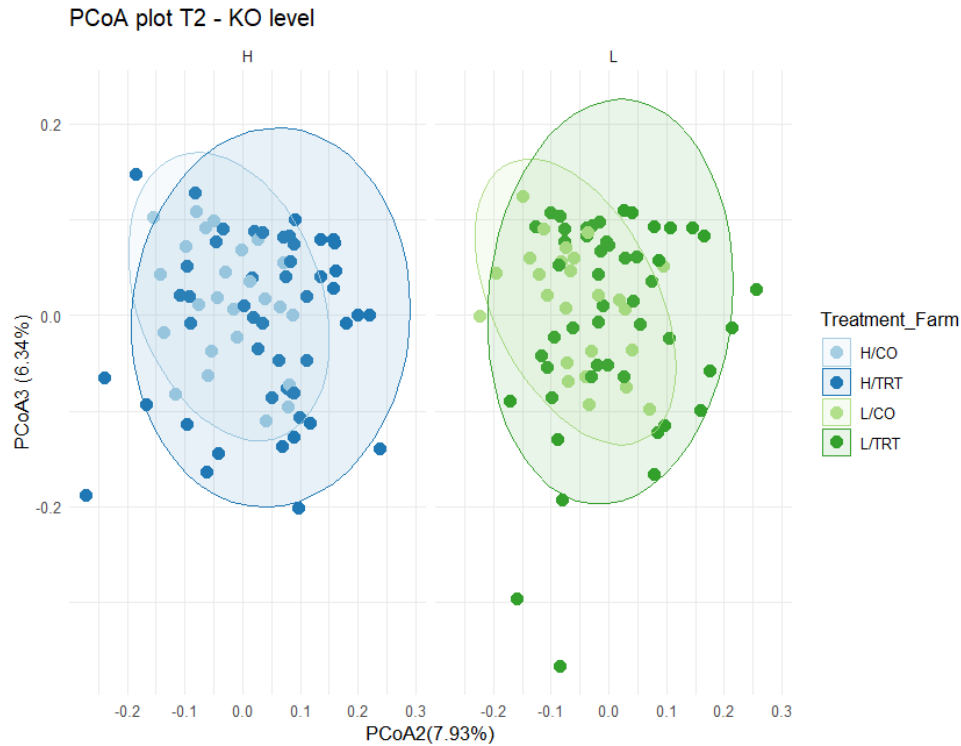
Term	Df	SumOfSqs	R2	F	Pr(>F)
Farm	1	2.081	0.06543	11.1392	0.001 ***
Diet	1	1.406	0.04420	7.5257	0.001 ***
Sow	17	3.600	0.11316	1.1333	0.051 .
Diet x Farm	1	0.621	0.01952	3.3235	0.001 ***
Residual	129	24.105	0.75769		
Total	149	31.814	1.00000		



Term	Df	SumOfSqs	R2	F	Pr(>F)
Farm	1	1.3516	0.06047	10.1914	0.001 ***
Diet	1	0.7840	0.03508	5.9120	0.001 ***
Sow	17	2.5090	0.11226	1.1129	0.149
Diet x Farm	1	0.5972	0.02672	4.5033	0.001 ***
Residual	129	17.1077	0.76546		
Total	149	22.3495	1.00000		



Results: microbiota metabolism and pathway, T2



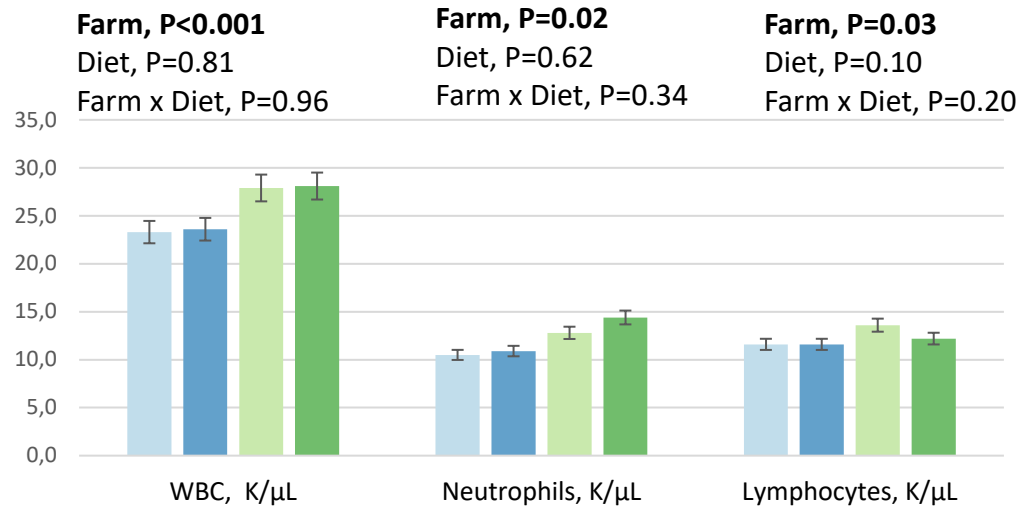
Term	Df	SumOfSqs	R2	F	Pr(>F)
Farm	1	0.356	0.024	3.95	0.009 *
Diet	1	0.707	0.048	7.85	0.002 ***
Sow	17	1.863	0.011	1.21	0.141
Diet x Farm	2	0.107	0.007	1.26	0.232
Residual	129	13.31	0.908		
Total	149	14.66	1.000		

