Pathogenesis, diagnosis and prevention of dysbiosis in broilers

IWT - 135067

Ghent University, Faculty of Veterinary Medicine

Department of Pathology, Bacteriology and Avian Diseases

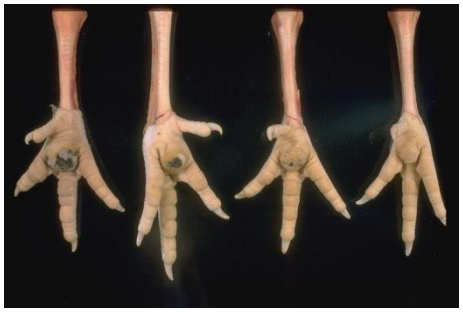
Department of Reproduction, Obstetrics, and Herd Health, Epidemiology Unit

15th January 2018

Challenges to the poultry industry that resulted in the project

Wet litter
Dysbiosis

Footpad lesions



Antimicrobial usage and AMR

Challenges to the poultry industry that resulted in the project

Wet litter
Dysbiosis

Footpad lesions

Antimicrobial usage
and AMR

Challenges to the poultry industry that resulted in the project

Wet litter
Dysbiosis

Footpad lesions

Antimicrobial usage and AMR

Intestinal Health

Strategic aims on long term

- Improve animal welfare
- Increase sustainability of poultry industry
- Prevent disease
- Diagnose gut health problems

Specific aims of the project

What is intestinal health?
What is dysbiosis?

What are factors that influence dysbiosis?

What are easy-to-measure markers for dysbiosis?

How can we prevent dysbiosis?

Workpackages

- WP1. Identification of risk factors in the field
- WP2. Description of microbiota composition and histology in field dysbiosis cases
- WP3. Development of diagnostic tools
- WP4. Model development and validation of preventive measures

Pathogenesis, diagnosis and prevention of dysbiosis in broilers

IWT - 135067

Fien De Meyer

Department of Pathology, Bacteriology and Avian Diseases
Faculty of Veterinary Medicine
Salisburylaan 133, 9820 Merelbeke
Ghent University, Belgium

15/01/2018

Table of contents

Development of an *in vivo* model for dysbiosis in broilers (WP4)

→ macroscopic lesion scoring parameters

→ morphological changes of the intestinal wall
→ intestinal inflammatory parameters

} WP2

→ markers for intestinal wall integrity
→ microbial marker populations

} WP3

WP4
Validation of diagnostic criteria

Aims

- Task 1) Development *in vivo* model dysbiosis

- Task 2) In field validation of prevention protocols

In vivo dysbiosis model: set-up

UNIVERSITEIT GENT

AGENTSCHAP INNOVEREN & ONDERNEMEN

Vlaanderen is ondernemen

- Control group

D1	D12	D12 - D18	D19 - D21	D20
Starter mash	**57% wheat + 5% rye**			

- Challenge group

D1	D12	D12 - D18	D19 - D21	D20
Starter mash	**57% wheat + 5% rye**	**Antibiotics**	**Bacterial challenge** - *E. coli* - *C. perfringens*	**Coccidiosis challenge** - *Eimeria acervulina* - *Eimeria maxima*

Sampling

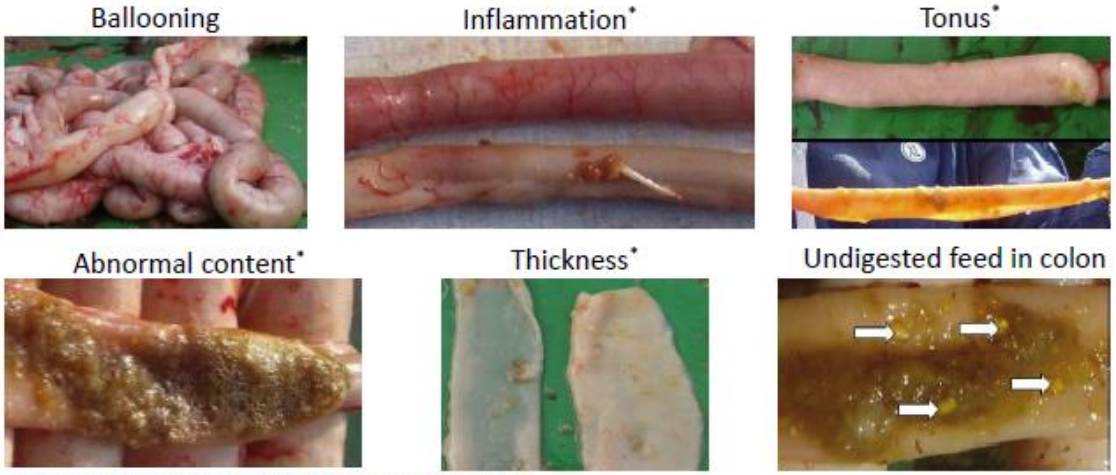
- D26 & D28
- 2 treatments, 9 replicates, 3 animals/group = 108 animals

Macroscopic lesion scoring of the intestine to identify dysbiosis

1) Body weight (BW)

2) Dysbiosis score (DS)

→ sum of 10 macroscopic parameters scored 0 (absent) or 1 (present)



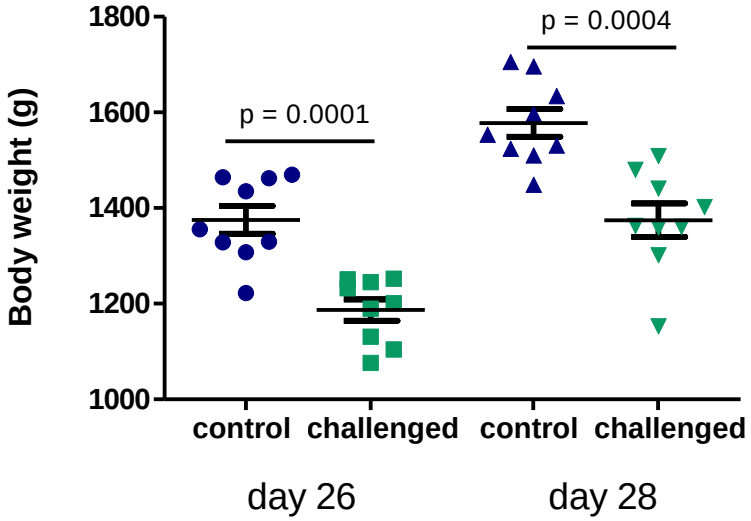
3) Coccidiosis score (CS) (Tellez *et al.*, 2014)

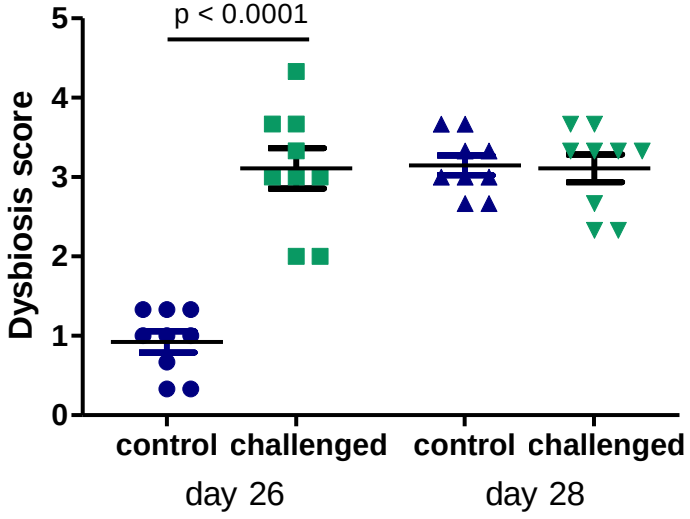
- *E. acervulina*
- *E. maxima*
- *E. tenella*

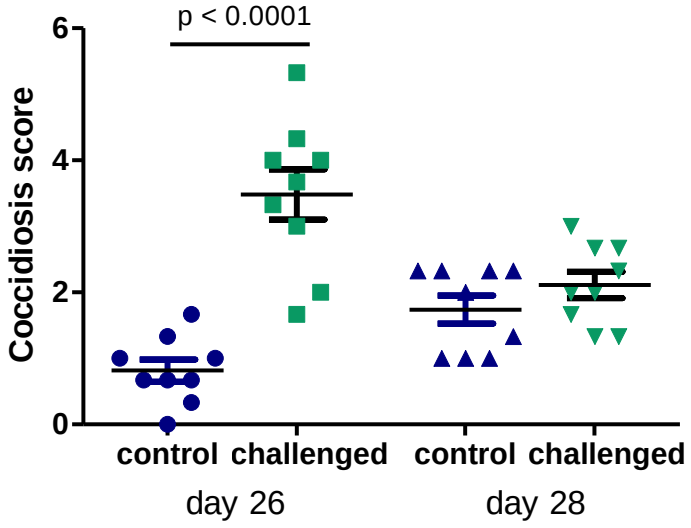
scored 0 (absent) to 4 (severe) → total score = sum

