Feed4Foodure; Effect of Antibiotics on Chicken Neonatal Intestinal Development

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Acknowledgements

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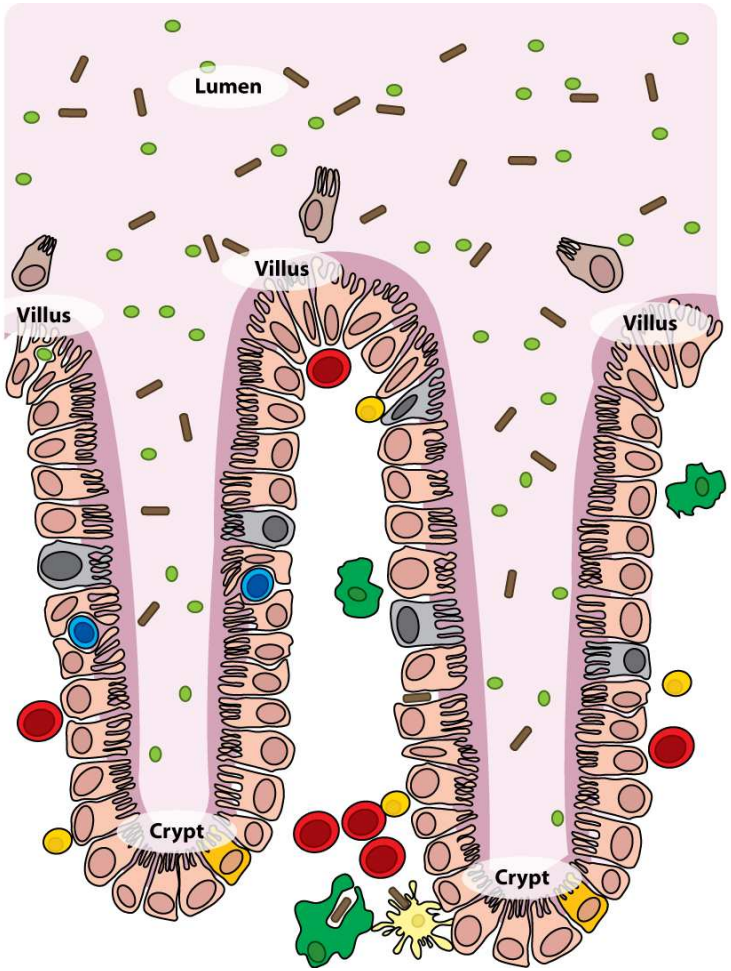
Feed4 Foodure

Voeding, Darmgezondheid, en Immuniteit

Ministerie van Economische Zaken
Productschap Diervoeder (PDV)
Productschap Pluimvee en Eieren (PPE)
Productschap Vee en Vlees (PVV)
Agrifirm Group
ForFarmers Hendrix
De Heus
Nutreco
VION Food Group
MSD - Animal Health
VanDrie Group
Denkavit

Intestinal functions

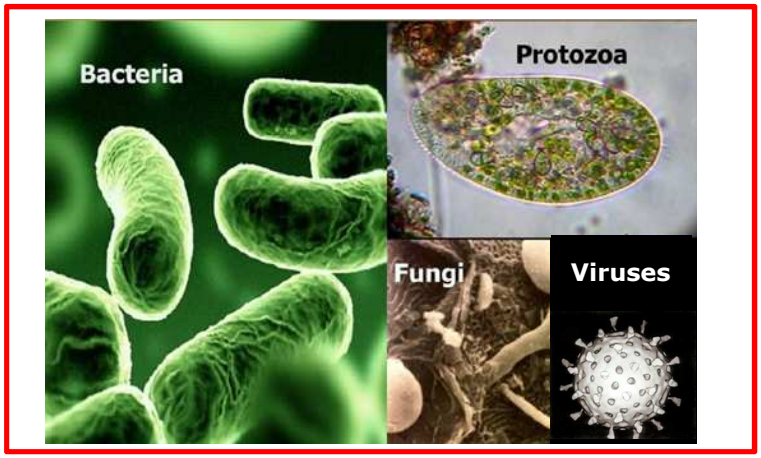


- **Feed uptake**
  - Converted to energy source
- **Microbiota**
  - Digestion, fermentation and metabolic conversions
- **Epithelial cell layer**
  - Frontline defense
- **Immune system**
  - Largest number of immune cells in the body
  - Constant monitoring

