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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Assessing differences in small intestinal function in pigs of low and high residual feed intake

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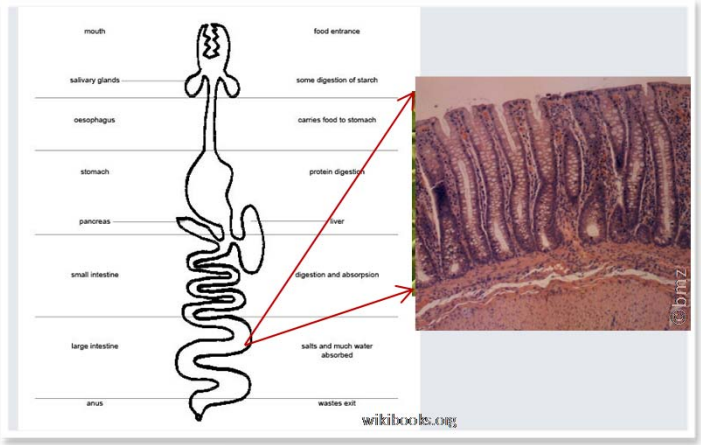
Introduction

Gastro-intestinal tract (GIT) and liver
- important energy sinks
- need disproportionate amounts of energy relative to their weight

Contribute to diverging residual feed intake (RFI) in pigs



<u>Low</u> <u>versus</u> **<u>high</u>** <u>RFI pigs:</u>

Controversial results for differences in small intestine
- digestive & absorptive function
- morphology
- barrier function
- mucosal immune response

Hypothesis

- Low RFI is linked to enhanced digestive and absorptive capacity in pigs.
- Low RFI is associated with enhanced intestinal barrier function and reduced expression of pathogen-recognition receptors and cytokines.

Objective

to investigate differences in size, structure and function...

- intestinal morphology
- duodenal disaccharidase activity
- jejunal permeability
- gene expression in relation to sugar and short-chain fatty acid transport, tight-junction proteins and innate immune response

... in finishing pigs of diverging RFI.

Pig trial and sample collection

- 6 litters (♂ and ♀), n = 12 per pen
- Weighing once a week
- Transponder feeding from day 42 postweaning
- Wheat-barley-soybean meal based diet
- Selection of pigs using feed intake and ADG from day 42 to 98 postweaning
  - 8 low RFI pigs and 8 high RFI pigs (4 ♂ and 4 ♀ each)
- Gut tissue sampling (postprandially): days 102-105 postweaning

Gut tissue	Duodenum	Jejunum	Ileum	Caeca
Length + weight	✓	✓	✓	✓
Histo-morphology	✓	✓	✓	✓
Mucosal disaccharidases activity	✓			
Ussing chamber experiment		✓		
Candidate gene expression		✓		

Lab analyses

Ussing chamber experiment:

Electrophysiological measurements:

- Continuous recording short-circuit current (Isc, µA/cm^2), and
- Tissue resistance (R_T, Ω × cm^2) -> tissue conductance (G_T, mS/cm^2) = 1 / R_T

Permeability marker measurements:

- Mucosal-to-serosal flux of fluorescein 5(6)-isothiocyanate (FITC) & horseradish peroxidase (HRP)

Histo-morphology:

- Formol-fixation and paraffin embedding
- Hematoxylin and eosin staining
- Villus height & width, crypt depth, goblet cells, intraepithelial lymphocytes

Mucosal disaccharidases activity:

- maltase (EC 3.2.1.20), sucrase (EC 3.2.1.48) and lactase (EC 3.2.1.23) activities in one gram protein (modified to Dahlquist, 1964)
- Released glucose was determined using glucose oxidase-peroxidase method
- Protein determination using Coomassie Blue dye-binding protein quantitation assay

