Exposure to antibiotics affects intestinal microbiota and immune development in broilers

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Acknowledgements

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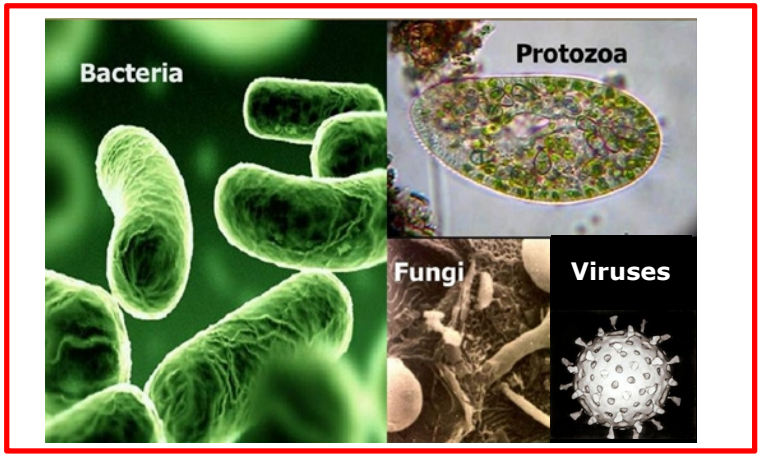
Feed4
Foodure

BREED4FOOD

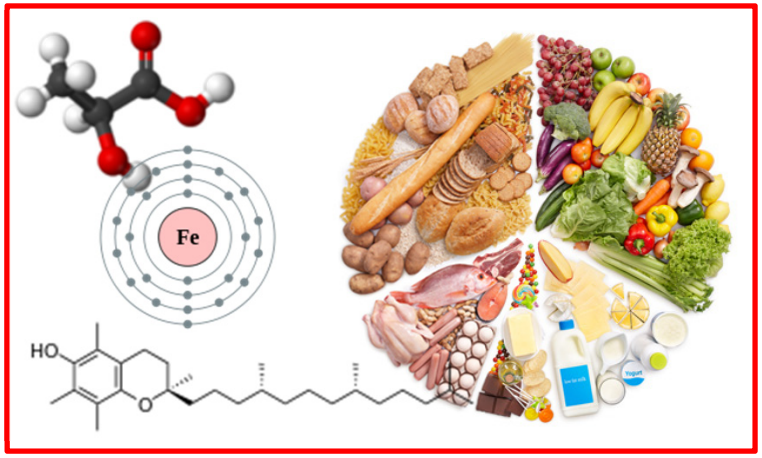
Host

interactions have effect on performance and health

Microbiota



Feed



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Background of study

- Focus on improving gut health (immune development)
- Approach
  - Antibiotics – known to have impact on the intestinal microbiota
  - proof of concept – modulation in early life affects both the intestinal microbiota and immune development
- Focus on neonatal period
  - Life long effect on immune status (development and programming of immune system)

Objective

- Investigate the effect of short-term administration of antibiotics, applied via the drinking water, at early life of chicken (day 1) on intestinal microbiota (composition and diversity) and intestinal gene expression later in life (days 1, 5, and 14)
    - Microbiota
        - Jejunum (MiSeq 16S rRNA sequencing)
    - Transcriptomics
        - Jejunum (Agilent microarray platform)

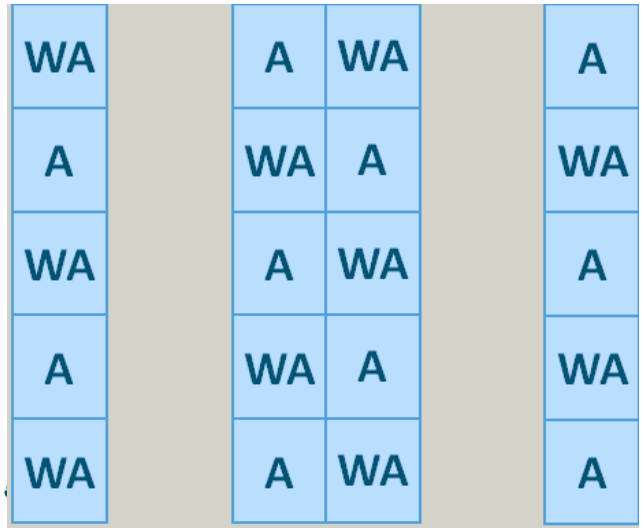
Study design

Without antibiotics (WA)

