



Digestive efficiency, Gut Microbiota and Genetics : Are These interrelated ?



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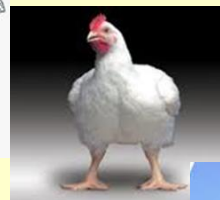
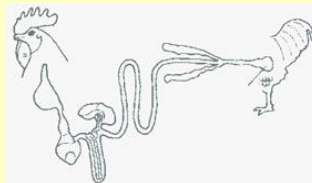
France



*Poultry beyond 2020 : 5th International Broiler Nutritionist's Conference
Queenstown, New Zealand, 13-17 april 2014*

Digestive efficiency : Sustainability of poultry breeding

↗ Digestive efficiency



ECONOMIC OBJECTIVES



Feed efficiency



Growth performance



PRESERVATION OF ENVIRONMENT



Animal wastes and manure



IMPROVEMENT OF ANIMAL HEALTH AND WELFARE



Nutrient content in the intestine



Microbiota development



Better equilibrium



Dysbacteriosis

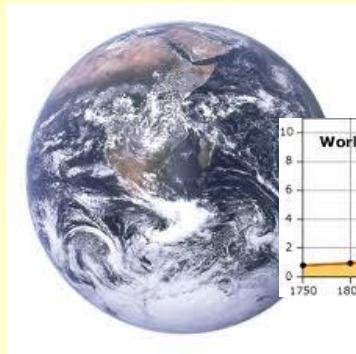


Quantity of fermentation substrates in the litter

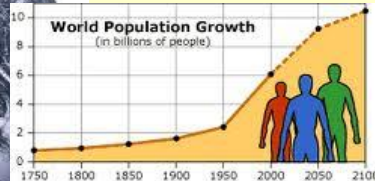


Frequency of pododermatitis





↗ Human population



→ ↗ Need for meat as poultry meat

→ ↗ Need for crop for animal and human

↗ Demand for biofuel → ↗ Need for crop

Conventional crop vegetal resources



Unconventional feedstuffs

Ex : Local feedstuffs
By-products



Variable or low nutritional value

→ ↘ Digestibility

→ Improvement of nutrient digestibility

Enzymes ...

Other Additives

→ Control of digestive microbiota

High individual variability



Reasons for ?

Environment ?

Genetics ?

Digestive efficiency, Digestive Microbiota and Genetics : Are These interrelated?

1. Digestive efficiency and digestive microbiota

1.1. Digestive microbiota : some reminders

1.2. Digestive efficiency and digestive microbiota composition relationship

1.2.1. Observation in chicken

1.2.2. Conclusion on these observed relationships and further works

2. Genetic control of digestive efficiency and digestive microbiota

2.1. Effect of genetics on digestive efficiency

2.2. Effect of genetics on digestive microbiota

2.2.1. In mammals / model animals

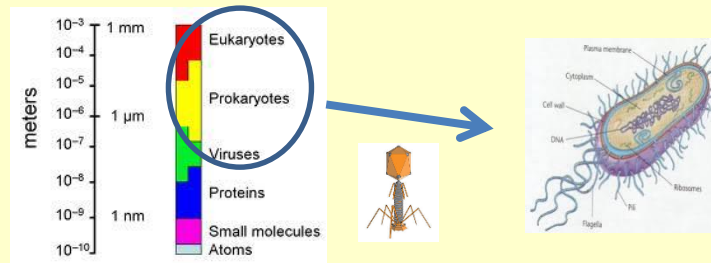
2.2.2. In birds

2.2.3. Assessment of present knowledge and hypotheses

3. Conclusion and further knowledge needed

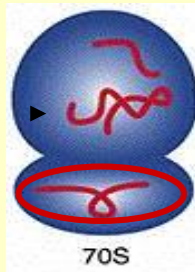
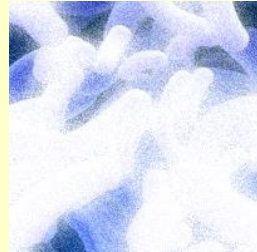
1.1. Digestive microbiota : some reminders

