An international, cross-functional and interdisciplinary AMR project

Preventing Infection in the Gut of developing Piglets – and thus Antimicrobial Resistance – by disentAngling the interface of DIet, the host and the Gastrointestinal Microbiome

PIG-PARADIGM

Pre-Symposium Session

THINK Piglet Health & Nutrition 2023

21-09-2023

PIG-PARADIGM at a glance

Project Sponsor

novo nordisk foundation

Research Institutions

AARHUS UNIVERSITY

UNIVERSITY OF COPENHAGEN

AALBORG UNIVERSITY

UCDAVIS UNIVERSITY OF CALIFORNIA

WAGENINGEN UNIVERSITY & RESEARCH

Industrial Partners

LANDBRUG FØDEVARER

Topigs Norsvin

novozymes

CHR. HANSEN Improving food & health

DSM

frouw nutrition a Nutreco company

Research Funding: DKK 150 million (€20.1 million)

Research Period: 2022 – 2026

Our Vision

Ultimate goal: To keep antimicrobials effective in 30 years from now, for both human and animals.

Mission

The mission of PIG-PARADIGM is to deliver fundamental science required to form solutions to reduce the overall need for antimicrobial treatments and mitigate the spread of AMR by researching the following questions:

- What defines healthy and robust intestinal function in pigs?
- What determines the host and microbial mechanisms leading to post weaning diarrhea (PWD) and subsequent antibiotic use?
- How can the intestinal microbiome and nutrition be modulated to prevent the need for antibiotic use by promoting resilience to early life stress and intestinal infections?
- How AMR can be minimized through increased intestinal resilience?

Impacts

PIG-PARADIGM works towards bringing long-lasting impact across multiple dimensions:

- Reducing the antibiotic use in livestock production, with a specific focus on pigs
- Mitigating the spread of antimicrobial resistance within livestock production
- Promoting One Health in animal science and veterinary education
- Elevating awareness regarding the challenge of antimicrobial resistance
- Advocating and promoting international cooperation to curtail antibiotic usage in livestock production

Project organization structure overview

Industrial Advisory Board

- Novozymes
- Danish Agriculture & Food Councils
- Chr. Hansen
- DSM Nutritional Products
- Trouw Nutrition
- Topics Norsvin

Project Sponsor

novo nordisk foundation

Steering Committee
project ultimate decision-making body

Project Management Support
daily project management, communication and outreach

Charlotte Lauridesen
PI, Chair of Steering Committee

Scientific Advisory Board

Anders Dalsgaard
Michael Bailey
Jürgen Zentek
Michel Georges
Sofia Forslund

Maria Marco
Co-PI, Chair of Nutrition Pillar

Merete Fredholm
Co-PI, Chair of Host Pillar

Hauke Smidt
Co-PI, Chair of Microbiome Pillar

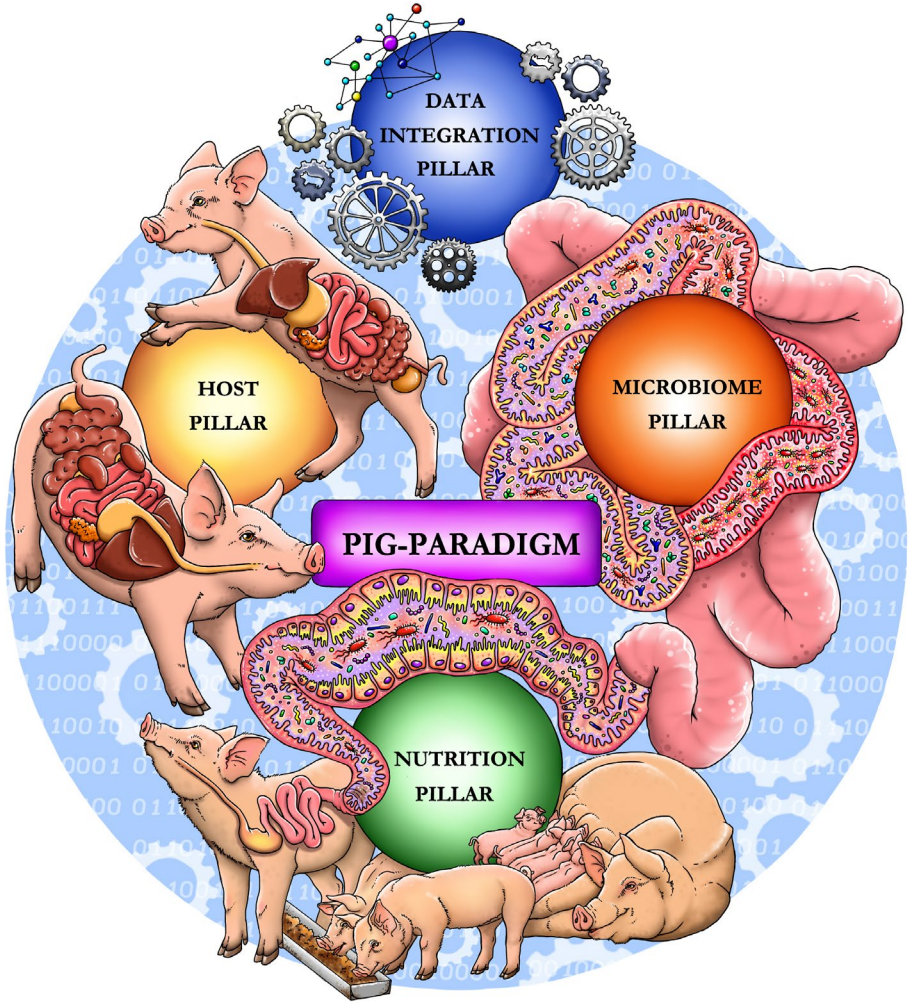
Mani Arumugan
Co-PI, Chair of Data Integration Pillar

Lasse Sommer Mikkelsen
Scientific Project Manager

Xi Song
Communication Officer

Pillar Leadership Committee
pillar decision-making body; cross-pillar coordination

Our Research

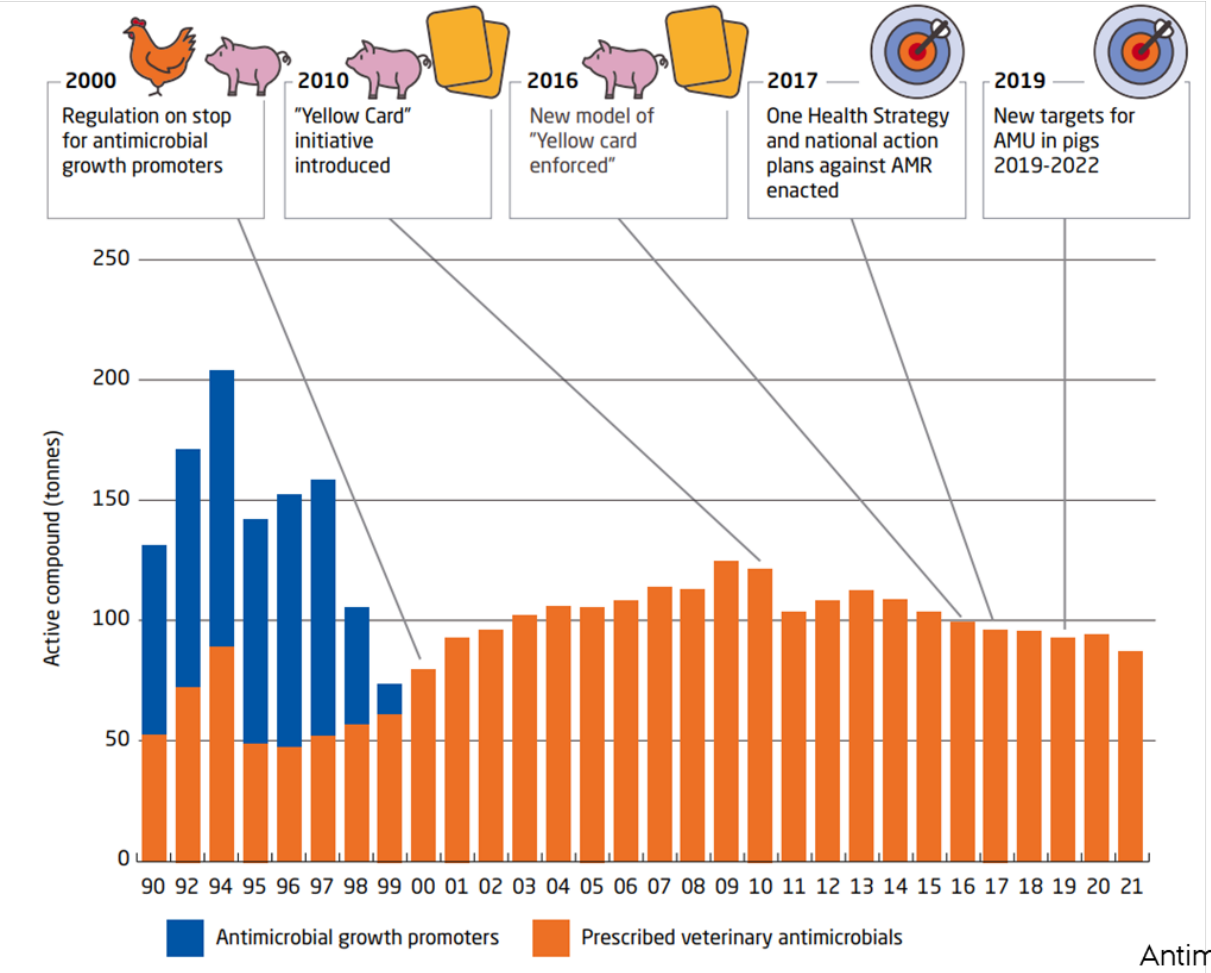


PIG-PARADIGM is organized into four research pillars, each of these pillars is led by dedicated scientists from esteemed institutions including Aarhus University, University of Copenhagen, Aalborg University, Wageningen University & Research, and University of California, Davis.

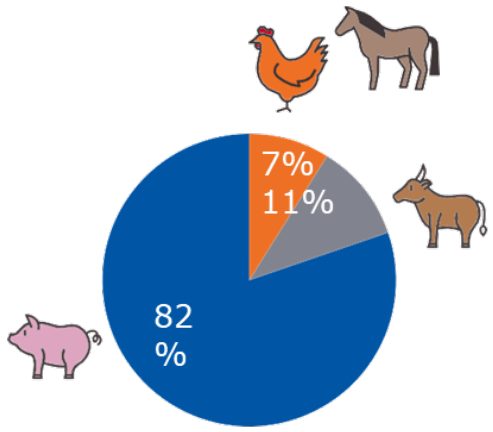
- **Host & Microbiome Pillar**: a large cohort study with pigs in a commercial production system to identify key attributes of robust pig gut health
- **Nutrition Pillar**: fundamental and practical knowledge of the pig diet and nutritional interventions to reduce the need for antibiotic use
- **Data Integration Pillar**: amalgamates all generated data from the other three pillars

Antimicrobial consumptions in animals Denmark

1990 - 2021

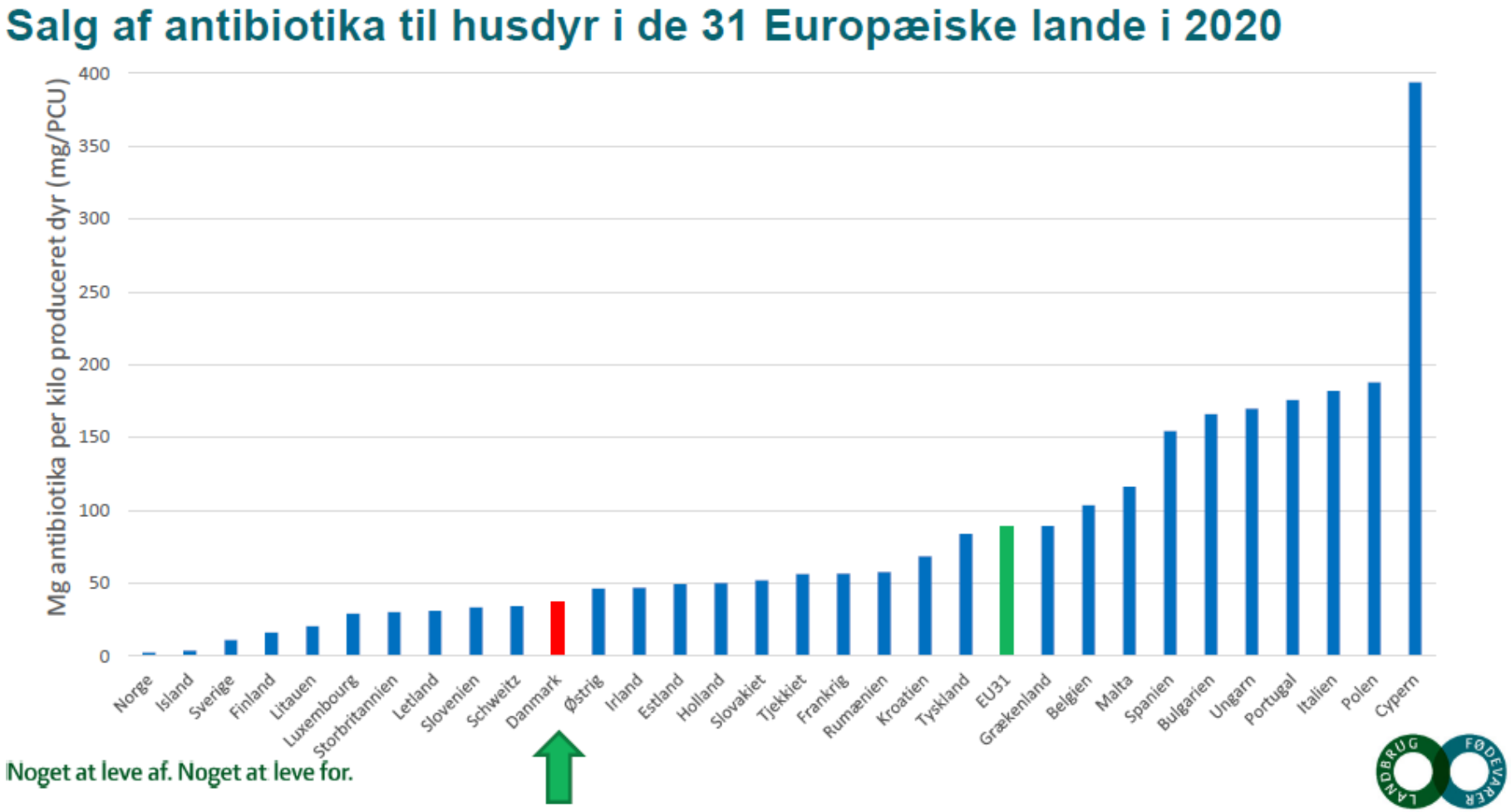


Important initiatives to reduce antimicrobial use in animals



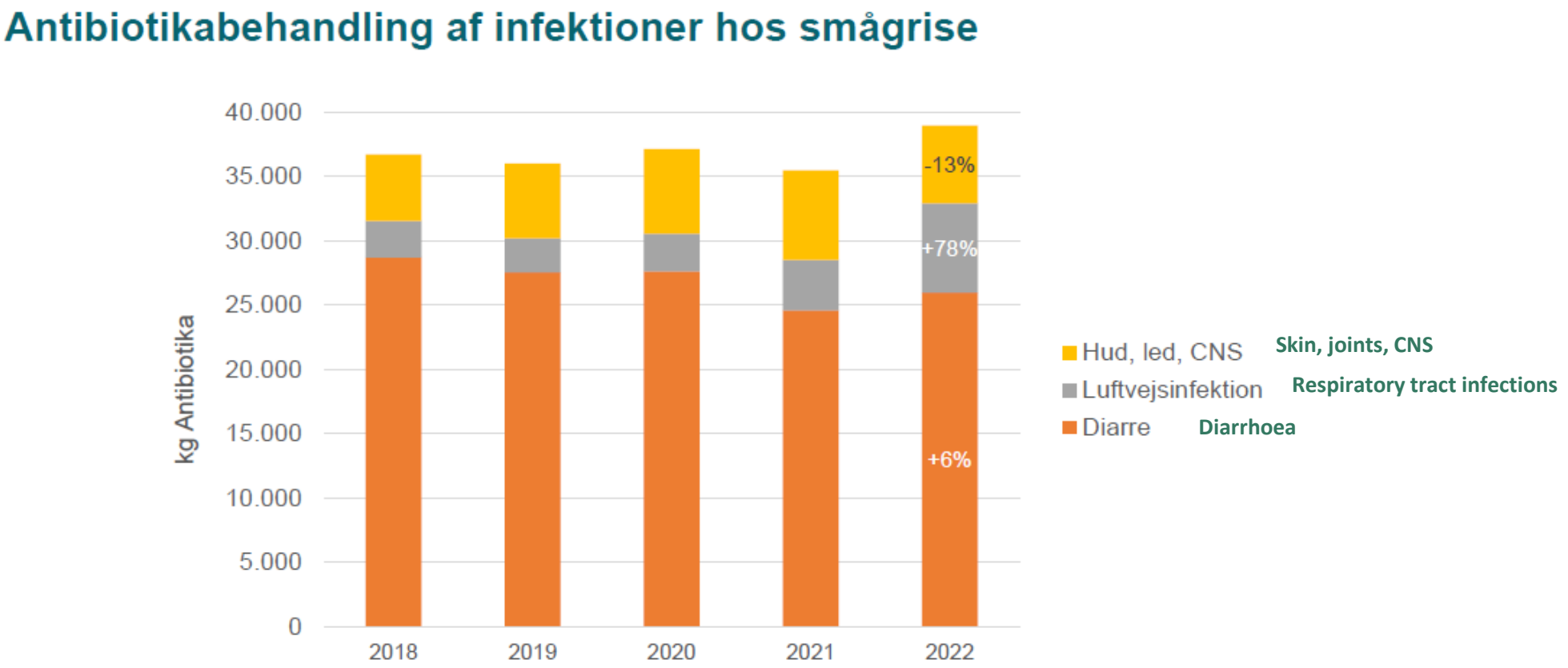
Antimicrobial use in main animal species

Antibiotics sale for livestock in 31 EU countries in 2020



Source: Christian Fink Hansen, Strategy seminar (26. January, 2023, Copenhagen) of the Pig Levy Foundation

Antibiotic treatment of infections in Danish piglets



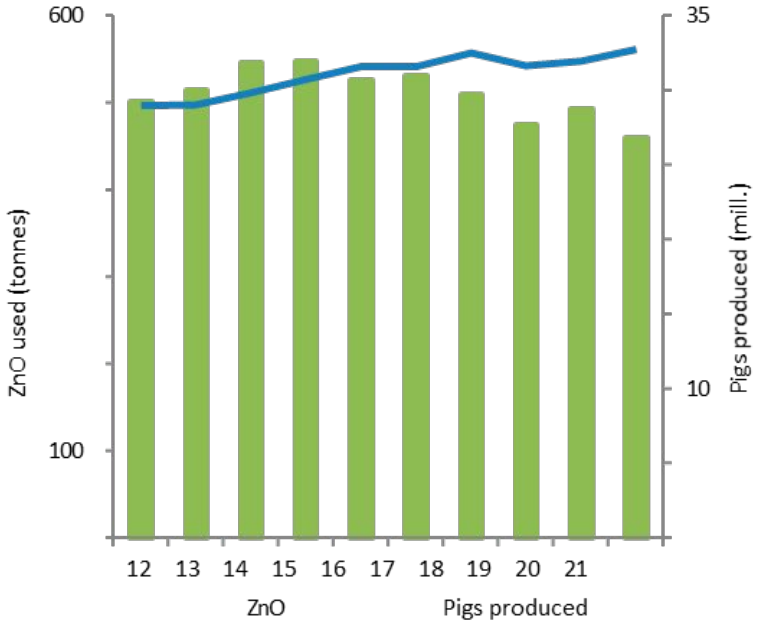
Noget at leve af. Noget at leve for.

