FUT1 GENE POLYMORPHISM: IMPACT ON GUT MICROBIOTA, IMMUNE RESPONSE AND METABOLOMIC PROFILE OF PIGLETS

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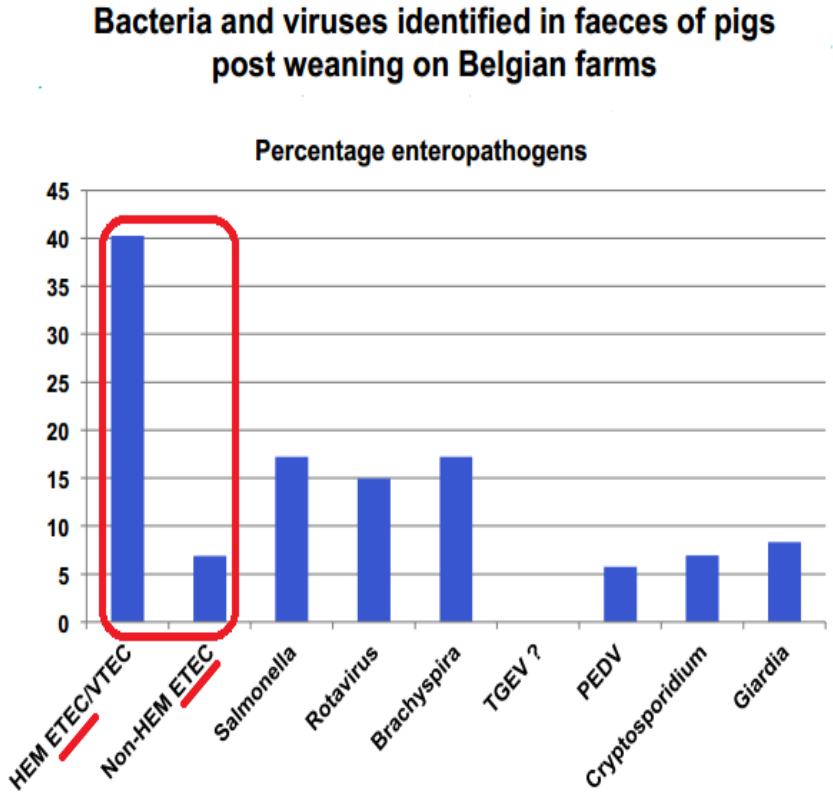
Genetic and environmental factors to understand dysbiosis in the GI tract of pigs (with PiGutNet)

DIARRHEA IN PIGS

› Most outbreaks occur during the first 2 wk post weaning

→ Morbidity may be over 50%
→ Losses of piglets can be 17%
→ Growth reduction (100-400 g/d)

› Enterotoxigenic *E. coli* (ETEC) is an important etiological agent
→ ~10 million piglets die annually worldwide due to diarrhea, 50% is caused by ETEC



Adopted from Cox (2013)

PATHOGENESIS OF PWD

ETEC

Stomach

Intestine

Adherence by fimbriae (F18)

Production of enterotoxins

Heat-labile toxin (LT)

Stimulates adenyl cyclase activity

Increases levels of cAMP in villous and crypt

Heat-stable toxin [ST (STa; STb)]