Complex community :
mainly bacteria



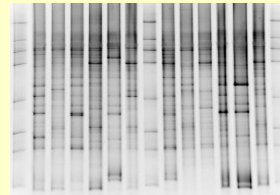
A large majority of species cannot be easily cultivated (70-90%)

Cultural approaches → Independent approaches to culture
Molecular methods based on 16S rDNA



Qualitative methods

Fingerprint techniques



Quantitative methods

Fluorescent in situ hybridization (FISH)

Quantitative PCR

Low throughput clone analysis

→ More precise image of the microbial community

More recently : High throughput sequencing of 16S rDNA
Pyrosequencing (Next Generation Sequencing, NGS)

→ More deep insight in digestive microbiota



1.1. Digestive microbiota in chicken



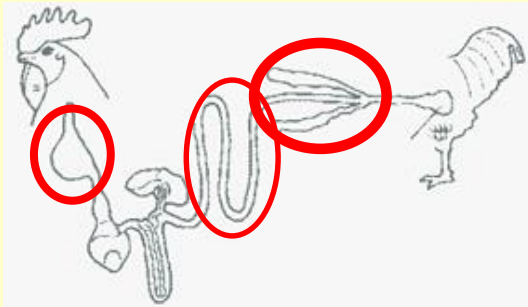
In digestive content : Most studied

Quantitative importance

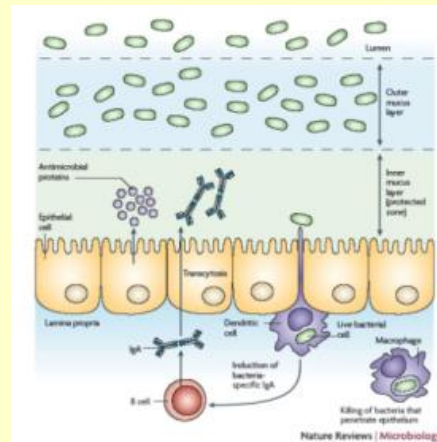
1. Caeca
2. Crop
3. Small intestine

Composition stability

Caeca : the most stable content



In digestive mucus



The mucus in the large intestine
From Hooper (2009)

Composition of mucosal microbiota \neq Composition of luminal microbiota

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1.2.1.1. Parallel change in digestive efficiency and digestive microbiota

Effect of microbiota on DE : germ-free (limited microbiota) / conventional chickens

Digestive microbiota → Change in digestibility of lipid / AMEn / protein

Boyd and Edwards (1967), Salter (1973), Salter and Fulford (1974), Kussaibati et al. (1982), Furuse and Okumura (1994),
Maisonnier et al. (2003), Garcia et al (2007),

Alternative feed management strategies to AGP or rearing conditions

Dietary factors

AGP (Virginiamycin)

Enzymes

Herbal products or essential oils

Insoluble fiber

Mannanooligosaccharide

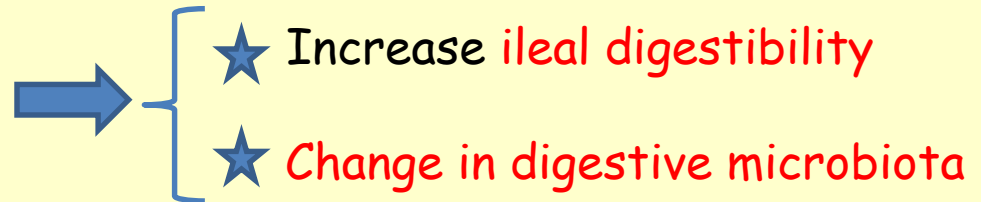
Type of cereal grains

Technological treatments of cereals

Rearing conditions

Delay feeding after hatching

Immunological stress



Choct et al. (1999), Yang et al. (2008), Amerah et al. (2009), Shakouri et al. (2009), Bhuiyan et al. (2010), Baurhoo et al. (2011); Nian et al. (2011), Amerah et al. (2011), Yang et al. (2011), Rodriguez et al. (2012), Zulkifli et al. (2012), Cowieson and Masey O'Neill (2013); Alloui et al. (2013); Gabriel et al. (2013), Sarica and Corduk (2013)