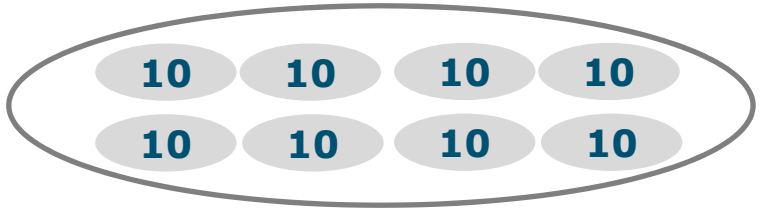
Amoxicillin; bacteriolytic, β-lactam antibiotic

Cobb500

Housing



Pools



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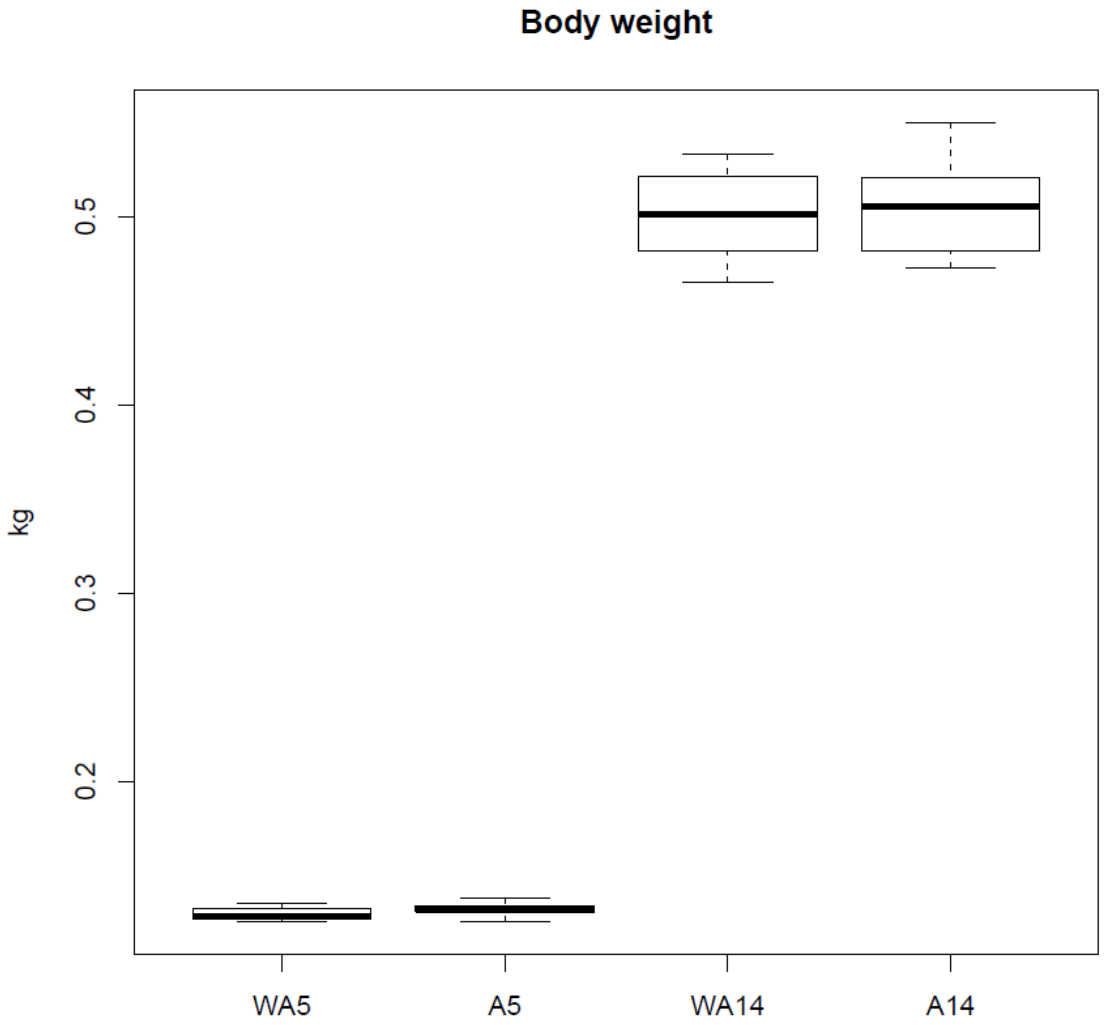
Performance results



