Nutrigenomics
Chances and challenges in animal production

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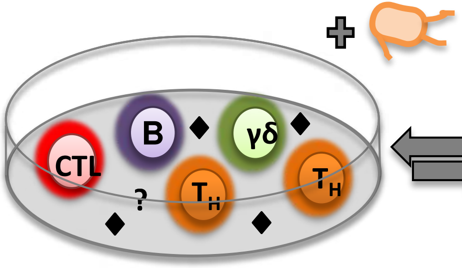
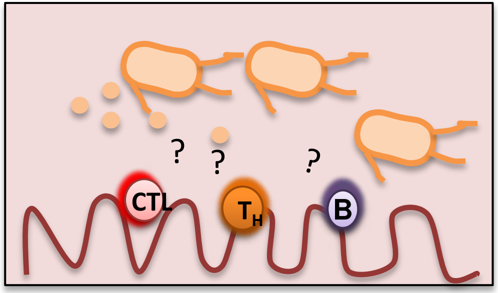
How can we improve animal health and performance?

FEEDING > functional feed additives as e.g. Probiotics

***In vitro* experiments based on primary cell culture**



***In vivo* experiments**



OMICS to investigate
Protein expression
Gene expression and regulation
Metabolism

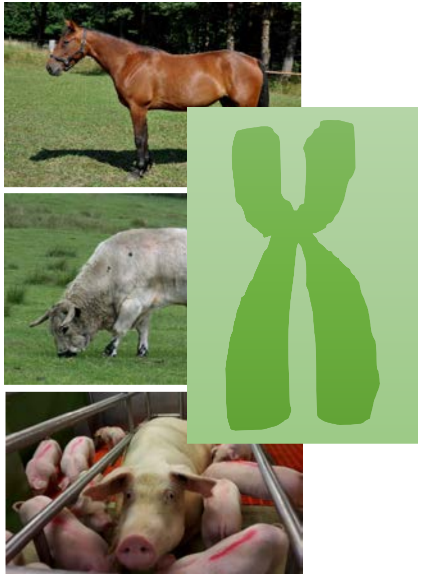
Aim

Elucidation of molecular mechanisms of physiological processes

NUTRIGENOMICS

The host genotype influences the effects of feed and feed additives

Knowledge about effects from feeds/feed formulation and feed additives on the host allow the development of precision diets/interventions

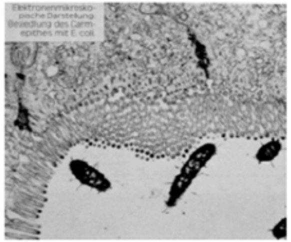


Knowledge about Genomics allow precision feeding strategies e.g. resource efficient feeding of monogastric animals

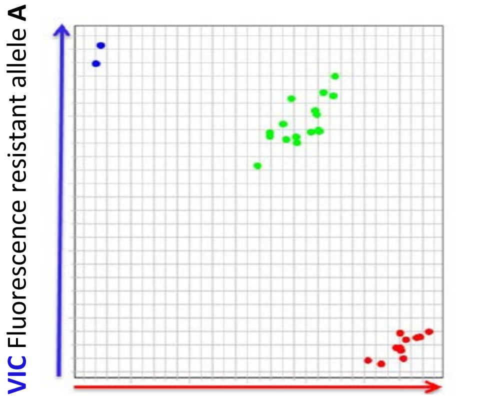
Effects of feed and feed additives on the host

The host genotype influences the effects of feed and feed additives

Example 1: of the influence of a genotype on the affectivity of feed additives during Enterotoxigenic Escherichia coli (ETEC) infection



- Pigs could be genetically resistant towards ETEC infection
- Associated SNP (G > A) within the Fucosyltransferase - gene (SSC6)



➢ Development of an allele-specific PCR assay to discriminate the receptor type

G = sensitive allele **S**, dominant; A = resistant allele **r**, recessive

Kreuzer et al. 2013b, *Vet. Microbiol*, 163:392-4

Application of developed gene test in feeding experiments

❖ Genetic testing of all animals used for feeding trails were possible

N = 430	ETEC F18 (SNP G > A)	ETEC F4 ab/ac (Haplotype CAT > TGC)
SS (sensitive)	371 (86 %)	185 (42 %)
Sr (sensitive)	56 (13 %)	212 (49 %)
rr (resistant)	3 (1 %)	33 (8 %)
allele S	798 (93 %)	582 (68 %)
allele r	62 (7 %)	278 (32 %)