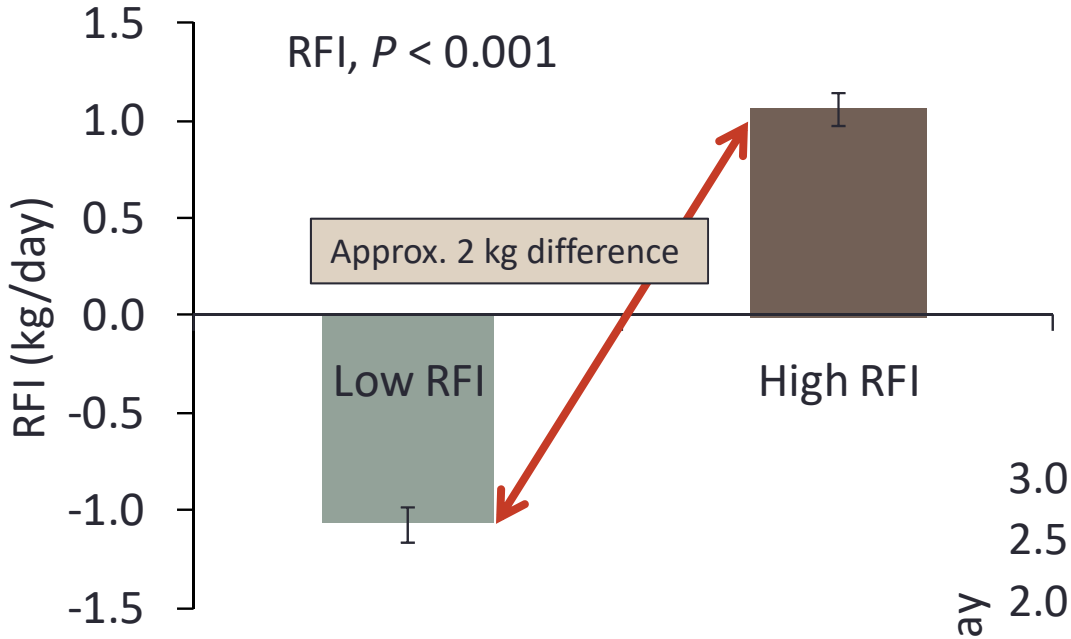
Candiate gene expression:

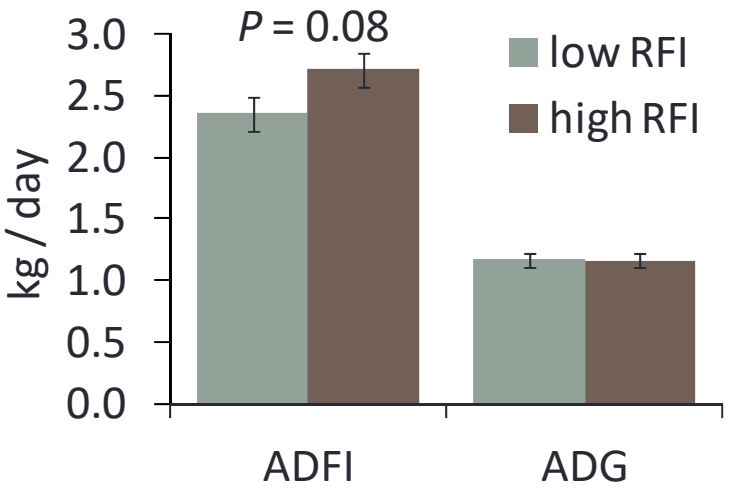
- RNA isolation using bead-beating and RNeasy Midi Kit (Qiagen)
- Reverse-transcription-quantitative PCR using EvaGreen chemistry

Statistical analysis:

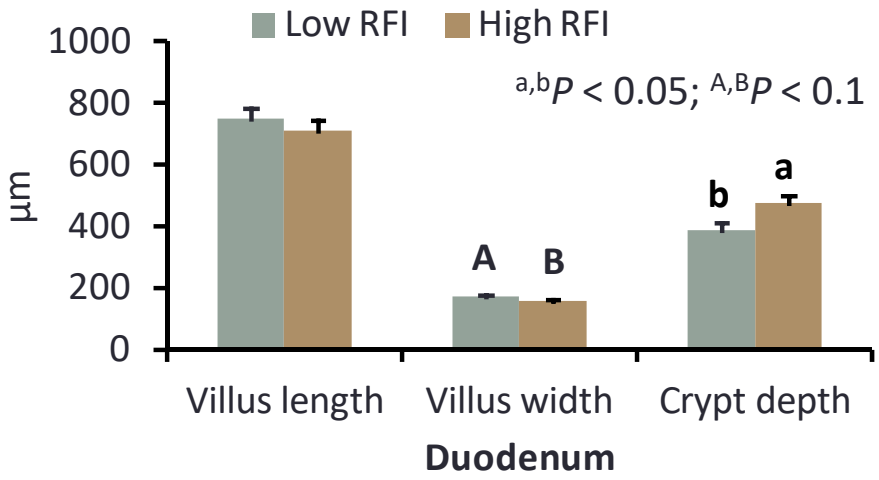
- ANOVA using PROC MIXED (SAS)
- least-squares means ± pooled SEM; significance: $P \leq 0.05$; trends: $0.05 < P \leq 0.10$

