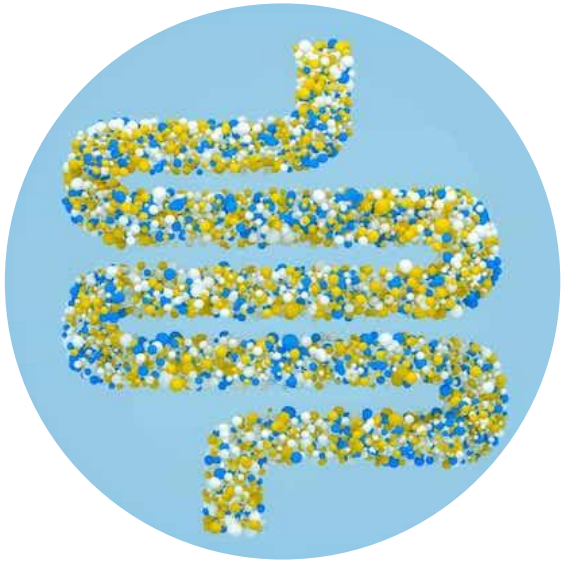
Microbiota and its role in intestinal health in livestock

April 2021, Dr. Astrid de Greeff

Wageningen Bioveterinary Research, part of WUR



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Top level veterinary and biomedical research for animal and public health

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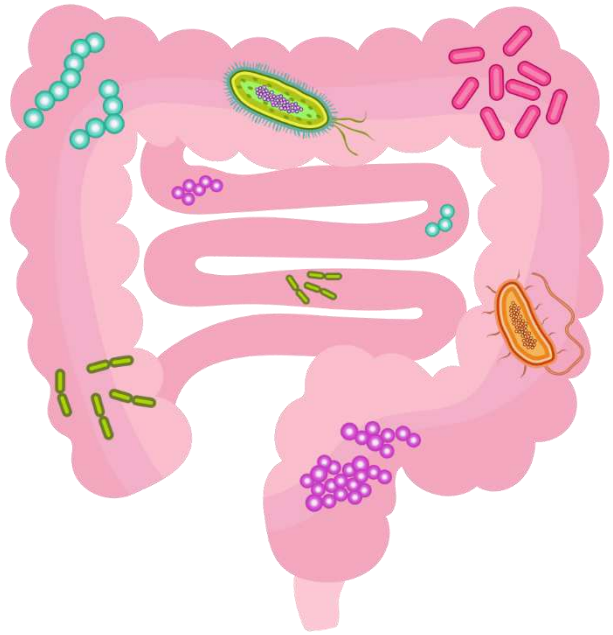
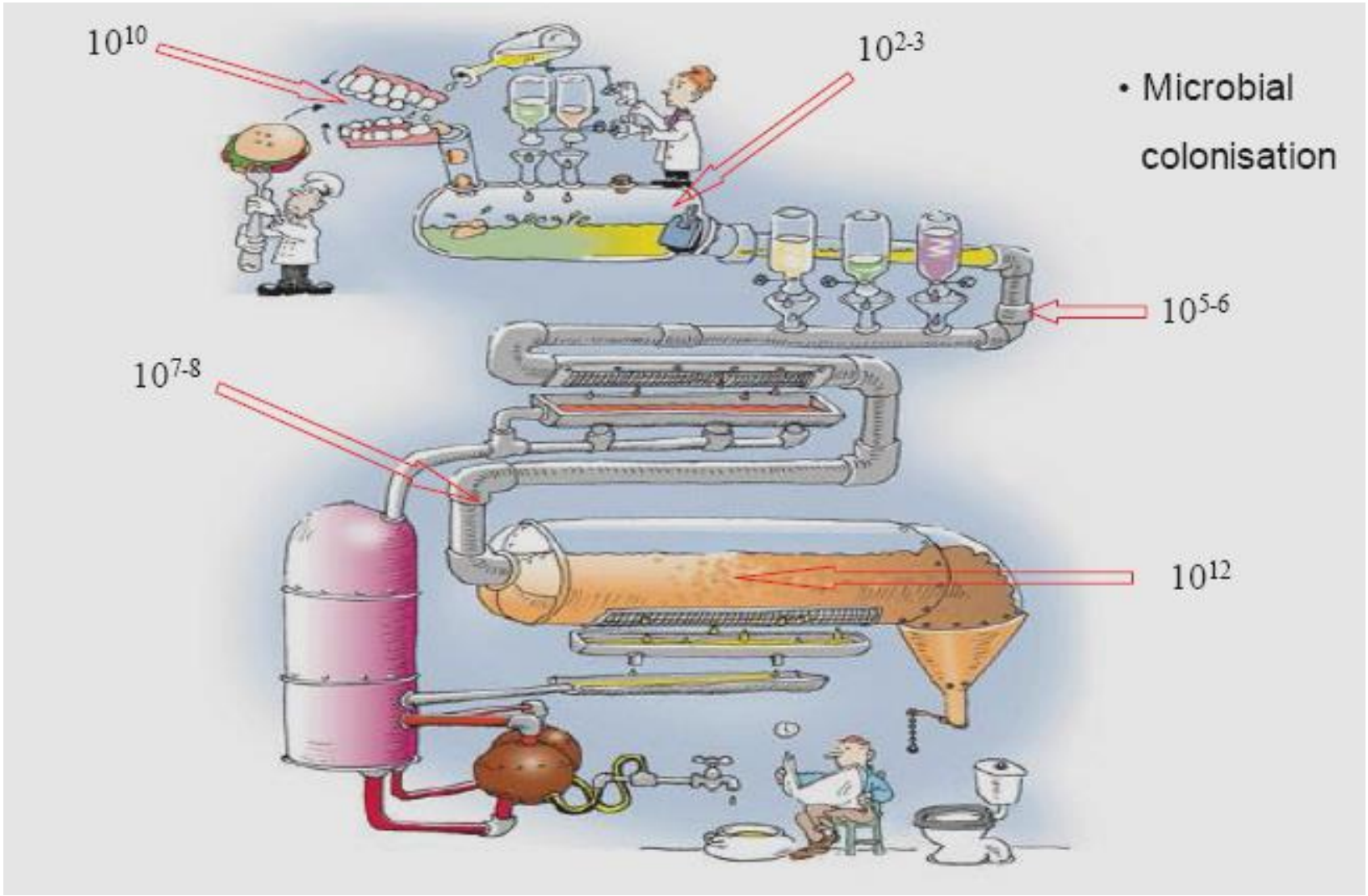
Outline

