Comparison of the digestive microbiota between two divergent lines of chickens selected based on digestive capacity

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A microbial world within us

Host

Feed

Digestive Tract

Microbiota

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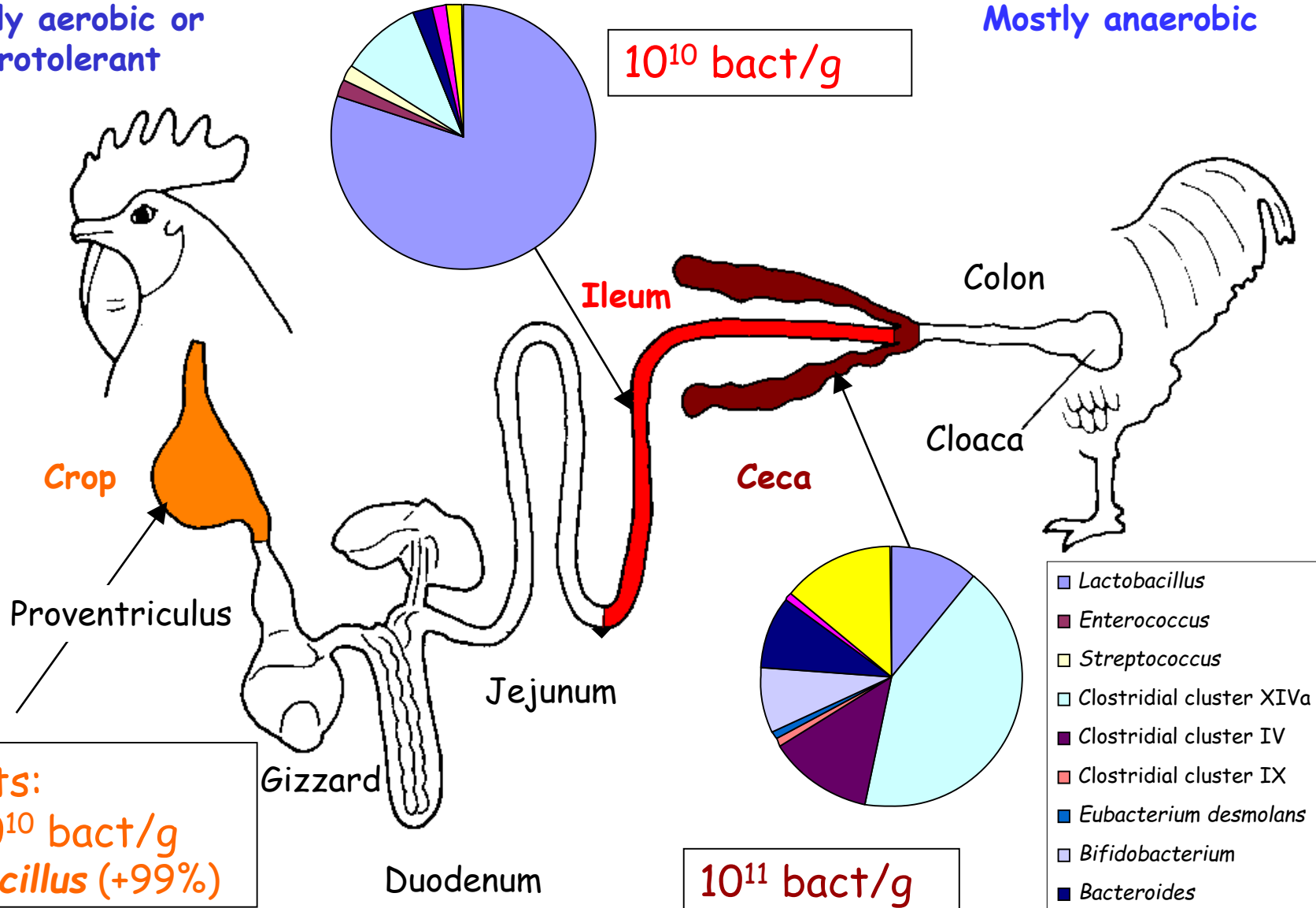
Digestive microbiota in the chicken

Mostly aerobic or aerotolerant

Mostly anaerobic

10^{10} bact/g

Colon

Ileum

Cloaca

Crop

Ceca

Proventriculus

Contents:
10^9 - 10^{10} bact/g
Lactobacillus (+99%)

Gizzard

Jejunum

Duodenum

10^{11} bact/g

- *Lactobacillus*
- *Enterococcus*
- *Streptococcus*
- Clostridial cluster XIVa
- Clostridial cluster IV
- Clostridial cluster IX
- *Eubacterium desmolans*
- *Bifidobacterium*
- *Bacteroides*
- *Escherichia*
- Others