- **Allele frequencies for the dominant sensitive allele were high**
- **Resistent animals could be excluded from the feed additive studies**

Kreuzer et al. 2013a, *Vet. Microbiol*, 162:293-5

Kreuzer et al. 2013b, *Vet. Microbiol*, 163:392-4

The host genotype influences the effects of feed and feed additives

Example 2: Genome wide association analyses of SARA traits in Holstein cows

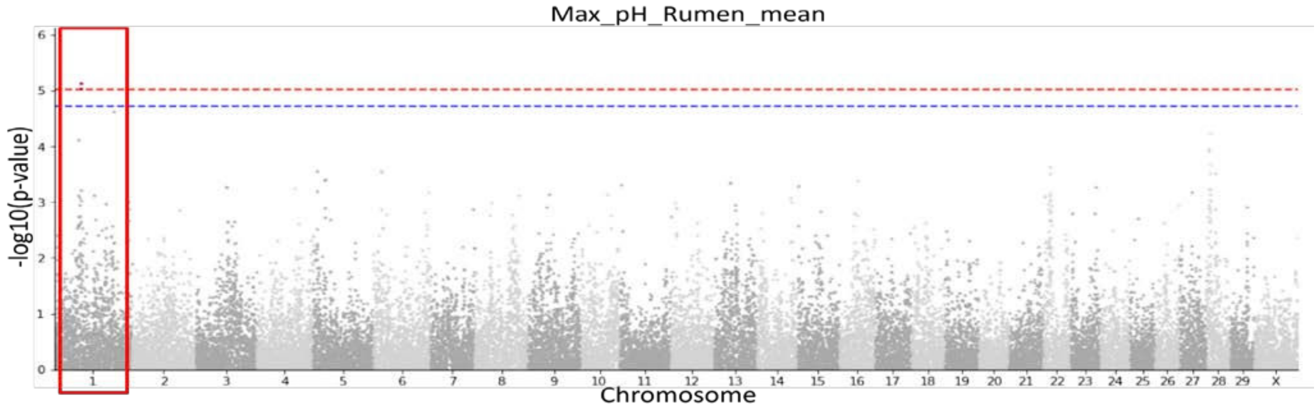
<u>Genotypes</u>

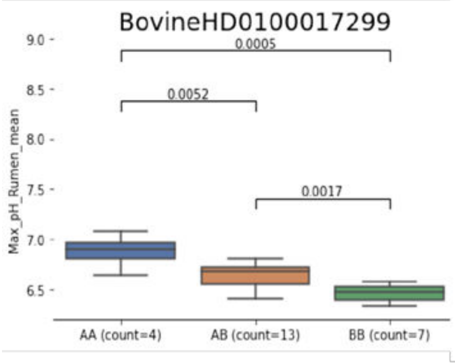
SNP-Chip (GGP Bovine 100k) analyses of DNA from the hair bulbs of 24 first-lactation Holstein cows

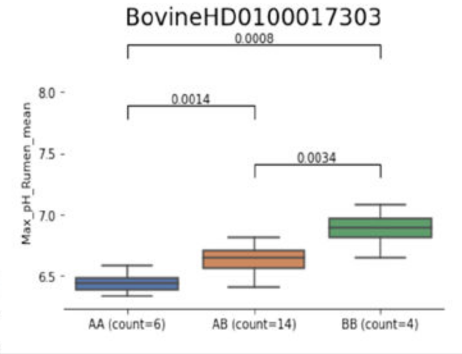
<u>Phenotypes</u>

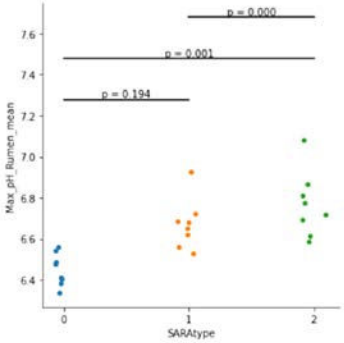
- Milking traits: Milk yield kg/d and ECM kg/d in week 2 and mean over the experiment (first 10 weeks in lactation)
- Rumen pH: Min pH, Max pH, Mean pH for week -3 (before calving) and week 2 (after calving), as well as mean over the whole experiment (13 weeks, week -3 until-week 10)
- Fecal pH: pH for week -3 (before calving) and week 2 (after calving), as well as mean over the whole experiment (13 weeks, week -3 until-week 10)
- SARA-types (HIGH, MOD, LOW). The SARA susceptibility was assessed by cluster analysis of several rumen pH metrics.