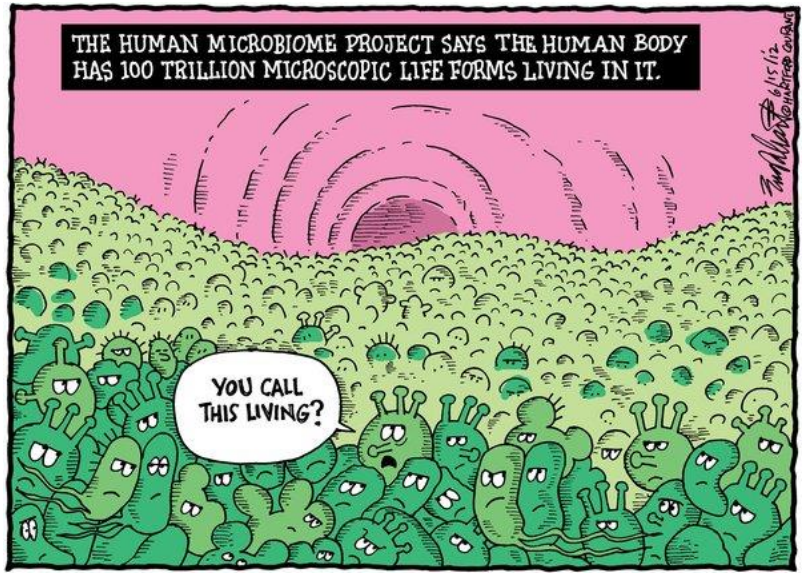
- Introduction microbiome
- Study: Probiotics in pigs
- Mucosal crosstalk gut – lung
- Study: Milk replacer in veal calves
- Conclusions



Gut microbiota important for gut health



Intestinal microbiome



1 - 2 kg bacteria

N = 10^{14} (human: 10^{13} cells)

800 - 1200 bacterial species

Additional organ

Function:

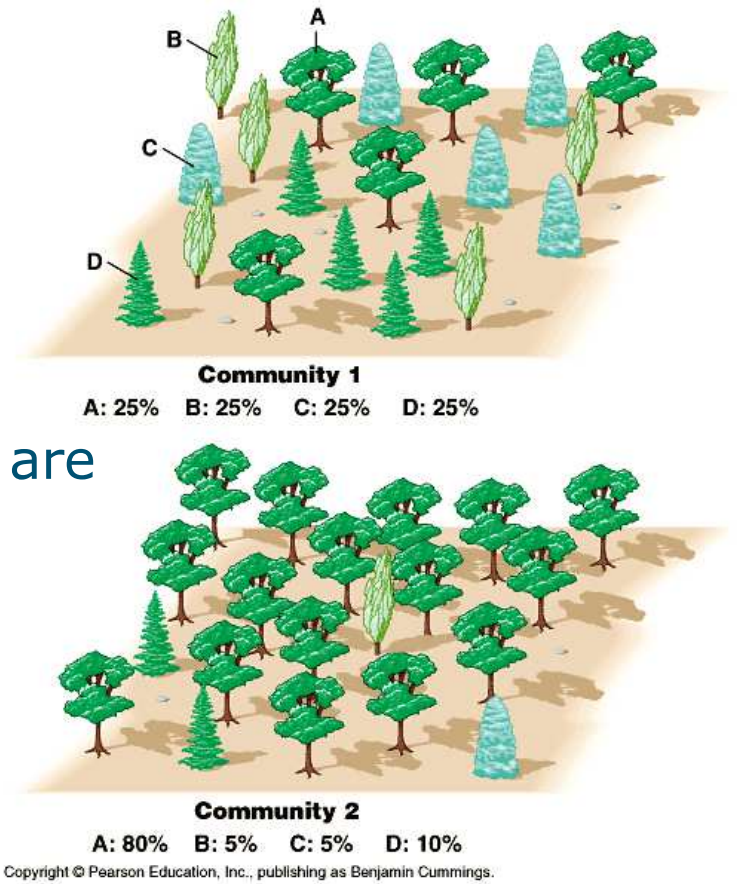
- Immunological development; 75% immune cells in GIT
- Digestive capacity
- Mental health (depression); gut-brain axis

5%

100%

How to measure microbiota?

- Sequencing
- Composition
  - 'Counting' the species
- Richness of population
  - How many different microbes are there?
- Diversity
  - Richness and distribution
- Function species
  - Largely unknown



Microbiome development in pigs

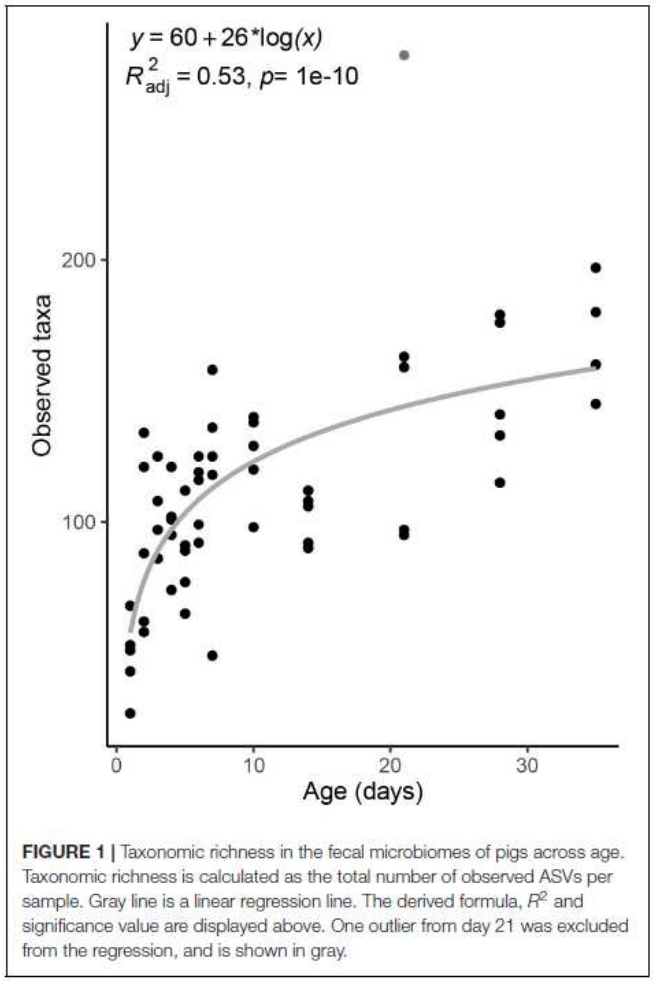
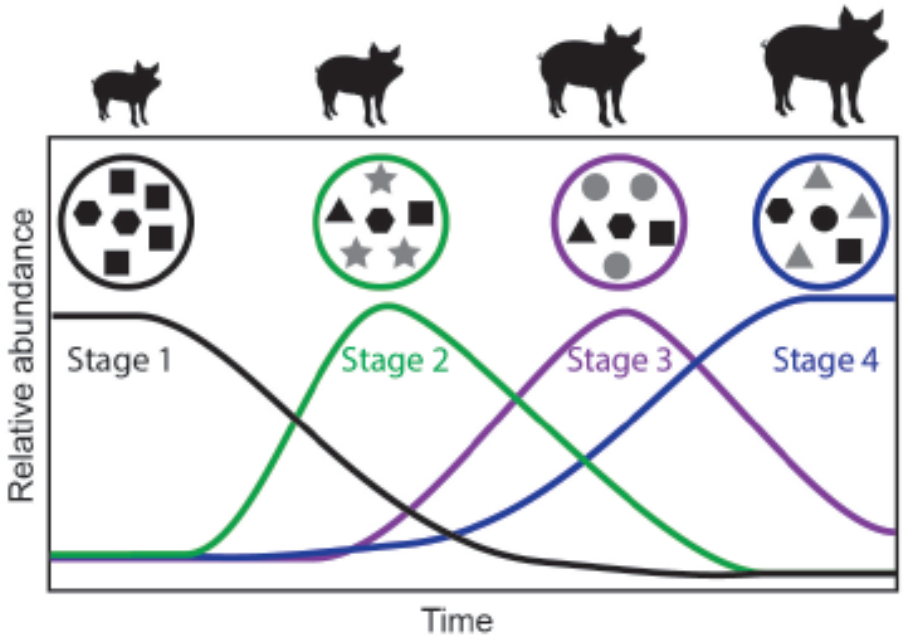