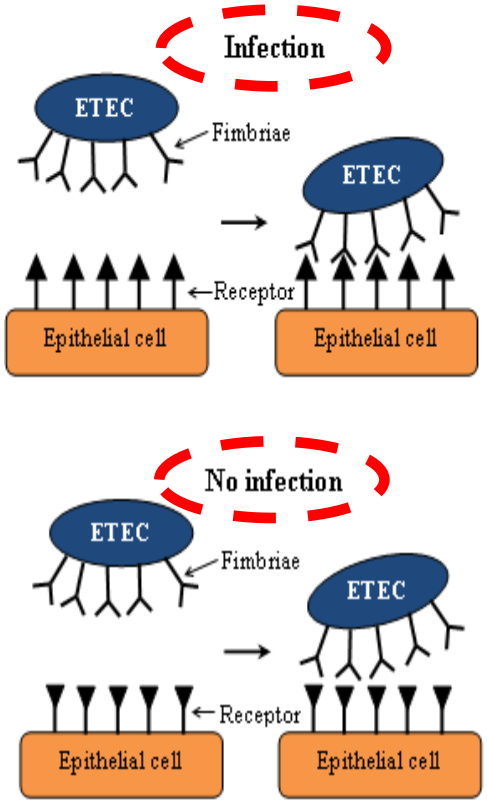
Stimulates guanylate cyclase

Increases levels of cCMP

Possibly increases cellular Ca^{++}

- Villous cells: inhibit the non-glucose-dependent pathway for Na^+, therefore also Cl^- and water absorption
- Crypt cells: stimulate Na^+ along with Cl^- and water secretion

Diarrhea



Modified from Fairbrother et al., (2005) and Heo et al. (2013)

Modified from Roubos-van den Hil (2010)

FUT-1 GENE IN PIGS

- A single guanine-to adenine mutation at nucleotide 307 in ±-(1,2)-fucosyltransferase (FUT-1) gene is determinant for the susceptibility of piglets to E. coli F-18 diarrhoea.

- What is the influence of the host genetics on the development of the gut microbiota, and the host responses (immune system and metabolome) under non-challenged conditions?

OBJECTIVE

To study the influence of FUT1 gene variants on:

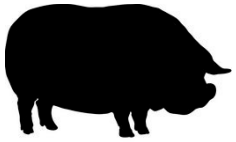
- Establishment and succession of the gut microbiota
- The intestinal binding of *E. coli* F18 and mucosal immune responses of piglets post weaning
- Expression of inflammatory genes and tight junctions in the gut mucosa
- Host metabolism and weight