Results





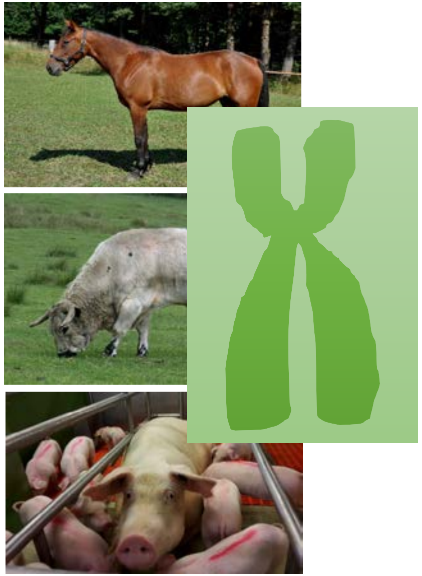




- → GWAS with trait data related to SARA revealed 20 significant or suggestive genetic markers when setting that there must be at least 3 individuals per genotype group.
- → From these, two SNPs located in the Intron of the *LSAMP gene* associated with max_pH_Rumen_mean are interesting candidates for further investigations >>> **genotype influenced effects of high grain feeding**

The host genotype can influence the effectivity of feed and feed additives

Knowledge about effects from feeds/feed formulation and feed additives on the host allow the development of precision diets/interventions

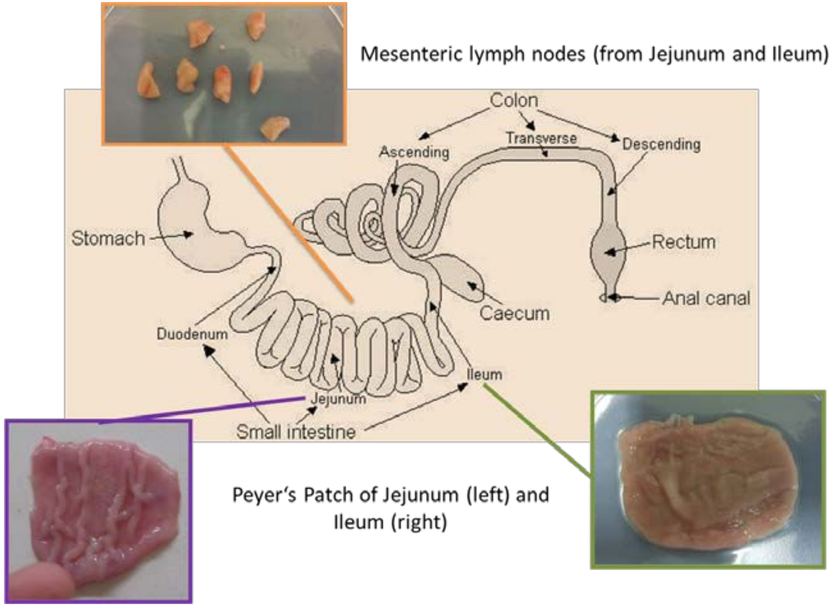


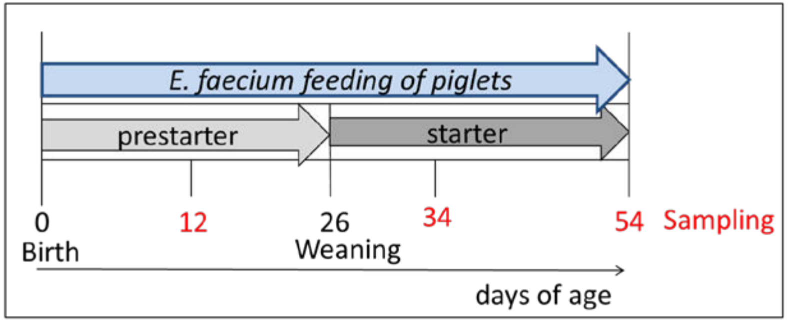
Knowledge about Genomics allow precision feeding strategies e.g. resource efficient feeding of monogastric animals