Pathway	Higher	coef	pval	qval
Linoleic acid metabolism	H	1.970	0.002	0.014
Bisphenol degradation	H	1.457	0.006	0.031
Lipopolysaccharide biosynthesis	H	1.190	0.007	0.038
Biotin metabolism	H	0.893	0.002	0.016
Toluene degradation	H	0.626	0.010	0.049
Phenylalanine metabolism	H	0.570	0.003	0.021
Pentose and glucuronate interconversions	H	0.488	0.006	0.032
Taurine and hypotaurine metabolism	H	0.467	0.003	0.021
Streptomycin biosynthesis	H	0.423	0.001	0.011
Polyketide sugar unit biosynthesis	H	0.354	0.009	0.045
Starch and sucrose metabolism	H	0.346	0.001	0.012
Nicotinate and nicotinamide metabolism	H	0.315	0.002	0.020
Citrate cycle TCA cycle	H	0.312	0.000	0.004
Pathway	Higher	coef	pval	qval
Retinol metabolism	L	2.842	0.000	0.000
Naphthalene degradation	L	2.082	0.000	0.004
Lysine degradation	L	1.543	0.002	0.014
Primary bile acid biosynthesis	L	1.393	0.006	0.031
Secondary bile acid biosynthesis	L	1.393	0.006	0.031
Ubiquinone and other terpenoid quinone biosynthesis	L	1.355	0.000	0.003
Fatty acid degradation	L	1.189	0.001	0.011
C5 Branched dibasic acid metabolism	L	0.597	0.003	0.021
Glutathione metabolism	L	0.565	0.005	0.030
Thiamine metabolism	L	0.216	0.001	0.008