FIGURE 1 | Taxonomic richness in the fecal microbiomes of pigs across age. Taxonomic richness is calculated as the total number of observed ASVs per sample. Gray line is a linear regression line. The derived formula, R^2 and significance value are displayed above. One outlier from day 21 was excluded from the regression, and is shown in gray.



- Pig microbiome develops over time
- Number of species and composition change
- Several developmental stages can be recognized until weaning

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Jurburg and Bossers, Front. Microbiol., 2021

Management of expectations

- Microbiome is relatively new topic
- Current science is very descriptive
- Literature indicate relation with infectious diseases and vaccines
- WBVR has done descriptive studies on microbiota in livestock
- Microbiota is no holy grail (yet.....)

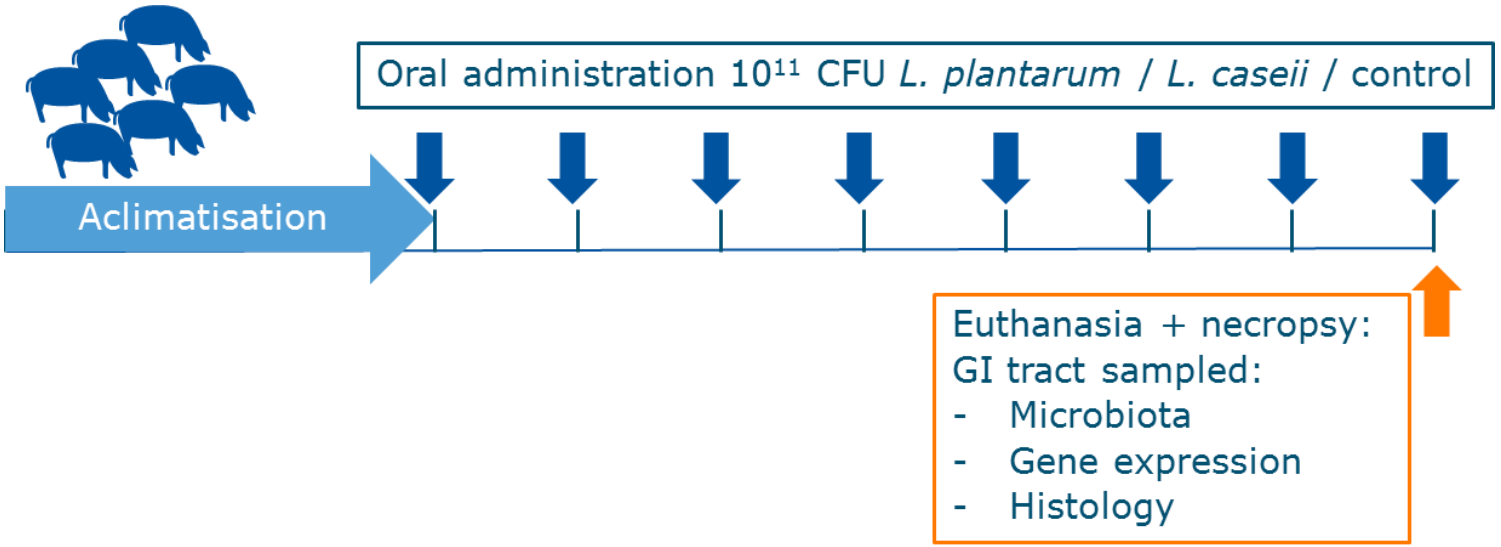
Microbiome affects immune responses to infections and / or vaccines

- Pre-vaccination microbiome affects vaccine response to *Mycoplasma hyopneumoniae* (Munyaka et al., Microorganisms 2020)
- Gut microbiome plays a role in PRRSV vaccine efficacy and in disease outcome of PRRSV / PCV-2 coinfections (Constance et al., Vet. Mic., 2021)(Ober et al., Vet. Mic., 2017)
- Probiotic treatment can ameliorate the effects of *Lawsonia intracellularis* infection (Muwonge et al., Animal Micorbiome, 2021)
- Probiotics can ameliorate effects of *Eschericha coli* challenge in weaned piglets (Zhang et al, Appl. Environm. Microbiol., 2017)