Effects of feed and feed additives on the host

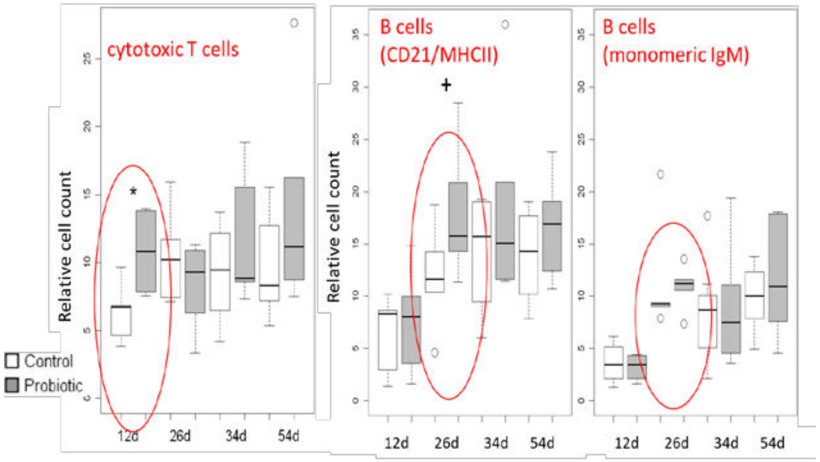
Example: Probiotic feed additives in swine nutrition, experimental design

- *Enterococcus faecium* (EF) dietary supplementation (4.2 x 10^6 cfu) for sows and piglets
- EF-feeding of sows beginning 4 wk before partum, piglets all time
- **6** individuals of each group (control, *E. faecium*)
- Mesenteric lymphnodes of Jejunum and Ileum, Peyer's patches of Jejunum and Ileum and blood