Residual feed intake, feed intake and growth of pigs of diverging feed efficiency

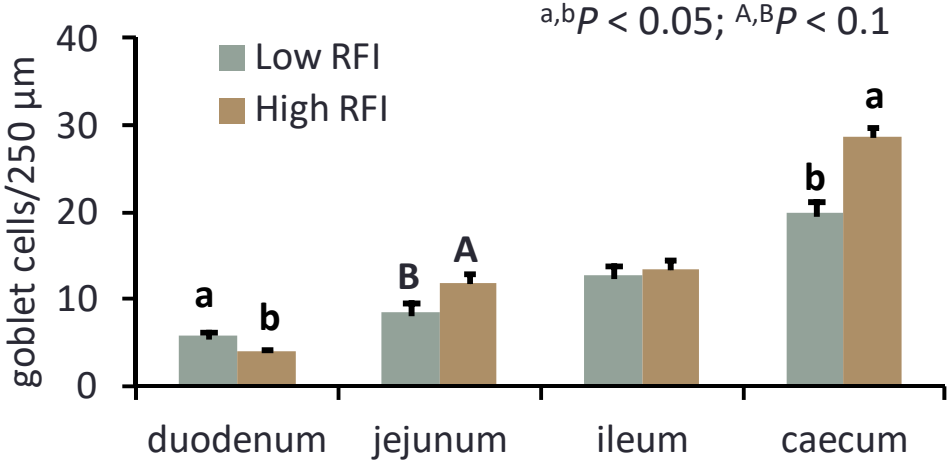




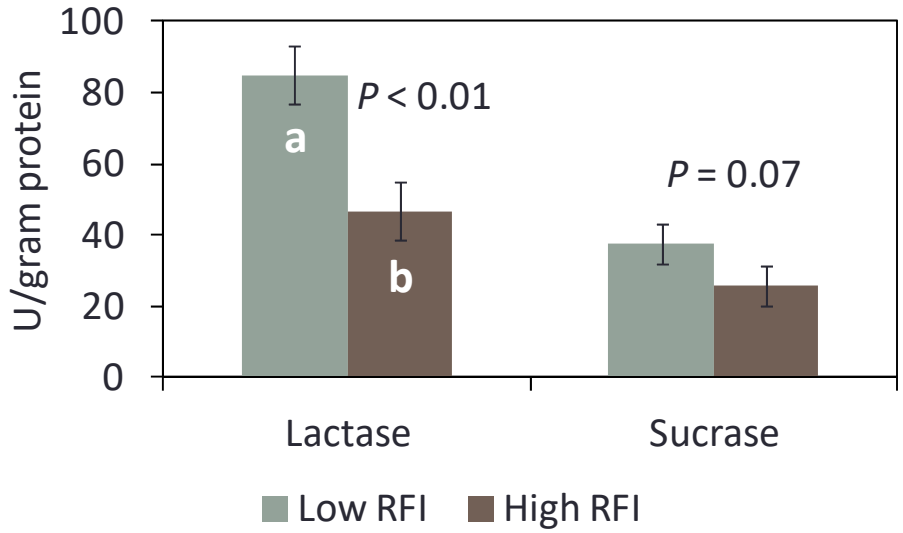
Histo-morphology in pigs of low and high RFI



Questions that arise:
- Effects related to feed intake ?
- Effects related to microbiota ?

