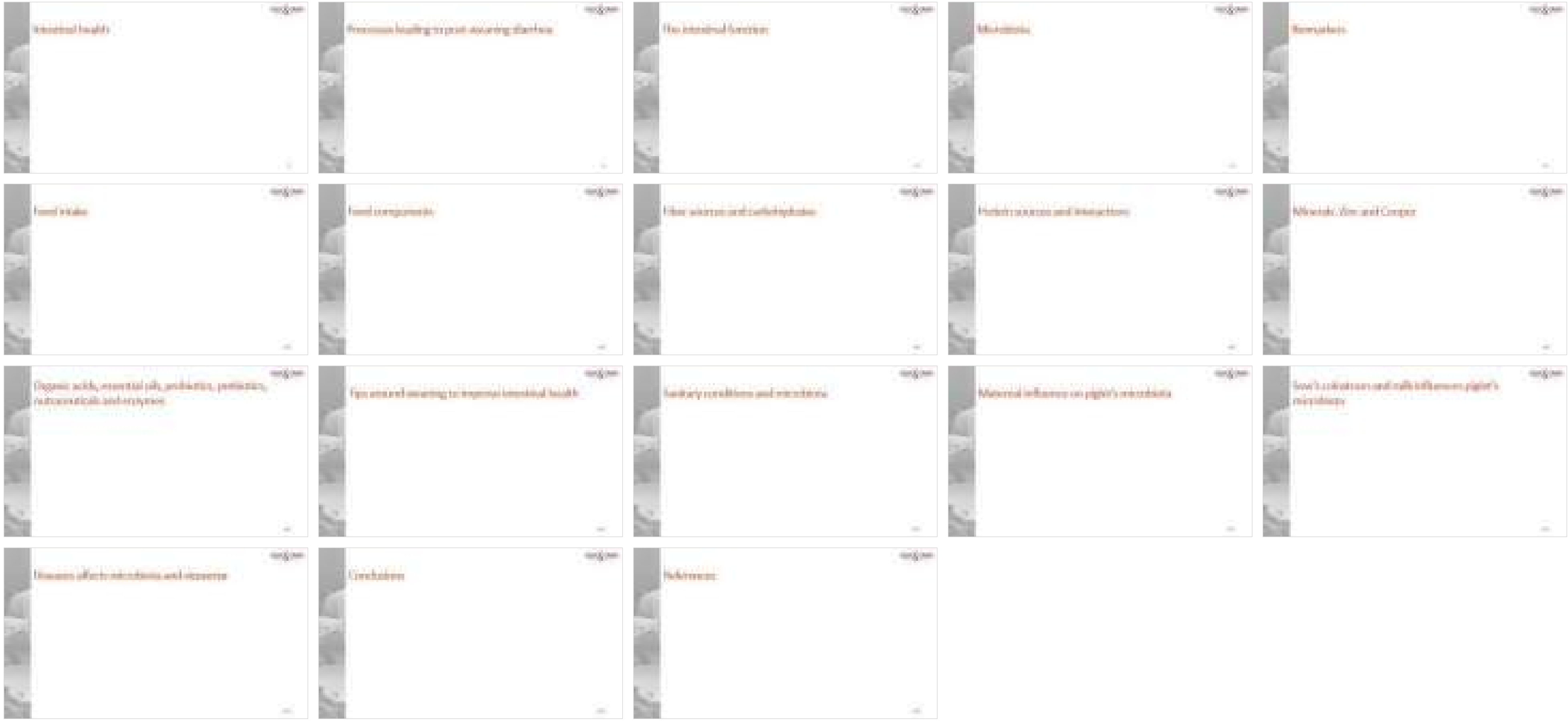
TESTS & TRIALS
exactitud precisión

MICROBIOTA AND INTESTINAL HEALTH IN PIGLETS

Alberto Morillo Alujas

Microbiota and intestinal health in the piglet



Intestinal health

What is intestinal health?

- The one in nature?: Feasts and famines
- Veterinarians?: Absence of disease
- Nutritionist?: Additives...
- Scientists?: We do not know yet...
- Animal production: high yield and productivity, economy

Bischoff *BMC M*
http://www.bio

DEBAT

'Gut

Stephan C

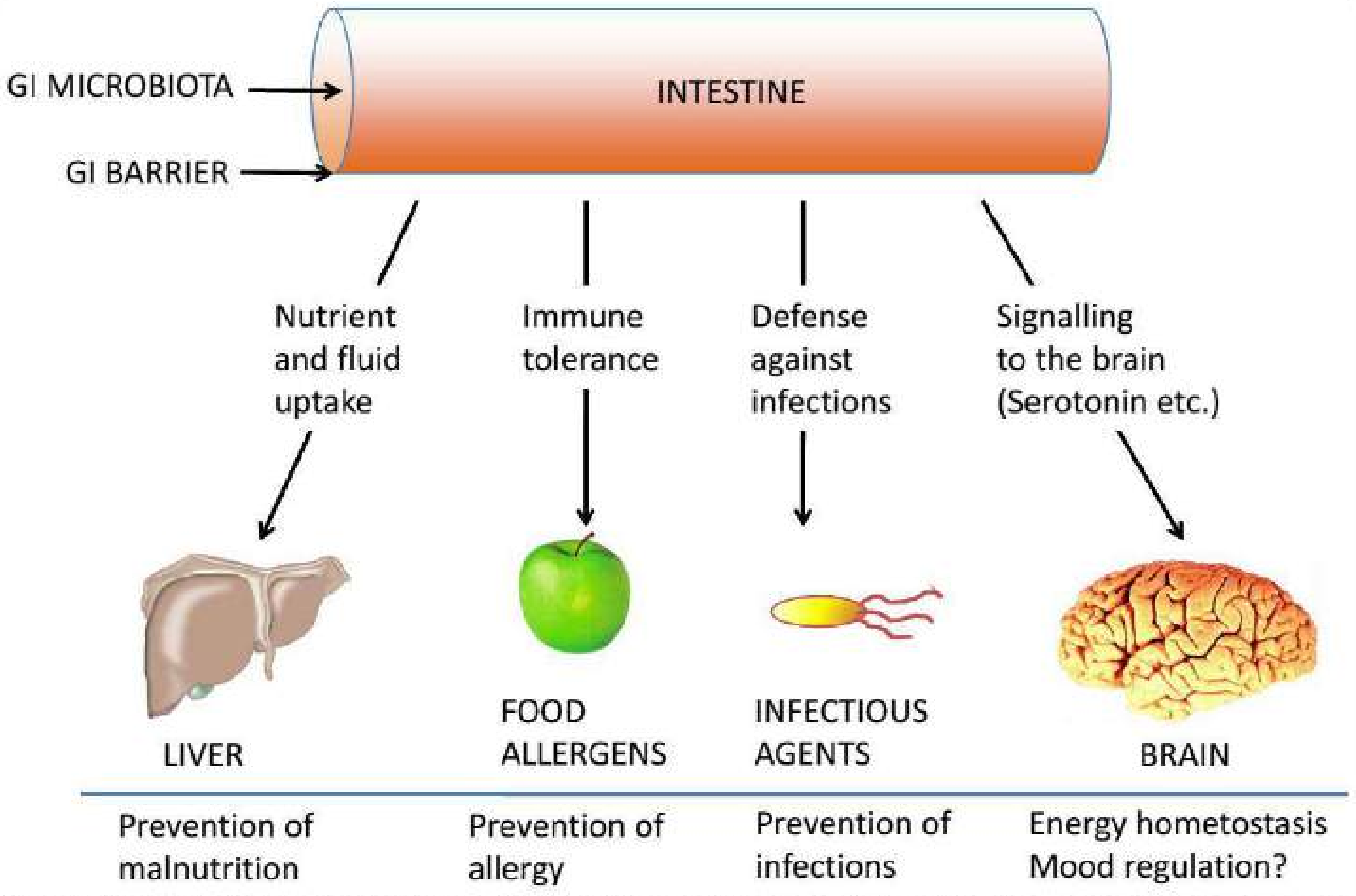


Figure 1 The intestine's impact on health. The gastrointestinal tract contributes to health by ensuring digestion and absorption of nutrients, minerals and fluids; by induction of mucosal and systemic tolerance; by defence of the host against infectious and other pathogens; and by signalling from the periphery to the brain. For details and references, see text 'Underlying mechanisms'.

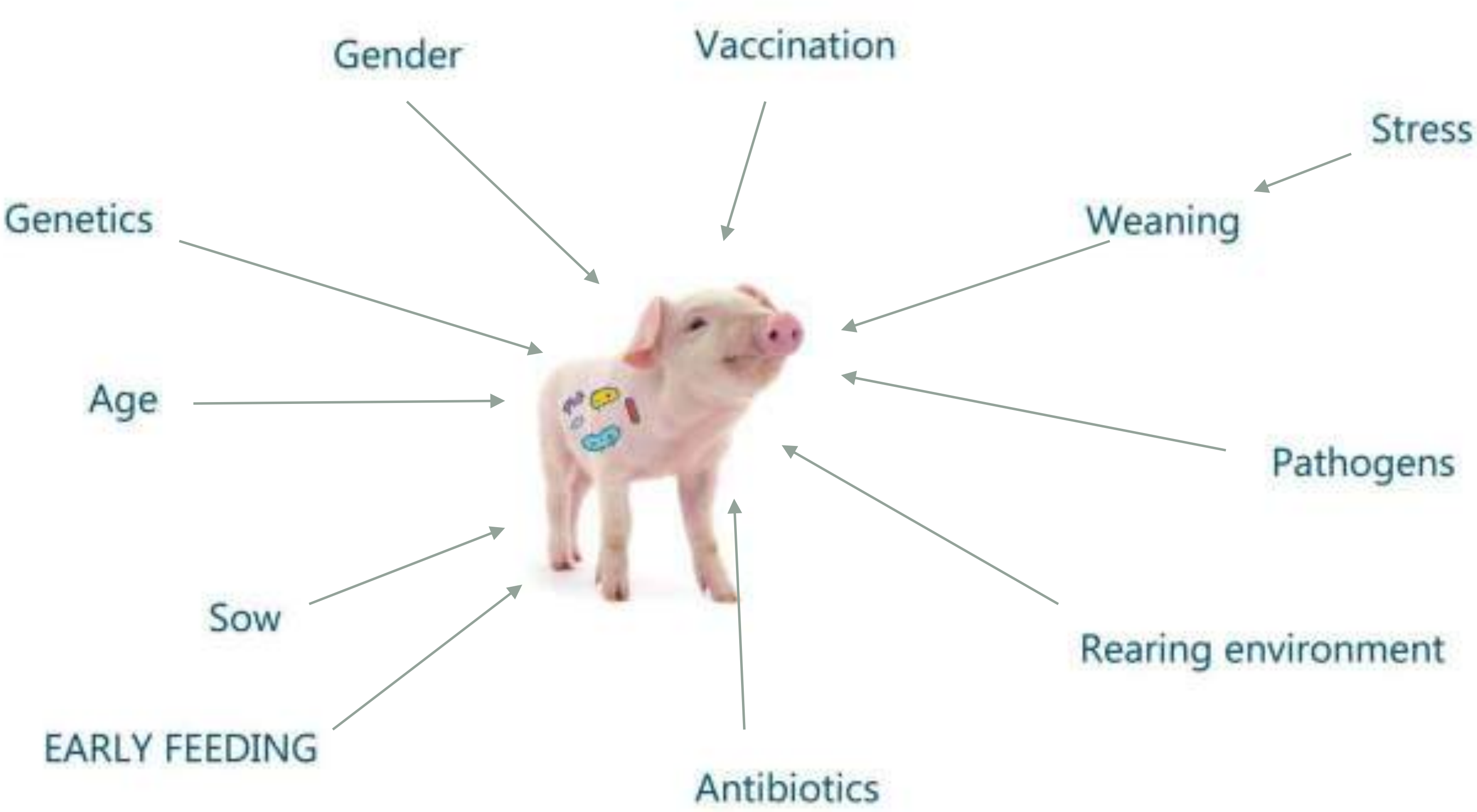
Bischoff, S.C. 201

What is intestinal health? Celi answers[32]

- Diet where macro and micronutrients, production-improving additives, anti-nutritional factors of the different ingredients and indigestible fractions must be considered.
- An effective immune system.
- Effective digestion and absorption.
- A stable and effective microflora without overgrowth.
- *An intact intestinal mucosa with its mucous layer, epithelium, and associated lymphoid tissue.*
- *Neuroendocrine and motor function of the intestine.*

Celi, P. et al. 2017. «Gastrointestinal Functionality in Animal Nutrition and Health: New Opportunities for Sustainable Animal Production». Animal Feed Science and Technology 234 (diciembre): 88-100.

What is intestinal health?



Gender

Vaccination

Stress

Genetics

Weaning

Age

Pathogens

Sow

Rearing environment

EARLY FEEDING

Antibiotics

Molist, F, 2013. Nutritional strategies to reduce AMU and ZnO.

Processes leading to post-weaning diarrhea

Process after weaning to develop post-weaning syndrome

Weaning

Diet changes
Inmature digestive system

Environmental & Social changes

Inmature Immune system

Stress
Fastening

Changes in microbiota
Bacterial overgrowth

Changes in intestinal morphology
Villi height and crypt depth

Opportunistic infections

Malapsorption
Mal digestion

Post weaning diarrhoea

POST WEANING SYNDROME

The intestinal function

The intestinal function[4]

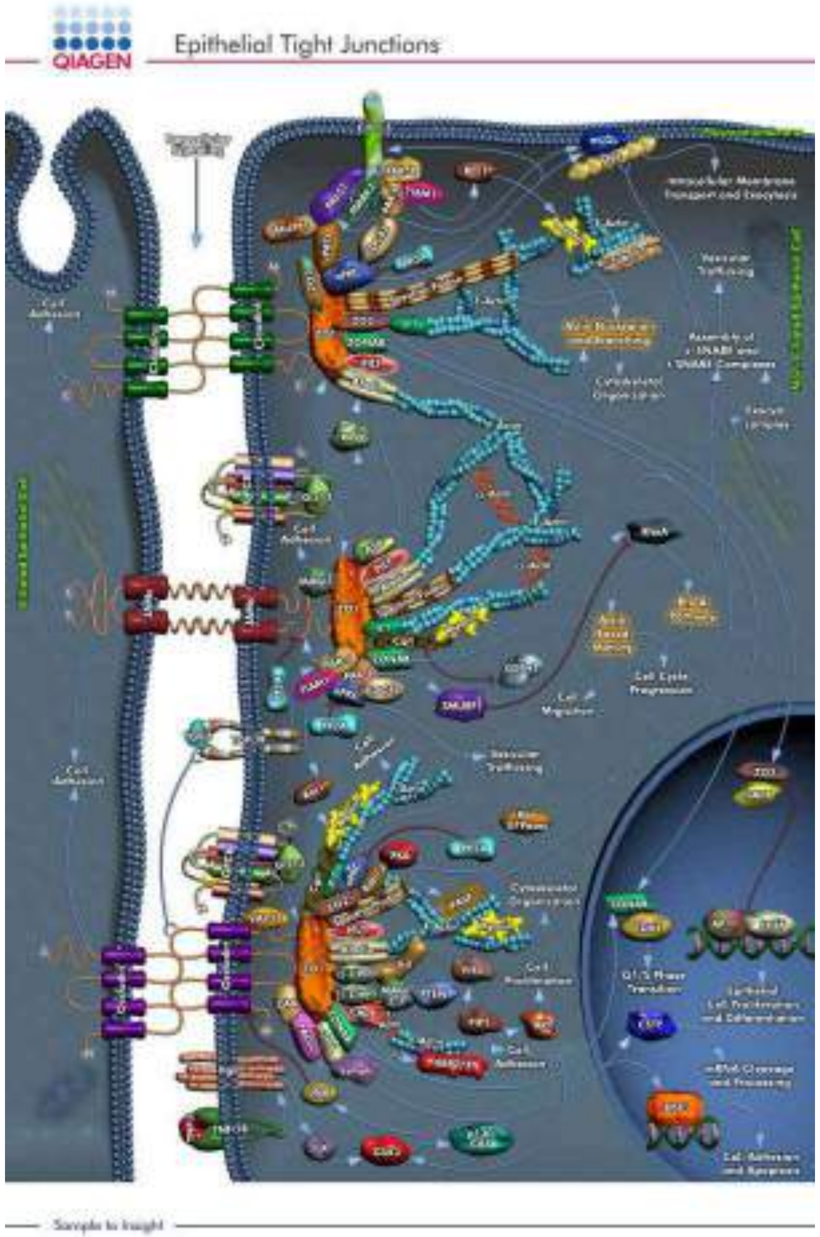
- Feed digestion
- Absorption of nutrients, electrolytes and water secretion
- Epithelial cell proliferation and differentiation
- Epithelial restitution after aggression and damage
- Protect the organism against:
  - Harmful feed constituent
  - Bacteria and viruses

The intestinal function[4,5]

- Epithelial layer is the major component of the gut barrier
  - Between (Tight Junctions, TJ)
  - Within cell protection systems (Heat Shock Proteins, HSP)

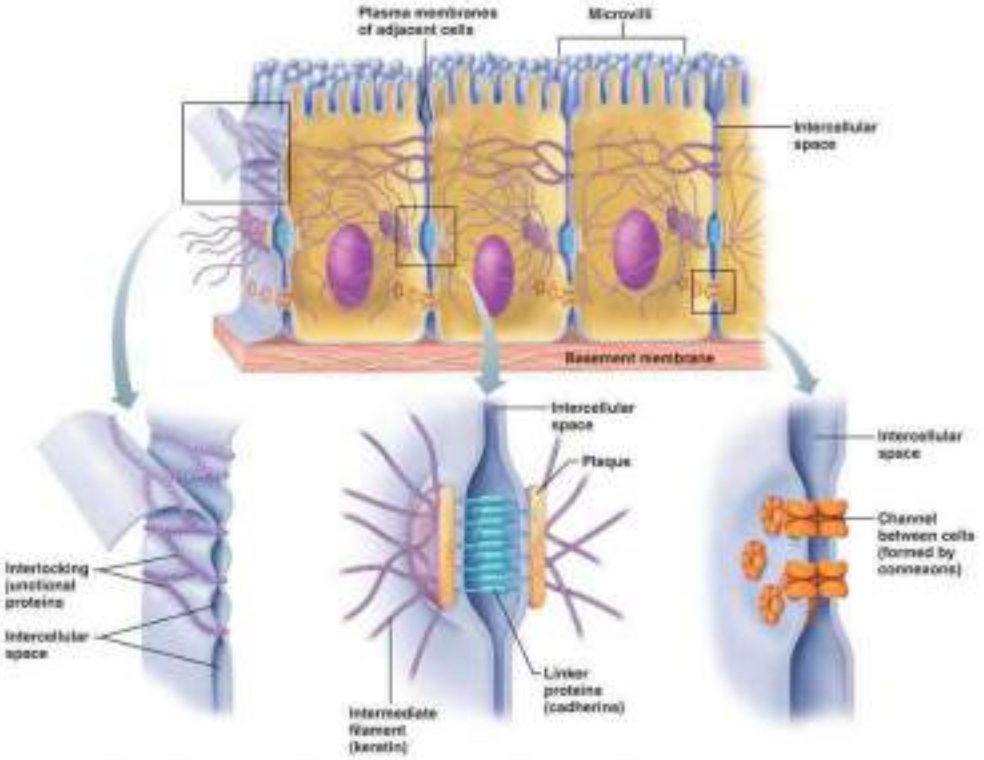
The intestinal function[4,5]

- Tight junctions:
  - Intercellular junctions to connect intestinal cells
  - Make links between the cytoskeleton and actin filaments
  - Controlling paracellular permeability
  - Types: > 40, Claudins, Occludins, scaffolding proteins of the zonula occludens (ZO family)



Pinto da Silva, P. and Kachar, B. (1982). «On tight-junction structure». *Cell* (Elsevier) **28** (3): 441--450.

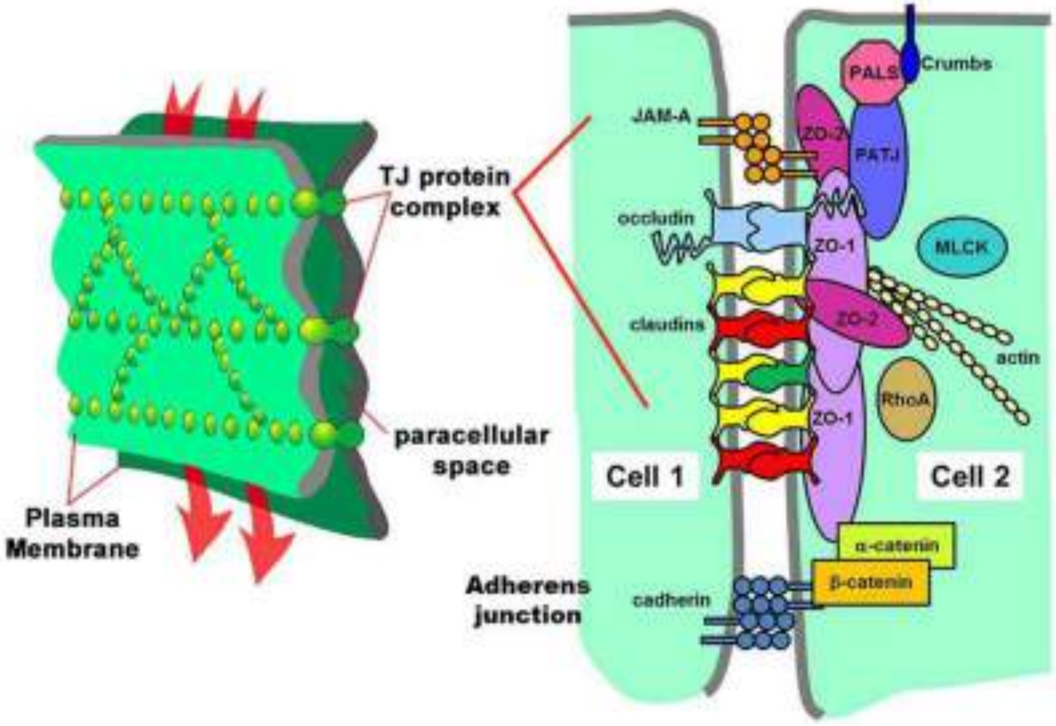
The intestinal function



(a) Tight junctions: Impermeable junctions prevent molecules from passing through the intercellular space.

(b) Desmosomes: Anchoring junctions bind adjacent cells together like a molecular "Velcro" and help form an internal tension-reducing network of fibers.

(c) Gap junctions: Communicating junctions allow ions and small molecules to pass for intercellular communication.



https://ruiabioanalyticalsciences.wordpress.com/2016/09/20/tight-junction-gap-junction/comment-page-1/

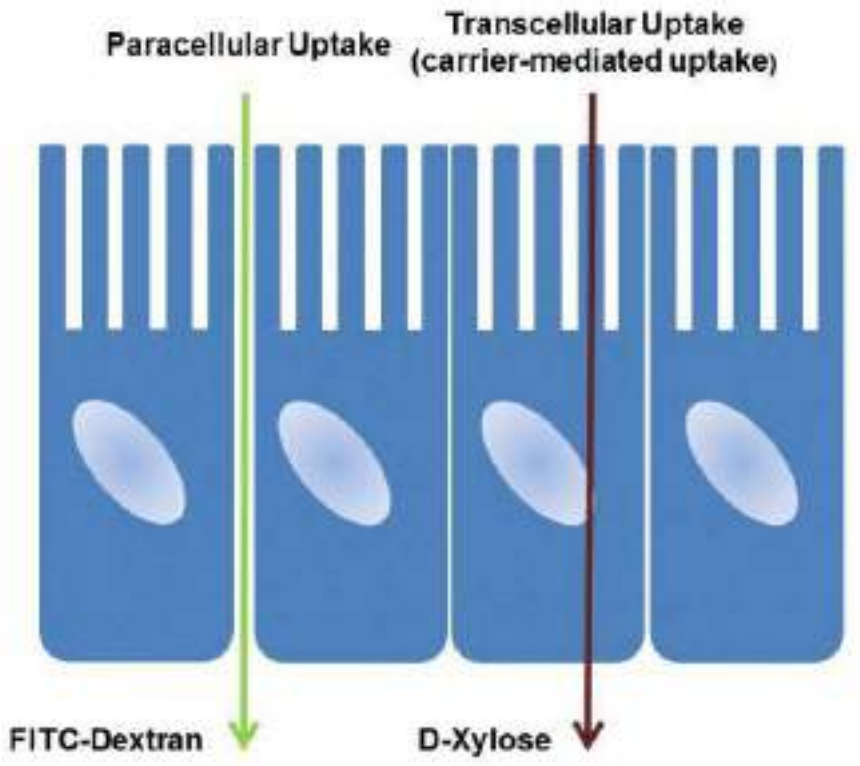
The intestinal function[4,5]

- Epithelial barrier function and its neuro-immune regulation
  - Direct cross-talk between the host and the microbiota
    - Integrate component of the brain-gut axis
  - Nervous system, mucosal mast cells and other mediators in the epithelium

The intestinal function[2,4]

- Gut barrier and mucosal mast cell
  - Activated by nervous pathways via CRF
  - Increasing epithelial permeability

- Cytokines
  - Major regulators of permeability
  - Inflammatory cytokines increases paracellular permeability
  - Anti-inflammatory cytokines decreases



https://www.chondrex.com/products/permeability-evaluation-solution-fitc-dextran

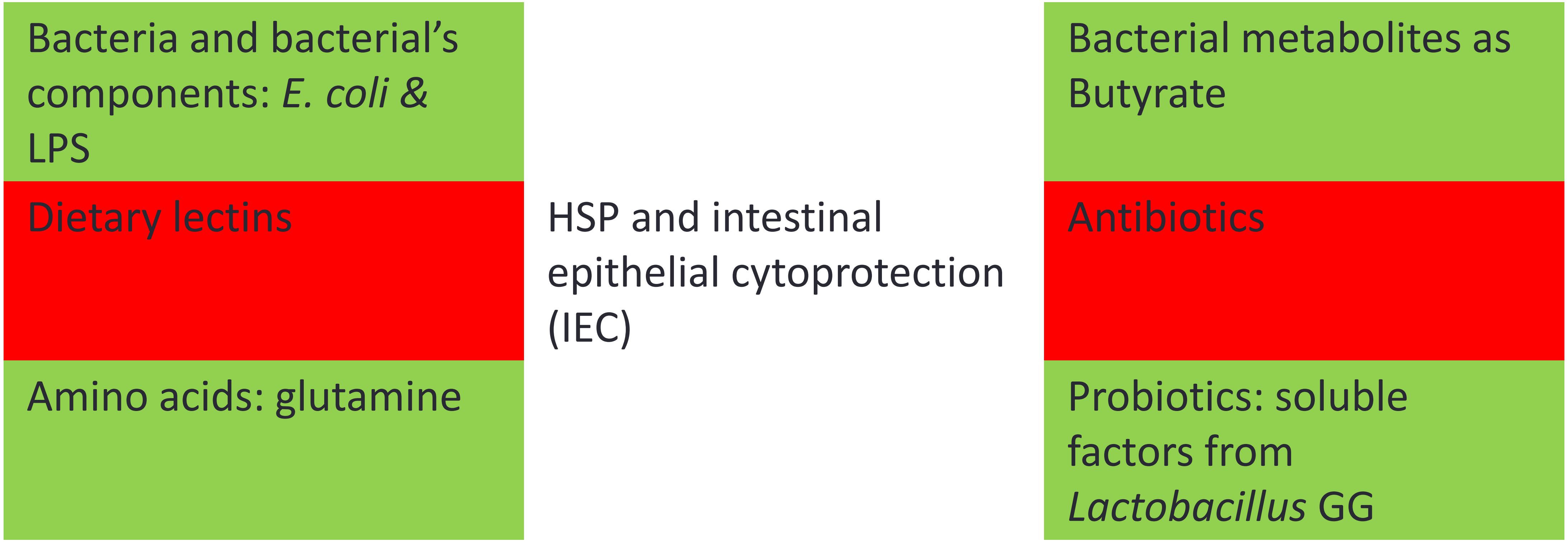
The intestinal function[4,5,6]

- HSP specialized in cell protection
  - HSP 25 actin cytoskeleton stabilizes cell to cell contacts including TJ
  - HSP 70 intra cellular protein chaperoning
- Glutamine, fermentation products as butyrate, bacterial products, inflammatory mediators induces HSP response

HSP: Heat Shock Protein

The intestinal function[4]

Modulation of the expression of HSP in IEC cells (modified from Lallès, 2010, p 38 in Dynamics in Animal Nutrition)



Bacteria and bacterial’s components: *E. coli &* LPS

Dietary lectins

Amino acids: glutamine

HSP and intestinal epithelial cytoprotection (IEC)

Bacterial metabolites as Butyrate

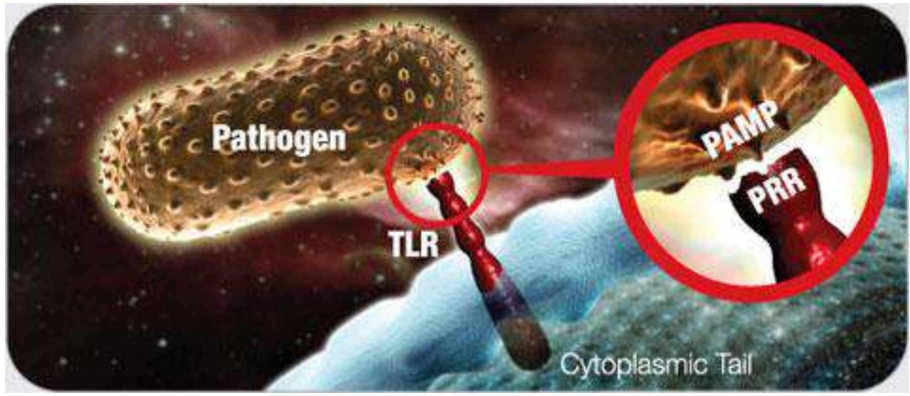
Antibiotics

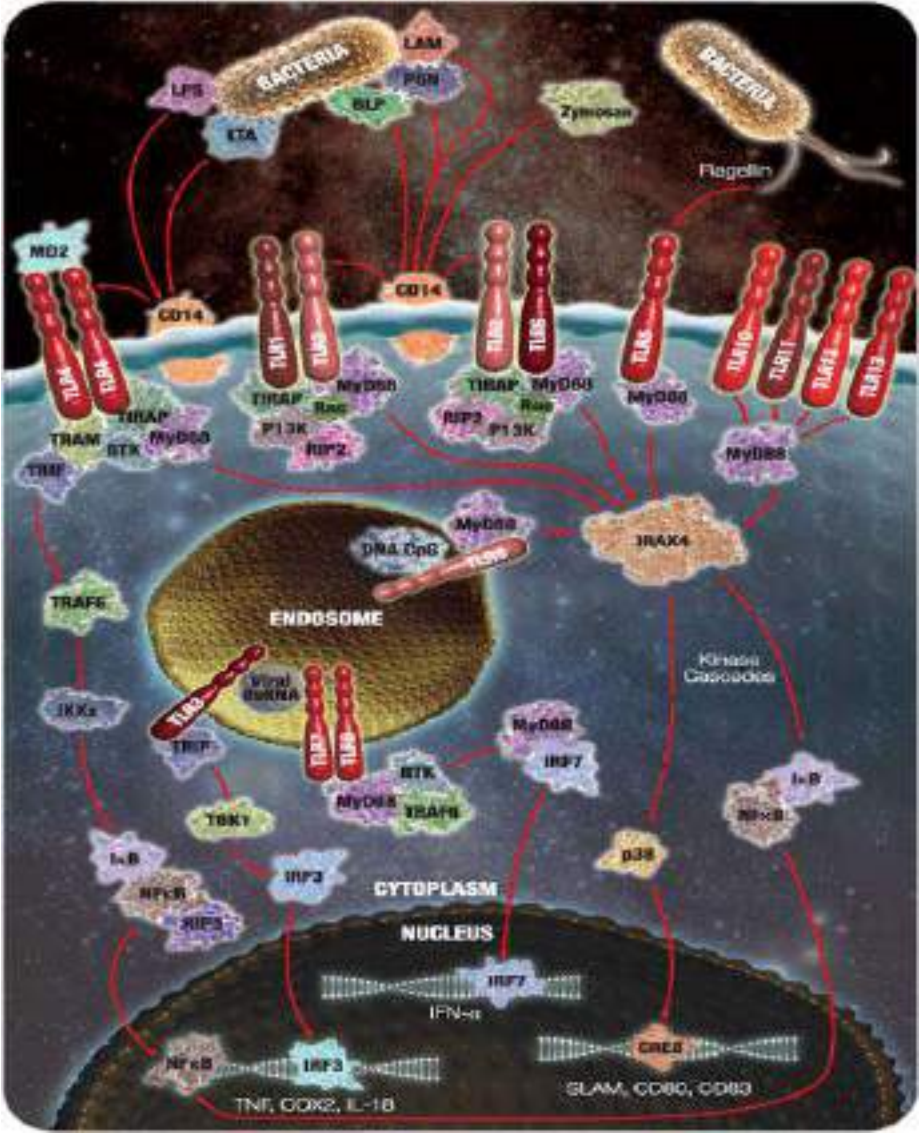
Probiotics: soluble factors from *Lactobacillus* GG