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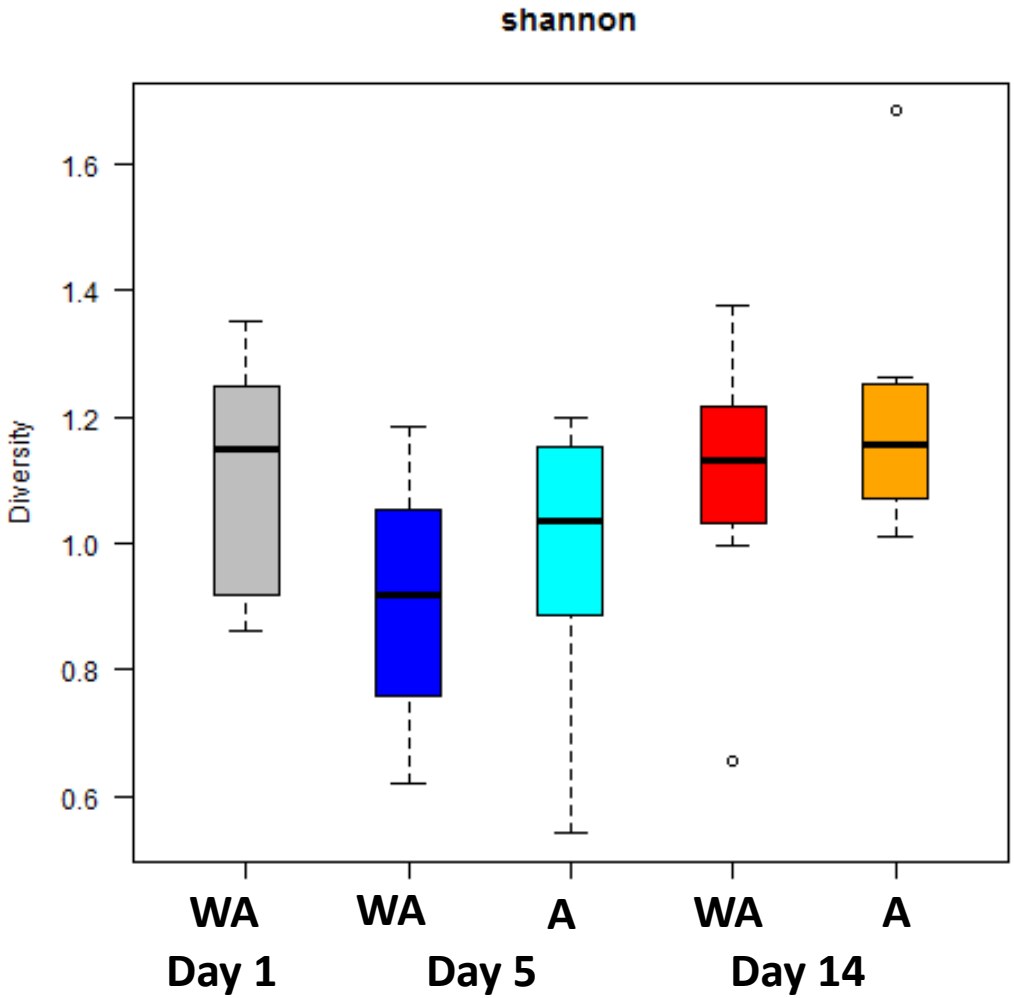
Results