Relationship between digestion and digestive microbiota



Poultry Research Unit of INRA



Experimental model of birds
selected on digestibility
estimated by AMEn

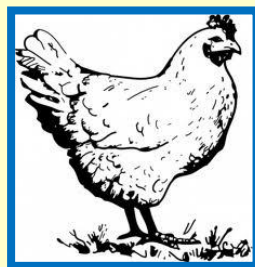
Mignon-Grateau *et al.*, 2004

Divergent selection on AMEn (3 weeks of age)

Wheat (525 g/kg) , high viscosity (Rialto)
High fat concentration (60-80 g/kg)



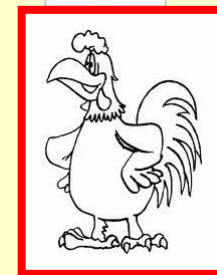
D+



Two lines :

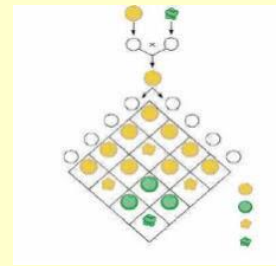
D+ (high digester) and D- (low digester)

Selection during 8 generations



D-

Relationship between digestion and digestive microbiota With divergent lines selected on AMEn



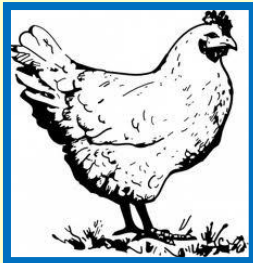
F2 cross between the D+ and D- lines (144 animals)

High range of AMEn (from 7.6 to 16.1 MJ/kg)

Digestive microbiota of caecal contents (qPCR of main groups)

D+

High digester



High AMEn associated with

Low amounts of *E. coli*

Low ratio of *E. coli* to all other bacterial groups

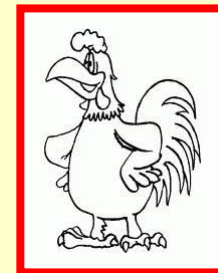
Low AMEn associated with

High amounts of *E. coli*

High ratio of *E. coli* / *Clostridium*

High amounts of *Lactobacillus salivarius*

High proportion of *L. salivarius* compared to *Lactobacillus* groups and *Clostridium* groups (*C. coccoides* and *C. leptum*).