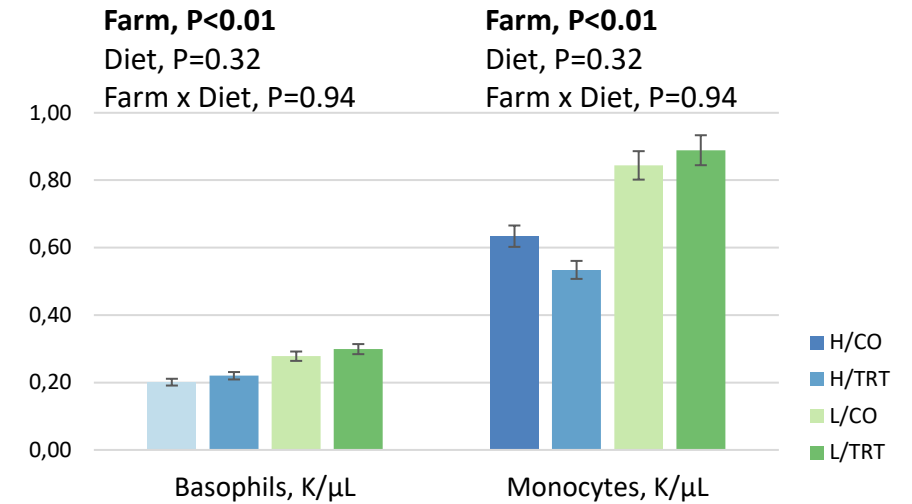


Results: blood formula and oxidative stress markers, T2 and T3

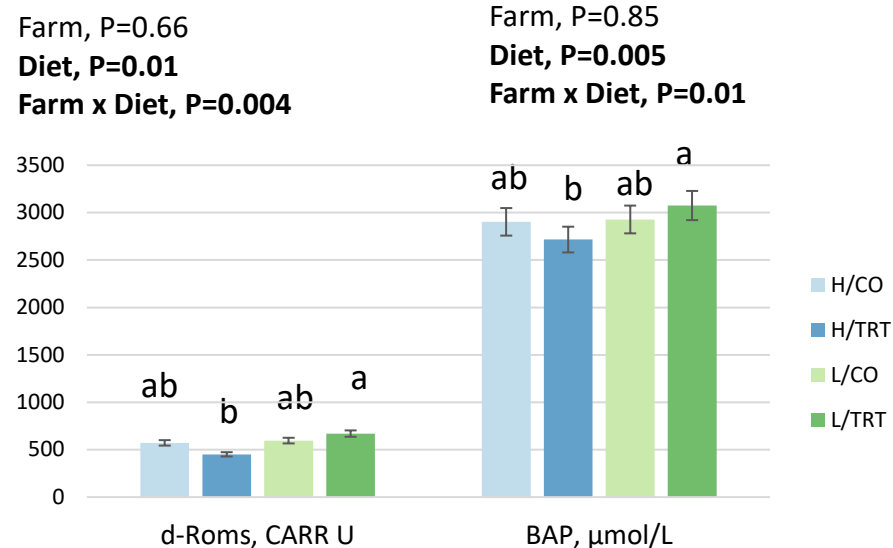
T2



T2



T2



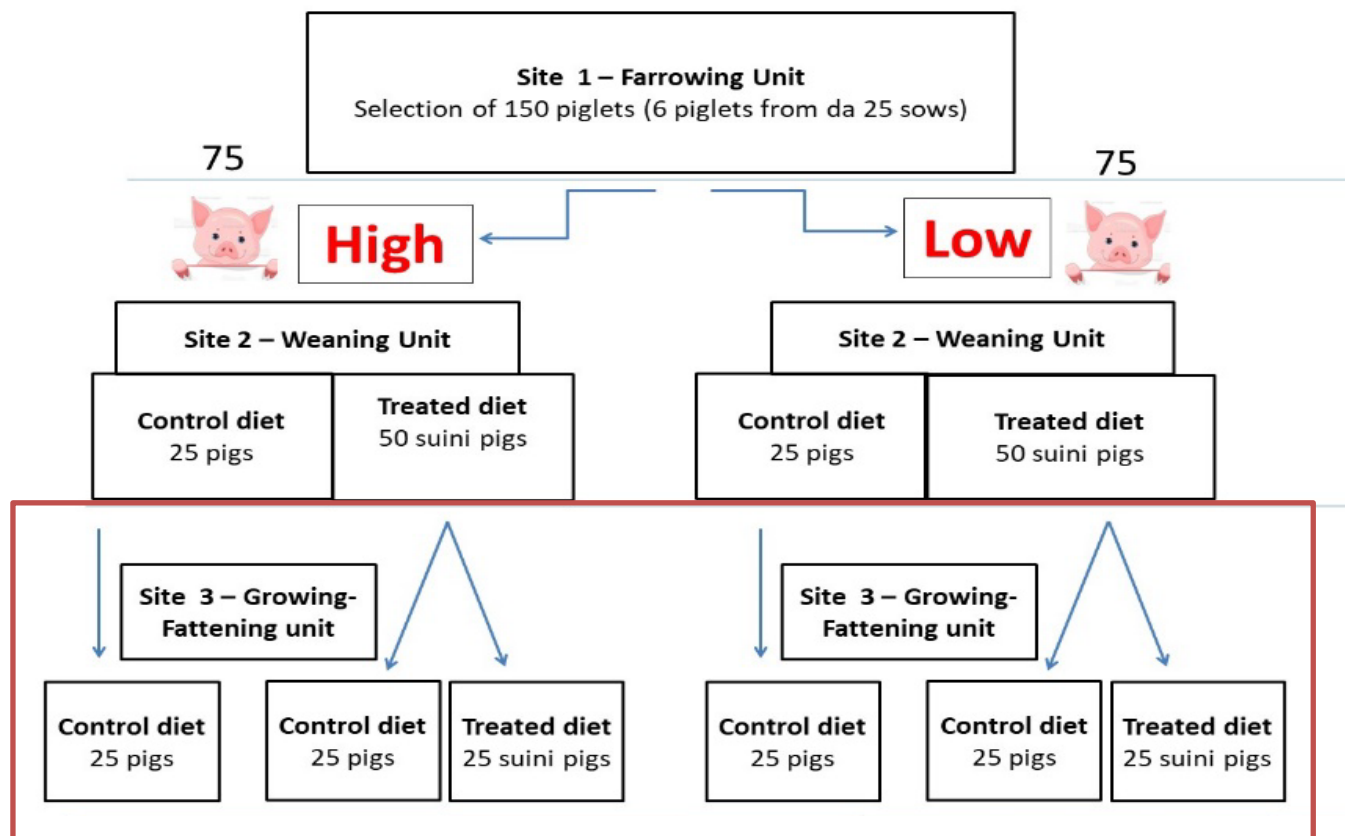
- No differences were observed at T3 for the white blood formula
- The farm confirms to play a strong stimulation of the immune system of the piglets ten days post- weaning
- Piglets can then adapt to the environmental and management stimuli
- Microbiota may contributed to stimulate the host immune response
- The oxidative status was affected by both the diet and the environment

Results: Growth performance, T2 and T3

Growth performance site 1 and 2	Farm x Dietary treatments								
	H		L		SEM	P-values			
	CO	TRT	CO	TRT		Farm	Diet	Sex	Farm X Diet
BW, kg									
At weaning (T1)	7.76	7.97	8.02	7.96	0.189	0.21	0.24	0.71	0.29
At two weeks post-weaning (T2)	10.15	9.96	10.61	10.82	0.269	0.22	0.55	0.50	0.37
At seven weeks post-weaning (T3)	33.60	33.40	33.40	33.50	0.757	0.89	0.84	0.48	0.82
Average daily gain (g/day)									
Weaning-Two weeks post-weaning (T1-T2)	165	143	173	191	14.15	0.70	0.19	0.66	0.09
Two weeks post-weaning-Seven weeks post-weaning (T2-T3)	669	670	645	646	17.7	0.33	0.98	0.50	0.99
Weaning-Seven weeks post-weaning (T1-T3)	525	519	507	510	14.6	0.37	0.74	0.55	0.72