Macroscopic scoring parameters

→ All animals





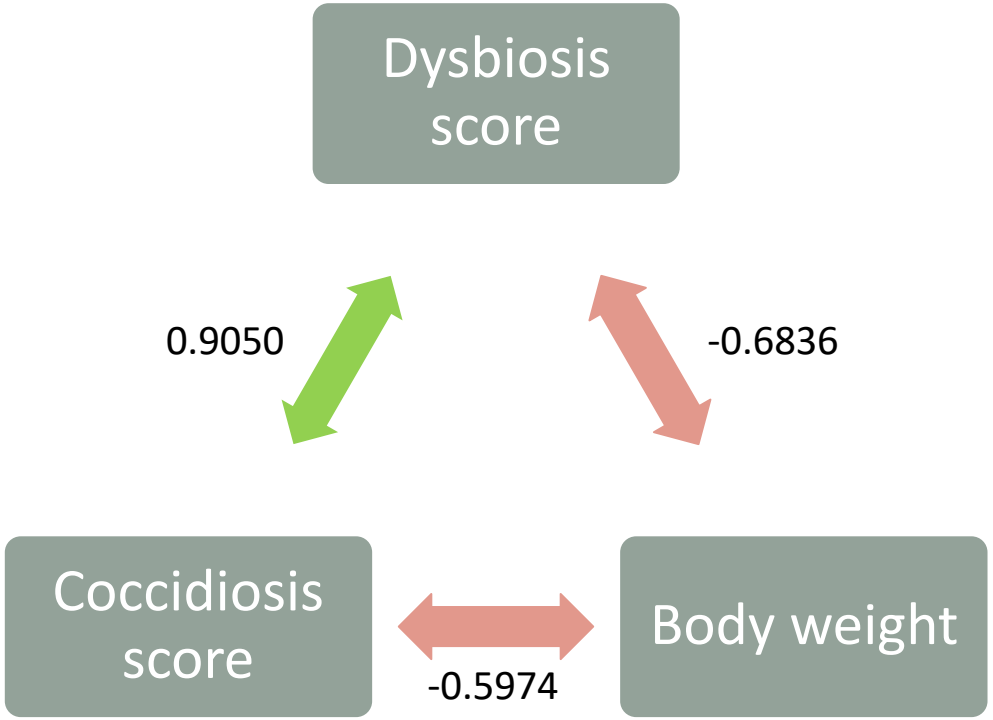


Macroscopic scoring parameters

D26 – associations

Positive (0 to 1)

Negative (0 to -1)



D28

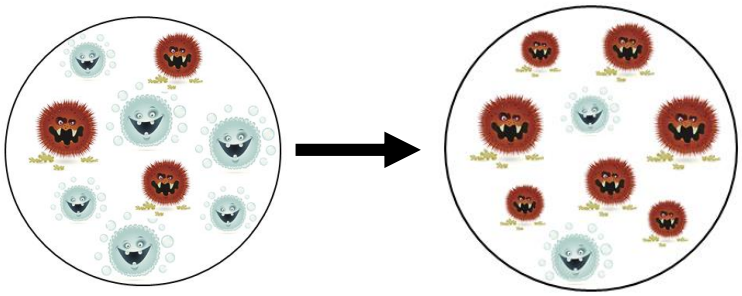
→ no significant associations

WP2

Description of morphological characteristics & gastrointestinal inflammatory parameters

WP2

Determination dysbiosis



- Definition still vague
- Macroscopic lesion scoring system = only method
- 1 study: scoring system ⟷ morphological parameters

Aims

- Task 1) Description of morphological changes of the intestinal wall
- Task 2) Description of intestinal inflammatory parameters

Description morphological changes

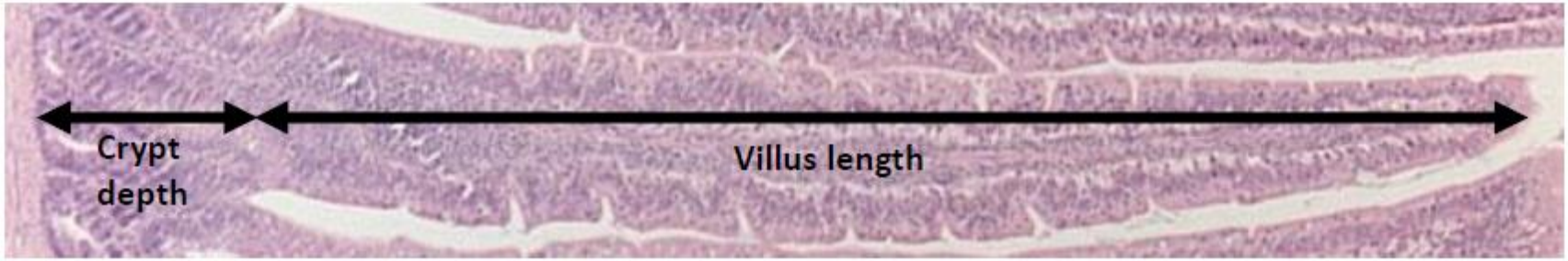
Duodenum (all animals)