The intestinal function[7]

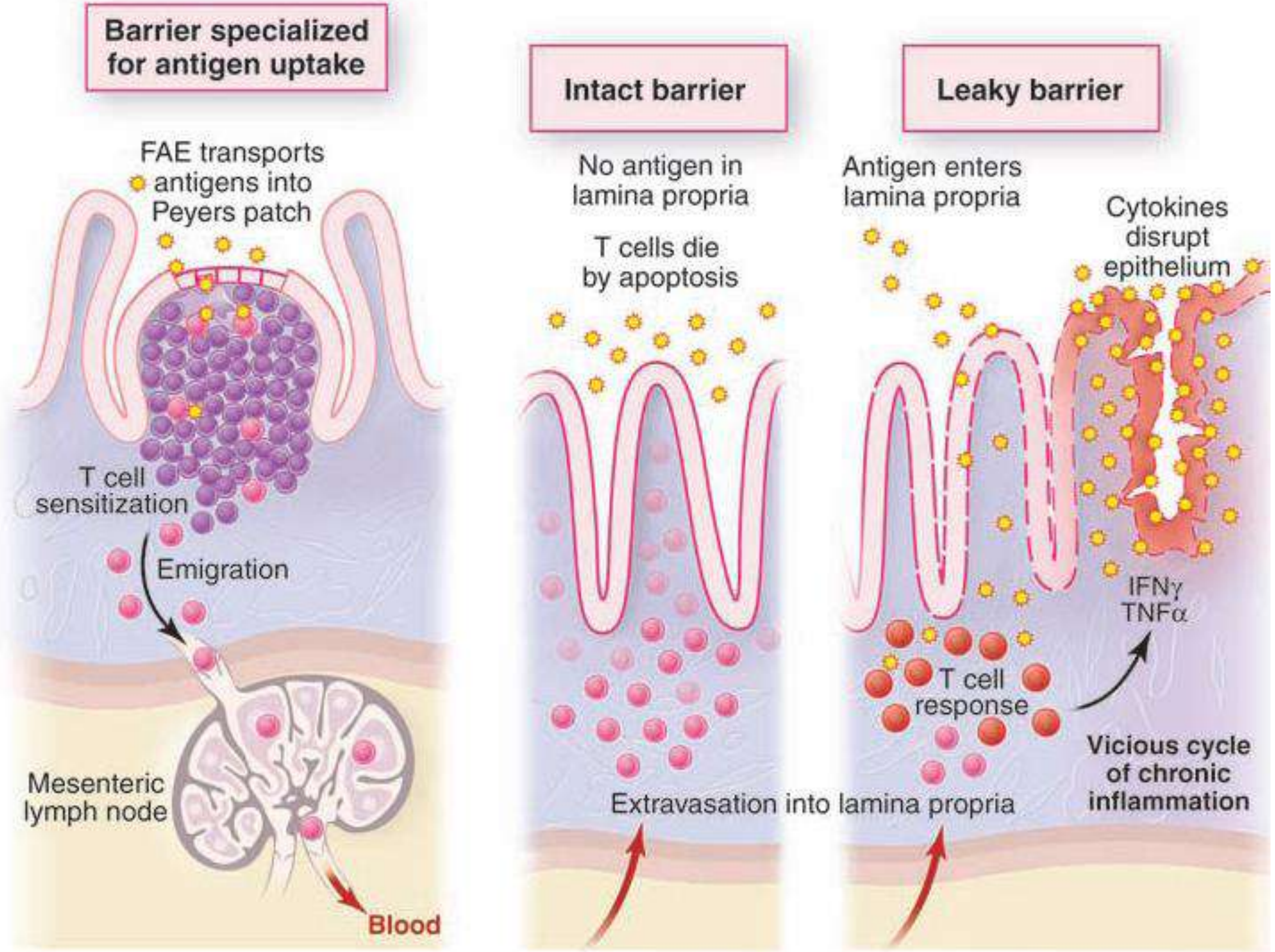
- Commensal and pathogenic bacteria are determinant in the development of the gut and the maintenance of its homeostasis
  - Via Toll-Like receptors (TLR)

The intestinal function: TLR[8]



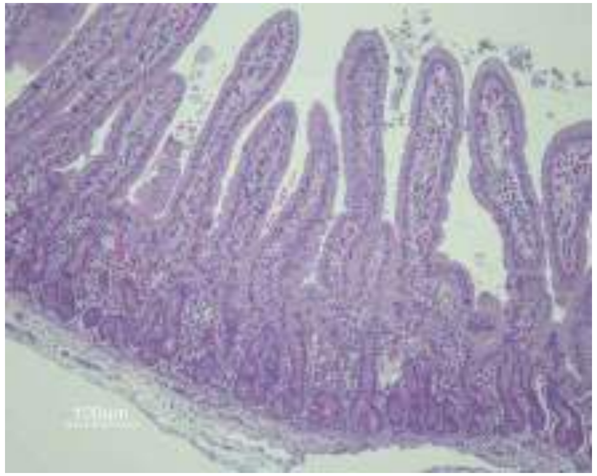


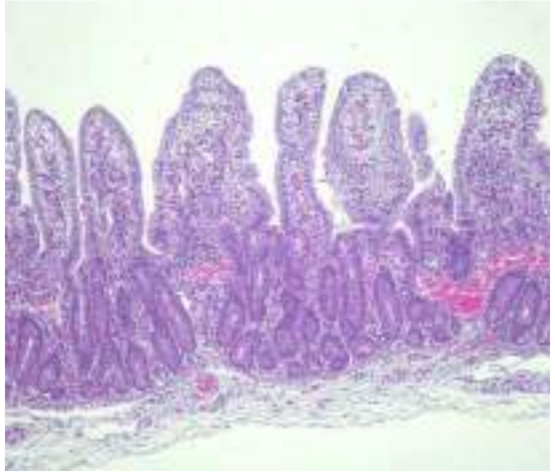
The intestinal function

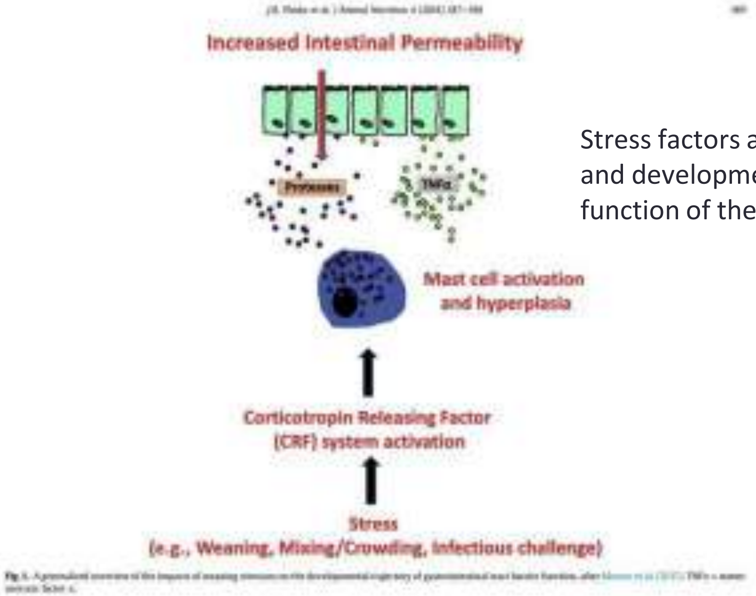


The intestinal function

- Loss of mature enterocytes rich in digestive enzymes
- Reduction of enzymatic activity of the brush edge of the intestinal epithelium







Stress factors after weaning in piglets and development of the barrier function of the gastrointestinal tract

Activation of immune system in post-weaned piglets

Table 2 - Performance of piglets from 30 to 64 days of age, vaccinated or non-vaccinated to *Haemophilus parasuis*, evaluated in different phases and fed diets with different levels of metabolizable energy

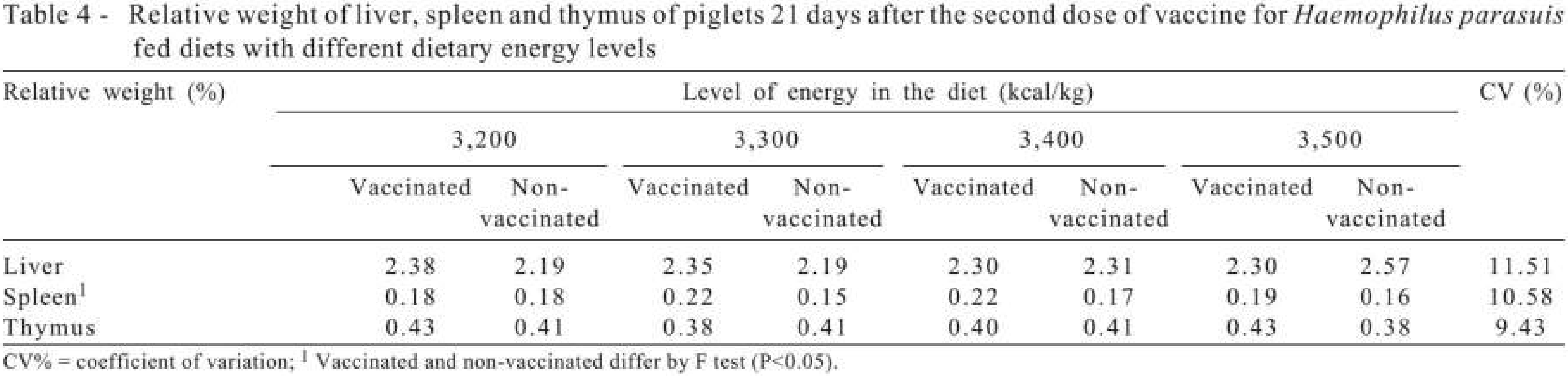
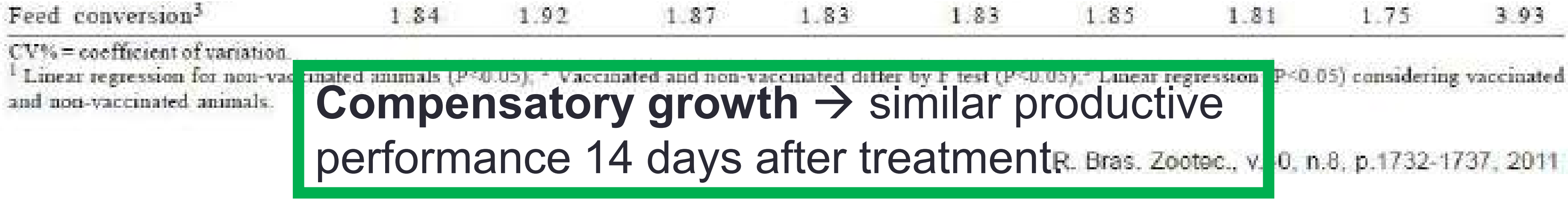


Table 4 - Relative weight of liver, spleen and thymus of piglets 21 days after the second dose of vaccine for *Haemophilus parasuis* fed diets with different dietary energy levels

Relative weight (%)	Level of energy in the diet (kcal/kg)								CV (%)
	3,200		3,300		3,400		3,500		
	Vaccinated	Non-vaccinated	Vaccinated	Non-vaccinated	Vaccinated	Non-vaccinated	Vaccinated	Non-vaccinated	
Liver	2.38	2.19	2.35	2.19	2.30	2.31	2.30	2.57	11.51
Spleen[1]	0.18	0.18	0.22	0.15	0.22	0.17	0.19	0.16	10.58
Thymus	0.43	0.41	0.38	0.41	0.40	0.41	0.43	0.38	9.43

CV% = coefficient of variation; [1] Vaccinated and non-vaccinated differ by F test (P<0.05).



Feed conversion[3]	1.84	1.92	1.87	1.83	1.83	1.85	1.81	1.75	3.93

CV% = coefficient of variation.

[1] Linear regression for non-vaccinated animals (P<0.05); [2] Vaccinated and non-vaccinated differ by F test (P<0.05); [3] Linear regression P<0.05) considering vaccinated and non-vaccinated animals.

Compensatory growth → similar productive performance 14 days after treatment

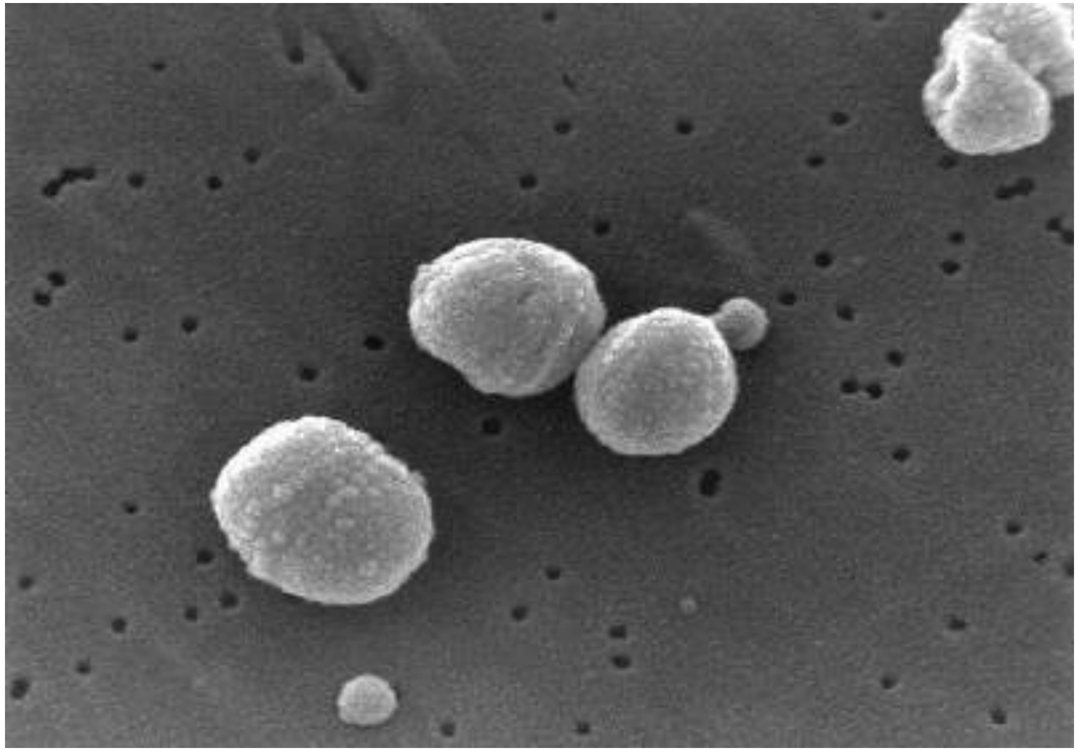
R. Bras. Zootec., v. 0, n.8, p.1732-1737, 2011

- Pereira, Leandro de Melo, et a., 2011. «Metabolizable Energy for Piglets in the Nursery Phase Submitted at Activation of Immune System».
- *Revista Brasileira de Zootecnia* 40 (8): 1732-37

Microbiota

Microbiota [35]

- Good, bad? Or rather optimal?



https://upload.wikimedia.org/wikipedia/commons/2/20/Streptococcus_pneumoniae.jpg

Microbiota[9, 10, 33]

- Intestinal porcine microflora:
  - 48 h after birth withstand
  - Maternal faeces
  - Autochthonous or indigenous
  - Allochthonous or non-indigenous
  - Colonisation
  - Pathogens: autochthonous or allochthonous

- It depends on:
  - The age of the animal
  - Of the environment
  - Anti-microbial agents
  - Diet
  - Stress
  - Genetic

Microbiota[9,10]

- Stomach and small intestine:
  - 10^3-10^5 bacteria/g, low pH and rapid flow
  - *Lactobacillus* and *Streptoccocus*
- Distal small intestine 10^8 bac/g
- Large intestine:
  - 400 different species
  - 10^{10} to 10^{11} bac/g

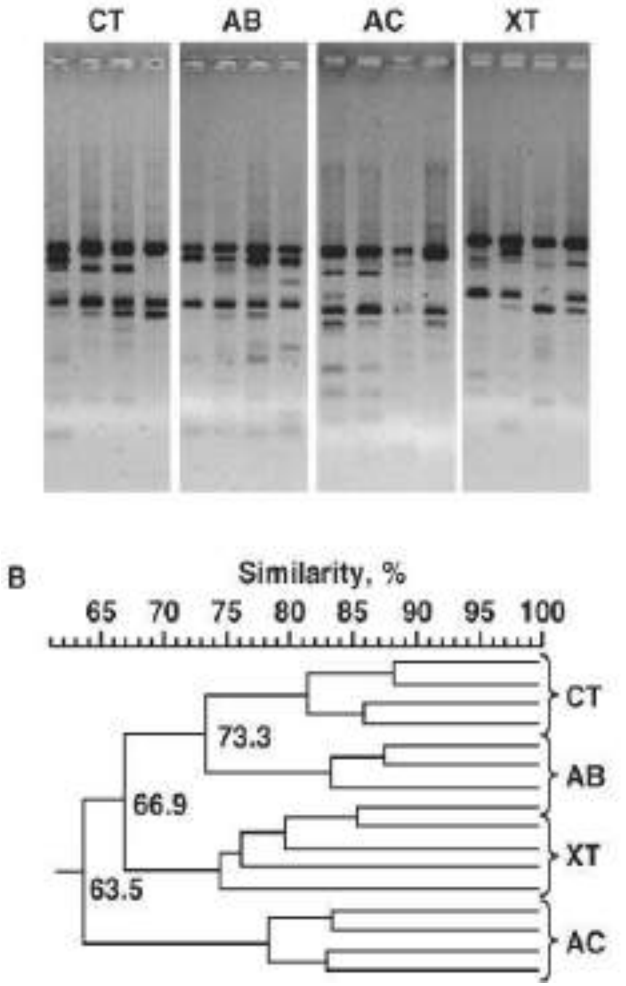
Microbiota[11]

- How to study:
  - Anaerobic culture techniques
    - Culture only if you know the requirements
    - Lack of phylogenetically classification
    - Different survival rate *in vitro*
  - Molecular techniques (see next slide)
    - Comparative sequence analysis of small subunit ribosomal RNA (16S mRNA)
    - Leser et al. (2002), 4270 cloned seq with 375 phylotypes

Leser TD, et al. 2002. Culture-independent analysis of gut bacteria: The pig gastrointestinal tract microbiota revisited. Applied and Environmental Microbiology. 68(2):673–90.

Microbiota

- How to study:
    - Culture-independent techniques based on the 16S rRNA gene
    - RFLP (Restriction Fragment Length Polymorphism)
    - DGGE (Denaturing Gradient Gel Electrophoresis)
    - TGGE (Temperature Gradient Gel Electrophoresis)
    - CE-CSSP (Capillary Electrophoresis single-strand conformation polymorphism)
- Non-cultivable bacteria can be identified
- Assess bacterial diversity
- No taxonomic assignment is performed



Leser TD, et al. 2002. Culture-independent analysis of gut bacteria: The pig gastrointestinal tract microbiota revisited. Applied and Environmental Microbiology. 68(2):673–90.

Microbiota

- How is the microbiota of a pig?

Feces GIT tracts

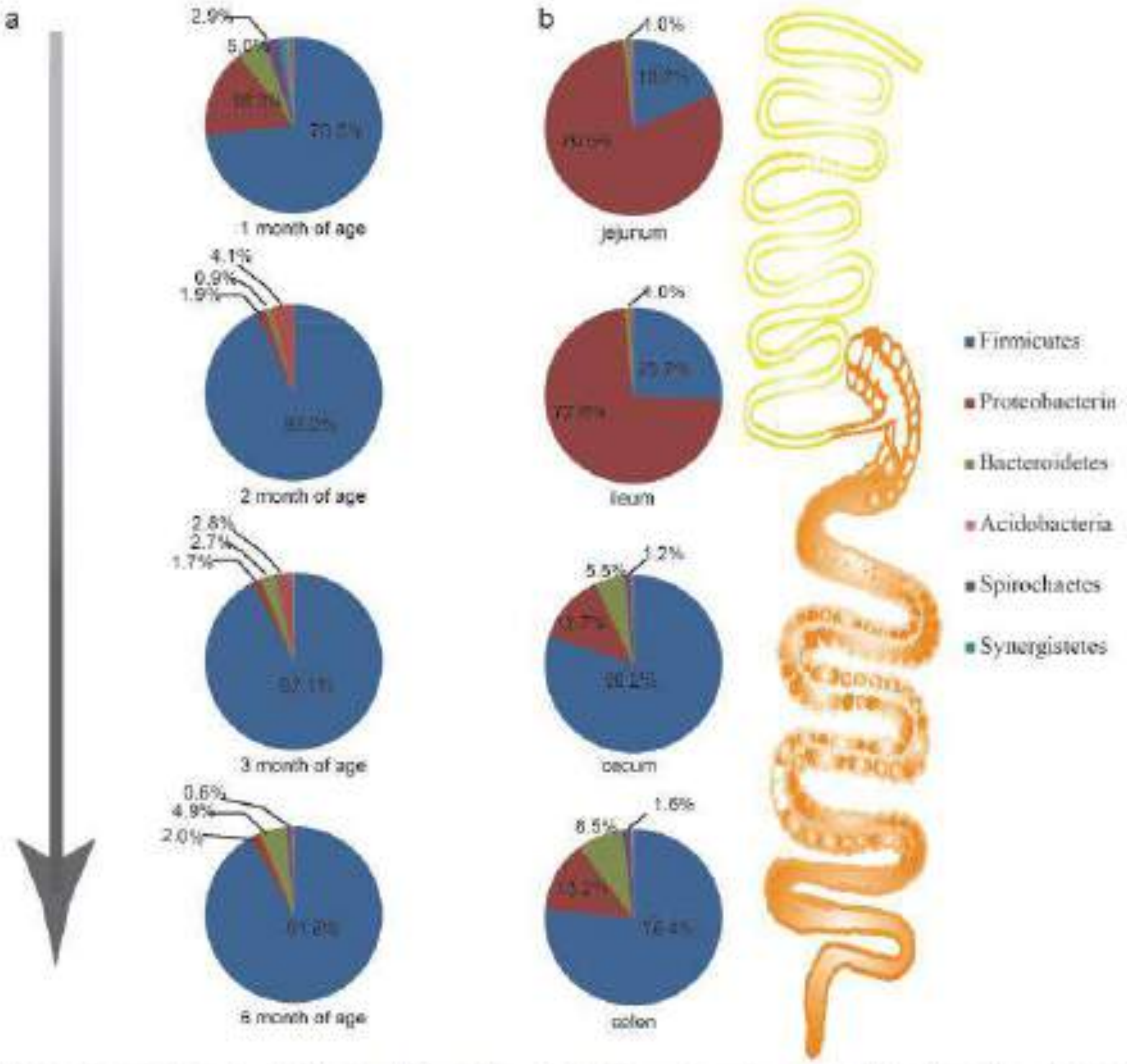
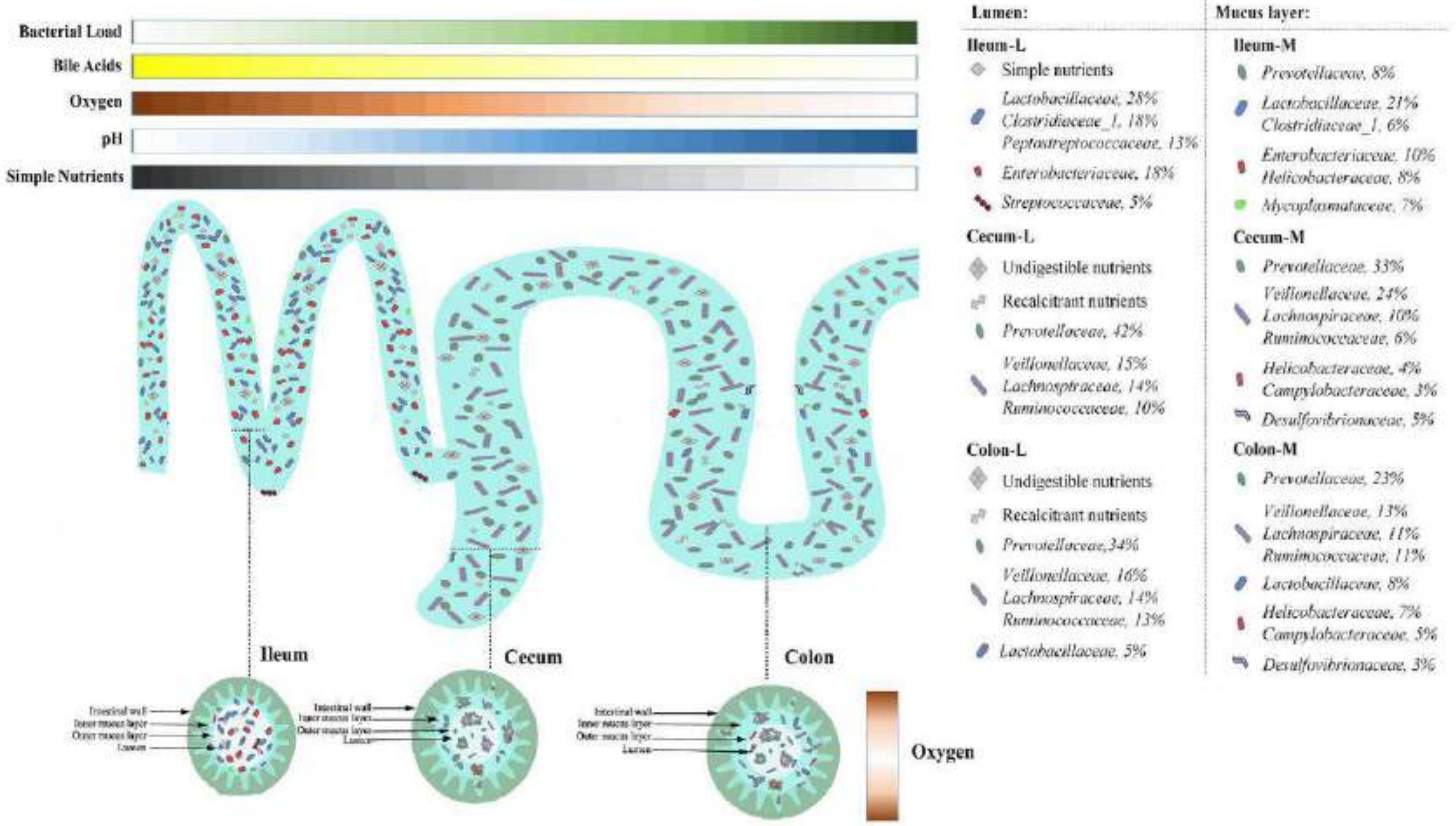


Fig 1. Profiles of gut microbes in GI tracts and feces at the rank of phylum. a, Composition structure of microbiome in feces at of 1, 2, 3, and 6 months of age. b, Profiles of microbes in different GI tract segments.

doi:10.1371/journal.pone.0117441.g001

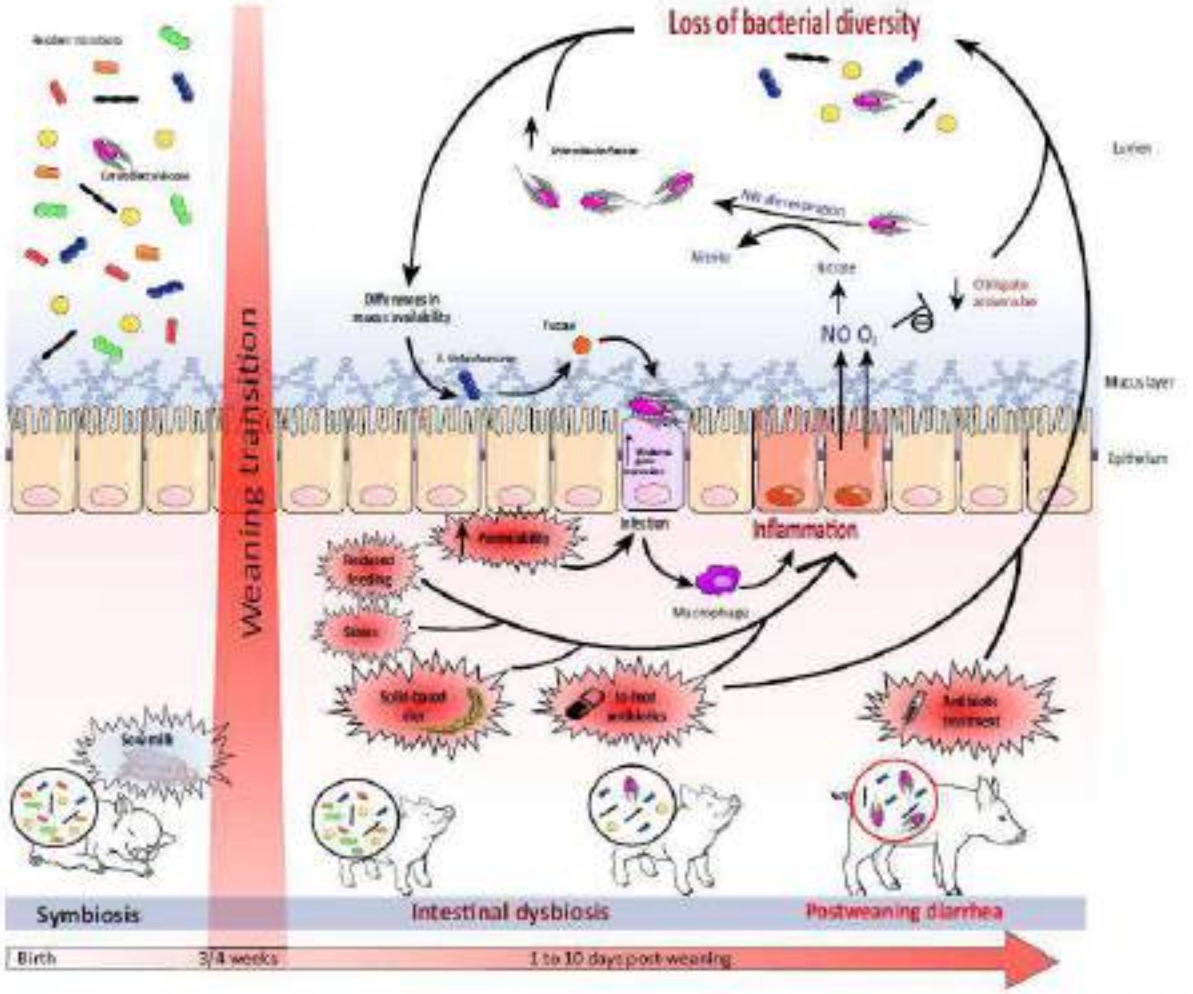
Zhao, W., et al. 2015. The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments. PloS one, 10(2).



Zhang, L., 2018. Spatial heterogeneity and co-occurrence of mucosal and luminal microbiome across swine intestinal tract. Frontiers in microbiology, 9, 48

Microbiota

- How is the microbiota of a pig at weaning?