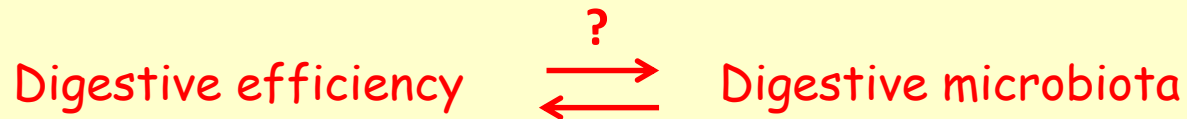
D-

Low digester

1.2. Digestive efficiency and digestive microbiota composition relationship

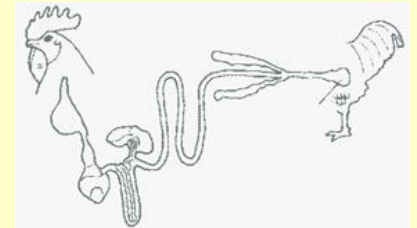
1.2.1. Observation in chicken

1.2.1.2. Hypotheses on the mechanisms implied in the relationships between digestive efficiency and digestive microbiota



Effect of DIGESTIVE EFFICIENCY on digestive microbiota

Change in digestive efficiency



→ Change in **undigested particles** in digestive biotopes

→ Substrates for microorganisms as bacteria

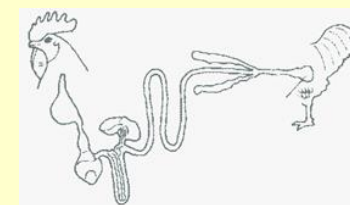


Effects of the DIGESTIVE MICROBIOTA on digestive efficiency



Positive effects

Negative effects



Crop

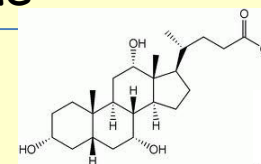
Small intestine

Caeca

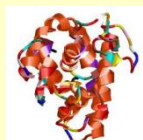
Lipids



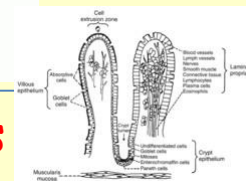
Deconjugation of bile salts



Proteins

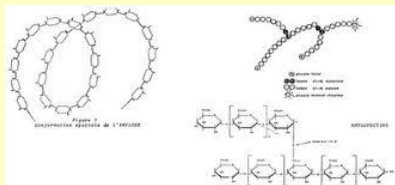


Endogenous production



Carbohydrates

Starch

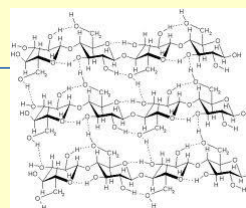
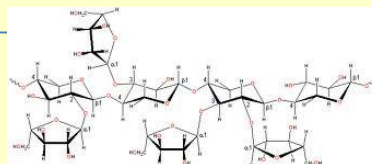


Bacterial amylases

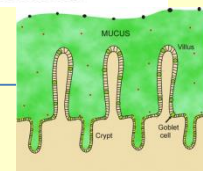
Competition

Hydrolysis
→ Carbohydrate,
→ Amino acids

Fiber



Fermentation
→ SCFA



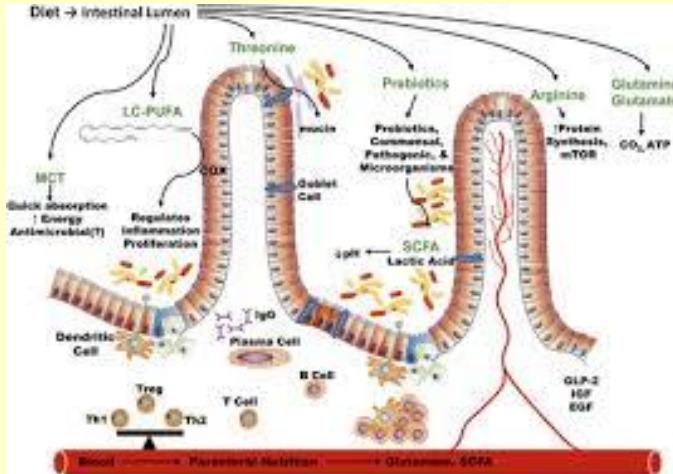
Effect of DIGESTIVE MICROBIOTA on digestive efficiency

Via modifications of the digestive physiology



Digestive microbiota

Bacterial products



Blood / Nervous system

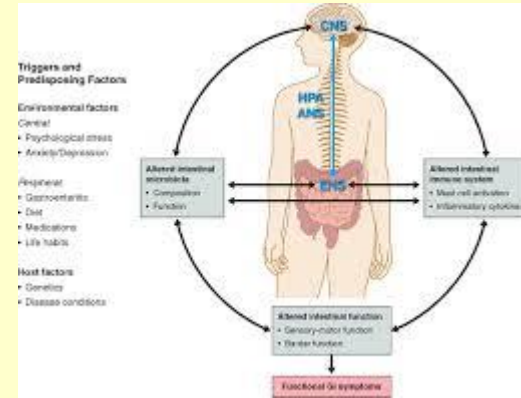
Systemic effect

Digestive physiology

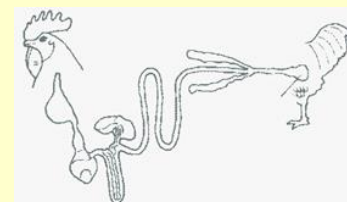
Enzymatic activities
Transit
...

