Gut microbial colonization in day old chicks

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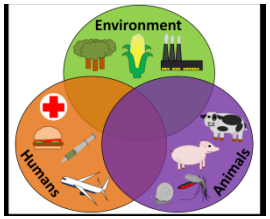
Trends

- Robustness; Resilience.
  - Ability to keep healthy, or recover from disease (with minimal human intervention).
  - Emphasis on Health, rather than on Disease

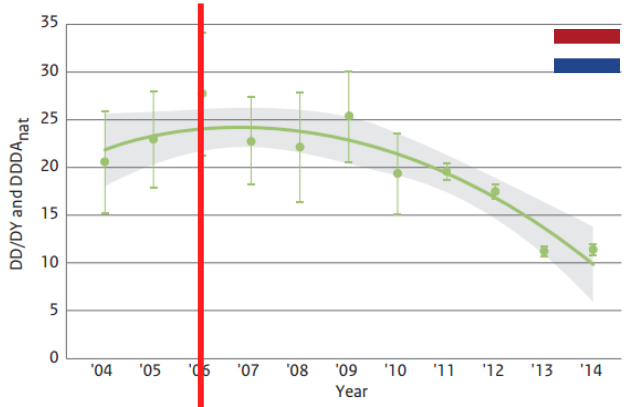
- Resource efficiency

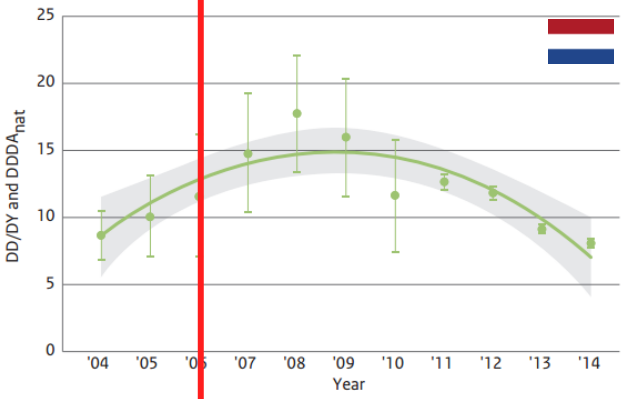
- One health concept:
  Interactions animal health – human health

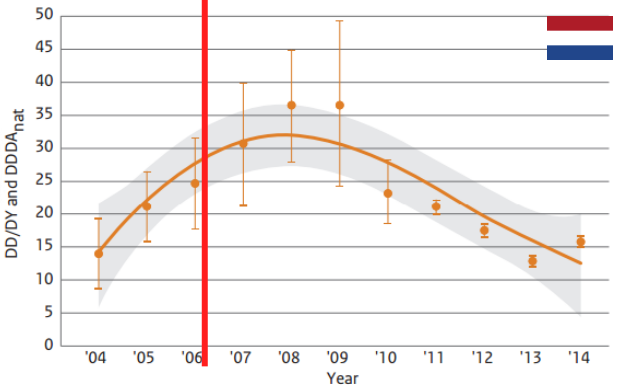


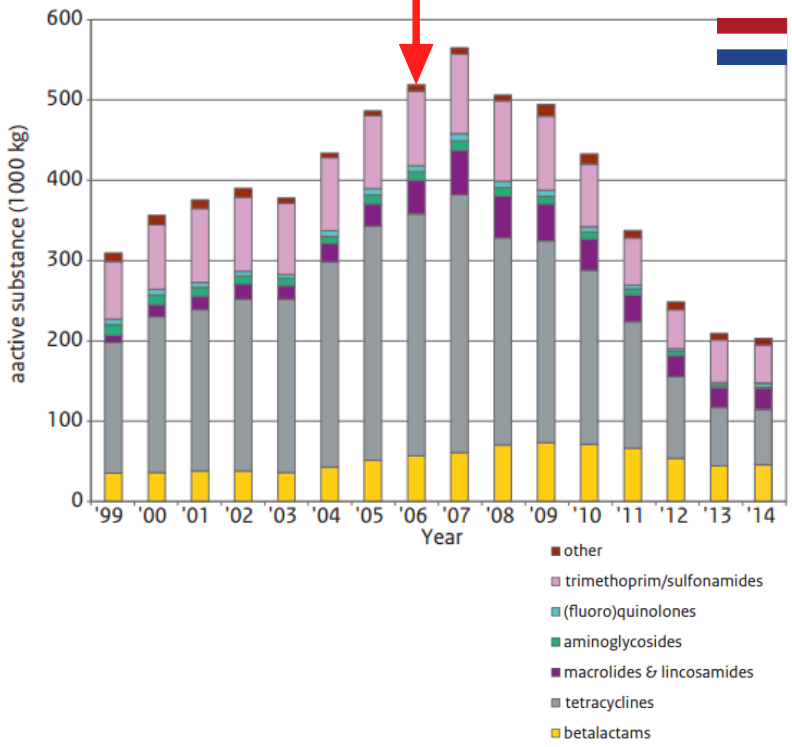
- Reduction of antibiotics use
  (and general veterinary interventions)

Antibiotics









Animal health and resilience:

Immuno-competence,

Role of the gut

Gastro-intestinal tract

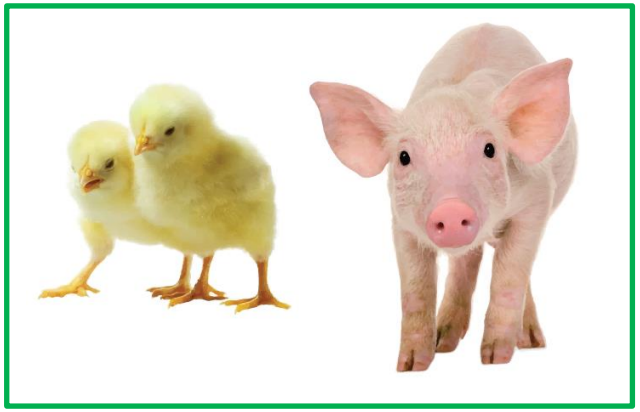
- **Gut is the gatekeeper of health**
  - ✓ 70% of the immune cells located in mucosal tissue