↓

Haematoxylin & eosin staining

↓

Measurement using computer based image analysis program



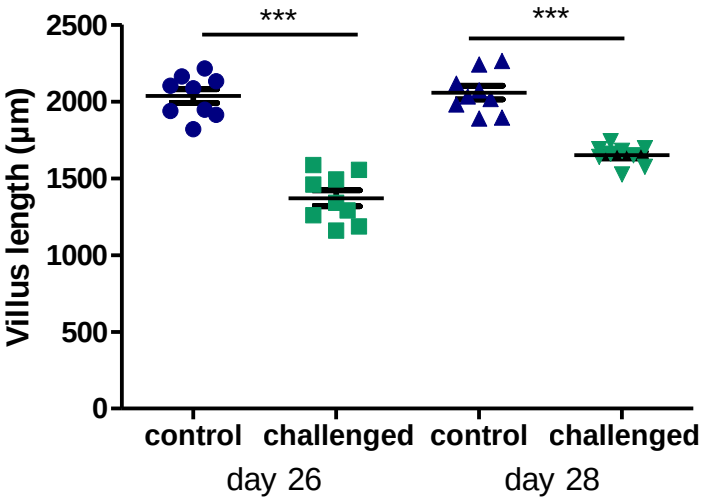
↓

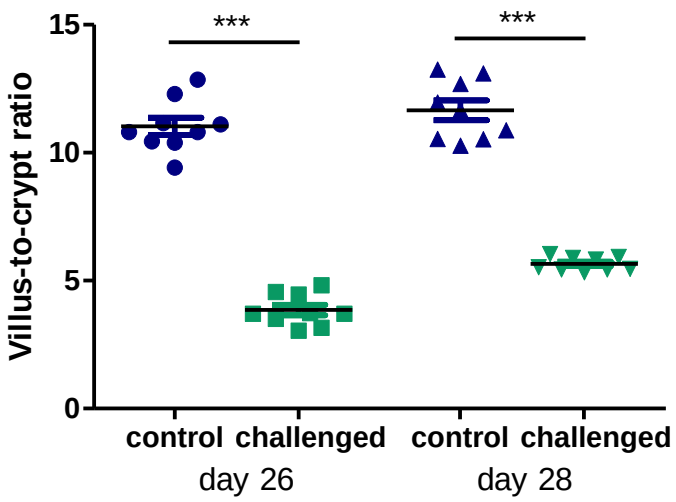
Histological parameters

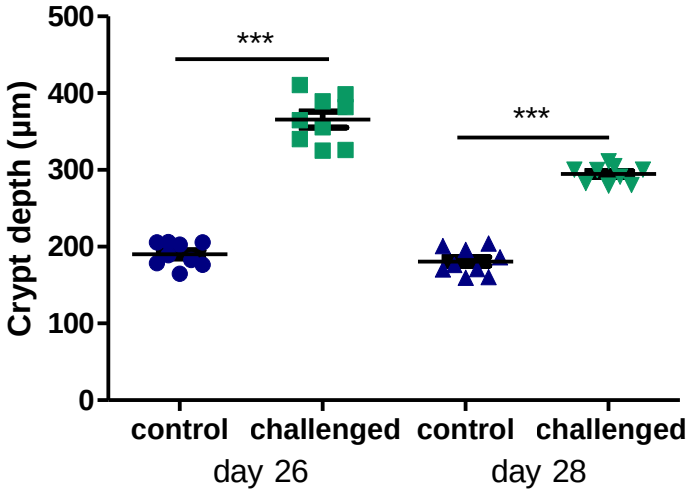
- villus length
- crypt depth

} Villus-to-crypt ratio

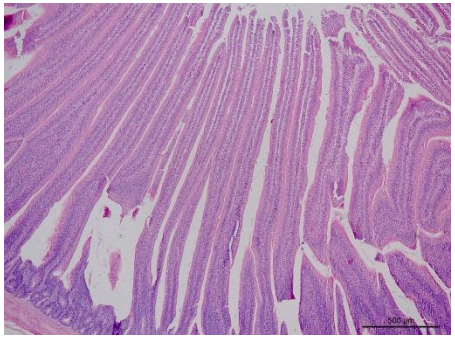
Description morphological changes







$***p < 0.0001$



Control

Challenged

Description inflammatory parameters

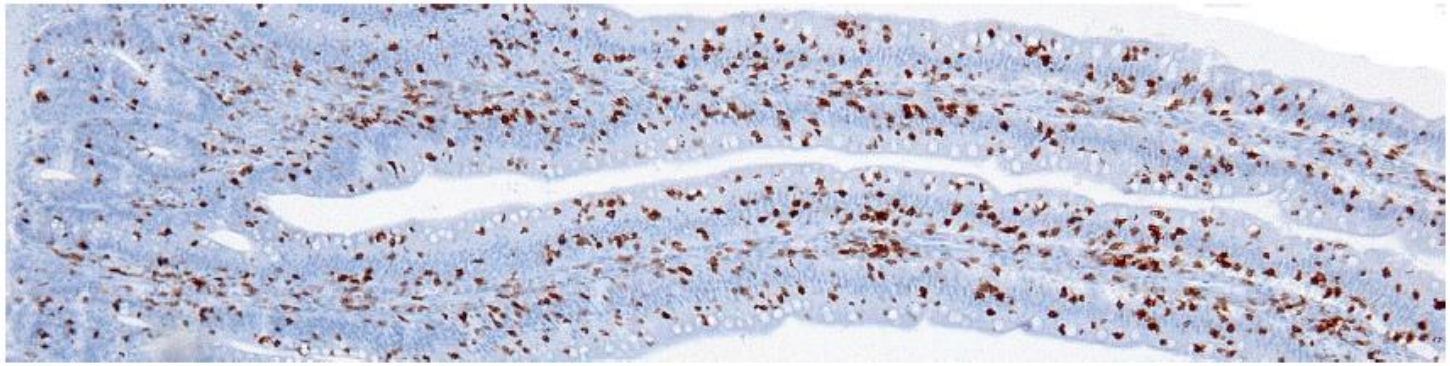
Duodenum (all animals)

↓

Immunohistochemistry (CD_3 staining)