Source: Christian Fink Hansen, Strategy seminar (26. January, 2023, Copenhagen) of the Pig Levy Foundation

Alternatives to antibiotics

Medical ZnO to treat diarrhea in pigs – EU-ban in June 2022

Figure 4.5 Usage (in tonnes) of medical zinc - zinc oxide (ZnO) and zinc (Zn) - in the pig production, Denmark DANMAP 2021

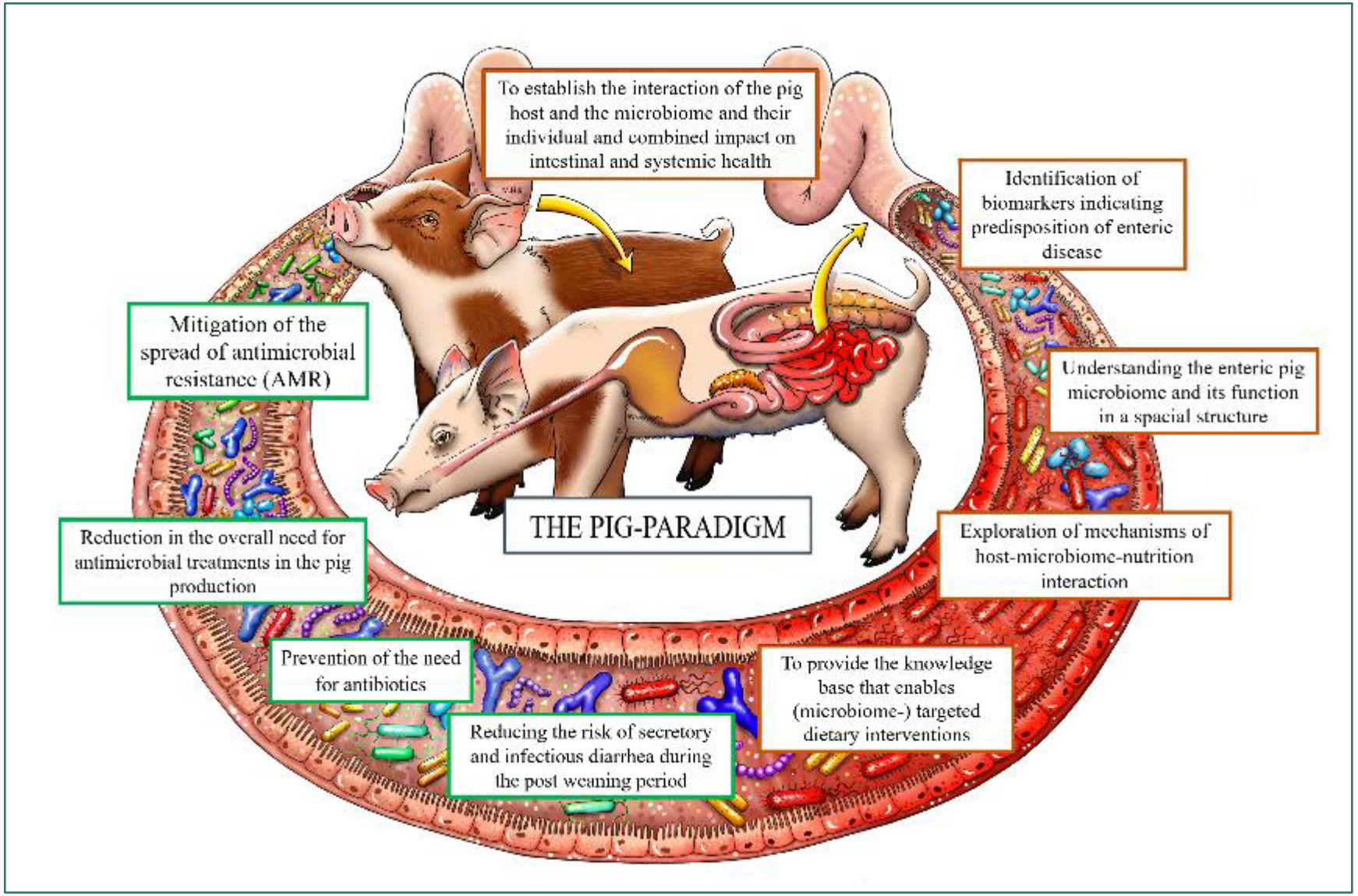


Note: The most commonly used product is zinc oxide (ZnO) which contains 80% zinc and which is largely insoluble in water

Medical zinc up to 2,500 ppm: ban

because of risk in AMR

Objectives and impacts of PIG-PARADIGM



Why are industrial stakeholders important to PIG-PARADIGM?

Expected results of our project

- Understanding the interactions between the nutrition, microbiome and host influencing the systemic health and intestinal resilience to reduce diarrheal disease incidence
- Methods for characterization and diagnostics of a healthy and diseased gut
- Methods for prognostic microbiota signature and prediction of robustness
- Disease management and treatment schemes to reduce antibiotics are developed

The Industry translates the knowledge on how to obtain robust pigs etc. into disease management with minimal use of antibiotics

Agenda for today

8:30 – 9:45 Presentations*5 (10 min presentation + 5 min Q&A)

9:45 – 10:00 General discussion

10:00 – 10:15 Networking

Why is pig production a major consumer of antibiotics? - **Jens Peter Nielsen (KU)**

The Host Pillar: current activities and aim of the studies - **Merete Fredholm (KU)**

The contradictionary effects of fibre in animal nutrition and health - **Knud Erik Bach Knudsen (AU)**

Focus on gut microbial metabolites in the weaning period - **Nuria Canibe (AU)**

Why is pig production a major consumer of antibiotics?

Jens Peter Nielsen

Professor of Pig Health
Department of Veterinary and Animal Science
Faculty of Health and Medical Sciences
University of Copenhagen

jpni@sund.ku.dk

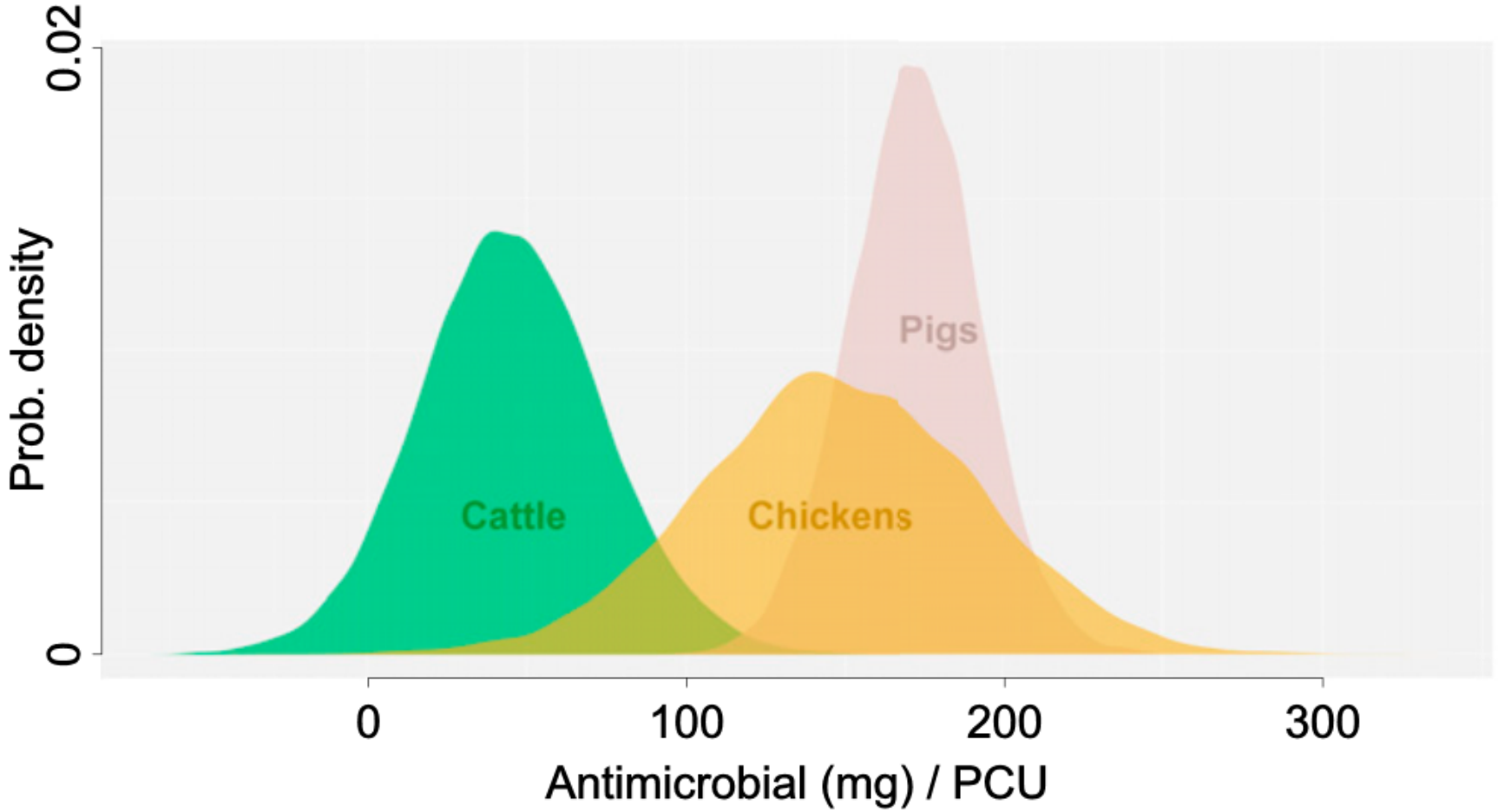


Fig. 2. Posterior distributions for estimates of antimicrobial consumption in cattle, chickens, and pigs in OECD countries.

Boeckel, PNAS, 2015

Pigs, Denmark, 42 mg/PCU

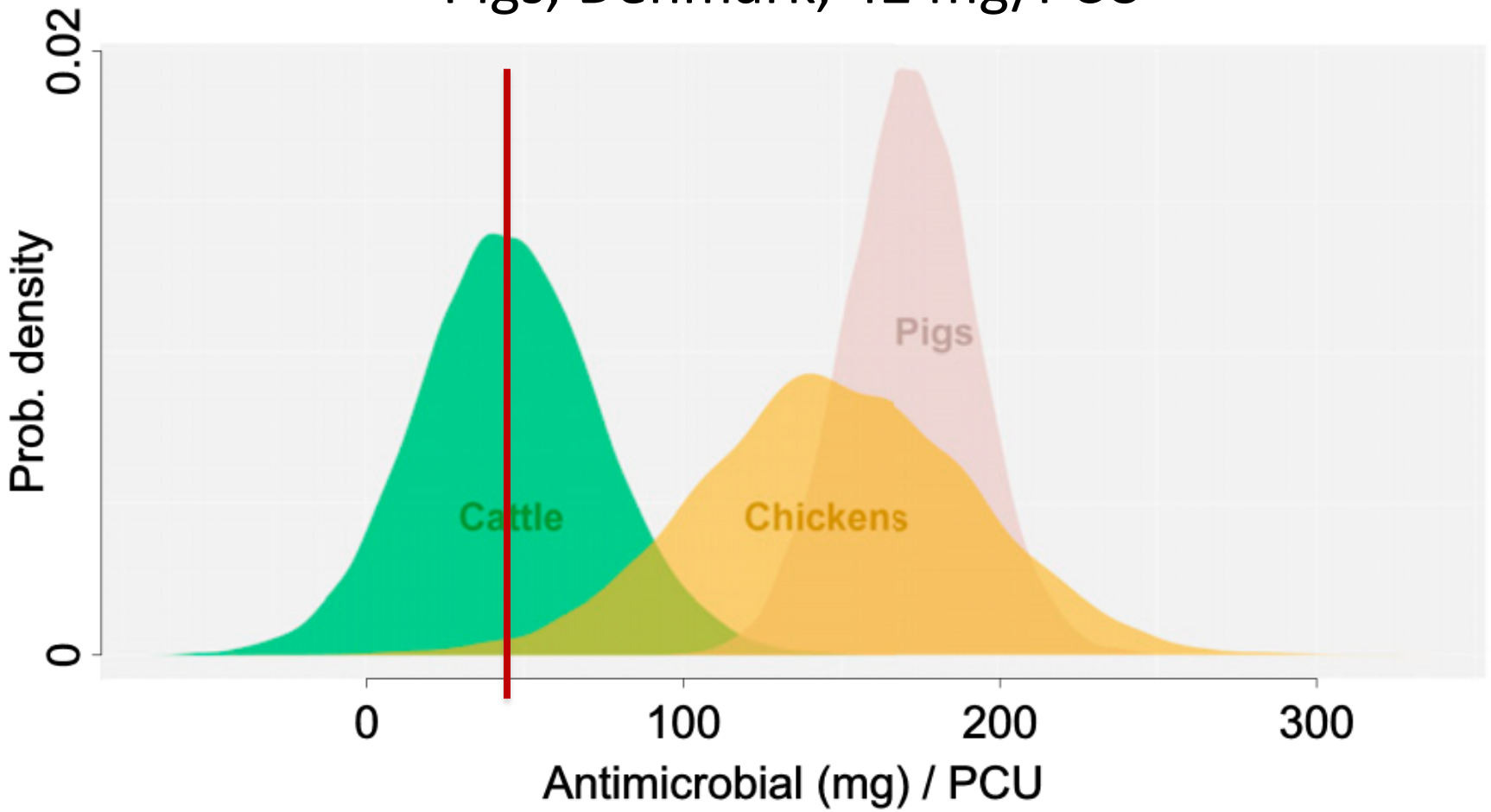
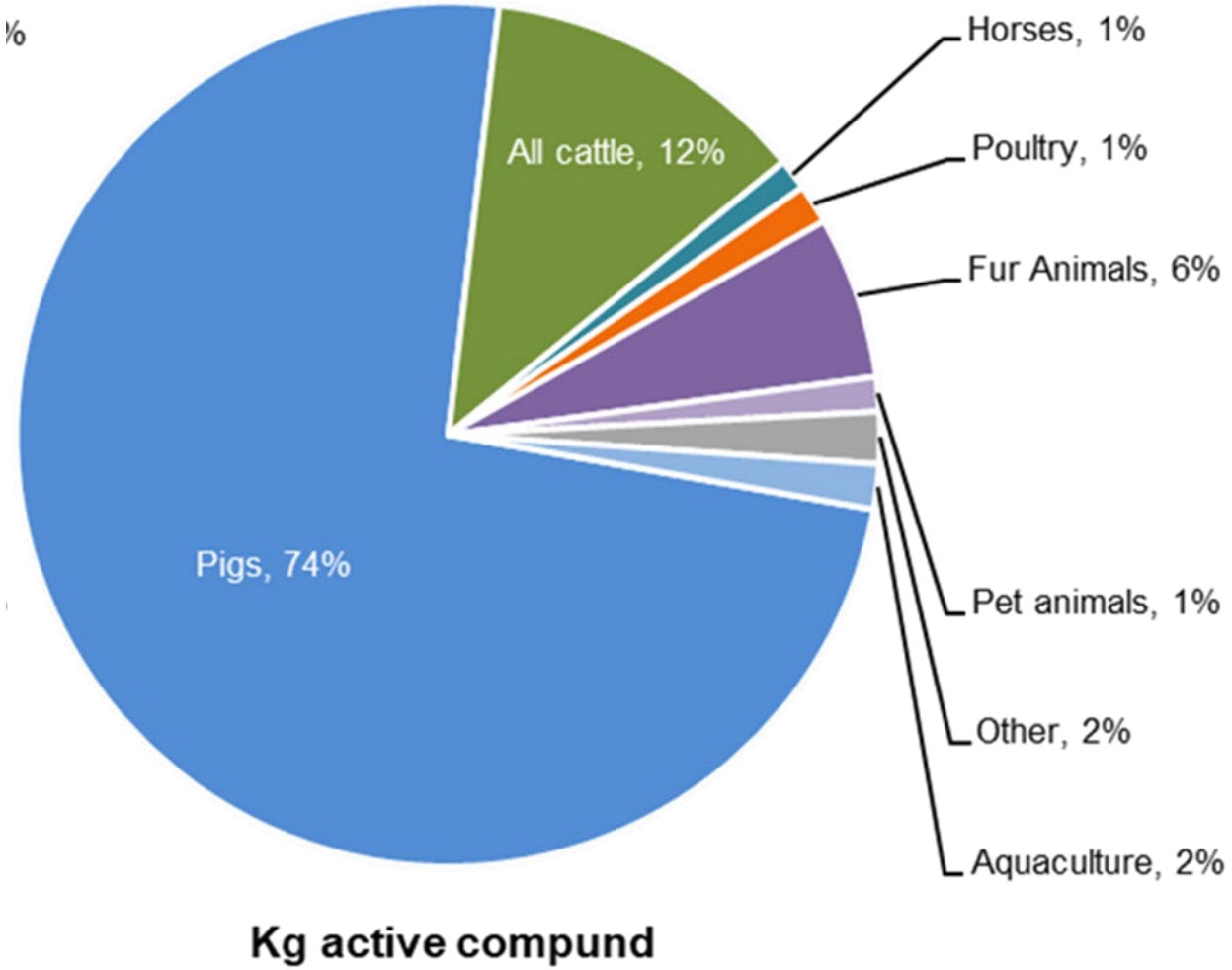