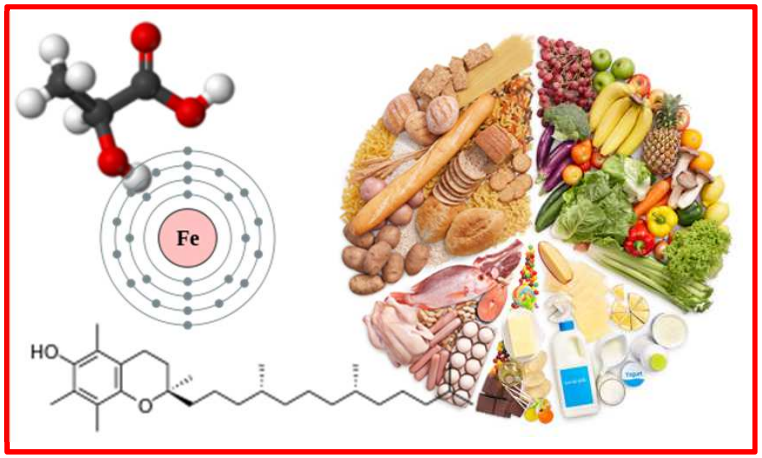
Host

interactions have effect on performance and health traits

Microbiota



Feed



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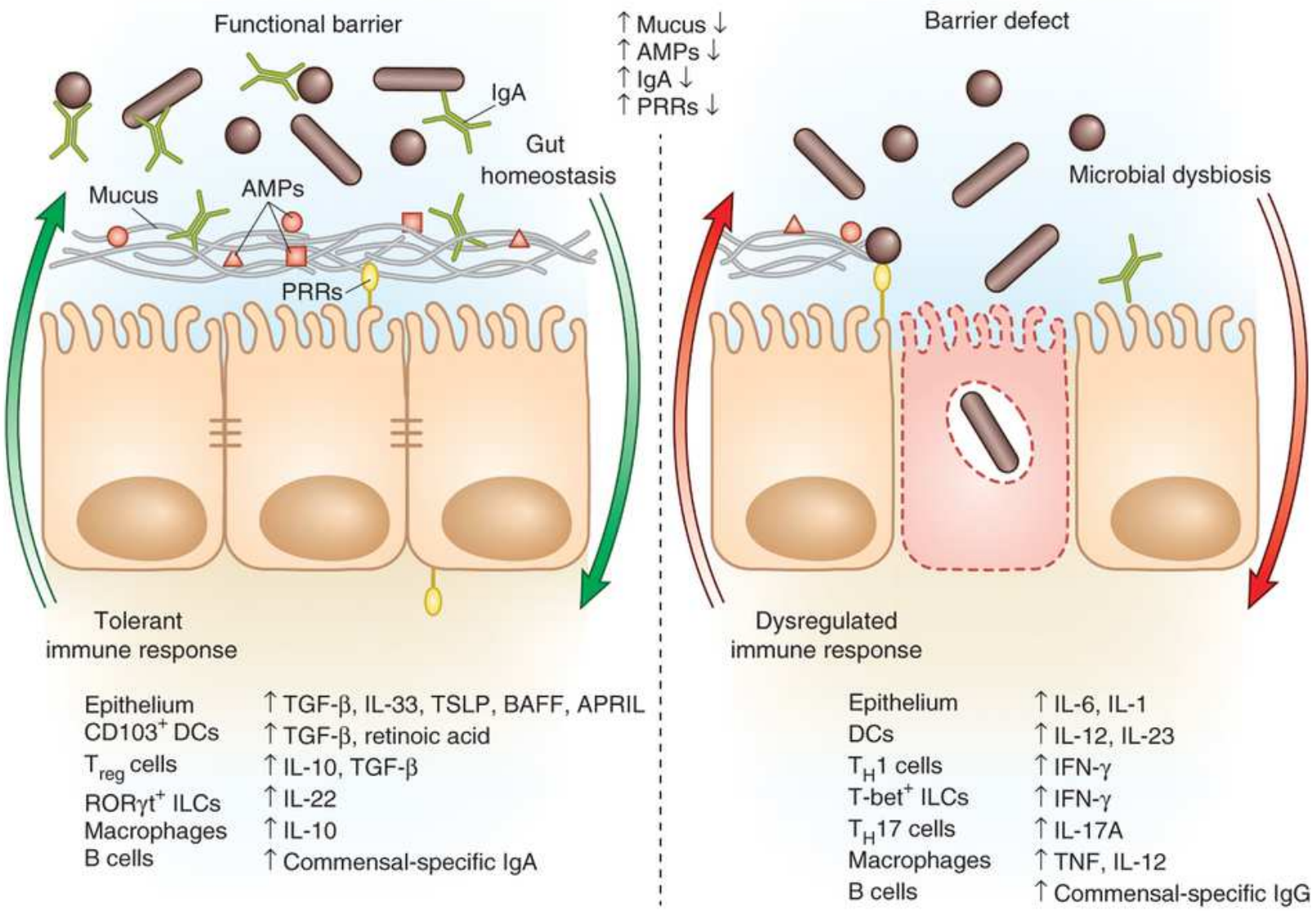
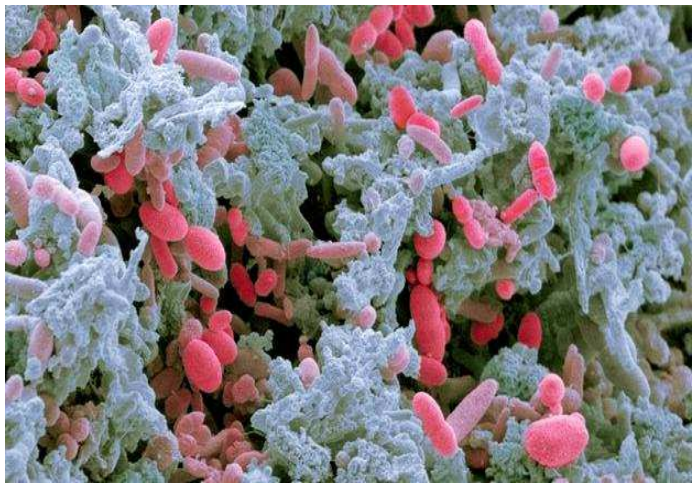
Host-Microbe Interactions



Important role for microbiota

Chicken

- 10^9/g ileal digesta
- Dominant and/or abundant species
  - *Lactobacillus* (very dominant)
  - *Streptococcus*
  - Coliforms
  - *Enterobacteriaceae*
  - *Clostridiaceae*



- **Constant evolution of microbiota composition!**

Aim talk

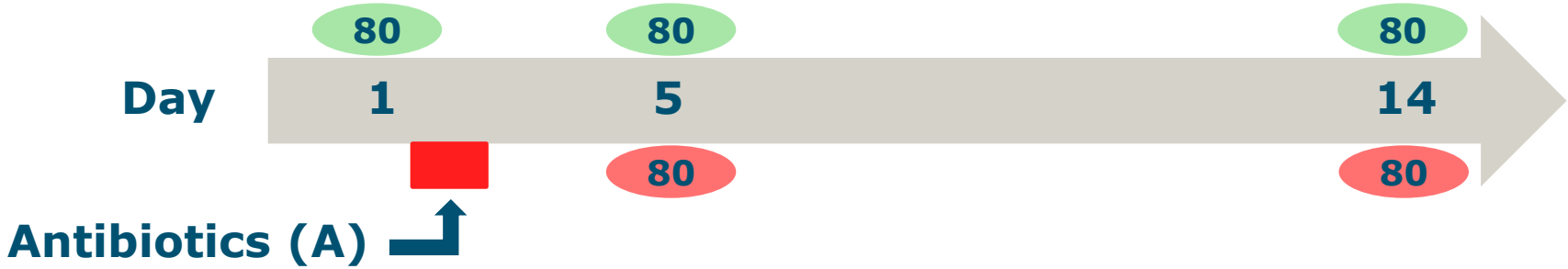
- Focus on improving gut health (immune development)
- Approach
  - Antibiotics (proof of concept – mechanism of immune development by changing microbiota composition and modulation in early life)
- Focus on neonatal period
  - Life long effect (programming of immune system)