- **Gut is important for animal performance**
  - ✓ Feed efficiency / growth

Healthy

Disturbed

Gut is the gatekeeper of health

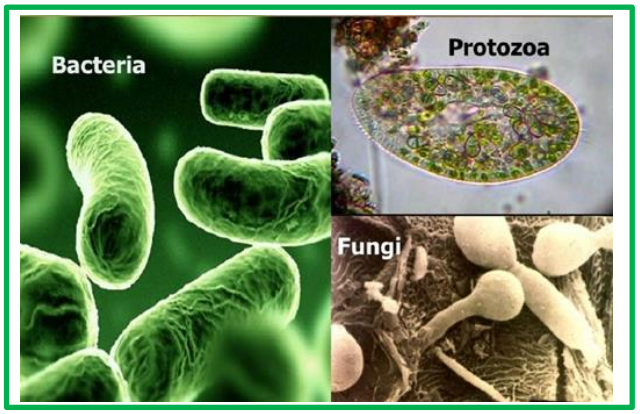


Host

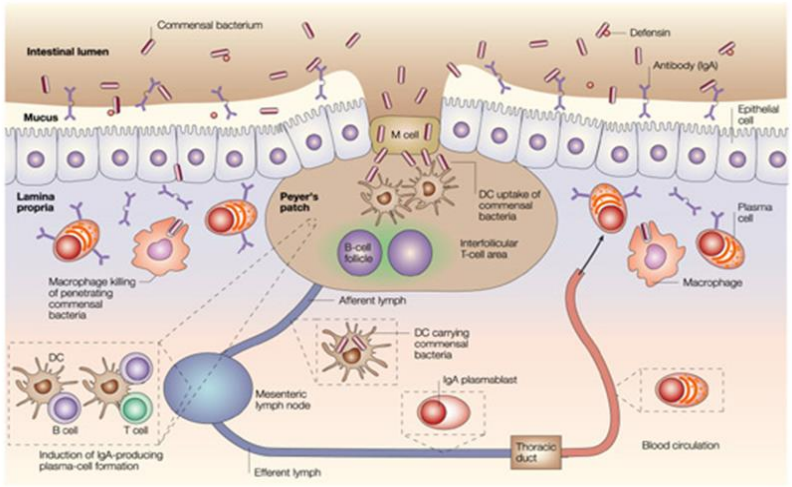
GUT

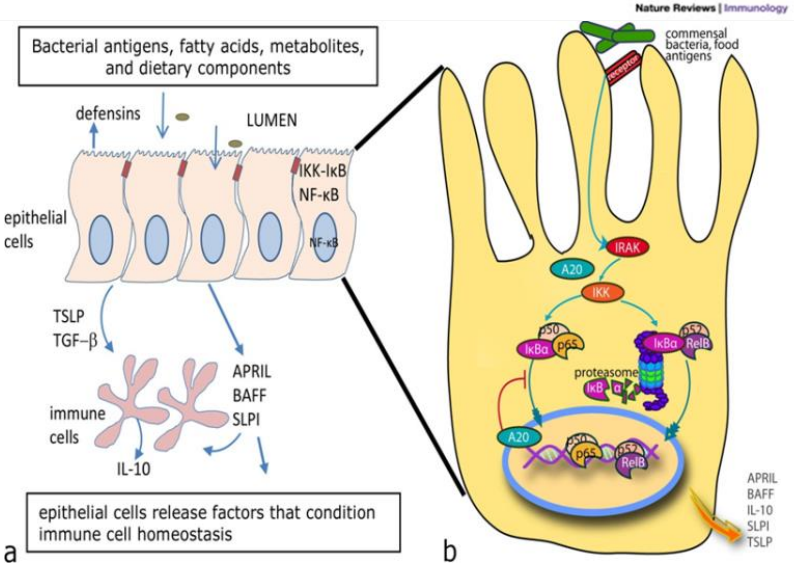
Microbiota

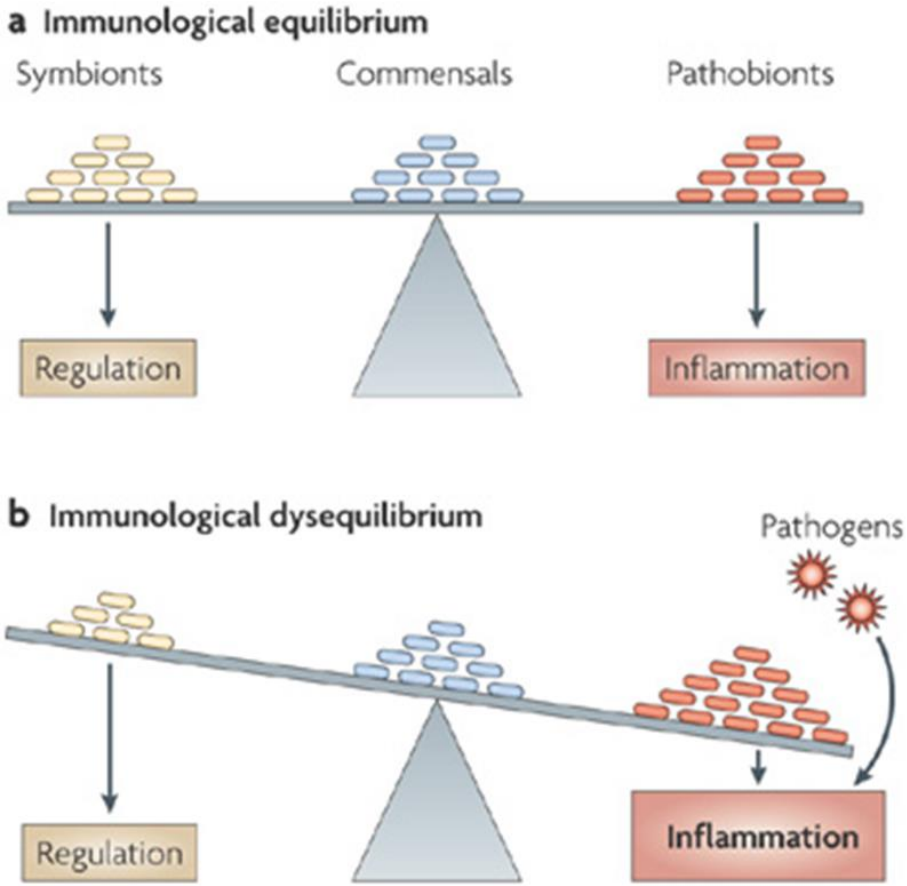
Environment



Interactions between intestinal microbiota and the immune system







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Factors influencing gut health

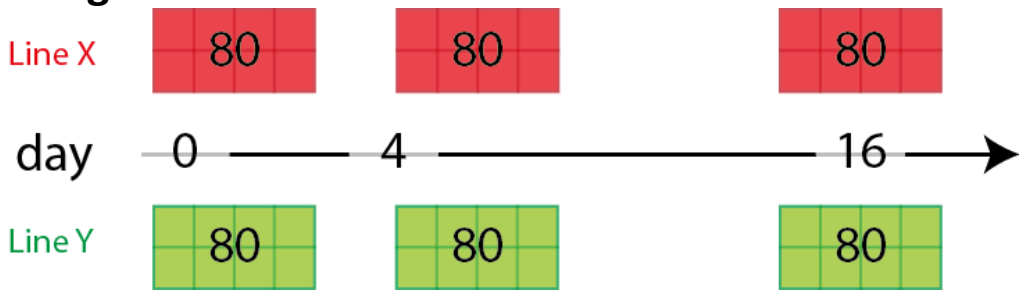
- **Host**
  - ✓ Genotype

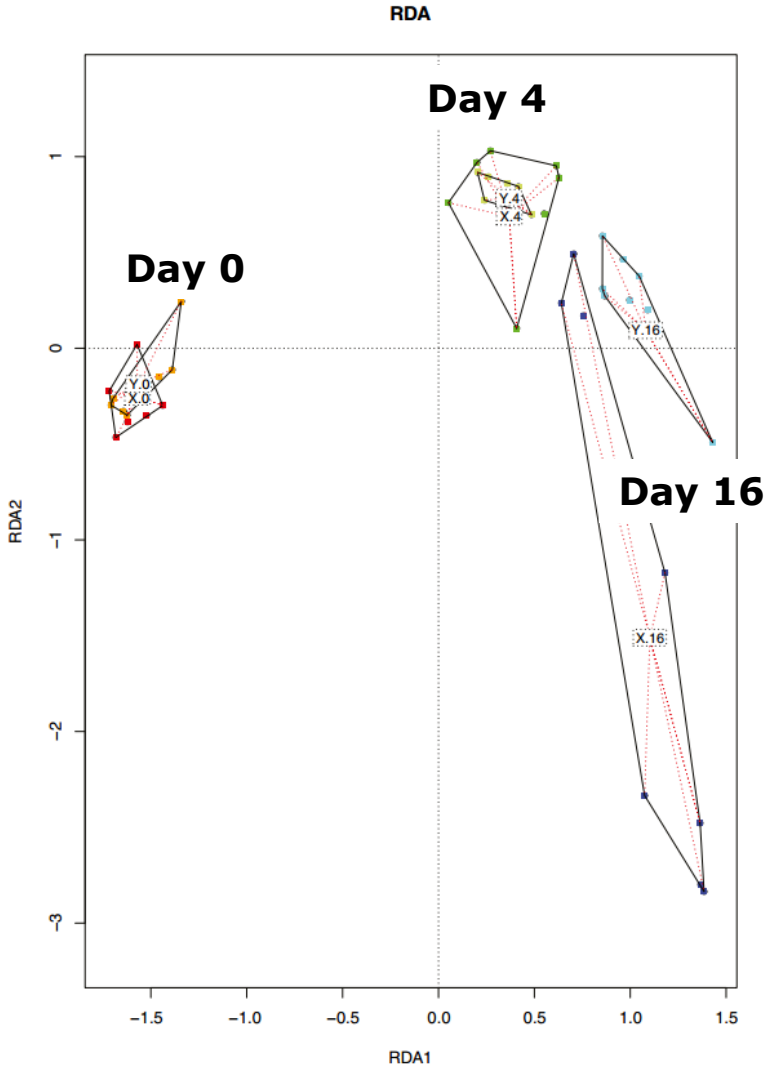
- **Environment / Management**
  - ✓ Early life antibiotic treatment

- **Nutrition**
  - ✓ Oligosaccharides

Microbiota composition in genetically divergent broiler lines

Design





- Divergence between groups in time
- Colonization of gut microbiota is influenced by the genetic background

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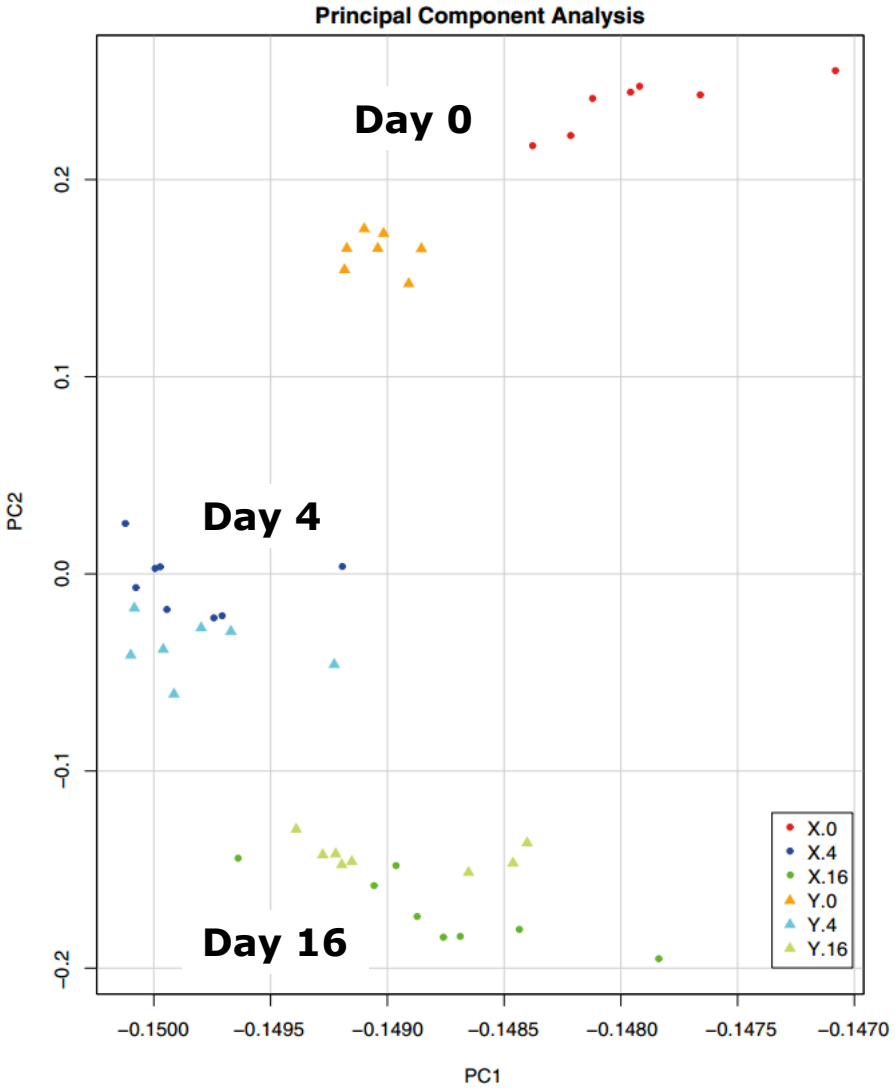
Host transcriptomic analyses

- Large differences at hatch between the groups
- Convergence between groups in time