ANIMAL MODEL

E. Coli
Sensitive

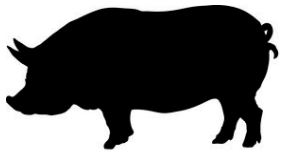
E. Coli
Resistant

♀ **M307$^{G/A}$**



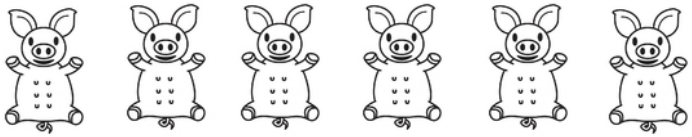
X

♂**M307$^{A/A}$**

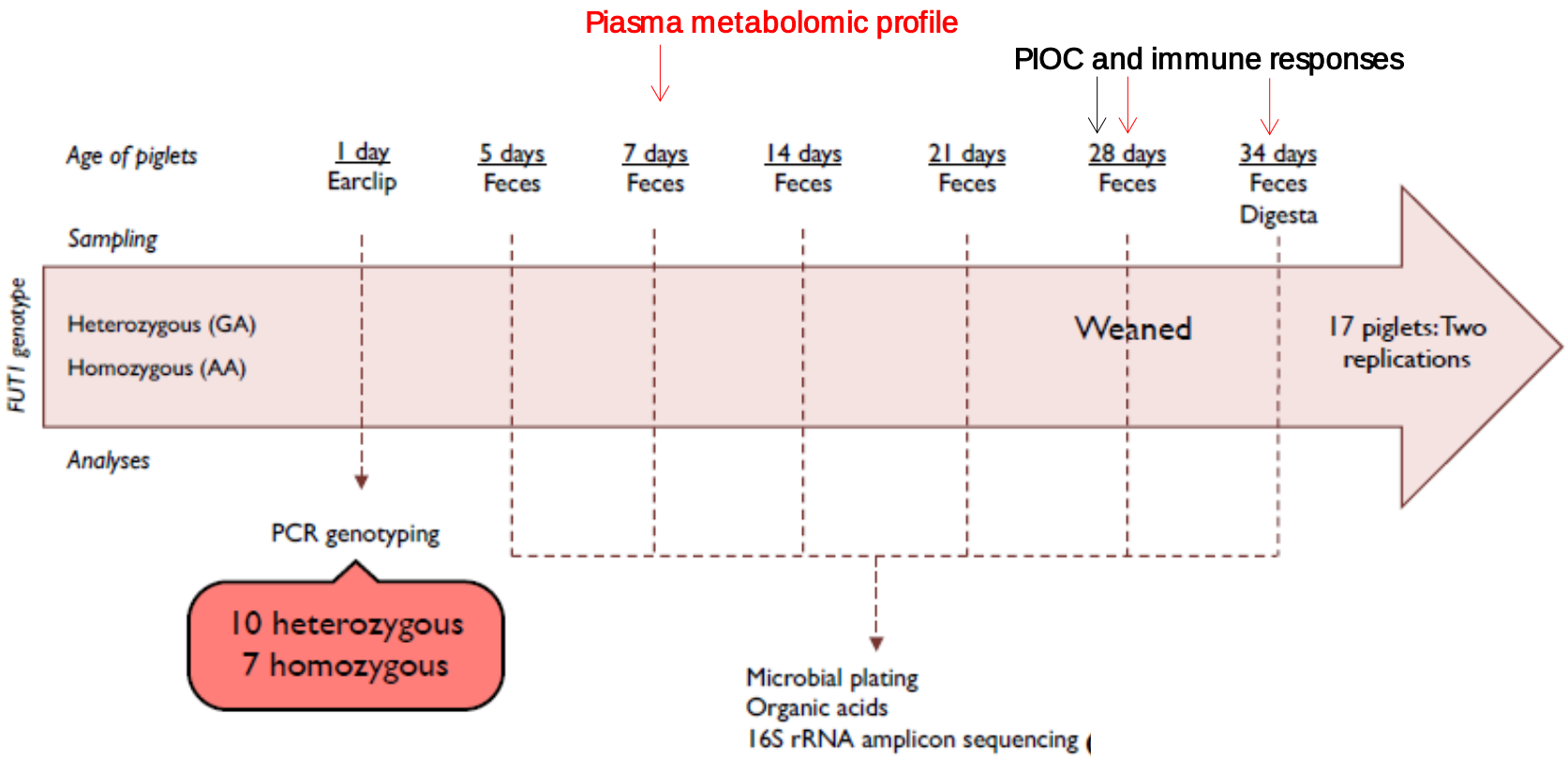


Sensitive

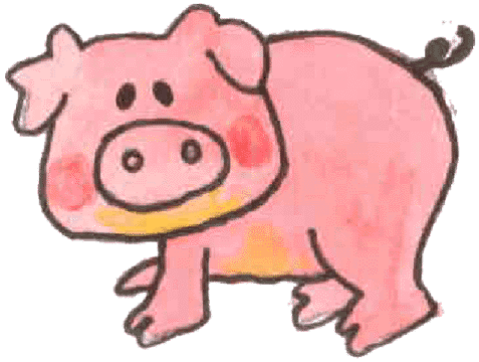
Resistent



EXPERIMENTAL SETUP



*Ex vivo (*PIOC*):* Adhesion of *E. coli* O138:F18 to gut epithelium

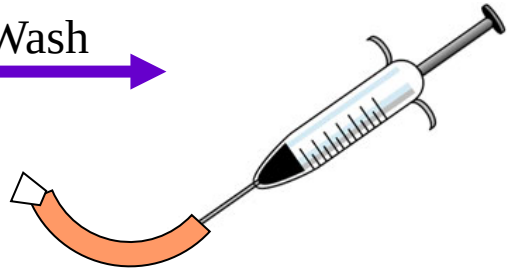


Pigs genotyped susceptible
or resistant to E. Coli F18

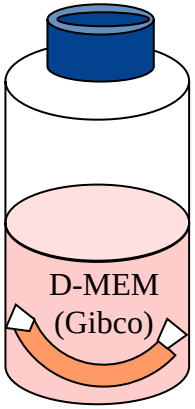
Slaughter

Pieces of ileum (~10 cm)
immediately retrieved

Wash

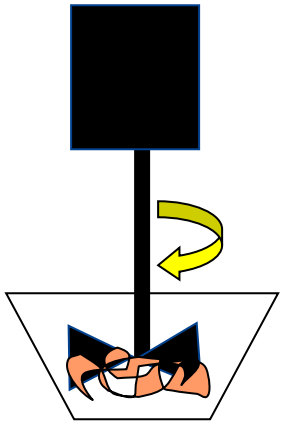


E. Coli ***F4*** in D-MEM (pH 7.0)



Incubation
(60 min, 37˚C)

Wash



Tissue homogenise

Suspension/
Lumen content

Numerically different:

SEN: 6.3 logCFU/g
RES: 6.0 logCFU/g — BI

SEN: 8.1 logCFU/g