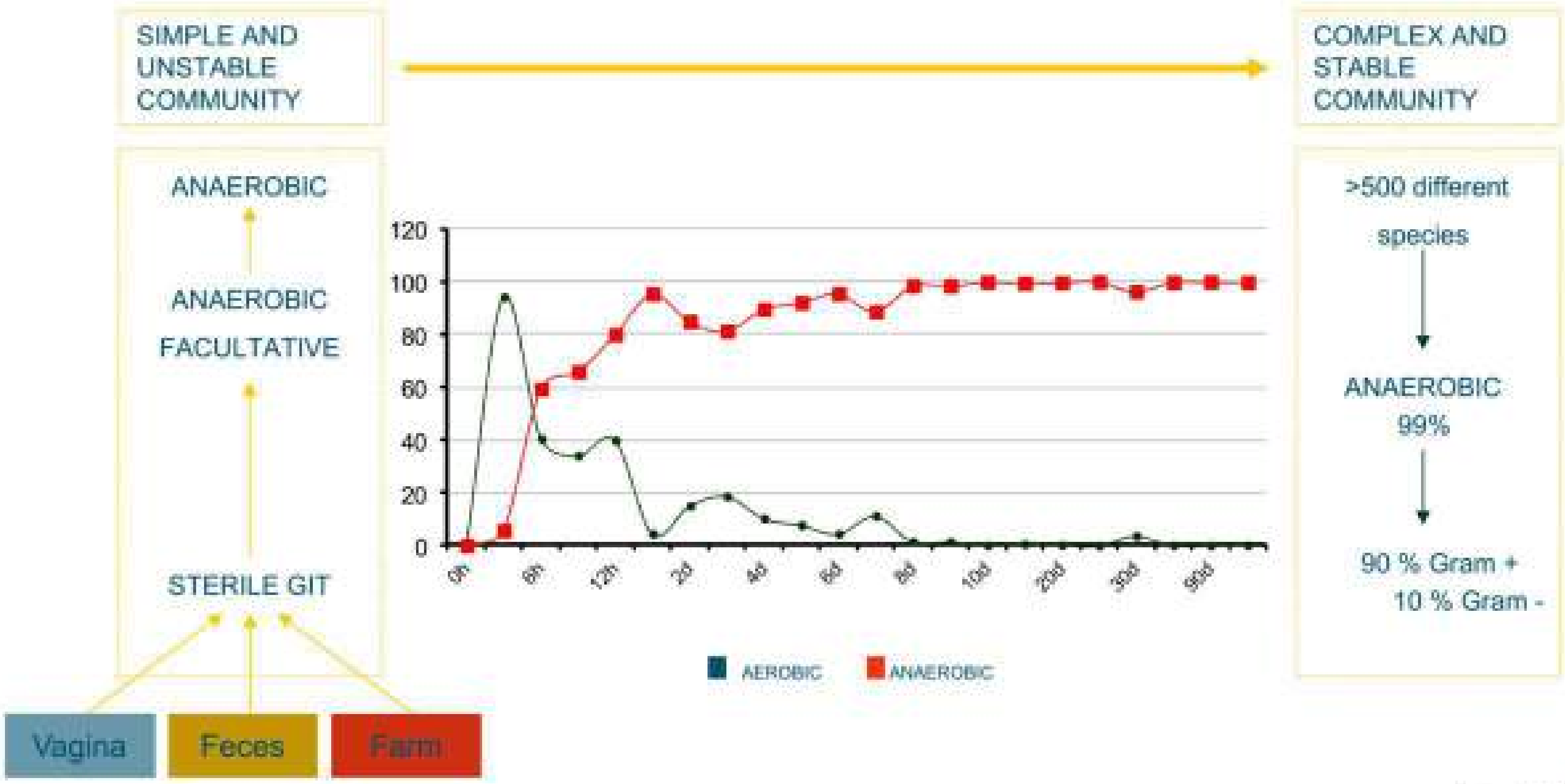
Trends in Microbiology

Figure 1. Impact of Weaning Transition on Piglet Gut Microbiota and Expansion of Infectious Agents. At weaning, piglets undergo the abrupt change from sow's milk to solid feed as well as social and environmental stresses. These modifications result in disruption of gut microbiota composition and intestinal inflammation that can lead to the expansion of enteric pathogens and postweaning diarrhea. Some hypotheses have been raised to explain underlying mechanisms. During weaning, the nutritional landscape of the piglet gut is modified, and mucus polysaccharides may be more available for commensal bacteria (such as Bacteroides thetaiotaomicron). Long-term feed antibiotics and therapeutic doses of antimicrobials may contribute to this vicious circle by decreasing bacterial diversity and increasing intestinal inflammation.

Gresse, R., et al. 2017. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends in microbiology, 25(10), 851-873

Microbiota

- How is the microbiota of a pig at weaning?



Swords, 1993

Microbiota[10,11]

- Interactions between intestinal bacteria and the gut epithelium
  - Mucin carbohydrates, repel or bind
  - Dietary proteolytic treatment of the glycoproteins receptors can prevent attachment: bromelain
  - Bacteria in the mucus layer prevent attachments
    - Mucolysis: use of energy

Microbiota[12]

- Functions:
  - Competitive exclusion for pathogenic bacteria
  - Produce some nutrients as vit B, K, VFA
  - Stimulate the development of intestinal protection
  - Immune system of intestinal mucosa depends on commensal and pathogenic bacteria colonization

Microbiota[32]

Animal Feed Science and Technology

Review article

Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production

P. Celi, A.J. Cowieson, F. Fru-Nji, R.E. Steinert, A.-M. Kluenter, V. Verlhac

"…the intestinal microbiota (or microbiome, representing the genomic information of the microbiota) represents a compromise between:

- intestinal barrier functionality,
- synthesis of beneficial nutrients and proteins and
- enhanced energy absorption from dietary components with low inherent potential,
- and the detrimental effects of inflammation and sub-clinical (and clinical) pathologies (Celi et al., 2017)"

Microbiota[36]

PLOS | ONE

RESEARCH ARTICLE

Characterisation of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhoea

Samir Dou[1], Pascale Gadonna-Widehem[1], Véronique Rome[2], Dounia Hamoudi[1], Larbi Rhazi[1], Lyes Lakhal[3], Thibaut Larcher[4], Narges Bahi-Jaber[1], Arturo Pinon-Quintana[5], Alain Guyonvarch[5], Isabelle L. E. Huërou-Luron[2☯]*, Latifa Abdennebi-Najar[1☯]*

1 UP 2012.10.101.EGEAL, Institut Polytechnique LaSalle Beauvais, rue Pierre Waguet Beauvais, France, **2** INRA, UR1341 ADNC, Domaine de la Prise, Saint-Gilles, France, **3** Institut polytechnique LaSalle Beauvais, Direction de la recherche, rue Pierre Waguet Beauvais, France, **4** INRA, UMR 703 APEX, Ecole Nationale Vétérinaire Agroalimentaire et de l'Alimentation Nantes-Atlantique (Oniris), Nantes, France, **5** INVIVO-NSA, Direction Scientifique Technique et Innovation, Département Innovation et Prospectives, Talhouët Saint Nolff, France

☯ These authors contributed equally to this work.

* latifanajar60@gmail.com (LAN); Isabelle.Luron@rennes.inra.fr (ILHL)

Check for updates

Microbiota[36]

- 5 sows between 3 to 5 parities
- 2 females and 2 males from each sow at median litter weight
- Weighed at 0 and 24 h
- Sampled at birth, 7, 14, 21, 28, 30, 38, 40 days of life

PLOS ONE

RESEARCH ARTICLE

Characterisation of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhoea

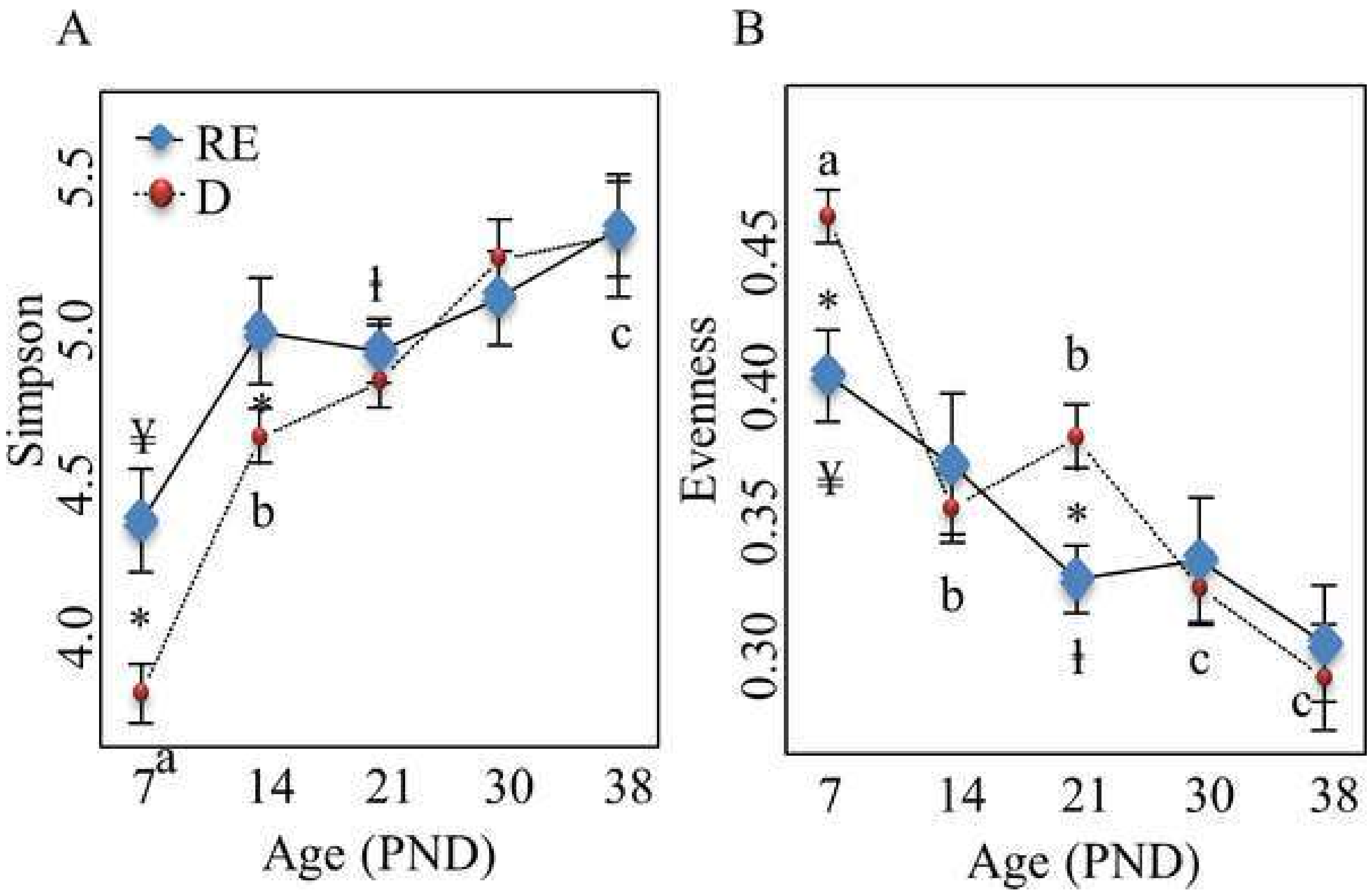
	Sow 1	Sow 2	Sow 3	Sow 4	Sow 5
D	4	4	3	1	1
H	0	0	1	3	3

Dou, S., P. Gadonna-Widehem, V. Rome, D. Hamoudi, L. Rhazi, L. Lakhal, T. Larcher, et al. 2017. «Characterisation of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhoea». *PloS one* 12 (1): e0169851.

Microbiota[36]

- Bacterial diversity during episodes of post-weaning diarrhea associated with piglet susceptibility
- Piglets with or without post-weaning diarrhea could be classified earlier as day 7 of life depending on the microbiota:
  - Healthy:
    - Abundance of *Prevotellaceae, Lachnospiraceae, Ruminococcaceae* and *Lactobacillaceae* on day 7 of life positively correlated with high levels of *Bacteroidetes* and negatively with low levels of *Enterobacteriaceae* after weaning
  - Piglets with diarrhea
    - On day 38 negative correlation between the abundance of *Enterobacteriaceae* and fecal dry matter (DM)

Fig 2. Dynamics o bacterial diversity (A) and evenness (B) in D and H (RE) pigs.

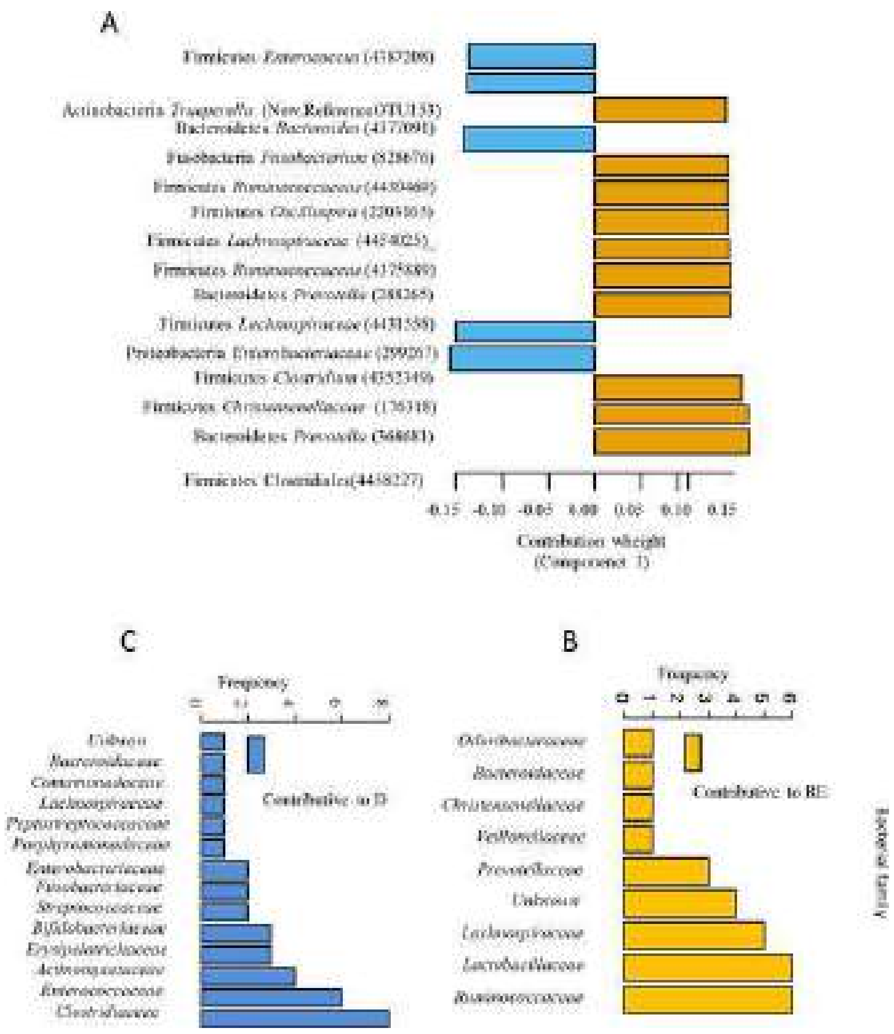


RE=H=Healthy; D=Diarrhoeic

No difference in the intake of colostrum or colostral components

Dou, S., P. Gadonna-Widehem, V. Rome, D. Hamoudi, L. Rhazi, L. Lakhal, T. Larcher, et al. 2017. «Characterisation of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhoea». *PloS one* 12 (1): e0169851.

Fig 5. Contribution plots.



(A) OTUs in feces to PND 7 that contribute mostly to the discriminating group and its frequency. OTUs ordered (Top 15 OTUs) according to their weighted contribution to the component 1.

i) Group H (healthy) or RE orange

ii) Group D (diarrheic) Blue

(B) Frequency of bacterial families between OTU of group H (with orange bars).

(C) Frequency of bacterial families between OTU of group D (with blue bars).

Diarrheal, D; H o RE, Healthy.

Dou, S., P. Gadonna-Widehem, V. Rome, D. Hamoudi, L. Rhazi, L. Lakhal, T. Larcher, et al. 2017. «Characterisation of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhoea». *PloS one* 12 (1): e0169851.

Fig 5. Contribution plots.

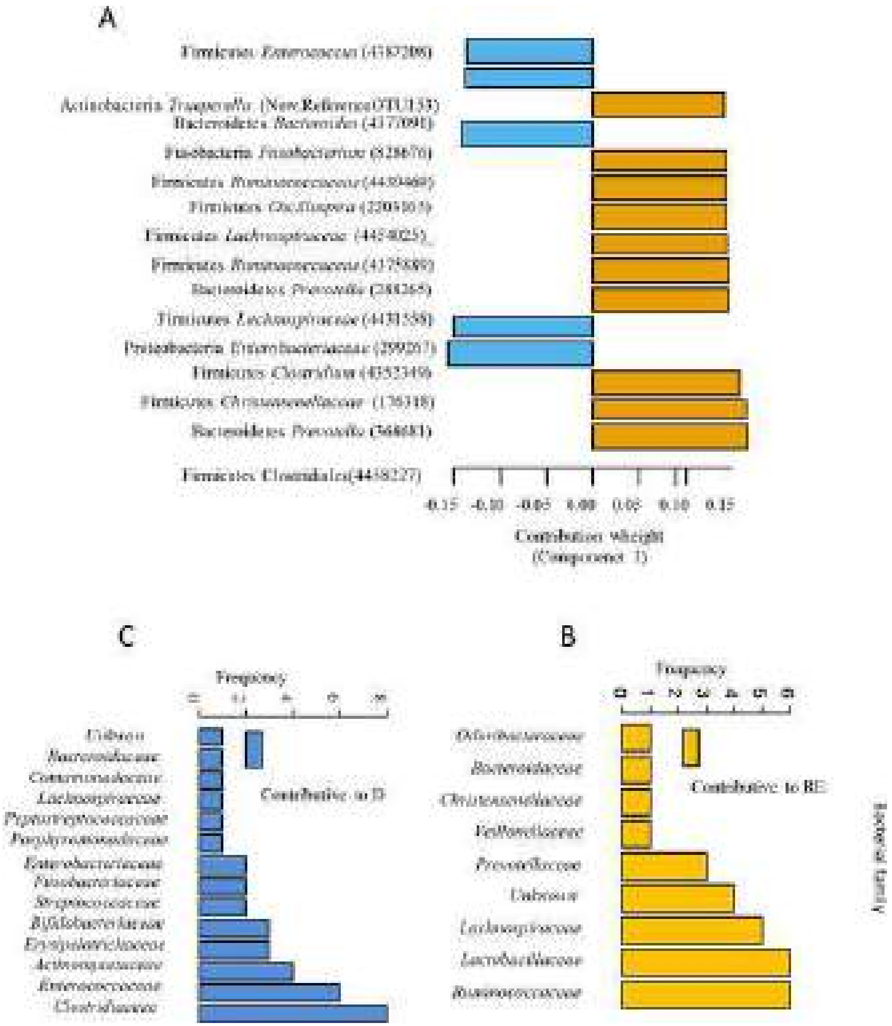
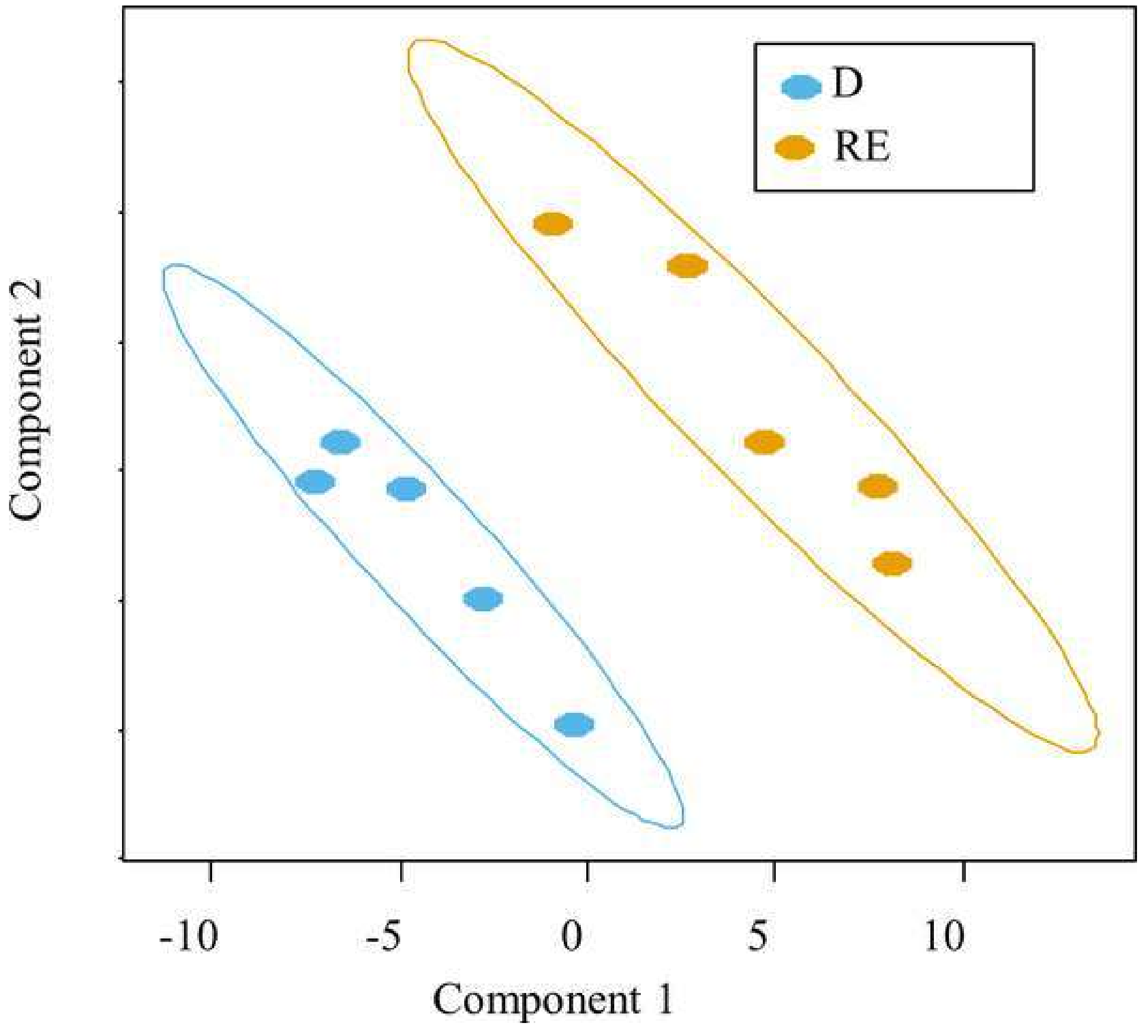


Fig 4. Individual plot of sPLS-DA classification model.



Dou, S., P. Gadonna-Widehem, V. Rome, D. Hamoudi, L. Rhazi, L. Lakhal, T. Larcher, et al. 2017. «Characterisation of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhoea». *PloS one* 12 (1): e0169851.

Microbiota

- Fecal transplantation

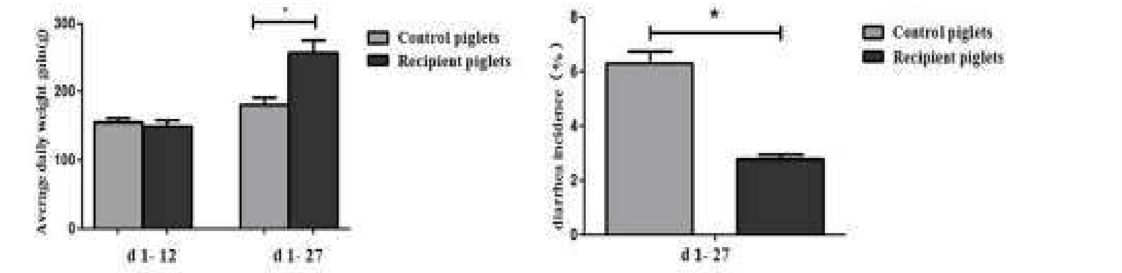


FIGURE 6 | Weight gain and the incidence of diarrhea in piglets orally treated with or without exogenous fecal microflora. Data are expressed as the mean ± SEM. *$P < 0.05$.

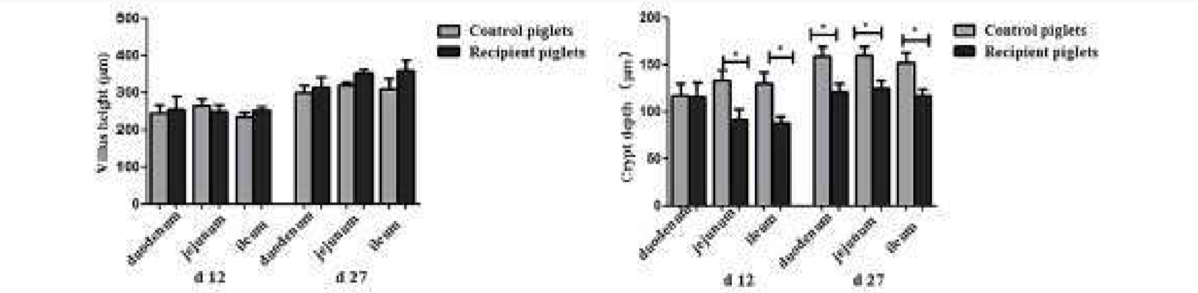


FIGURE 7 | Duodenal, jejunal and ileal morphology of piglets orally treated with or without exogenous fecal microflora. Data are expressed as the mean ± SEM. *$P < 0.05$.

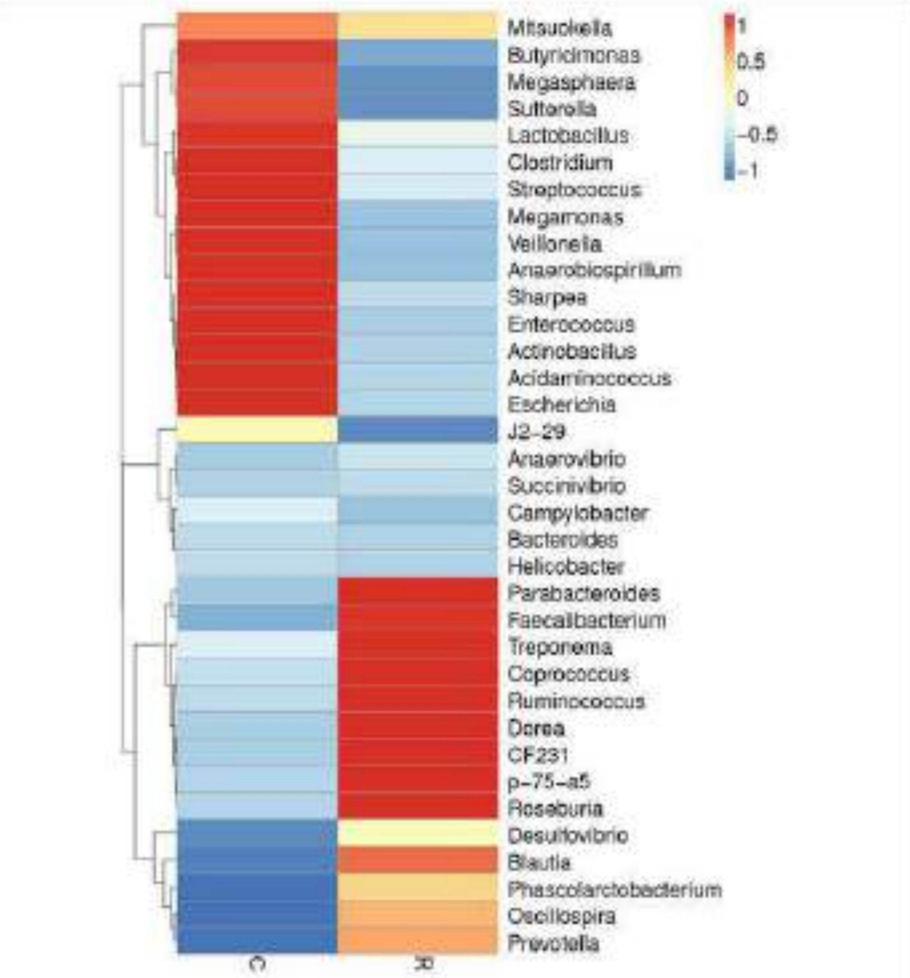


FIGURE 5 | The distribution of luminal bacteria in the colonic digesta at day 27. Taxonomic profiles of the microbial communities of the colon were analyzed from genera. Pigs with the highest and lowest bacterial loads are red and green, respectively. C, Control piglets. R, Recipient piglets.

↑ ↑

Control **Recipient**

Hu, L., et al.2017. Exogenous Fecal Microbiota Transplantation from Local Adult Pigs to Crossbred Newborn Piglets. Frontiers in microbiology 8: 2663.

Biomarkers

How to measure intestinal health?

- M. Varley, 2017 (commentary on Pig progress), high correlation between general health and intestinal health:
    - ADG from 30 kg to slaughterhouse
    - Number of days in antibiotic treatment
    - Lung Injury Score
    - Acute protein measurement
    - Mortality

How to measure intestinal health?[46]

- How to measure it:
  - Celi et al., 2018, Biomarkers:
    - The establishment of biomarkers of intestinal function is crucial for the advancement in understanding the functioning of the intestinal barrier, its ecology and intestinal microbiota
    - We know how nutrients are absorbed
    - But not about:
      - Intestinal permeability, function of the intestinal barrier, endocrine system of the intestine and microbiota and its metabolites

Animal Feed Science and Technology

Biomarkers of gastrointestinal functionality in animal nutrition and health

Celi Pietro, Verlhac Viviane, Pérez Calvo Estefania, Schmeisser Jerome, Kluenter Anna-Maria

Biomarkers[46]

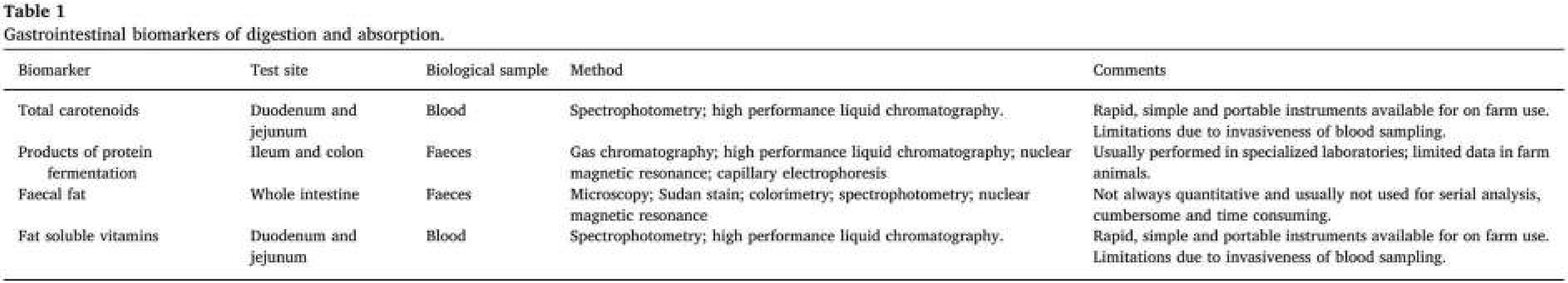


Table 1
Gastrointestinal biomarkers of digestion and absorption.

Biomarker	Test site	Biological sample	Method	Comments
Total carotenoids	Duodenum and jejunum	Blood	Spectrophotometry; high performance liquid chromatography.	Rapid, simple and portable instruments available for on farm use. Limitations due to invasiveness of blood sampling.
Products of protein fermentation	Ileum and colon	Faeces	Gas chromatography; high performance liquid chromatography; nuclear magnetic resonance; capillary electrophoresis	Usually performed in specialized laboratories; limited data in farm animals.
Faecal fat	Whole intestine	Faeces	Microscopy; Sudan stain; colorimetry; spectrophotometry; nuclear magnetic resonance	Not always quantitative and usually not used for serial analysis, cumbersome and time consuming.
Fat soluble vitamins	Duodenum and jejunum	Blood	Spectrophotometry; high performance liquid chromatography.	Rapid, simple and portable instruments available for on farm use. Limitations due to invasiveness of blood sampling.