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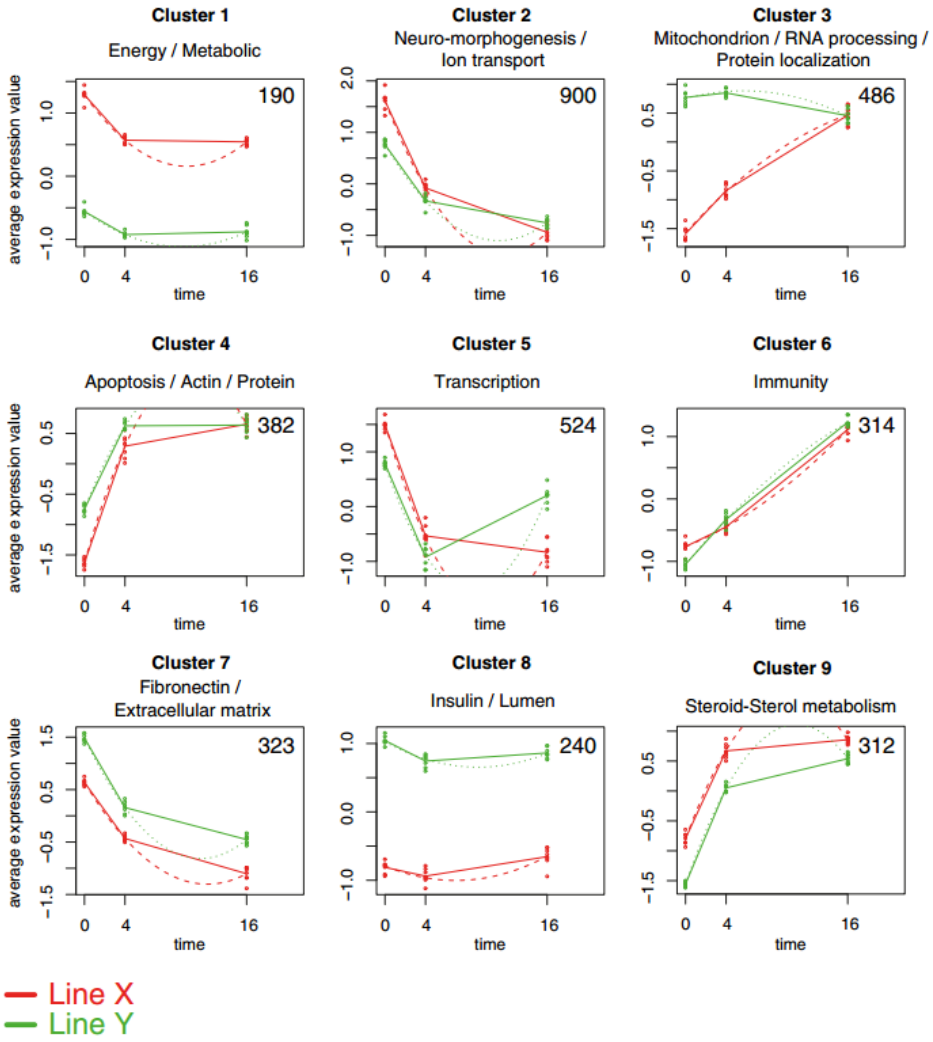
Temporal differences



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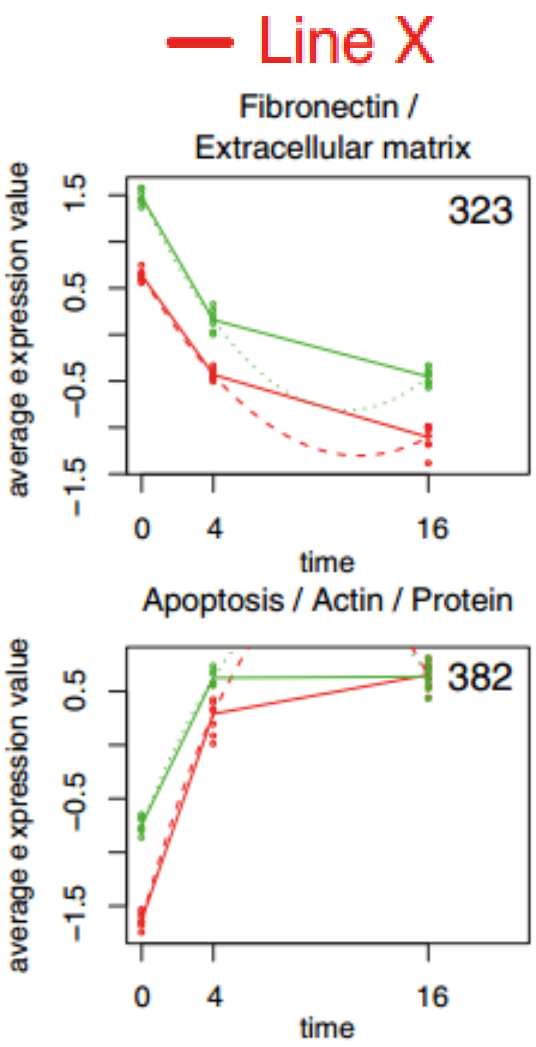
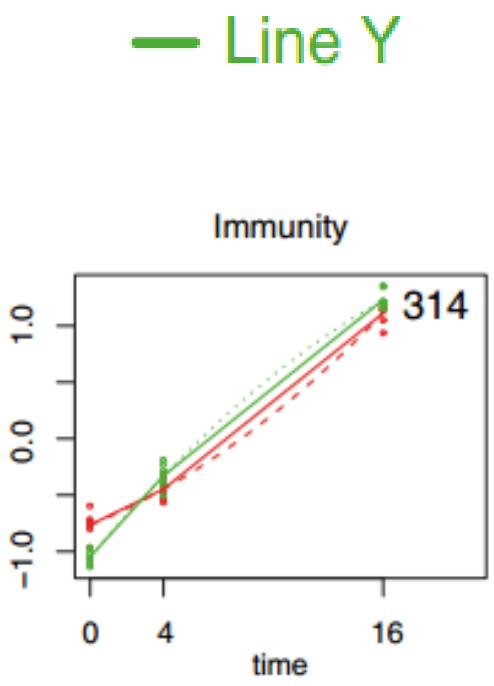
Focus on specific clusters





➢Temporal differences between these two lines suggest different coping mechanisms in early life

Events in early life are important (Walstra)

- Incubation and hatching as well as rearing conditions affected resistance against Eimeria challenge. But no mechanisms are known.
- Similar results in piglets: Enriched housing improved later resistance against App and PRRSv.

Focus on gut development in early life

Functional differentiation

Morphological development

Immune system programming

Microbiota colonization

Nutritional factors

Management factors

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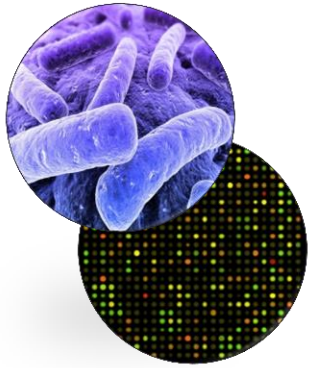
Perturbation during early life

- Study the effects of antibiotic usage during early life stages

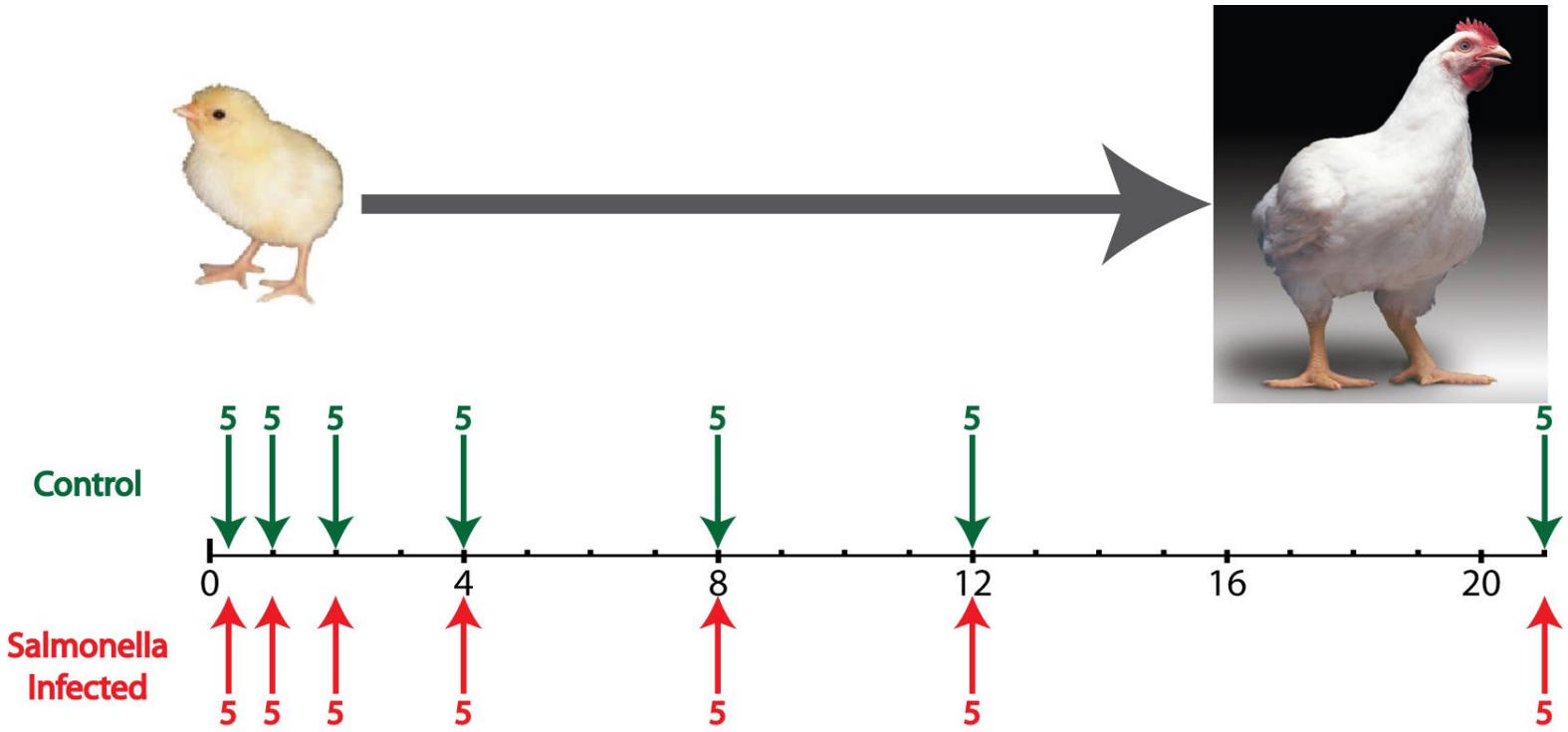
- Analysed effects at the level of intestinal microbiota and mucosal functions