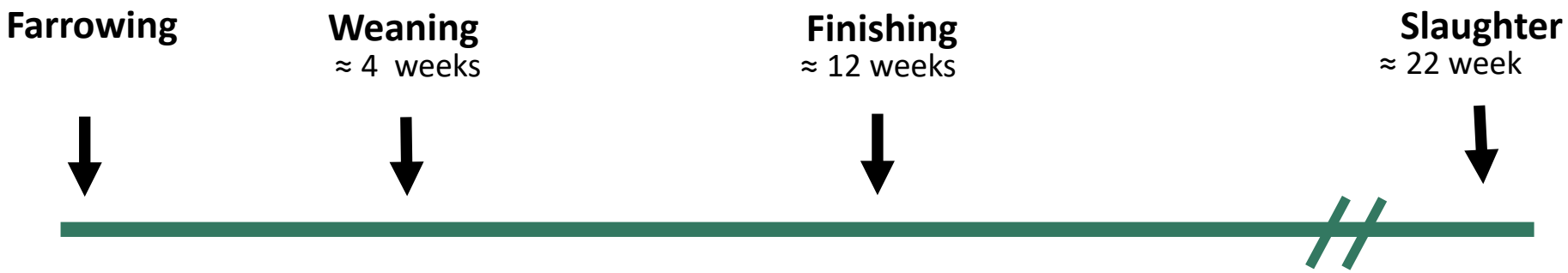
Fig. 2. Posterior distributions for estimates of antimicrobial consumption in cattle, chickens, and pigs in OECD countries.

Boeckel, PNAS, 2015

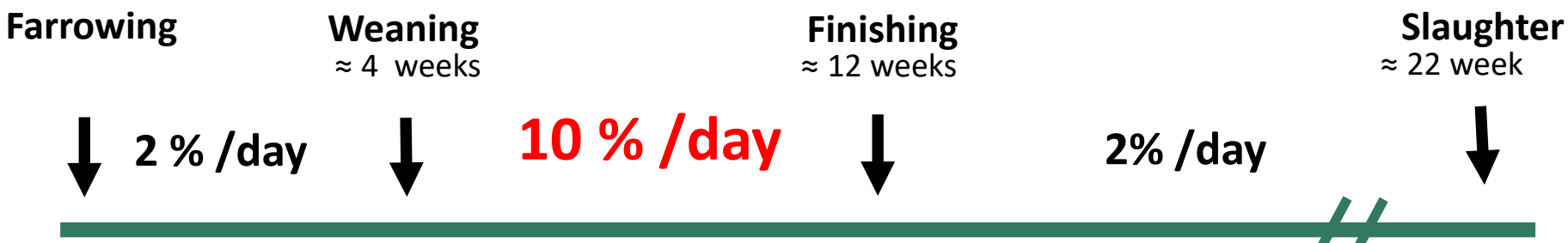
Antimicrobial consumption (kg) in main animal species, DANMAP 2017

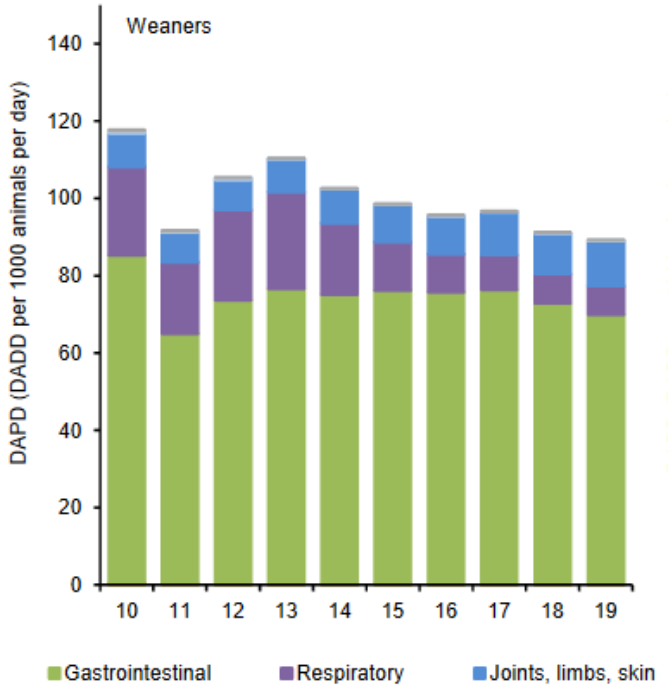


The lifetime of a pig

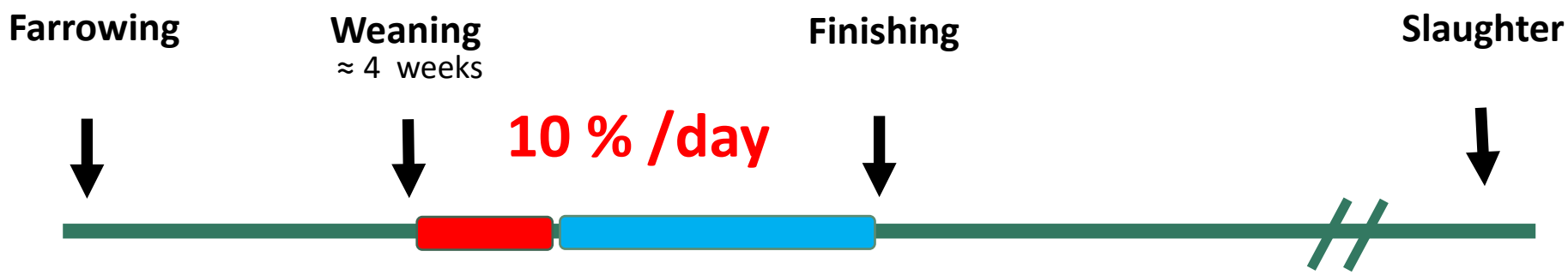


Diarrhoea/enteritis in the nursery period (7-30 kg, 4-12 weeks)

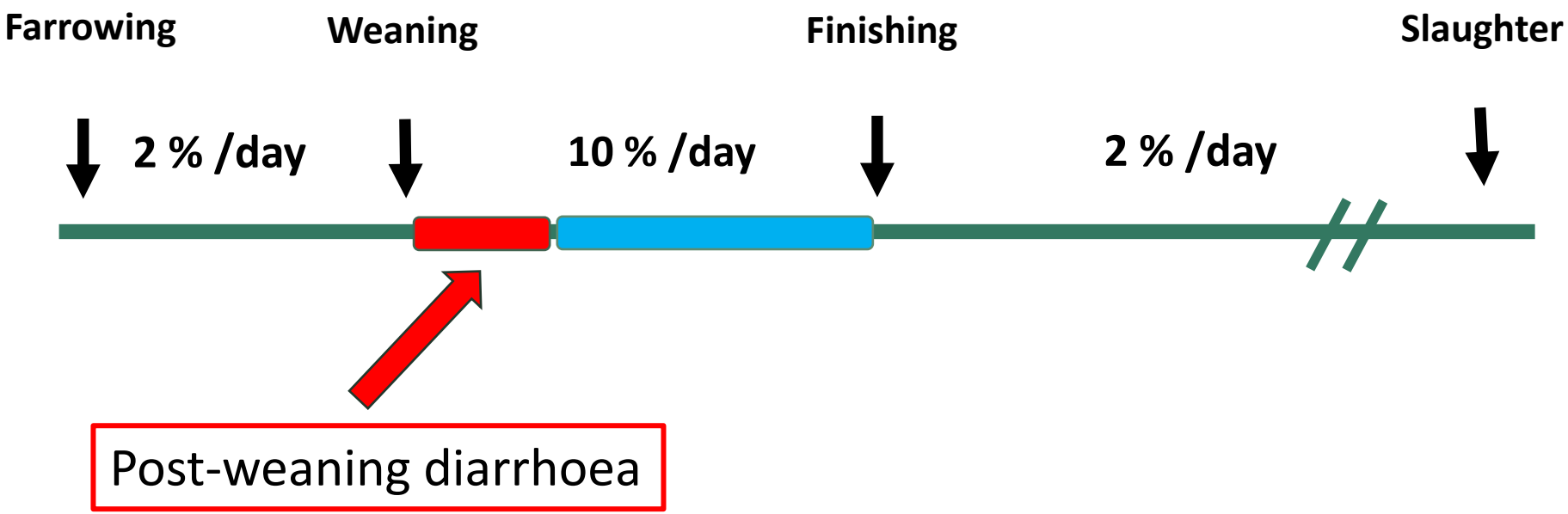


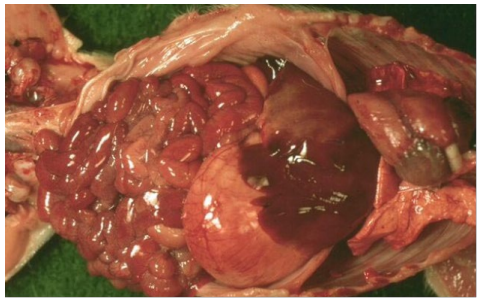


Diarrhoea/enteritis in the nursery period



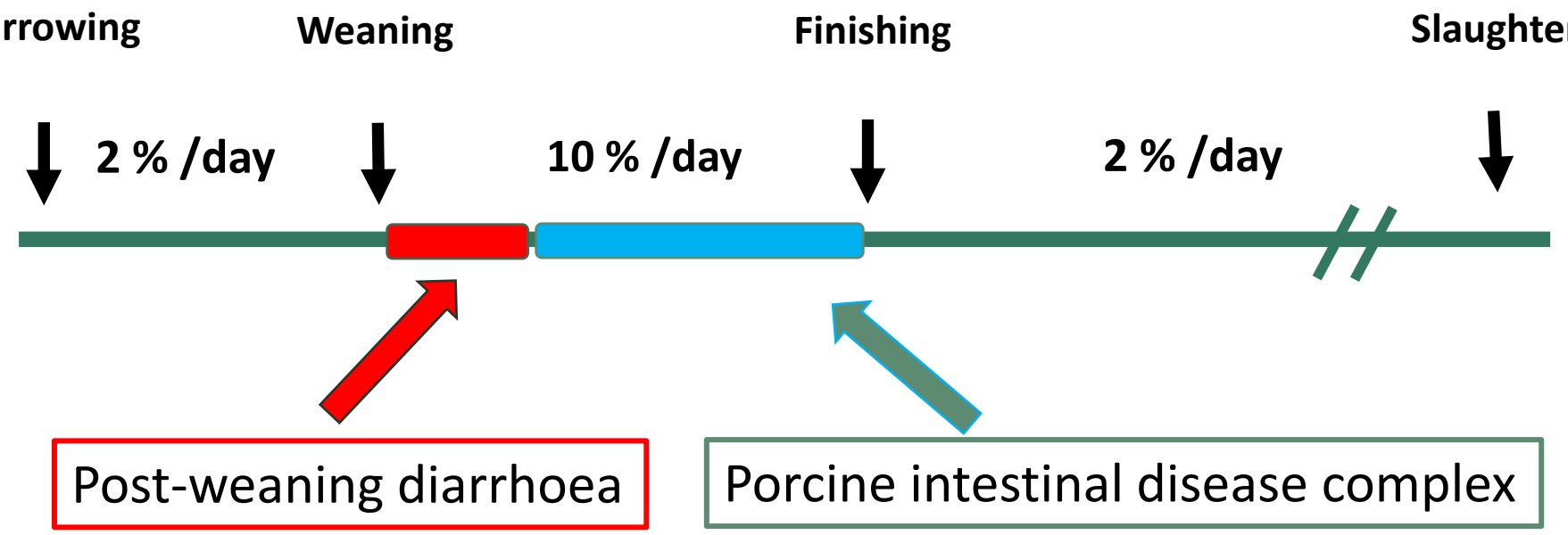
Diarrhoea/enteritis in the nursery period

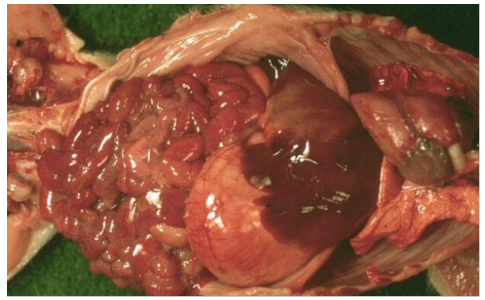




E. coli etc.

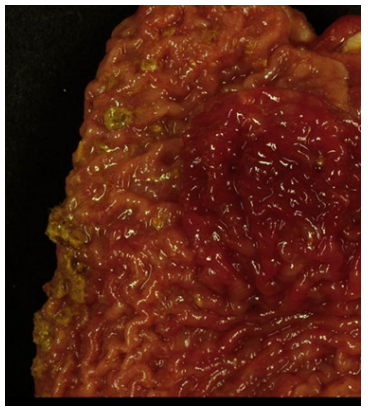
Diarrhoea/enteritis in the nursery period