Objective

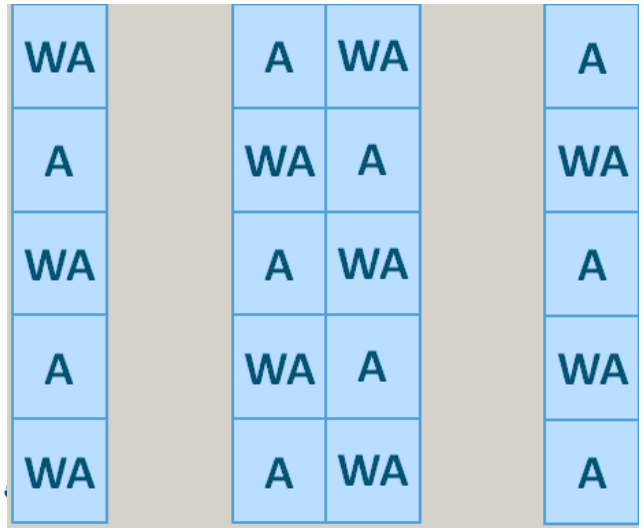
- Investigate the effect of the use of antibiotics, via the drink water, at early life of chicken (day 1) on the microbiota (composition and diversity) and host gene expression later in life (day 1, 5 and 14)
    - Microbiota
        - Jejunum (MiSeq)
    - Transcriptomics
        - Jejunum (Agilent)

Study design

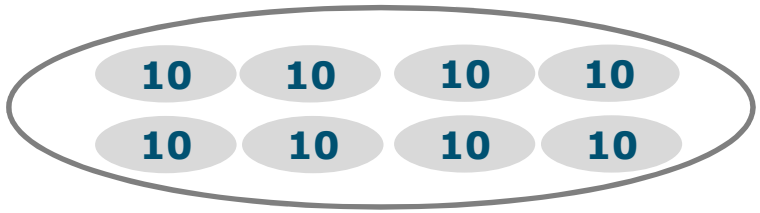
Without antibiotics (WA)



Housing



Pools



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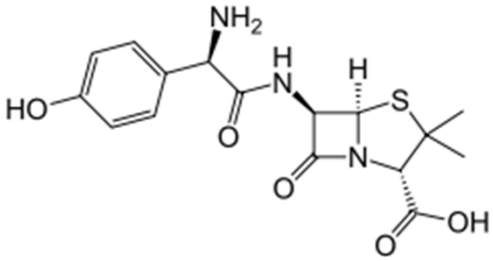
Antibiotic

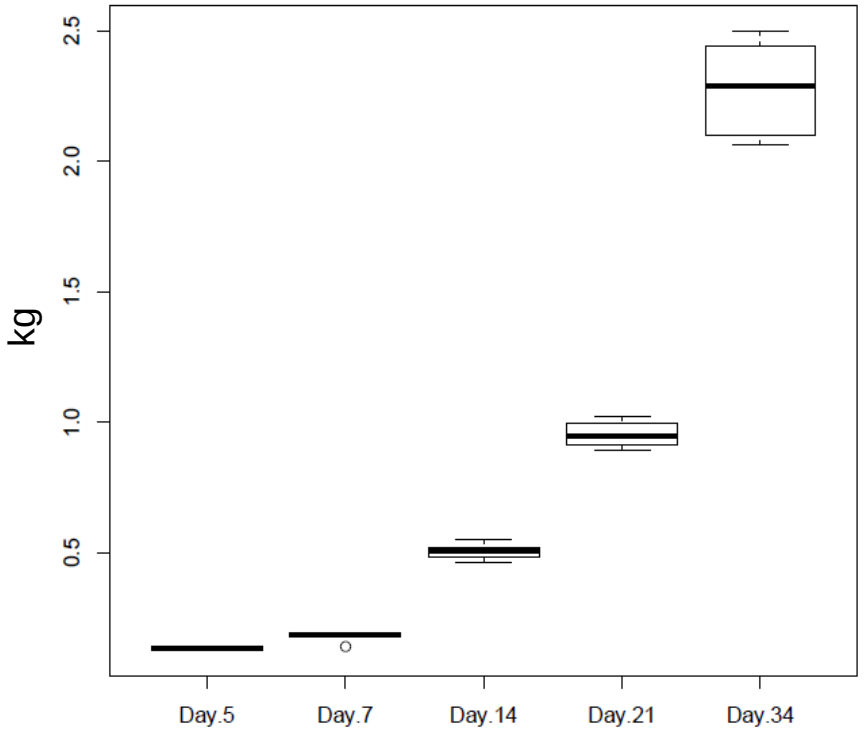
- Amoxicillin
- Bacteriolytic, β-lactam antibiotic
  - Prevents synthesis of cell walls



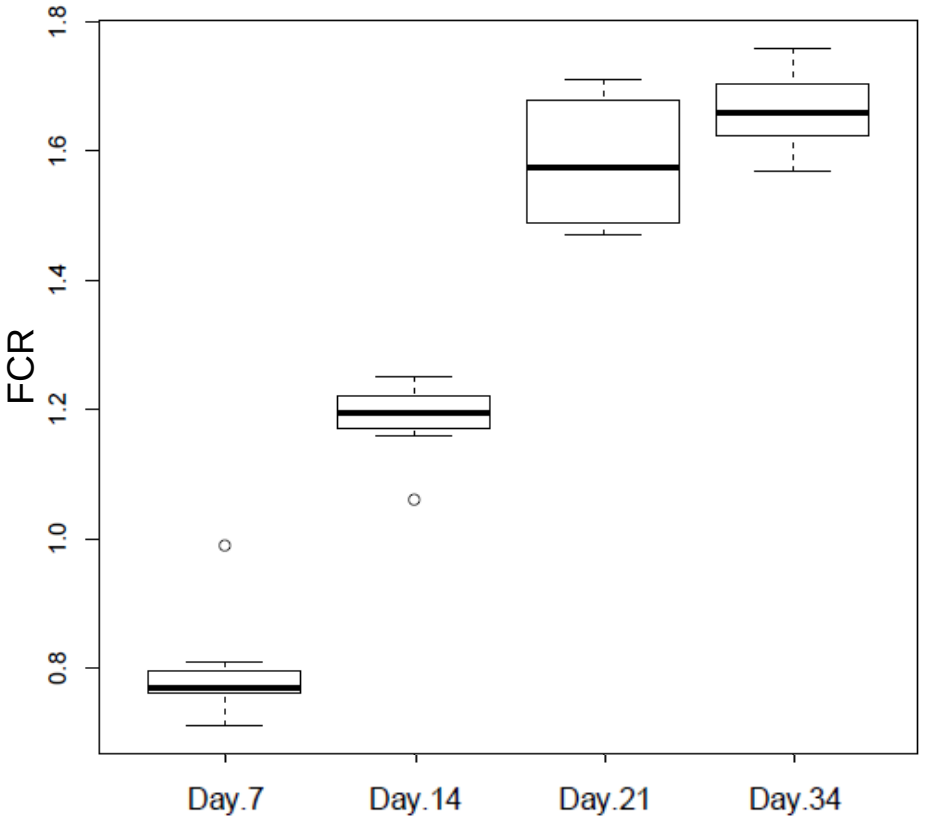
- Susceptible bacteria to amoxicillin include
  - *Streptococcus*
  - *Bacillus subtilis*
  - *Enterococcus*
  - *Haemophilus*
  - *Helicobacter*
  - *Moraxella*