Results: Growing unit

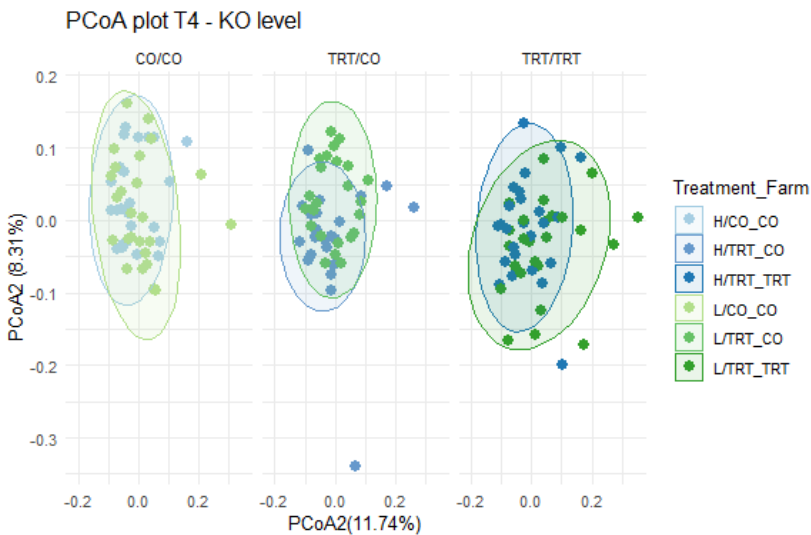
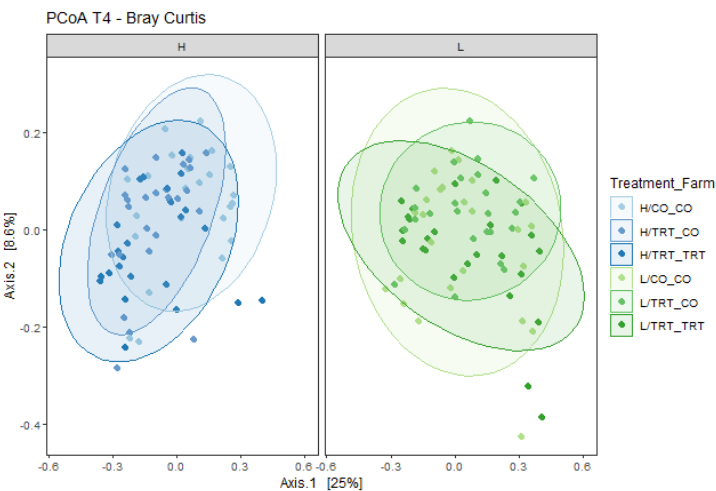
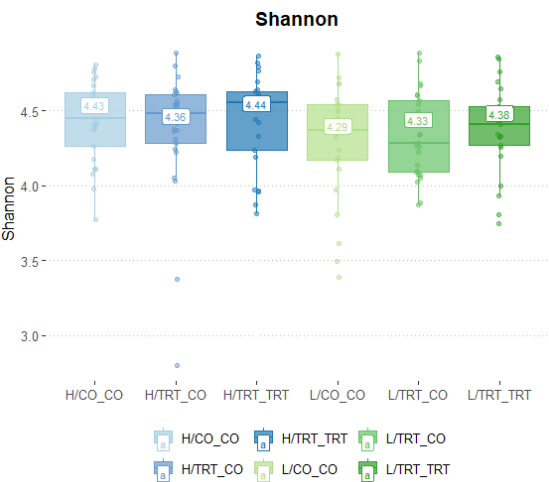


Sampling

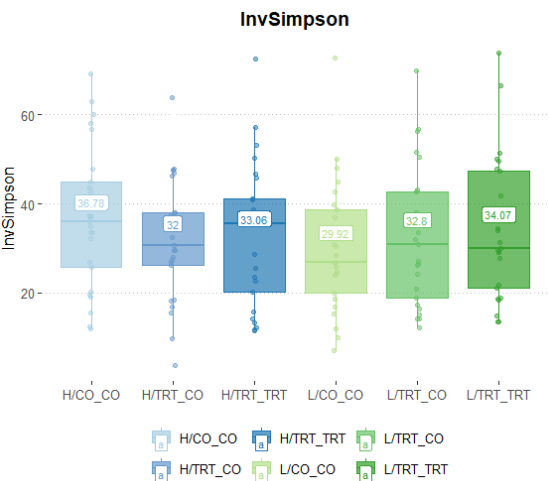
Time	Age	Site	BW	Faeces	Blood
T1 ¹	d 28	Site 1	150	150	
T2 ²	d 42	Site 2	150	150	150
T3 ²	d 80	Site 2	150	150	150
T4 ²	d 98	Site 3	150	150	150

Results: Metageomics of faecal samples T4

Farm, P = N.S. | Diet, P =N.S. |
Diet x Farm, P= N.S.



Farm, P = N.S. | Diet, P
=N.S. | Diet x Farm, P= N.S.