The effect of probiotics in pigs

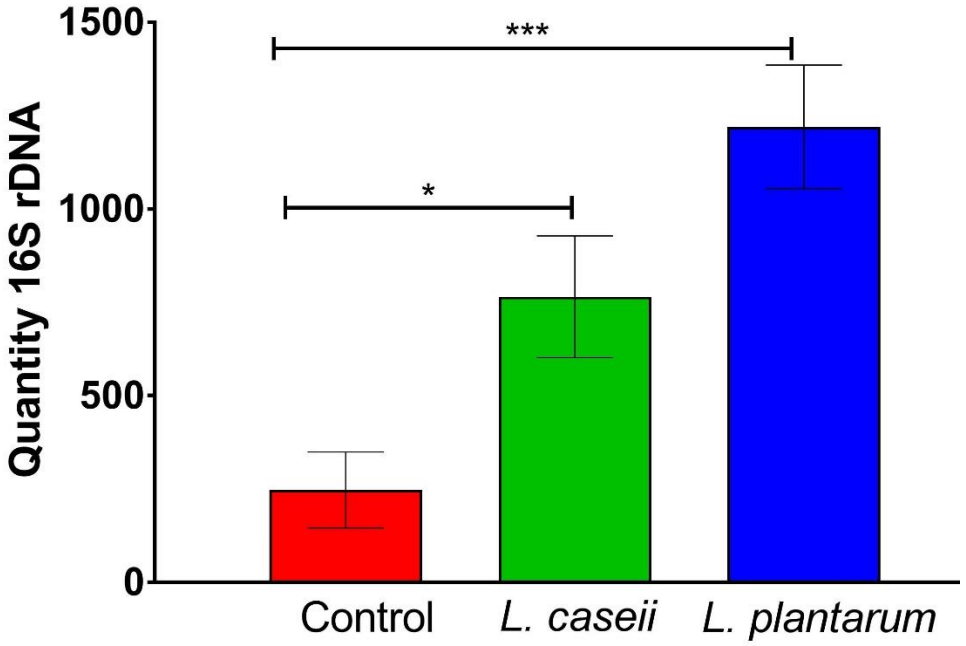
- Descriptive study to test if human probiotics affect pigs

- Three groups of six piglets
- 1. *L. plantarum*; 2. *L. caseii*; 3.Control
- Daily probiotic treatment
- Animals euthanized after 8 days and GI tract sampled



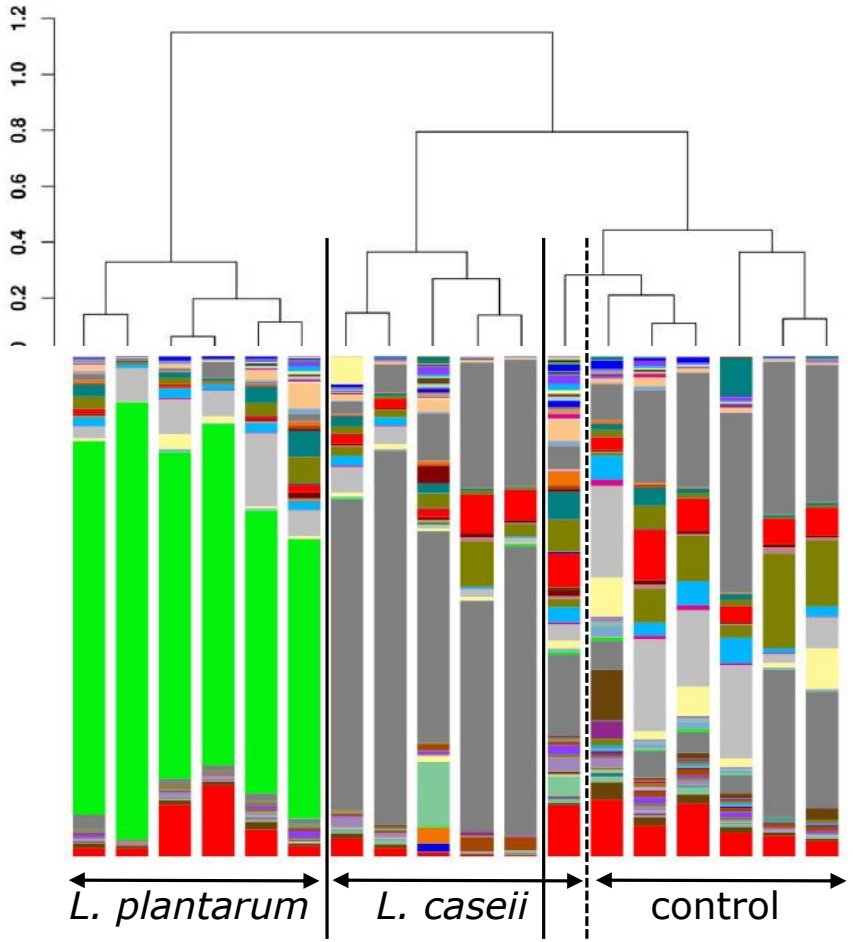
Probiotics affect microbiota quantitatively

- Amount of microbiota in jejunum determined using quantitative PCR on V3 of 16S rDNA
- Probiotic treatment significantly increased amount of microbiota
- *L. plantarum* stronger effect than *L. caseii*

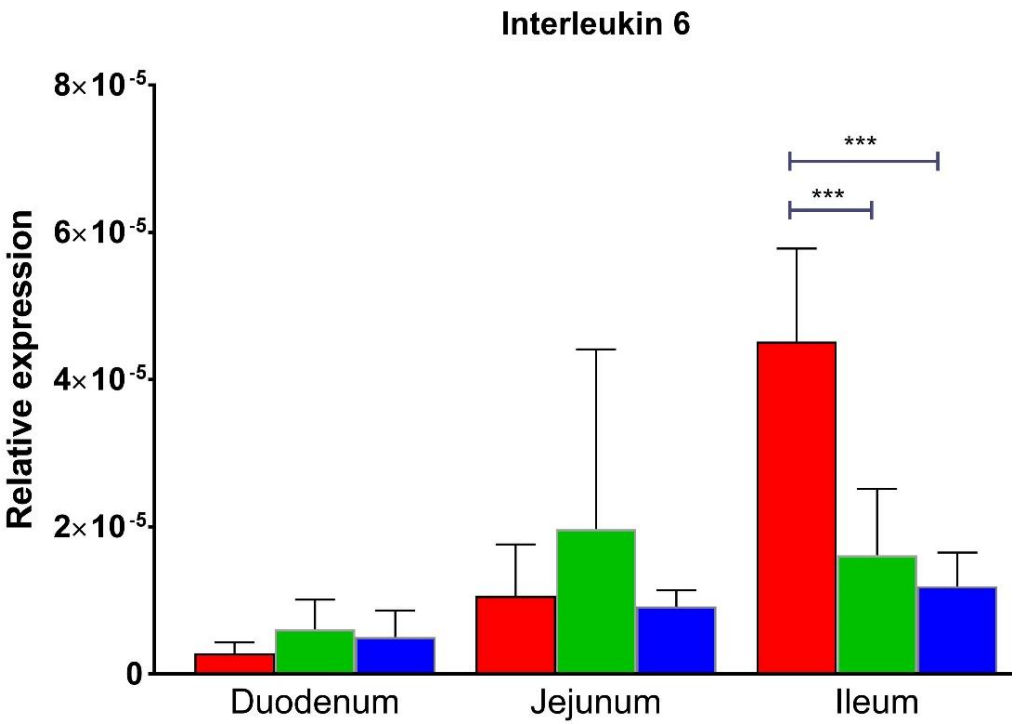


Probiotics affect microbiota composition

- Microbiota composition of jejunum samples
- Treatments can be recognized
- Lactobacilli dominantly present in microbiota
- If lactobacilli are removed from analysis → treatment groups can still be distinguished