Biomarkers[46]

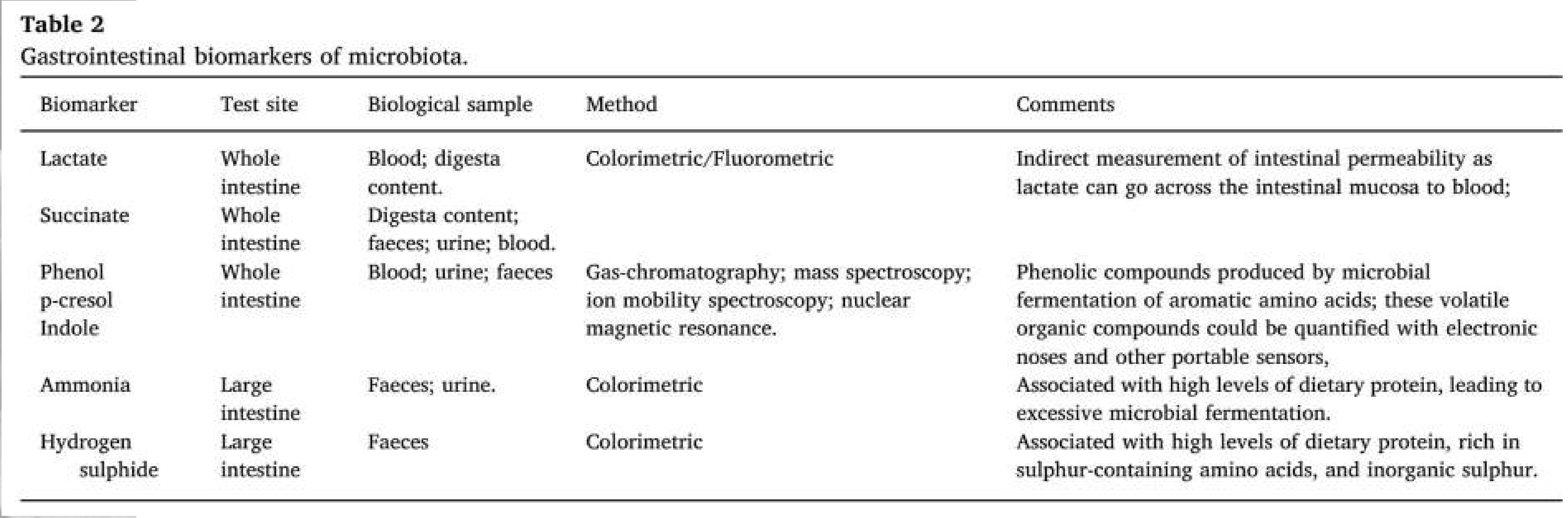


Table 2

Gastrointestinal biomarkers of microbiota.

Biomarker	Test site	Biological sample	Method	Comments
Lactate	Whole intestine	Blood; digesta content.	Colorimetric/Fluorometric	Indirect measurement of intestinal permeability as lactate can go across the intestinal mucosa to blood;
Succinate	Whole intestine	Digesta content; faeces; urine; blood.		
Phenol p-cresol Indole	Whole intestine	Blood; urine; faeces	Gas-chromatography; mass spectroscopy; ion mobility spectroscopy; nuclear magnetic resonance.	Phenolic compounds produced by microbial fermentation of aromatic amino acids; these volatile organic compounds could be quantified with electronic noses and other portable sensors,
Ammonia	Large intestine	Faeces; urine.	Colorimetric	Associated with high levels of dietary protein, leading to excessive microbial fermentation.
Hydrogen sulphide	Large intestine	Faeces	Colorimetric	Associated with high levels of dietary protein, rich in sulphur-containing amino acids, and inorganic sulphur.

Biomarkers[46]

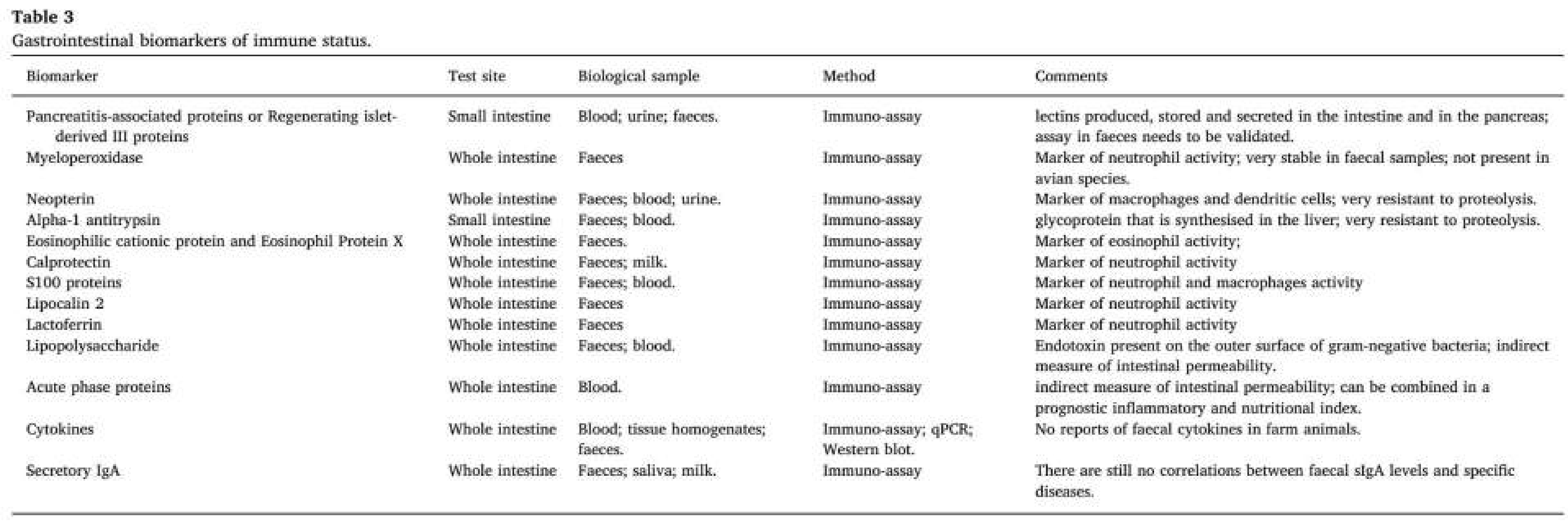


Table 3
Gastrointestinal biomarkers of immune status.

Biomarker	Test site	Biological sample	Method	Comments
Pancreatitis-associated proteins or Regenerating islet-derived III proteins	Small intestine	Blood; urine; faeces.	Immuno-assay	lectins produced, stored and secreted in the intestine and in the pancreas; assay in faeces needs to be validated.
Myeloperoxidase	Whole intestine	Faeces	Immuno-assay	Marker of neutrophil activity; very stable in faecal samples; not present in avian species.
Neopterin	Whole intestine	Faeces; blood; urine.	Immuno-assay	Marker of macrophages and dendritic cells; very resistant to proteolysis.
Alpha-1 antitrypsin	Small intestine	Faeces; blood.	Immuno-assay	glycoprotein that is synthesised in the liver; very resistant to proteolysis.
Eosinophilic cationic protein and Eosinophil Protein X	Whole intestine	Faeces.	Immuno-assay	Marker of eosinophil activity;
Calprotectin	Whole intestine	Faeces; milk.	Immuno-assay	Marker of neutrophil activity
S100 proteins	Whole intestine	Faeces; blood.	Immuno-assay	Marker of neutrophil and macrophages activity
Lipocalin 2	Whole intestine	Faeces	Immuno-assay	Marker of neutrophil activity
Lactoferrin	Whole intestine	Faeces	Immuno-assay	Marker of neutrophil activity
Lipopolysaccharide	Whole intestine	Faeces; blood.	Immuno-assay	Endotoxin present on the outer surface of gram-negative bacteria; indirect measure of intestinal permeability.
Acute phase proteins	Whole intestine	Blood.	Immuno-assay	indirect measure of intestinal permeability; can be combined in a prognostic inflammatory and nutritional index.
Cytokines	Whole intestine	Blood; tissue homogenates; faeces.	Immuno-assay; qPCR; Western blot.	No reports of faecal cytokines in farm animals.
Secretory IgA	Whole intestine	Faeces; saliva; milk.	Immuno-assay	There are still no correlations between faecal sIgA levels and specific diseases.

Biomarkers[46]

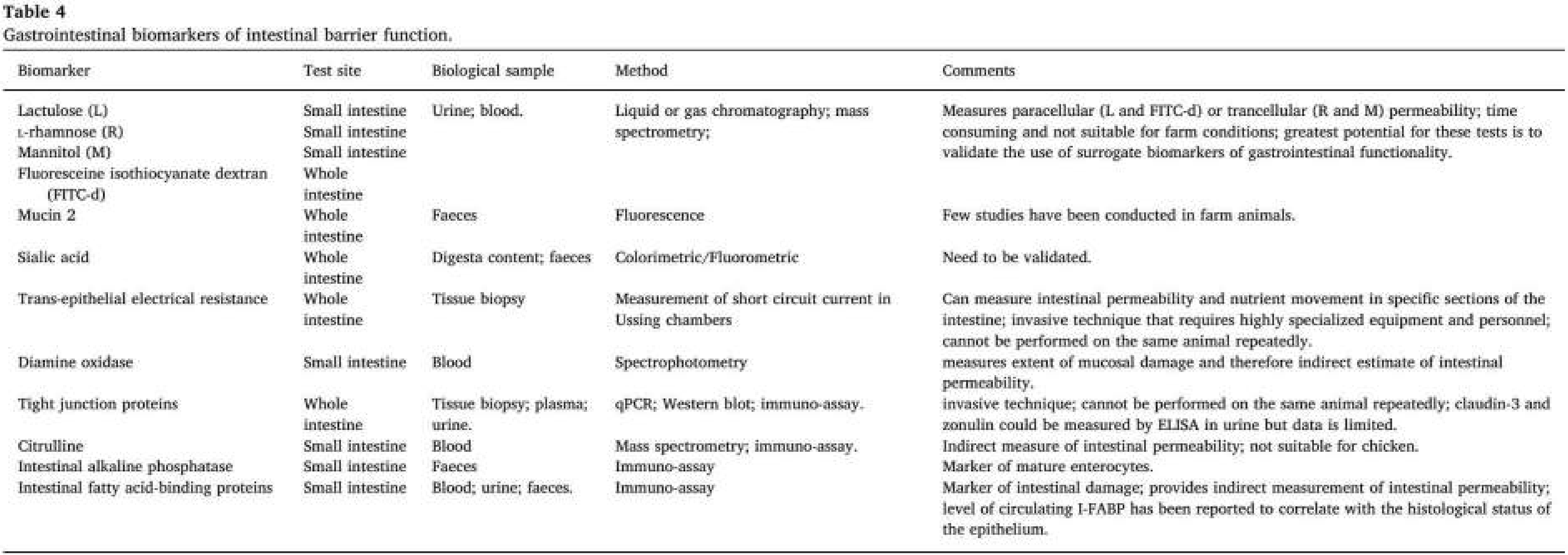


Table 4
Gastrointestinal biomarkers of intestinal barrier function.

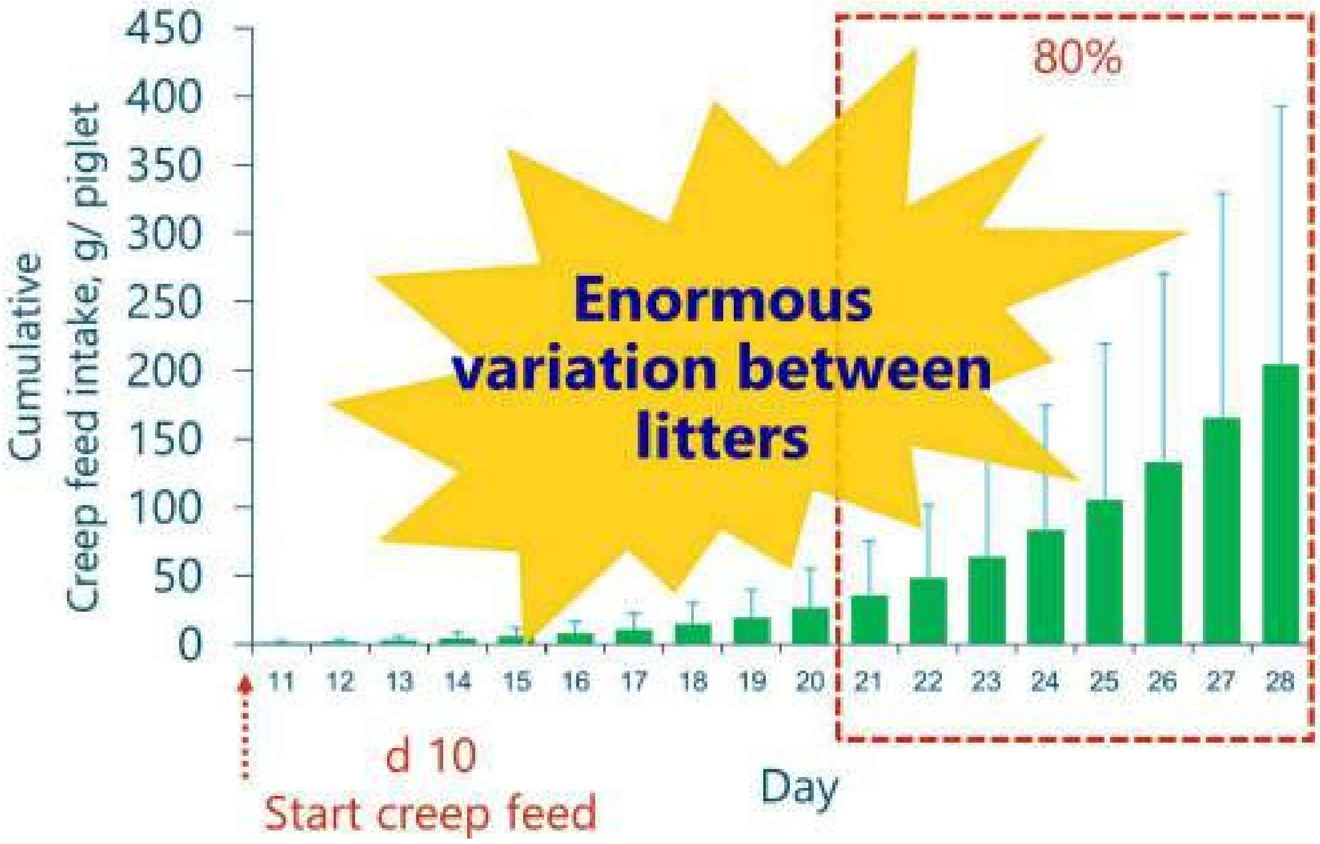
Biomarker	Test site	Biological sample	Method	Comments
Lactulose (L)	Small intestine	Urine; blood.	Liquid or gas chromatography; mass spectrometry;	Measures paracellular (L and FITC-d) or trancellular (R and M) permeability; time consuming and not suitable for farm conditions; greatest potential for these tests is to validate the use of surrogate biomarkers of gastrointestinal functionality.
L-rhamnose (R)	Small intestine			
Mannitol (M)	Small intestine			
Fluoresceine isothiocyanate dextran (FITC-d)	Whole intestine			
Mucin 2	Whole intestine	Faeces	Fluorescence	Few studies have been conducted in farm animals.
Sialic acid	Whole intestine	Digesta content; faeces	Colorimetric/Fluorometric	Need to be validated.
Trans-epithelial electrical resistance	Whole intestine	Tissue biopsy	Measurement of short circuit current in Ussing chambers	Can measure intestinal permeability and nutrient movement in specific sections of the intestine; invasive technique that requires highly specialized equipment and personnel; cannot be performed on the same animal repeatedly.
Diamine oxidase	Small intestine	Blood	Spectrophotometry	measures extent of mucosal damage and therefore indirect estimate of intestinal permeability.
Tight junction proteins	Whole intestine	Tissue biopsy; plasma; urine.	qPCR; Western blot; immuno-assay.	invasive technique; cannot be performed on the same animal repeatedly; claudin-3 and zonulin could be measured by ELISA in urine but data is limited.
Citrulline	Small intestine	Blood	Mass spectrometry; immuno-assay.	Indirect measure of intestinal permeability; not suitable for chicken.
Intestinal alkaline phosphatase	Small intestine	Faeces	Immuno-assay	Marker of mature enterocytes.
Intestinal fatty acid-binding proteins	Small intestine	Blood; urine; faeces.	Immuno-assay	Marker of intestinal damage; provides indirect measurement of intestinal permeability; level of circulating I-FABP has been reported to correlate with the histological status of the epithelium.

Feed intake

"Why do piglets eat very little or nothing at weaning, while chicks tend to overeat?"

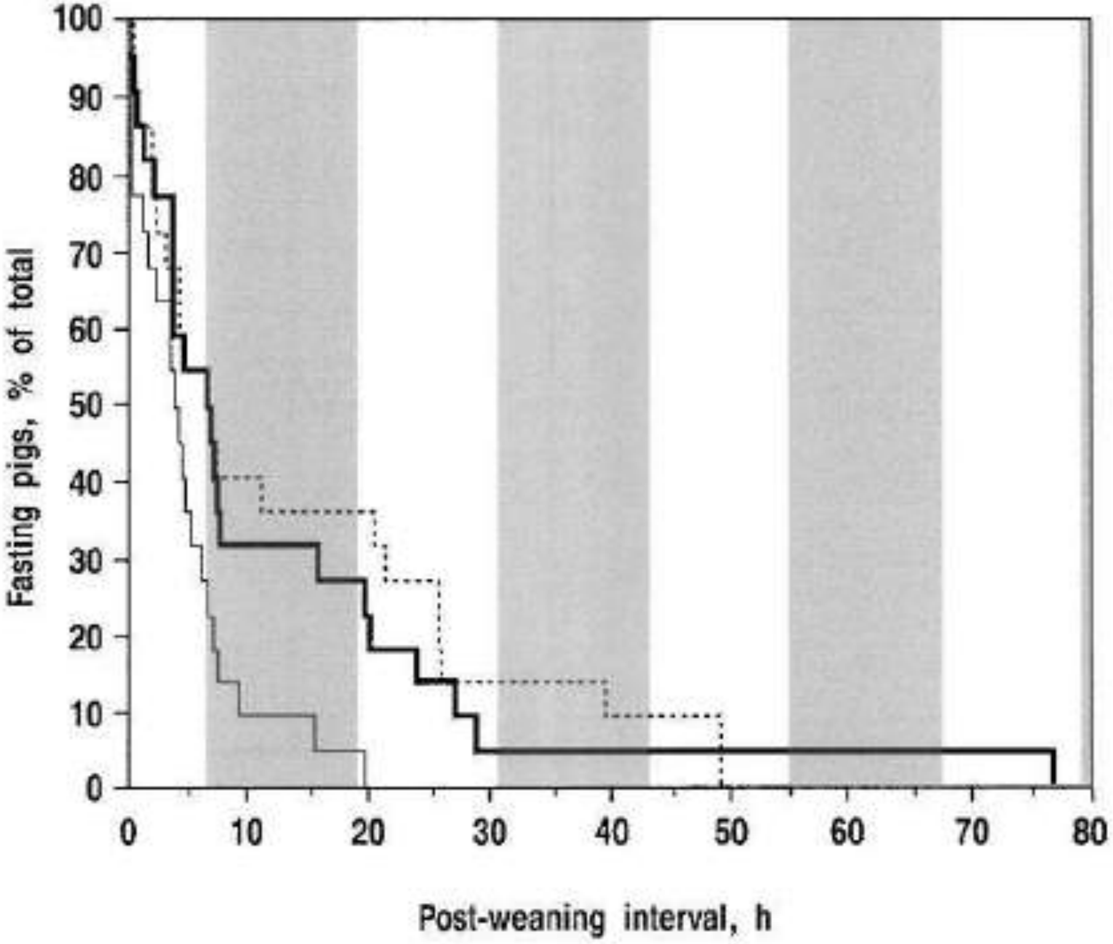
"Why do piglets eat very little or nothing at weaning, while chicks tend to overeat?"

- Pre-weaning creep feed intake the higher the better, is kg of intake realistic?



Huting, A. 2021. «Using Nutritional Strategies to Shape the Gastro-Intestinal Tracts of Suckling and Weaned Piglets». *Animals* 11 (2): 402..

Why do piglets at weaning eat little or nothing?[50]



The percentage of weaned piglets that did not eat after weaning as a function of the interval between weaning and the time they start eating

(average =10.7 h; SD=1.73 h).

The curves are for:

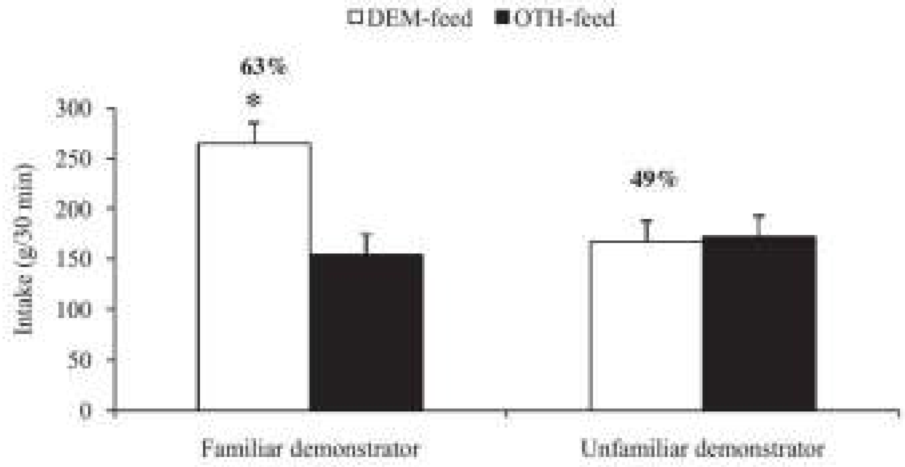
- those that ate before weaning (—),
- for those who did not eat, (—)
- and for those who did not have access to the feed (----).

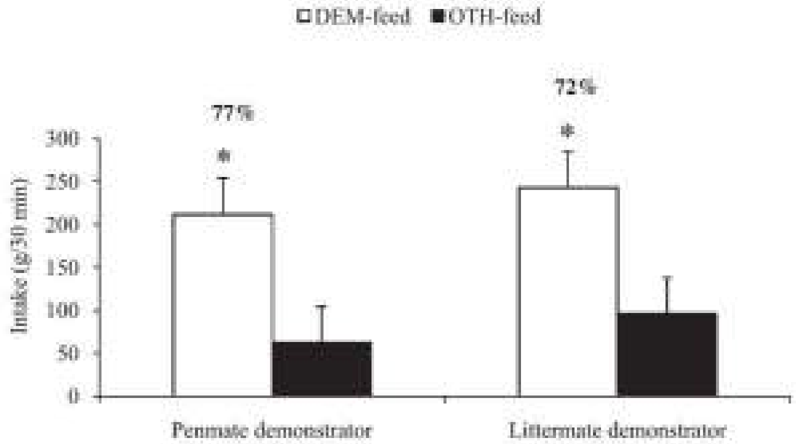
Bruininx, E.M.A.M., et al., 2002. «Effect of creep feed consumption on individual feed intake characteristics and performance of group-housed weanling pigs». Journal of Animal Science 80 (6): 1413-18

Why do piglets at weaning eat little or nothing?[37]

- Increase in consumption when the demonstrator and the observer were of the same litter or pen

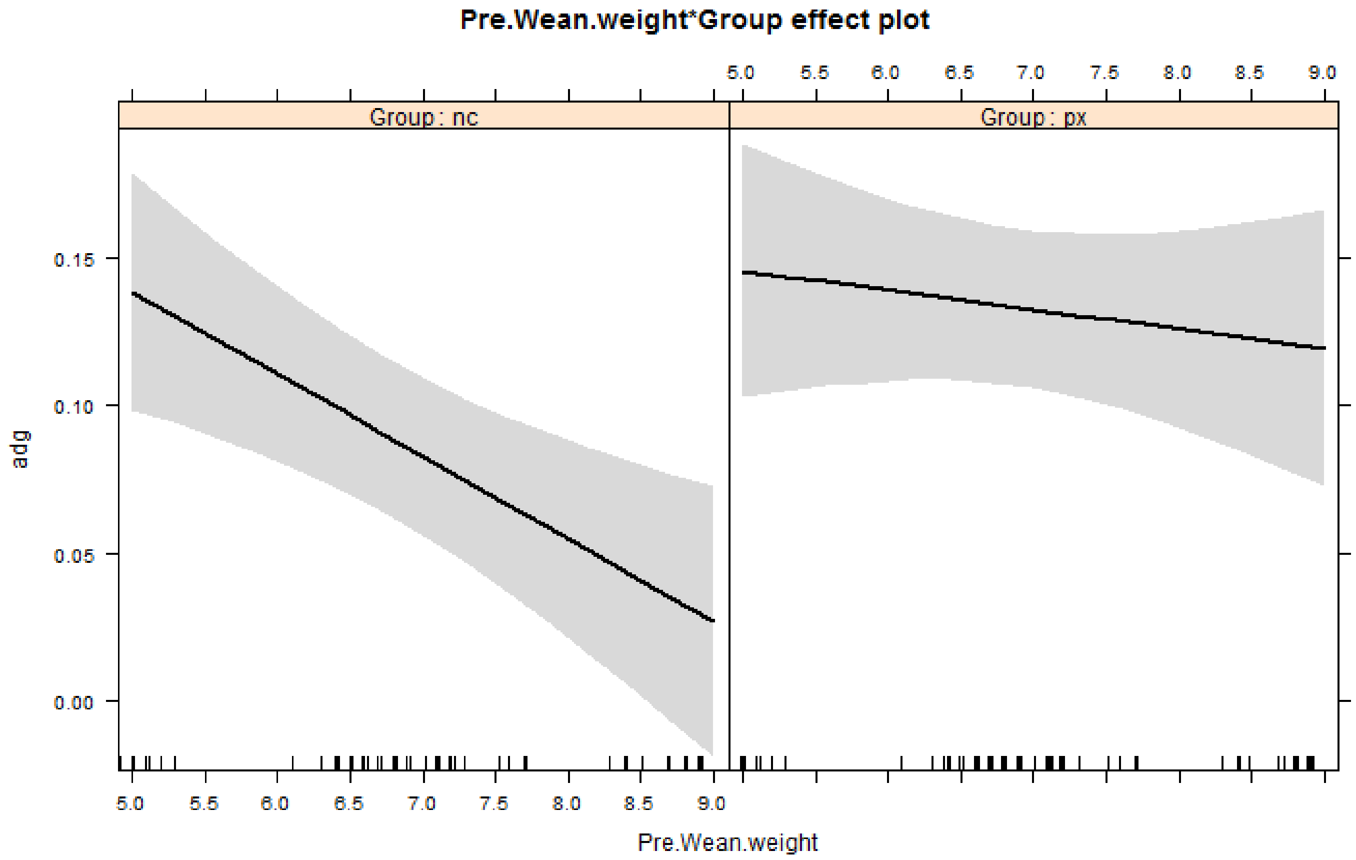
- **DEM-feed**: Flavored feed previously eaten by demonstrator
- **OTH-feed:** Other flavored feed





Feed intake

- Different patterns of feed intake



Feed components

Feed components

- Fiber sources and carbohydrates
- Protein sources and interactions
- Minerals: Zinc and Cooper
- Organic acids, essential oils, probiotics, prebiotics, nutraceuticals and enzymes

Fiber sources and carbohydrates

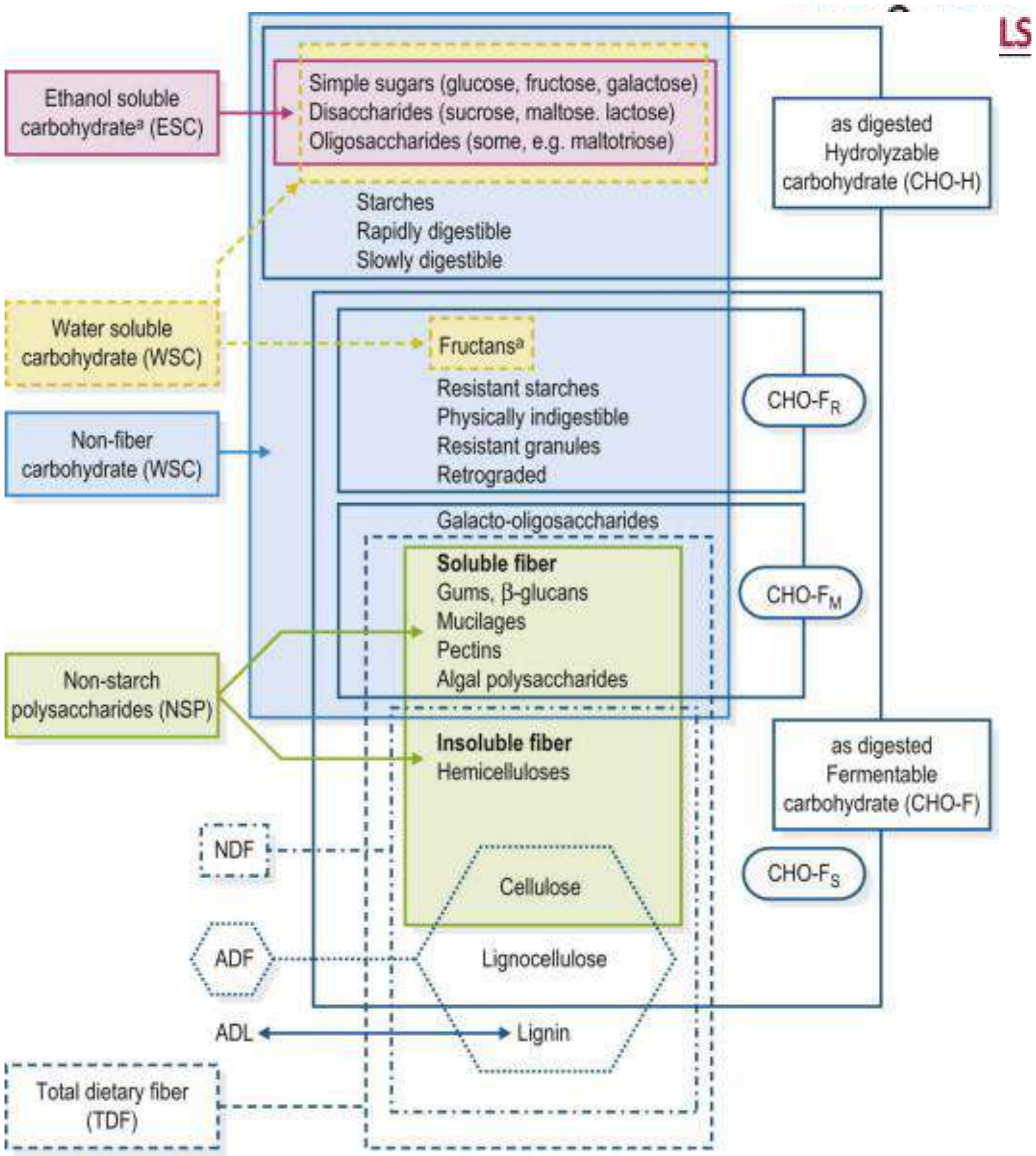
Carbohydrates in feed

<u>Abbreviations:</u>

ADL, acid detergent lignin;
ADF, acid detergent fiber;
NDF, neutral detergent fiber;

CHO-F_{S}, slowly fermentable carbohydrate
(yielding mainly acetate and butyrate);
CHO-F_{M}, moderately rapid fermentable carbohydrate
(yielding mainly propionate and acetate),
CHO-F_{R}, rapidly fermentable carbohydrate
(yielding mainly lactate).

Adapted and updated from Hoffman et al 2001.