RES: 7.8 logCFU/g — AI

PIGLET WEIGHT

Day	SENSITIVE (SEN)	RESISTANT (RES)
0	1.3	1.7
7	2.9	2.4
14	4.0	3.8
21	5.9	5.2
28	8.1[a]	6.8[b]
34	7.8[a]	6.7[b]

[a,b]Values are significantly different ($p<0.05$)

MICROBIAL GROUPS IN FAECES

Day	Hemolytic bacteria Log CFU/ g sample		Clos. Perfringens Log CFU/ g sample		Lactic acid bacteria Log CFU/ g sample	
	SEN	RES	SEN	RES	SEN	RES
5	<6.2	6.3	7.7	7.5	9.7	9.4
7	<6.2	6.3	<8.0	7.8	9.6	9.4
14	<6.3	6.3	7.5	<7.4	8.9	8.7
21	<6.3	6.2	<6.6	6.5	9.1	8.8
28	<6.3	6.3	5.2	<5.0	9.0	8.7
34	8.7	<7.5	<3.0	<2.8	8.8	8.6

No influence of genotype or age on entero- or anaerobic bacteria

SHORT-CHAIN FATTY ACIDS* IN FAECES

Day	SEN	RES	
5	61.7	55.4	A
7	56.0	49.7	A
14	49.4	43.0	A
21	54.3	48.0	A
28	63.8	57.4	Ab
35	87.1	80.7	B

*Sum of acetic, propionic, butyric acid (mmol/kg sample)