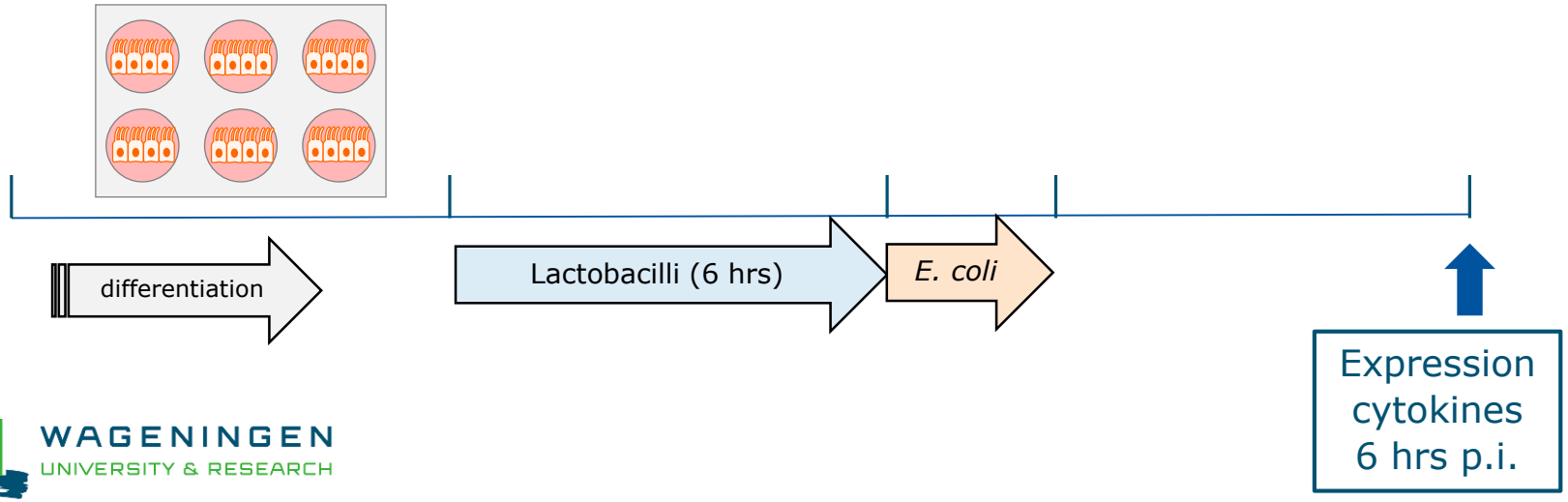
Probiotics decreases IL-6 expression



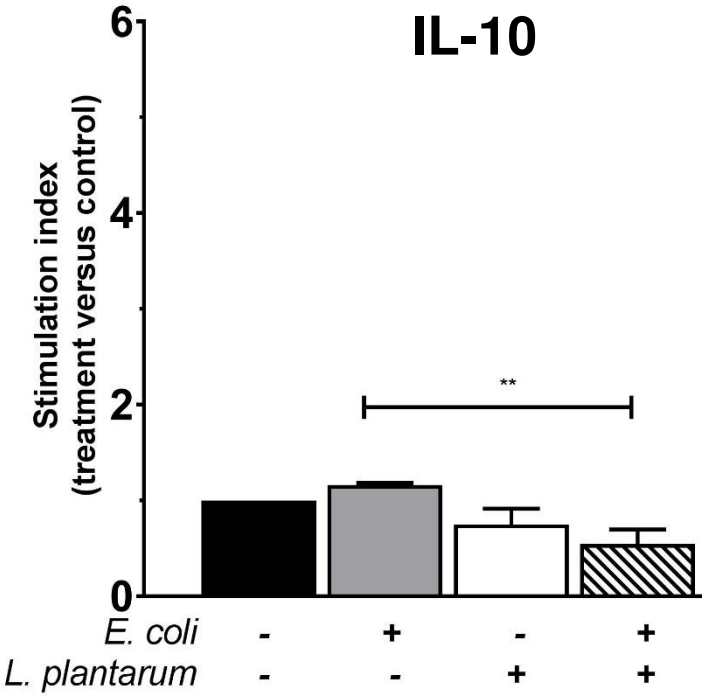
- IL-6 involved in pro-inflammatory process
- Significant decrease of gene expression compared to the control animals
- Only in ileum → Peyer's patches?

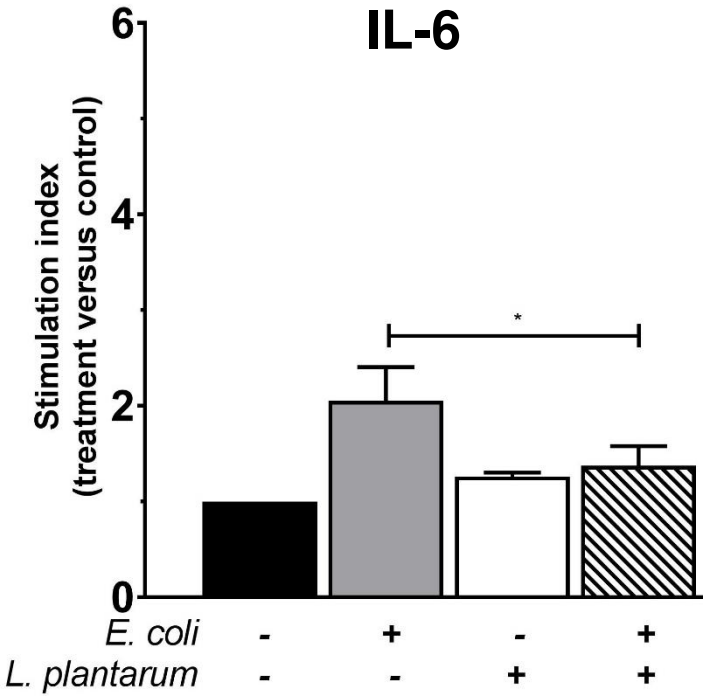
In vitro analysis of probiotics in combination with *Escherichia coli*

- Intestinal porcine epithelial cells (IPEC-J2)
- *In vitro* cultured mature intestinal cells
- Stimulated with Lactobacilli
- Infected with *Escherichia coli* (ETEC)
- Immune gene expression measured