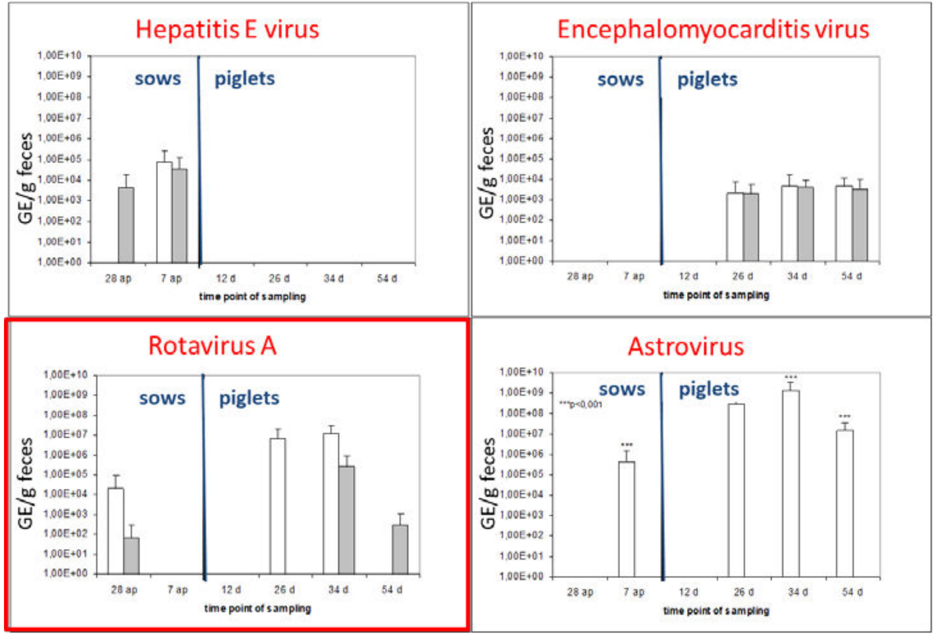




Example: Probiotic feed additives in swine nutrition



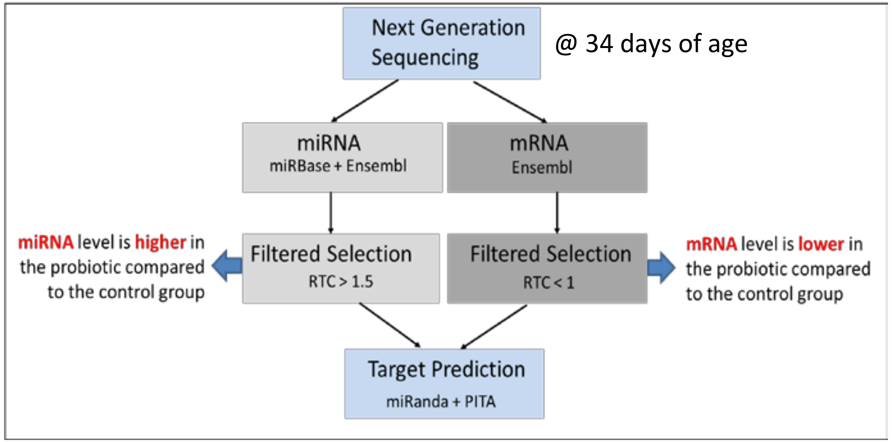
Preweaning increase of cytotoxic T cells and B cells in peripheral blood mononuclear cells

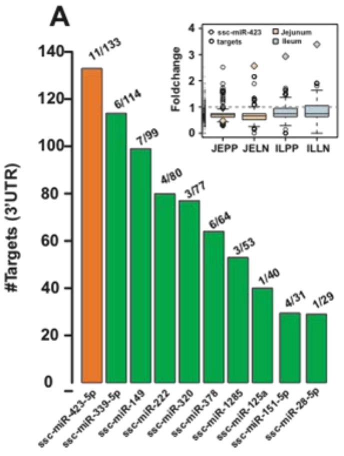


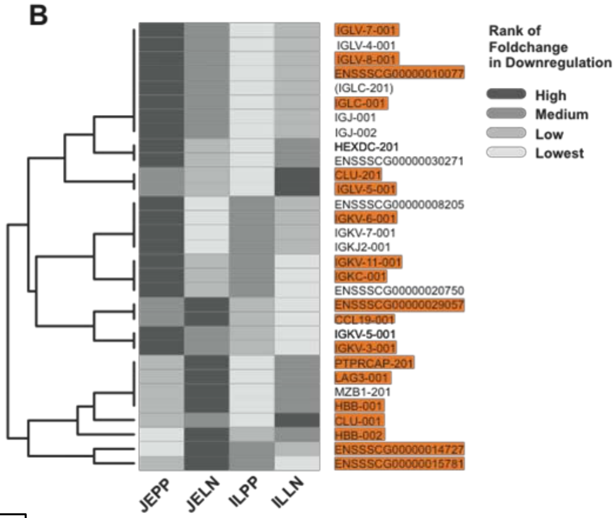
- ➢ Results indicate that probiotic bacteria can affect virus shedding **dependent on the type of virus**.
- ➢ Although this effect seems to be caused by changes in the immune status of pigs, mechanisms have to be further elucidated.

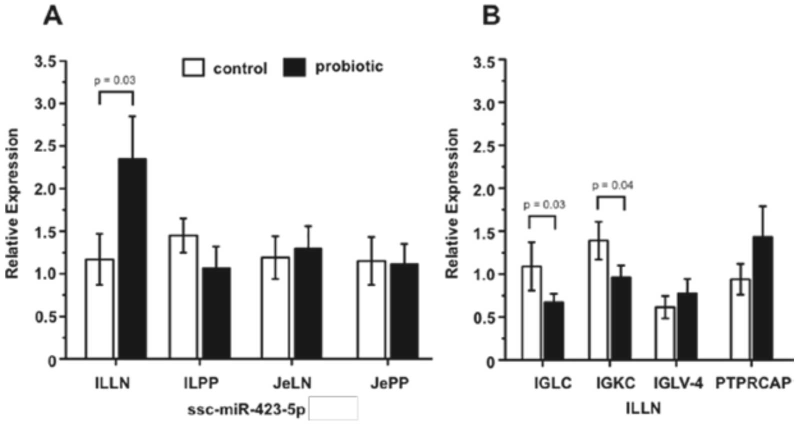
Kreuzer et al. *Veterinary Research* 2012, 43: 58