No effect of genotype

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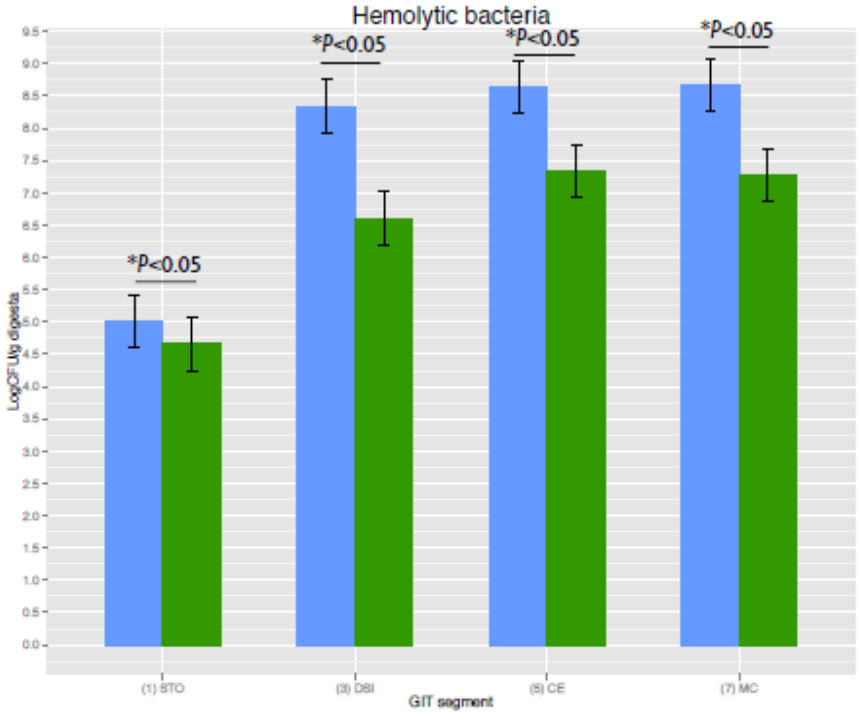
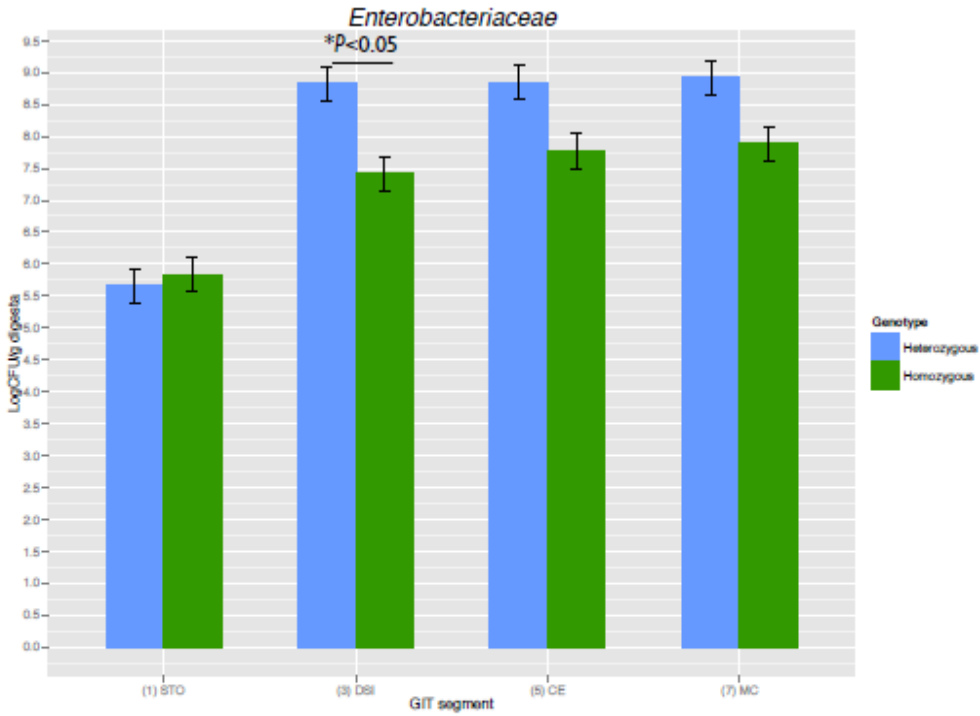
BACTERIAL ENUMERATIONS - DIGESTA





Blue=SEN
Green=RES

(1) STO = stomach
(3) DSI = distal small intestine
(5) CAE = caecum
(7) MC = mid colon

ORGANIC ACID IN DIGESTA (NMOL/KG WET SAMPLE)

	SEN	RES
Acetic acid	GEN*SEG: P=0.01	
Stomach	21.1	26.8
Small intestine	4.2	5.4
Caecum	47.6	39.0
Mid colon	54.5a	39.4b
Propionic acid	GEN*SEG: P=0.01	
Stomach	8.1	11.7
Small intestine	2.3	13.2
Mid colon	1.9	11.9