Microbiota Jejunum

Microbiota diversity in jejunum



T-test treatment effect on specific day

Day 5

p = 0.45

Day 14

p = 0.33

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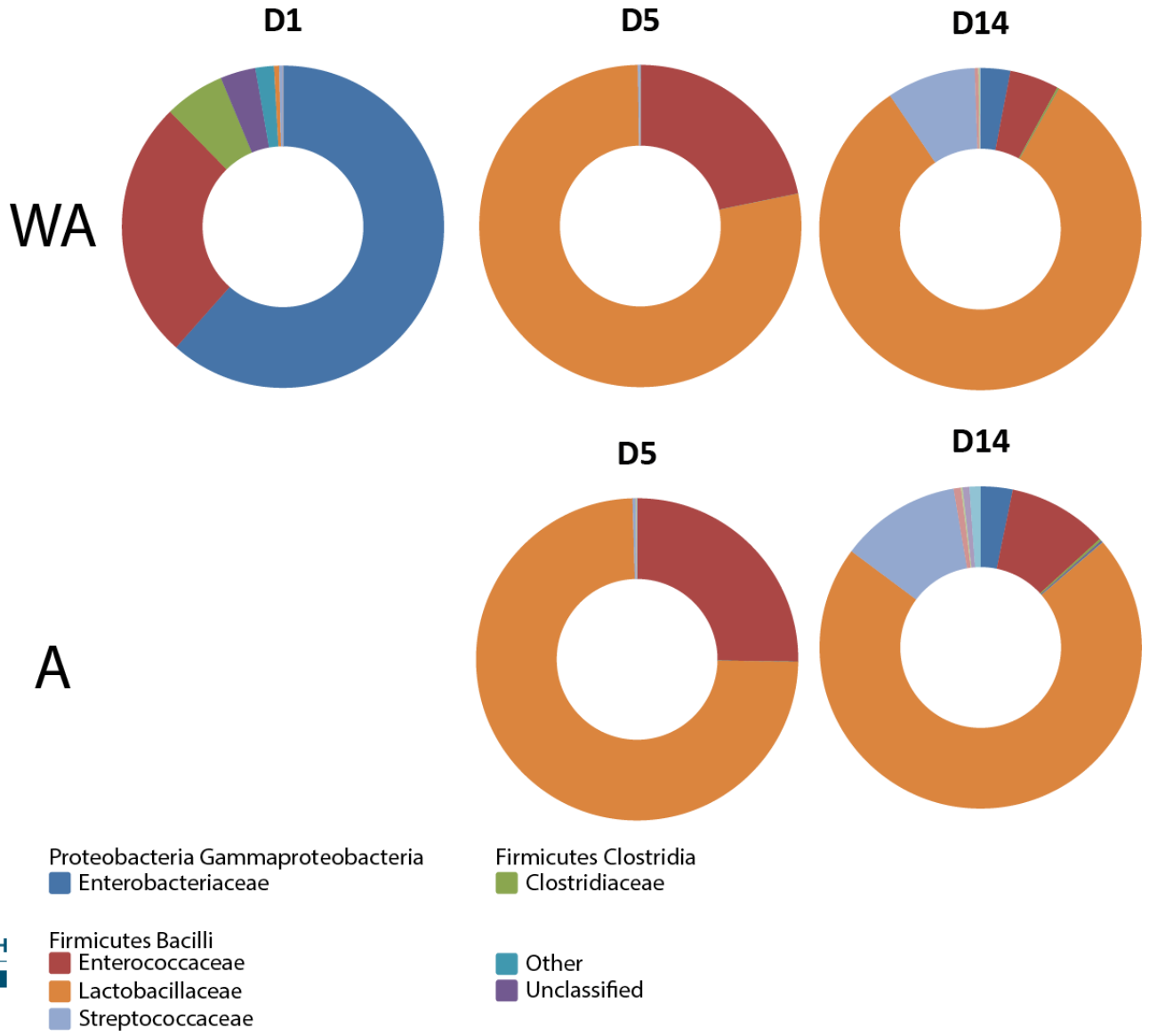
Comparison of microbiota composition between days 1, 5, and 14



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Statistical analysis of jejunal microbiota

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	21.7	25.2	4.9	**9.9**
		Lactobacillaceae	77.9	74.2	**82.2**	70.5
	Clostridia	Other	0.004	0.02	0.37	**0.73**