(Apajalahti & Kettunen 2006)

The effects of genetics on the digestive microbiota

Genotype impacts the composition of microbiota

Greater similarity between microbiota of identical twins
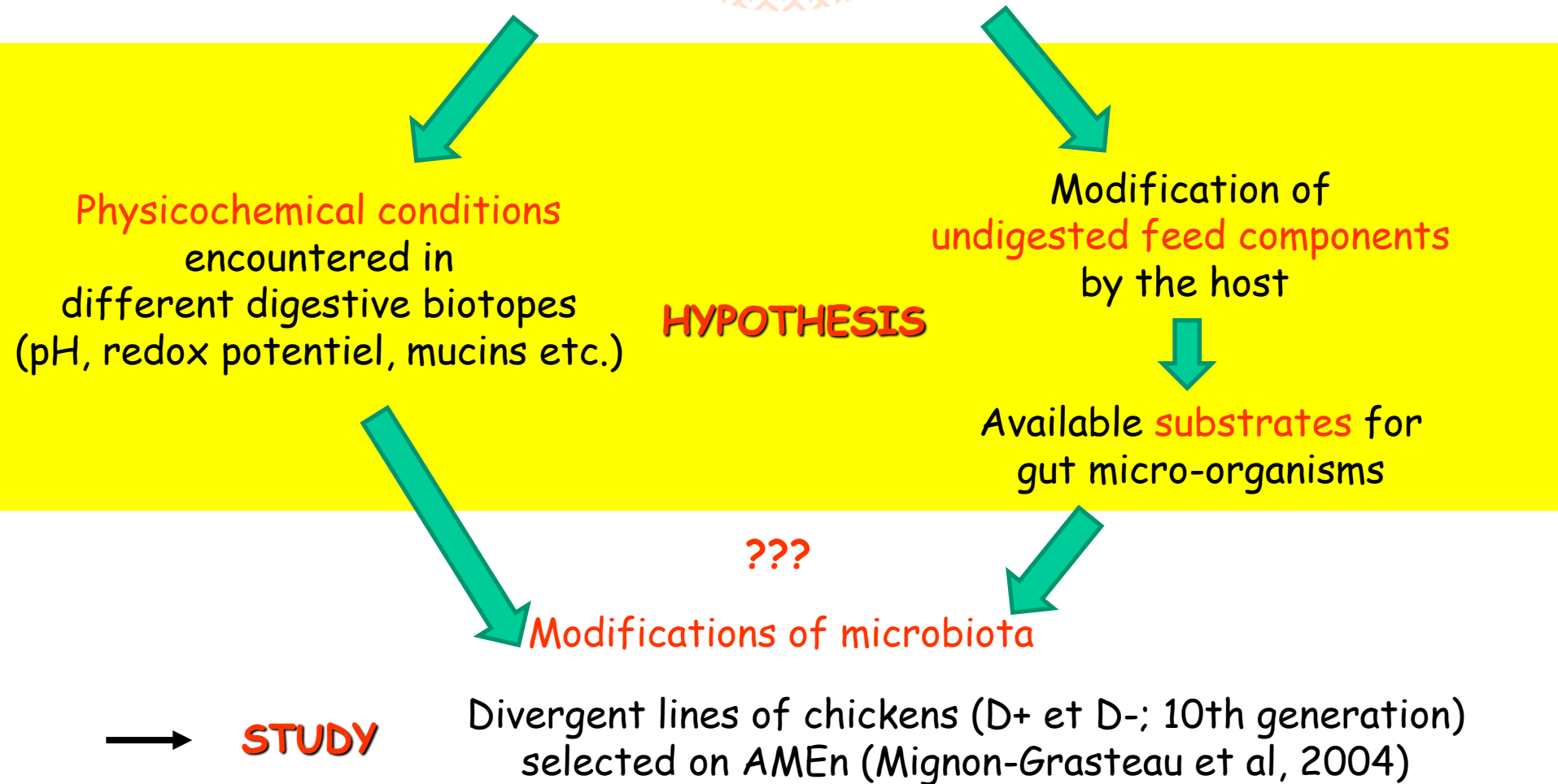(Stewart 2005)

MHC and other genetic factors have an effect on the gut microbiota
(Toivanen et al, 2001; Vaahtovuo et al, 2003)

Differences in microbiota observed between different strains
(Peters et al, 2010; Limpkins et al, 2010)

Genetic selection of animals based on digestive capacity

Physicochemical conditions
encountered in
different digestive biotopes
(pH, redox potentiel, mucins etc.)

HYPOTHESIS

Modification of
undigested feed components
by the host

Available **substrates** for
gut micro-organisms

???