Digestive efficiency

Brain-Gut axis



Ringel and Maharshak (2013)



1.2. Digestive efficiency and digestive microbiota composition relationship

1.2.1.3. No parallel change

Improvement in ileal digestibility, but not in digestive microbiota

- AGP (avilamycin)
- Enzyme
- Products of essential oils



Change in digestive microbiota, but no change in ileal digestibility

Germ-free / conventional chickens

Dietary factors

- Enzyme
- Vegetal products
- Prebiotic
- Probiotic
- Cereal structure and type



1.2. Digestive efficiency and digestive microbiota composition relationship

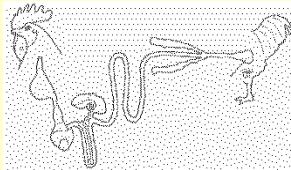
1.2.2. Conclusion on these observed relationships and further works

Various results that may be explained by different experimental conditions

Animal experiment



Digestive samples



Sample analyses



Digestibility efficiency
Microbiota analysis



Absence of observed effect \neq No effect

Parallel modifications of digestive microbiota and digestive efficiency



Links between them



Environmental factors : dietary factors / rearing conditions
Animal **genetics**

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2.1. Effect of **genetics** on **digestive efficiency** In chicken

First observations

With wheat diets (≠ maize diets)

High **difference** in ME **between lines** of chicken

Low DE and **high variability between birds**

→ Suggestion of a **genetic factor in digestibility**

(Pym et al., 1984; Hughes and Choct, 1997; Carré et al., 2002)



Selections for digestive efficiencies

Dep. Poultry Sci, Univ. Georgia (Zhang et al., 2003, 2005)

Selection for phytate phosphorous (P) bioavailability in a randomly bred chicken population

→ Selection for 3 generations improved P bioavailability

Significant heritability although very low ($h^2=0.05$)

Poultry Research Unit of INRA (Nouzilly, France)



(Mignon-Grasteau et al, 2004)

Selection with a diet rich in wheat (8 generations)

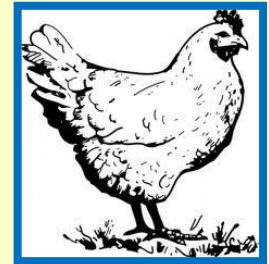
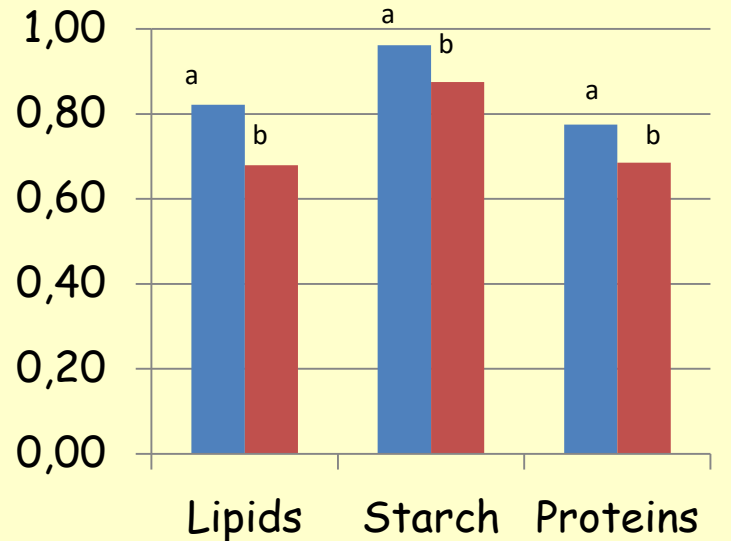
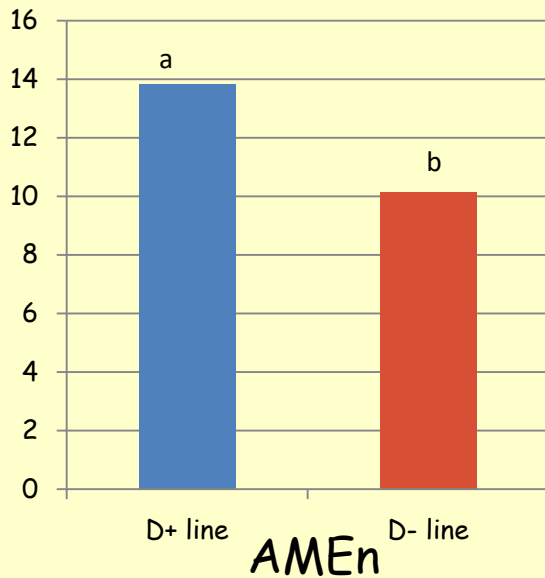
AMEn and fecal digestibility