L. plantarum reduces immune response to *E. coli*



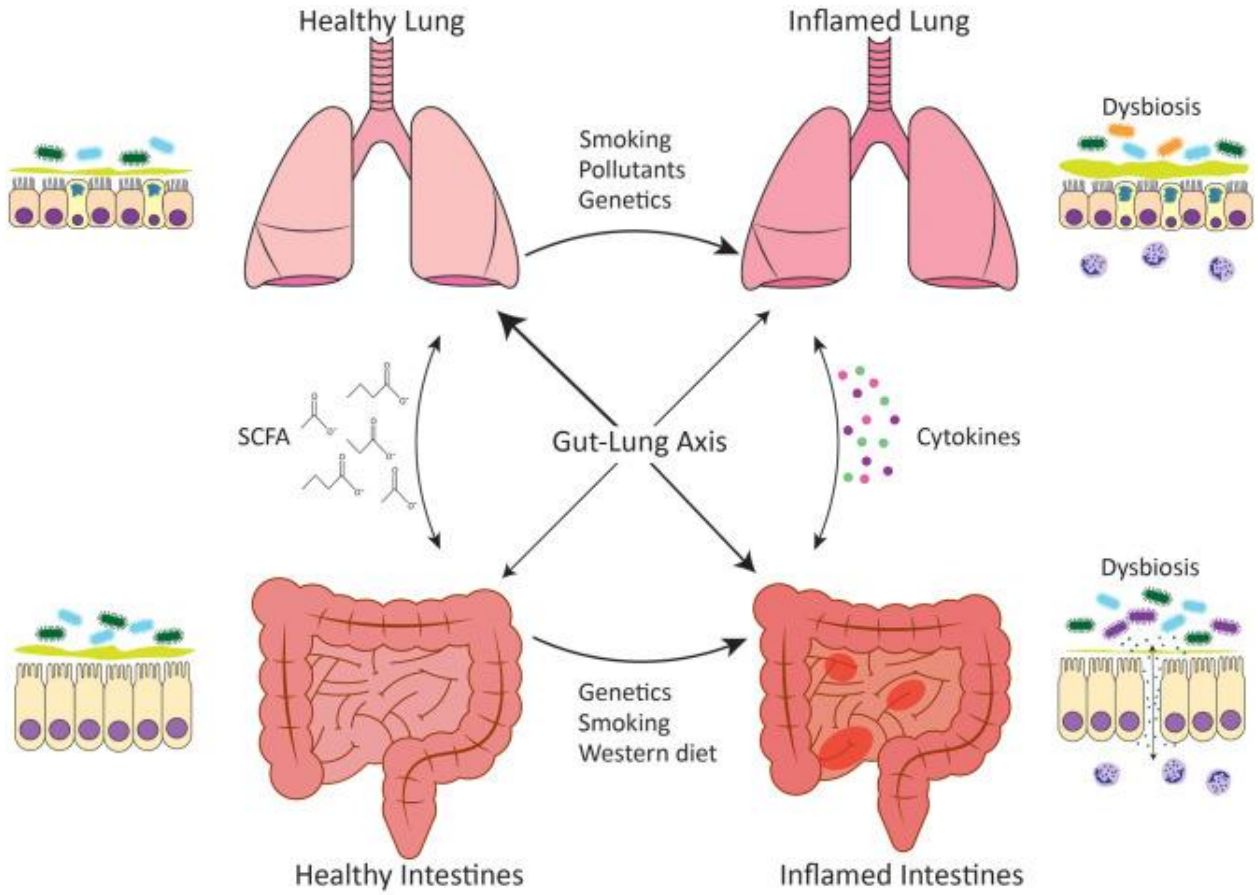


- *E. coli* induces IL-6 production
- *L. plantarum* significantly lowers expression of IL-6 and IL-10
  - Both in presence and absence of *E. coli*

Conclusions

- Probiotics change intestinal microbiota and affect the immune status of pigs
- Preliminary *in vitro* results indicate that probiotics affect the immune responses induced by *E. coli*
- Biological relevance yet unknown, but in humans probiotics dampen proinflammatory responses to promote health
- Current data suggest that probiotics in pigs might have beneficial effects on disease outcome

Mucosal cross-talk



Gut-lung axis

Communication between the intestines and the lungs occurs in both healthy situations and disease settings.

A healthy, fiber-rich diet promotes intestinal, and respiratory health.

Front. Immunol., 11 September 2020 | https://doi.org/10.3389/fimmu.2020.02144

Resilience to infection in veal calves

- Animals with problems in the lung often have problems in the gut
- Veal calves face a lot of disease problems in the first 12 weeks of life, either intestinal or respiratory
- In an environment with pathogens, **resilience** is critical to cope with infections and maintain health
- Innate and acquired **immunity** are key players in resilience
- Commensal **microbiota** shape the immunological development

AIM: To study the effect of milk replacer composition on gut ang lung health parameters including microbiota to improve resilience of veal calves.

Effects of milk replacer composition on gut and lung health in veal calves

	Experimental study		Field Study	
Milk Replacer	Plant Based	Milk Based	Plant Based	Milk Based
Age	1 – 49 days		Production Cycle	
No. of animals	30 (n = 15 per group)		200 (n = 100 per group)	
Parameters	Overall Health Immunology Lung Mucosal Development Gene Expression Lung / Gut Microbiota Gut		Health Parameters Performance Gene Expression Lung	

Composition milk replacers

	Milk Replacer Start		Milk Replacer Finish	
Milk replacer	Plant Based	Milk Based	Plant Based	Milk Based
Whey powder conc.	17	50		29
Whey powder	17		47	23
Fat filled whey powder (palm- & coconut-oil)	38	35	35	34
Delactosed whey	13	13	3	12
Wheat protein	6		6	
Soya conc.	4		4	
Starch	3		3	
Premix	2	2	2	2
TOTAL	100	100	100	100