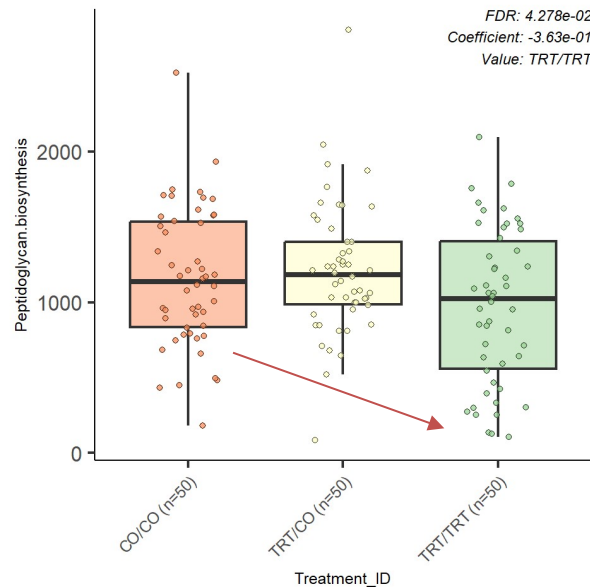
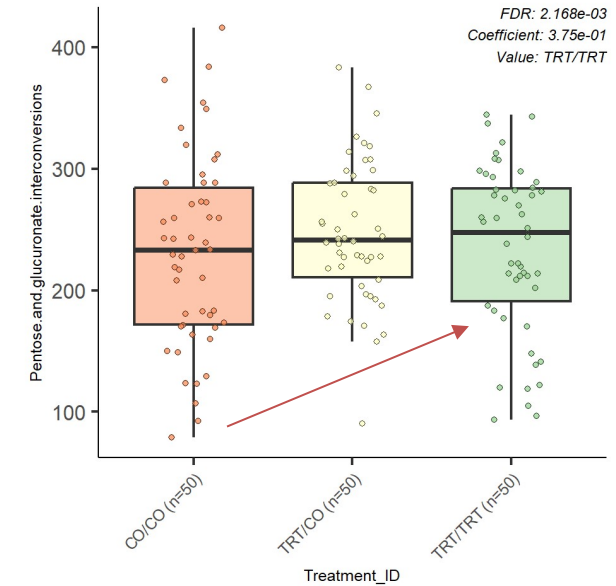
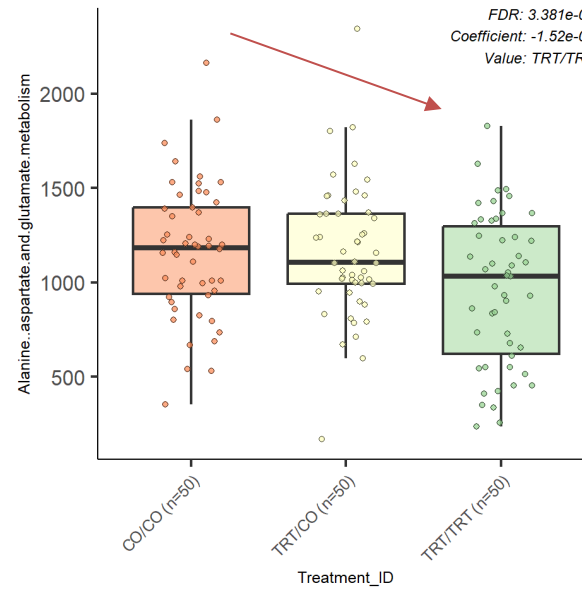
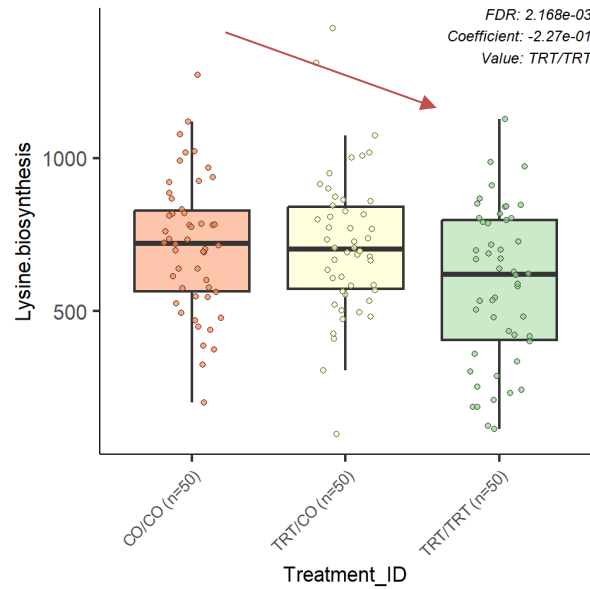


Term	Df	SumOfSqs	R2	F	Pr(>F)
Farm	1	0.783	0.032	5.32	0.001 ***
Diet	2	0.789	0.032	2.68	0.001 ***
Sow	17	3.339	0.136	1.33	0.001 ***
Diet x Farm	2	0.810	0.033	2.75	0.001 ***
Residual	127	18.69	0.765		
Total	149	24.42	1.000		

Term	Df	SumOfSqs	R2	F	Pr(>F)
Farm	1	0.100	0.011	1.794	0.116
Diet	2	0.408	0.044	3.646	0.004 **
Sow	17	1.1483	0.125	1.205	0.138
Diet x Farm	2	0.365	0.038	3.169	0.006 **
Residual	127	7.115	0.779		
Total	149	9.127	1.000		