Wheat diet (1-8th generations)
(Mignon-Grasteau et al, 2010)



D+



D+ line

D- line



D-

D+/D-

+ 36%

+ 21 %

+ 10 %

+ 13 %

H²

0,32

0,25

0,28

0,29

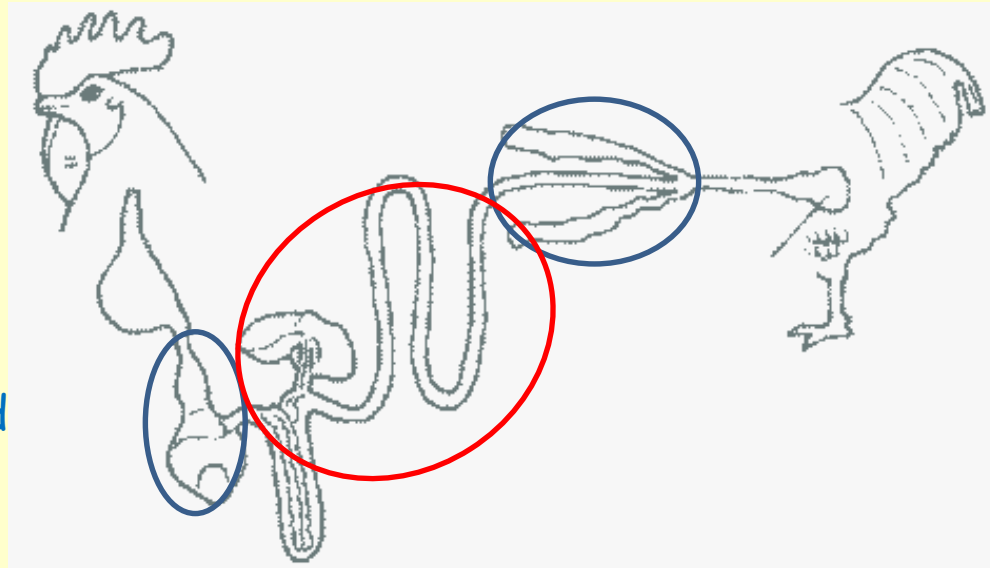
Differences in digestive efficiency

↔ Anatomical and physiological differences

D+

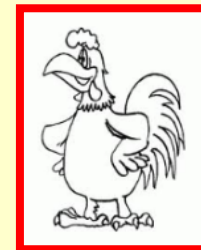


Caeca



Proventriculus-gizzard complex

Small intestine



D-



Detection of QTLs controlling DE

F2 cross D+ and D- lines
820 chickens

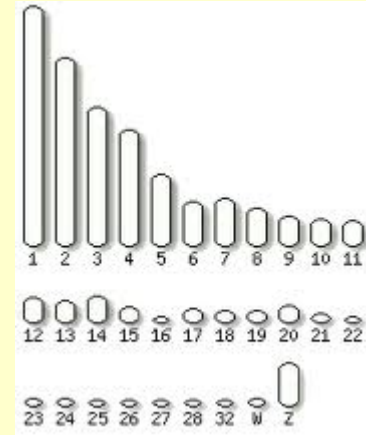
Tran et al. (2014)

Number of QTLs detected : 9

They were mainly found for components of AMEn (mainly DE_S^*)

One for AMEn itself

*Starch : the highest proportion in dietary content



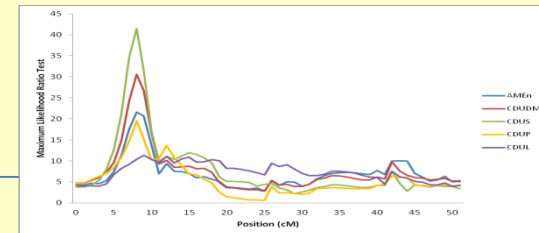
Chromosome	QTLs
n° 16*	AMEn, DE_S , DE_{DM} and DE_P^{**} (the same region)
n° 20	DE_S and DE_{DM} (the same position)
n° 23	DE_S
n° 26	DE_S^{**}
n° 27	DE_{DM}

S : Starch
DM : Dry matter
P : Protein

*Role in immunity

** Co-localization with QTL controlling the ratio of intestine length to BW

Maximum likelihood ratio tests



Chromosome 20

➡ Refining the position of these QTLs ➡ Candidate genes

➡ Validation in commercial populations and breeding environments

QTLs controlling digestive anatomy-related traits were observed : 11 QTL

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2.2. Effect of **genetic** on **digestive microbiota**