↓

Computer based image analysis system

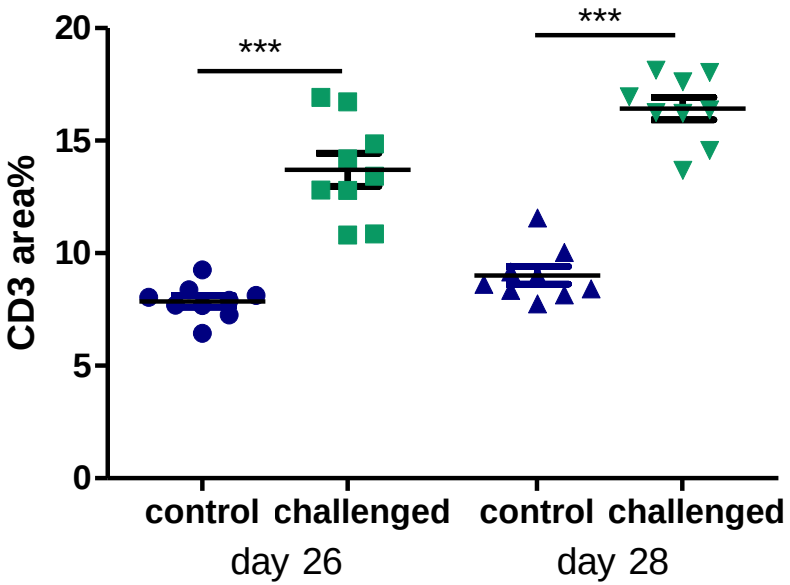


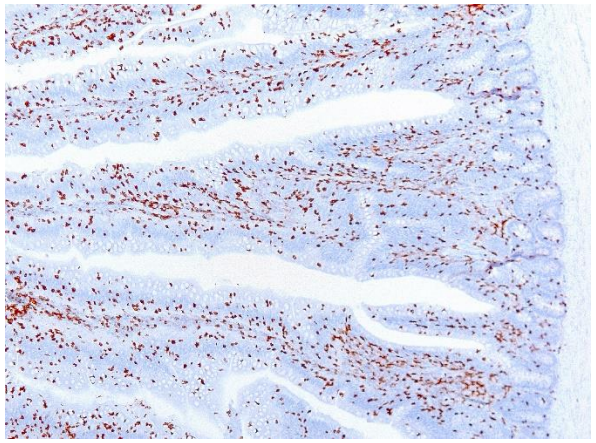
↓

Measurement area% of CD_3^+ T-cells

→ quantification infiltration immune cells

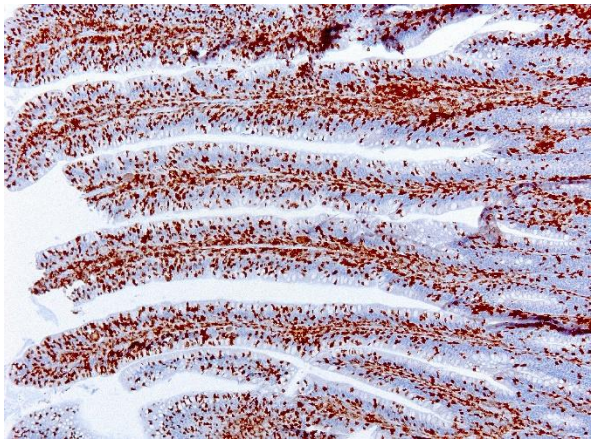
Description inflammatory parameters





Control

$***p < 0.0001$



Challenged

Description morphological changes & inflammatory parameters

UNIVERSITEIT GENT

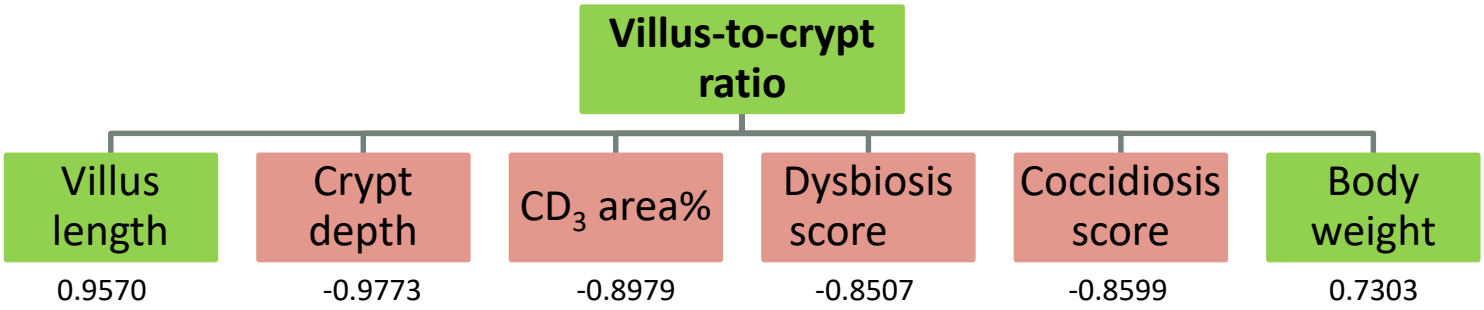
AGENTSCHAP INNOVEREN & ONDERNEMEN

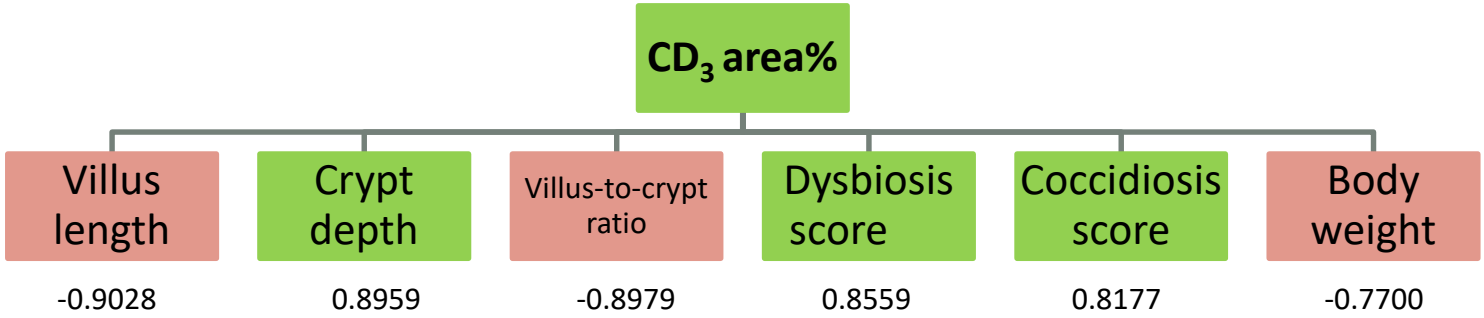
Vlaanderen is ondernemen

Associations D26

Positive: p1 ↑ then p2 ↑

Negative: p1 ↑ then p2 ↓





Description morphological changes & inflammatory parameters

UNIVERSITEIT GENT

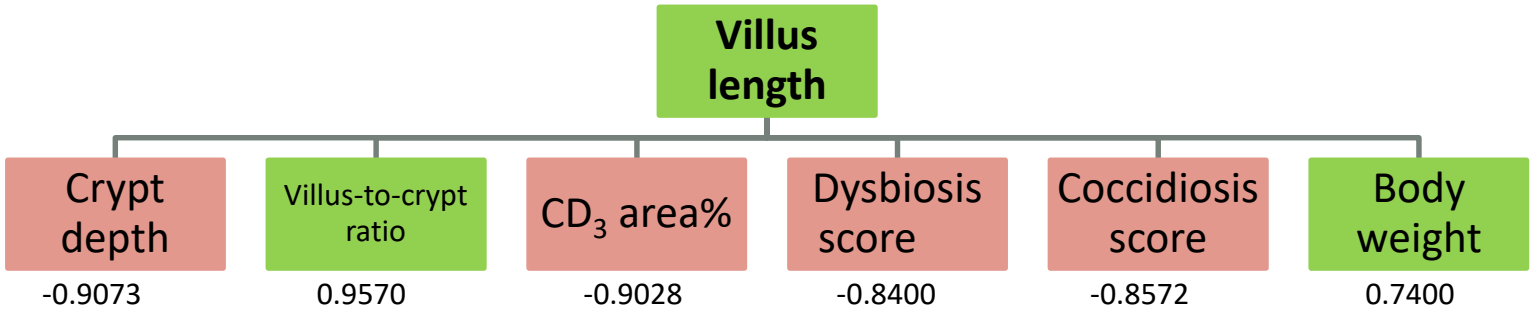
AGENTSCHAP INNOVEREN & ONDERNEMEN

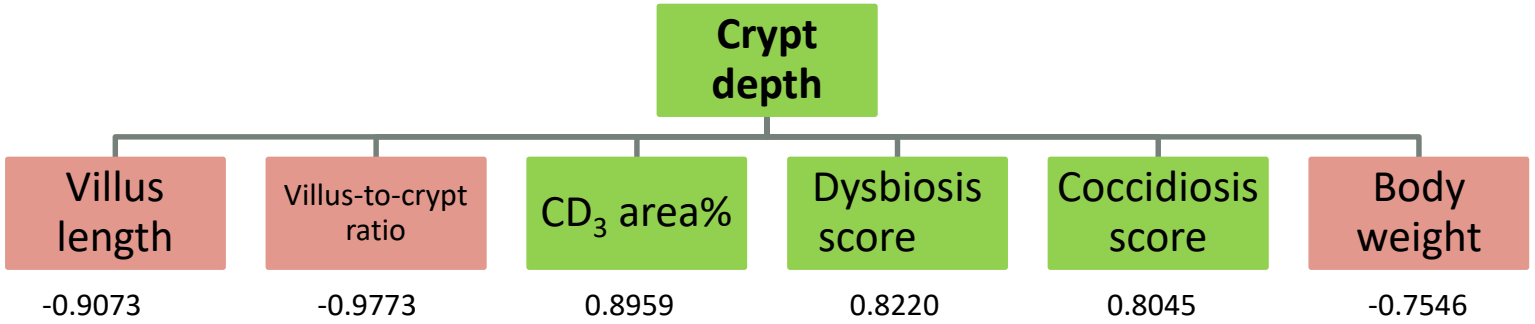
Vlaanderen is ondernemen

Associations D26

Positive: p1 ↑ then p2 ↑

Negative: p1 ↑ then p2 ↓





Description morphological changes & inflammatory parameters

UNIVERSITEIT GENT

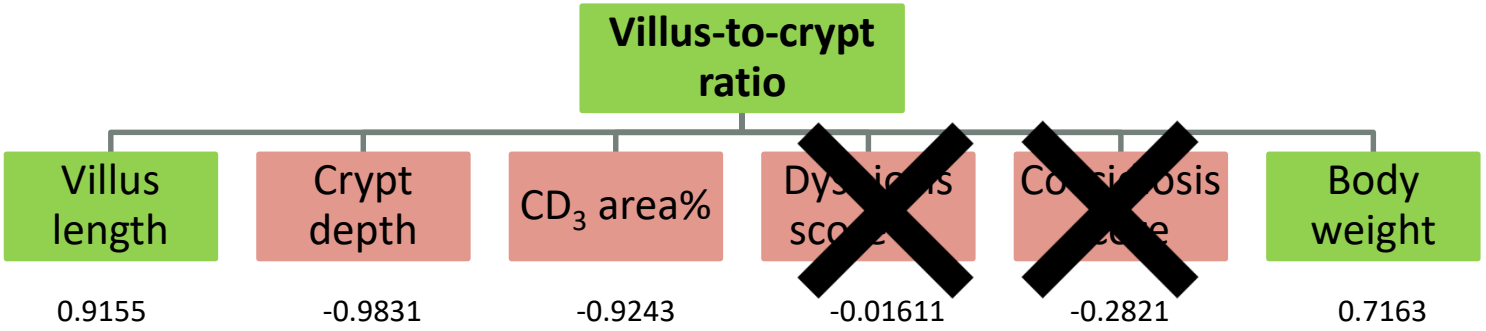
AGENTSCHAP INNOVEREN & ONDERNEMEN