- Applied metagenomics, transcriptomic and immunologic analytical approaches



Gene expression in jejunum

Chick development day 0-day 21



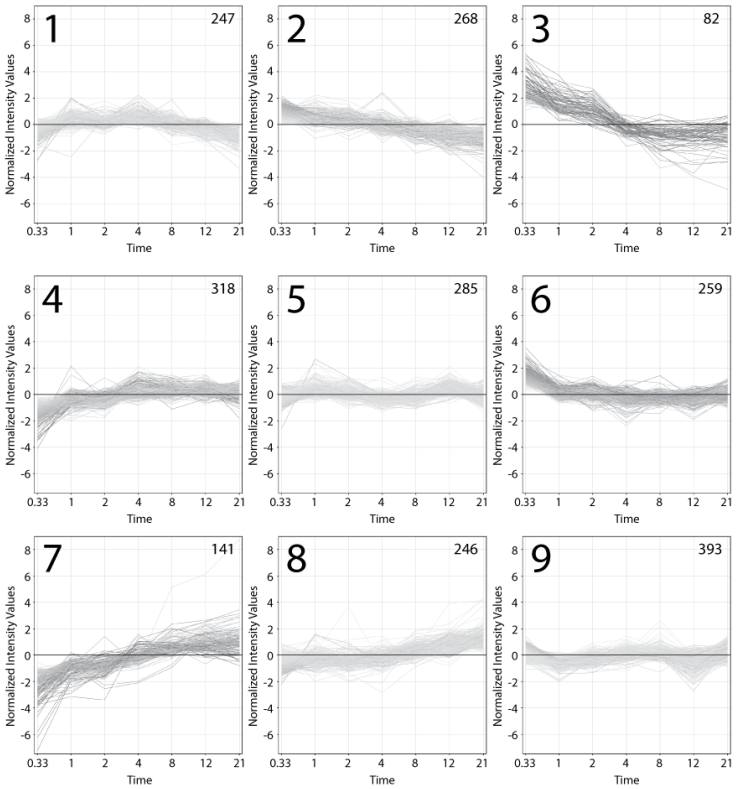
- Control chicks
- Perturbed chicks: *Salmonella enterica* ser. Enteritidis day 0

Agilent microarray – single color

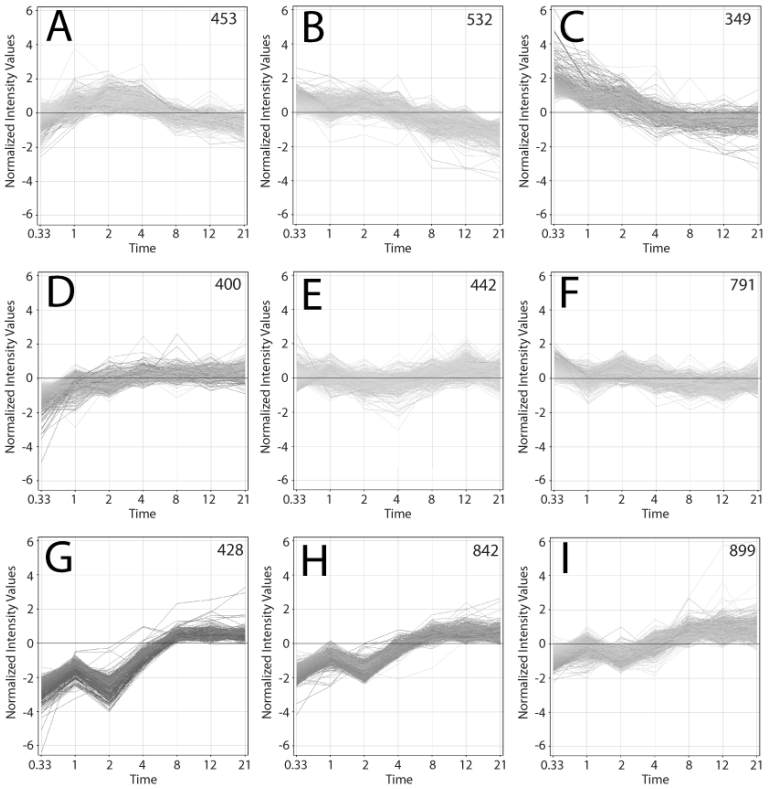
Clustering genes

according to time pattern of gene expression

Control



Perturbed



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Functional analysis

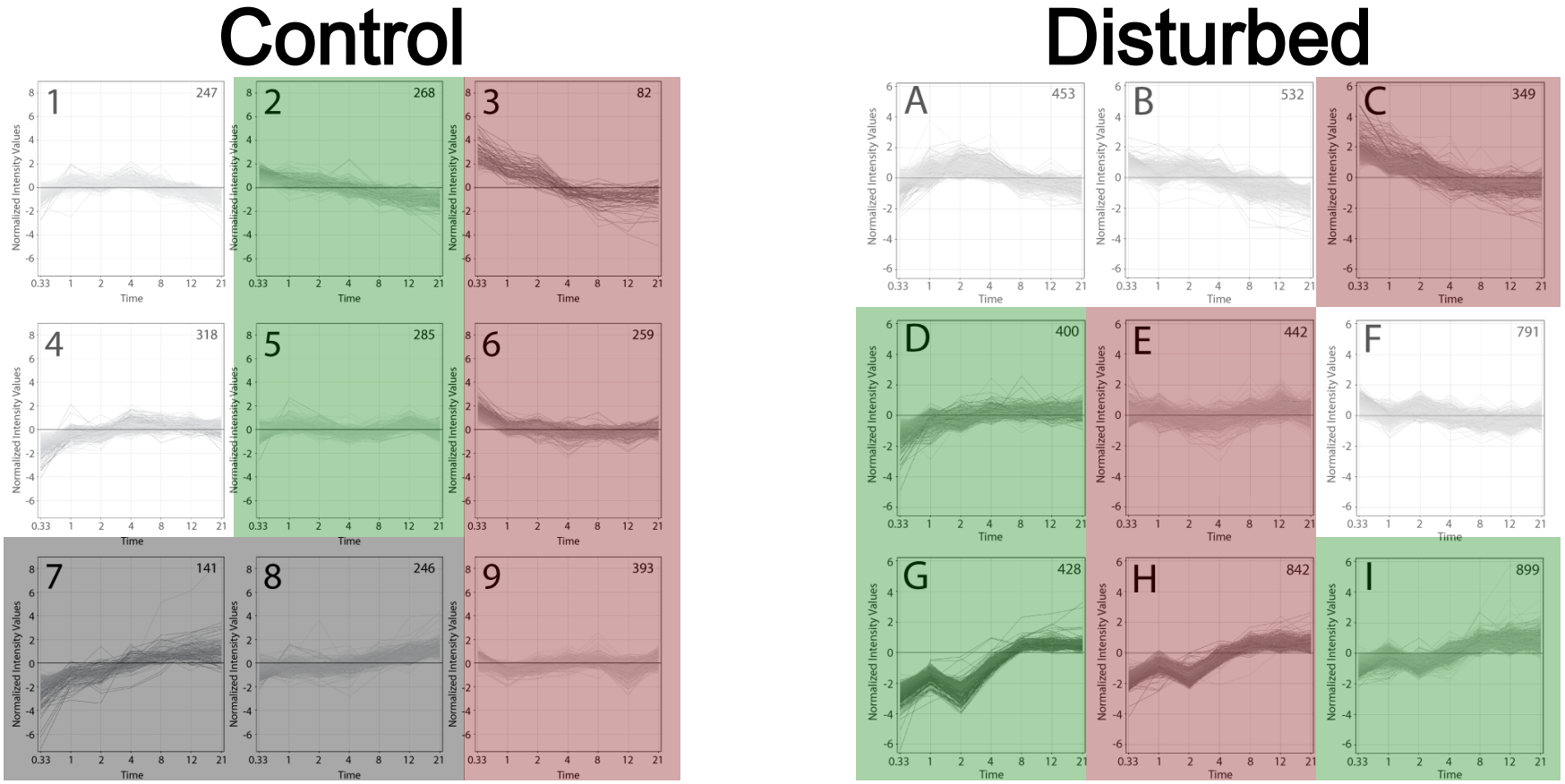
Control

1	Cell cycle Chromosome Reproduction/gamete generation
2	Ankyrins Reproduction/gamete generation (neuron) Cell morphogenesis
3	Metabolic process Metabolic process (acids) Transporter activity
4	Induction apoptosis Regulation apoptosis (innate) Immunity
5	Adhesion Development (general) Lumen
6	Kinase activity Nucleotide metabolic process Metabolic process
7	Signal/receptor activity Immune system development Scavenger receptor
8	Plasma membrane Response (defense) Signal/receptor activity
9	Biosynthesis Organelle/intracellular membrane Response (toxin/xenobiotic)

Perturbed

A	M-phase (cell cycle) Cytoskeleton DNA damage/repair
B	M-phase (cell cycle) Cell cycle process DNA damage/repair
C	Metabolic process (nitrogen/amine) Localization/transport Metabolic process (nitrogen/amine)
D	ANK domain Plasma membrane Coagulation (blood)
E	Vitamin binding (pyridoxal phosphate) Metabolic process (nitrogen/amine) Oxido-reductase activity
F	Thrombospondin Localization/transport Thrombospondin
G	Fibronectin Cell development/differentiation Development (general)
H	Cell migration/motility Cytokine biosynthetic process Negative regulation biosynthetic processes
I	Cell development/differentiation Extra cellular matrix Protein modification