2.2.1. In **mammals / model animals**



Human : twins or varying degrees of genetic relatedness → **Conflicting results**

Animal population : Related animals , twin, animal species more or less related



More conclusive results



Natural mutation of one gene

Candidate gene approaches :

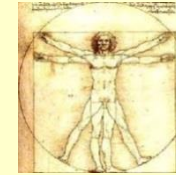
Monogenic models (knockout mice)

Genetically inbred mouse lines

Transplantations of microbiota

Quantitative genetics (QTL mapping)

Genome-wide association studies (GWAS)



Genome-wide linkages



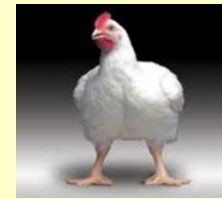
Genes potentially implied **in immunity**

in **metabolism / digestion (enzymes)**

in the regulation of **developmental processes**

2.2. Effect of **genetic** on **digestive microbiota**

2.2.3. In **birds**



First studies with AGP (Nordskog et Johnson, 1953)

Their effects on growth was **dependent** on the **animal strain**

→ Genetics may have an effect on digestive microbiota

Comparison of 3 genetic lines of chickens (Lumpkins et al. 2010)

Different molecular fingerprint

Comparison of two genotypes of ducks (Pekin and Muscovy) (Vasai et al., 2014)

Fed ad libitum or overfed

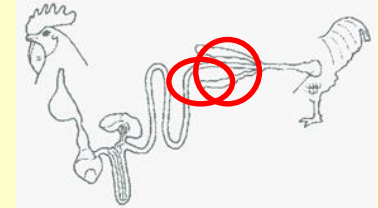
Microbiota (Pyrosequencing) of caecal content more affected by **genetics** than by overfeeding