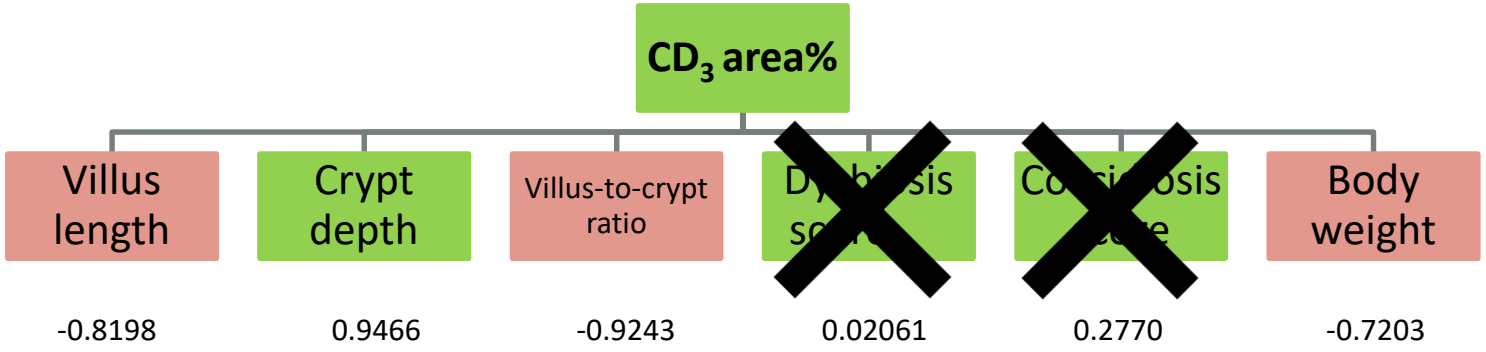
Vlaanderen is ondernemen

Associations D28

Positive: p1 ↑ then p2 ↑

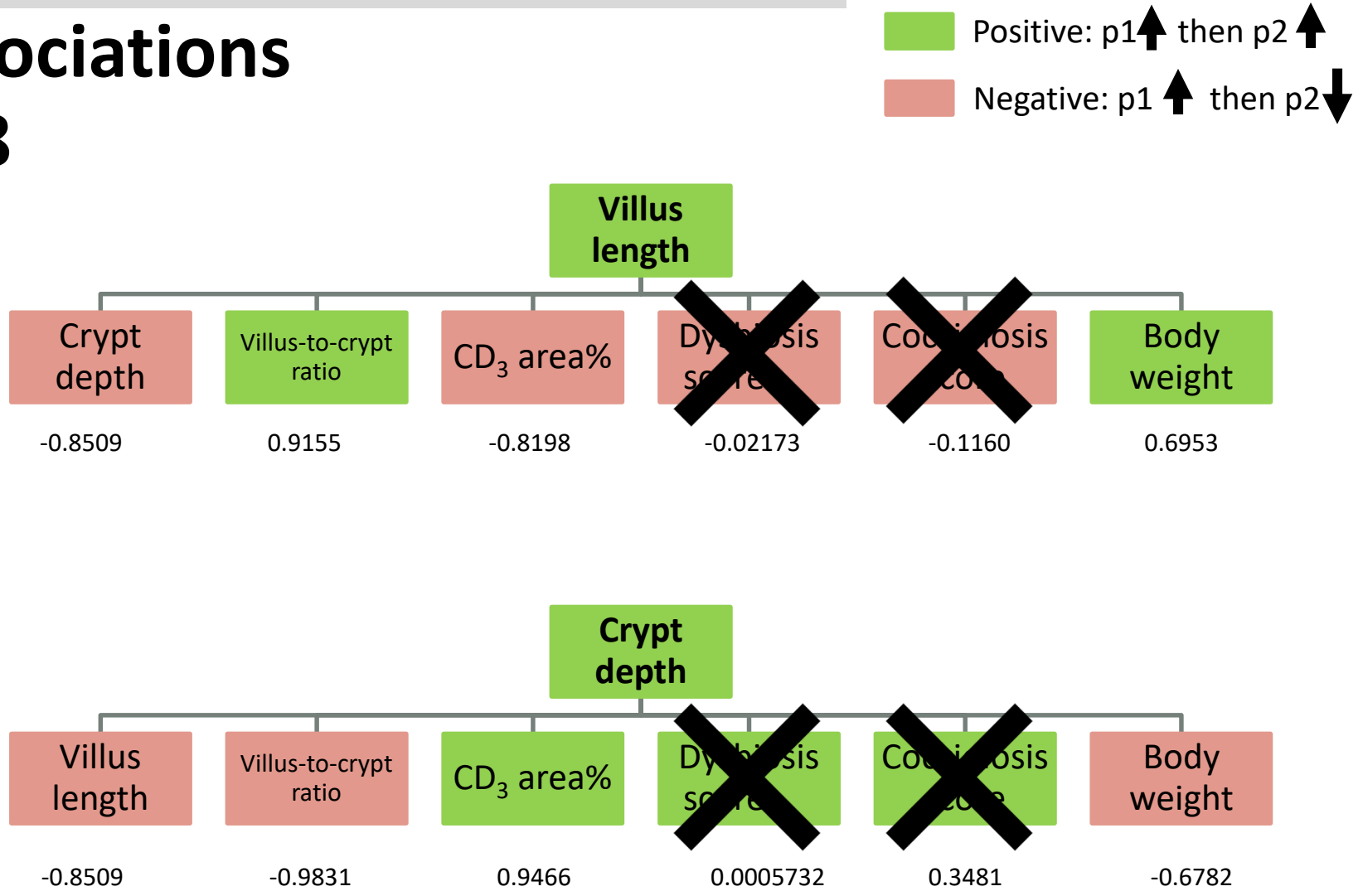
Negative: p1 ↑ then p2 ↓





Description morphological changes & inflammatory parameters

Associations D28



Overview

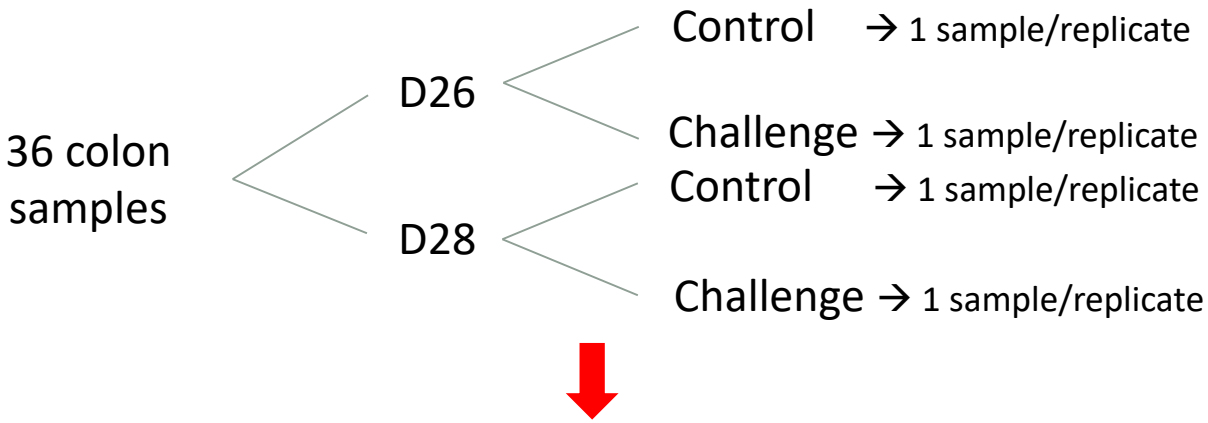
Dysbiosis score dependent of coccidiosis score
→ not identical

Day 26
→ Associtation between all macroscopic and histological parameters

Day 28
→ No more association with dysbiosis & coccidiosis score

WP3
Development of diagnostic criteria for nonspecific gastrointestinal problems in broilers

Proteomics



Discovery proteomics

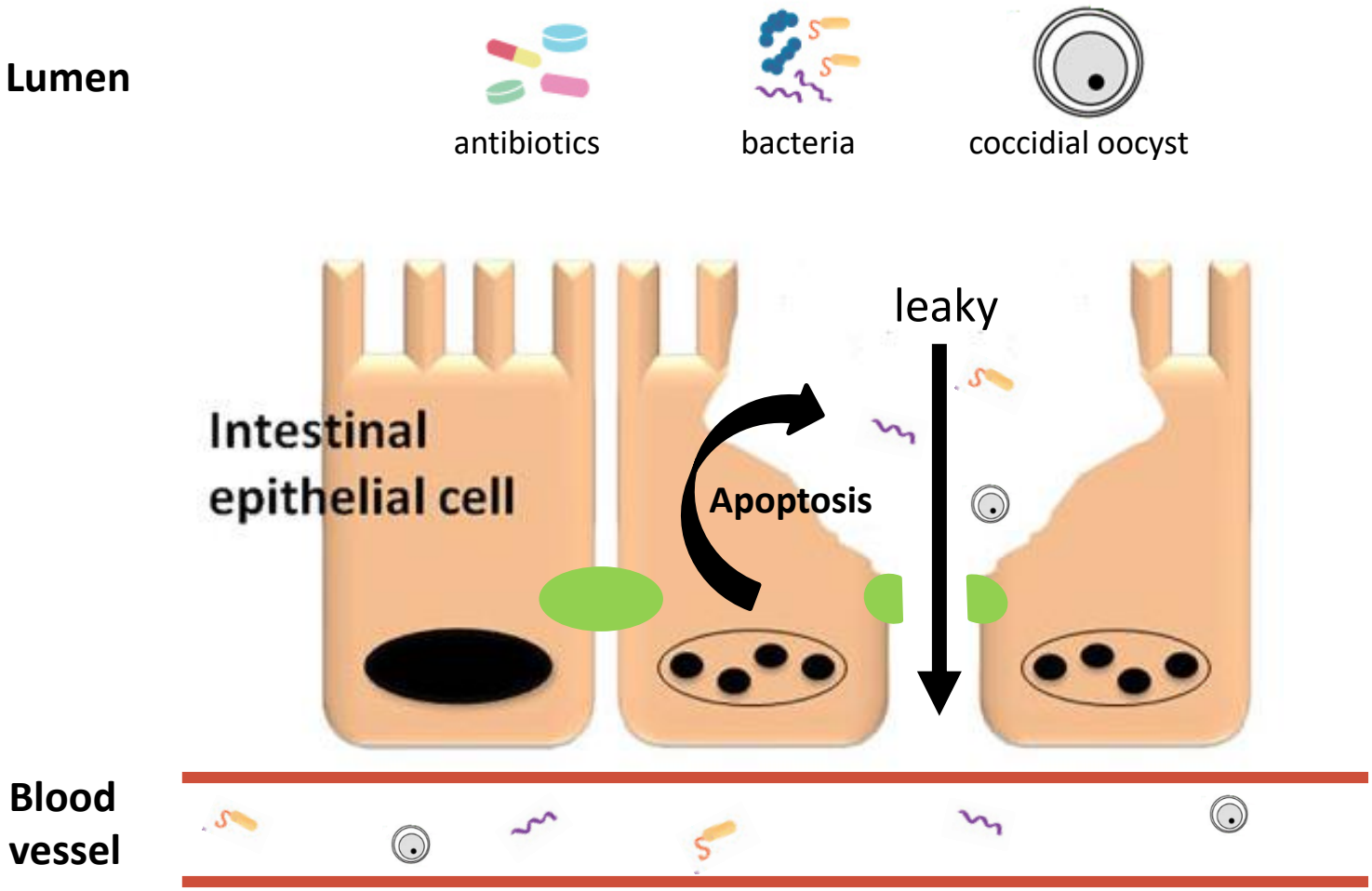
Liquid chromatography–mass spectrometry (LC-MS)

Analysis with Progenesis

Checked associations between proteins and

- villus length
- crypt depth
- villus-to-crypt ratio
- CD_3 area%
- dysbiosis score

Proteomics


Lumen
antibiotics
bacteria
coccidial oocyst
leaky
Intestinal epithelial cell
Apoptosis
Blood vessel

Proteomics

Inflammation marker	Villus length	CD_3	Dysbiosis	Function
Myeloid protein 1	-	+	+	granulocyt