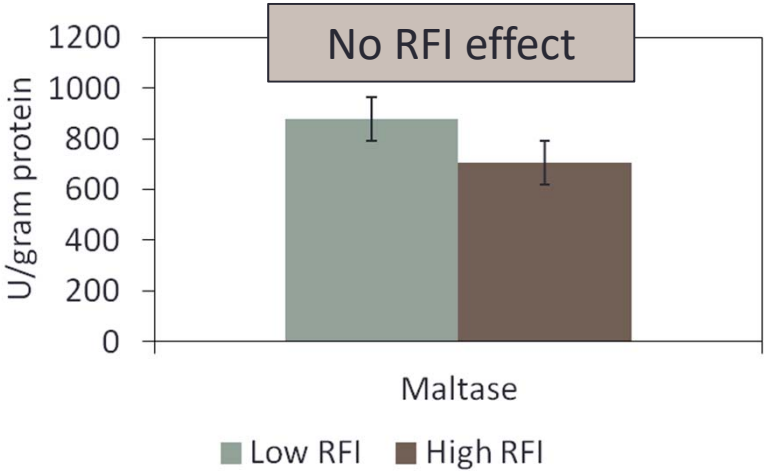


Disaccharidase activities in duodenum of pigs of low and high RFI



Low RFI in pigs:

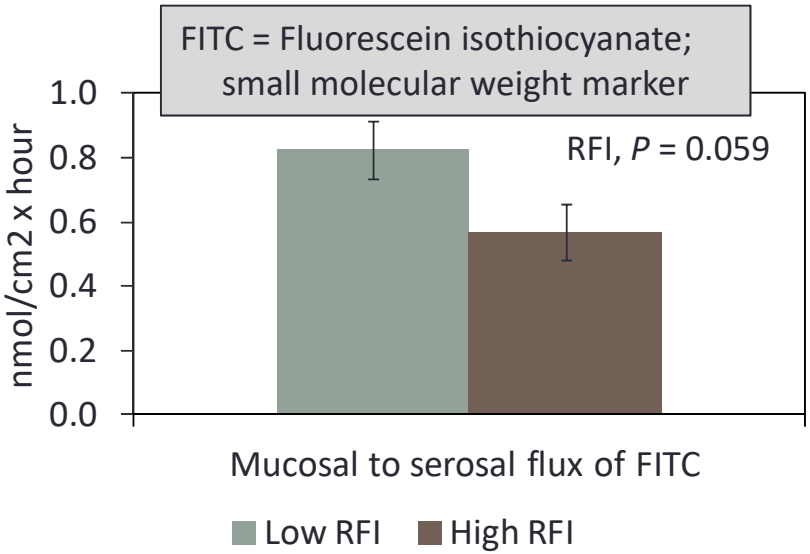
- Does the greater lactase activity have relevance for RFI gain ?

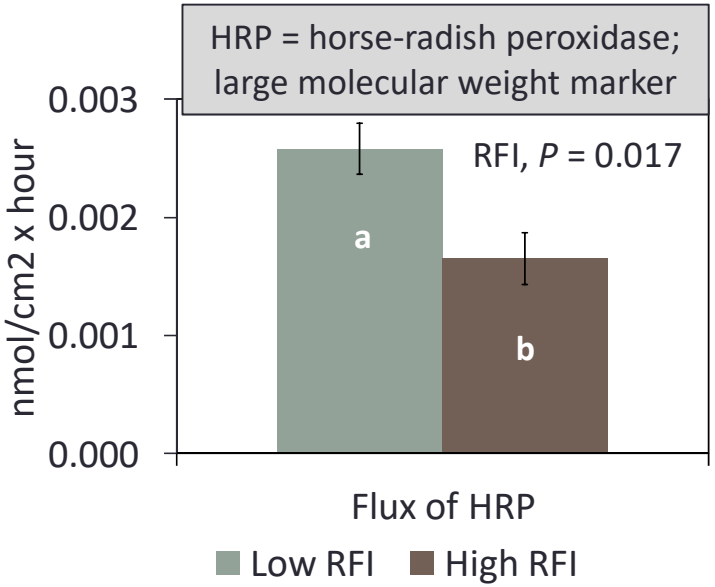


Ussing chamber results of low and high RFI pigs

Distal jejunum (2.5 m crandial to ileo-cecal junction)

Parameter	Low RFI	High RFI	SEM	RFI, P-value
Short-circuit current (mA/cm^2)	54.9	55.7	13.77	0.97
Tissue conductance (mS/cm^2)	16.9	17.5	1.90	0.81