Performance Data

Weight



FCR



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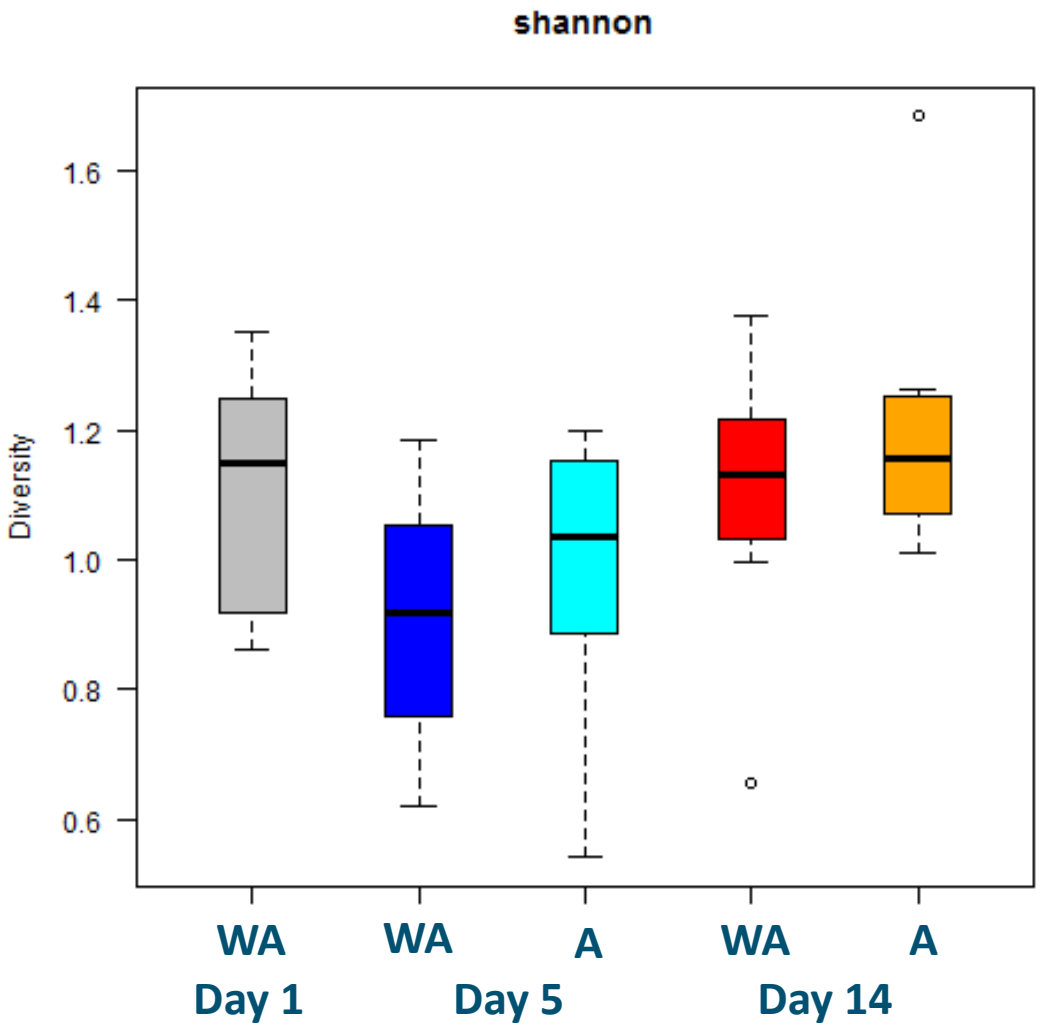
Microbiota
Small Intestine

Microbiota diversity



T-test treatment on specific day

Day 5

p = 0.45

Day 14

p = 0.33

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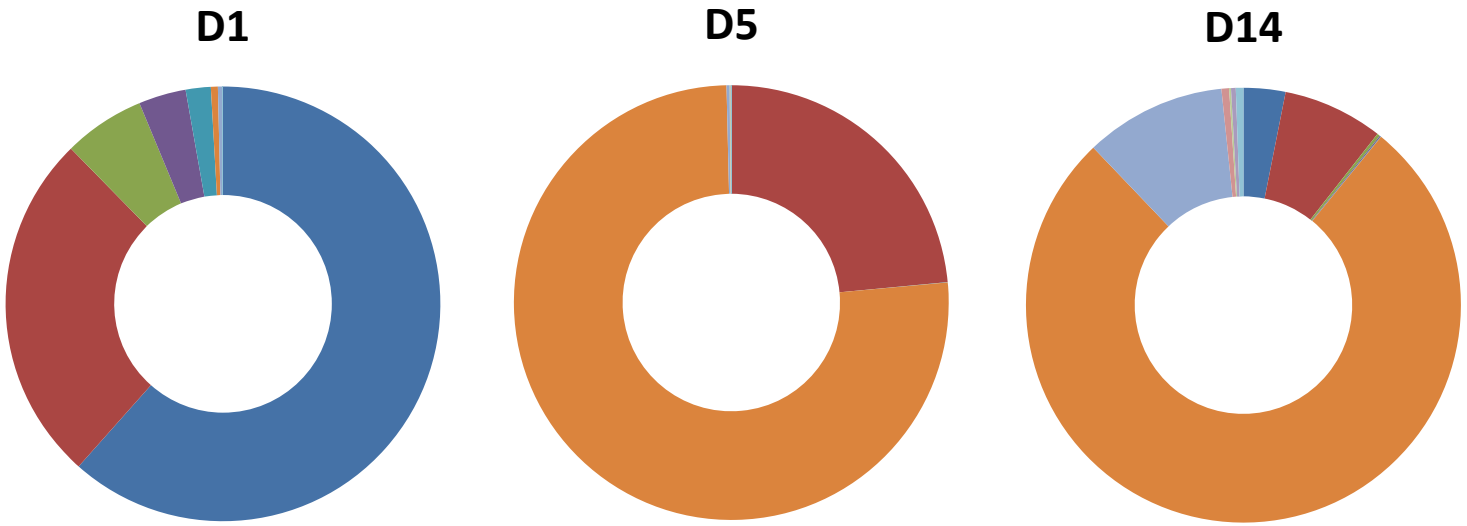
Comparison between days 1,5, and 14