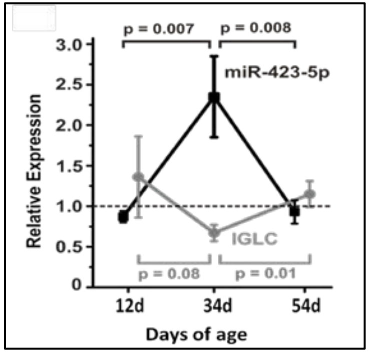
Example: Probiotic feed additives in swine nutrition, applying OMICs











- ➢ Additional examination of 12 and 54 days old piglets showed an anti-correlation of **miR-423-5p and IGLC**

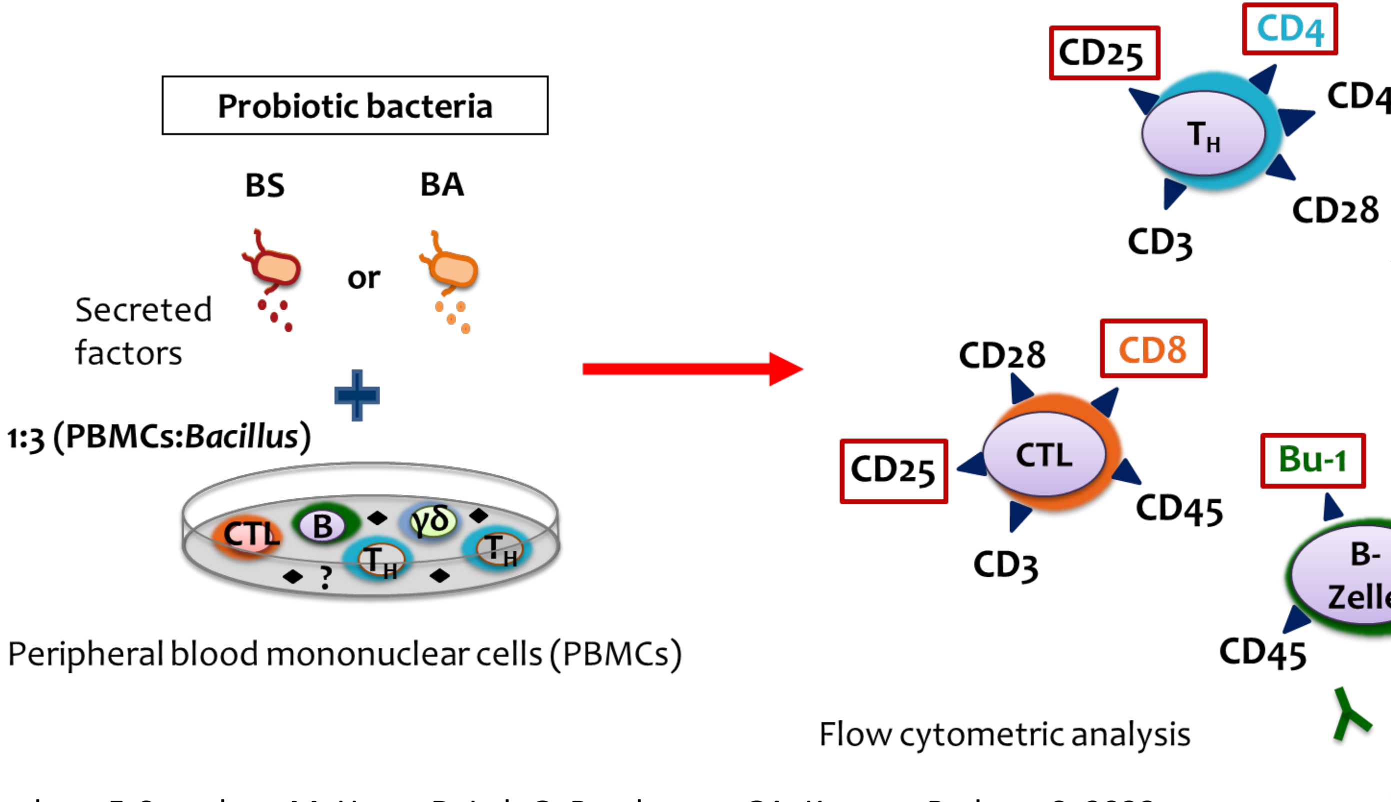
➔There seem to be miRNA mediated effects through *E. faecium*