Low RFI in pigs: permeability ↑ in distal jejunum

Expression of target genes at jejunal mucosa in pigs of low and high RFI pigs

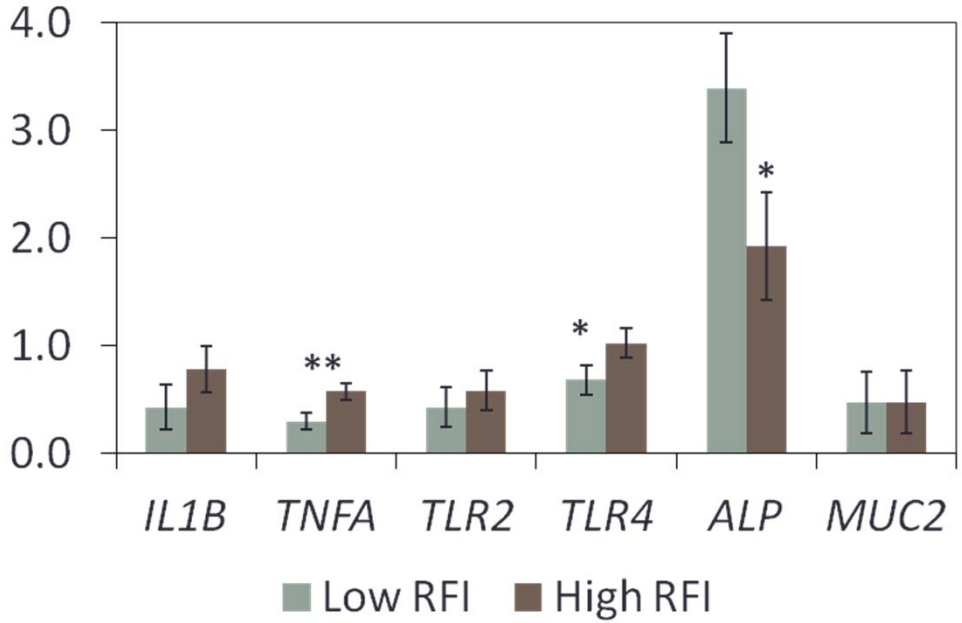
Distal jejunum (2.5 m crandial to ileo-cecal junction)

Candidate genes:

IL1B	=	interleukin 1β
TNFA	=	tumor necrosis factor-α
TLR2	=	Toll-like receptor 2
TLR4	=	Toll-like receptor 4
ALP	=	intestinal alkaline phosphatase
MUC2	=	mucin 2
MCT1	=	monocarboxylate transporter 1
SGLT1	=	sodium/glucose-co-transporter 1
OCLN	=	occludin
ZO1	=	zona occludens 1

*RFI, $P < 0.1$
**RFI, $P < 0.05$

Genes related to innate immune response



Differences in mucosal response to luminal lipopolysaccharides in distal jejunum

Fold changes were calculated using the 2^{-ddCq} method

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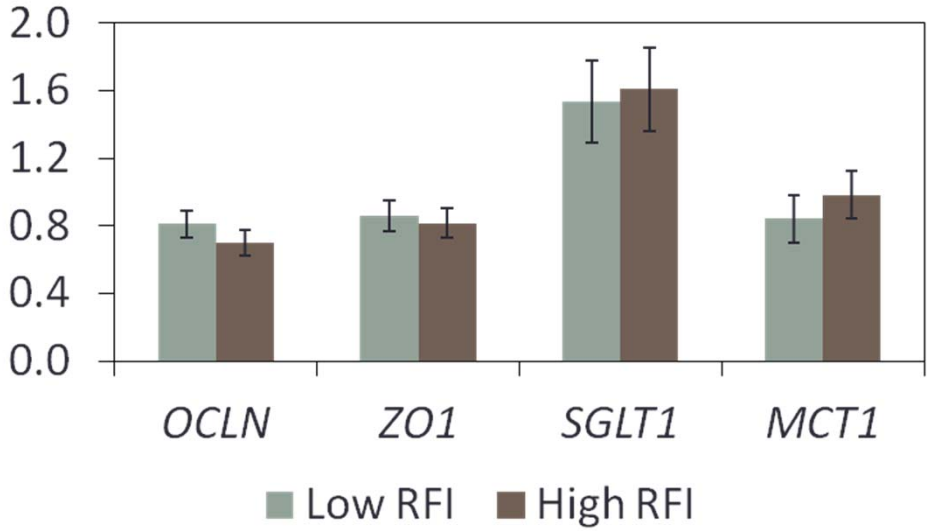
No RFI effect



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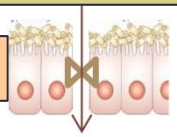
Summary and Conclusion