E. coli etc.

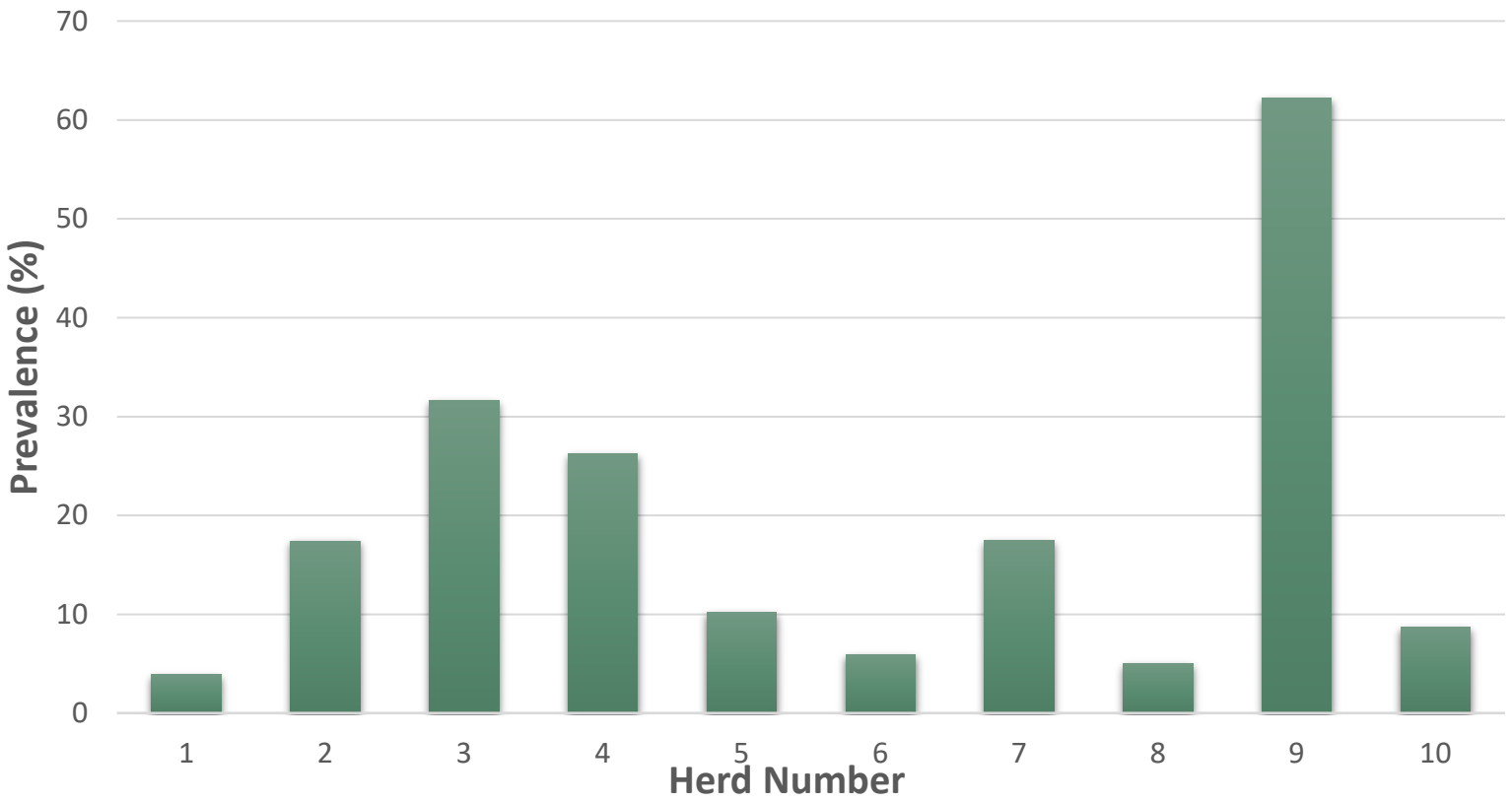
Lawsonia intracellularis



Brachyspira pilosicoli

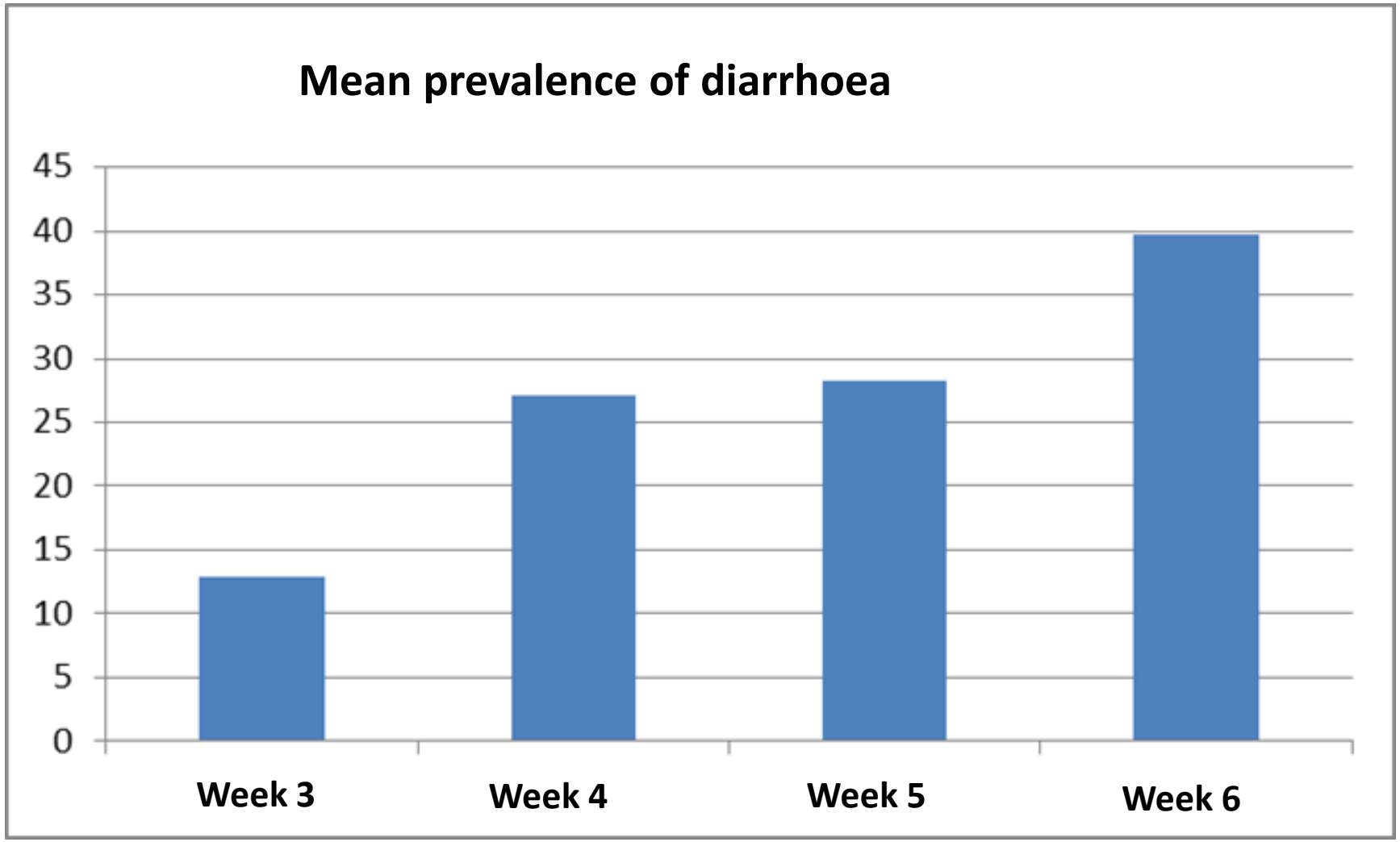
Post-weaning diarrhoea at time of AB treatment

(day 4-6 pw, 10 herds, N=4055)



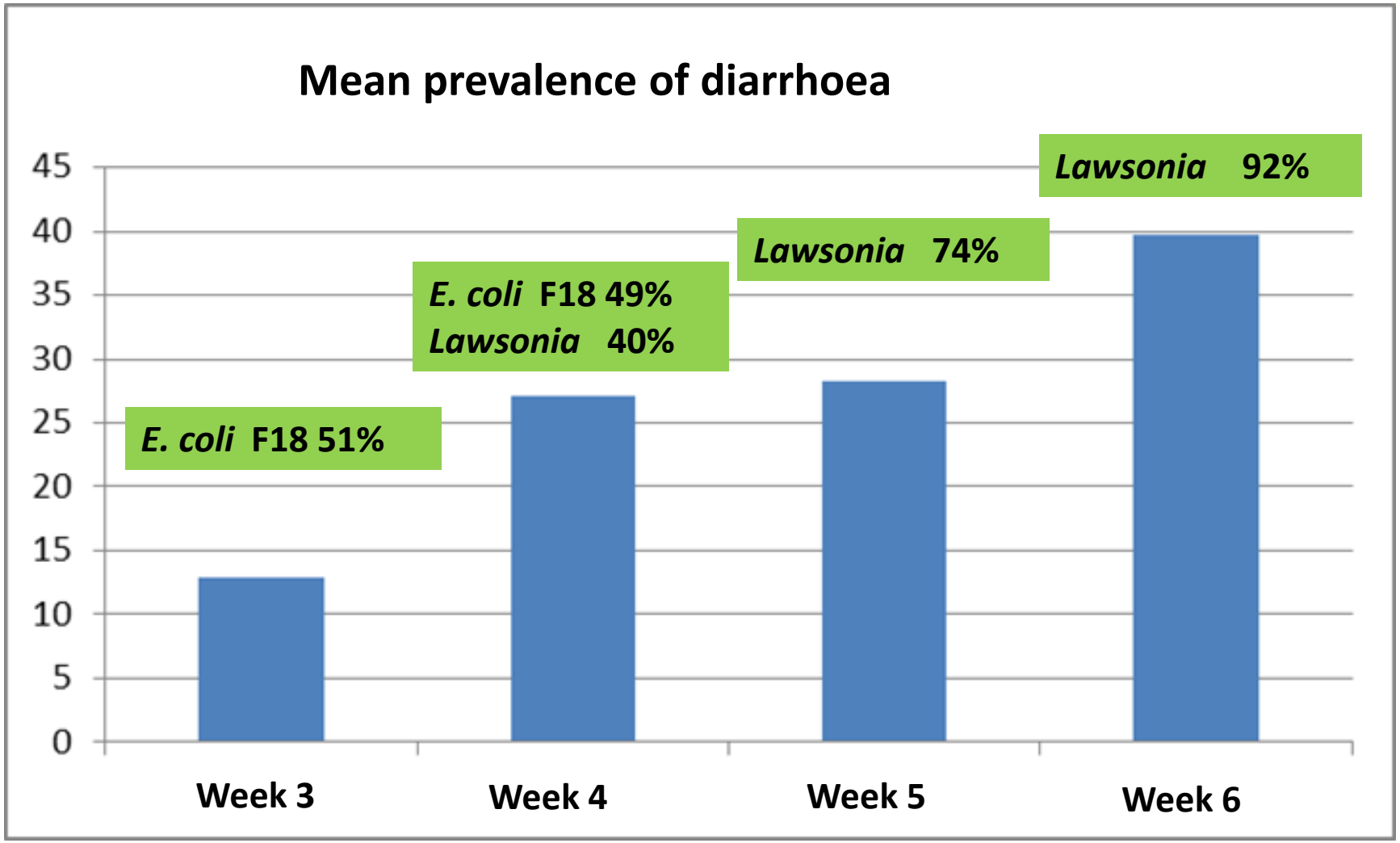
ETEC caused diarrhoea in 30 % of pigs (N=97)

Porcine intestinal disease complex



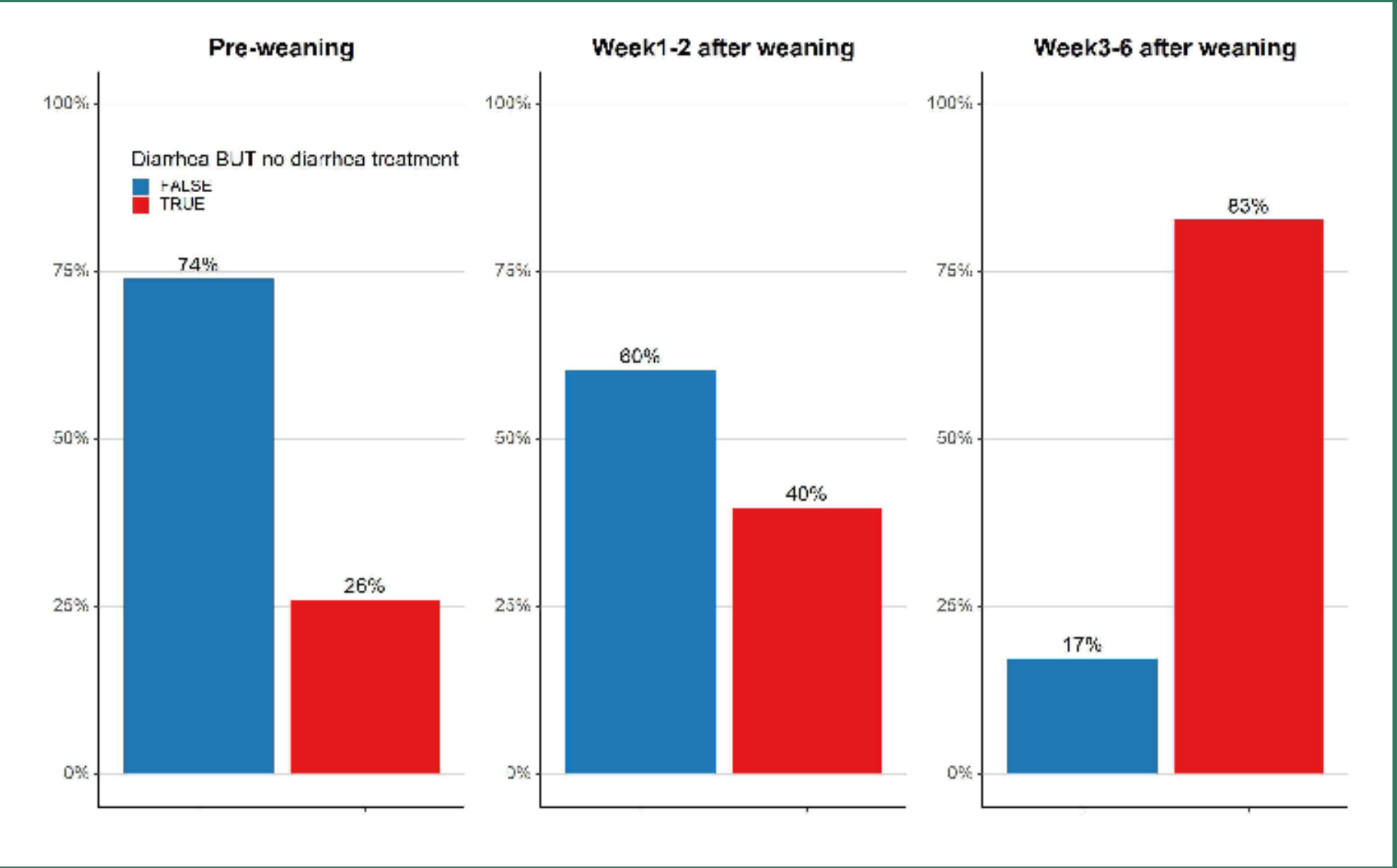
(Weber et al 2017)

Porcine intestinal disease complex



(Weber et al 2017)

PIG-PARADIGM cohort study



Why is pig production a major consumer of antibiotics ?

Pig factors

Microbiome robustness

Genetic robustness

Immunity (specific/nonspecific)

Why is pig production a major consumer of antibiotics ?

System factors

Lack of hygiene, mixing and crowding

Feed and feeding system

Stressors (weaning, competition, boredom)

Breeding program

Questions?

Host Pillar: Current activities and aim of the studies

Merete Fredholm

Professor
Institute of Veterinary and Animal Sciences
Faculty of Health and Medical Sciences
University of Copenhagen

mf@sund.ku.dk

Herd Trail

Professor Jens Peter Nielsen, Post Doc Malene Kjelin Morsing

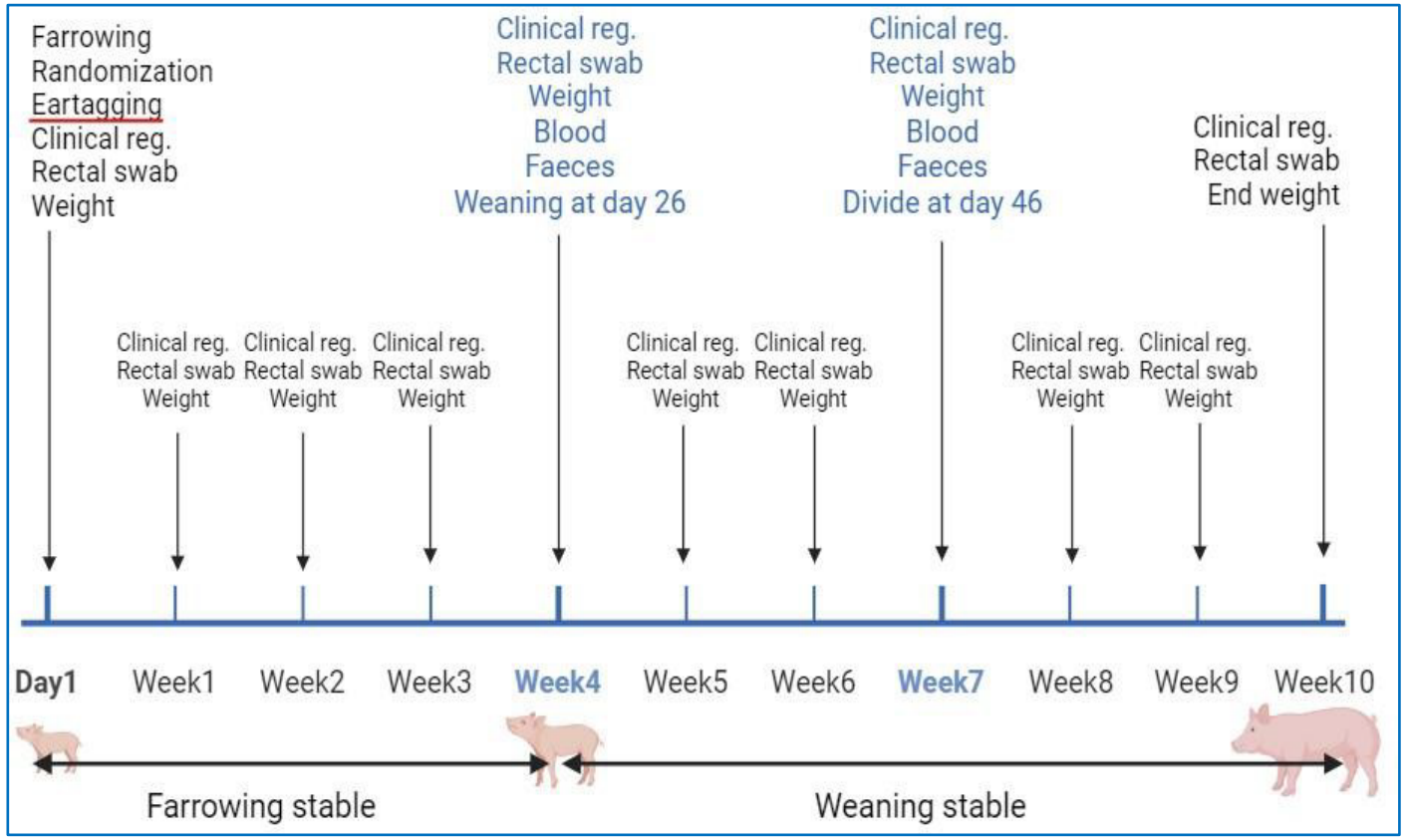
Sows at inclusion

- ~18 sow parity 1-6 included every sixth week
- Finished farrowing between Sunday mid day and Monday morning
- No antimicrobial treatment up to farrowing
- Min. 15 pigs

Piglets at inclusion

- 15 random pigs stay with the sow
- Min. 600 g
- Warm and able to walk

Herd trail overview



Rectal swabs:

- 16S seq
- qPCR diagnostics

Faecal samples:

- metagenomics

Blood and serum samples:

- Genetics
- CBC and diff
- metabolomics
- transcriptomics

Clinical observations

Daily:

- Alertness in pigs
- Untrifty/thin pigs
- Treatment and cause

Weekly:

- Perianal fecal staining, fecal score, wounds, lameness, body condition, coat, umbillical hernia

0-14 days post weaning:

- Perianal fecal staining

Treatment of diarrhea (NB! no batch treatment)