- p__Proteobacteria;c__Gammaproteobacteria;f__Enterobacteriaceae
- p__Firmicutes;c__Bacilli;f__Enterococcaceae
- p__Firmicutes;c__Clostridia;f__Clostridiaceae
- Unclassified
- Other
- p__Firmicutes;c__Bacilli;f__Lactobacillaceae
- p__Firmicutes;c__Bacilli;f__Streptococcaceae
- p__Firmicutes;c__Clostridia;Other
- p__Firmicutes;c__Bacilli;f__Leuconostocaceae
- p__Firmicutes;c__Erysipelotrichi;f__Erysipelotrichaceae
- p__Tenericutes;c__Mollicutes;f__

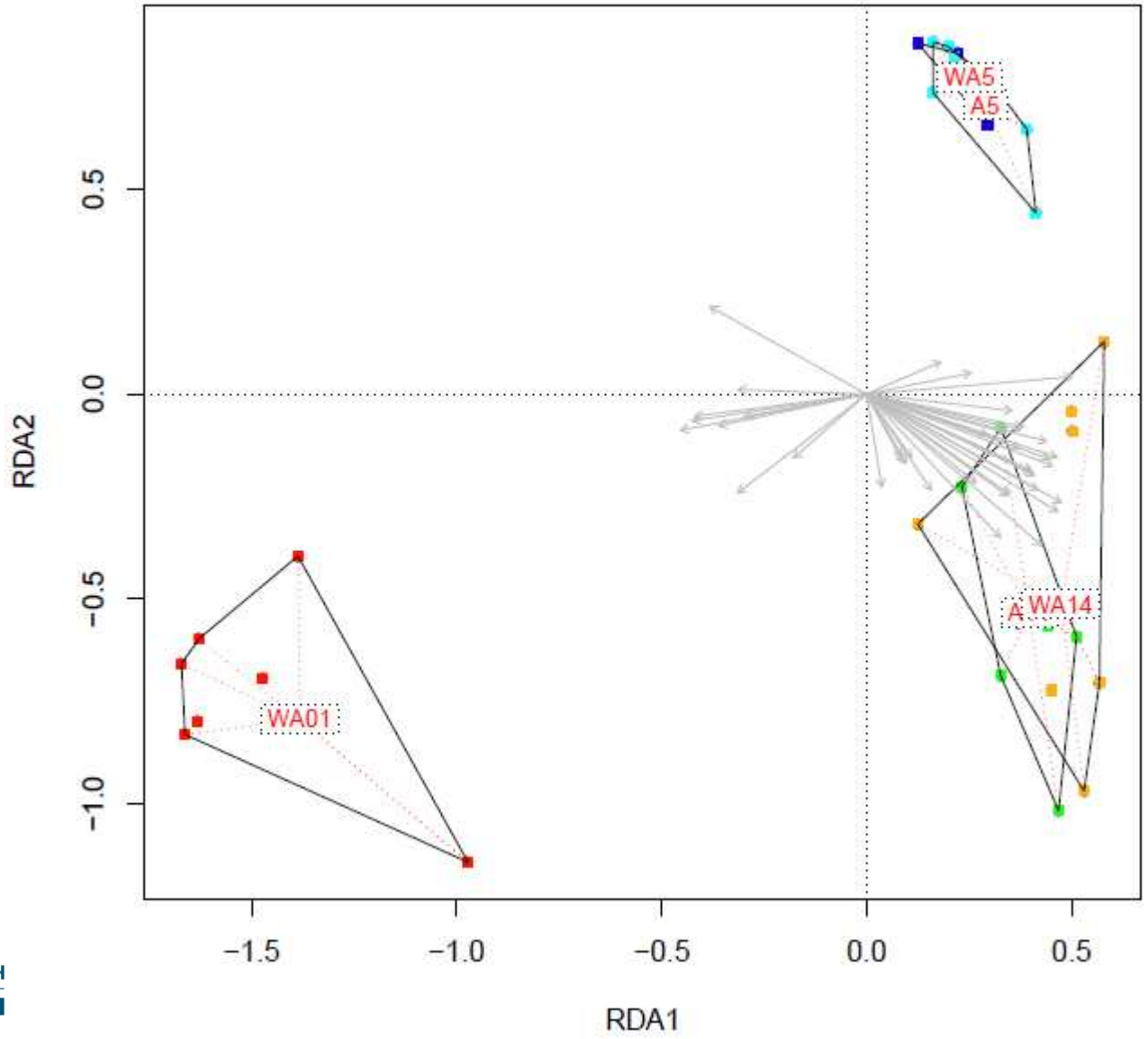
Microbiota composition

Redundancy analysis

Statistical model

y = Day + Group + Day*Group

Day	p<0.05
Group	p<0.05
Day*Group	p<0.05



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Statistical analysis

Phylum	Class	Family	Average relative contribution (%) WA5	A5	WA14	A14
Firmicutes	Bacilli	Bacillaceae	0.002	**0.006**	0.012	0.305
		Carnobacteriaceae	0.003	**0.007**	0.013	0.016
		Leuconostocaceae	0.043	**0.082**	0.122	0.156
		Thermoactinomycetaceae	<0.001	**0.001**	0.003	0.002
	Clostridia	Ruminococcaceae	<0.001	**<0.001**	0.002	0.002
Actinobacteria	Other		**0.002**	0.001	0.007	0.381
	Actinobacteria	Nocardiodaceae	0.001	**0.002**	0.002	0.003
		Nocardiaceae	0.001	**0.003**	0.004	0.147
Unclassified			0.002	**0.006**	0.012	0.305
Firmicutes	Bacilli	Enterococcaceae	21.689	25.153	4.906	**9.890**
		Lactobacillaceae	77.891	74.208	**82.156**	70.480
	Clostridia	Other	0.004	0.019	0.369	**0.732**