Tissue destruction markers	Villus length	CD_3	Dysbiosis	Funtion
Fibronectin	-	+	+	Component of extracellular matrix
Annexin A5	-			Calcium-channel activity
Nucleophosmin	-	+		Activates tumor suppressor gene p53

Epithelial cell markers	Villus length	CD_3	Dysbiosis	Function
Carbonic anhydrase 2	-		+	Alkaline secretion
Aminopeptidase Ey	+	-	-	Secreted for protein digestion

Leakage markers	Villus length	CD_3	Dysbiosis	Function
Transthyretin	-	+		Acute phase protein
Ovotransferrin	-			Acute phase protein
Ovoinhibitor	-			Serine protease inhibitor
Apolipoprotein A1	-			Component of high-density lipoprotein

Tight junction marker	Villus length	CD_3	Dysbiosis	Function
Alpha-actinin	-		+	Actin-binding protein

Ovotransferrin ELISA

Colon content (all animals)

↓

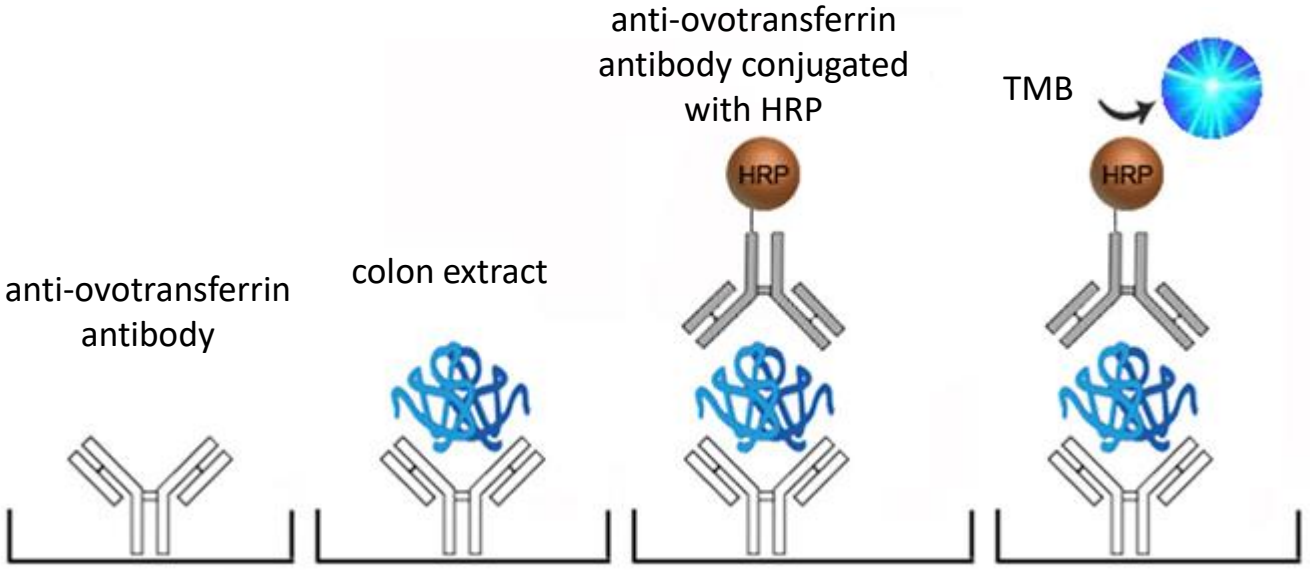
Extraction in PBS

↓

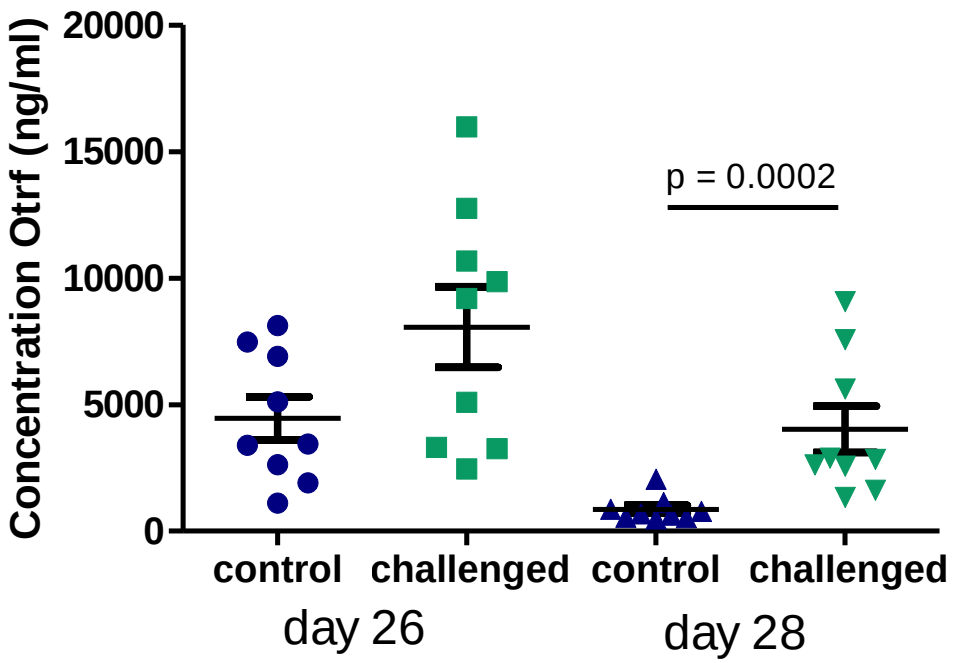
Double antibody sandwich ELISA

↓

Concentration determination ovotransferrin



Ovotransferrin ELISA



Ovotransferrin ELISA

UNIVERSITEIT GENT

AGENTSCHAP INNOVEREN & ONDERNEMEN

Vlaanderen is ondernemen

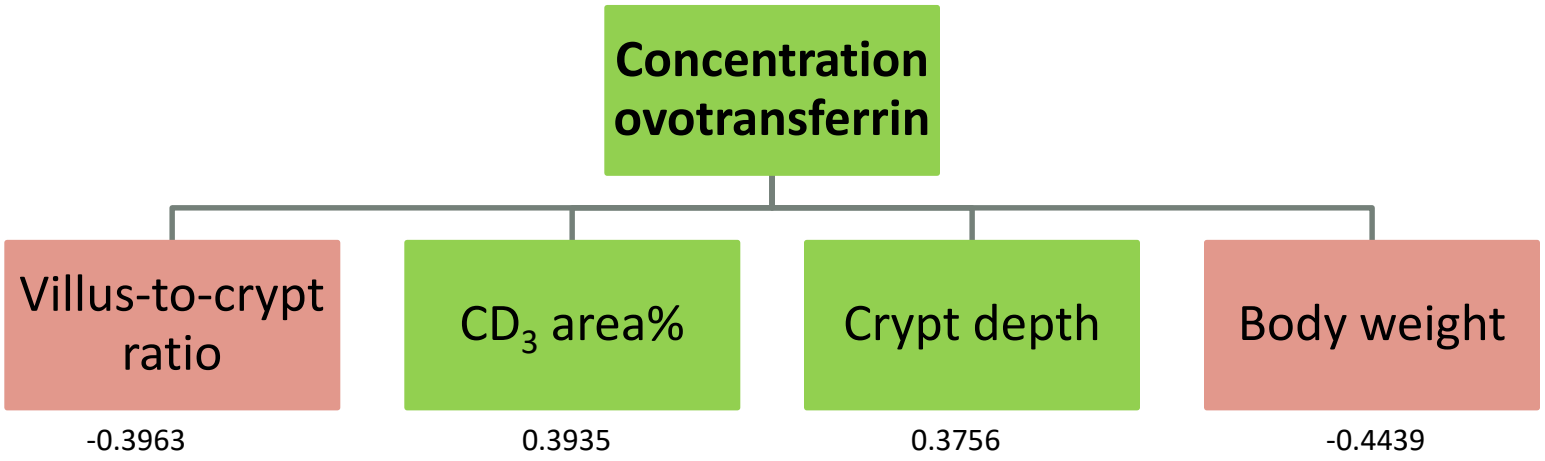
Associations

Positive: p1 ↑ then p2 ↑

Negative: p1 ↑ then p2 ↓

D26

D28



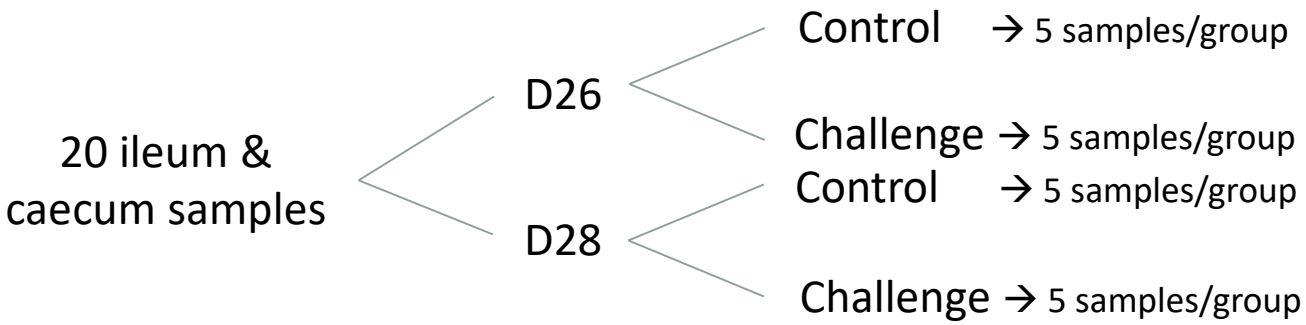
Overview

- D26 > protein expression

- Identification of protein markers
  - fibronectin
  - alpha-actinin
  - myeloid protein 1
  - carbonic anhydrase 2
  - aminopeptidase