Perianal fecal staining Fecal score 3-4

AND

Losing body condition and/or depressed

Data management

Post Doc Martin Peter Rydal

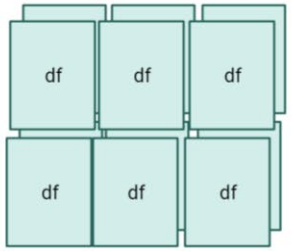
Data collection

First QC at the herd

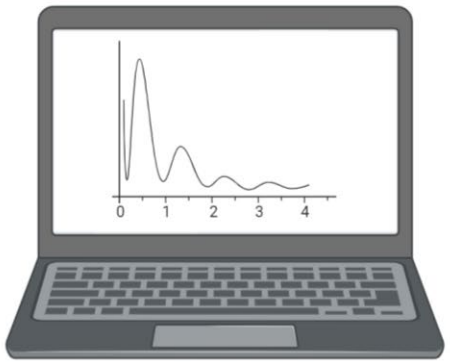
Second QC: Raw data cleaned

Database creation

Assembling clinical data

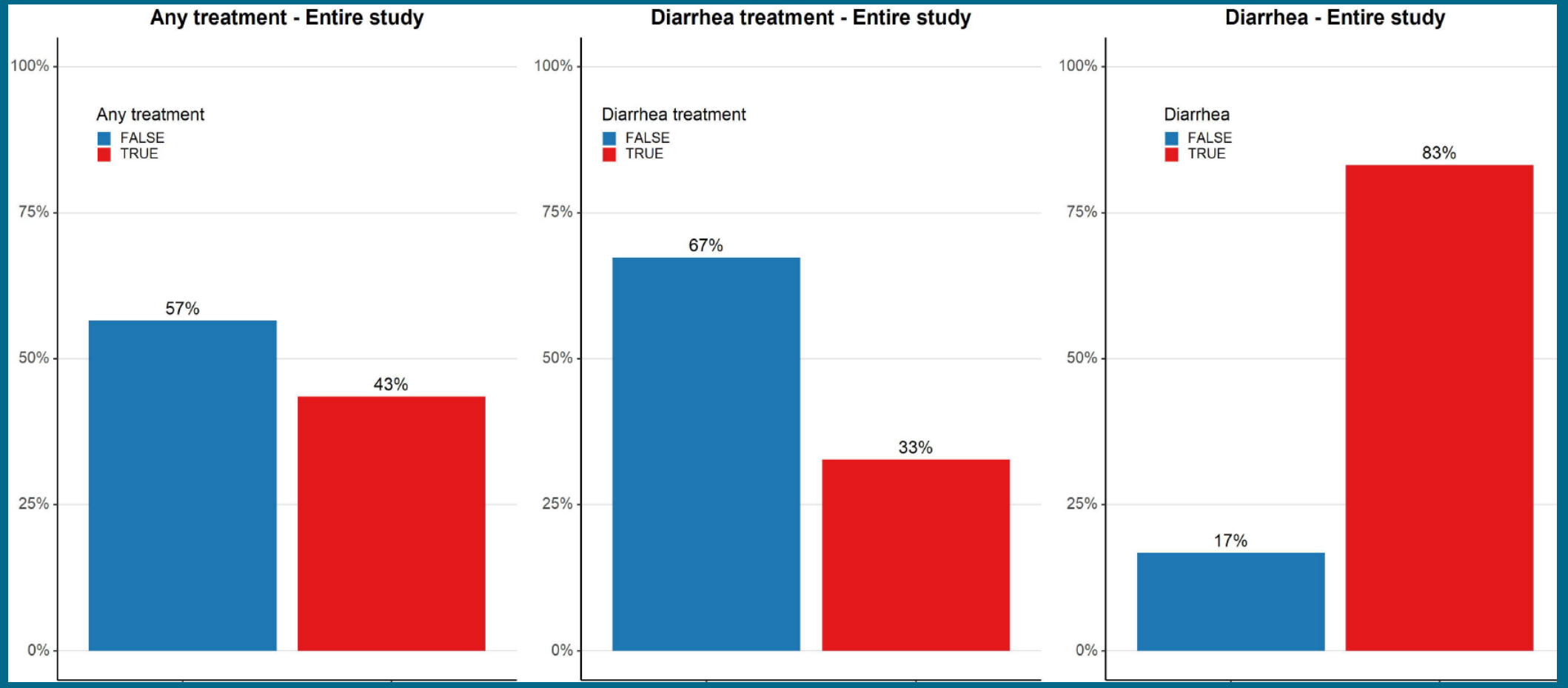


Data description and analysis



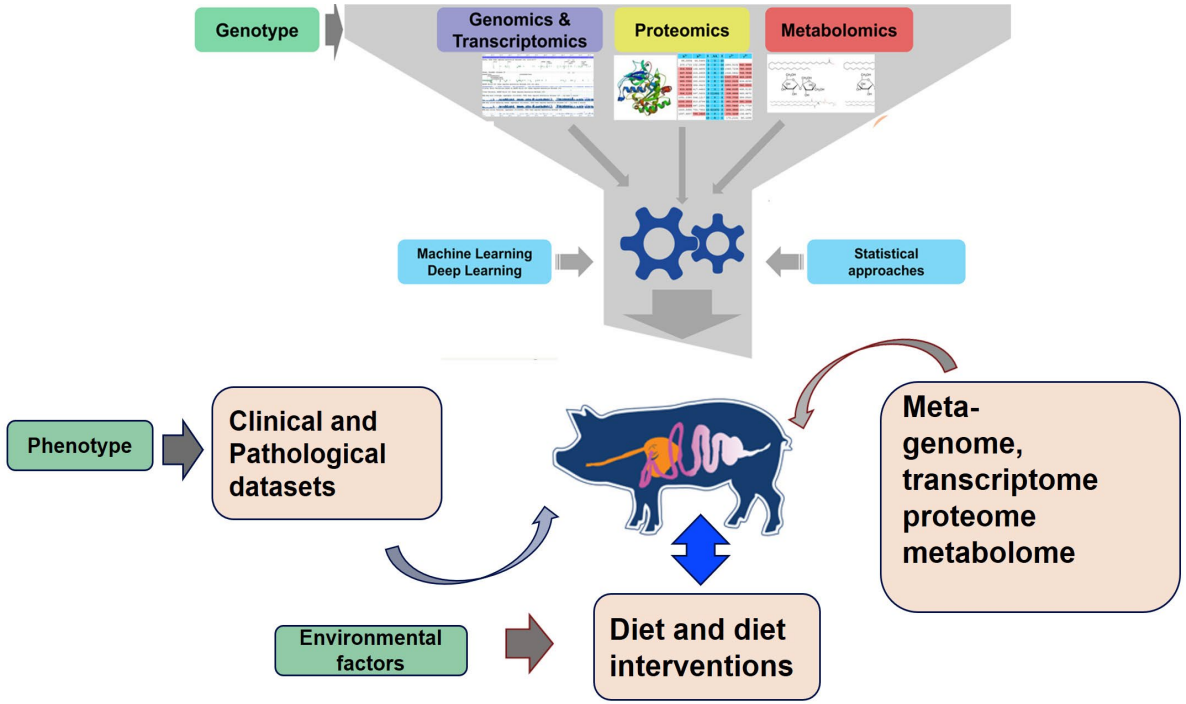
Preliminary data on diarrhea and treatment

Post Doc Martin Peter Rydal



Aim

- Establish biomarkers for intestinal health
- Establish a robustness score for intestinal and systemic health
- Elucidate the complex interactions between the intestinal microbiome and host factors and determine their separate and combined influence on intestinal health and resilience



People involved at present

Copenhagen University

Postdoc Malene Kjelin Morsing

Postdoc Martin Rydal

Research assistant Amanda Andersen

Animal caretaker Rasmus Syhler

5-10 students

Associate professor Ken Steen Pedersen

Professor Jens Peter Nielsen

Research assistant Frederik Scharling

Lab technician Tina Mahler

Lab technician Minna Jakobsen

Post doc Emil Ibragimov

AC TAP Christian Anton

PhD student Cecilie Brandt Becker

Associate professor Mette Sif Hansen

Research assistant Benjamin Meyer Jørgensen

Professor Henrik Elvang Jensen

Professor Merete Fredholm

Wageningen University & Research

Professor Hauke Schmidt

Professor Michiel Kleerebezem

PhD student Mohak Gujare

PhD student Baris Osdinc

Questions?

The contradictionary effects of fibre in animal nutrition and health

Knud Erik Bach Knudsen

Professor

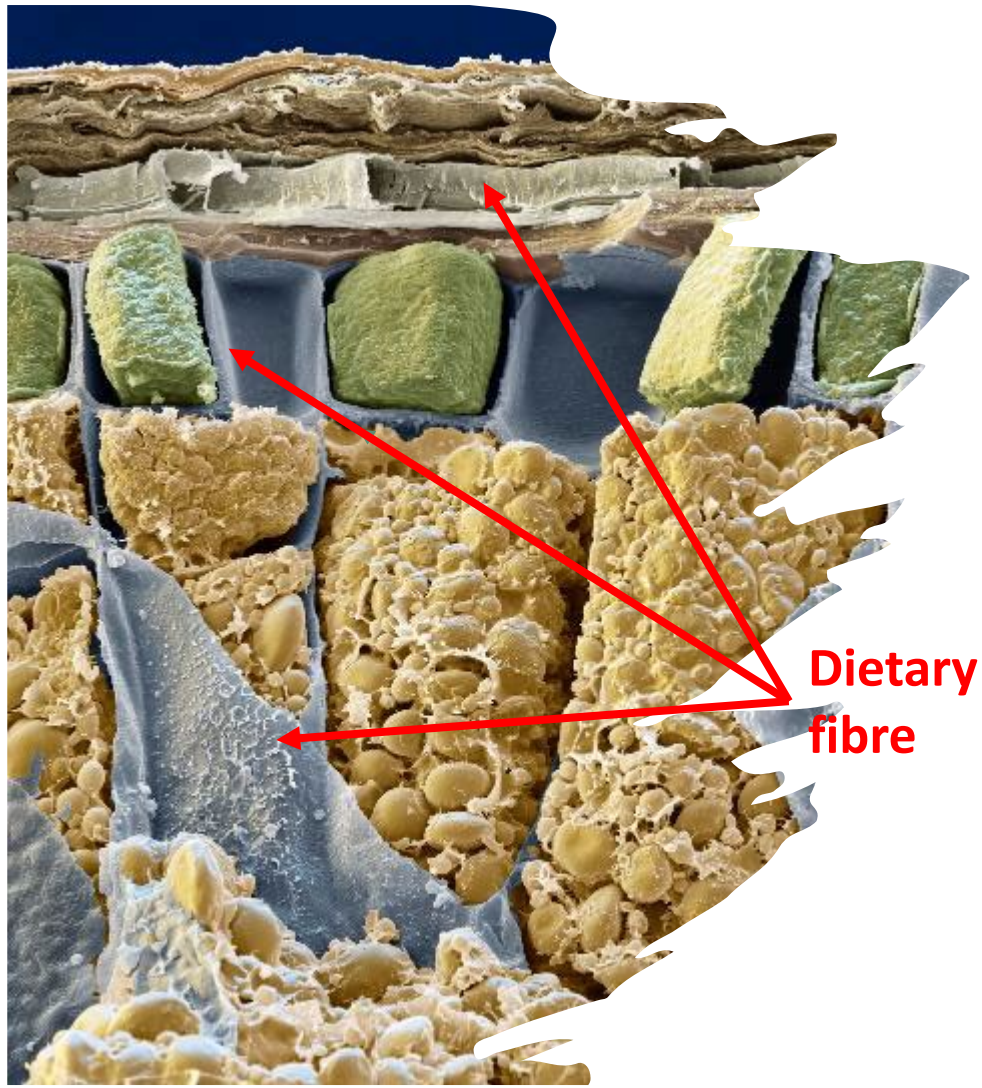
Department of Animal and Veterinary Sciences

Aarhus University

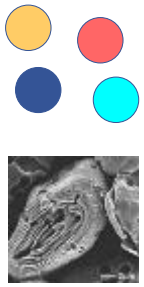
knuderik.bachknudsen@anivet.au.dk

General introduction

- Dietary fibre (DF) represent carbohydrates and lignin that cannot be digested by endogenous enzymes but can be fermented to a variable extent by the microbiota mostly in the large intestine
- Because of its influence on nutrient digestibility, DF it is mostly considered negative from a nutritional point of view
- Some DF components and functional properties of DF may, however, influence gastrointestinal health in a positive way



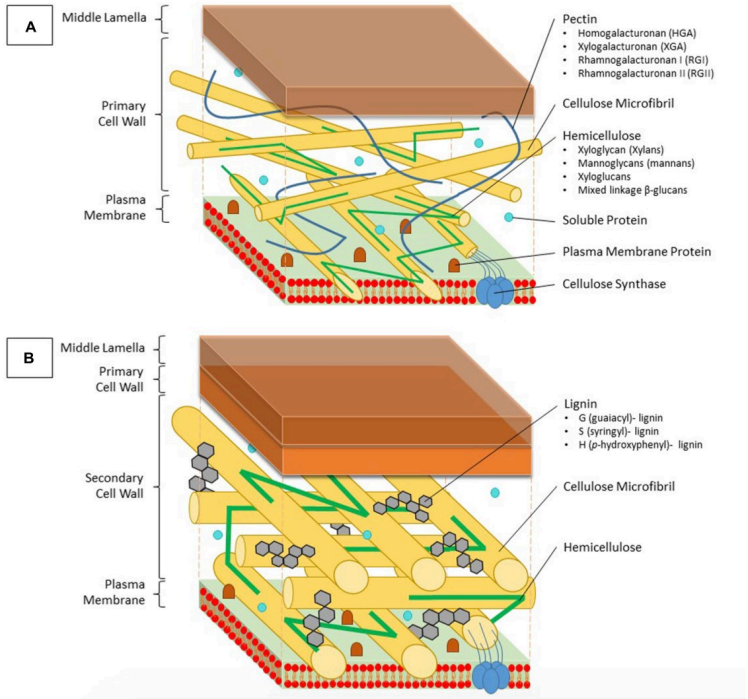
The main classes of dietary fibre and potential effects



Oligosaccharides

Resistant starch

Prebiotic effects on microbiota



Non-lignified

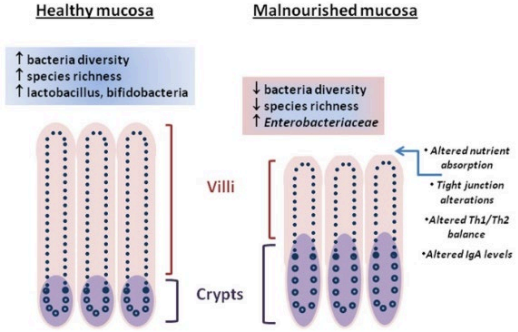
Lignified

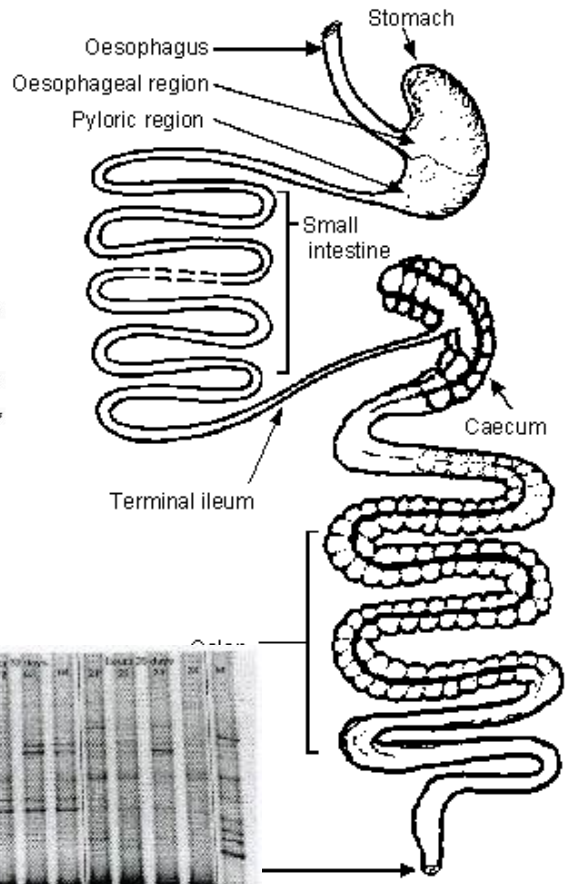
Cell walls

Effects on luminal content and epithelium

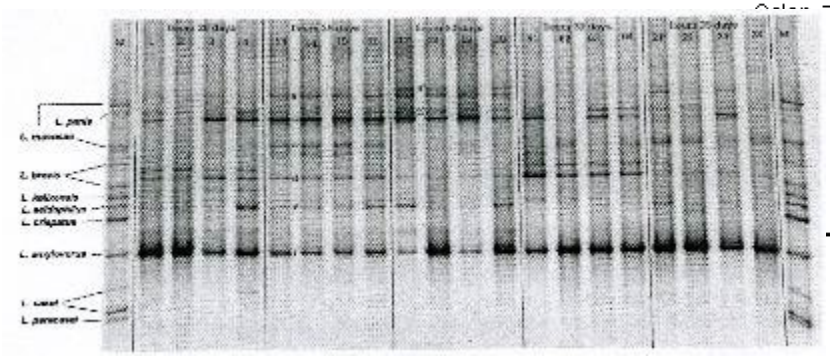
Physiological consequences of the challenging weaning process

Alteration in morfologi



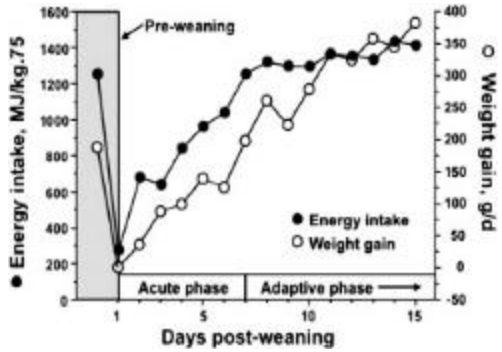


Unstable microbiota



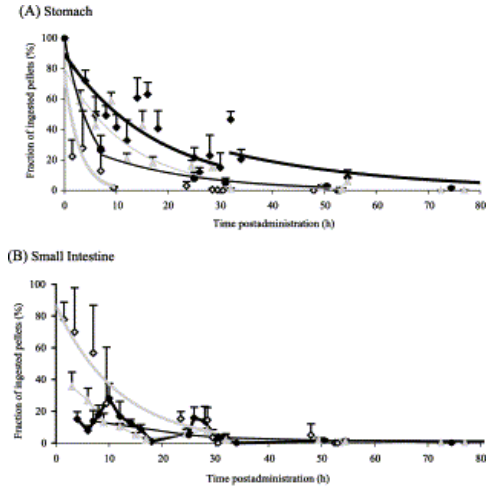
Janczyk et al. (2007).