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* p-value < 0.05

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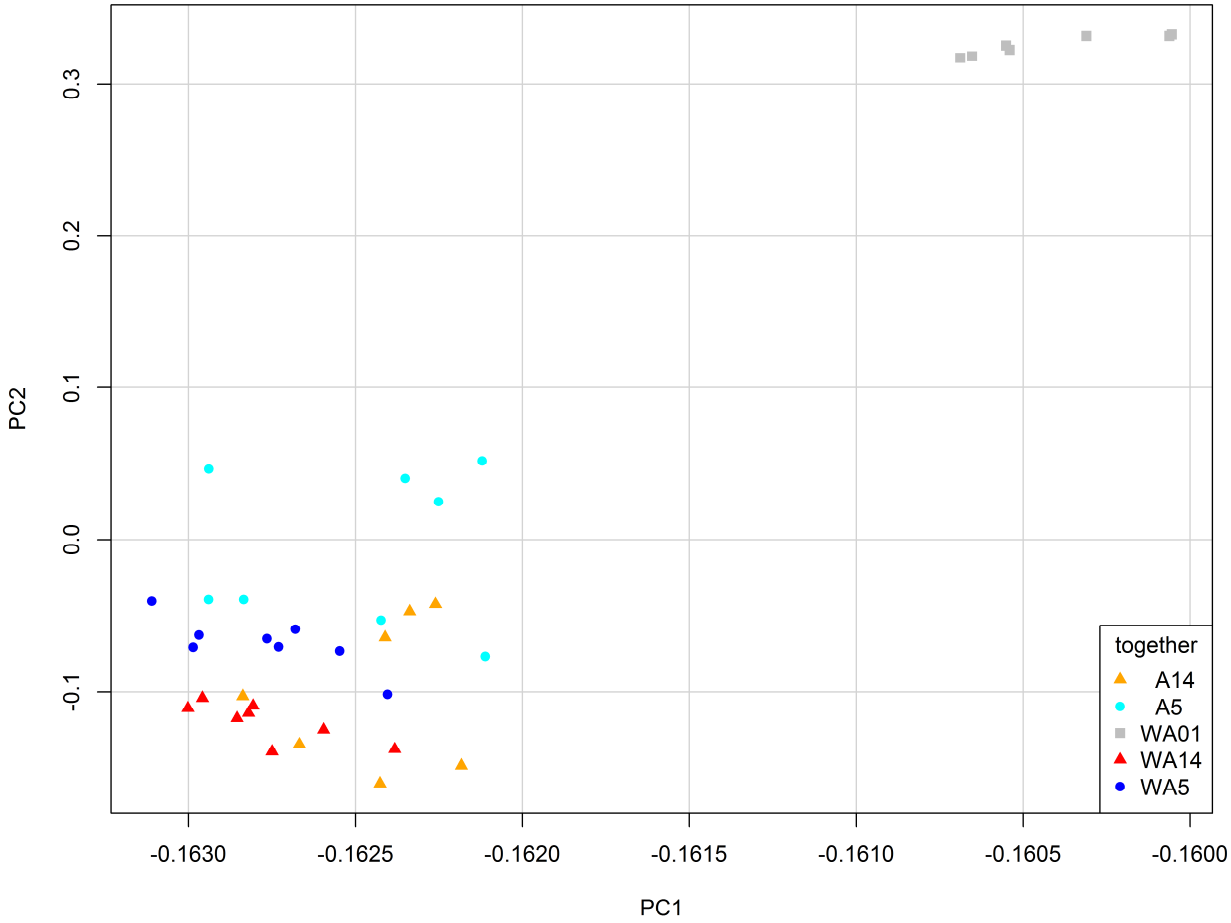
Conclusions microbiota

- ↑ microbial diversity in antibiotic treated birds
- Development in time clearly visible
- Time-effect greater than treatment effect
  - Although small changes per treatment at specific time-points

Gene Expression
Small Intestine

Differences and similarities in time

identify the trends or patterns in your data, experimental bias, 'naturally' clustering according to the biological conditions



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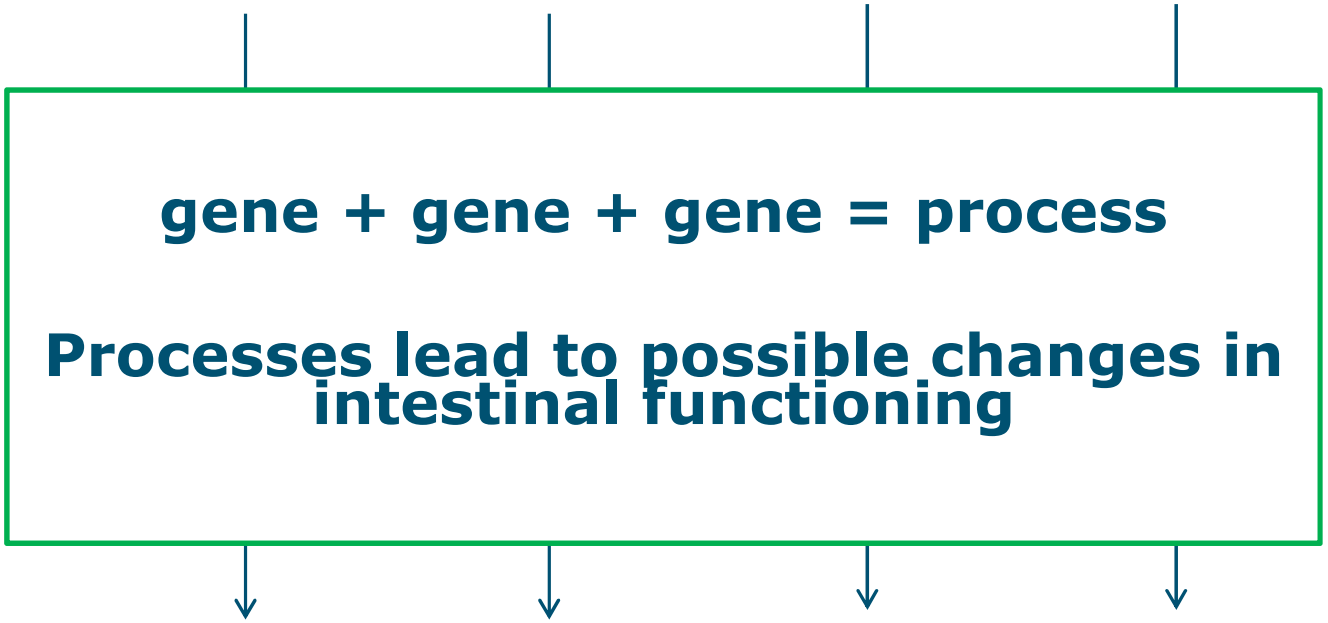
Statistical Analysis - Treatment