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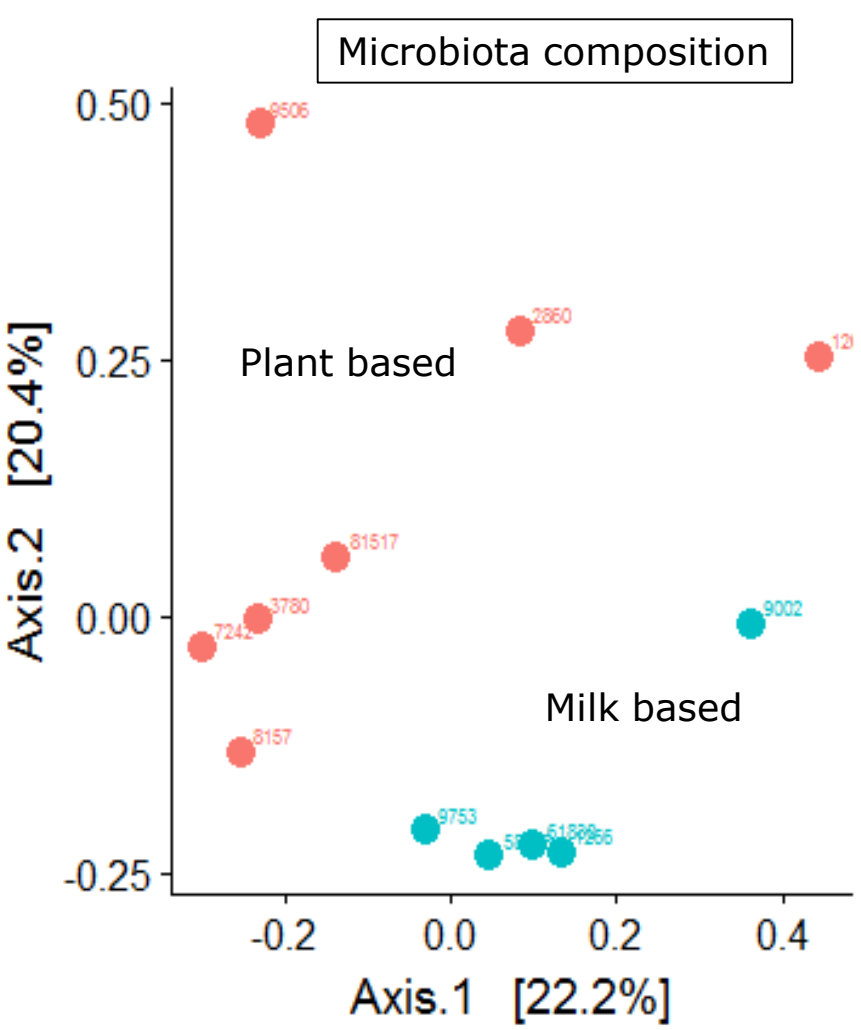
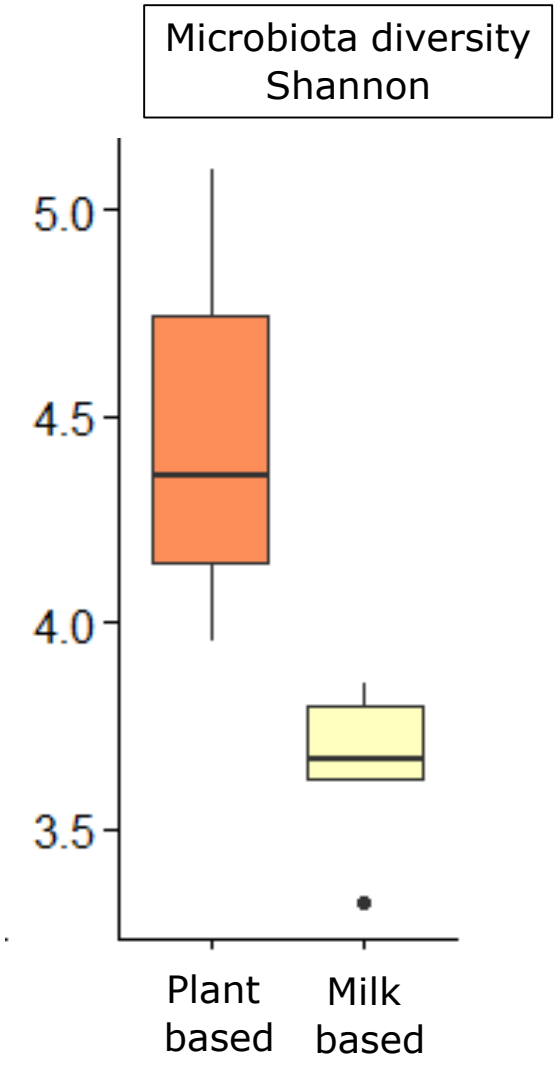
Milk replacer affects jejunum microbiota