Modifications of microbiota

→ **STUDY**

Divergent lines of chickens (D+ et D-; 10th generation)
selected on AMEn (Mignon-Grasteau et al, 2004)

Methods

Rearing conditions

Floor pens from 1 to 12d (Acquisition of digestive microbiota)
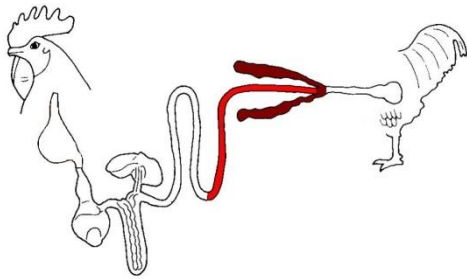Cages from 13 to 23d (Measurement of individual feed intake)

Feed

Degraded feeding conditions : high viscosity wheat
(Rialto, 52.5%)
No antibiotic compounds (Coccidiostat: Clinacox)
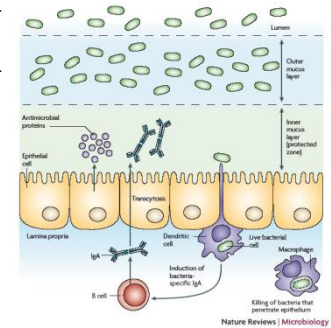
Animals

Sampling: 3 wks of age

F0

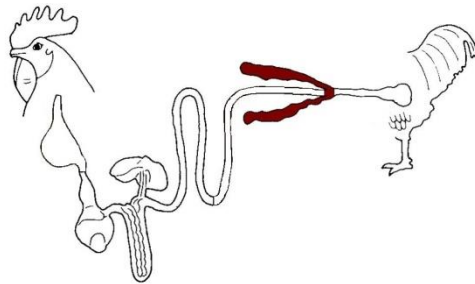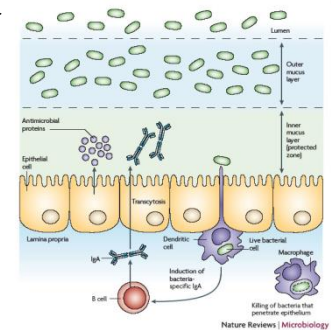6 pools of 5 animals/strain → DNA extraction

Contents →
Mucosa →

TTGE

qPCR

F2

Contents →

72 animals/strain → DNA extraction → qPCR

Major bacterial groups investigated: qPCR

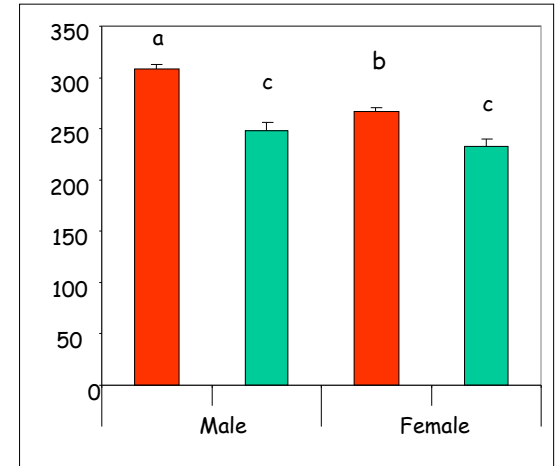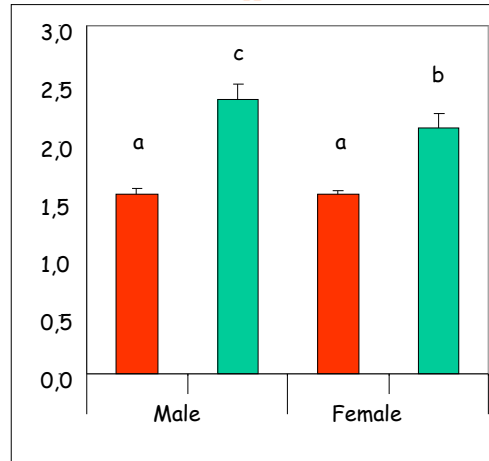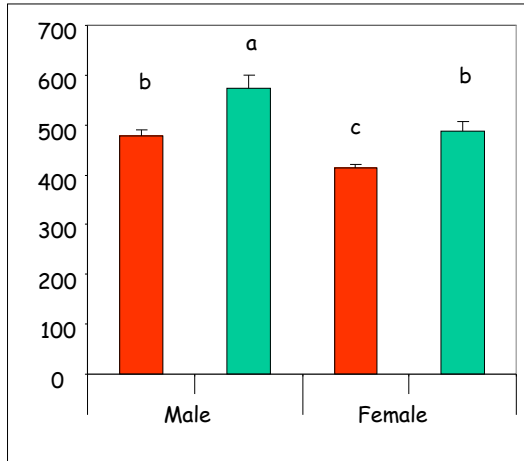| Division	Family	Genus	Species
Firmicutes	Lactobacillaceae	Lactobacillus	
	Clostridiaceae	Clostridium Cluster IV (*C. leptum*)	
		Clostridium cluster XIVa (*C. coccoides*)	
Proteobacteria	Enterobacteriaceae	*Escherichia* *E. coli*	
Bacteroidetes	Bacteroidaceae	Bacteroides	