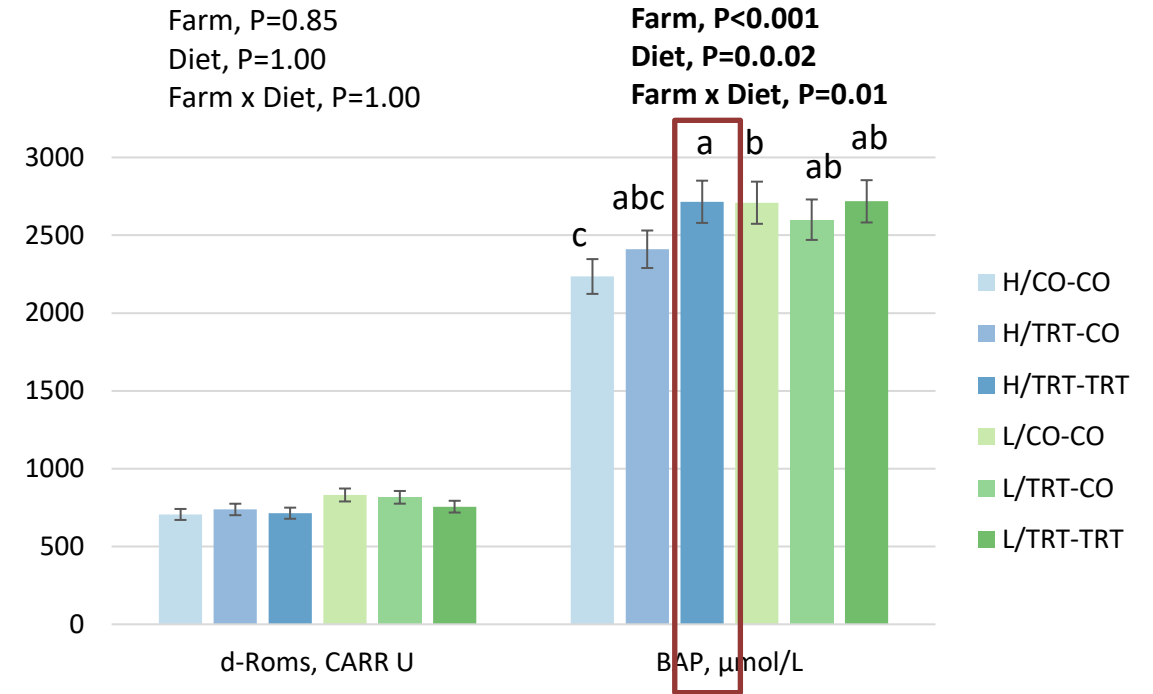
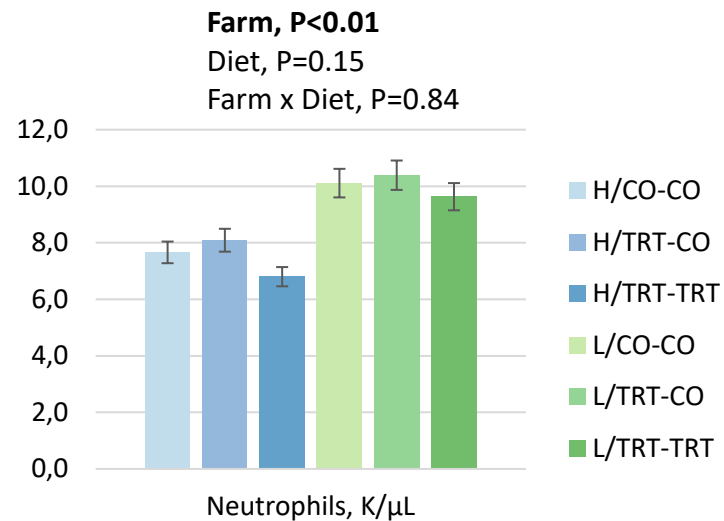
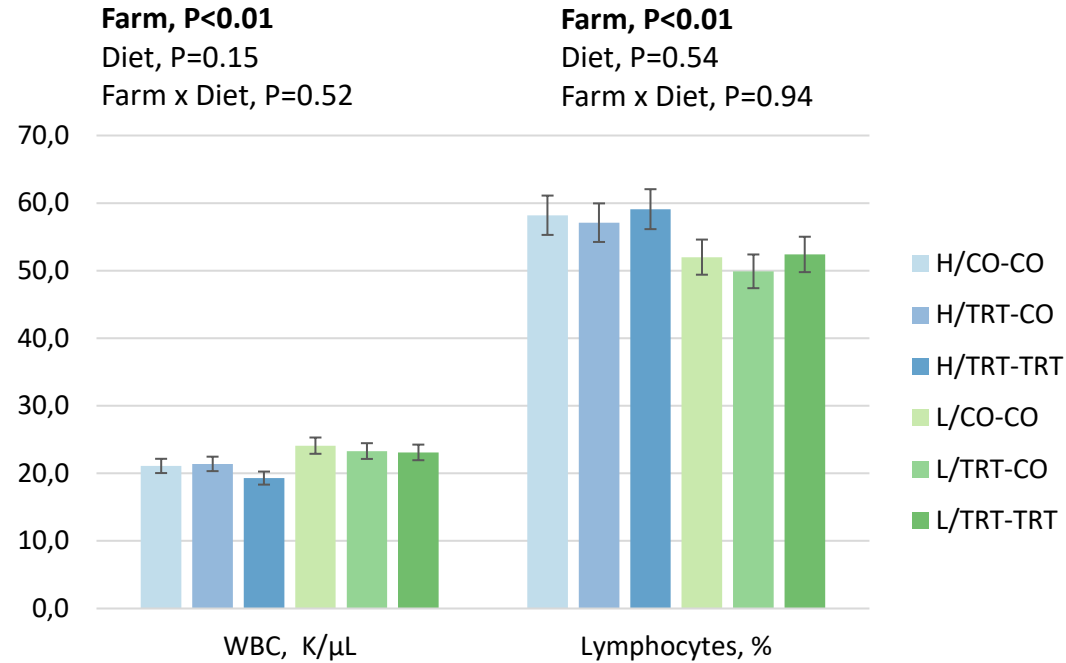