Kreuzer-Redmer et al. Appl Environ Microbiol 2016, 82: 2263-2269

Current Research – Motivation

- It is **not known**, if the observed *E. faecium* effects are based on direct or indirect signaling mechanisms >>> as well as for many different functional feed additives as phytogenics
- For mechanistic studies: simplified cell culture experiments are necessary to exclude uncontrollable conditions within the animal; additionally, animal experiments can be reduced
- Primary cultured immune cells can serve as a model. Already established for:
  - **<u>Chicken</u>**
  - **<u>Pig</u>**
  - Horse
  - Cow
- Additionally, the role of **<u>miRNAs</u>** involved in the action of feed additives on signaling pathways in the host should be investigated.

Application of chicken immune cell assay



Cell surface marker:

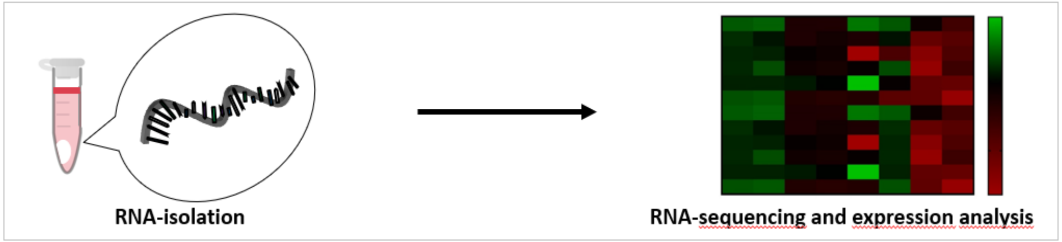
CD4 – T-helper cells
CD8 – cytotoxic T cells
CD25 – activated T cells
Bu-1 – B cells

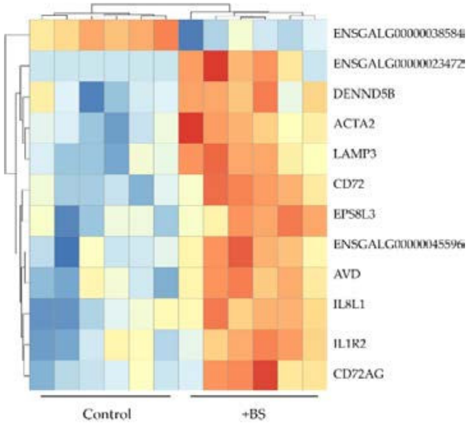
Larsberg F, Sprechert M, Hesse D, Loh G, Brockmann GA, Kreuzer-Redmer S. 2023.
Probiotic Bacillus Strains Enhance T Cell Responses in Chicken. *Microorganisms*, 11(2):269.

Application of chicken immune cell assay applying OMICS

RNA-sequencing:

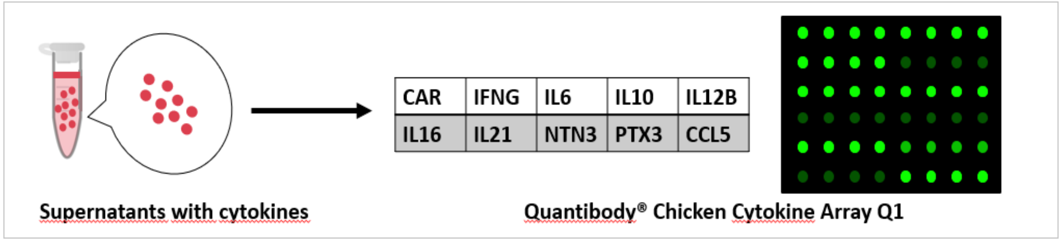
- Library preparation (10 ng RNA): SMART-Seq Stranded Kit (Takara)
- Sequencing parameters: 2x 100 bp, NovaSeq6000





Chicken Cytokine Array:

- Quantibody® Chicken Cytokine Array Q1 (RayBiotech)