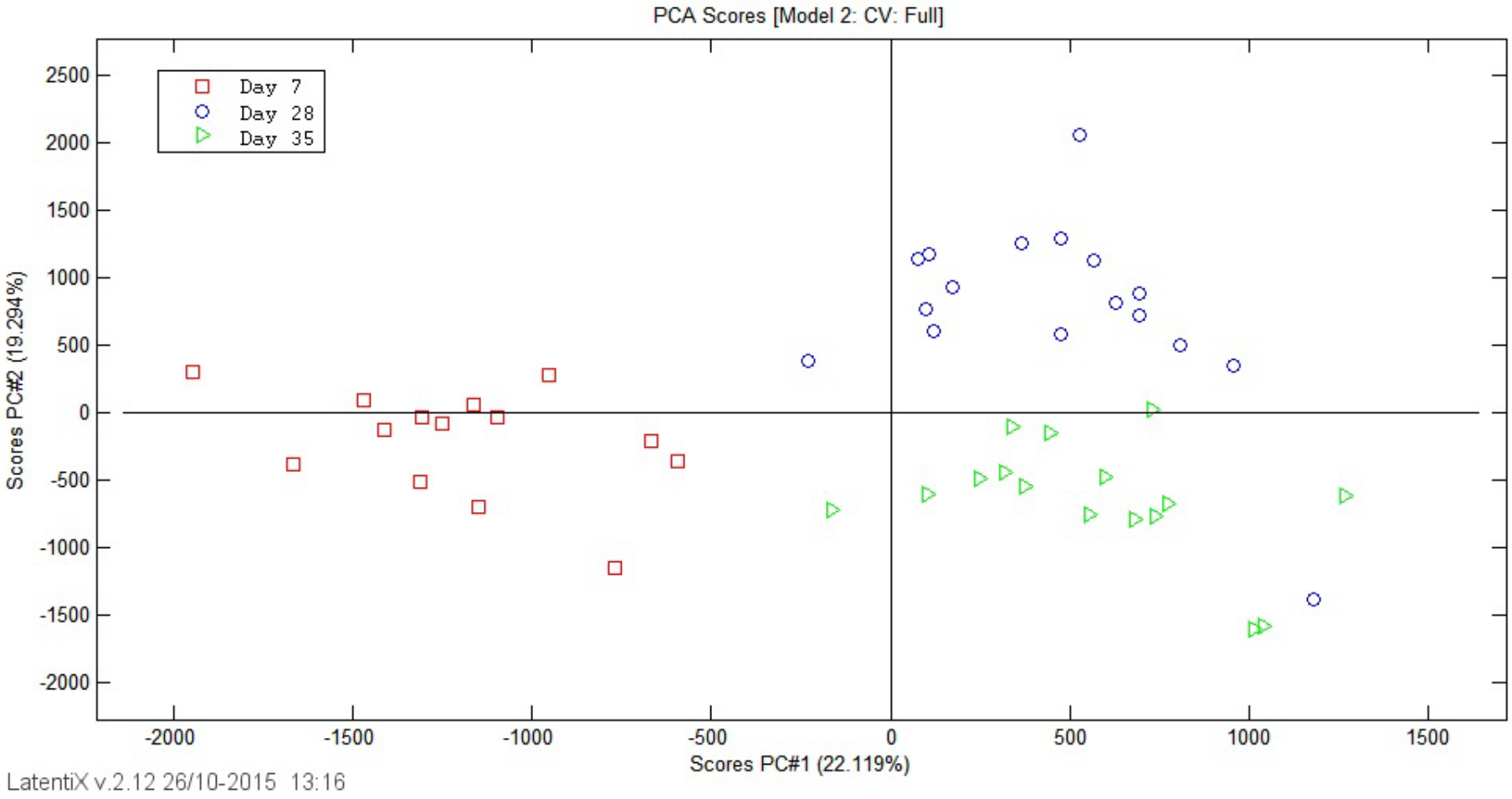
No influence of genotype on lactic acid and butyric acid