➔ Description of gut health
  - intestinal damage
  - inflammation

16S sequencing



16S sequencing via Illumina MiSeq technology

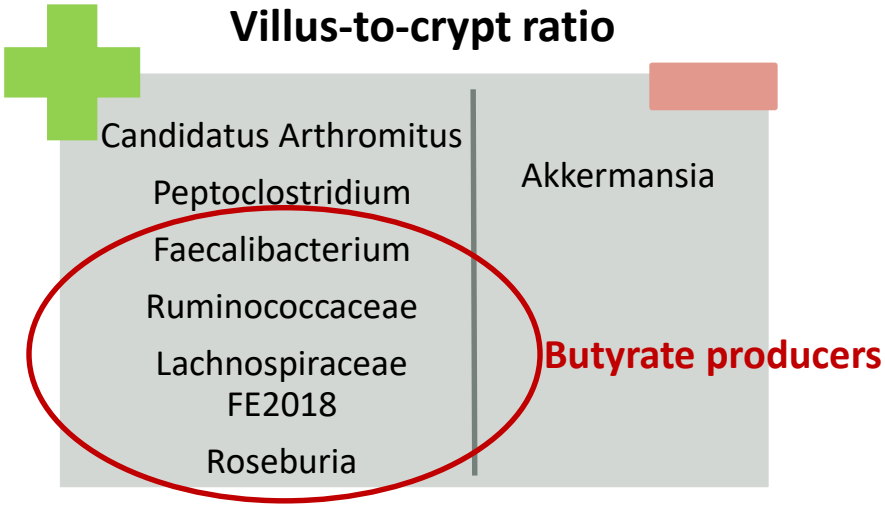
Checked associations between 16s data and

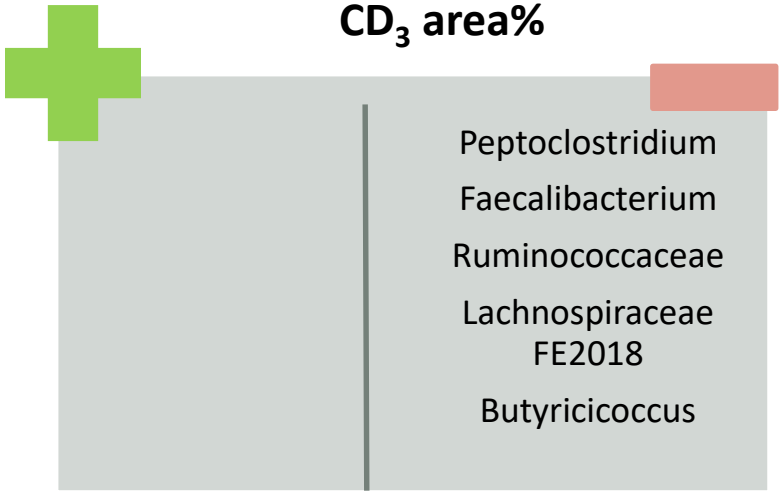
- macroscopic parameters
- histology parameters