Reduced feed intake



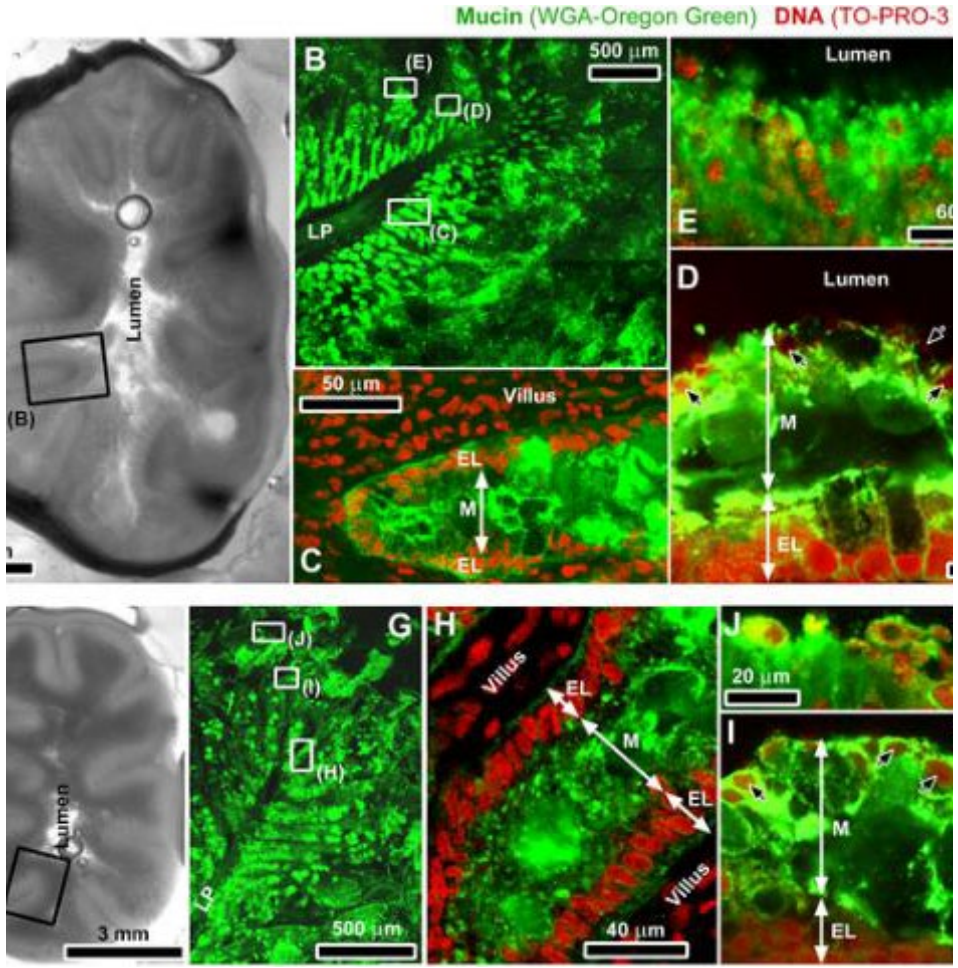
Burrin & Stoll (2003)

Alteration in GI transit



Snoeck et al. (2004).

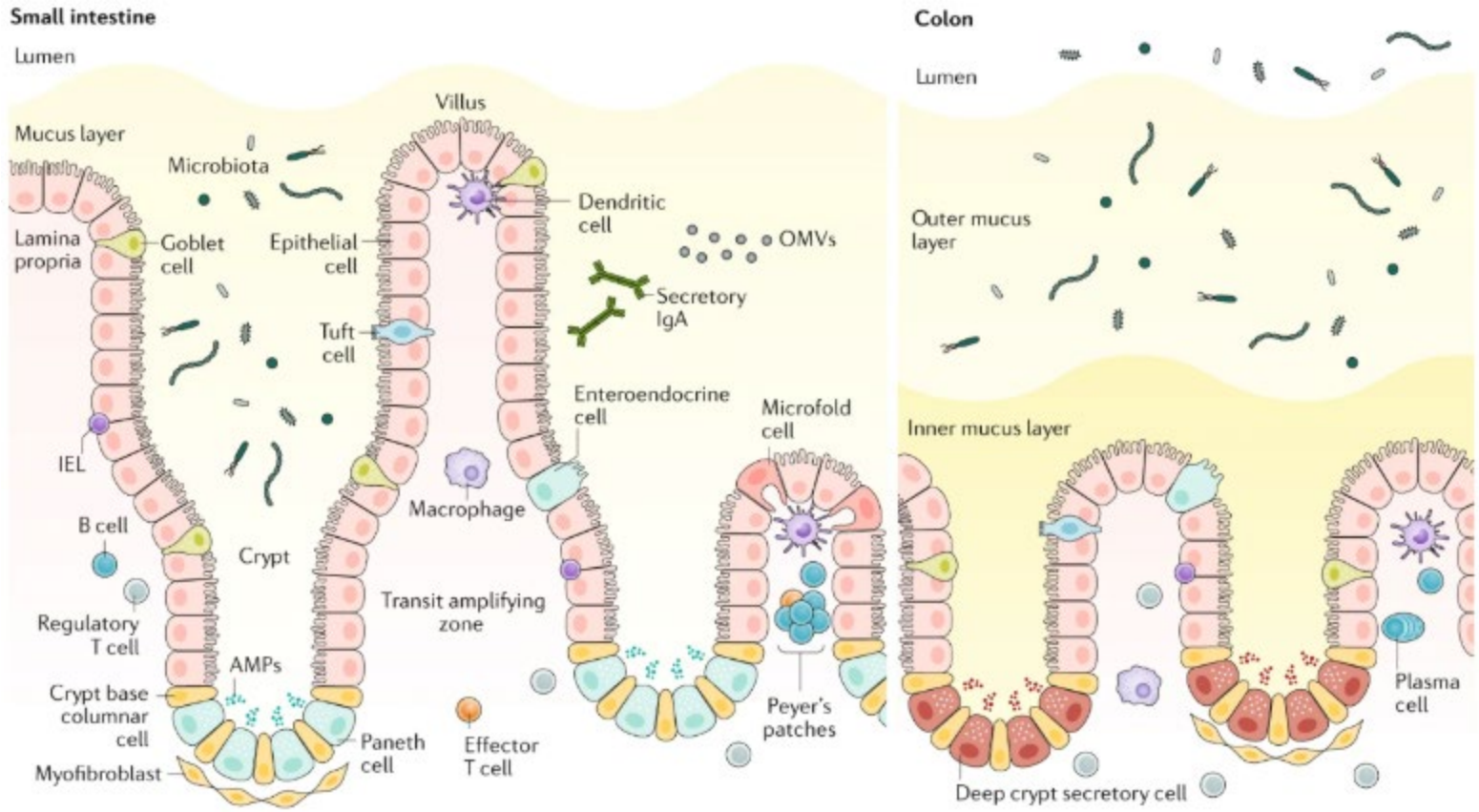
Findings in the literature



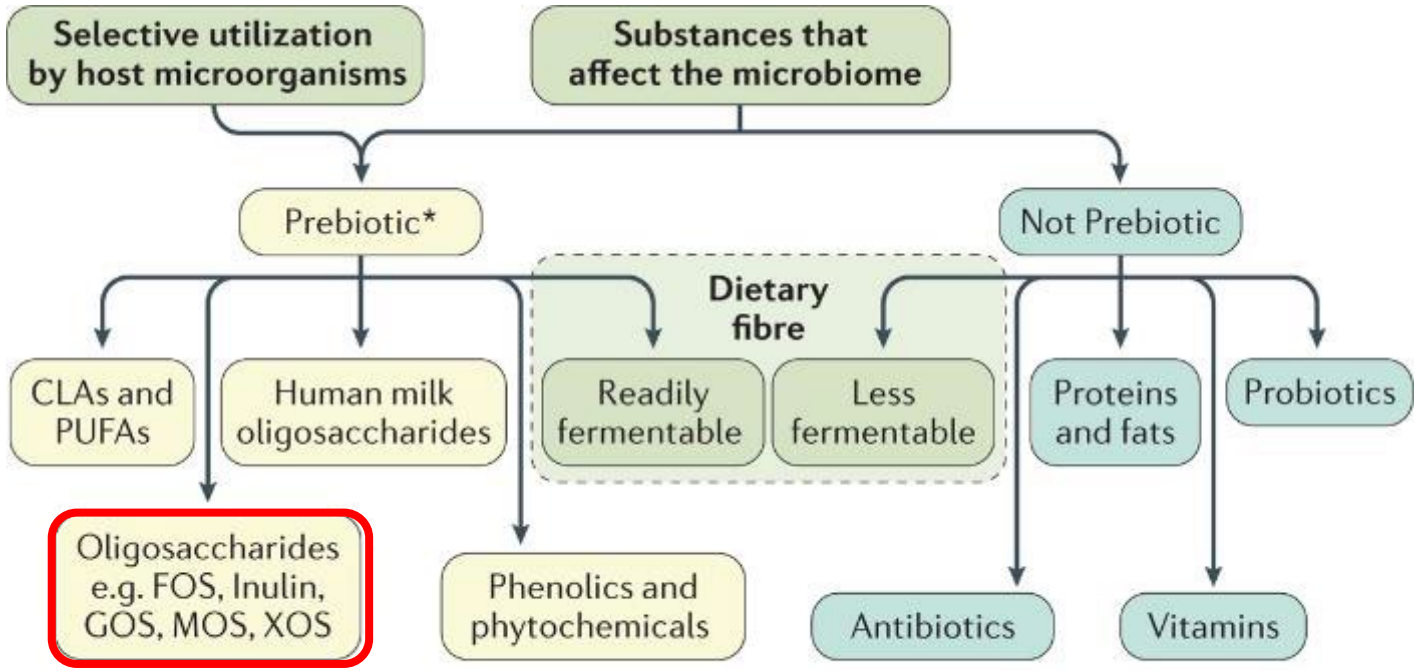
- The environment in the gastrointestinal tract is unstable in the immidiate post-weaning period
- Soluble DF is a risk factor for post weaning enteric disorders (PWD) because soluble DF can:
  - Influence digestion and absorption processes in the small intestine
  - interact with the intestinal mucus layer and promote the hydrolysis of mucus by sialidase to sialic acid that promote ETEC proliferation
- Insoluble fibre types, on the other hand, seems to stabilise the luminal environment

What do we want to achieve?

Keep the homeostasis and avoiding dysbiosis and inflammation by stabilising the luminal environment and minimizing interference of the mucus layer with soluble fibre



Distinguishing prebiotics from dietary fibre

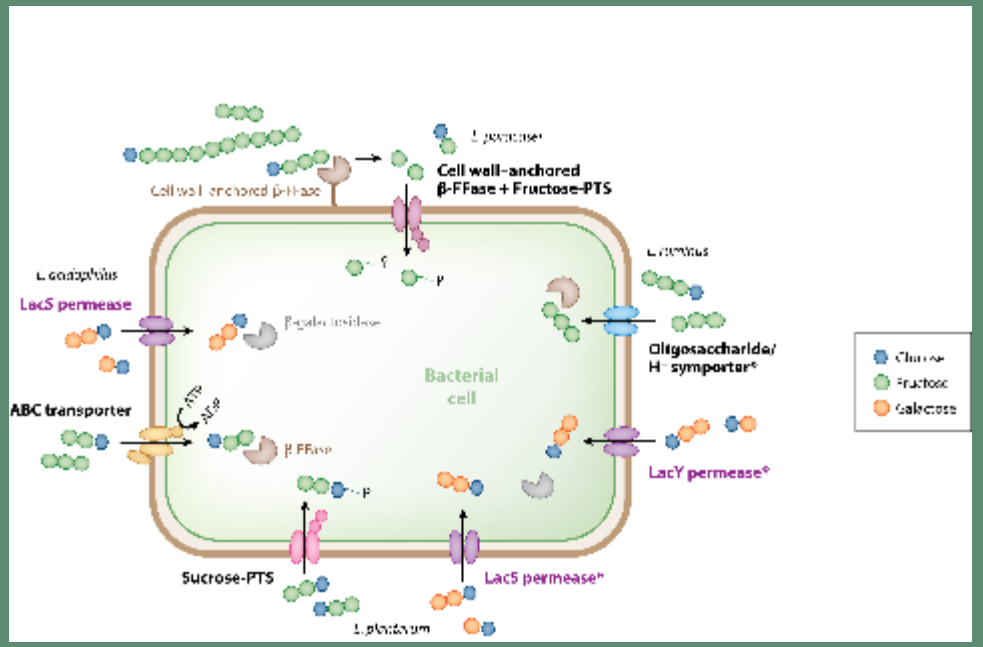


CLA, conjugated linoleic acid;
PUFA, polyunsaturated fatty acid;
FOS, fructooligosaccharides;
GOS, galactooligosaccharides;
MOS, mannanoligosaccharide;
XOS, xylooligosaccharide

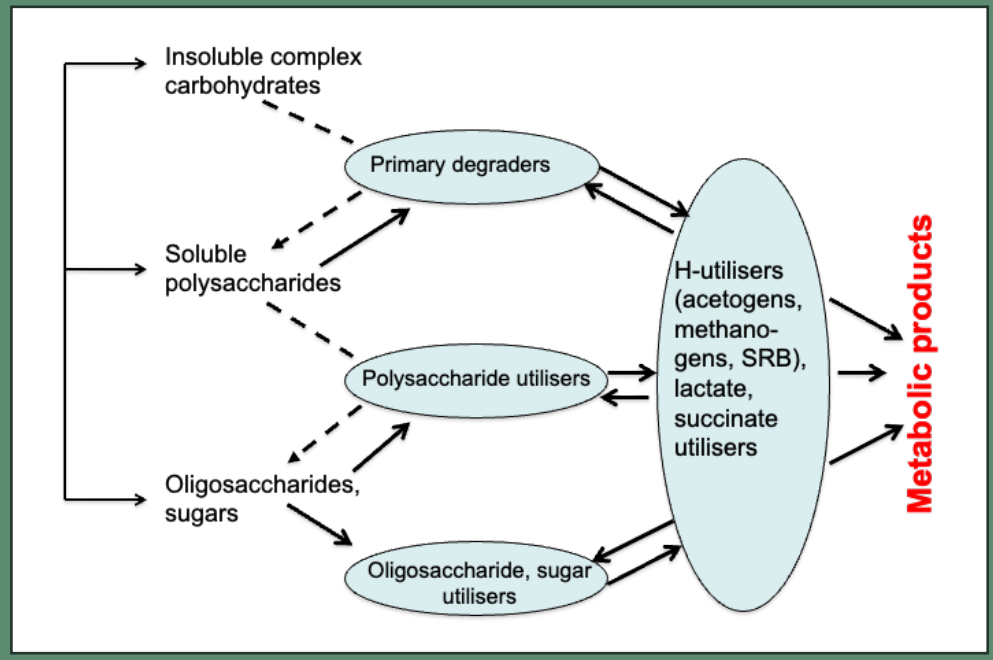
Gibson, G. R. *et al.* (2017) The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics *Nat. Rev. Gastroenterol. Hepatol.* doi:10.1038/nrgastro.2017.75

Fermentation of prebiotics vs. Dietary fibre

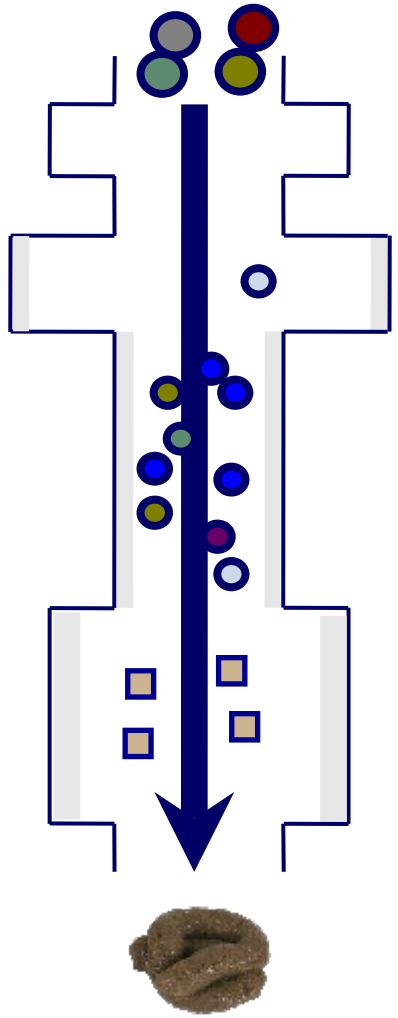
Prebiotics

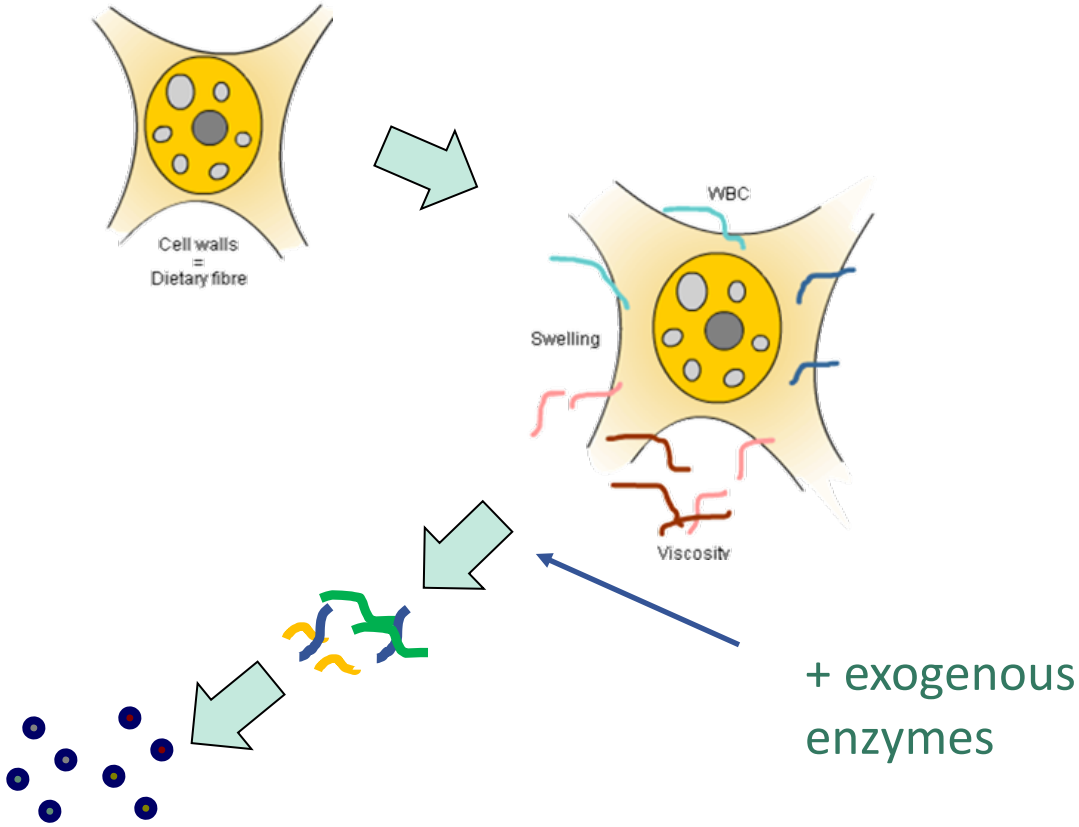


Dietary fibre



The use of exogenous enzymes to reduce luminal viscosity and produce prebiotics in situ





Questions?