Ethanol soluble carbohydrate[a] (ESC)

Simple sugars (glucose, fructose, galactose)
Disaccharides (sucrose, maltose. lactose)
Oligosaccharides (some, e.g. maltotriose)

Starches
Rapidly digestible
Slowly digestible

as digested Hydrolyzable carbohydrate (CHO-H)

Water soluble carbohydrate (WSC)

Non-fiber carbohydrate (WSC)

Fructans[a]
Resistant starches
Physically indigestible
Resistant granules
Retrograded

CHO-F_{R}

Galacto-oligosaccharides

Soluble fiber
Gums, β-glucans
Mucilages
Pectins
Algal polysaccharides

CHO-F_{M}

Non-starch polysaccharides (NSP)

Insoluble fiber
Hemicelluloses

as digested Fermentable carbohydrate (CHO-F)

NDF

Cellulose

CHO-F_{S}

ADF

Lignocellulose

ADL

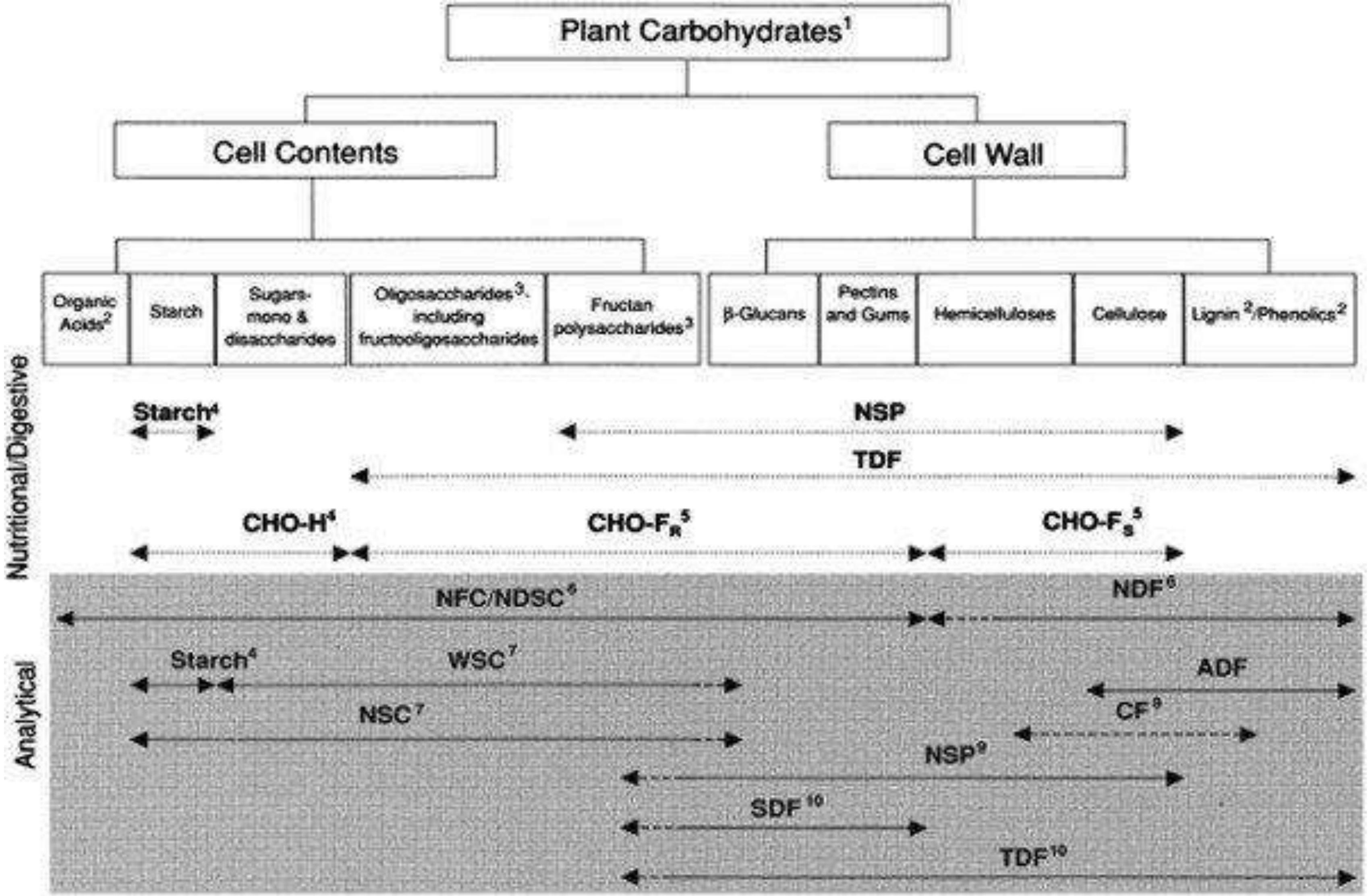
Lignin

Total dietary fiber (TDF)

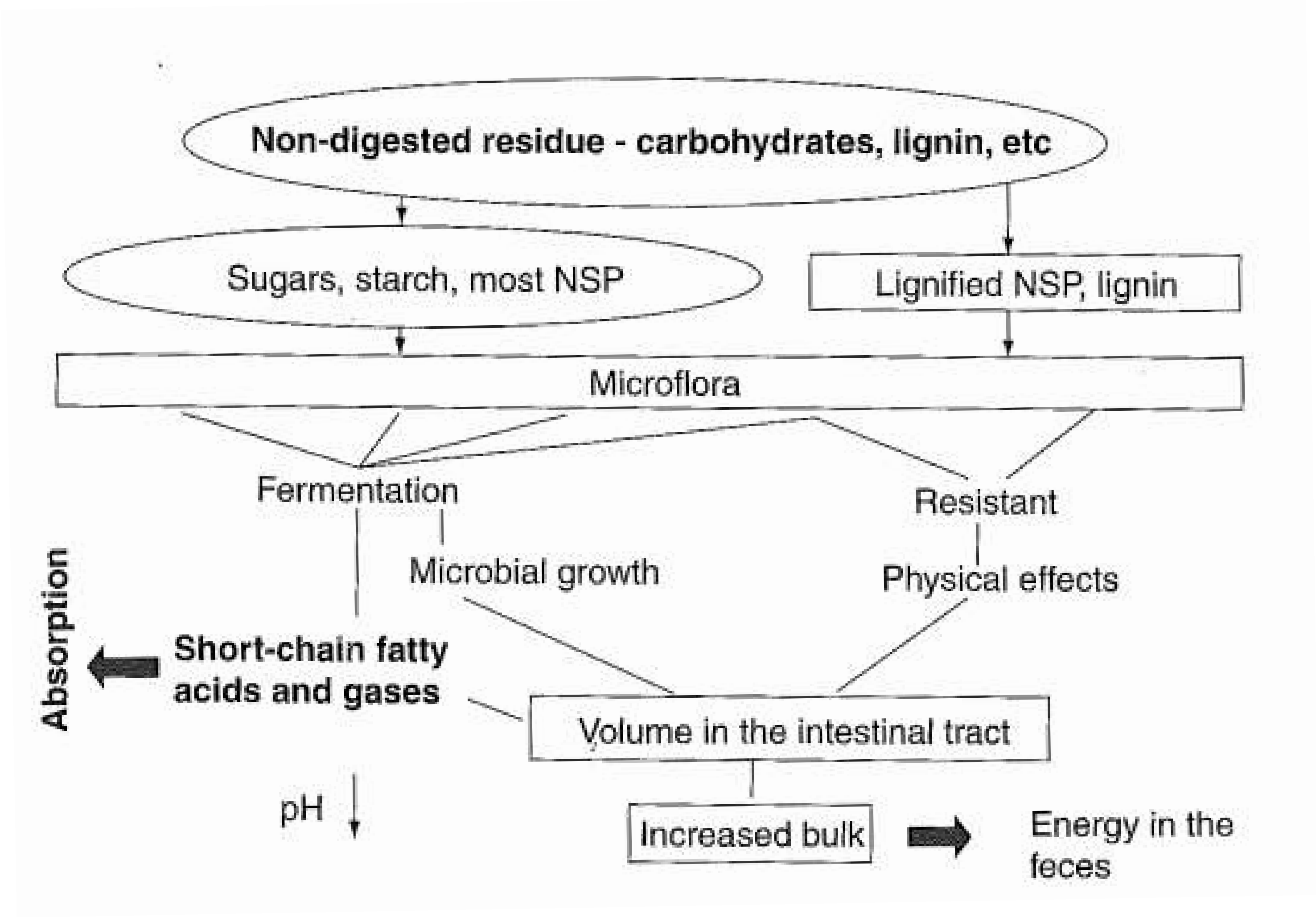
Hoffman, Rhonda M. 2013. «8 - Carbohydrates». In *Equine Applied and Clinical Nutrition*, edited by Raymond J. Geor, Patricia A. Harris, and Manfred Coenen, 156-67. W.B. Saunders

Kerr, Brian J, y Gerald C Shurson. 2013. «Strategies to Improve Fiber Utilization in Swine». *Journal of Animal Science and Biotechnology* 4 (1).

Carbohydrates in feed



Carbohydrate degradation in the large intestine



Non-digested residue - carbohydrates, lignin, etc

Sugars, starch, most NSP

Lignified NSP, lignin

Microflora

Fermentation

Resistant

Microbial growth

Physical effects

Absorption

Short-chain fatty acids and gases

Volume in the intestinal tract

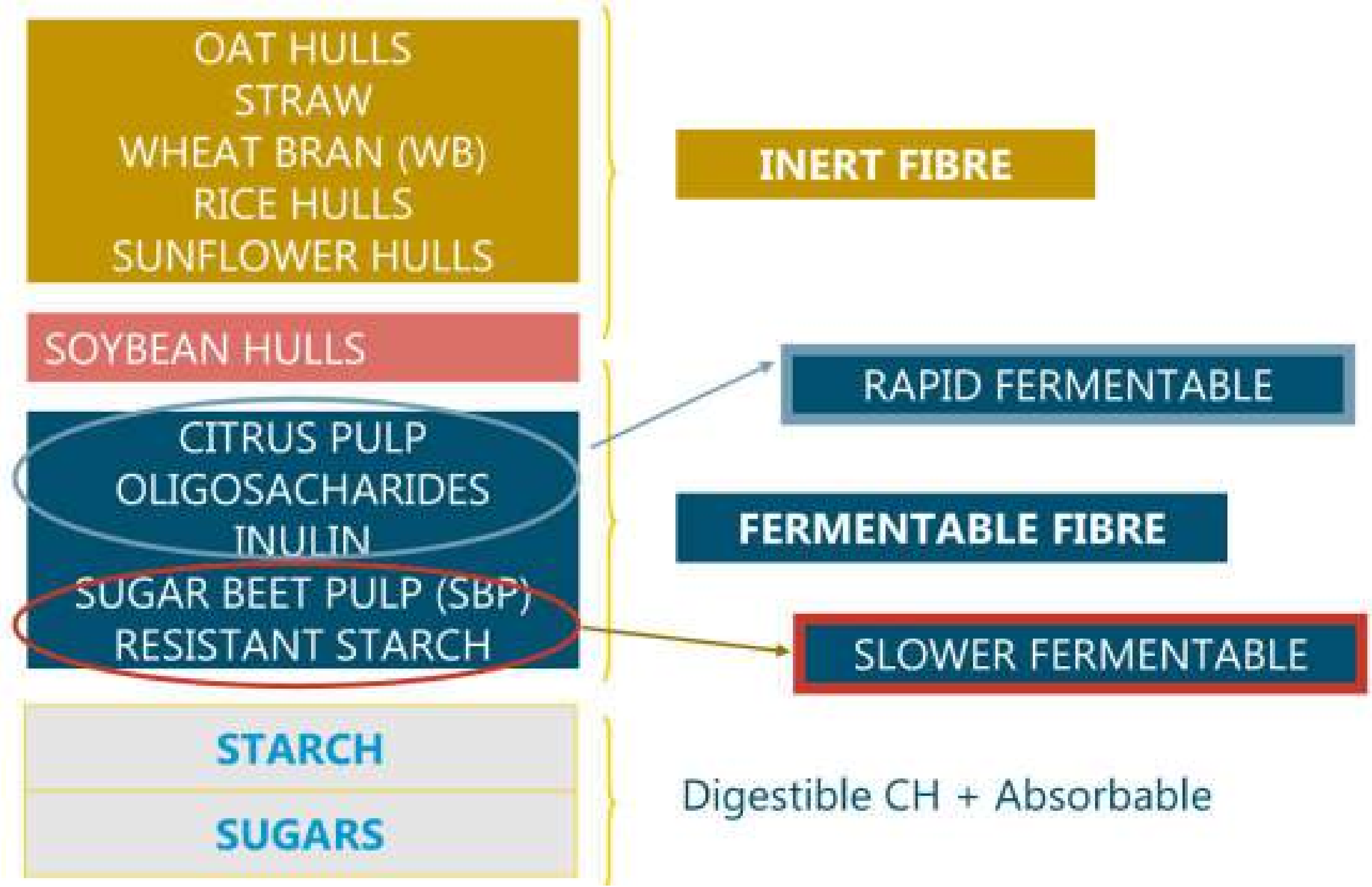
pH ↓

Increased bulk

Energy in the feces

Bach Knudsen, E.K. et al. 2012. «Carbohydrates and Carbohydrate Utilization in Swine». In *Sustainable Swine Nutrition*, edited by Lee I. Chiba, 109-37. Oxford, UK: Blackwell Publishing Ltd.

Fibre fermentability and solubility



OAT HULLS
STRAW
WHEAT BRAN (WB)
RICE HULLS
SUNFLOWER HULLS

SOYBEAN HULLS

CITRUS PULP
OLIGOSACHARIDES
INULIN
SUGAR BEET PULP (SBP)
RESISTANT STARCH

STARCH

SUGARS

INERT FIBRE

RAPID FERMENTABLE

FERMENTABLE FIBRE

SLOWER FERMENTABLE

Digestible CH + Absorbable

Molist, F, 2013. Nutritional strategies to reduce AMU and ZnO.

Processed cereals

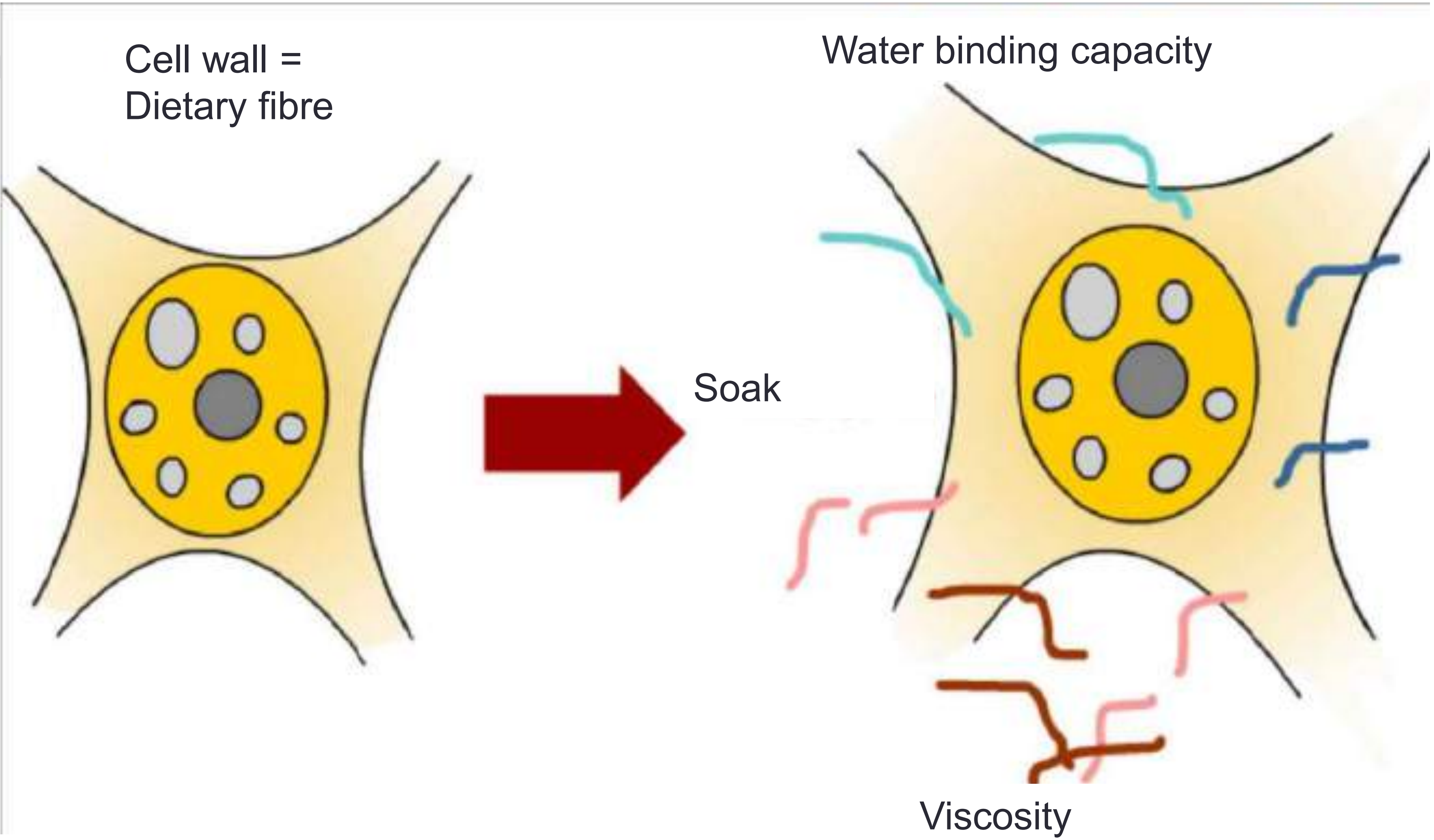
* OHC: oat hulls cooked

	OHC*	Days 21-35			Days 35-49			Days 21-49		
		ADFI	ADG	FCR	ADFI	ADG	FCR	ADFI	ADG	FCR
Maize										
Raw	0	330	240	1.42	672	443	1.52	501	341	1.48
	20	336	241	1.41	666	433	1.54	501	337	1.49
Cooked	0	339	247	1.37	639	415	1.55	490	331	1.49
	20	323	232	1.41	623	406	1.53	473	319	1.49
Rice										
Raw	0	101	277	1.49	813	520	1.56	607	398	1.49
	20	388	297	1.33	706	471	1.51	547	384	1.43
Cooked	0	420	304	1.38	797	490	1.63	609	397	1.53
	20	379	269	1.41	734	480	1.53	557	375	1.59

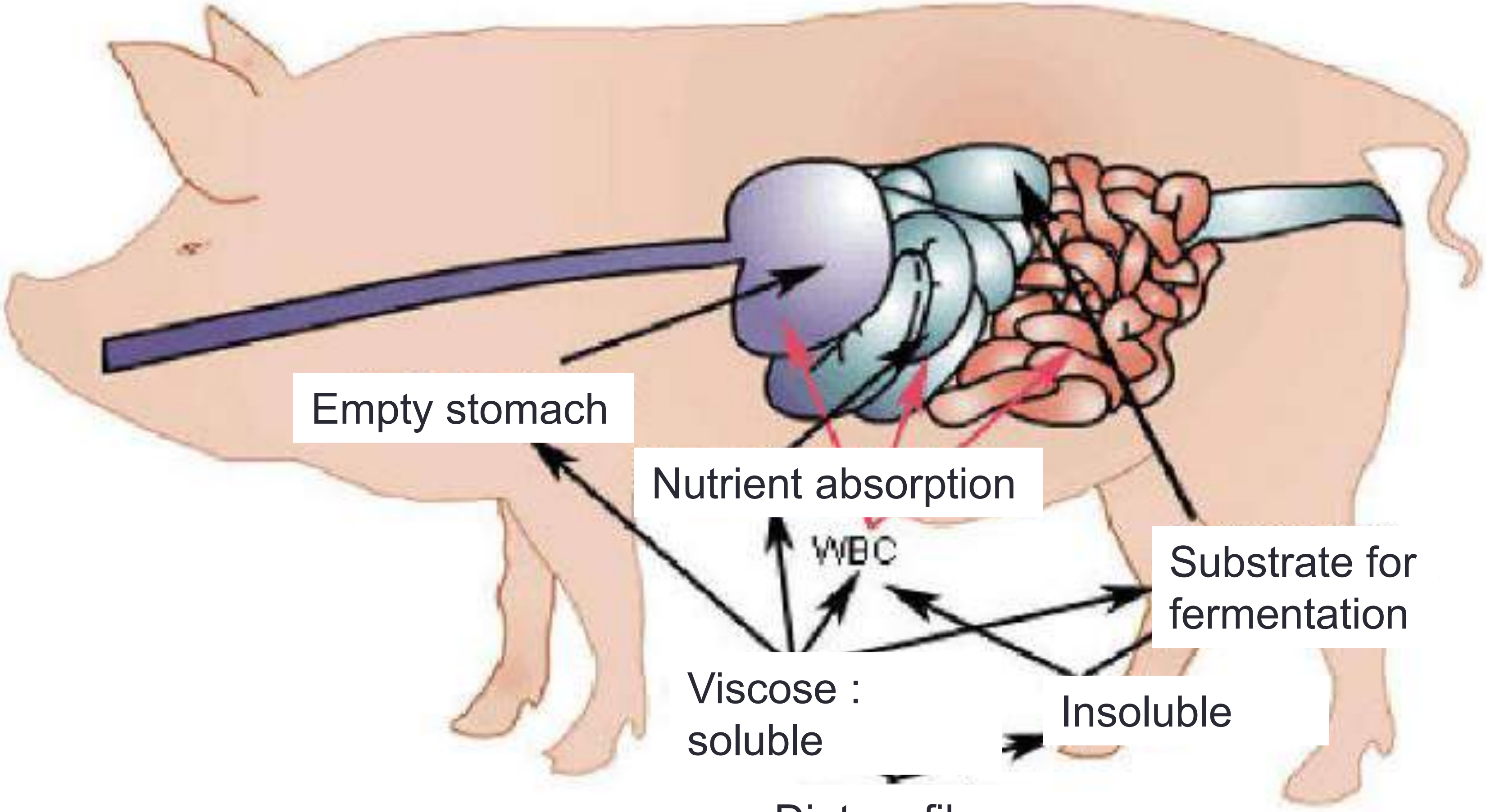
Effect of thermal processing of cereals and oat hulls inclusion on growth performance (Mateos, 2006)

Fibre sources

- In



Fibre sources



Empty stomach

Nutrient absorption

Substrate for fermentation

Viscose : soluble

Insoluble

Dietary fibre

Fibre sources[13]

- Influence of fibre sources
  - Insoluble fibre sources (cereals's husk) reduce the excretion of haemolytic *E. coli*
  - Soluble NSP stimulate proliferation of *E. coli* in the small intestine
  - Components in boiled rice inhibit electrolyte secretions in small intestine

Modified from McDonald, 2001	**Non-infected Piglets**		**Infected Piglets**			**Significance**	
	Rice	Barley	Rice	Barley	SEM	Diet	Health
Empty body weight gain (g/d)	74	26	-28	-56	36.3	*	***
Large intestine (% live weight)	2.7	3.8	2.6	3.2	0.62	**	NS
VFA in the distal colon (Mm)	84	114	60	78	20.4	**	**
pH in the colon distal	6.8	6.1	6.8	6.5	0.37	**	NS
***E. coli j*ejunum (log$_{10}$)**	0	0	0.9	4.2	2.44	*	
***E. coli* colon (log$_{10}$)**	0	0	3.2	6.2	1.89	**	
Ileal viscosity (cP)	2.1	2.8	1.6	2.3	1.13	*	*

Infected at 48, 72 y 96 h post weaning (21 days) with E. coli enterotoxigenic.
Culled at 7-9 days post weaning.

Fibre sources[14]

- Fermentability or viscosity

Proportion of pigs in groups with diarrhoea

Diet	Day 7	Day 8	Day 9	Day 10
Rice	$0/8^{a}$	$1/8^{a}$	$0/8^{a}$	$0/8^{a}$
Rice + low viscosity CMC	$5/8^{b}$	$3/8^{b}$	$4/8^{b}$	$4/8^{b}$
Rice + high viscosity CMC	$7/7^{b}$	$7/7^{b}$	$7/7^{b}$	$5/7^{b}$
P-value	< 0.05	< 0.05	< 0.05	< 0.05

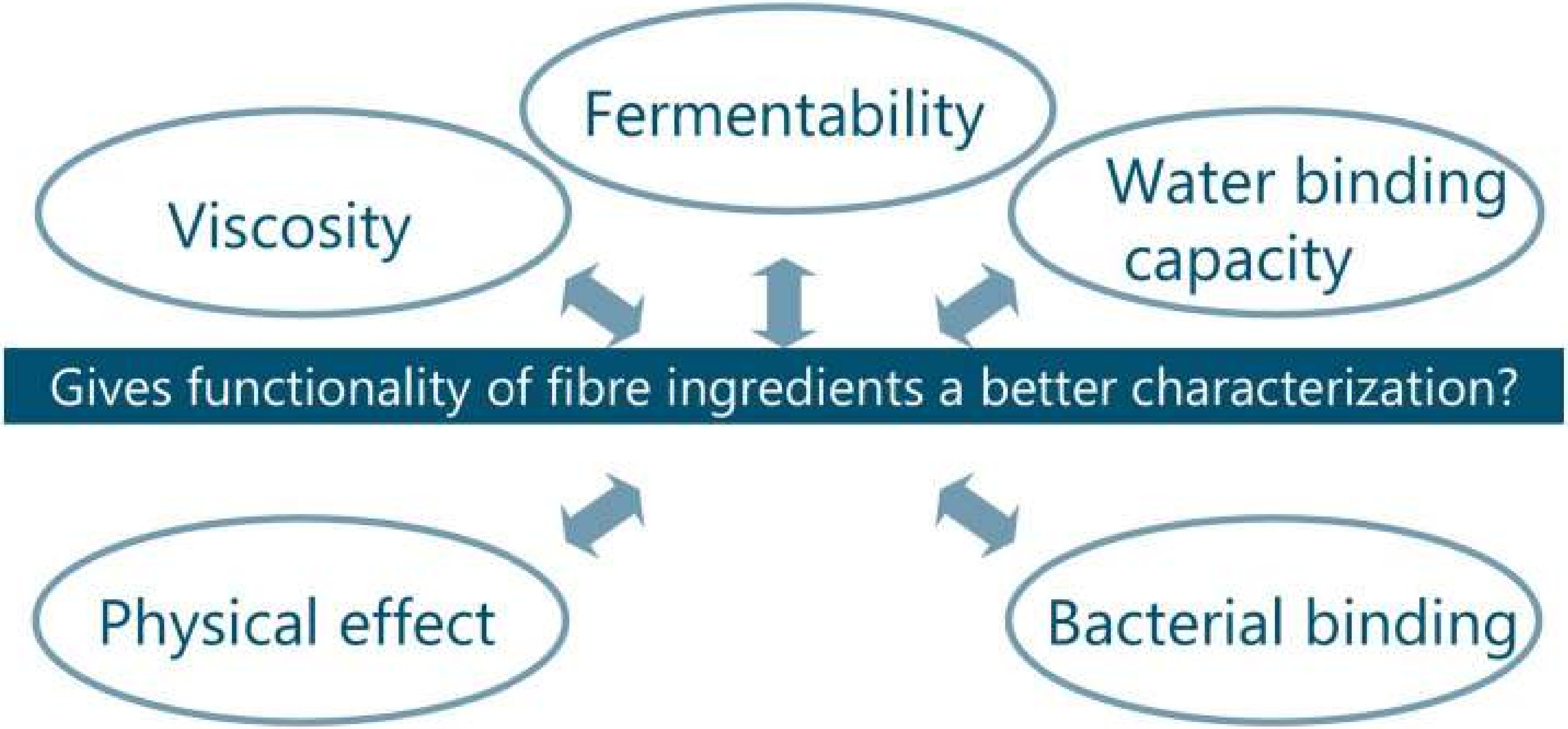
McDonald DE, Pethick DW, Mullan BP, Hampson DJ. Increasing viscosity of the intestinal contents alters small intestinal structure and intestinal growth, and stimulates proliferation of enterotoxigenic *Escherichia coli* in newly-weaned pigs. British Journal of Nutrition. 2001;86(4):487–98.

Fibre sources^{15}

Concentration (micromol/g DM) of SCFA on colon digesta and bacterial populations on caecum digesta of piglets 15 days after weaning (modified after Molist, 2007)

	Diets				**SEM**	**Diet p-value**
	Control	**Wheat Bran**	**Sugar Beet Pulp**	**WB-SBP**		
Butyric	11.7y	35.9x	12.2y	31.3x	10.83	0.027
Enterobacteria	11.1x	10.0xy	10.8xy	8.3y	1.14	<0.05
Lactobacilli	11.7	12.0	11.9	11.5	0.53	0.572

Role of fiber: functionality or chemical composition?



Fermentability

Viscosity

Water binding capacity

Gives functionality of fibre ingredients a better characterization?

Physical effect

Bacterial binding

From Molist, F. 2012, in Nutritional Strategies to reduce AMU and ZnO

Role of fiber: Inert or Fermentable?

	Positive	Negative
Inert	• Improve digestion function • Modifies microbiota • Enhances microbial fermentation	• Reduces nutrient digestibility • Penalizes animal performance
Fermentable	• Slows gastric emptying • Proximal fermentation in the hindgut	• Increases luminal viscosity

Role of fiber: Inert or Fermentable, sanitary conditions and diarrhoea

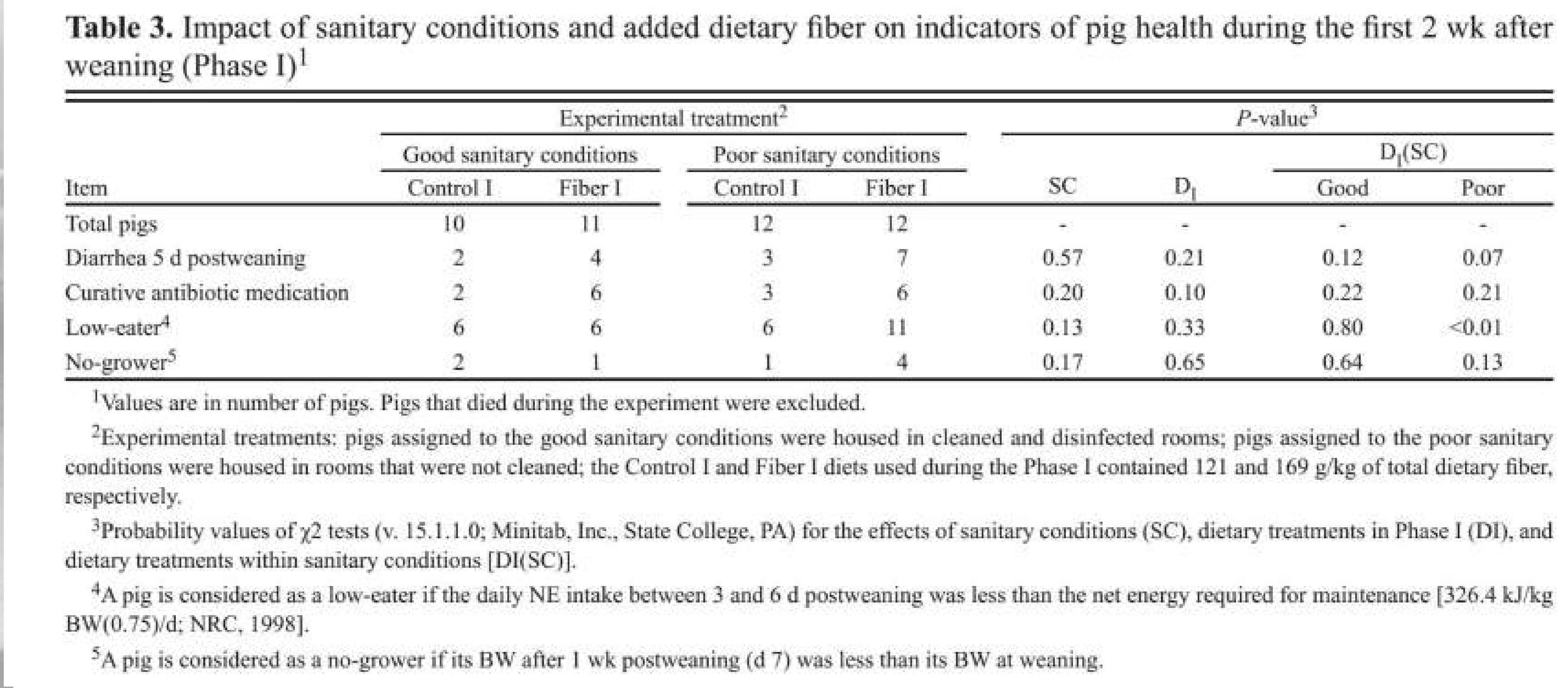


Table 3. Impact of sanitary conditions and added dietary fiber on indicators of pig health during the first 2 wk after weaning (Phase I)[1]

	Experimental treatment[2]				*P*-value[3]			
	Good sanitary conditions		Poor sanitary conditions				$D_I(SC)$	
Item	Control I	Fiber I	Control I	Fiber I	SC	D_I	Good	Poor
Total pigs	10	11	12	12	-	-	-	-
Diarrhea 5 d postweaning	2	4	3	7	0.57	0.21	0.12	0.07
Curative antibiotic medication	2	6	3	6	0.20	0.10	0.22	0.21
Low-eater[4]	6	6	6	11	0.13	0.33	0.80	<0.01
No-grower[5]	2	1	1	4	0.17	0.65	0.64	0.13

[1] Values are in number of pigs. Pigs that died during the experiment were excluded.