	A5vsWA5		A14vsWA14	
#genes	**DOWN**	**UP**	**DOWN**	**UP**
p_{adj}<0.01	489	556	182	234



Functional analysis (DAVID) day 5

(A5-WA5) Down low(er) in antibiotic treatment			(A5-WA5) Up high(er) in antibiotic treatment		
ES	**Genes**	**General Term**	**ES**	**Genes**	**General Term**
4.83	30	intracellular organelle lumen	7.86	72	extracellular matrix
4.77	11	protein transport/localization	5.25	44	triple helix (hydroxyproline,hydroxylysine)
3.26	13	domain: BTB/POZ-like (transcriptional repression)	5.16	14	Collagen triple helix repeat (hydroxyproline,hydroxylysine)
3.09	26	macromolecule/protein catabolic process	4.47	35	cell projection morphogenesis (neuron, differentiation)
2.65	5	immune response-regulating signal transduction	3.66	9	Fibrillar collagen
2.39	18	nuclear envelope-endoplasmic reticulum network	3.56	18	regulation of cell development (neuronal)
2.33	42	positive regulation of immune system process	3.08	15	positive regulation of transcription/macromolecule
2.27	20	cellular protein localization	3.07	21	EGF-like domain
2.19	12	adaptive immune response	2.57	8	response to steroid hormone stimulus (cortico/glucocortico)
2.08	5	Protease/peptidase activity	2.57	24	thrombospondin-type (Laminin G)

Metabolic / generic
Transcription
Immune
Cell (structure)
Development

ES; enrichment score

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Functional analysis (DAVID) day 14

(A14-WA14) Down low(er) in antibiotic treatment		
ES	Genes	General Term
2.49	19	positive reg. of biosynthetic process/transcription
2.00	8	epithelium morphogenesis/development
1.60	15	macromolecule/protein catabolic process
1.48	24	intracellular organelle lumen
1.47	5	blood vessel development

(A14-WA14) Up high(er) in antibiotic treatment		
ES	Genes	General Term
4.51	40	organelle lumen (intracellular)
2.38	18	transit peptide:Mitochondrion
1.84	4	sterol/steroid biosynthesis
1.68	6	Heat shock protein (DnaJ)
1.53	7	RNA recognition motif (RNP-1)
1.51	4	translation initiation factor activity
1.48	3	(negative) regulation of lipid storage
1.43	3	Signaling Pathways (EPO/IGF1/IL6/TPO/IL2/PDGF/EGF)
1.39	10	cellular protein localization/targeting
1.32	4	zinc-binding (LIM domain)

Metabolic / generic
Transcription
Immune
Cell (structure)
Development

ES; enrichment score

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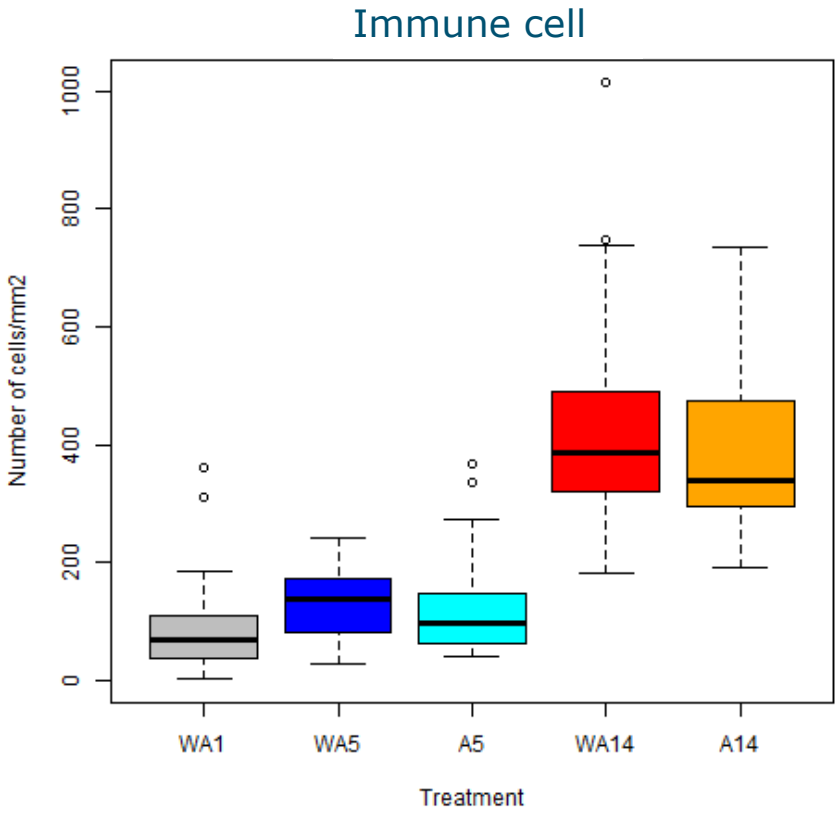
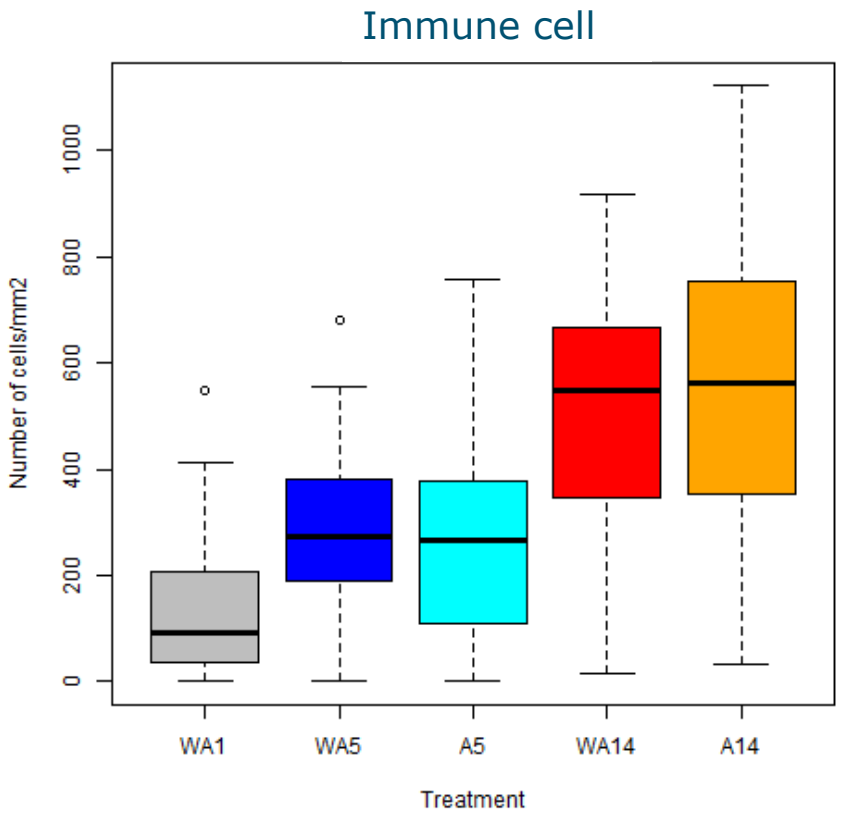
Conclusions gene expression