FO Zootechnical results

D+ ■ D- ■

Feed intake d12-22 (g) **Feed conversion d12-22** **WG d12-22 (g)**

Digestive organs

Weight of organs (g/kg)

Results: F0-Ileal contents

(1) Similarity between profiles: Pearson's coefficient of similarity

D+ : 49.7%   D- : 73.5%   (p=0.0003)

(2) Analysis of the microbiota structure   Two dimensional nMDS plot of the TTGE profiles

Non-metric multidimensional scaling

Stress= 14.442

Distance between microbial profiles (ANOSIM, Pearson distance matrix)

	P value	R value
Strain	0.006	0.2936
Sex	0.963	-0.1352
Pool	0.296	0.0671

(3) Identification of bands:

D+

Division	Order	Family	Species
Firmicutes	Clostridiales	Clostridiaceae	Candidatus Arthromitus
Firmicutes	Clostridiales	Peptostreptococcaceae	

D-

Division	Order	Family	Species
Firmicutes	Lactobacillales	Lactobacillaceae	Lactobacillus crispatus

Major band

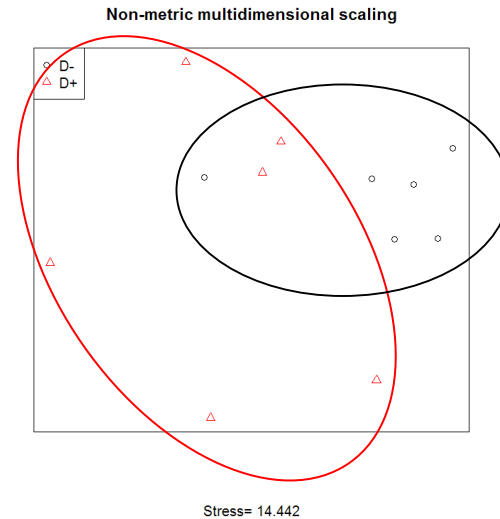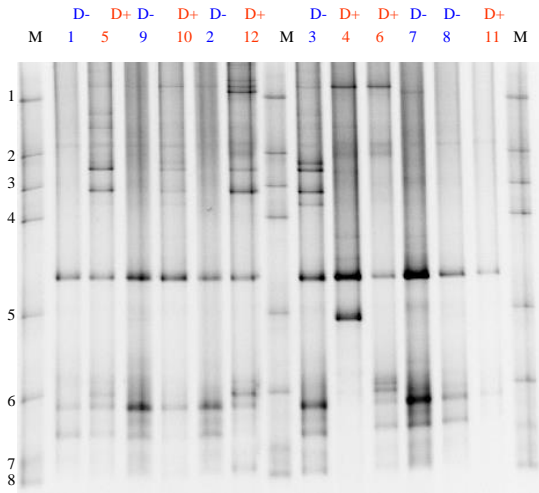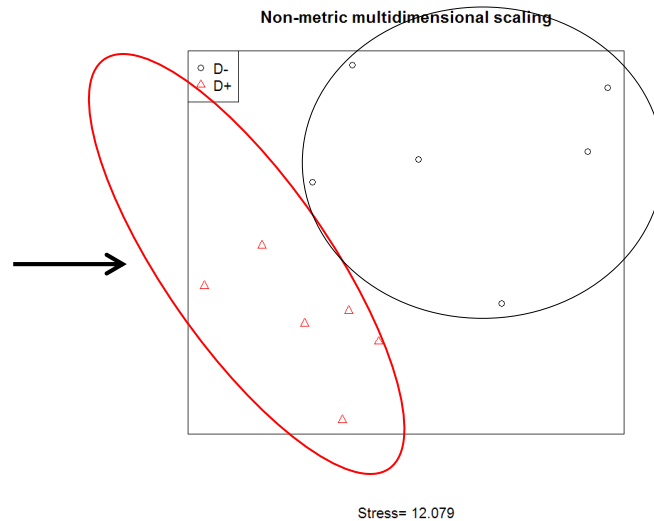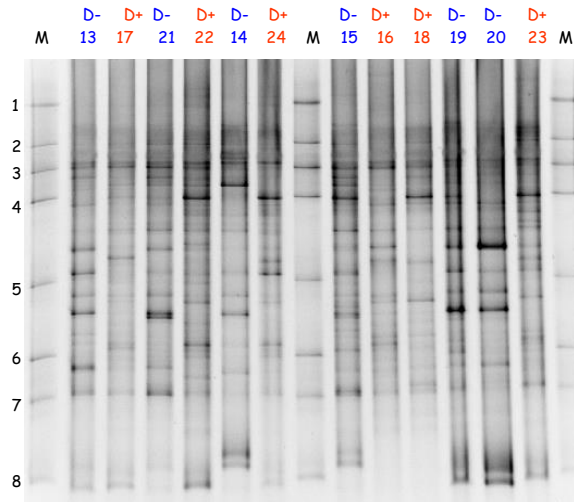| Division	Order	Family	Species
Firmicutes	Lactobacillales	Lactobacillaceae	Lactobacillus salivarius