[2] Experimental treatments: pigs assigned to the good sanitary conditions were housed in cleaned and disinfected rooms; pigs assigned to the poor sanitary conditions were housed in rooms that were not cleaned; the Control I and Fiber I diets used during the Phase I contained 121 and 169 g/kg of total dietary fiber, respectively.

[3] Probability values of $\chi2$ tests (v. 15.1.1.0; Minitab, Inc., State College, PA) for the effects of sanitary conditions (SC), dietary treatments in Phase I (DI), and dietary treatments within sanitary conditions [DI(SC)].

[4] A pig is considered as a low-eater if the daily NE intake between 3 and 6 d postweaning was less than the net energy required for maintenance [326.4 kJ/kg BW(0.75)/d; NRC, 1998].

[5] A pig is considered as a no-grower if its BW after 1 wk postweaning (d 7) was less than its BW at weaning.

Montagne, L. 2012. «Comparative Effects of Level of Dietary Fiber and Sanitary Conditions on the Growth and Health of Weanling Pigs1». *Journal of Animal Science* 90 (8): 2556-69

Role of fiber: Inert or Fermentable, sanitary conditions and diarrhoea

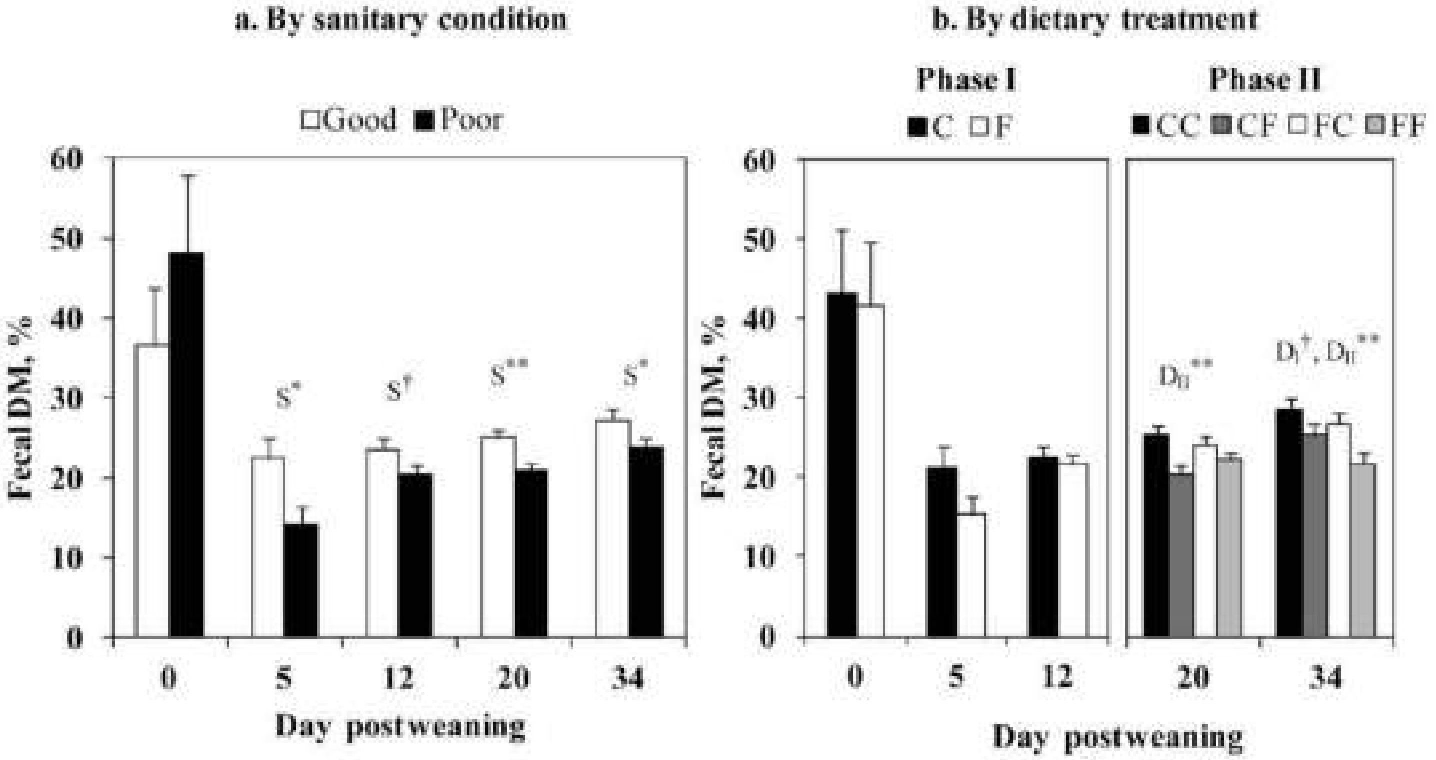
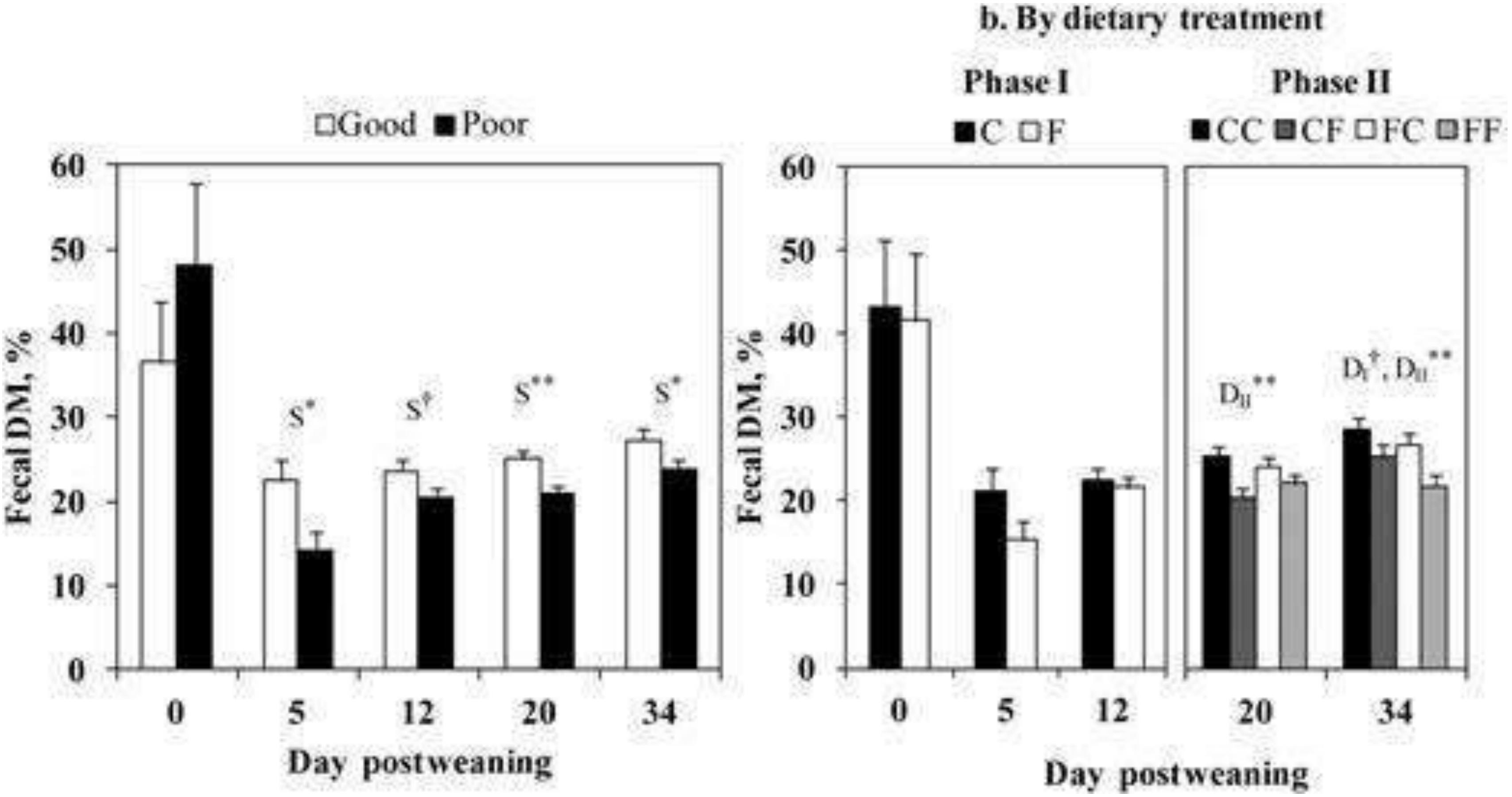


Figure 2. Impact of (a) sanitary conditions (good or poor) or (b) dietary treatments (C for control and F for fiber diets; first letter for Phase I and second for Phase II) on the DM content of feces from pigs collected on d 0, 5, 12, 20, and 34 postweaning. Values are least squares means; n = 21 and 24 for good and poor sanitary conditions, respectively; n = 22 and 23 in the control and fiber treatments during Phase I (d 0, 5, and 12, C and F groups); n = 11 in the CC (Control I-Control II), CF (Control I-Fiber II), FC (Fiber I-Control II) groups, and n = 12 in the FF group (Fiber I-Fiber II) during Phase II (d 20 and 34). Probability values for the effects of sanitary conditions (SC) or dietary treatments in Phase I and II (DI and DII): $^{**}P < 0.01$, $^{*}P < 0.05$, and $^{\dagger}P < 0.10$. Interactions between sanitary conditions and dietary treatments and between the dietary treatments applied in Phases I and II were not significant.

Montagne, L. 2012. «Comparative Effects of Level of Dietary Fiber and Sanitary Conditions on the Growth and Health of Weanling Pigs1». *Journal of Animal Science* 90 (8): 2556-69

G/F: Worse in Poor
Diarrhea: More in Poor in Fase I
Enteroccocus in diarrhea
End of treatment:
Poor: + Lactobacillus, + Enterobacteria, - sulfito reductoras
Poor: More VFA independently of the diet

Item	Experimental Diet			
	Phase I		Phase II	
	Control I	Fiber I	Control II	Fiber II
Chemical composition, g/kg DM				
Ash	64.5	64.9	58.8	60.1
CP (N × 6.25)	219.1	212.3	220.2	213.0
Ether extract	47.2	46.0	31.6	32.2
Starch	381.3	341.5	488.8	425.9
GE, MJ/kg	18.77	18.65	18.55	18.41
Crude fiber	32.5	48.9	35.8	63.9
NDF	109.6	112.5	122.3	153.2
ADF	34.6	50.1	39.3	69.0
ADL	2.1	8.6	2.9	9.9
Total dietary fiber	120.9	169.1	145.8	216.8
Water insoluble fiber	102.6	140.7	122.7	186.1



S: sanitary conditions; D: diet

Montagne, L. 2012. «Comparative Effects of Level of Dietary Fiber and Sanitary Conditions on the Growth and Health of Weanling Pigs1». *Journal of Animal Science* 90 (8): 2556-69

Take home messages for fiber

Weaning age		
(-5 days) to (+5 to 10 days)	(+5 to 10 days) to (+10 to 21 days)	(+10 to 21 days) to (+21 to 35 days)
Acute phase: Focus on GIT health	Maturation phase: Health and ADG	Maturation phase: Prepare for G/F
• Low CP & ABC-4 • Functional AA • Low FCHO • High ICHO	• High Lys/NE ratio • High CP & low ABC-4 • Medium FCHO • Medium ICHO	• Appropriate Lys/NE • U/S ratio • High FCHO • Low ICHO

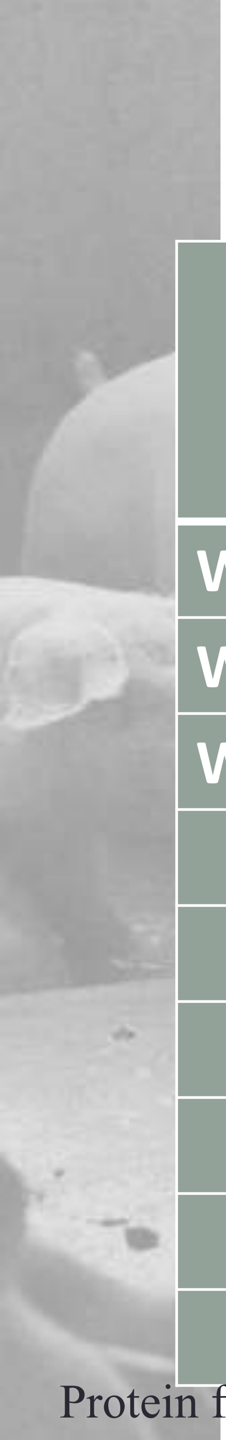
Protein sources and interactions

Protein content and its source[18]

Reduction of diarrhea with low protein diets and its interaction with lactose (Modified from Pierce, 2007)

CP, %	**16**		**21**		**SEM**	**Lactose*CP**
Lactose, %	12,5	21,5	12,5	21,5		
ADFI, g/d	830	820	840	950	0.028	*
ADG, g/d	440	420	510	580	0.017	**
Lactobacilli	8.0	8.5	7.2	8.3	0.21	*
E. coli	6.8	6.8	8.0	7.1	0.22	*

Milk protein and lactose: Replacement



	Whey	Casein from whey	Permeate+ Soybean	Lactose + Soybean
Weight 4 d post-weaning	6.2	6.2	6.2	6.2
Weight 15 d post-weaning	8.7^{ab}	8.4^{b}	8.9^{a}	8.9^{a}
Weight 40 d post-weaning	23.4^{ab}	23.2^{b}	24.4^{a}	24.4^{a}
ADG 4-15d (g/d)	225^{ab}(100)	201^{b}(89)	256^{a}(114)	249^{a}(111)
ADFI 4-15d (g/d)	252^{ab}(100)	227^{b}(90)	272 (108)	256^{b}(102)
FCR	1.12^{a}(100)	1.13^{a}(101)	1.07^{ab}(95)	1.03^{b}(92)
ADG 15-40d (g/d)	505 (100)	508 (101)	540 (107)	532 (105)
ADFI 15-40d (g/d)	790 (100)	789 (100)	828 (105)	821 (104)
FCR	1.57 (100)	1.55 (99)	1.53 (98)	1.55 (99)

Protein fraction (SCA Iberica, 2003)

Protein restriction(1/2)

Item	NP (20% protein)	LP (16% protein)	SEM	p value
Restriction phase (0 to 14 days post weaning)				
Initial weight, kg	6.39	6.38	0.02	0.861
Average daily gain, g/day	324	261	18.1	0.041
Average daily consumption, g/day	418	372	17.0	0.093
Conversion rate, feed/live weight	1.30	1.43	0.02	0.022
Incidence of diarrhea, %	2.00	0.29	0.55	0.060
Weight at the end of the restriction phase	10.9	10.0	0.26	0.043

Hou , L. et al. 2021. « Effects of Protein Restriction and Subsequent Feedback on Bodysuit Composition , Gut Microbiota and Metabolite Profiles in Weaned Piglets » Animals 11 (3): 686.

Protein restriction

Refeeding phase (15 days post weaning up to 25 kg live weight)				
	NP (20% protein)	LP (16% protein)	SEM	p value
Average daily gain, g/day	524	543	7.9	0.153
Average daily consumption, g/day	859	888	10.1	0.086
Conversion rate, feed/live weight	1.64	1.64	0.01	1
Incidence of diarrhea, %	5.33	2.61	1.21	0.151
Weight at the end of the refeeding phase, end of study	24.9	25.1	0.23	0.579
The entire study				
Average daily gain, g/day	455	452	9.5	0.814
Average daily consumption, g/day	708	718	8.2	0.413
Conversion rate, feed/live weight	1.56	1.59	0.01	0.283
Incidence of diarrhea, %	3.76	1.56	0.82	0.095
Study days	40.8	41.5	0.81	0.537

Minerals: Zinc and Cooper

Zinc[47]

Variable	Mean effect size	95% CI	Number of studies	Comparisons
ADG	1.086	0.905 a 1.266	26	72
ADFI	0.794	0.616 a 0.971	25	71
G/F	0.566	0.422 a 0.710	24	70

$$d = \frac{\overline{x}_t - \overline{x}_c}{s_{pooled}}$$

Effect size: 0.3 Small; 0.5 Medium; 0.8 large

Zinc[19, 20, 21, 41, 43]

- Increased results due to improved intestinal integrity and morphology:
    - Increases the height of the intestinal villi and the height/depth ratio of the crypts
    - Decreases crypt’s depth
- Recovering damaged tissue from the epithelium
- Increased glucose absorption capacity
- Stimulates enzymatic production at the pancreatic and intestinal level
- Promotes intestinal absorption of nutrients

Copper[42, 43]

- Hemoglobin synthesis and oxidative enzymes
- Independent action of AGP
- Bactericidal and bacteriostatic properties
- Reduction of *Enterobacteriaceae* and *Lactobacilli* in the caecum
- Increase of VFA

Copper[42]

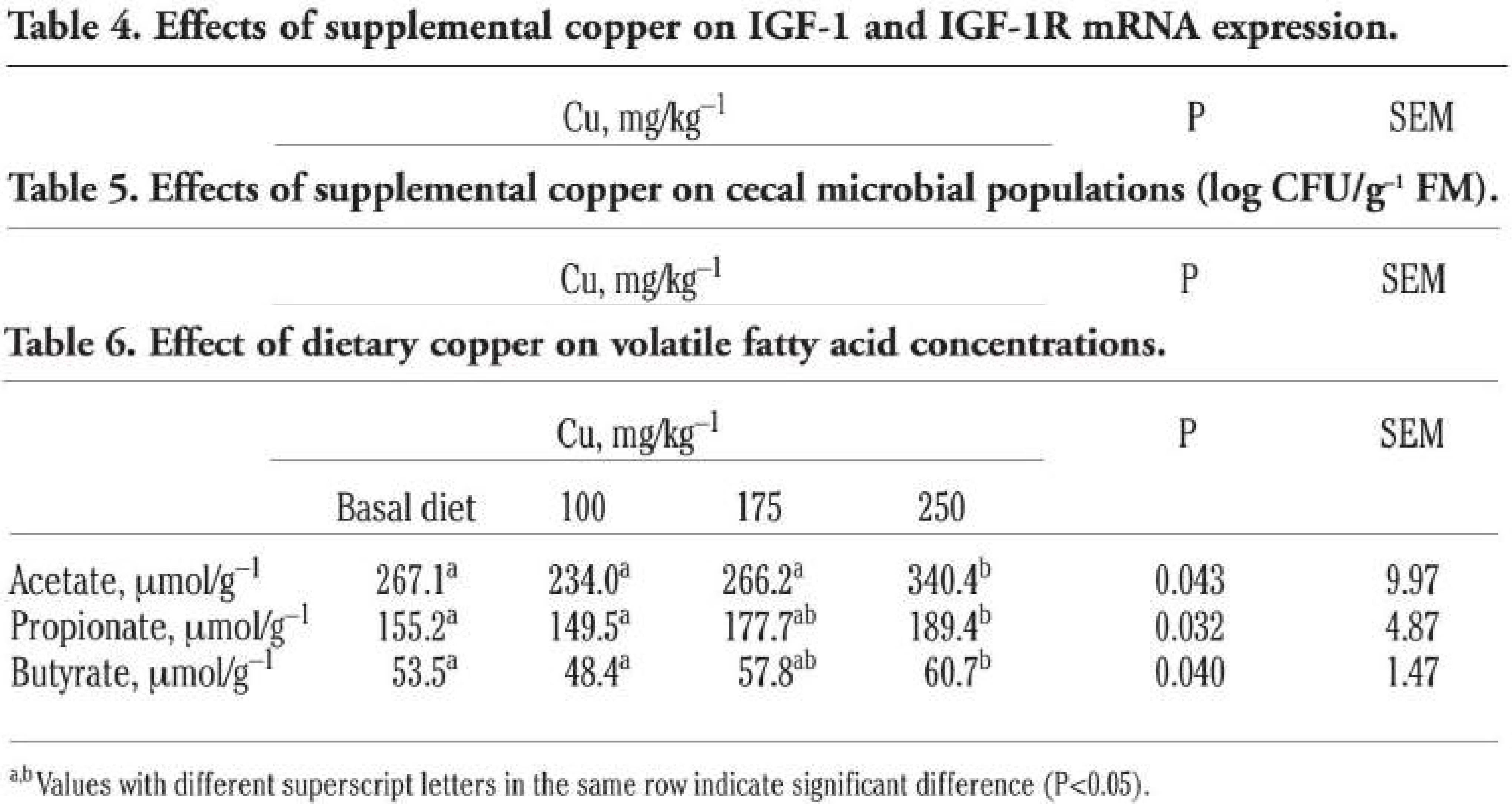


Table 4. Effects of supplemental copper on IGF-1 and IGF-1R mRNA expression.

	Cu, mg/kg^{-1}	P	SEM

Table 5. Effects of supplemental copper on cecal microbial populations (log CFU/g^{-1} FM).

	Cu, mg/kg^{-1}	P	SEM

Table 6. Effect of dietary copper on volatile fatty acid concentrations.

	Cu, mg/kg^{-1}				P	SEM
	Basal diet	100	175	250		
Acetate, µmol/g^{-1}	267.1^{a}	234.0^{a}	266.2^{a}	340.4^{b}	0.043	9.97
Propionate, µmol/g^{-1}	155.2^{a}	149.5^{a}	177.7^{ab}	189.4^{b}	0.032	4.87
Butyrate, µmol/g^{-1}	53.5^{a}	48.4^{a}	57.8^{ab}	60.7^{b}	0.040	1.47

[a,b] Values with different superscript letters in the same row indicate significant difference ($P<0.05$).

Organic acids, essential oils, probiotics, prebiotics, nutraceuticals and enzymes

Probiotics

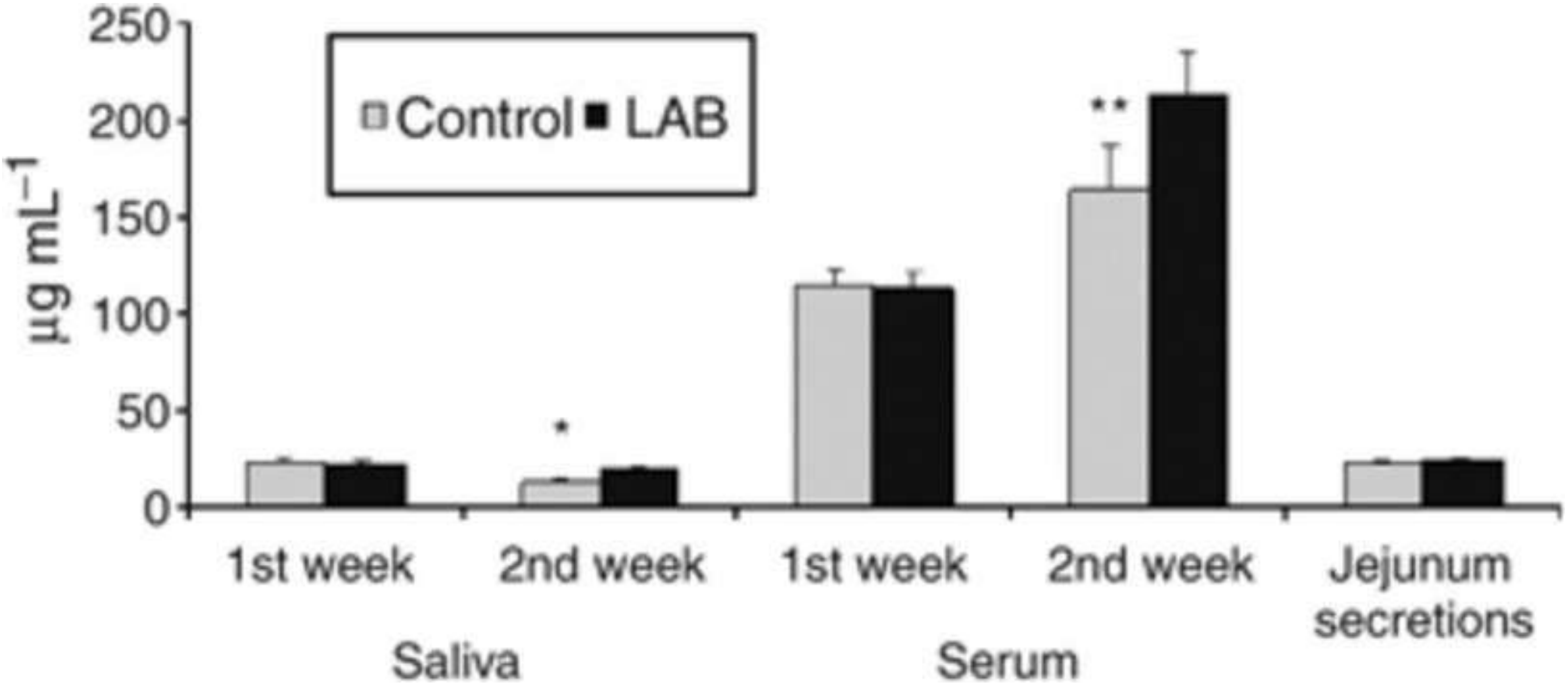
- Probiotics
- Specific and viable microorganisms
  - Implantation and colonization
  - Alters microflora
- Connected with human health but not consistent
- Piglets:
  - Microbiota balance, integrity of epithelium, maturation of tissues GIT and neuroendocrinous functions

Probiotics[27-29]

- Probiotics:
  - *Lactobacillus sobrius* reduce ETEC adhesion to IEC lines and epithelial damage:
    - Inhibit TJ ZO-1 delocalization
    - Reduce occludin concentration and dephosphorylation
    - Rearrangement of actin filaments

Probiotics[29]

Effect of dietary supplementation with *Lactobacillus sobrius* DSM 16698 on total IgA in saliva, blood serum, and jejunum secretions of ETEC-challenged pigs
(least squares means ± SEM); *effect of diet, P<0.05; **effect of diet, P=0.10.



Prebiotics

- Prebiotics:
  - Non digestible feed ingredients
  - Stimulate selectively growth or activity of a colon bacteria improving animal health
    - Substrate for *Bifidobacteria* and *Lactobacilli*
  - Oligosaccharides: inulin and FOS and MOS
  - Non starch polysaccharides (NSP), soluble or insoluble

Non digestible Oligosaccharides

- Inulin and oligofructose
  - Stimulate growth of *Bifidobacterium*
  - Suppress proliferation of pathogens
  - Modulate a variety of human enteric conditions and diseases
- The effectiveness depends on the environment

Nutraceuticals, botanics and fatty acids

- Essential plant oils and extracts: carvacrol, timolol, cinnamaldehydes, coumarins
- Anti inflammatory and immunological actions
- Conjugated linoleic acid: enhanced cellular immunity (CD8+)

Exogenous enzymes

- Enzymes have no effect *per se* on the microflora
- Effect as consequence of breaking branched NSP as arabinoxylans and xylanase or beta galactomannans and mannanase
- Change the ratio of *Bifidobacteria* and *Lactobacilli*
- Reduction of viscosity

Non antibiotics feed additives diets for pigs: Phytase instead zinc

Boost starter pig performa
phytase

by Dr Pete Wilcock
Global Technical M

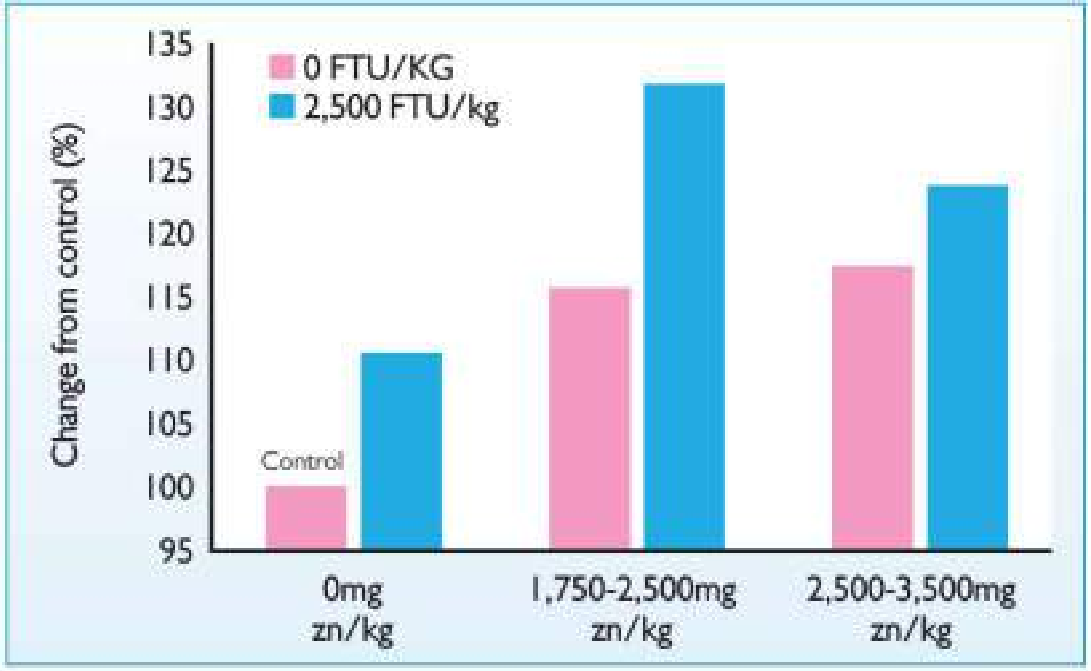
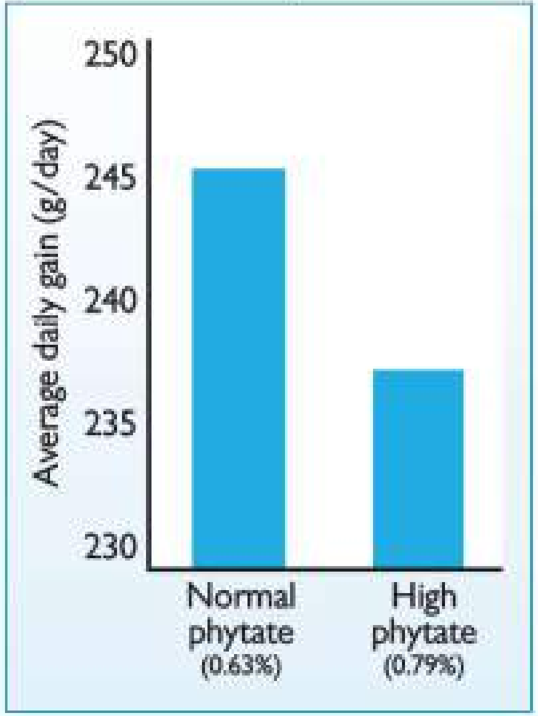


Fig. 2. Influence of zinc level (from zinc oxide) on growth rate response to phytase superdosing with Quantum Blue in starter pig (average of five trials) up to 21 days post-weaning (AB Vista, 2014).

Fig. 1. Performance of piglets fed typical starter feeds with (high phytate) and without (low phytate) 2.5% rice bran from weaning to 21 days post-weaning (Walk et al., 2014).