Clustering + functional annotation



Immunological	Turn-over
Morphological	Functional

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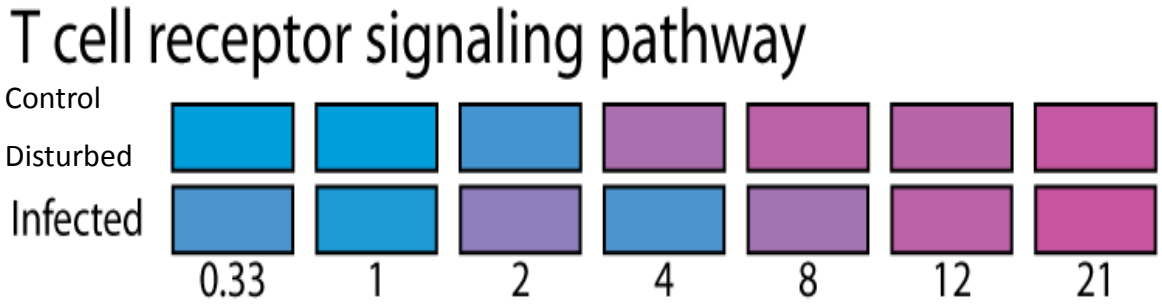
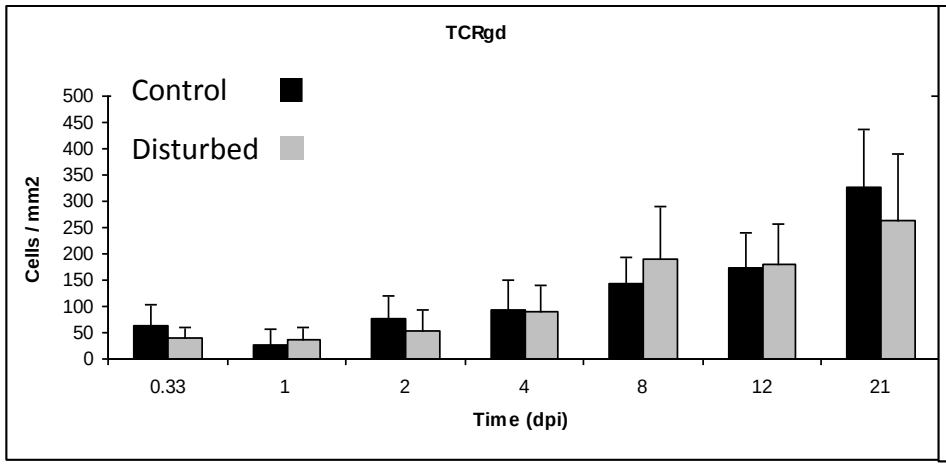
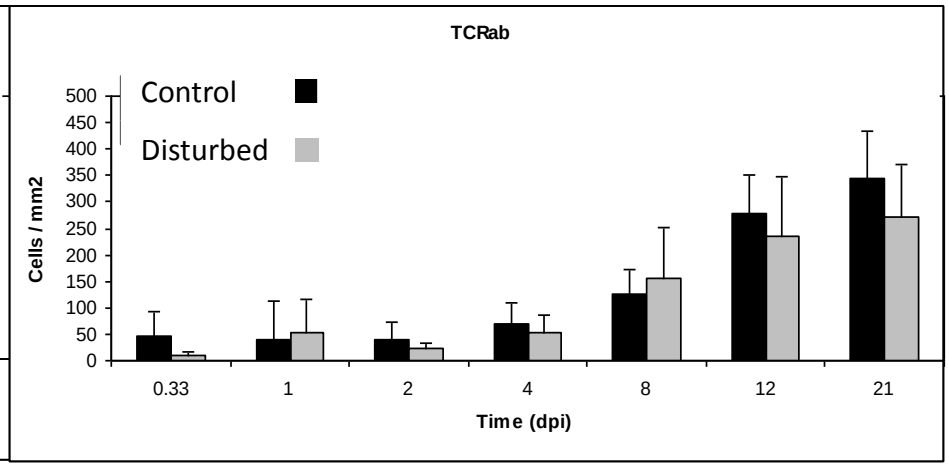
Gene expression correlates with immunological development and response







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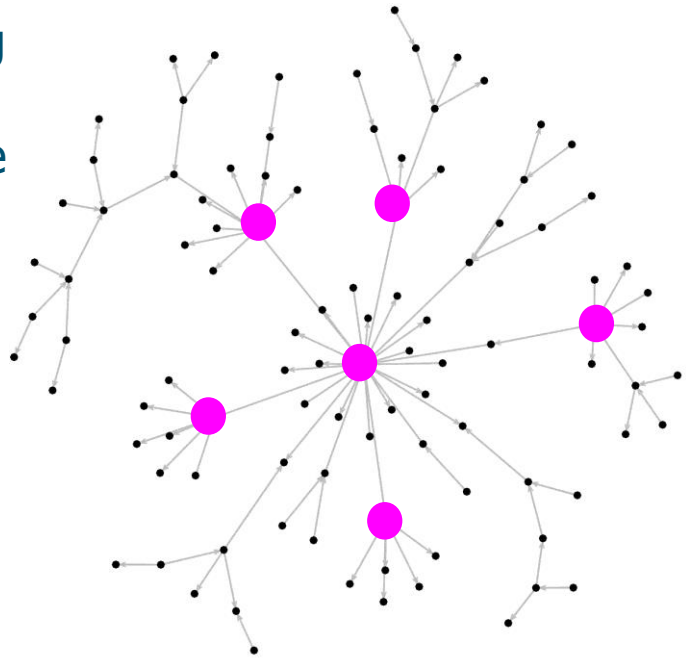
Conclusions “gene expression”

- Specific gene expression patterns coincide with the immunological, morphological, and functional processes
- Due to the disturbance → well-organized spatial-temporal development altered
  - Morphological processes are delayed
  - Processes related to immunological development are scattered
- Demonstrate the flexibility of developmental processes in the broiler chicken intestine
- Still lacking information about the regulation / modulation of intestinal processes

Gene network analysis

- Gene network
  - Complex, interconnected system of g
  - Representing behavior of system at transcriptional level, interaction of ge with each other in time

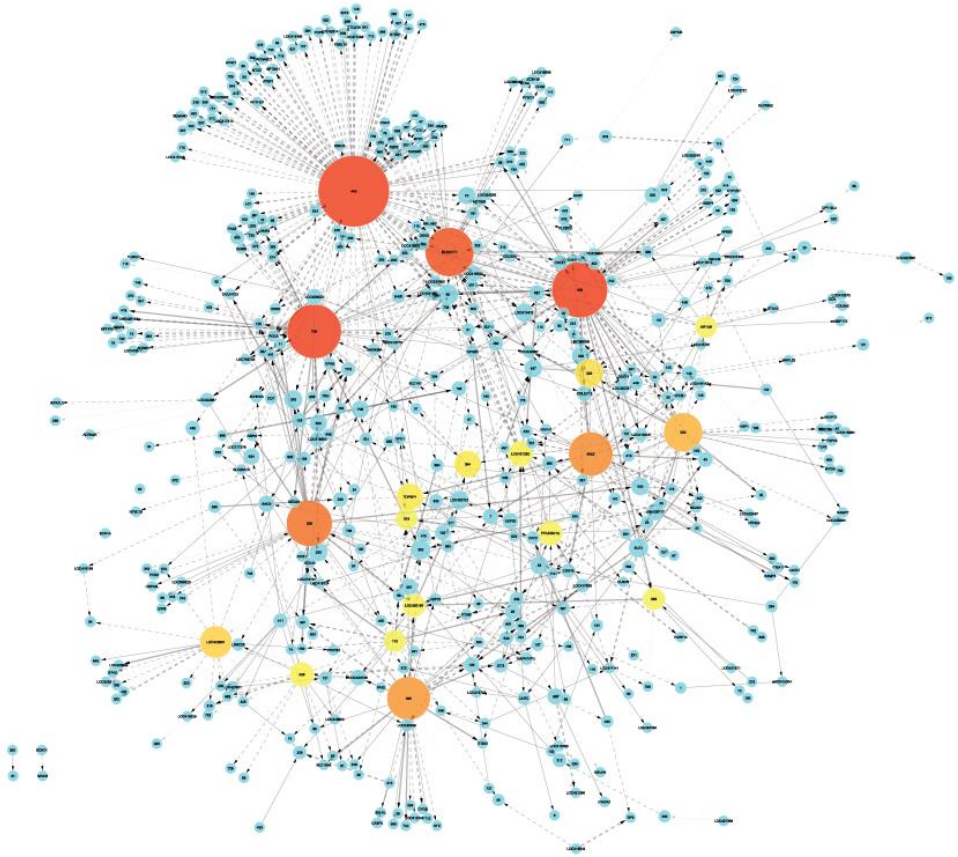


- Hubs
  - Highly connected nodes
  - Key regulators that modulate systems behavior
  - Potential targets to modulate systems outcome

  - Focus on interaction time-group → **759** probes as input - selected based on p-value and not 'feel good' genes

Gene association networks (GANs)

Control



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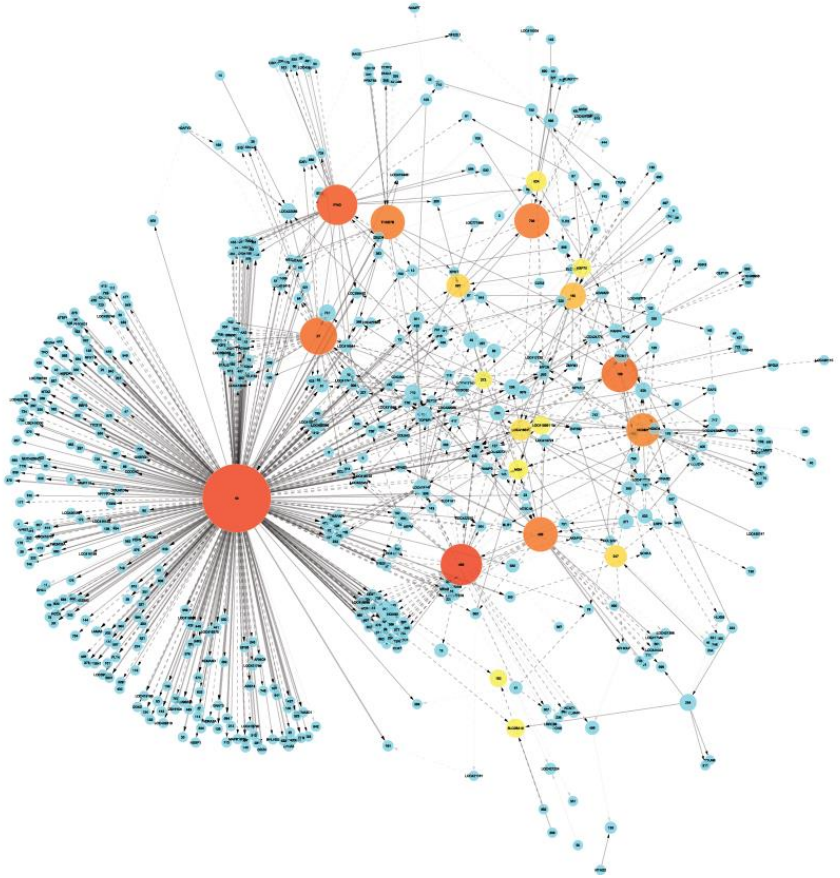
Infected



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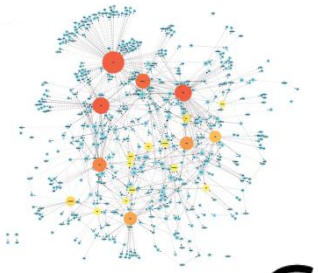
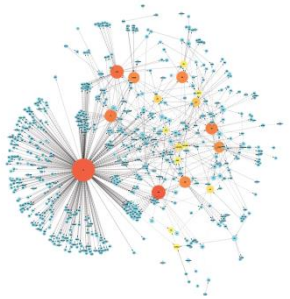
Hub network