16S sequencing

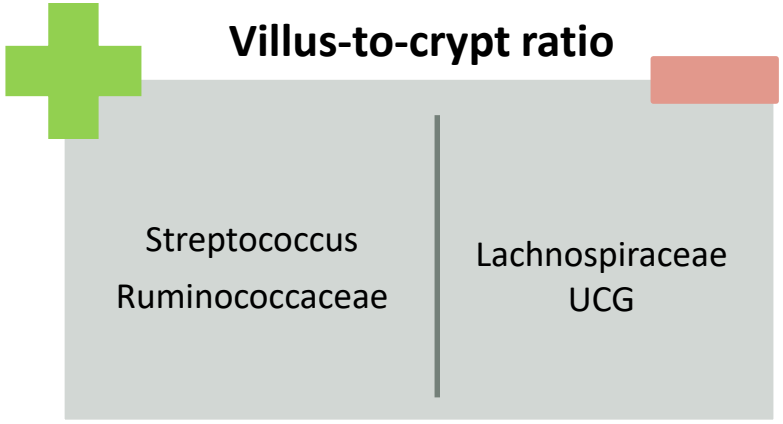
Associations – caecum

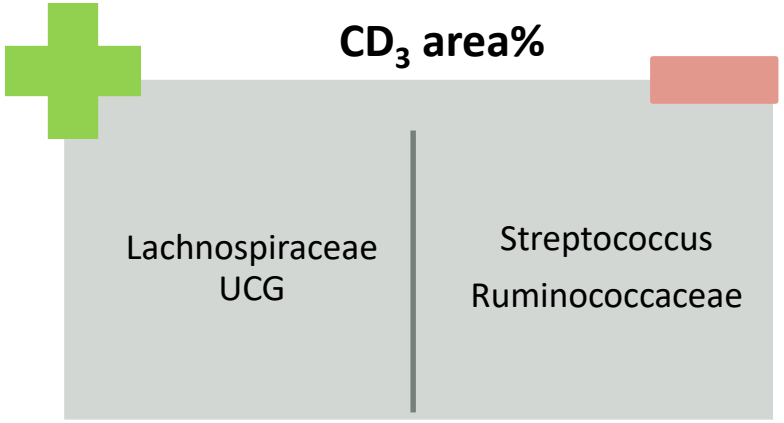
D26





D28



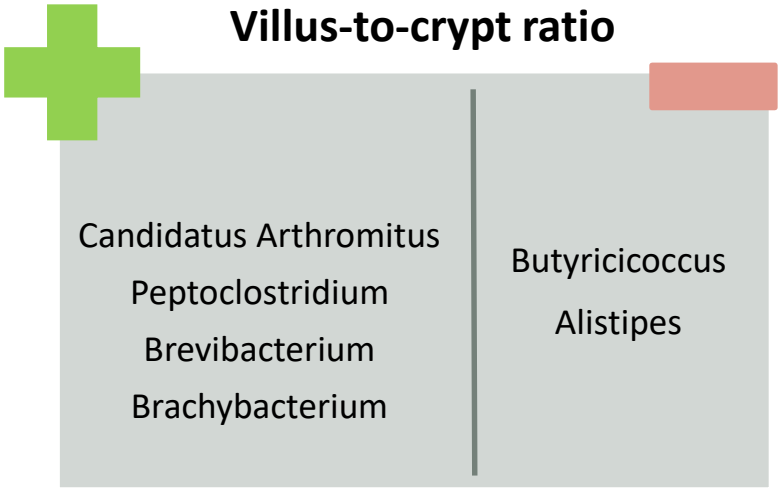


16S sequencing

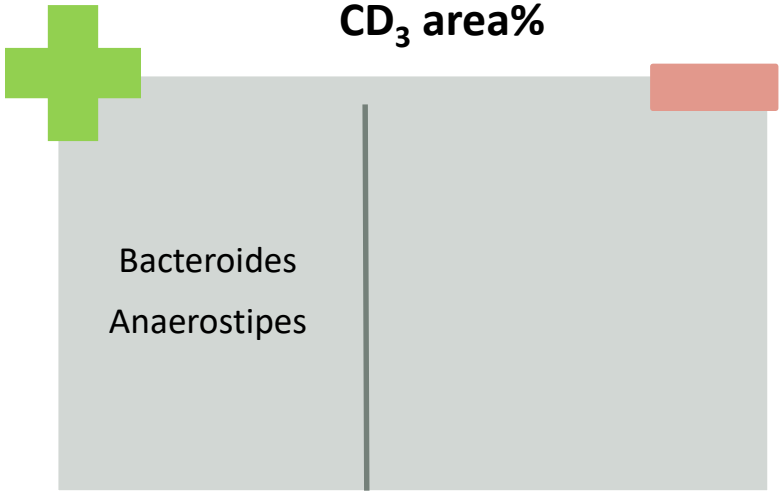
Associations – ileum

D26

Villus-to-crypt ratio

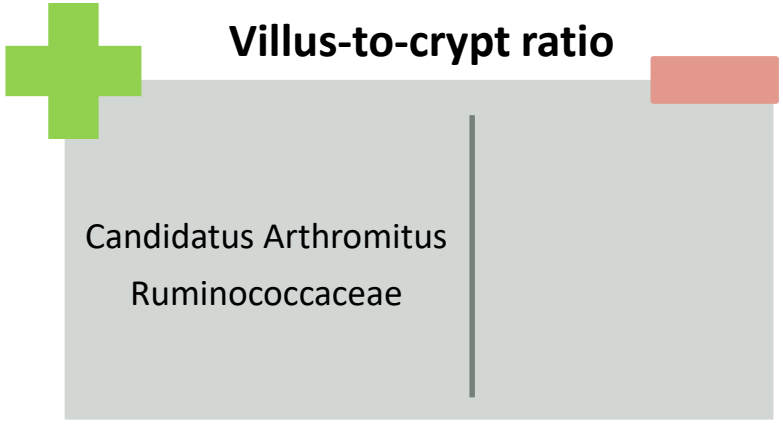


CD_3 area%

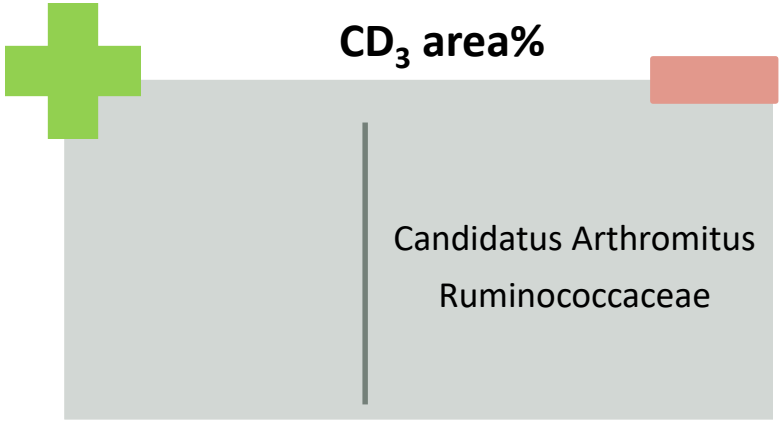


D28

Villus-to-crypt ratio



CD_3 area%



qPCR

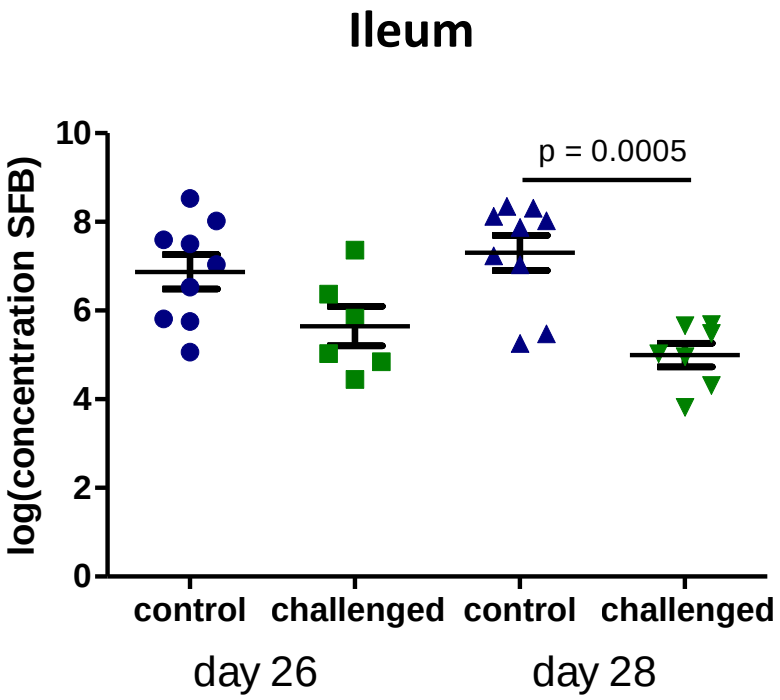
Ileum & caecal samples (1 replicate/group at D26 & D28)

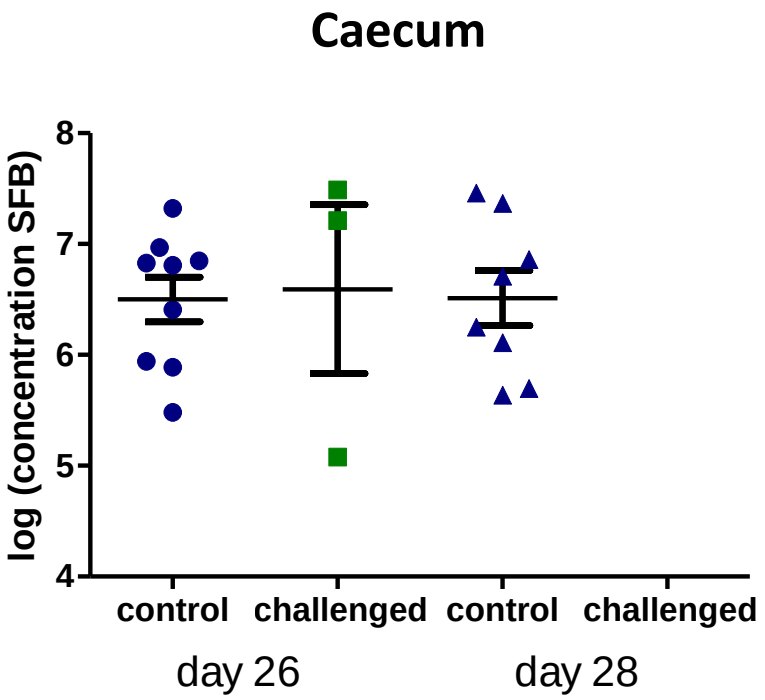
Quantification with qPCR

Segmented filamentous bacteria (Candidatus Arthromitus)

Check with 16s data

qPCR


Ileum
log(concentration SFB)
10
8
6
4
2
0
p = 0.0005
control
challenged
control
challenged
day 26
day 28

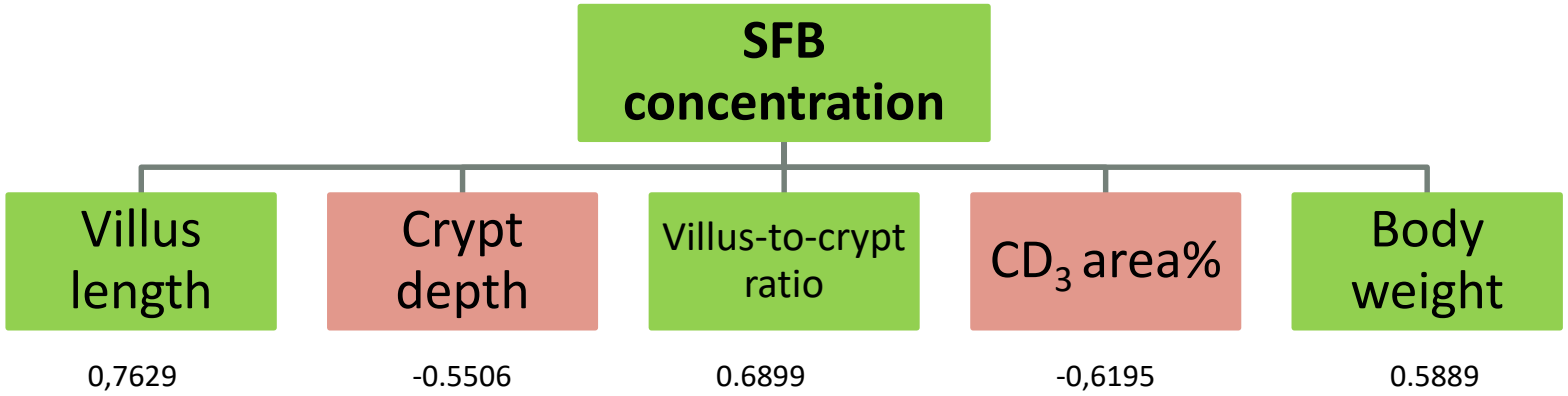

Caecum
log (concentration SFB)
8
7
6
5
4
control
challenged
control
challenged
day 26
day 28

qPCR

D26

→ No associations

D28



➔ Same observations as 16S data

Conclusion

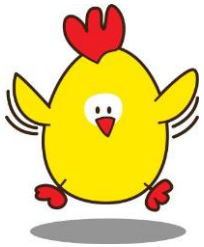
D26

- Association between macroscopic and histology parameters
- > protein expression
- > microbial diversity

➔ Model for evaluation of gut health

Future activities

- Validation model
- Analysis field samples
- Proteomics of ileal samples
- ELISA development for quantification protein markers
- qPCR microbial data



UNIVERSITEIT GENT

AGENTSCHAP INNOVEREN & ONDERNEMEN

Vlaanderen is ondernemen

Thanks for your attention!