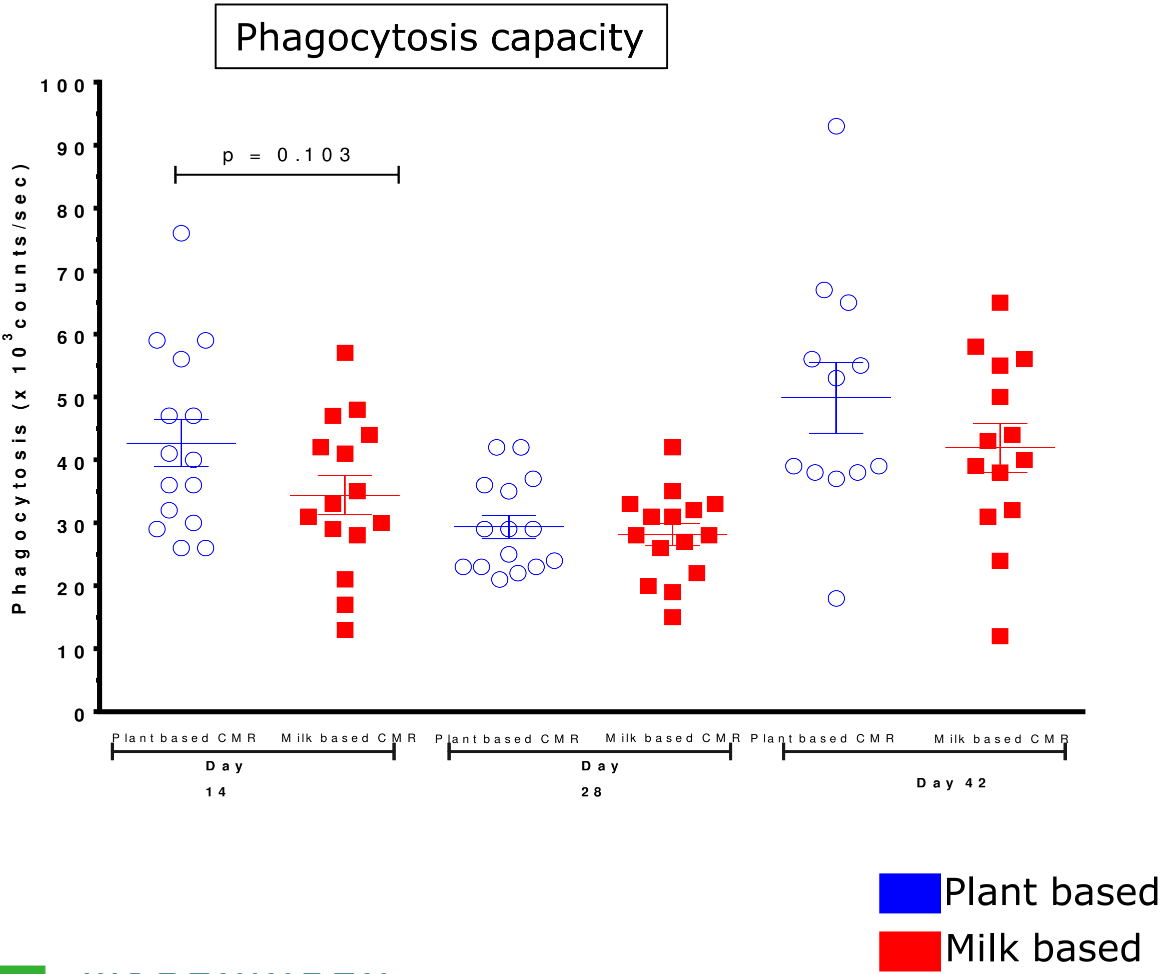




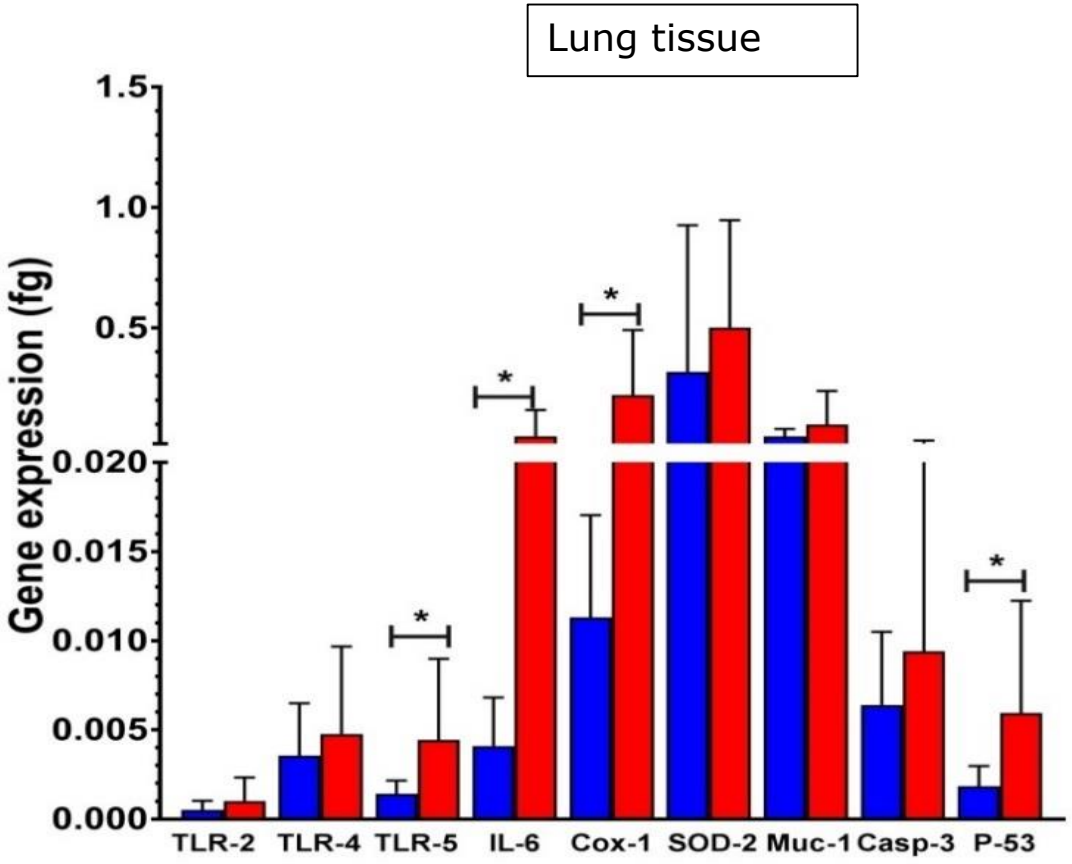
Milk replacer induced changes in lungs

- Broncheo-alveolar fluid (BALF) was collected
- BALF contains alveolar macrophages and other cells
  - Number of cells
  - Functionality of cells
  - Composition of cells
  - Gene expression of cells

Functionality of alveolar macrophages affected by milk replacer



Milk replacer affects respiratory immune gene expression



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Conclusions

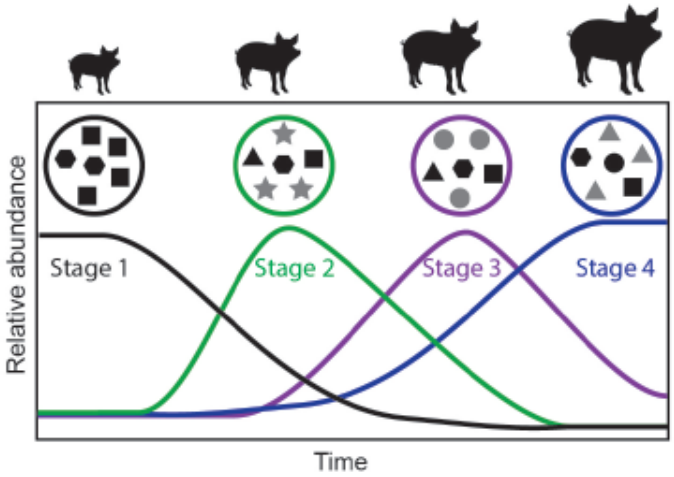
- Milk replacer has significant effects on **intestinal** development
  - Large changes in microbiota composition
  - No morphological changes in intestine
  - Changes in immune gene expression observed
- Milk replacer affected immune parameters in **lungs**
  - Functionality of cells in BALF differ
  - Immune gene expression in lungs differ
- Data are confirmed in larger field study

Perspective

- Feed composition strongly modifies microbiota development in the intestine of veal calves
- Feed composition changes functionality of immune cells in the lung of veal calves; thus lung health might be modified by feed composition
- A better understanding of the interaction between feed – microbiota – respiratory health (via mucosal cross-talk) could lead to improved health of calves

Microbiome management

- Microbiota affects immune responses
- Relevant for infection control
    - Less symptoms
    - Enhancing vaccine efficacy
- Unknown if there is a "window of opportunity": at which age does it work?
    - Only during development microbiota?
    - Only in young animals?
- New field of research with large implications
    - Vaccine strategy
    - Antibiotic management
    - Feed strategy
    - Infection control



Questions

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