Organic acids

- Acids for piglets

Tips around weaning to improve intestinal health

Pre-weaning

- Colostrum intake
- Modifying microbiota has long-lasting effects: take care
- Creep-feed supplementation
- Hygiene to help and develop a stable microflora
- Minimize negative effects associated to weaning
- Complex and simple diets

Post weaning

- Importance of control feed intake
- Phase feeding according of nutrient’s requirements and quality of feed ingredients
- Reduce stress
- Nutrition and vaccination
- Health affects microbiota

Sanitary conditions and microbiota

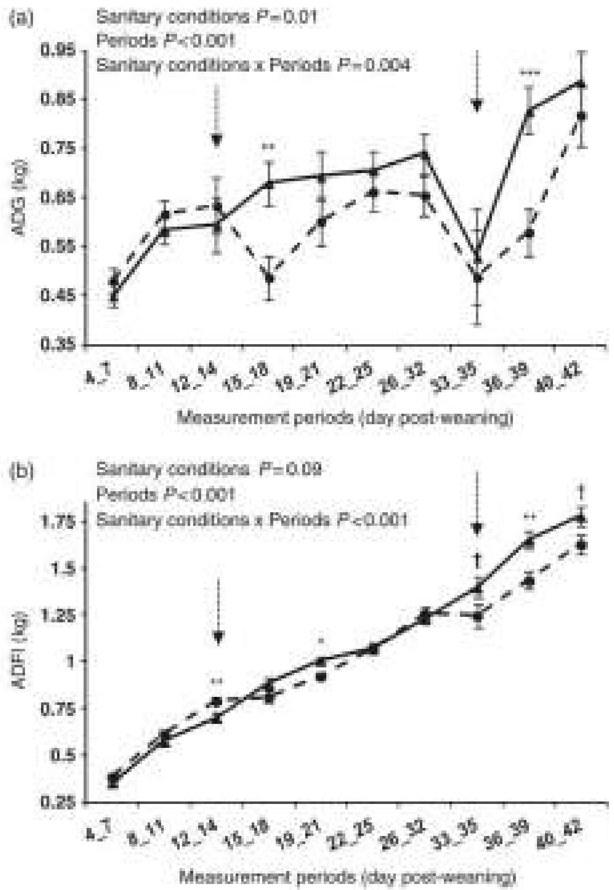
Sanitary conditions, fiber and microbiota

	Sanitary conditions and diets				
	Adequate		Deficient		SEM[1]
	Control diet	High fiber diet	Control diet	High fiber diet	
Total VFA, µ mol/g DM [2]	225^{c}	349^{bc}	496^{ab}	554^{a}	
Mole proportion, %					
Acetate	62.5^{ab}	64.2^{a}	58.3^{b}	59.2^{ab}	1.34
Butyrate	5.8^{b}	5.7^{b}	11.1^{a}	10.7^{a}	0.85
Productive parameters					
ADG 0-14 days [3, 4]	128	125	132	84	17.6
ADFI 0-14 days [3, 5]	228	217	276	227	18.9

1, SEM: standard error of the mean;
2, DM: dry matter;
3, Statistical trend (0.05≤p≤0.10);
4, ADG: Average daily gain in g/piglet/day;
5, ADFI: Average daily consumption in g/piglet/day

Montagne, L.,et al. 2010. «Effect of sanitary conditions and dietary fibre on the adaptation of gut microbiota after weaning». Livestock Science 133 (1-3): 113-16.

Sanitary conditions and performance



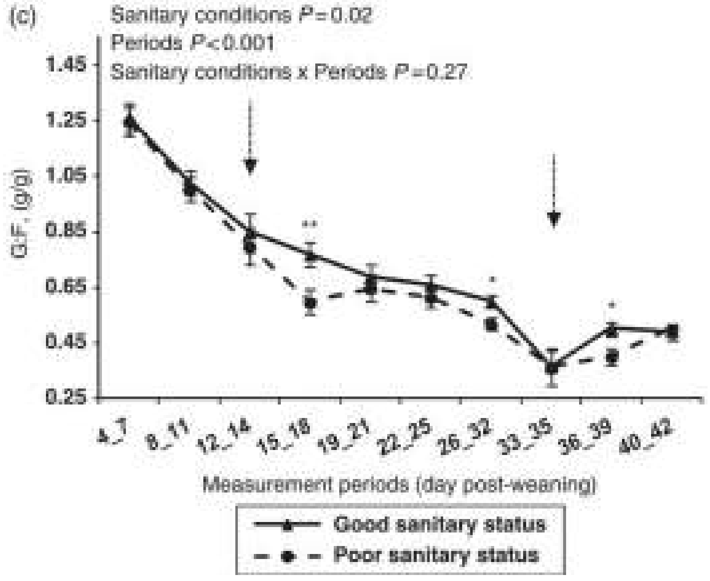


Figure 1 Average daily gain (a), in kg/d, average daily feed intake (b), in kg/d, and gain to feed ratio (c), in g/g, measured in pigs housed in poor or good sanitary conditions during 42 days post weaning (day 0). The two arrows indicate successively the diet change (starter to weaner diet) and the housing change (weaning to grower unit). Values are least-squares means and their respective standard error (LS means ± s.e.) calculated for 10 pigs per sanitary condition. Within a period, symbols †, *, ** and *** denote an effect of sanitary conditions, $P < 0.10$, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

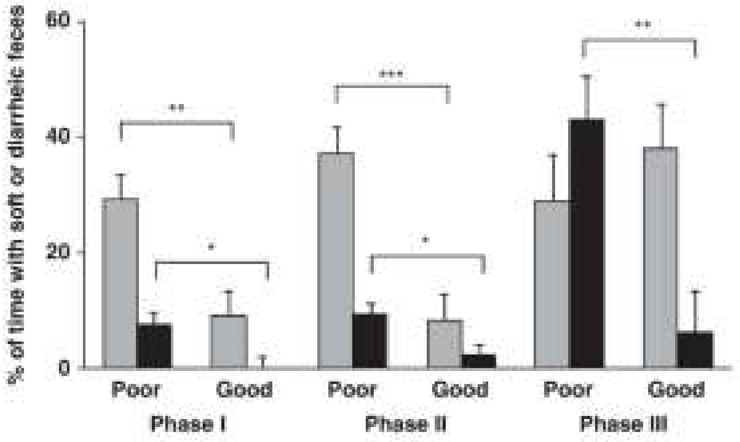
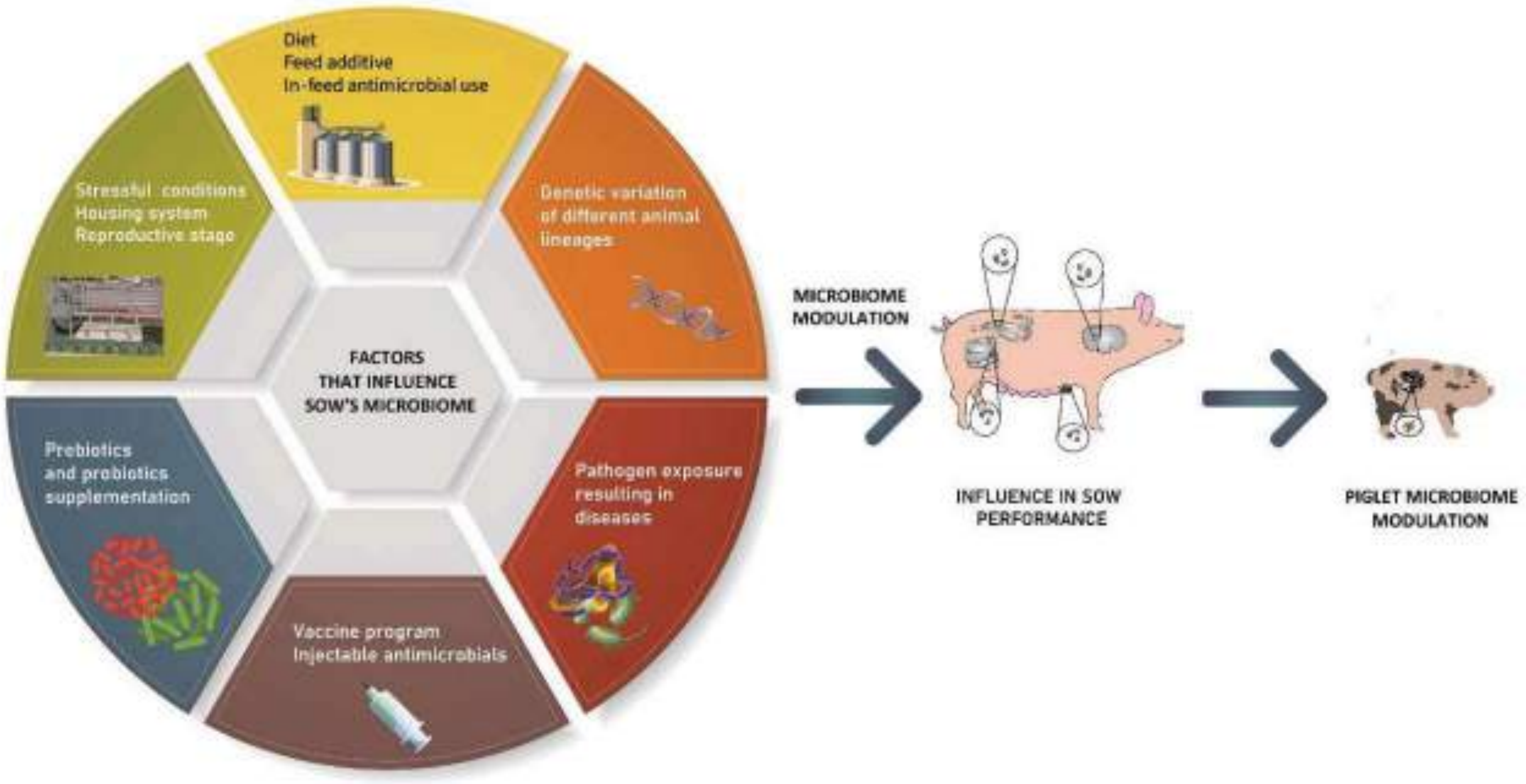


Figure 2 Percentage of time with soft (grey) and diarrhoeic faeces (dark bar) in each sanitary condition (poor and good) during the three phases of experiment: Phase I from day 0 to 11 post weaning; Phase II from day 12 to 32 post weaning; Phase III from day 33 to 42 post weaning. Values are least-squares means and their respective standard error (LS means ± s.e.) of percentage calculated for 10 pigs per sanitary condition. Within a phase, symbols *, ** and *** denote an effect of sanitary conditions, $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Pastorelli, H., et al. 2012. «Sanitary Housing Conditions Modify the Performance and Behavioural Response of Weaned Pigs to Feed- and Housing-Related Stressors» Animal 6 (11): 1811-20.

Maternal influence on piglet’s microbiota

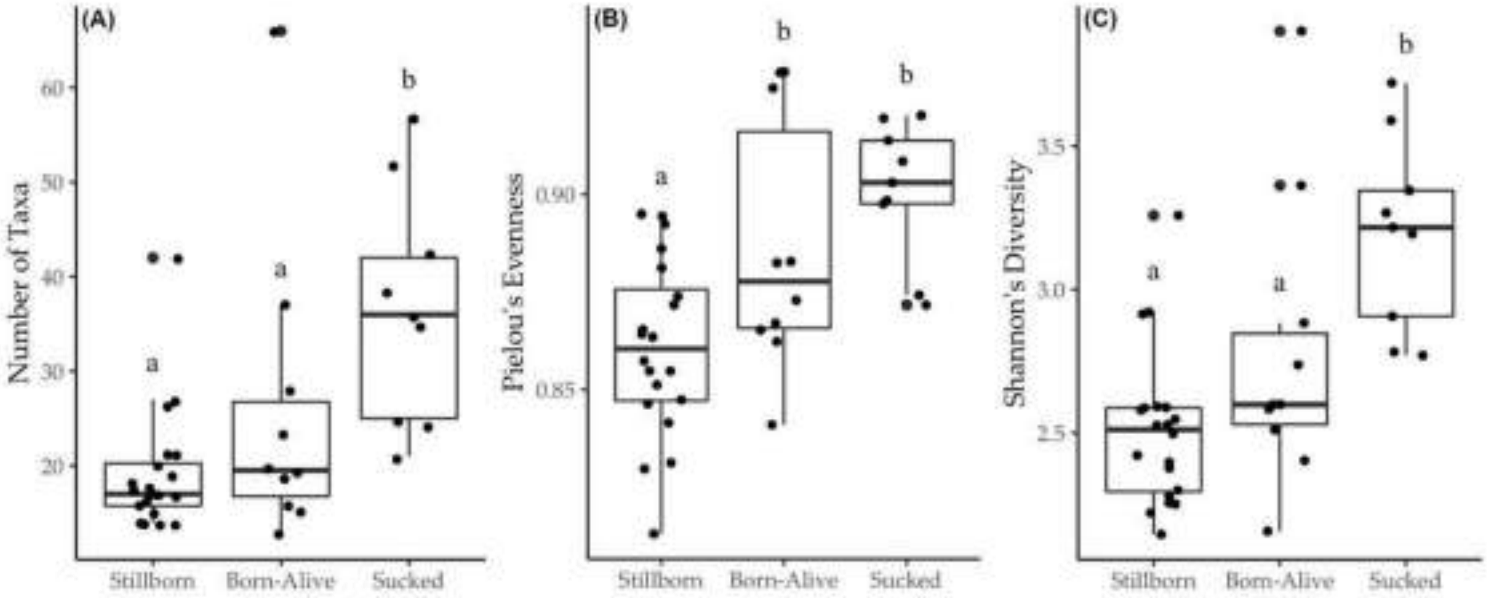
Maternal influence on piglet’s microbiota



Monteiro, M S. 2022. "The sow microbiome: Current and future perspectives to maximize the productivity in swine herds." *Journal of Swine Health and Production* 30 (4): 238-50

Maternal influence on piglet's microbiota

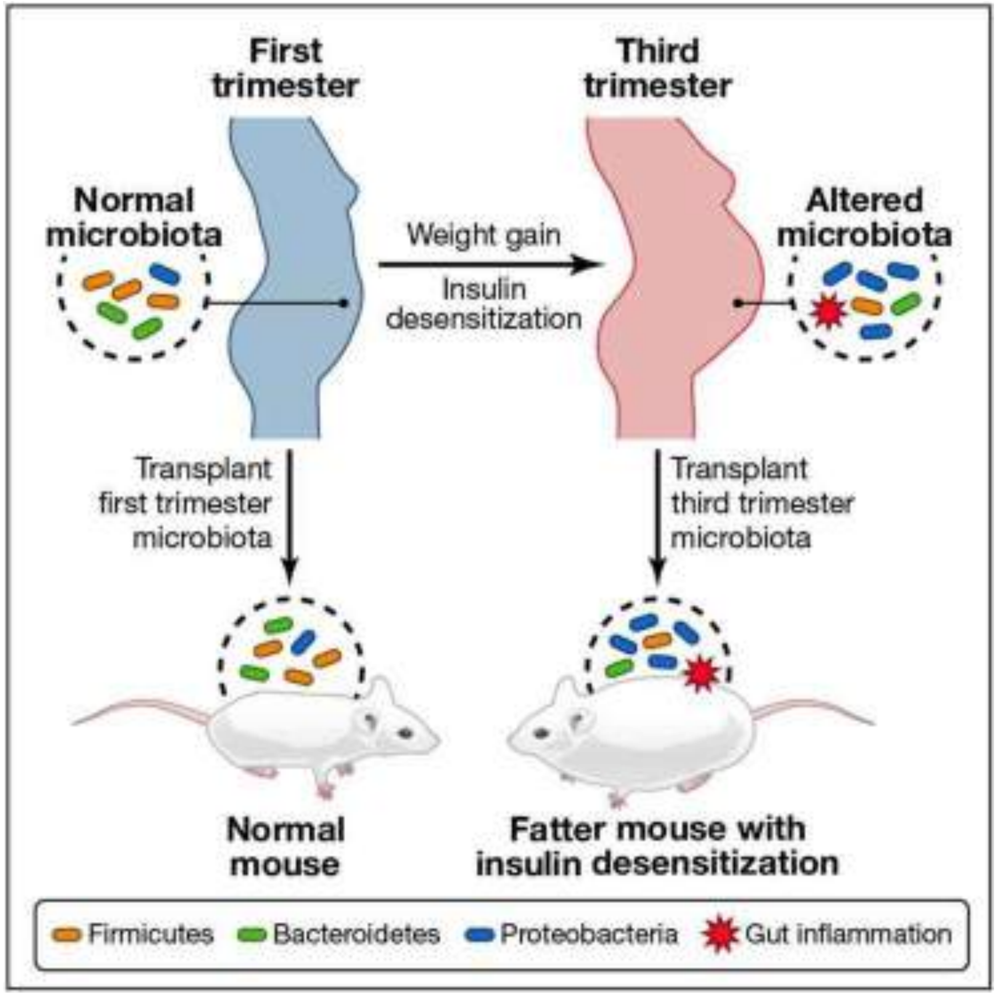
- Bacteria in the spiral colon of stillborn in birth channel
- Abundance, and diversity of the microbiota that colonized the spiral colon could increase after birth due to exposure to the environment and the intake of colostrum



Nowland, T L., et al. 2021. "Characterization of Early Microbial Colonizers within the Spiral Colon of Pre- and Post-Natal Piglets." *Life* 11 (4): 312

Maternal influence on piglet’s microbiota

- Can we modify the microbiota by modifying sow’s diet or the environmental farm conditions?



Koren, O. et al. 2012. “Host Remodeling of the Gut Microbiome and Metabolic Changes During Pregnancy.” *Cell* 150 (3): 470-80

Maternal influence on piglet’s microbiota

Concentrations of organic acids in fresh feces from two groups of high and low productivity sows. Modified from Uryu et al. 2020.

Organic acids, µmol/g fresh feces	High productivity sows	Low productivity sows	p value
Acetate	86.53 ± 2.43	81.00 ± 2.73	0.04
Propionate	37.49 ± 1.27	33.28 ± 1.61	0.01
n-Butyrate	16.11 ± 0.79	14.48 ± 0.96	0.045

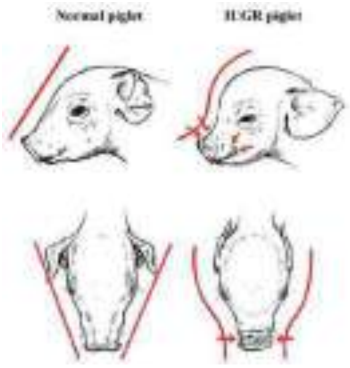
- Oxidative stress in sows: microbiota impact
- Sows fed diets that favored the abundance of fiber-degrading and acetate, propionate, and butyrate-producing bacteria, such as *Ruminoccocus*, *Fibrobacter* and *Butyricicoccus*, were the most productive in terms of number of farrows per sow per year since reductions in oxidative stress were observed.

Uryu, Het al. 2020. “Comparison of Productivity and Fecal Microbiotas of Sows in Commercial Farms.” *Microorganisms* 8 (10): 1469.

Maternal influence on piglet’s microbiota

Percentage of IUGR piglets in the different diets. Modified from Liu et al. 2021

	Control diet	Alfalfa diet	Beet pulp diet	Soy hull diet
IUGR, %	9.48 ± 0.07^{a}	2.08 ± 0.04^{b}	11.21 ± 0.08 ^{a}	8.94 ± 0.06^{a}

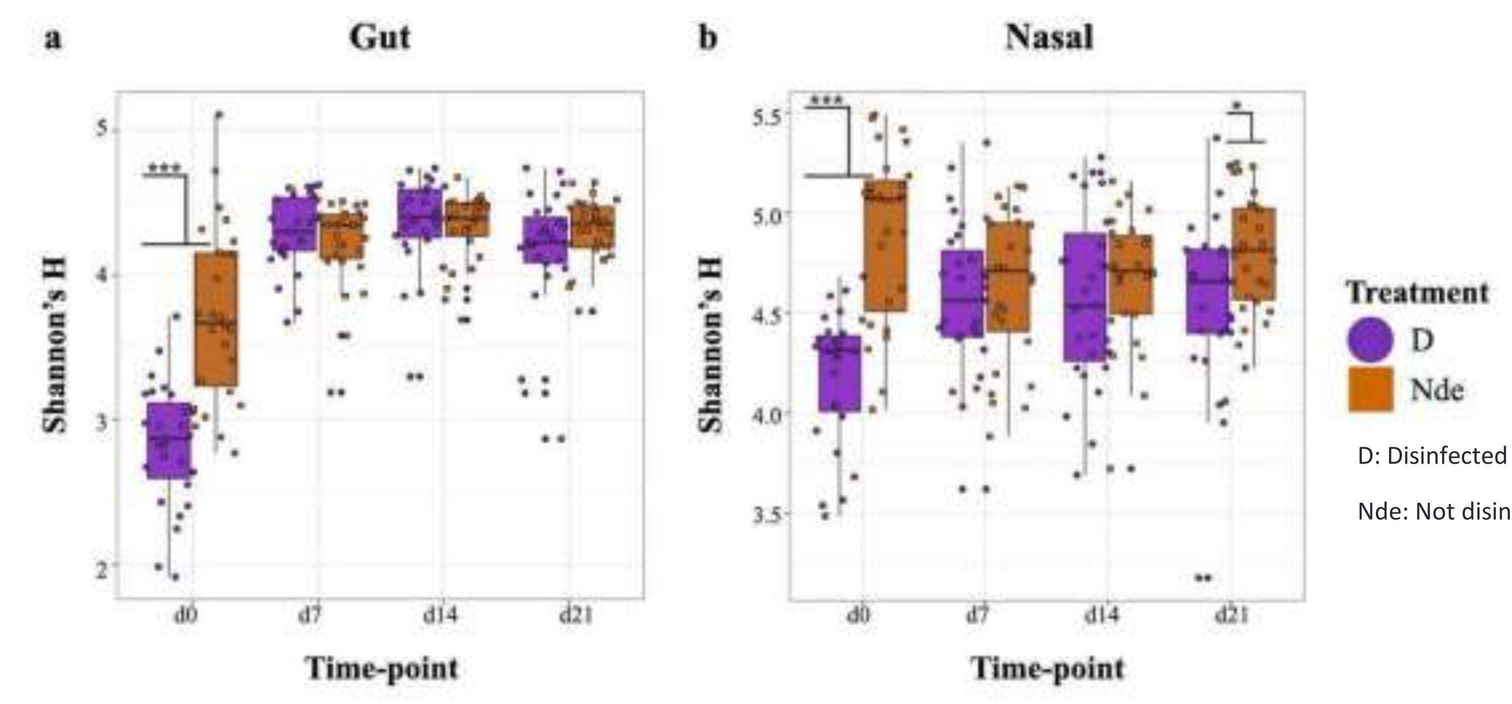


- The alfalfa flour reduced the number of low-birth-weight piglets,
- This study was carried out with only 48 sows in total.
- These effects, according to the authors, were due to the decrease in inflammatory factors in the sows

- Pictures and draw from Chantal Farmer
- Liu, B., et al. 2021. "Consumption of Dietary Fiber from Different Sources During Pregnancy Alters Sow Gut Microbiota and Improves Performance and Reduces Inflammation in Sows and Piglets." *mSystems* 6(1): e00591-20.

Maternal influence on piglet’s microbiota

Microbiota diversity of intestinal contents and nasal samples of piglets. Taken from Law et al. 2021.



D: Disinfected

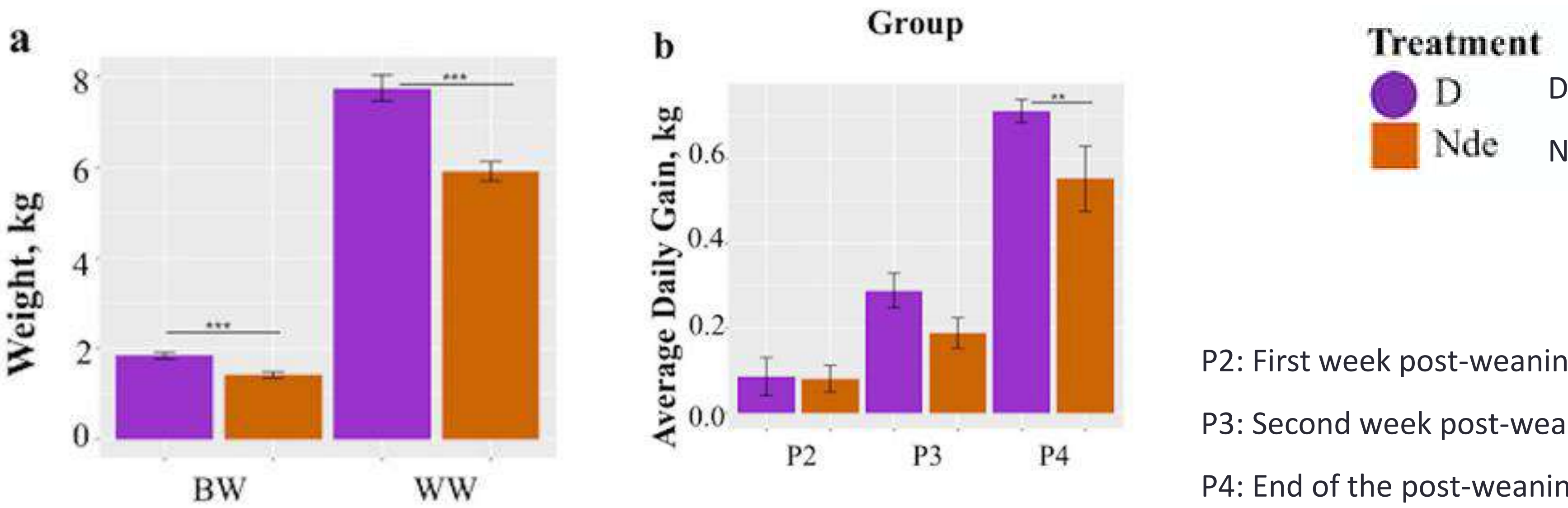
Nde: Not disinfected

- Sows from 3 days before farrowing in the farrowing rooms with <u>two cleaning and disinfection status</u>, one disinfected and washed and the other without
- No differences in the intestinal microbiota of the sows, nor in vaginal samples, milk, or skin
- Microbiota of their piglets was modified in both intestinal and nasal content samples

Law, K.et al.. 2021. “Disinfection of Maternal Environments Is Associated with Piglet Microbiome Composition from Birth to Weaning.” mSphere 6(5): e0066321

Maternal influence on piglet’s microbiota

Weight and ADG of piglets whose mothers were kept in farrowing rooms with differences in terms of cleaning and disinfection 3 days before farrowing and during lactation. Modified from Law et al. 2021.



D: Disinfected

Nde: Not disinfected

P2: First week post-weaning

P3: Second week post-weaning

P4: End of the post-weaning period

BW: birth weight

WW: Weaning weight

Law, K.et al.. 2021. “Disinfection of Maternal Environments Is Associated with Piglet Microbiome Composition from Birth to Weaning.” mSphere 6(5): e0066321

Sow’s colostrum and milk influences piglet’s microbiota

Sow’s colostrum and milk influences piglet’s microbiota

- Skin of the udder
- Teat channel
- Up to 77 colostrum samples, 16 were completely negative
- *Staphylococcaceae* was isolated from 96.9% of the skin samples and 75.4% of the positive colostrum samples
- *Streptoccocus spp* seems to be only incidental findings from the skin of the sow's breast, as *Enterobacteriaceae* species that are part of the fecal flora and are contaminations of the mammary skin

Kemper, Nicole. 2011. "Bacterial flora on the mammary gland skin of sows and in their colostrum." Journal of Swine Health and Production 19 (2): 112-15

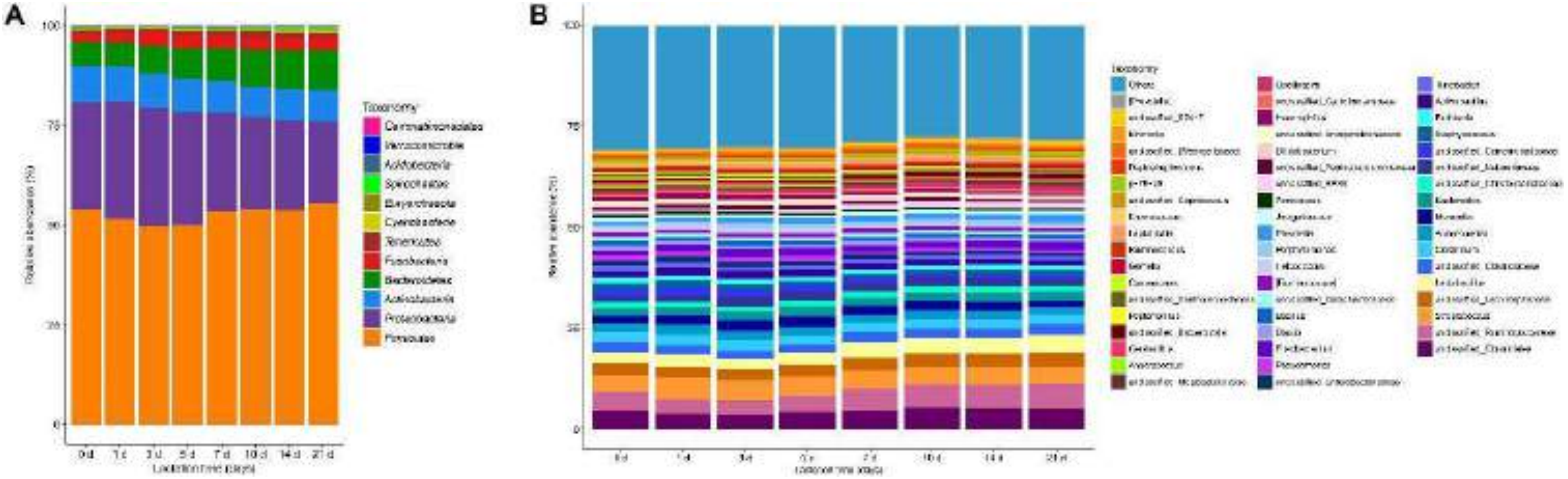
Sow’s colostrum and milk influences piglet’s microbiota

- Sows with post partum agalactia syndrome:
  - *Enterobacteriaceae*
  - Anaerobic bacterial genera associated with intestinal contents such as *Bacteroides, Blautia, Ruminococcus,* and *Bifidobacterium*

Kemper, Nicole. 2020. "Update on Postpartum Dysgalactia Syndrome in Sows." Journal of Animal Science 98 (Supplement_1): S117-25

Sow’s colostrum and milk influences piglet’s microbiota

Taxonomic composition of milk samples by Chen et al. in 2018 throughout lactation



Graph A: Abundance of milk microbiota composition.

Graph B, relative abundance of microbiota genera

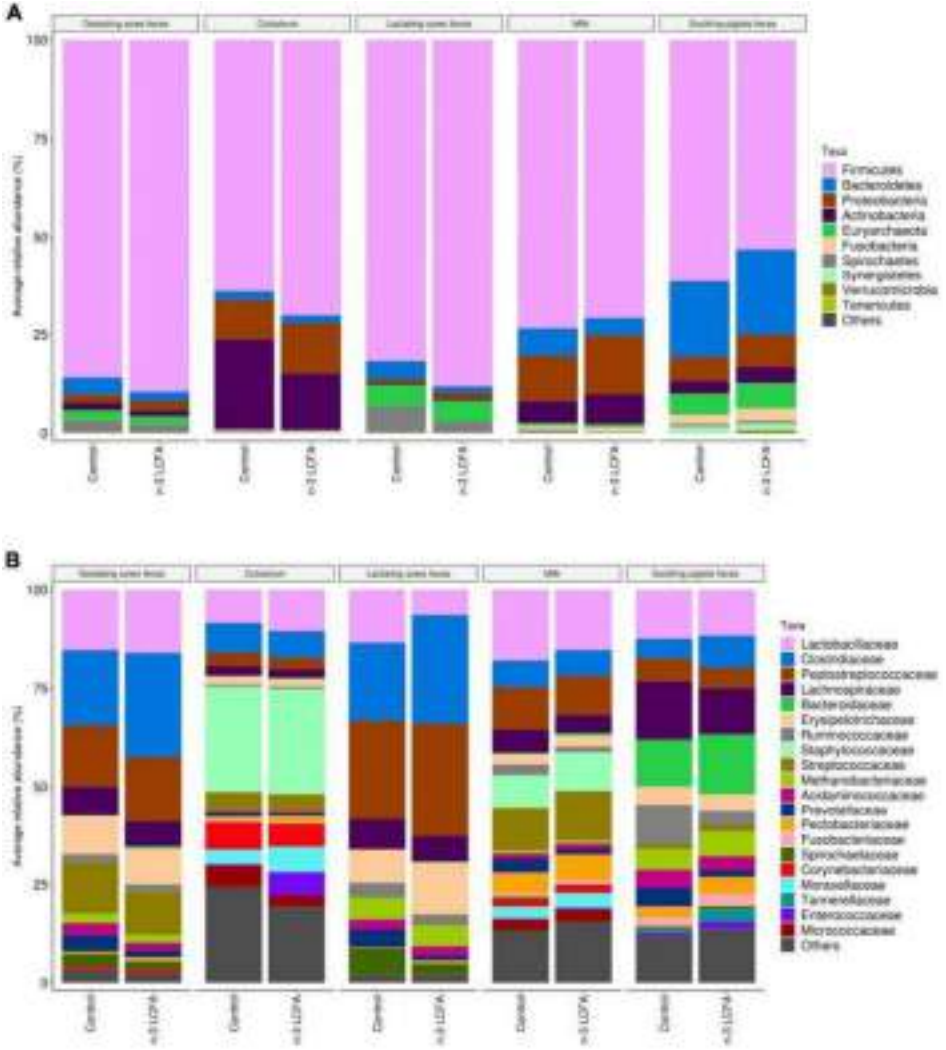
Chen, Wet al.. 2018. “Lactation Stage-Dependency of the Sow Milk Microbiota.” Frontiers in Microbiology 9.