Results: F0-Cecal contents

(1) Similarity between profiles: Coefficient of similarity (Pearson)

D+ : 60.3%   D- : 45.0%   (p=0.0039)

(2) Analysis of the microbiota structure   Two dimensional nMDS plot of the TTGE profiles

Non-metric multidimensional scaling

Stress= 12.079

Distance between microbial profiles (ANOSIM, Pearson distance matrix)

	P value	R value
Strain	0.005	0.6074
Sex	0.1828	0.0982
Pool	0.0663	-0.0602

(3) Identification of specific bands:

D+

Division	Order	Family	Genus	Species
Firmicutes	Clostridiales	Ruminococcaceae	Faecalibacterium	Faecalibacterium prausnitzii
Firmicutes	Clostridiales	Ruminococcaceae	Subdoligranulum	
Firmicutes	Clostridiales	Lachnospiraceae	Roseburia	

D-

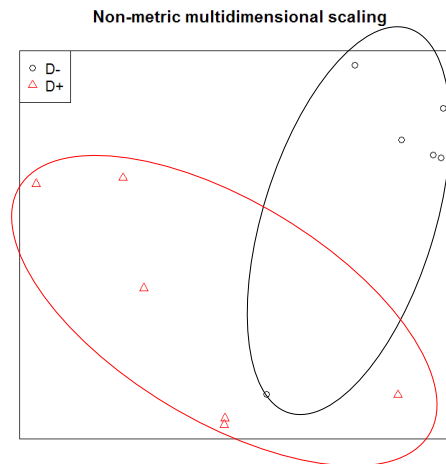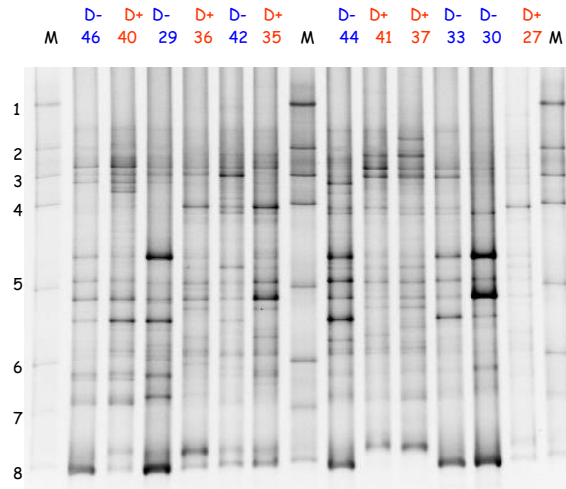| Division	Order	Family	Genus	Species
Firmicutes	Clostridiales	Incertae Sedis XIV	Blautia	Blautia coccoides
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella	

Results: F0–Cecal mucosa

(1) Similarity between profiles: Coefficient of similarity (Pearson)

D+ : 46.4%   D- : 56.9%   (p=0.2127)

(2) Analysis of the microbiota structure

Two dimensional nMDS plot of the TTGE profiles

Non-metric multidimensional scaling

○ D-
△ D+

Stress= 6.721

Distance between microbial profiles (ANOSIM, Pearson distance matrix)

	P value	R value
Strain	0.009	0.5407
Sex	0.1328	0.1537
Pool	0.9878	-0.2546

(3) Identification of specific bands:

D+

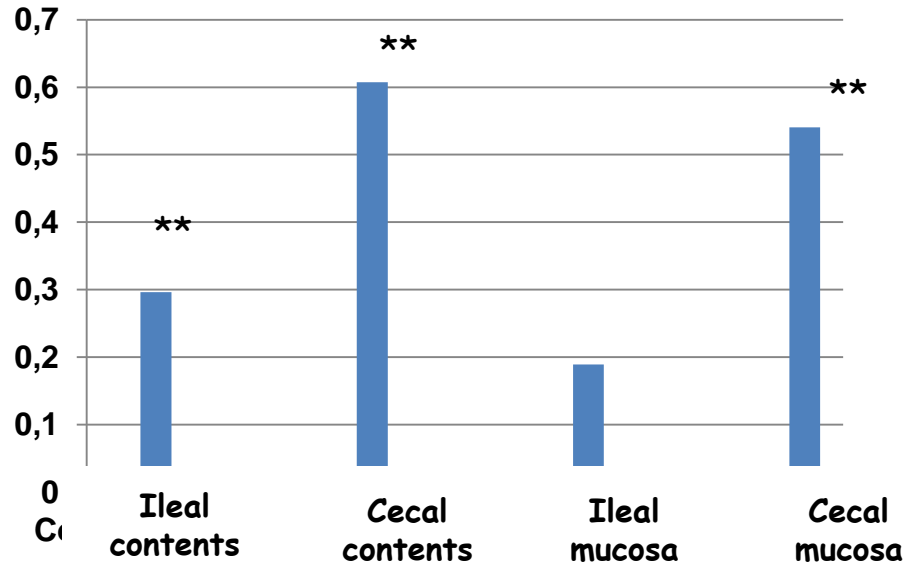| Division	Order	Family	Genus	Species
Firmicutes	Clostridiales	Ruminococcaceae	Faecalibacterium	Faecalibacterium prausnitzii
Proteobacteria	Enterobacteriales	Enterobacteriaceae	Escherichia-Shigella	

D-

Division	Order	Family	Genus	Species
Firmicutes	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus salivarius
Firmicutes	Clostridiales	Incertae Sedis XIV	Blautia	Blautia coccoides

Results F0: All bacteria- strain effect

Distance between profiles (TTGE)
(Pearson's correlation coefficient)

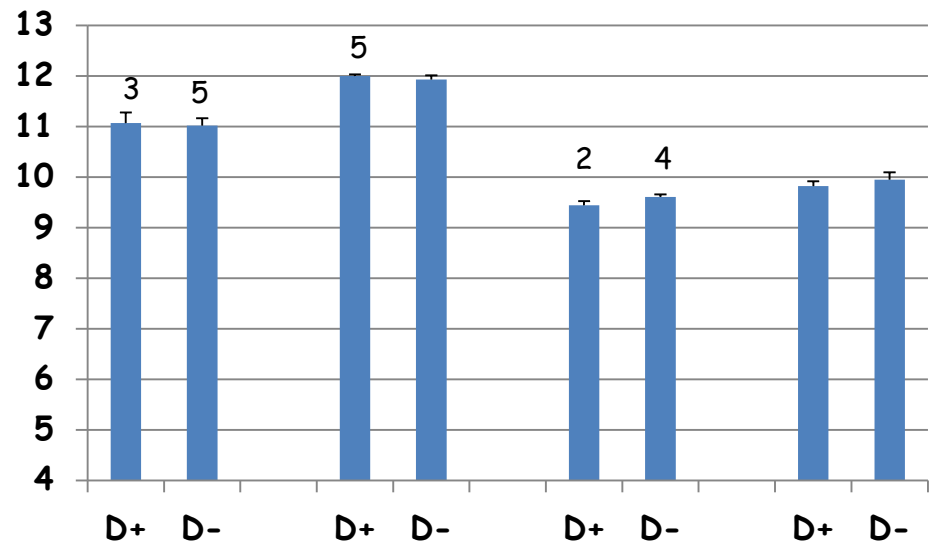* P and R significance
- If p>0.05, the profiles of the two groups are not separated
- If p<0.05 and R>0.75, the groups are separated well
- If p<0.05 and 0.75>R>0.5, the groups are separated with overlap
- If p<0.05 and 0.5>R>0.25, the groups are separated with strong overlap
- If p<0.05 and R<0.25, profiles of the two groups are not separated