Results: microbiota metabolism and pathway, T4



- The TRT/TRT diet reduced the microbial metabolism of AAs biosynthesis → less use of this substrates in the large intestine
- The TRT/TRT diet increased the pentose and glucuronate interconversion pathway, which is a branch pathway for complex carbohydrates catabolism → selected for bacteria capable of using complex carbohydrates.
- The reduction in peptidoglycan biosynthetic activity in the TRT/TRT diet could confirm a muramidase mechanism of action (Wang et al., 2021); the fact that it is particularly evident in the TRT/TRT group, which received only benzoic acid supplementation in the final phase, suggests a residual effect and possible synergism with benzoic acid.

Results: blood formula and oxidative stress markers, T4



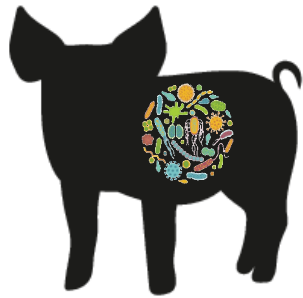
An increase of neutrophil:lymphocyte ratio is a marker of stress in response to adrenocortical activity (Widowsk et al., 1989)



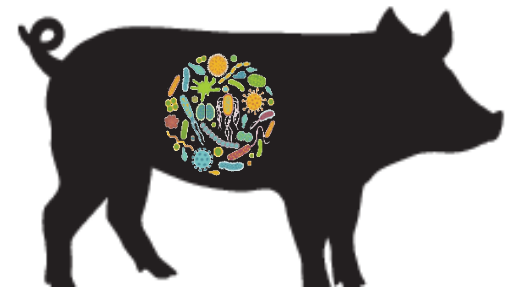
Results: Growth performance, growing phase

Growth performance site 3	H			L			SEM	P-values			
	CO-CO	TRT-CO	TRT-TRT	CO-CO	TRT-CO	TRT-TRT		Farm	Diet PW-Diet Fattening	Sex	Farm:Diet PW-Diet Fattening
BW, kg											
At nine weeks post-weaning (T4)	42.20	42.70	45.40	41.90	42.20	40.60	1.03	0.850	0.05	0.02	0.04
Average daily gain (g/day)											
Seven weeks post-weaning-nine weeks post-weaning (T3-T4)	505 ^b	565 ^{ab}	690 ^a	499 ^b	496 ^b	463 ^b	31.4	0.88	<0.01	<0.01	<0.01
Two weeks post-weaning-nine weeks post-weaning (T2-T4)	615 ^{ab}	634 ^{ab}	679 ^a	597 ^b	600 ^{ab}	572 ^b	16.9	0.43	0.02	<0.01	0.01
Weaning-nine weeks post-weaning (T1-T4)	520 ^{ab}	529 ^{ab}	566 ^a	505 ^{ab}	510 ^{ab}	487 ^b	14.7	0.45	0.05	0.01	0.04