Focus on gut microbial metabolites in the weaning period

Nuria Canibe

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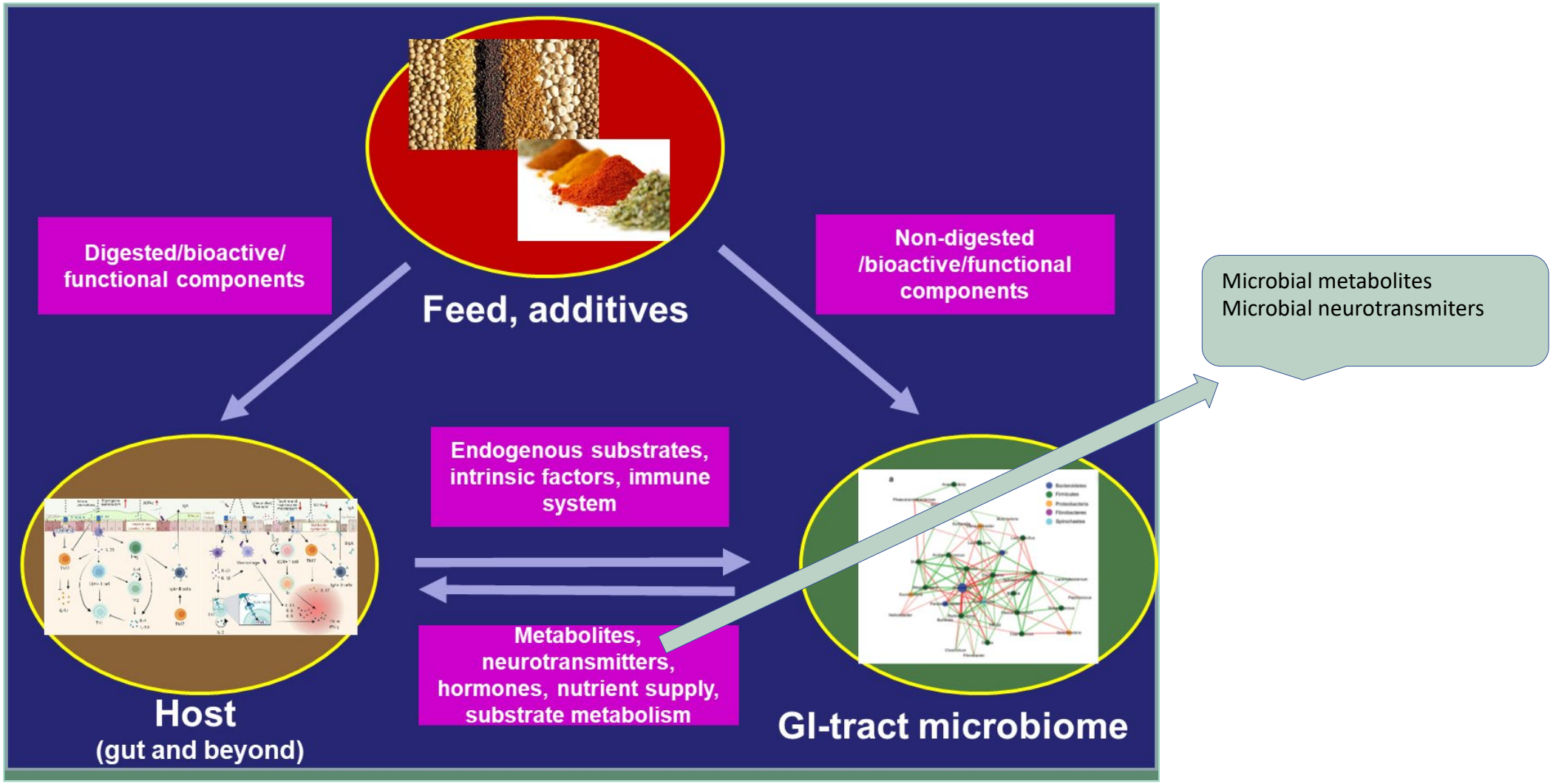
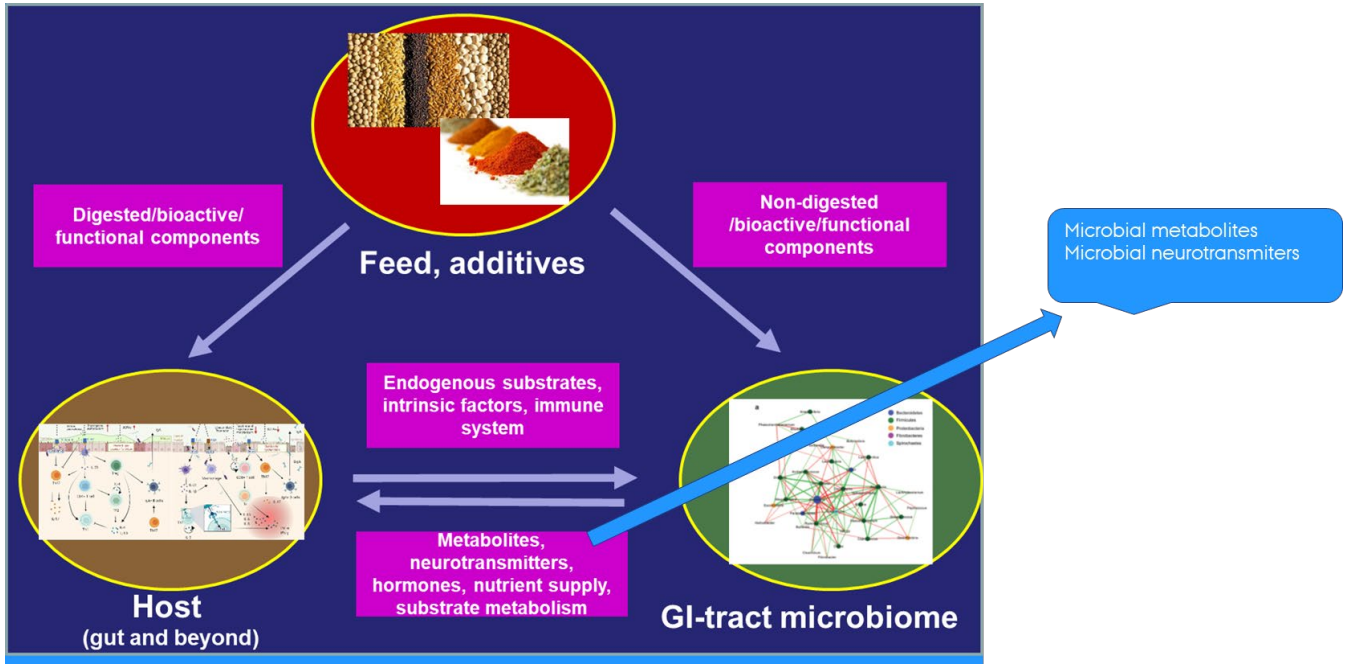
Introduction



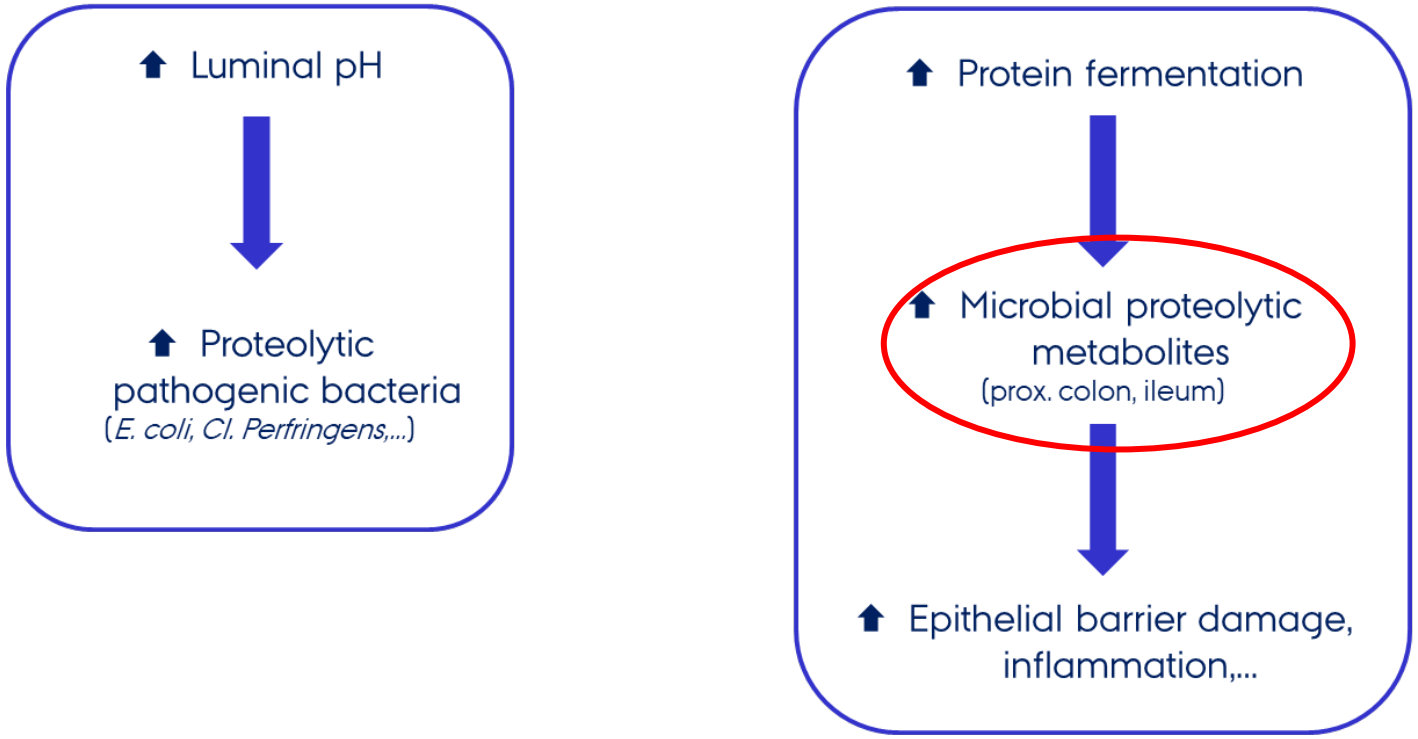
Introduction

- ❑ Dietary protein fermentation
- ❑ Microbiota-gut-brain axis

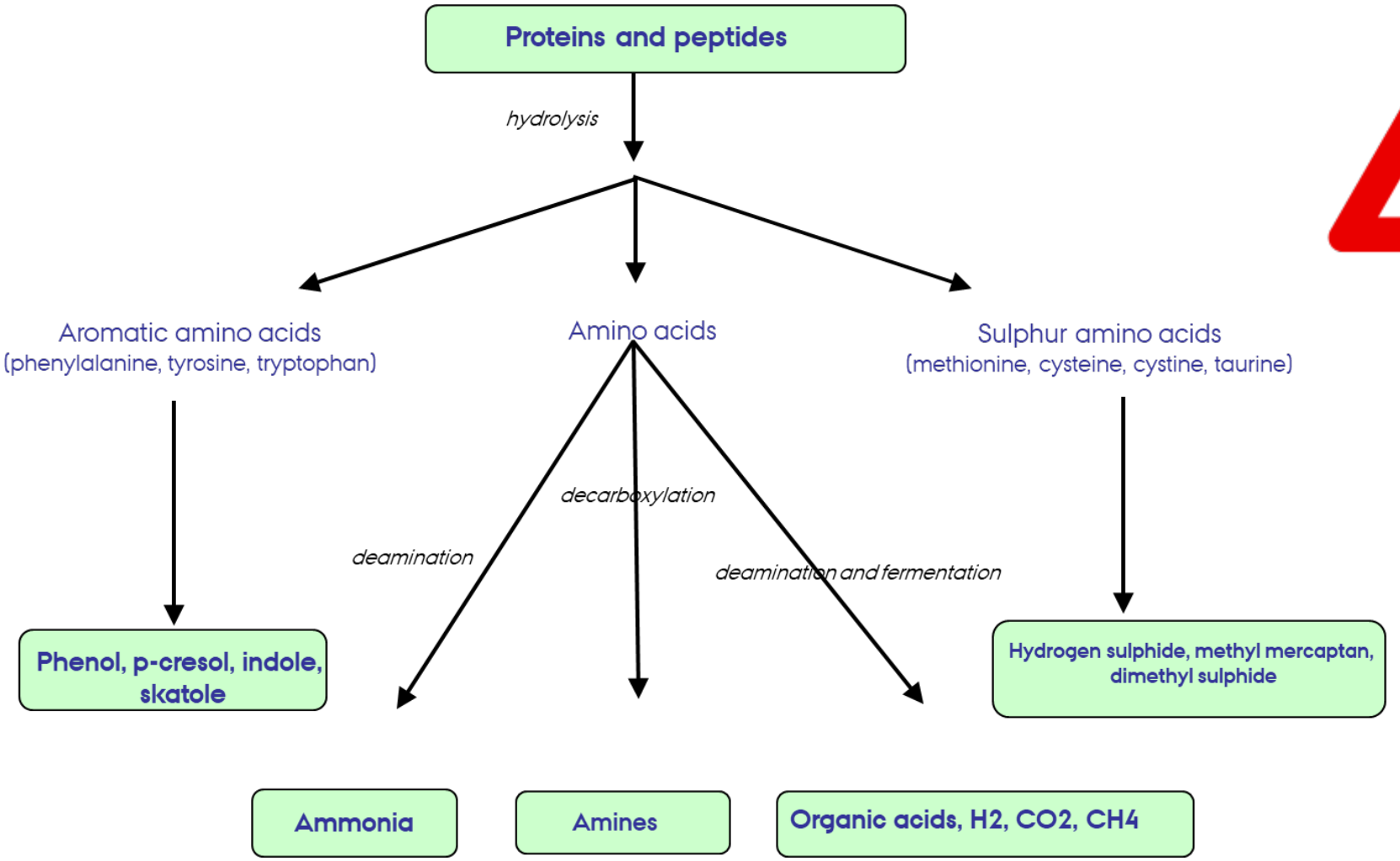


Dietary protein fermentation in the gut- metabolites

High protein level predisposes to diarrhea:



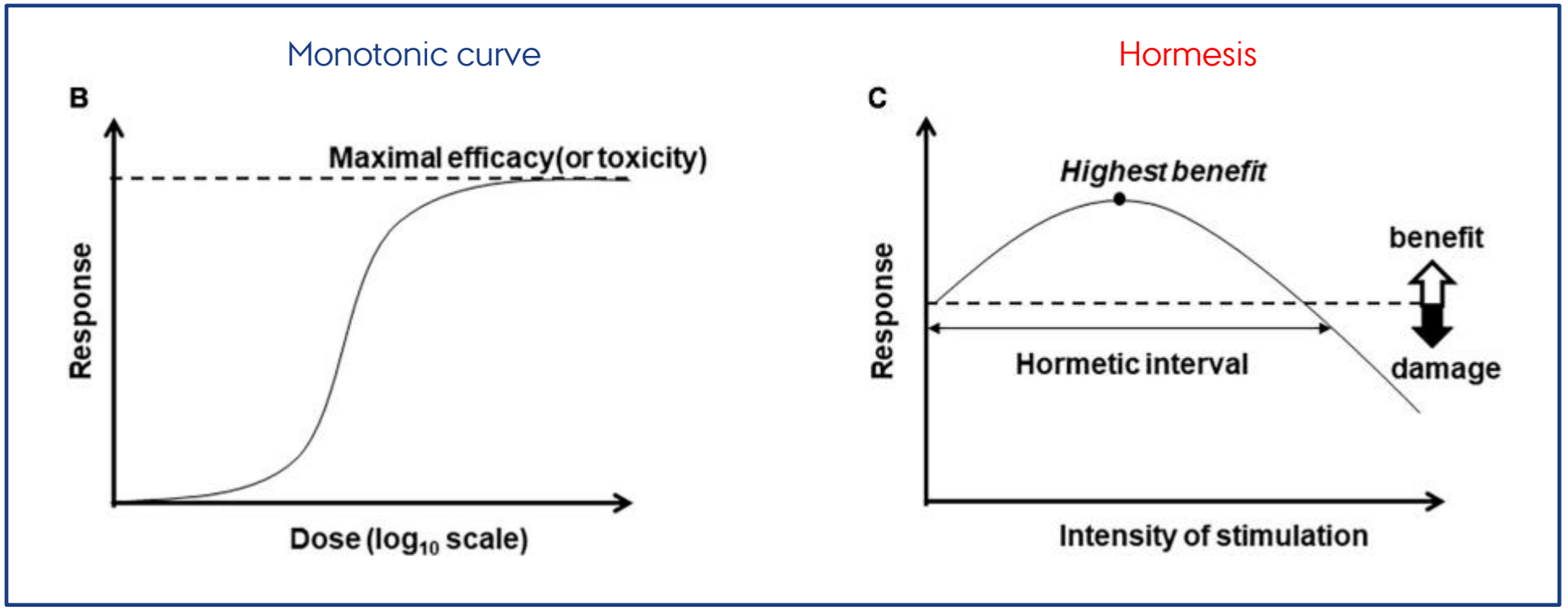
Dietary protein fermentation in the gut- metabolites