(*) $P < 0.10$; * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$

qPCR

Log number of copies of 16S rDNA
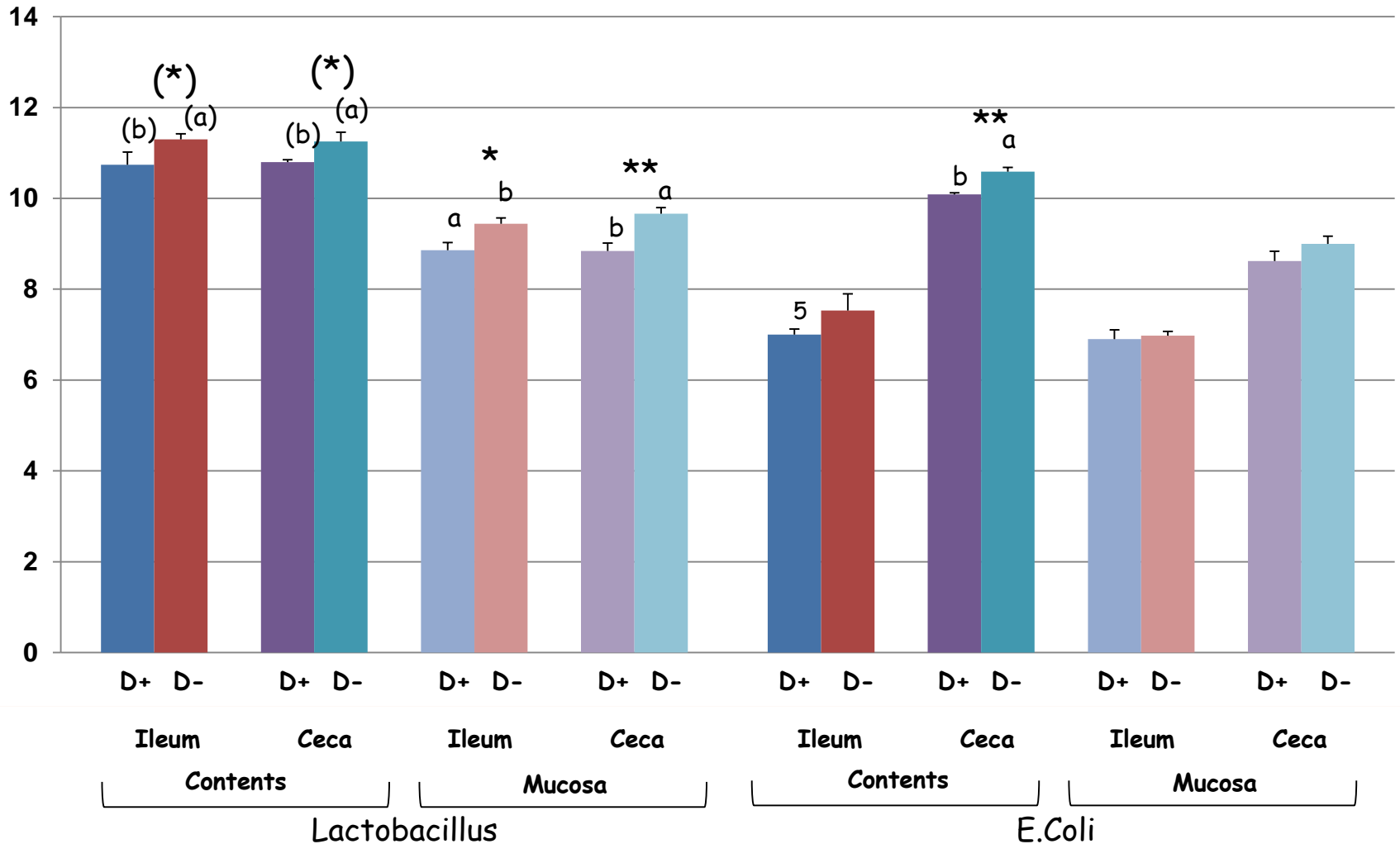per g of contents
or
per digestive segment

N=6 pools/group (unless otherwise indicated)