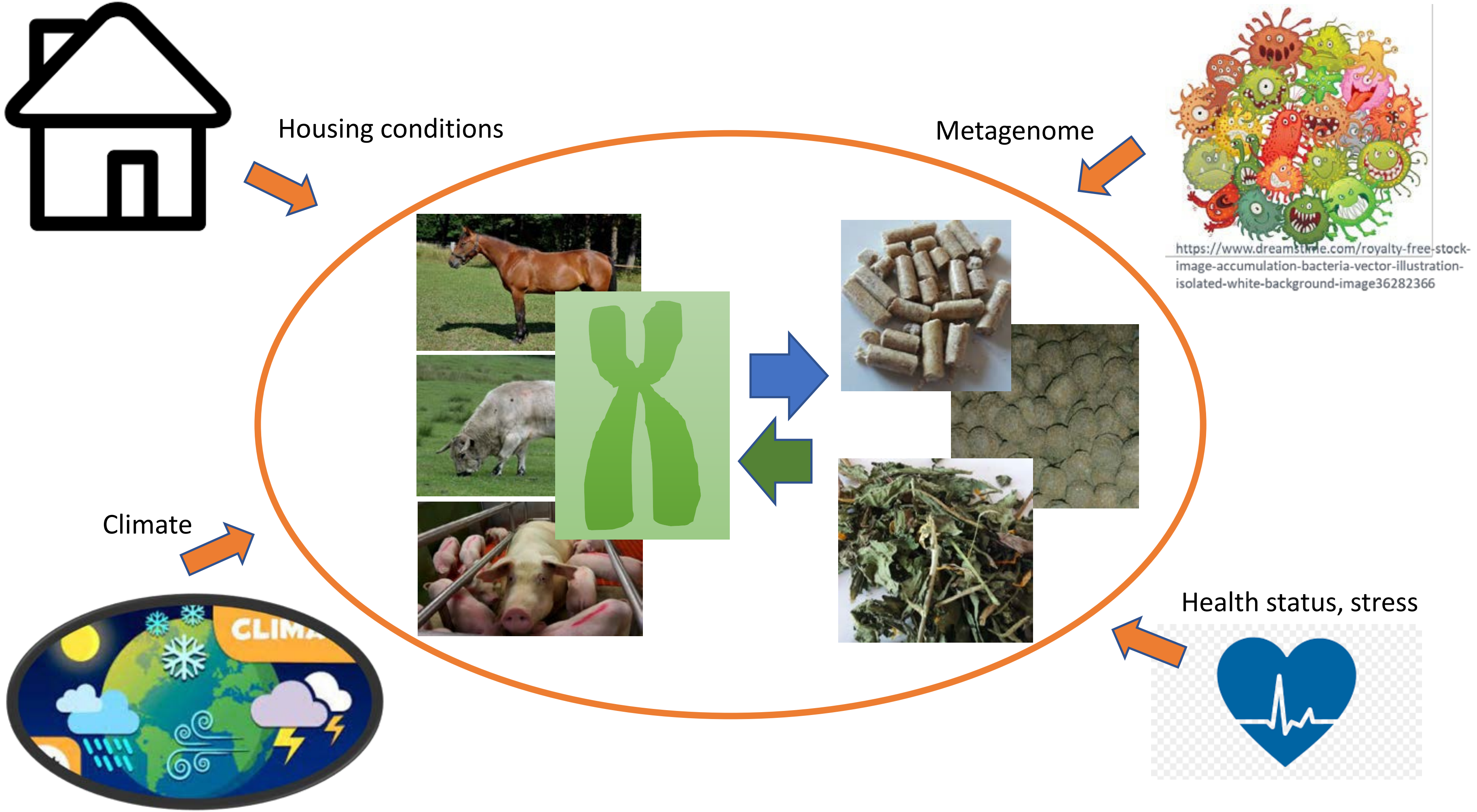


- ➢ BS and BV stimulated cell surface marker and surface marker gene expression of T-helper cell
- ➢ BS most likely stimulated Tregs

Larsberg F, Sprechert M, Hesse D, Falker-Gieske C, Loh G, Brockmann GA, **Kreuzer-Redmer S**. 2024. In vitro assessment of the immunomodulatory effects of probiotic Bacillus strains on chicken PBMCs. *Frontiers in Immunology* 15:1415009.

Future Research Perspective

Identification of the (bi) directional interaction of the host with feeding



Climate for Kids - Types of Climate, Youtube Channel Smile and Learn

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