- Antibiotic treated birds
  - ↑ Cell structure/Development
  - ↓ Immune
- Effect most prominent on day 5

Immunohistochemistry
Small Intestine

IHC (1)





	p value
Time:Treatment	**0.755**

	p value
Time:Treatment	**0.477**

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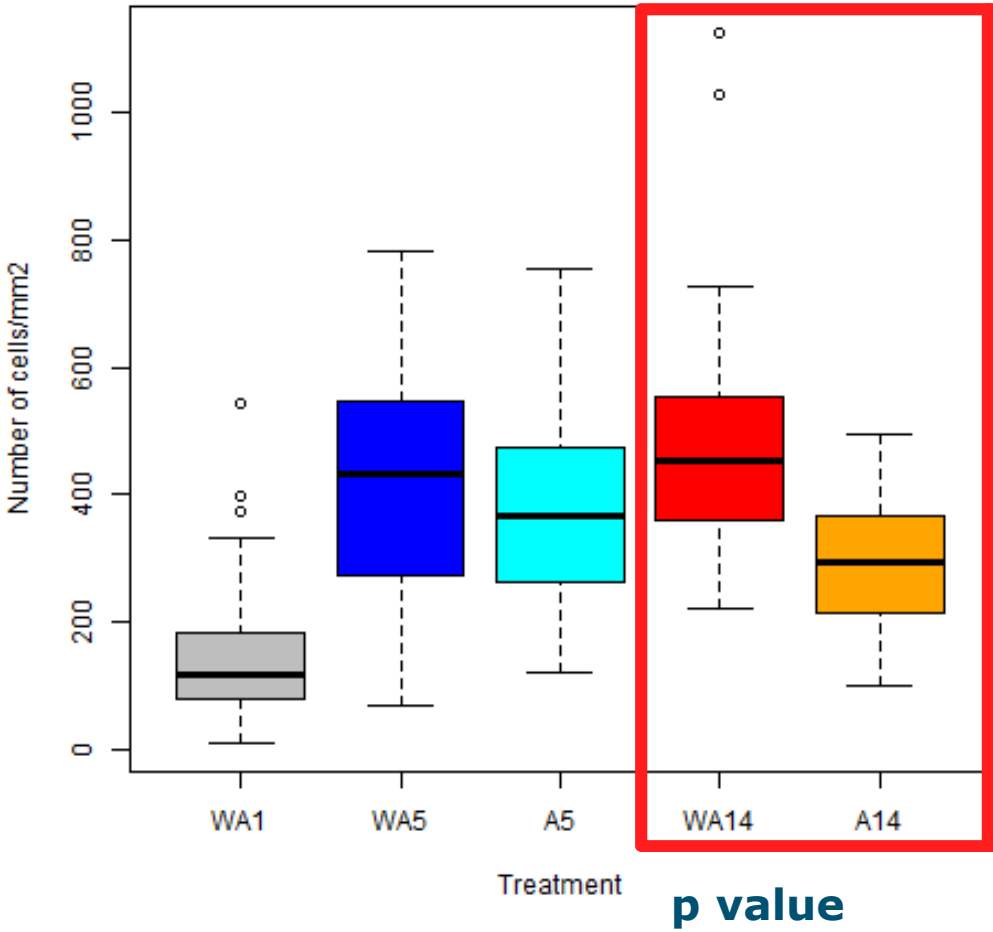
IHC (2)

Macrophage-like cells



T-test treatment effect on specific day

day5	day14
0.57	5.56e-07

p value

Time:Treatment **1.48e-10**

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Conclusions immunohistochemistry

- Development in time clearly visible
- Treatment effect in macrophage-like cells on day 14
  - ↓ in antibiotic treated birds

Discussion

- Modulation of microbiota and intestinal (immunological) development is possible
- To gain more insight on intestinal health parameters
  - (Pathogenic) challenge are necessary in future studies
- Search for feed interventions which also show modulation of microbiota and intestinal (immunological) development

Thanks for your attention

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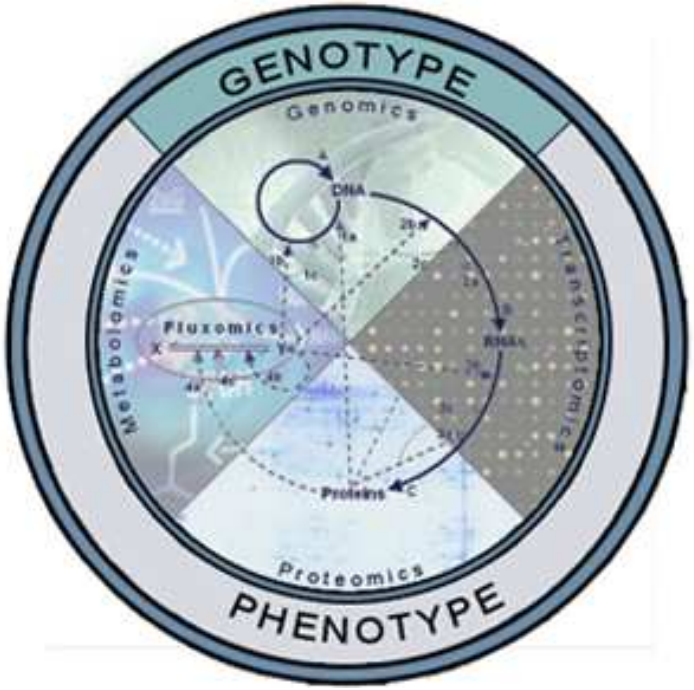

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