Control

Infected



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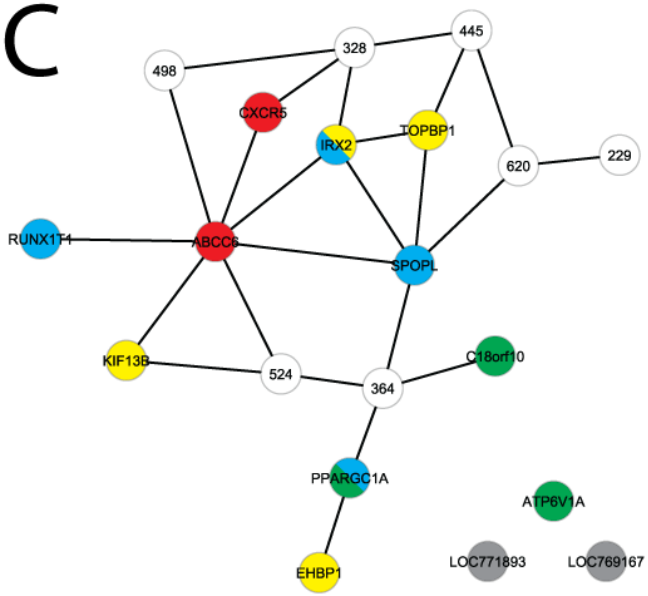

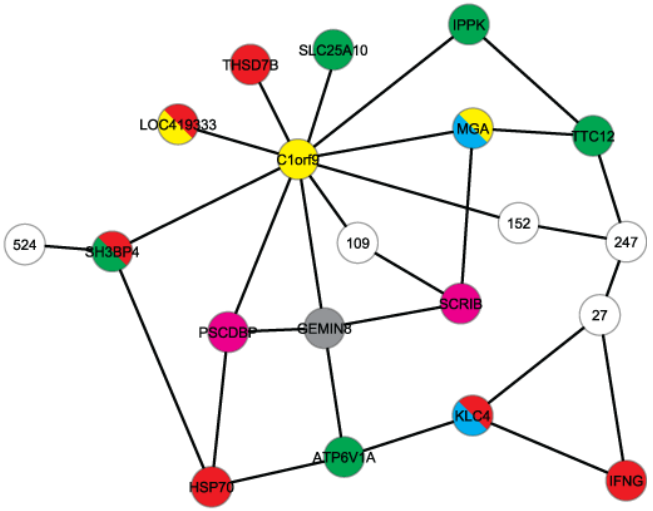

Metabolic	Defense / Host Response
Transcriptional Regulation	Communication
Development	No conserved domains

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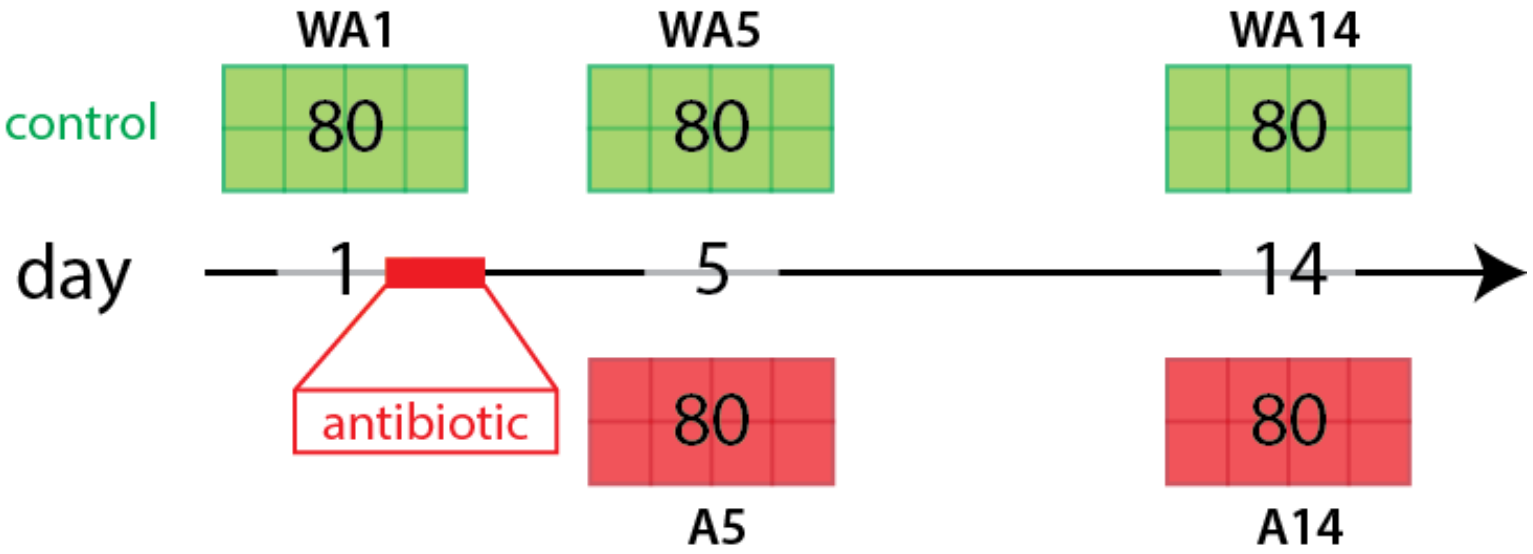
Conclusions – Network Approach

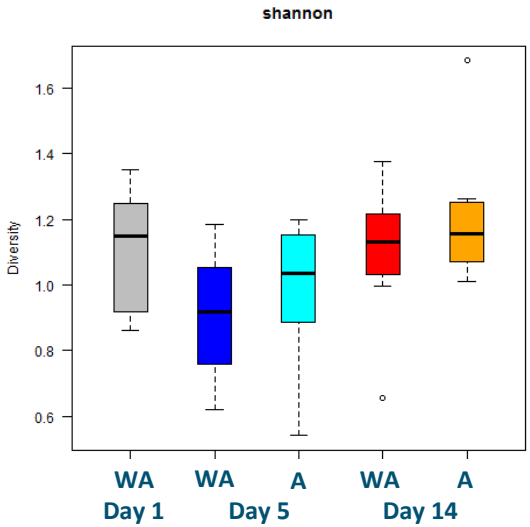
- **Shift in behavior system translated in networks**
  - Starting with same 759 nodes / probes
    Completely different networks
- **Function genes in GRNs**
  - Control → Developmental related processes well orchestrated
  - Salmonella → Pathogen response / host defense well orchestrated
- **Differences in hubs**
  - Different hubs in GRNs (except ATP6V1A)
  - Shift from 'transcriptional regulation' to 'communication signaling'
  - Key regulators of systems behavior
  - Potential targets to modulate systems behavior