Sow’s colostrum and milk influences piglet’s microbiota

Composition of the microbiota in relation to the abundance of

- phyla (A),
- family (B)

in the feces of pregnant and lactating sows, colostrum, milk and feces of piglets by treatment



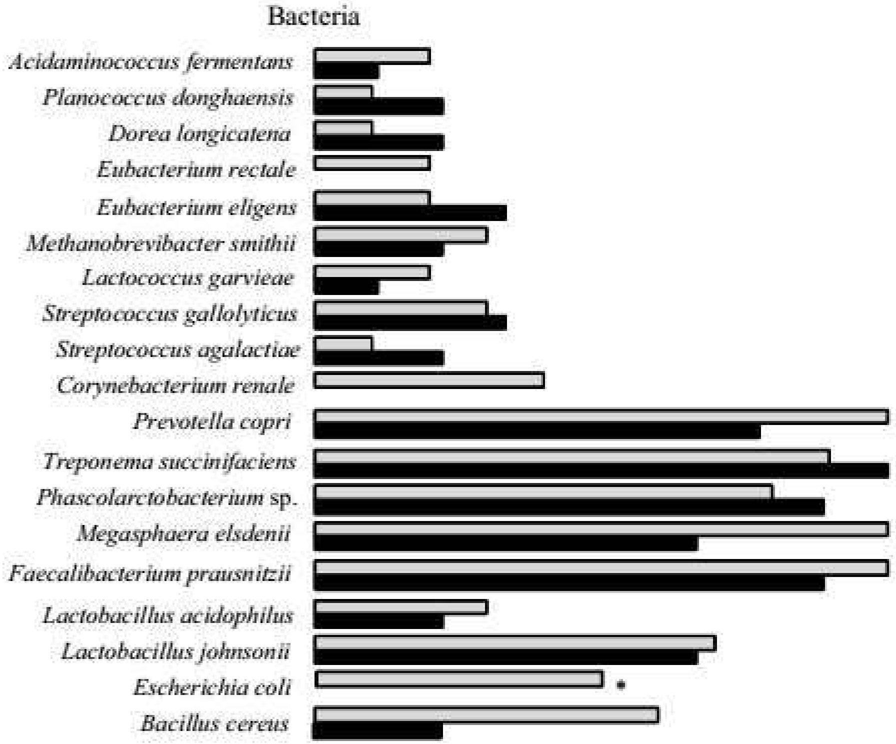
Llauradó-Calero, Eet al. . 2022. "Influence of dietary n-3 long-chain fatty acids on microbial diversity and composition of sows' feces, colostrum, milk, and suckling piglets' feces." Frontiers in Microbiology 13.

Diseases affects microbiota and *viceversa*

Previous microbiota affects the development of PRRSv and PCV2 infection

Pathogenic bacteria detected in the 20 pigs slaughtered in the study.

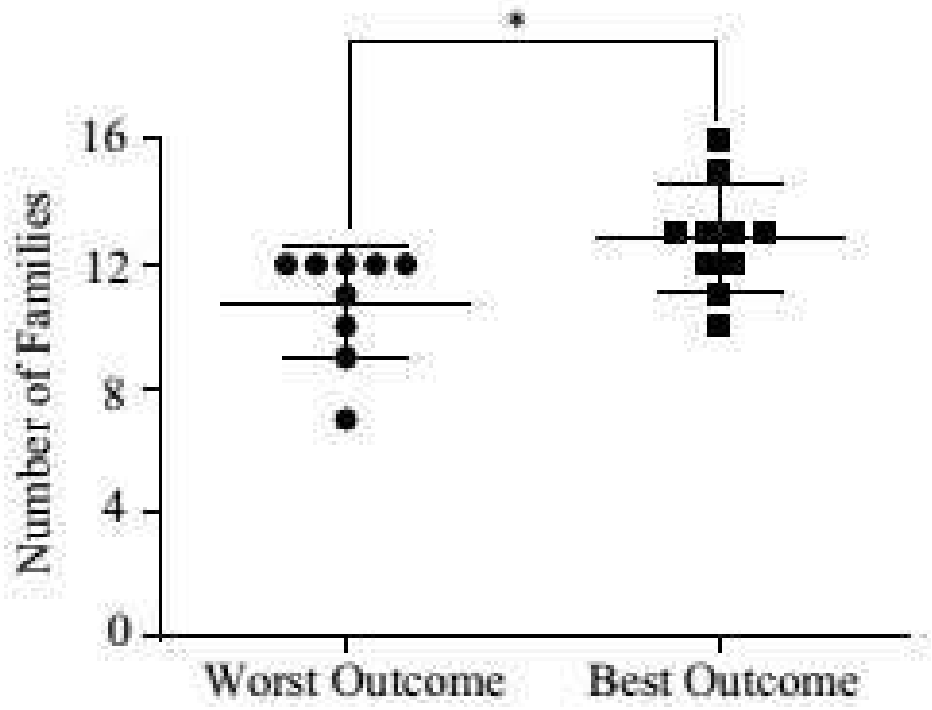
Piglets with the best ADG are shown in light color and the piglets with the worst ADG are shown in dark color (modified from Niederwerder et al. in 2016)



Niederwerder, M et al.. 2016. “Microbiome Associations in Pigs with the Best and Worst Clinical Outcomes Following Co-Infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Circovirus Type 2 (PCV2). Veterinary Microbiology 188 (May): 1-11.

Previous microbiota affects the development of PRRSv and PCV2 infection

Number of microbial families detected in the 10 best and 10 worst pigs in the study from Niederwerder et al. 2016.

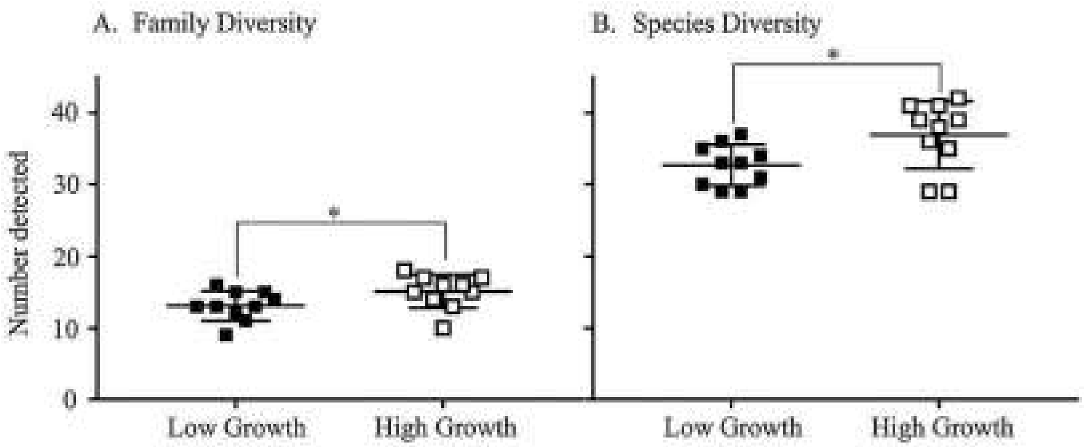


Niederwerder, M et al.. 2016. “Microbiome Associations in Pigs with the Best and Worst Clinical Outcomes Following Co-Infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Circovirus Type 2 (PCV2). Veterinary Microbiology 188 (May): 1-11.

Previous microbiota affects the development of PRRSv and PCV2 infection

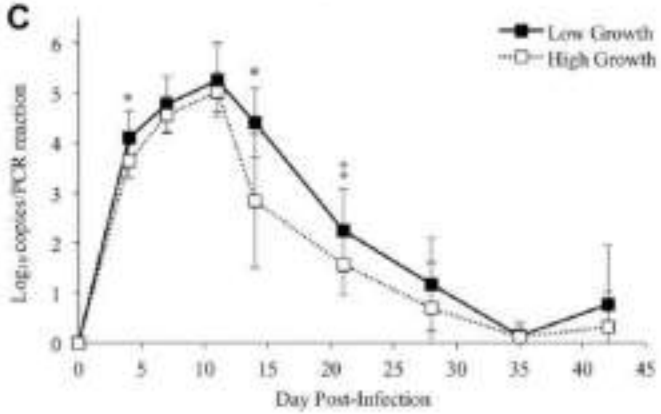
Microbiota diversity before infection in pigs with high and low ADG after coinfection with PRRSV and PCV2 (Mean and standard deviation).

A) Total number of microbial families
B) Number of microbial species before coinfection (taken from Ober et al., 2017)



Evolution of PRRS viremia of the two groups of piglets

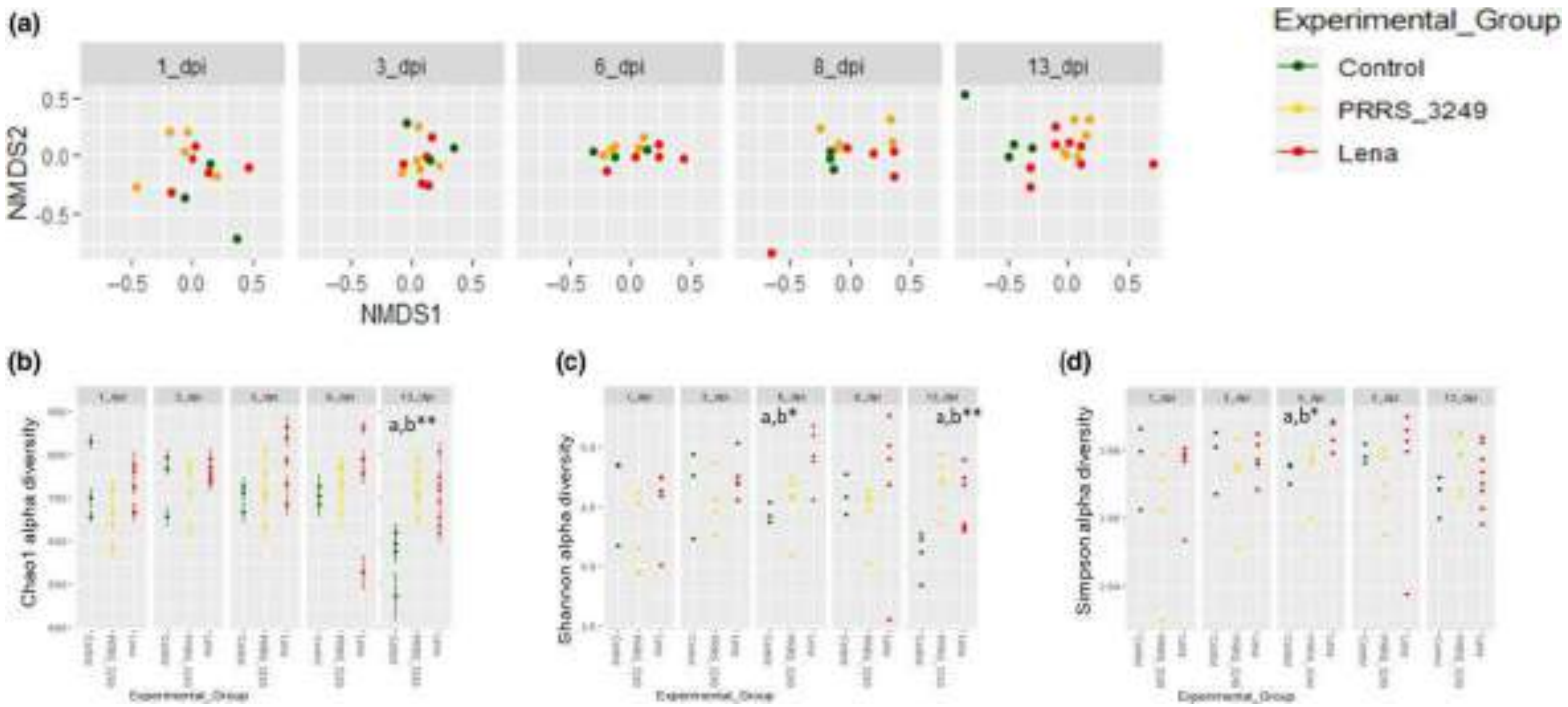
(mean ± standard deviation)
(taken from Ober et al., 2017).



Ober, R et al.. 2017. “Increased Microbiome Diversity at the Time of Infection Is Associated with Improved Growth Rates of Pigs after Co-Infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Circovirus Type 2 (PCV2).” Veterinary Microbiology 208 (September): 203-11.

PRRSv strain affects the development of the microbiota in piglets

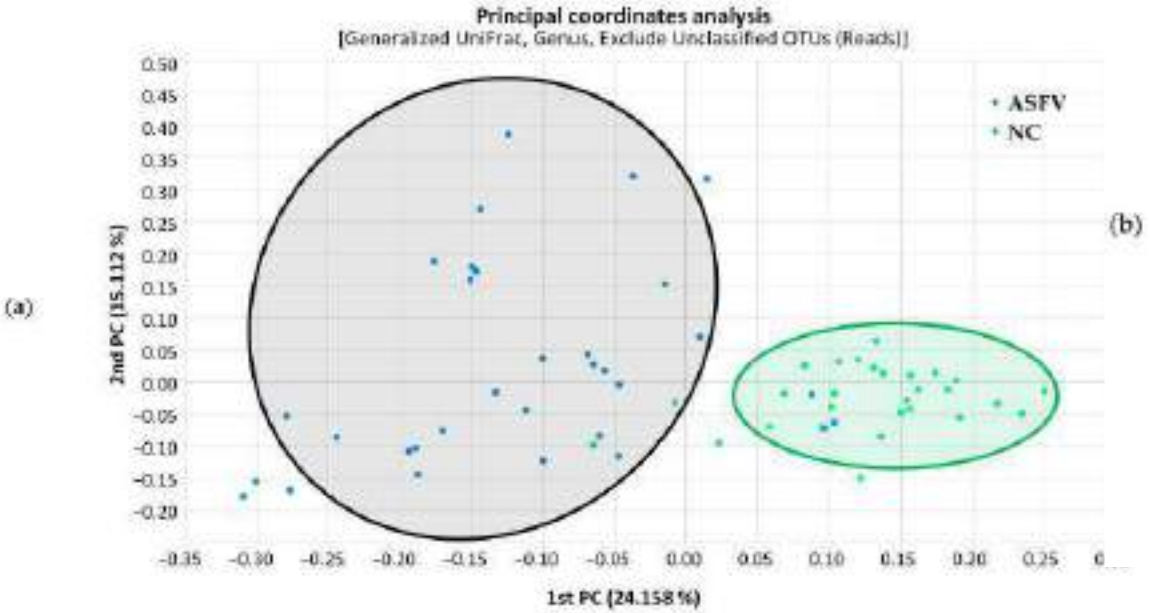
Diversity analysis in fecal samples from pigs infected with PRRSv in the study from Argüello et al. 2021.



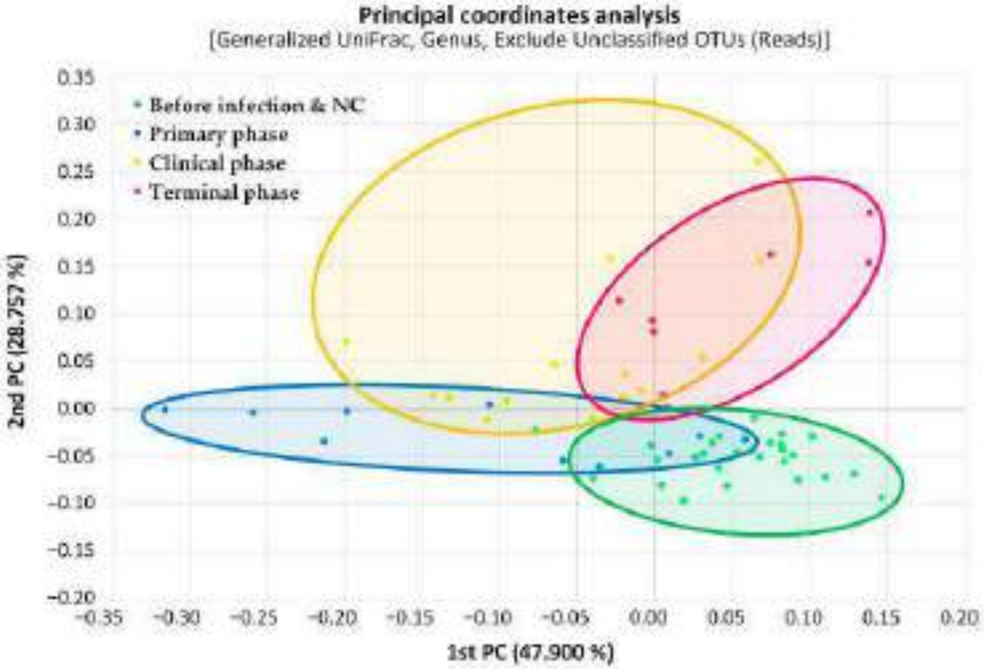
Argüello, H, et al. 2021. “Porcine reproductive and respiratory syndrome virus impacts on gut microbiome in a strain virulence-dependent fashion.” Microbial Biotechnology 15 (3): 1007-16.

ASFv affects the microbiota even in the different phases of the disease

Principal coordinate analysis of pigs infected with ASF virus. (Wang, et al., 2021)



Principal coordinate analysis of the microbiota of piglets infected by the ASFv in the different phases of the disease. (Wang, et al., 2021)



Wang, S, et al. 2021. “Cytokine Storm in Domestic Pigs Induced by Infection of Virulent African Swine Fever Virus.” Frontiers in Veterinary Science 7.

Conclusions

Conclusions

- Molecular technology, genetics and new statistical tools as well as big data, will help us to understand better how microbiota interacts with the digestive system to increase piglet productivity

- The knowledge of the composition of the ingredients will facilitate the understanding of digestive fermentations

- The manipulation of the immune system will help us to modulate the exacerbated response to challenges that we submit to the intestine

Definition of gut health

‘Gut health is a state of physical and mental well-being in the absence of gastrointestinal complaints that require the consultation of a doctor, in the absence of indications or risks of bowel disease, and in the absence of confirmed bowel disease’

www.who.int/governance/b/who_constitution_en.pdf

Definition of gut health

‘A steady state where the microbiome and the intestinal tract exist in symbiotic equilibrium and where the welfare and performance of the animal is not constrained by intestinal dysfunction’

Celi, P. et al. 2017, in “Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production” Animal Feed Science and Technology 234 (2017) 88-100

Thanks for your attention and questions

References

- 1. Lallès J-P, Boudry G, Favier C, Le Floc'h N, Luron I, Montagne L, et al. Gut function and dysfunction in young pigs: Physiology. Animal Research. 2004;53(4):301–16.
- 2. Moeser AJ, Klok CV, Ryan KA, Wooten JG, Little D, Cook VL, et al. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. American Journal of Physiology - Gastrointestinal and Liver Physiology. 2007;292(1):G173–G181.
- 3. Kraehenbuhl J-P, Corbett M. Keeping the Gut Microflora at Bay. Science. 2004 Mar 12;303(5664):1624–5.
- 4. Lallès JP. Basis and regulation of gut barrier function and epithelial cell protection: Applications to the weaned pig. Dynamics in Animal Nutrition. 2010. p. 31–51.
- 5. Ma TY, Anderson JM. Tight Junctions and the Intestinal Barrier. 2006. 1559-1594 p.
- 6. Otaka M, Odashima M, Watanabe S. Role of heat shock proteins (molecular chaperones) in intestinal mucosal protection. Biochemical and Biophysical Research Communications. 2006;348(1):1–5.
- 7. Cario E, Gerken G, Podolsky DK. Toll-Like Receptor 2 Controls Mucosal Inflammation by Regulating Epithelial Barrier Function. Gastroenterology. 2007;132(4):1359–74.
- 8. SABioscience. Toll-like Receptors and Innate Immunity. Pathways. Issue 7. 2008.
- 9. Reis de Souza TCR, Landín GM, García KE. Algunos factores fisiológicos y nutricionales que afectan la incidencia de diarreas posdestete en lechones Some physiological and nutritional factors affecting the incidence of post-weaning diarrhea. Vet Méx. 2010;41:4.
- 10. Pluske JR, Pethick DW, Hopwood DE, Hampson DJ. Nutritional influences on some major enteric bacterial diseases of pigs. Nutrition Research Reviews. 2002;15(2):333–71.

- 11. Leser TD, Amenuvor JZ, Jensen TK, Lindecrona RH, Boye M, Moøller K. Culture-independent analysis of gut bacteria: The pig gastrointestinal tract microbiota revisited. Applied and Environmental Microbiology. 2002;68(2):673–90.
- 12. Perez, JF, Nofrarías, M. Influencia de la nutricion sobre la patologia digestiva del lechon. XXIV Curso de especializacion Fedna. Madrid; 2008. p. 81–105.
- 13. McDonald DE, Pethick DW, Mullan BP, Pluske JR, Hampson DJ. Soluble non-starch polysaccharides from pearl barley exacerbate experimental post-weaning colibacillosis. Proceedings of the 8th Symposium on Digestive Physiology in Pigs. 2001;280–2.
- 14. McDonald DE, Pethick DW, Mullan BP, Hampson DJ. Increasing viscosity of the intestinal contents alters small intestinal structure and intestinal growth, and stimulates proliferation of enterotoxigenic Escherichia coli in newly-weaned pigs. British Journal of Nutrition. 2001;86(4):487–98.
- 15. Molist F, de Segura AG, Gasa J, Hermes RG, Manzanilla EG, Anguita M, et al. Effects of the insoluble and soluble dietary fibre on the physicochemical properties of digesta and the microbial activity in early weaned piglets. Animal Feed Science and Technology. 2009 Mar;149(3-4):346–53.
- 16. Honig DH, Rackis JJ. Determination of the total pepsin-pancreatin indigestible content (dietary fiber) of soybean products, wheat bran, and corn bran. J Agric Food Chem. 1979 Nov 1;27(6):1262–6.
- 17. Nyachoti CM, Omogbenigun FO, Rademacher M, Blank G. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. Journal of Animal Science. 2006;84(1):125–34.
- 18. Pierce KM, Callan JJ, McCarthy P, O'Doherty JV. The interaction between lactose level and crude protein concentration on piglet post-weaning performance, nitrogen metabolism, selected faecal microbial populations and faecal volatile fatty acid concentrations. Animal Feed Science and Technology. 2007 Enero;132(3–4):267–82.
- 19. Finamore A, Massimi M, Devirgiliis LC, Mengheri E. Zinc deficiency induces membrane barrier damage and increases neutrophil transmigration in Caco-2 cells. Journal of Nutrition. 2008;138(9):1664–70.
- 20. Davin R, Manzanilla E g., Klasing K c., Pérez J f. Effect of weaning and in-feed high doses of zinc oxide on zinc levels in different body compartments of piglets. J Anim Physiol Anim Nutr. 2013 May 1;97:6–12.

- 21. Liu P, Pieper R, Rieger J, Vahjen W, Davin R, Plendl J, et al. Effect of Dietary Zinc Oxide on Morphological Characteristics, Mucin Composition and Gene Expression in the Colon of Weaned Piglets. PLoS ONE. 2014 Mar 7;9(3):e91091.
- 22. Schroeder B, Duncker S, Barth S, Bauerfeind R, Gruber AD, Deppenmeier S, et al. Preventive effects of the probiotic Escherichia coli strain Nissle 1917 on acute secretory diarrhea in a pig model of intestinal infection. Digestive Diseases and Sciences. 2006;51(4):724–31.
- 23. Kleta S, Steinrück H, Breves G, Duncker S, Laturnus C, Wieler LH, et al. Detection and distribution of probiotic Escherichia coli Nissle 1917 clones in swine herds in Germany. Journal of Applied Microbiology. 2006;101(6):1357–66.
- 24. Duncker SC, Lorentz A, Schroeder B, Breves G, Bischoff SC. Effect of orally administered probiotic E. coli strain Nissle 1917 on intestinal mucosal immune cells of healthy young pigs. Veterinary Immunology and Immunopathology. 2006;111(3-4):239–50.
- 25. Barth S, Duncker S, Hempe J, Breves G, Baljer G, Bauerfeind R. Escherichia coli Nissle 1917 for probiotic use in piglets: Evidence for intestinal colonization. Journal of Applied Microbiology. 2009;107(5):1697–710.
- 26. Trebichavsky I, Splichal I, Rada V, Splichalova A. Modulation of natural immunity in the gut by Escherichia coli strain Nissle 1917. Nutrition Reviews. 2010;68(8):459–64.
- 27. Roselli M, Finamore A, Britti MS, Konstantinov SR, Smidt H, De Vos WM, et al. The novel porcine Lactobacillus sobrius strain protects intestinal cells from enterotoxigenic Escherichia coli K88 infection and prevents membrane barrier damage. Journal of Nutrition. 2007;137(12):2709–16.
- 28. Siggers RH, Siggers J, Boye M, Thymann T, Mølbak L, Leser T, et al. Early administration of probiotics alters bacterial colonization and limits diet-induced gut dysfunction and severity of necrotizing enterocolitis in preterm pigs. Journal of Nutrition. 2008;138(8):1437–44.
- 29. Konstantinov SR, Smidt H, Akkermans ADL, Casini L, Trevisi P, Mazzoni M, et al. Feeding of Lactobacillus sobrius reduces Escherichia coli F4 levels in the gut and promotes growth of infected piglets. FEMS Microbiology Ecology. 2008;66(3):599–607.
- 30. Moran CA, Ward G, Beal JD, Campbell A, Brooks PH, Miller BG. Influence of liquid feed, fermented liquid feed, dry feed and sow’s milk fed ad libitum, on the “ecophysiology” of the terminal ileum, caecum and colon of the postweaned pig. Proceedings of the 8th Symposium on Digestive Physiology in Pigs. 2001;266–8.

- 31. Slide 4. Castillo, M. S. Development of gut microbiota in the pig: modulation of bacterial communities by different feeding strategies. phD Dissertation. 2006
- 31. Slide 17 Tight Junctions. https://www.qiagen.com/au/shop/genes-and-pathways/pathway-details/?pwid=157
- 32, Celi, P., A.J. Cowieson, F. Fru-Nji, R.E. Steinert, A.-M. Kluenter, y V. Verlhac. 2017. «Gastrointestinal Functionality in Animal Nutrition and Health: New Opportunities for Sustainable Animal Production». *Animal Feed Science and Technology* 234 (diciembre): 88-100.
- 33, Holman, Devin B., Brian W. Brunelle, Julian Trachsel, y Heather K. Allen. 2017. «Meta-Analysis To Define a Core Microbiota in the Swine Gut». MSystems 2 (3): e00004-17.
- 34, Bischoff, S.C. 2011. «"Gut health": A new objective in medicine?» BMC Medicine 9.
- 35, Pluske, John R., Diana L. Turpin, y Jae-Cheol Kim. 2018. «Gastrointestinal tract (gut) health in the young pig». Animal Nutrition 4 (2): 187-96
- 36, Dou, S., P. Gadonna-Widehem, V. Rome, D. Hamoudi, L. Rhazi, L. Lakhal, T. Larcher, et al. 2017. «Characterisation of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhoea». *PloS one* 12 (1): e0169851.
- 37, Figueroa, Jaime, David Solà-Oriol, Xavier Manteca, y José Francisco Pérez. 2013. «Social learning of feeding behaviour in pigs: Effects of neophobia and familiarity with the demonstrator conspecific». *Applied Animal Behaviour Science* 148 (1–2): 120-27.
- 38, Molist, F., M. van Oostrum, J. F. Pérez, G. G. Mateos, C. M. Nyachoti, y P. J. van der Aar. 2014. «Relevance of functional properties of dietary fibre in diets for weanling pigs». Animal Feed Science and Technology 189 (marzo): 1-10
- 39. Montagne, L., N. Le Floc'h, M. Arturo-Schaan, R. Foret, M.C. Urdaci, y M. Le Gall. 2012. «Comparative effects of level of dietary fiber and sanitary conditions on the growth and health of weanling pigs». Journal of Animal Science 90 (8): 2556-69.
- 40., Montagne, L., F.S. Cavaney, D.J. Hampson, J.P. Lallès, y J.R. Pluske. 2004. «Effect of diet composition on postweaning colibacillosis in piglets». *Journal of Animal Science* 82 (8): 2364-74.

- 41. Yu, Ting, Cui Zhu, Shicheng Chen, Lei Gao, Hang Lv, Ruowei Feng, Qingfeng Zhu, Jinsong Xu, Zhuang Chen, y Zongyong Jiang. 2017. «Dietary High Zinc Oxide Modulates the Microbiome of Ileum and Colon in Weaned Piglets». Frontiers in Microbiology 8. 42.
- 42, Mei, Shao-Feng, Bing Yu, Cui-Fang Ju, Dan Zhu, y Dai-Wen Chen. 2010. «Effect of different levels of copper on growth performance and cecal ecosystem of newly weaned piglets». Italian Journal of Animal Science 9 (4): e71.
- 43. Liu, Yanhong, Charmaine D. Espinosa, Jerubella J. Abelilla, Gloria A. Casas, L. Vanessa Lagos, Su A. Lee, Woong B. Kwon, et al. 2018. «Non-antibiotic feed additives in diets for pigs: A review». Animal Nutrition 4 (2): 113-25.
- 44. Pearce, S. C., M.-V. Sanz Fernandez, J. Torrison, M. E. Wilson, L. H. Baumgard, y N. K. Gabler. 2015. «Dietary Organic Zinc Attenuates Heat Stress–Induced Changes in Pig Intestinal Integrity and Metabolism». Journal of Animal Science 93 (10): 4702-13.
- 45. Zhu, Cui, Hang Lv, Zhuang Chen, Li Wang, Xiuju Wu, Zhongjian Chen, Weina Zhang, Rui Liang, y Zongyong Jiang. 2017. «Dietary Zinc Oxide Modulates Antioxidant Capacity, Small Intestine Development, and Jejunal Gene Expression in Weaned Piglets». Biological Tracelement Research 175 (2): 331-38.
- 46. Celi, Pietro, Viviane Verlhac, Estefania Pérez Calvo, Jerome Schmeisser, y Anna-Maria Kluenter. 2018. «Biomarkers of gastrointestinal functionality in animal nutrition and health». *Animal Feed Science and Technology*, julio.
- 47. Sales, James. 2013. «Effects of Pharmacological Concentrations of Dietary Zinc Oxide on Growth of Post-Weaning Pigs: A Meta-Analysis». *Biological Trace Element Research* 152 (3): 343-49.
- 48. Blavi, L., D. Sola-Oriol, J. F. Perez, y H. H. Stein. 2017. «Effects of Zinc Oxide and Microbial Phytase on Digestibility of Calcium and Phosphorus in Maize-Based Diets Fed to Growing Pigs». *Journal of Animal Science* 95 (2): 847
- 49. Walk, C. L., P. Wilcock, y E. Magowan. 2015. «Evaluation of the Effects of Pharmacological Zinc Oxide and Phosphorus Source on Weaned Piglet Growth Performance, Plasma Minerals and Mineral Digestibility». *Animal* 9 (07): 1145-52.
- 50. Bruininx, E.M.A.M., et al., 2002. «Effect of creep feed consumption on individual feed intake characteristics and performance of group-housed weanling pigs». Journal of Animal Science 80 (6): 1413-18
- 51 Ducatelle, Richard, Evy Goossens, Fien De Meyer, Venessa Eeckhaut, Gunther Antonissen, Freddy Haesebrouck, y Filip Van Immerseel. 2018. «Biomarkers for monitoring intestinal health in poultry: present status and future perspectives». *Veterinary Research* 49 (1): 43.
- 52 Apajalahti, J., y K. Vienola. 2016. «Interaction between chicken intestinal microbiota and protein digestion». *Animal Feed Science and Technology*, Protein nutrition in non-ruminants and the implications for exogenous protease use, 221 (noviembre): 323-30.