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* p-value < 0.05

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Conclusions microbiota

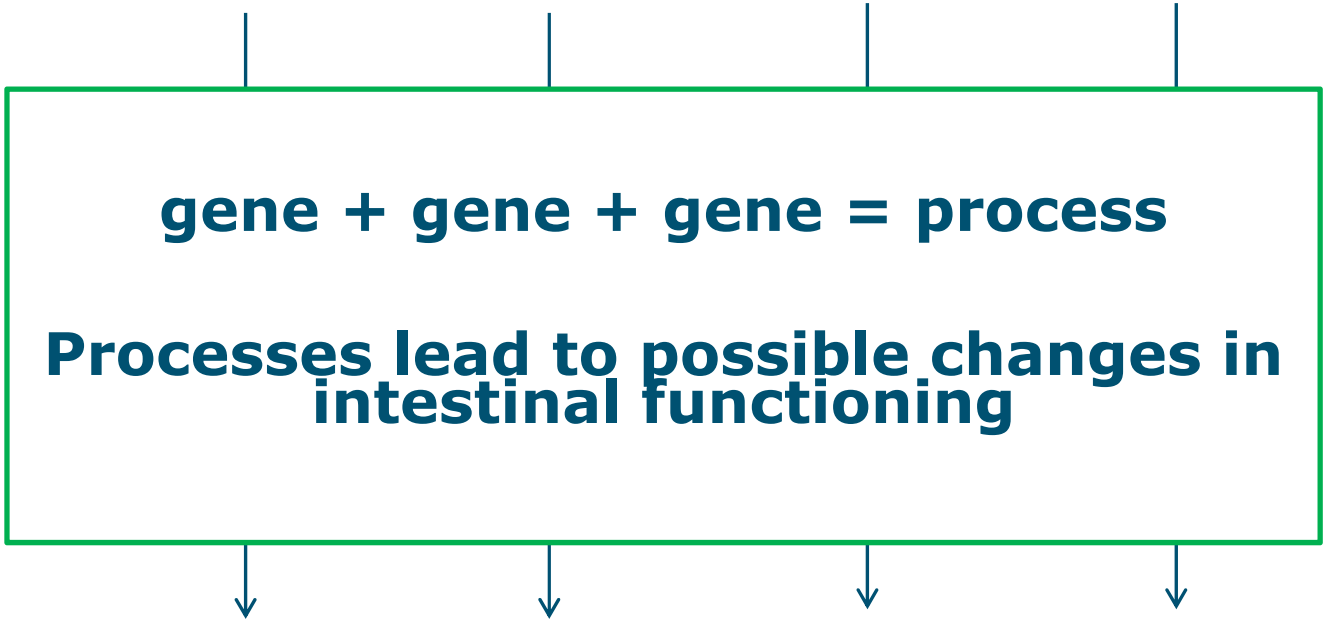
- Early life (day 1) antibiotic treatment increases jejunal microbiota diversity on days 5 (and 14)
- Major changes in jejunal microbiota composition during development
- Age-effect greater than antibiotic treatment effect

Results

Jejunal Gene Expression

Statistical Analysis – Antibiotic effects

	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

MS9

(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
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Cell (structure)
Development

ES; enrichment score

Dias nummer 16

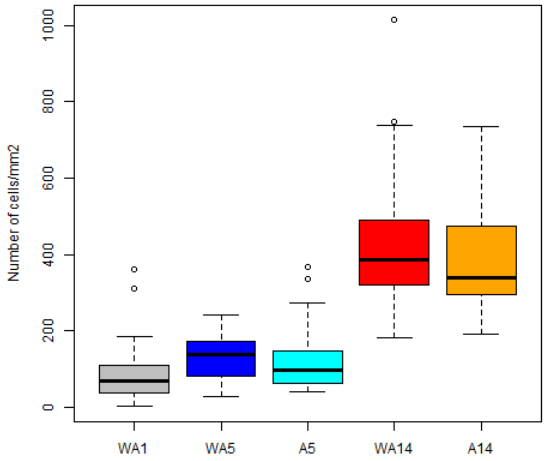
MS9 teksten erg klein
Smits, Mari, 20/08/2014

Conclusions gene expression

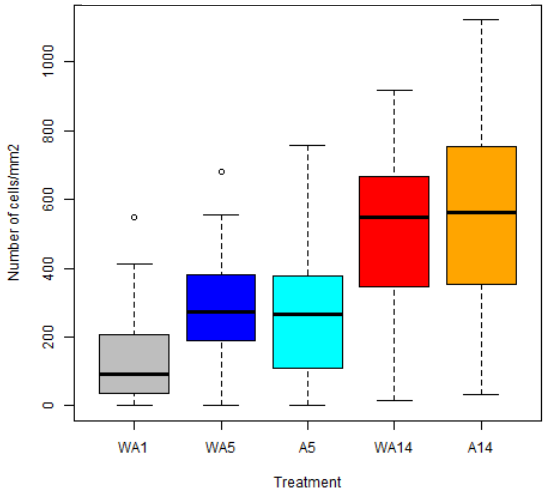
- Early life antibiotic treatment affects gene expression of jejunal mucosa
  - Increase in processes related to cell structure / development
  - Decrease in a variety of immune related processes
- Effect of antibiotic treatment is most prominent on day 5 compared to day 14