Early life perturbation in broilers

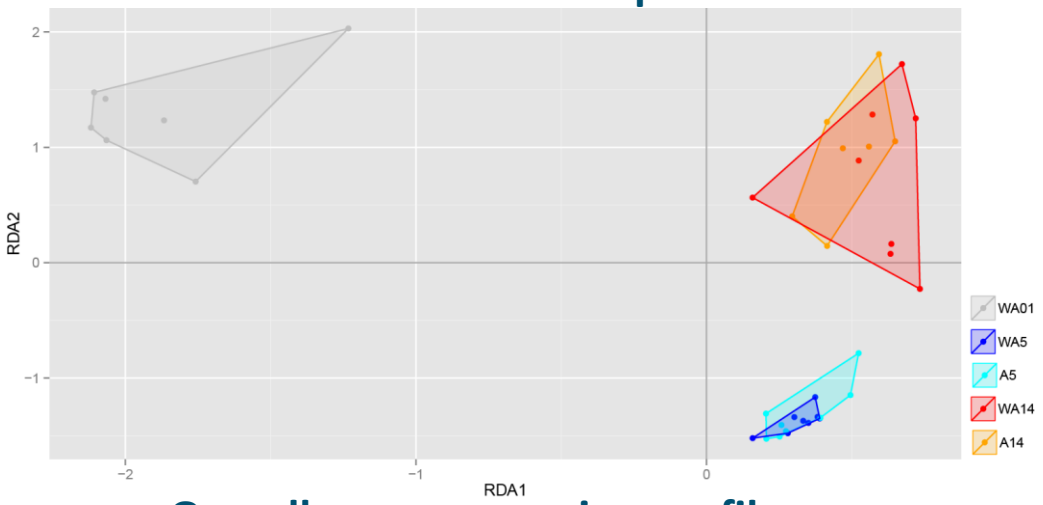


- Antibiotic = therapeutic dose of amoxicillin for 24h

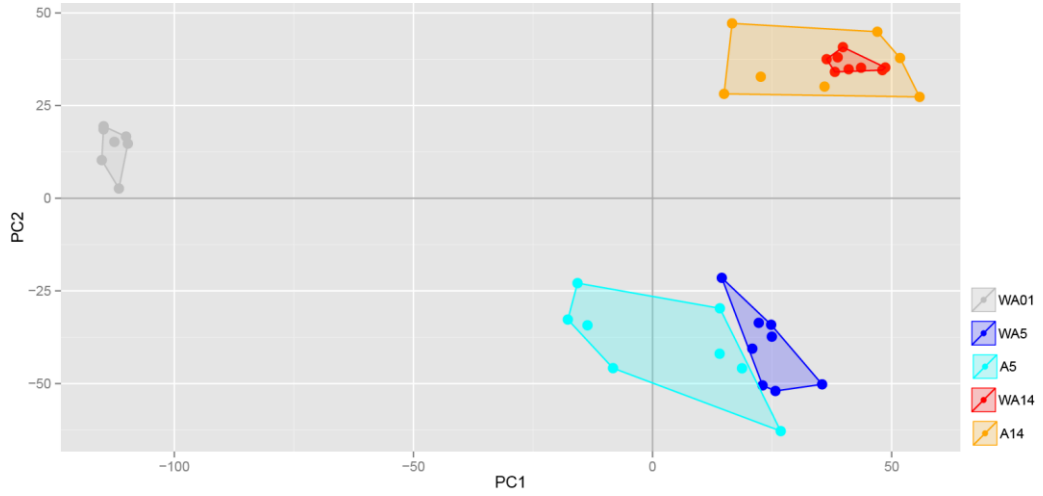
Results microbiota and transcriptomics



Overall microbiota composition



Overall gene expression profiles



- Numerically higher Shannon diversity

- Gene expression differs at day 5

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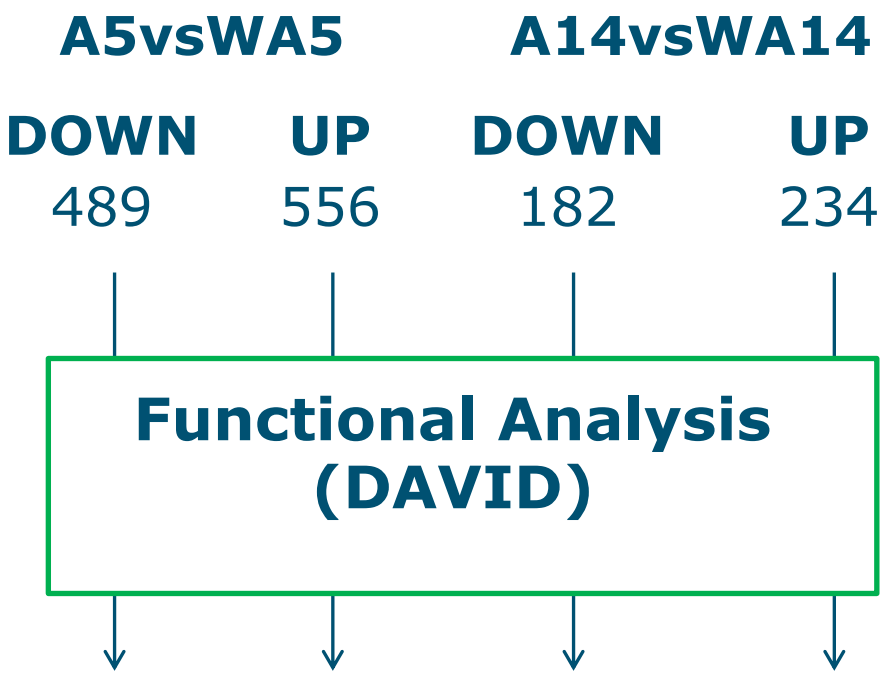
Statistical Analysis - Treatment

#genes
p_{adj}<0.01



Functional analysis (DAVID) day 5

(A5-WA5) Down low(er) in antibiotic treatment		(A5-WA5) Up high(er) in antibiotic treatment	
ES	**General Term**	**ES**	**General Term**
4.83	intracellular organelle lumen	7.86	extracellular matrix
4.77	protein transport/localization	5.25	triple helix (hydroxyproline,hydroxylysine)
3.26	domain: BTB/POZ-like (transcriptional repression)	5.16	Collagen triple helix repeat (hydroxyproline,hydroxylysine)
3.09	macromolecule/protein catabolic process	4.47	cell projection morphogenesis (neuron, differentiation)
2.65	immune response-regulating signal transduction	3.66	Fibrillar collagen
2.39	nuclear envelope-endoplasmic reticulum network	3.56	regulation of cell development (neuronal)
2.33	positive regulation of immune system process	3.08	positive regulation of transcription/macromolecule
2.27	cellular protein localization	3.07	EGF-like domain
2.19	adaptive immune response	2.57	response to steroid hormone stimulus (cortico/glucocortico)
2.08	Protease/peptidase activity	2.57	thrombospondin-type (Laminin G)

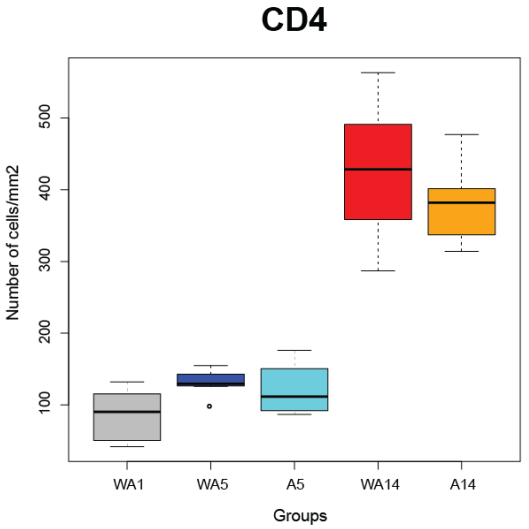
Metabolic / generic
Transcription
Immune
Cell (structure)
Development

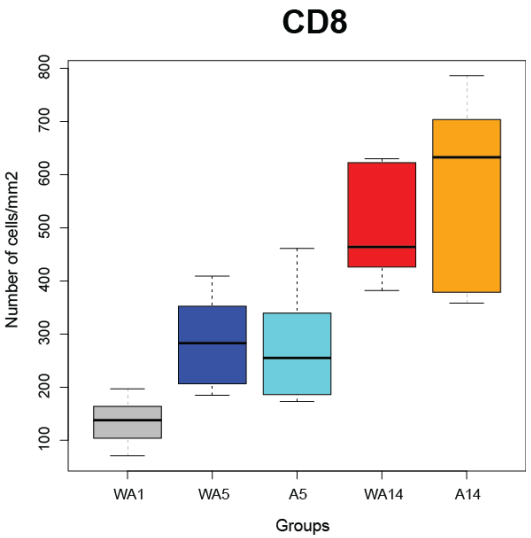
Functional analysis (DAVID) day 14

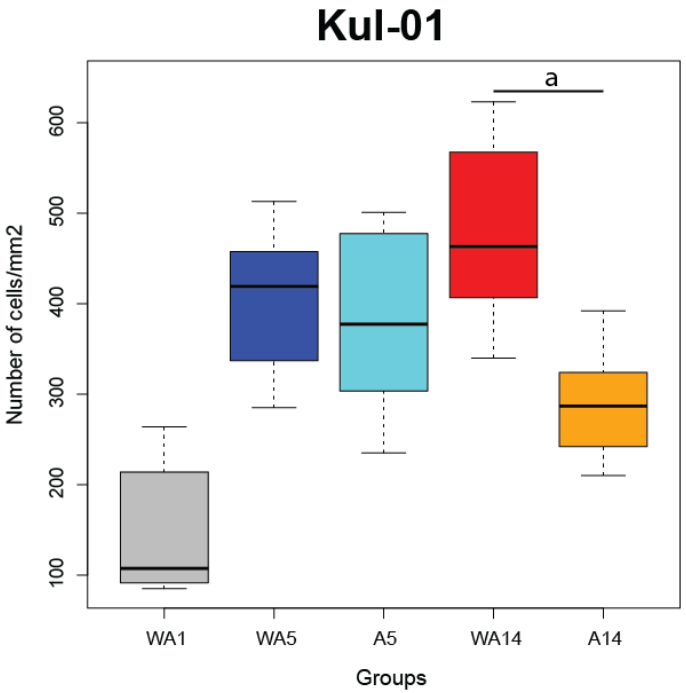
(A14-WA14) Down low(er) in antibiotic treatment		(A14-WA14) Up high(er) in antibiotic treatment	
ES	**General Term**	**ES**	**General Term**
2.49	positive reg. of biosynthetic process/transcription	4.51	organelle lumen (intracellular)
2.00	epithelium morphogenesis/development	2.38	transit peptide:Mitochondrion
1.60	macromolecule/protein catabolic process	1.84	sterol/steroid biosynthesis
1.48	intracellular organelle lumen	1.68	Heat shock protein (DnaJ)
1.47	blood vessel development	1.53	RNA recognition motif (RNP-1)
		1.51	translation initiation factor activity
		1.48	(negative) regulation of lipid storage
		1.43	Signaling Pathways (EPO/IGF1/IL6/TPO/IL2/PDGF/EGF)
		1.39	cellular protein localization/targeting
		1.32	zinc-binding (LIM domain)

Metabolic / generic
Transcription
Immune
Cell (structure)
Development

Gut immunology





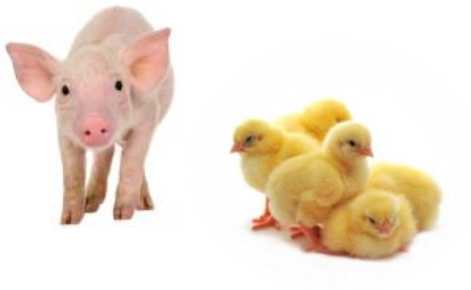


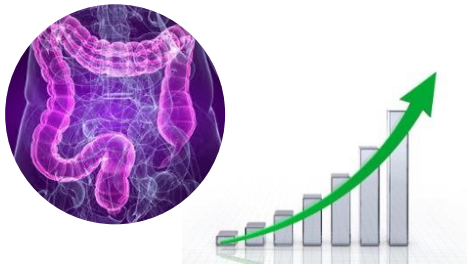
- **Development in time**
- **Lower KUL-01^{+} cells in antibiotic treated birds day 14**

Conclusions

- Microbiota
  - Higher diversity in antibiotic treated birds
  - Time-effect greater than treatment effect
- Gene expression
  - Antibiotic treated birds; ↑ cell structure/development, without antibiotics; ↑ immune
  - Effect most prominent on day 5
- Relevance for industry
  - Antibiotics affect immune programming
  - Need alternatives to improve gut colonization and therefore development of immune system

Overall conclusions





- Antibiotics in early life have negative consequences for the programming of the host immune system
- Alternatives to improve gut health should primarily focus on early life
- Candidates for antibiotic alternatives
  - Host genotype
  - E.g. Prebiotics
  - .....