Dietary protein fermentation in the gut- metabolites

Hormesis

is an adaptive response characterized by biphasic dose response affected by an active compound

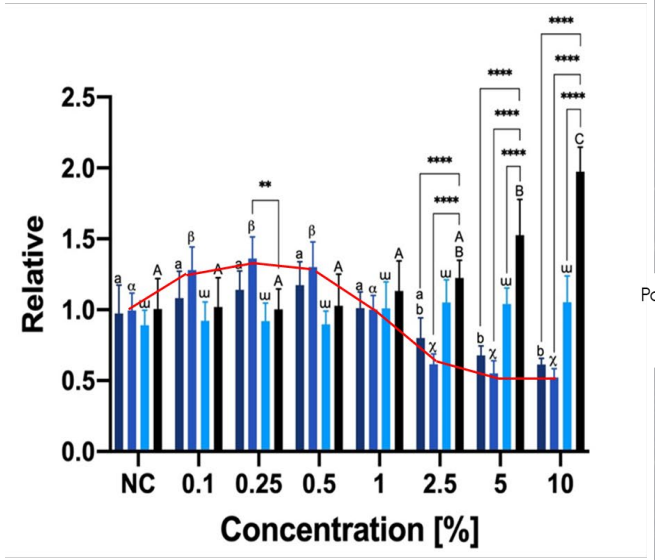


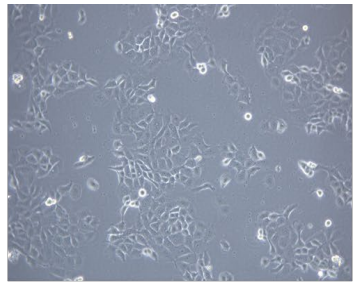
doi.org/10.3389/fnut.2022.969823

Dietary protein fermentation in the gut- metabolites

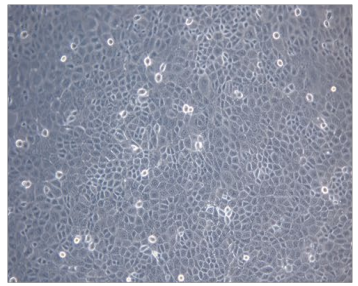
What is the impact (beneficial/detrimental) of individual metabolites and in combination on the gut?
(Ranking)

Epithelial cell lines



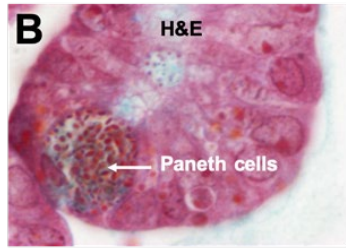


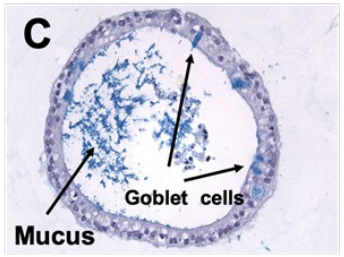
Porcine intestinal epithelial cells at **day 1** of culture

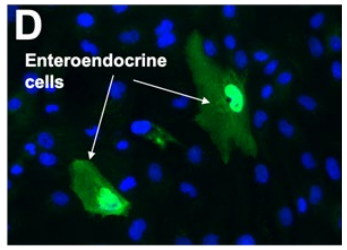


Porcine intestinal epithelial cells at **day 3** of culture

Intestinal organoids

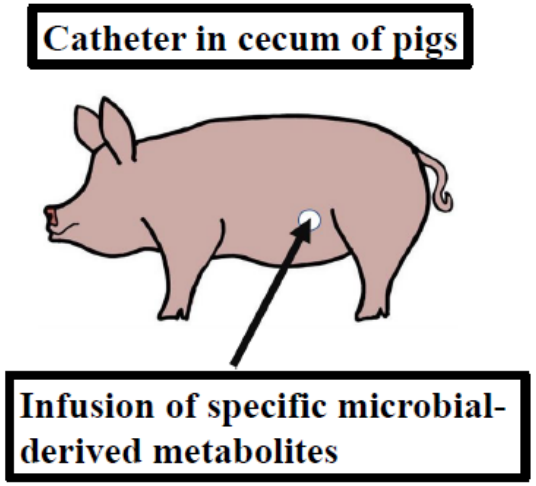






- ETEC adherence
- Intestinal permeability
- Innate immunity redox balance
- Nutrient active transport
- Wound repair

In vivo



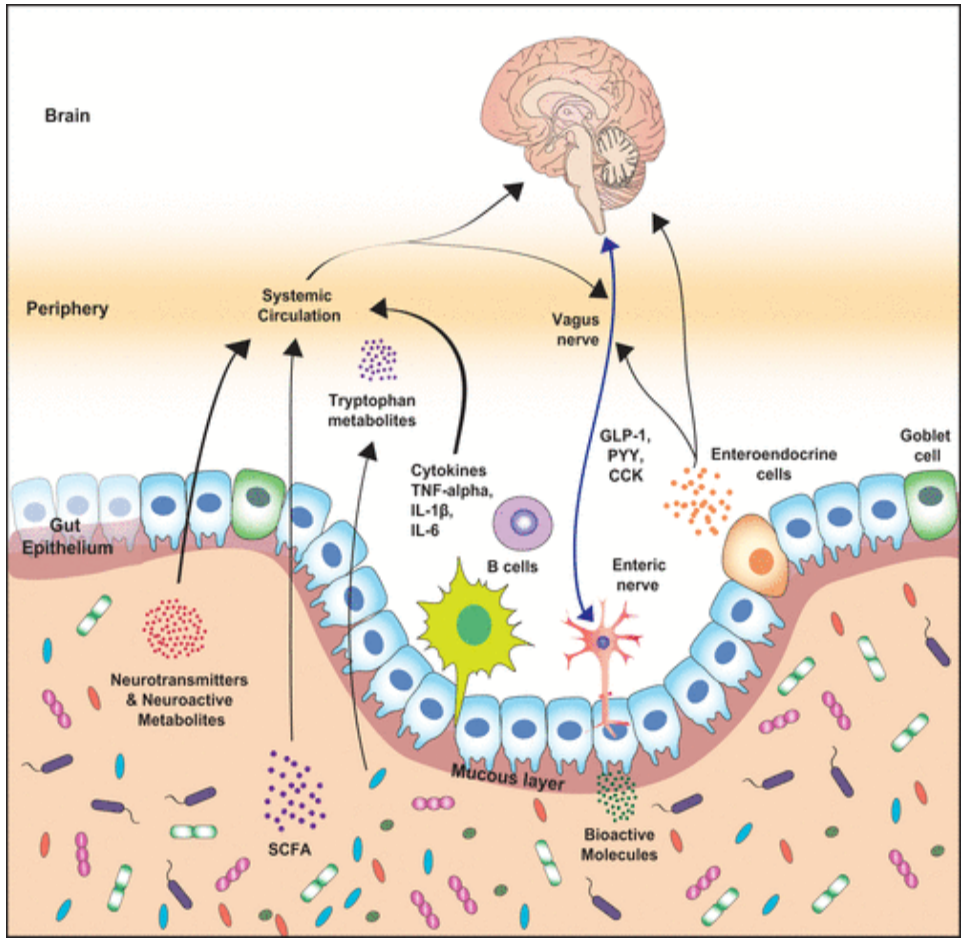
Dietary protein fermentation in the gut- metabolites

Experimental groups:

1) Low fermentable protein (~15 CP)
2) High fementable protein (~25% CP)
3) High fermentable protein added Lys, Val, Met, Trp, & Thr (35% above requirements)

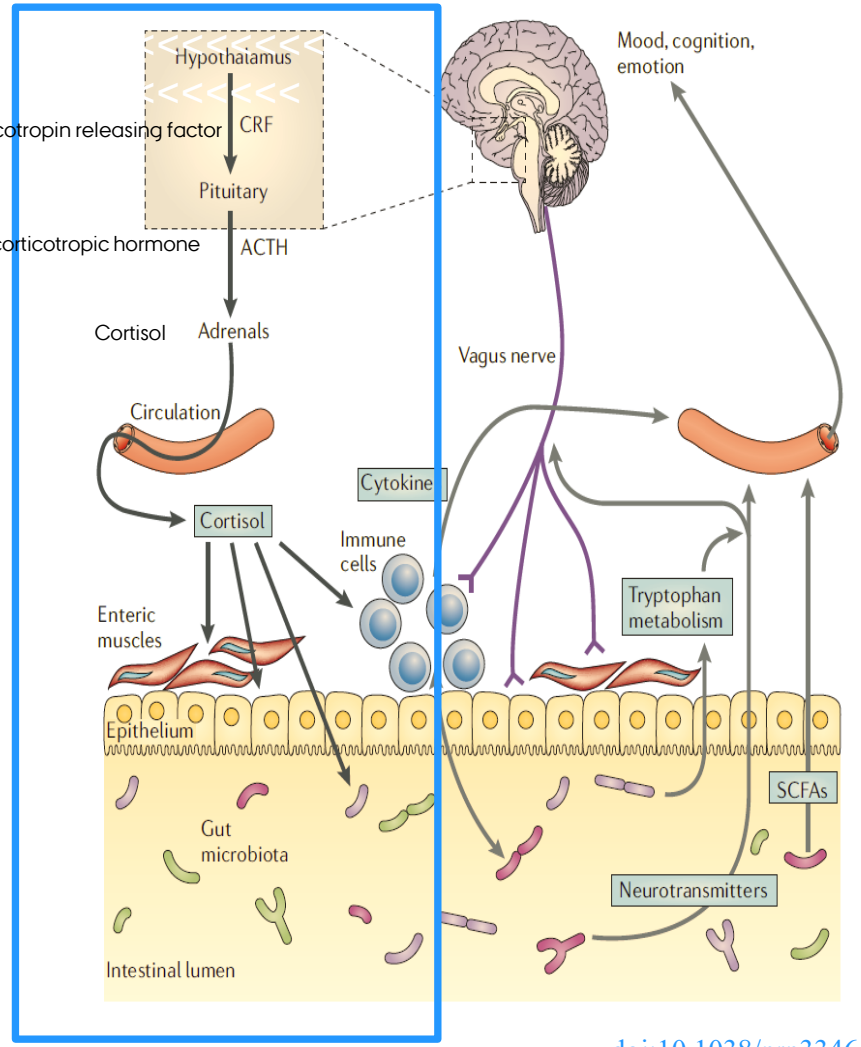
Microbiota-gut-brain axis

Gut to brain



doi:10.1152/physrev.00018.2018

Brain to gut



doi:10.1038/nrn3346

Microbiota-gut-brain axis

Neuroactive compounds (neurotransmitters) involved in MGBA communication:

- Glutamate: exitatory
- γ-aminobutyric acid (GABA): inhibitory
- Dopamine: inhibitory
- Noradrenaline : exitatory
- Serotonin (5-HT): inhibitory
- Histamine: exitatory

Important intermediate compounds:

- SCFA
- Tryptophan
- Secondary bile acids

Host and microbiota derived neurotransmitter	Bacteria producing neurotransmitter
GABA	Bifidobacterium, Lactobacillus
Serotonin	Enterococcus, Escherichia, Lactobacillus, Spectrococcus
Dopamine	Bacillus, Escherichia, Lactobacillus, Lactococcus, Spectrococcus
Noradrenaline	Bacillus, Escherichia
Acetylcholin	Bacillus, Lactobacillus

Microbiota-gut-brain axis
Stress and post-weaning diarrhoea

- 2x2 factorial design
  - Control pigs
  - ACTH pigs (simulated long-term stress)
  - ETEC pigs (induced dysbiosis)
  - ACTH + ETEC
- Weaned piglets (ca.7kg, 28 days of age)

Samples collected at slaughter:

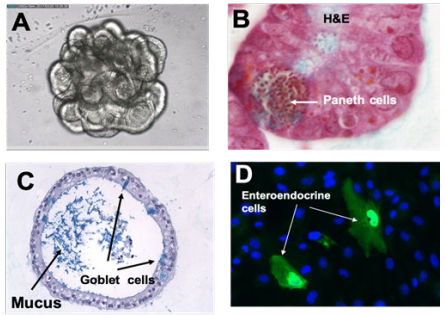
- Mucus & digesta from GI sections
- Blood
- Feces

Phenotypic endpoints:

- Incidence and severity of PWD
- Behavioural endpoints (e.g., activity, startle-and novel object response)

- Investigate effects of bacterial-derived neurotransmitter metabolites (responding to ACTH/cortisol challenge) on intestinal cell function :
  - Epithelial interaction (e.g., TER, gene expression)
  - Epithelial translocation
  - Immunomodulatory effects

Intestinal organoids



Questions?



General Discussion

Get connected with us

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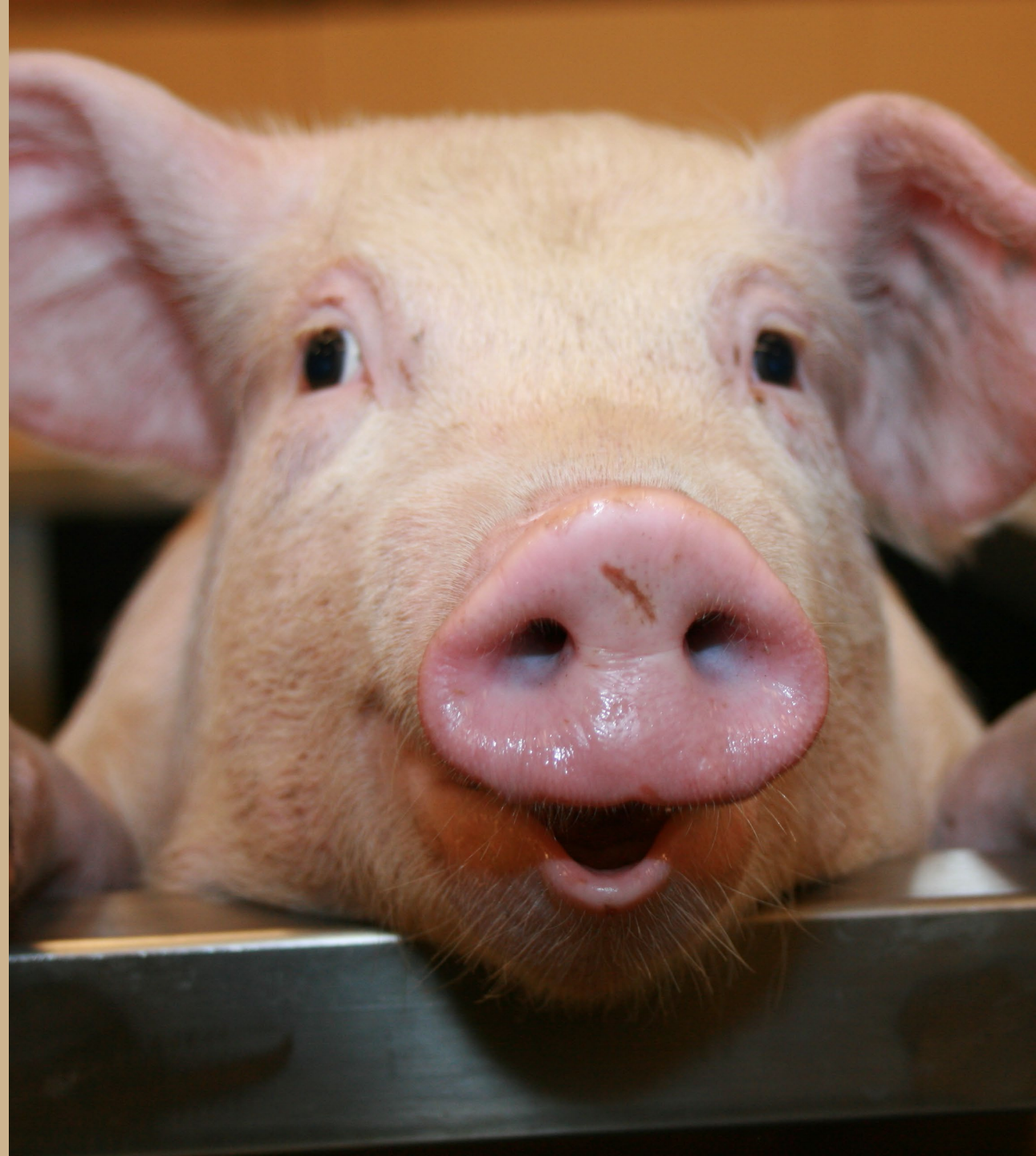

Thank you!