Number of immune cells in jejunum

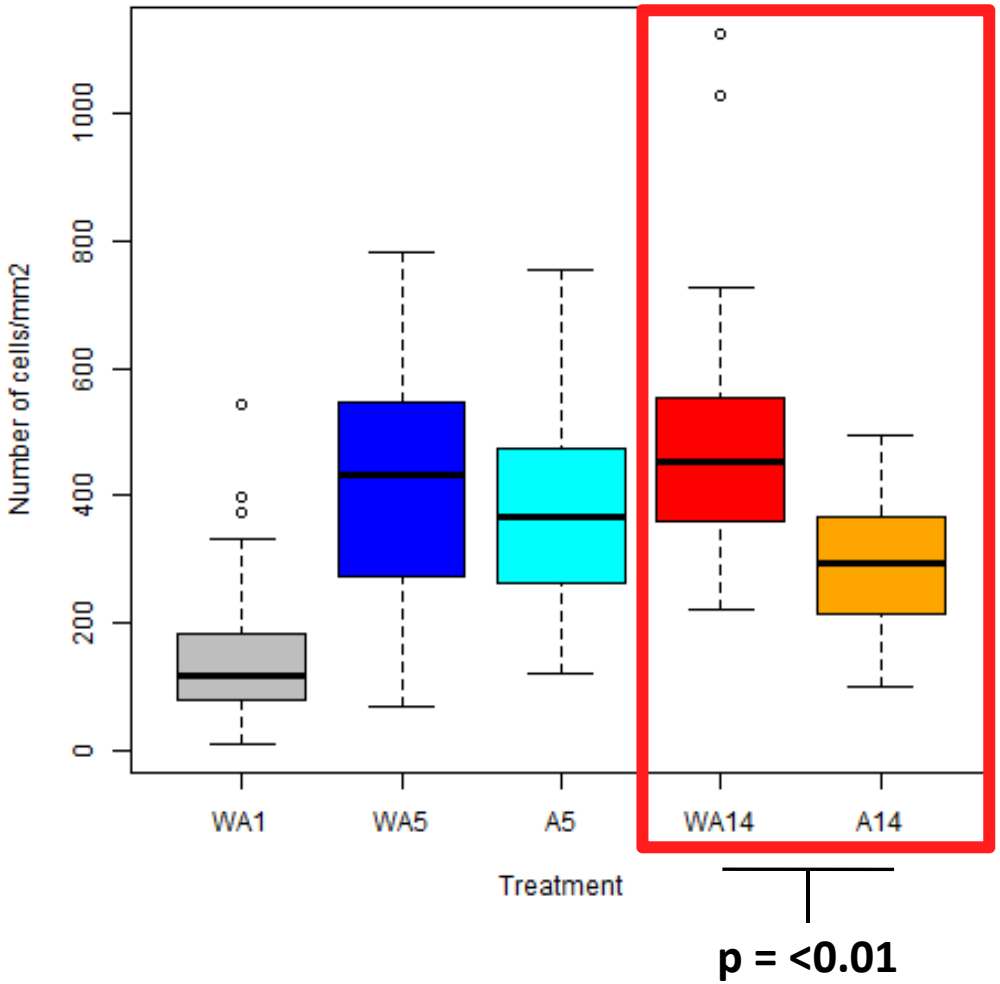
CD4⁺ cells



CD8⁺ cells



Macrophage-like cells



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Discussion

- Modulation of microbiota and jejunal immunological development is possible, both at the molecular as well as the cellular level
- To gain more insight into the relevance of the inducible immune parameters for health, future challenge studies are required
- Investigate dietary interventions which show modulation of microbiota and intestinal immunological development and programming that contribute to improvement of functionality of the immune system and animal health

Thanks for your attention

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