EFFECT OF FUT1 ON METABOLOME PROFILE

- Non-targeted LC-MS
- Total dataset: 16 animals for 3 times sampling
- Day 7, Day 28, Day 35
- Raw data: more than 1,000 compounds

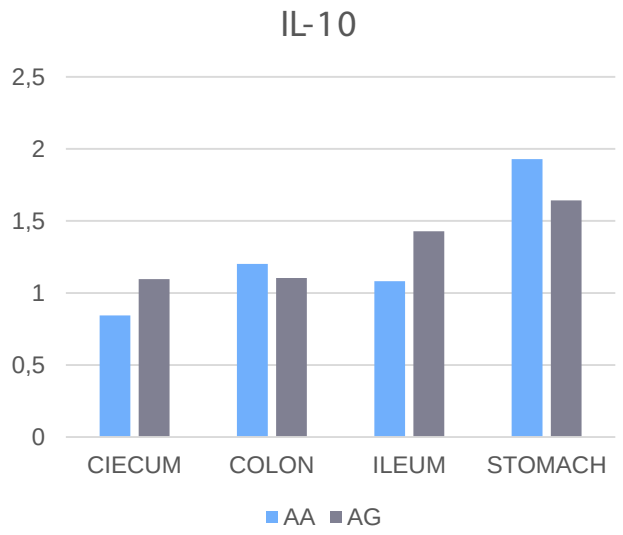
- Age rather than genotype influenced metabolomic profile of plasma

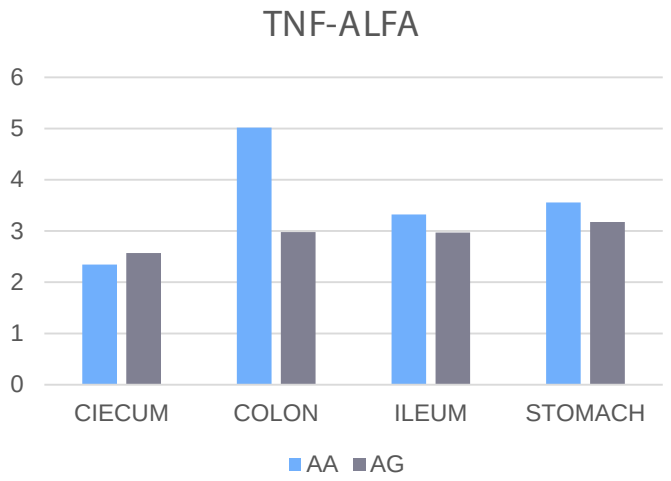
PCA PLOT OF TOTAL PLASMA SAMPLES



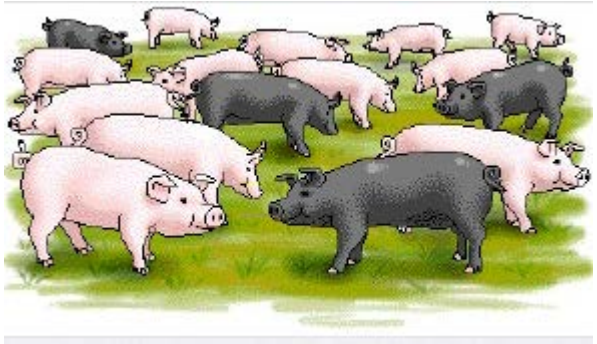
GENE EXPRESSION IN GI-TRACT

› Tissues selected: cecum, colon, ileum, stomach
› Expression of TNF-±, IL-10, COX-2, ZO-1, Occludin
› Preliminary results showed little influence of genotype effect. Tendencies (P<0.1) on IL-10 and TNF-±:





CONCLUSIONS



- FUT1 genotype does seem to influence the colonisation of the GI tract
- Same age-dependent GI microbiota development
- Ex vivo model showed more binding of E. coli to intestinal epithelium in sensitive pigs.
- Preliminary data showed that age rather than genotype affected metabolomic profile.
- Difference in piglet weight (?) – limited number of piglets