Results F0: Major bacteria groups qPCR

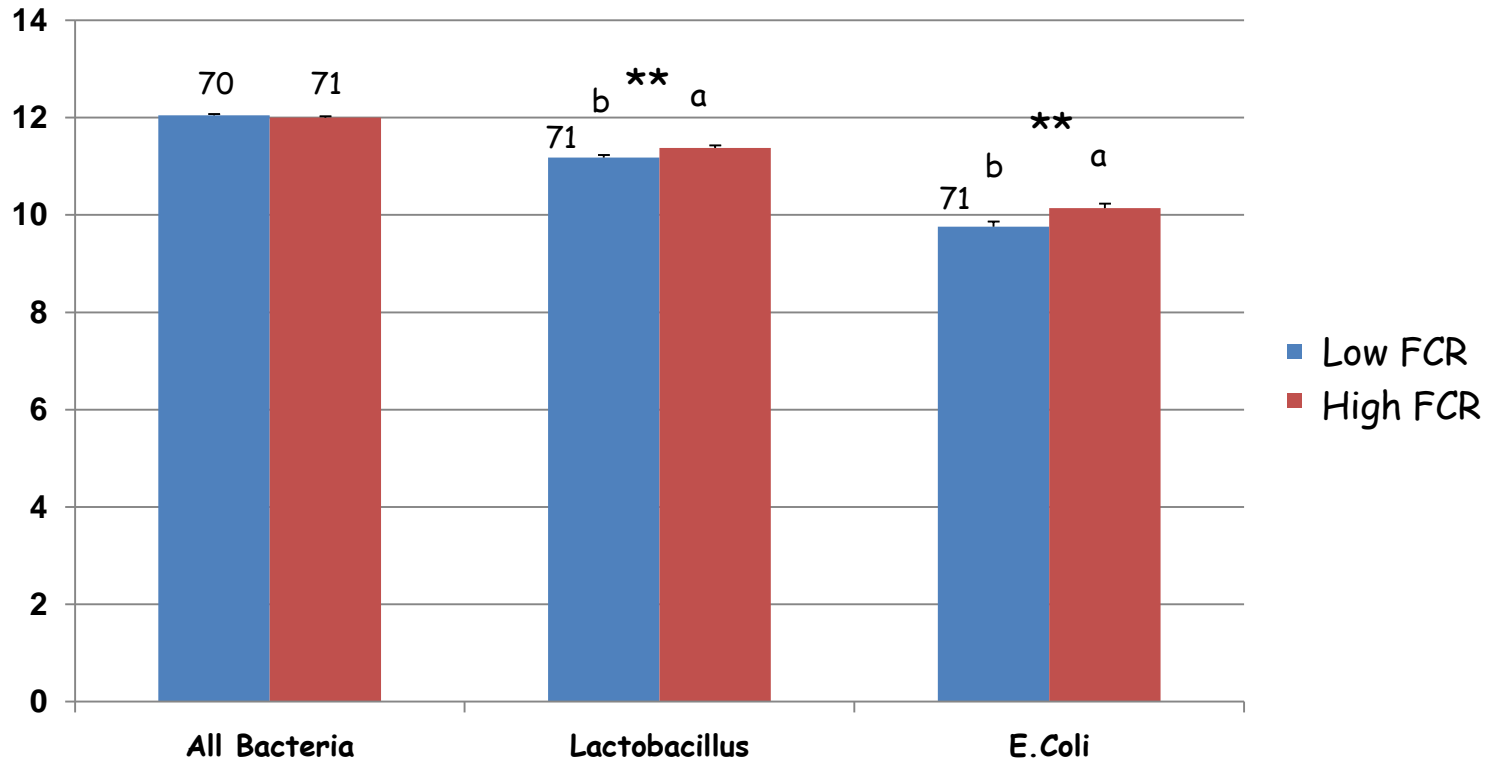Log number of copies of 16S rDNA/ g of contents or / digestive segment (n= 6)

(*) P < 0.10; * P < 0.05; ** P < 0.01 and *** P < 0.001

Results- F2

Log number of copies rDNA16S/ g of contents (n=72)

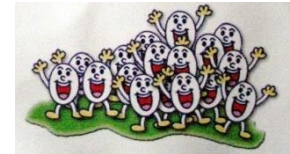(*) P < 0.10; * P < 0.05; ** P < 0.01 and *** P < 0.001

Summary of results

| | D+/D- | | | | Low FCR / high FCR |
| | Contents | | Mucosa | | Contents |
	Ileum	Ceca	Ileum	Ceca	Ceca
All Bacteria (TTGE)	R=0.30	R=0.61	(R=0.19)	R=0.54	-
Lactobacillus (qPCR)	(x 0.28)	(x 0.35)	x 0.26	x 0.15	x 0.63
E. Coli (qPCR)	(x 0.30)	x 0.32	ns	ns	x 0.42

Diff stat : p<0.05; (p<0.10); ns (non significative)

Discussion

Lactobacillus

Considered beneficial
Certain *Lactobacillus* can have a negative
effect on the animal (eg: *L. salivarius*)

E.coli

Generally considered harmful

But not all E.coli strains are harmful
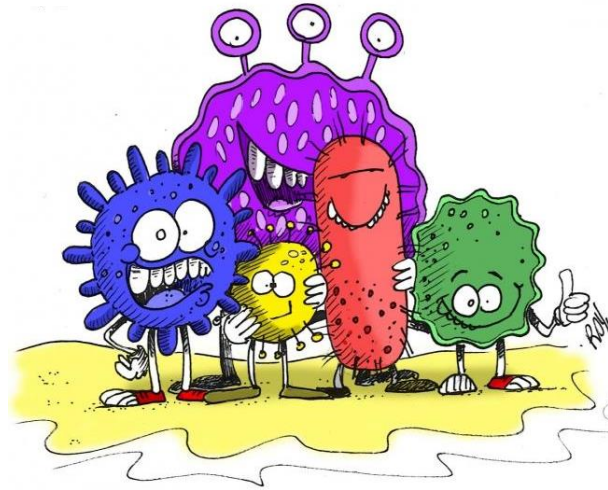
Further work

Quantification of other major groups:

- *Bacteroides*
- *C. leptum* and *C. coccoides*

- *L. salivarius, L. crispatus, F. prausnitzii* and *C. arthromitus*

Thank-you

INRA, Tours

Equipe: Dynamiques Nutritionnelles

Equipe: Sélection Avicole pour la Qualité, la Sécurité alimentaire et l'Environnement

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