Thank you for your attention

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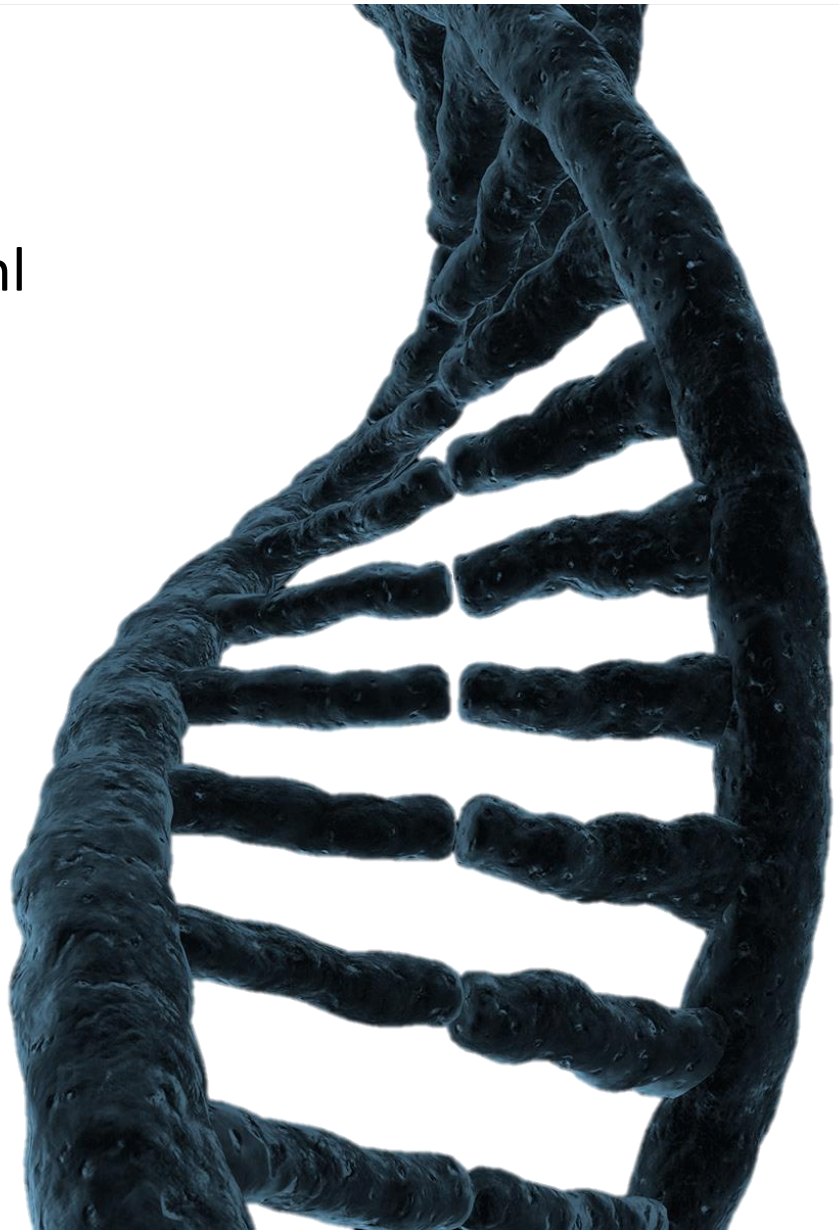

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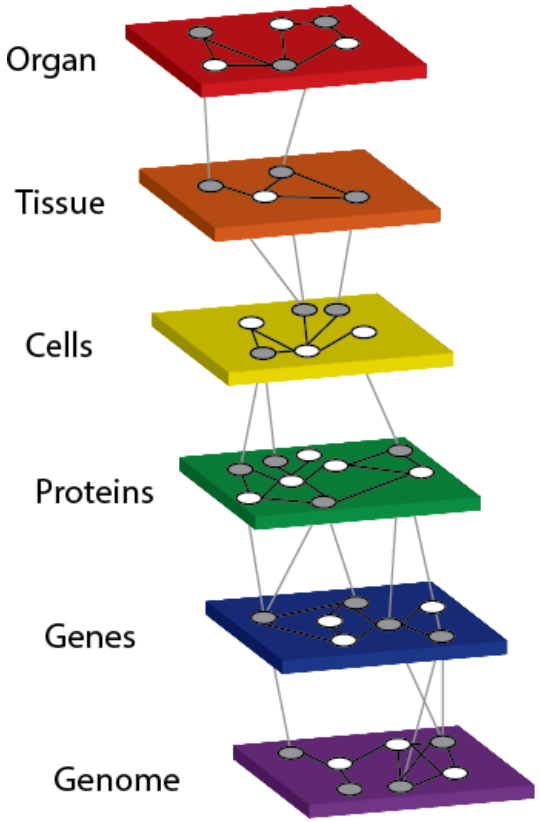
Integration of multiple biological levels

Host



Environment

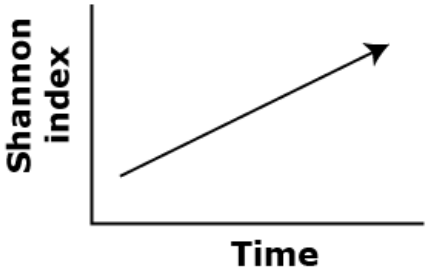
Nutrition

Stressors

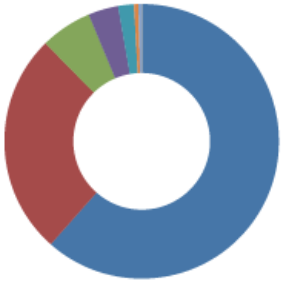
Antibiotics

Microbiota

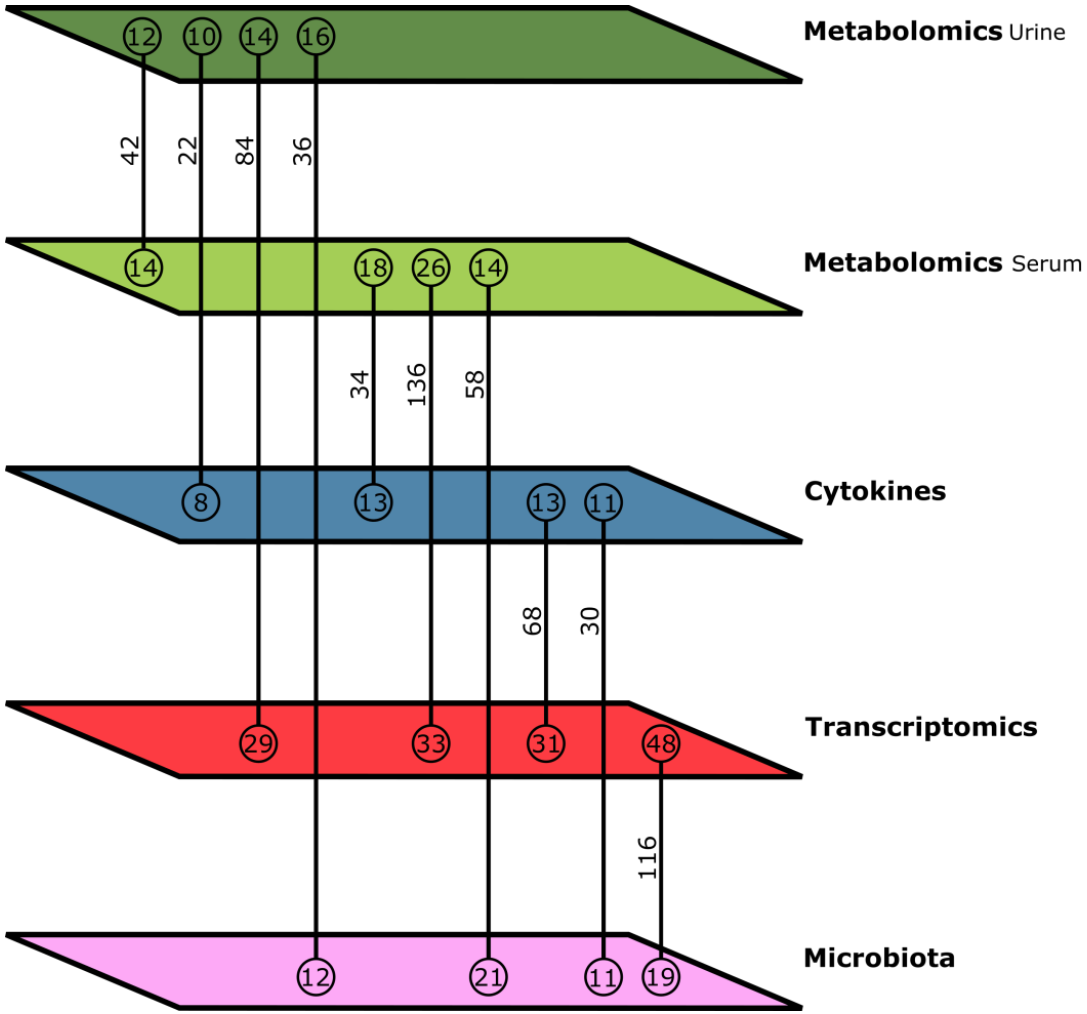
Diversity



Composition



Integration of multiple data types



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Microbiome develops over time

- Videnska et al., 2014

Early life differences in microbiota composition

				Day 0		4		16	
P[b]	Class	Family	Genus	X	Y	X	Y	X	Y
F	Bacilli	Enterococcaceae	Enterococcus	**83.5**	**71**	0.16	1.33	0.09	**0.72**
			Other	1.12	1.28	**7.74**	**8.6**	**3.2**	**6.12**
		Lactobacillaceae	Lactobacillus	0.12	0.02	**88**	**84.5**	**68.8**	**72.9**
		Streptococcaceae	Streptococcus	0	0	1.6	2.45	**14**	**15.1**
		Bacillaceae	Other	0	0	0	0	**1.48**	0.01
	Clostridia	Other	Other	0	0	0.14	0.09	**0.926**	0.04
		Lachnospiraceae	Other	0	0	0.12	0.02	**1.44**	0.11
		Ruminococcaceae	Faecalibacterium	0	0	0	0	**0.741**	0.01
	Erysipelotrichi	Erysipelotrichaceae	Coprobacillus	0	0	0.02	0	**1.5**	0.01
Pr	Gammaproteobacteria	Enterobacteriaceae	Escherichia	**12.6**	**26.8**	1.47	**2.69**	**1.98**	**0.84**
T	Mollicutes			0	0	0	0	**1.26**	0.03

- **Differences at day 0**

  Escherichia trend p=0.08

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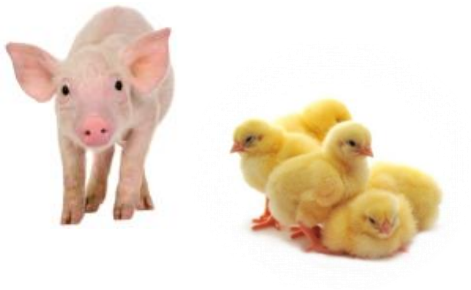
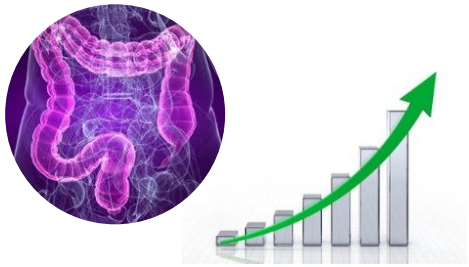
Overall conclusions





- Antibiotics in early life have negative consequences for the programming of the host immune system
- Alternatives to improve gut health should primarily focus on early life
- Candidates for antibiotic alternatives
  - Host genotype
  - E.g. Prebiotics
  - .....

Colonization may affect development of microbiome

- Vazunova et al., 2016

On farm hatching

- May give colonization a head start

Acknowledgements

WAGENINGEN UNIVERSITY
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CENTRAL VETERINARY INSTITUTE
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