Finishing pigs → RFI-related differences at gut level

Duodenum
- Goblet cells
- Villus width
- Crypt depth
- Lactase & sucrase activities

Distal jejunum



- Goblet cells
- Paracellular permeability
- Gene expression
  - *TNFA, TLR4, ALP*

Cecum
- Goblet cells

Low vs. high RFI:
- Differences in intestinal **structure** and **function**
- Jejunal gene expression ≠ observations at functional protein level

→ RFI gain was related to differences in innate immune response including jejunal barrier function
→ effect of the **intestinal microbiota**
→ effect of host-nutrient absorption
→ To clarify: effect of feed intake level and intestinal nutrient flow

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Determination of feed efficiency

Selection of high and low feed efficient animals - based on **Residual Feed Intake**

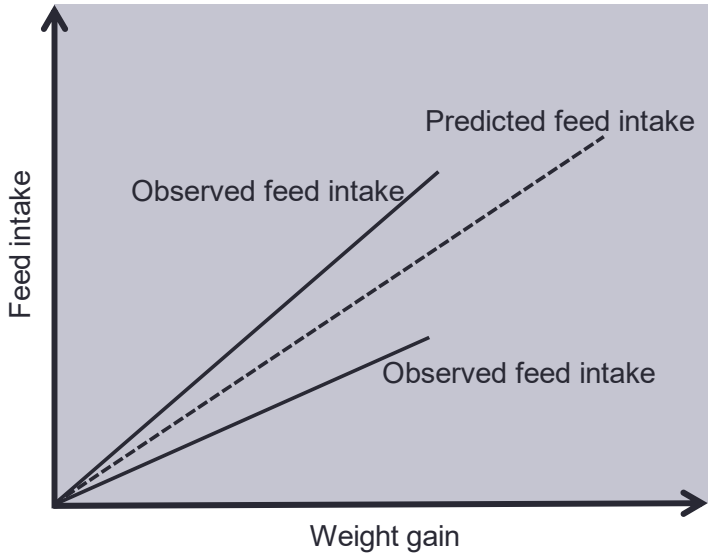
Residual Feed Intake (RFI) = difference between observed and predicted feed intake, with lower RFI values indicating greater energy efficiency

Other measures of feed efficiency

Feed conversion ratio

Residual body weight gain

Residual feed intake and body weight gain



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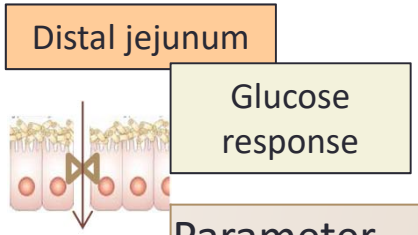
Ussing chamber experiment

- The tissue was alternatively pulsed with a positive or negative pulse of 20 µA and 100 ms duration.
- The displacement in PD caused by the current pulse was measured, and the transepithelial tissue resistance (R_T) was calculated from the change in PD using the law of Ohm.
- After the equilibration period, the tissue was short-circuited by clamping the voltage to zero.

- Transepithelial potential difference (dP0, mV), I_{sc} (µA/cm^2) and R_T ($\Omega \times cm^2$) were continuously recorded using a microprocessor-based voltage-clamp device and software (version 9.10; Mussler, Microclamp, Aachen, Germany).
- The magnitude of the clamp current is determined from PD and the series resistance of the circuit plus mucosa, and it was applied continuously by the automatic voltage clamp

I_{sc} = net sum of electrogenic charge transfer by the epithelium, which has the same magnitude but opposite direction to the externally applied clamp current.

Ussing chamber results of low and high RFI pigs



Parameter	Low RFI	High RFI	SEM	RFI, *P*-value
Basal short-circuit current (mA/cm²)	55.8	61.7	10.72	0.71
Δ short-circuit current (mA/cm²)	0.39	1.40	0.32	0.05
Basal tissue conductance (mS/cm²)	15.8	16.2	2.01	0.90
Δ tissue conductance (mS/cm²)	0.89	0.83	0.10	0.70

Basal measurements were performed 1 min before glucose addition (concentration at mucosal chamber side: 10 mmol/L)

Δshort-circuit current and Δ tissue conductance = max. values obtained from 2 min after glucose addition minus basal values 1 min before glucose addition.