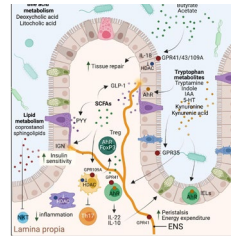
Discussion and conclusion



Gut microbiota and host immune status co-evolution



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Environment

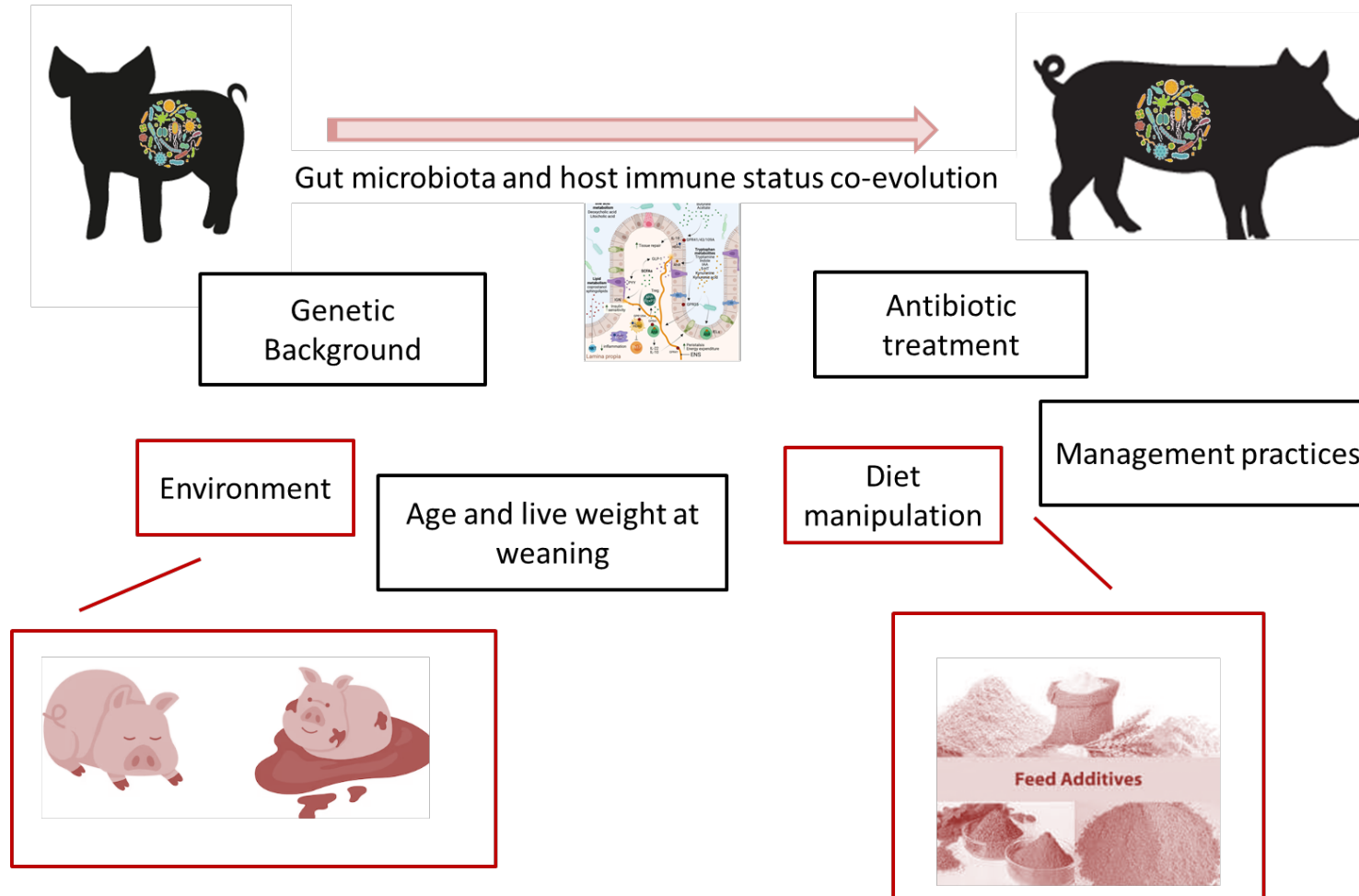
Age and live weight at
weaning

Diet
manipulation

Management practices



Discussion and conclusion



The use of additives under different conditions can have different effects, highlighting the importance of evaluating feeding strategies tailored to specific farm conditions.



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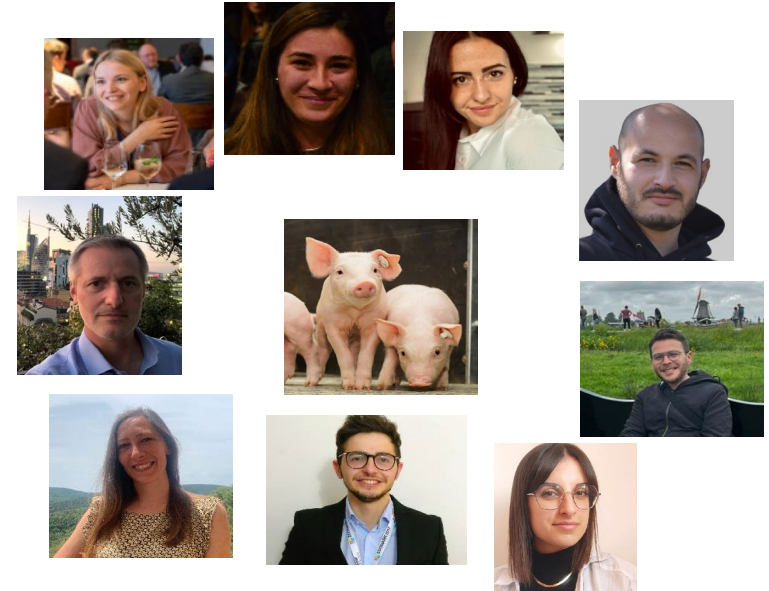


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CircuLations for bEtter food Systems
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Results: metagenomics of faecal samples, T2 and T3