Experimental model of birds selected on digestibility estimated by AMEn



Population F0



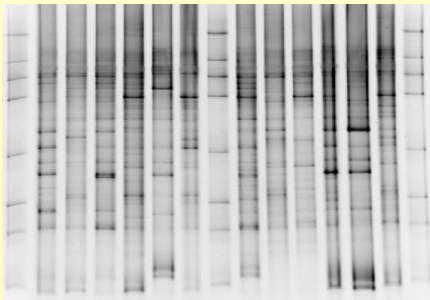
Bacteria { in ileum
 in caeca
 { in digestive contents
 in mucus

6 pools of 6 birds / line

Fingerprint / qPCR of main groups

Significant different fingerprint

Mainly in caecal content (R=0.61)



Significant different bacterial number in main groups

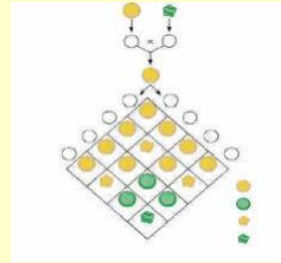
D+ and **D-** lines

Mainly in caeca : content and mucus



Population F2

(144 animals)



High range of AMEn (from 7.6 to 16.1 MJ/kg)

Digestive microbiota of **caecal contents** (qPCR of main groups)

Significant heritability estimates (H^2) (Gabriel et al., 2012)

Bacterial numbers Genus *Lactobacillus* (*L. salivarius*) : 0.11 to 0.14

Bacterial ratios

L. salivarius / *C. leptum* and *C. leptum* / *C. coccoides* : 0.20 - 0,22

C. coccoides / *Lactobacillus* : 0.34

→ The **microbiota balance** is partly controlled by the **genetics** of the host



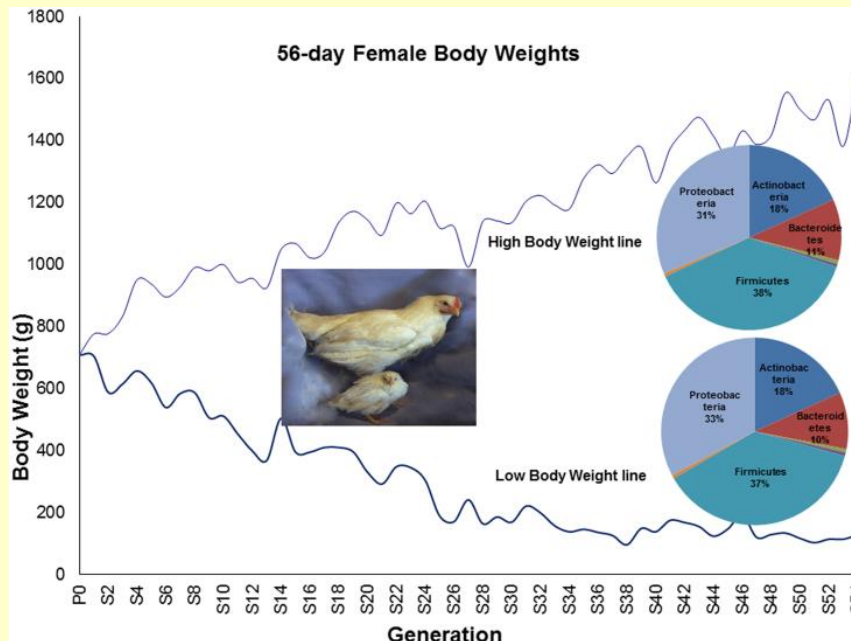
Implied genes

15 QTLs regions → **Under analysis** to detect candidate genes

Effect of genetic on digestive microbiota in bird

School of Agriculture and Biology, Shanghai Jiaotong University, China /
Virginia Bioinformatics Institute, Dep. Anim. Poult. Sci., Virginia, USA
(Zhao et al., 2013)

Two lines of chickens selected for high or low 56-day body weight (54 generations)
56 animals: 2 chicken strains, 2 sexes, 12-15 animals per group



- ➔ Difference in microbiota at the genera and species level (29 species)
- ➔ Genetic background of the host influences gut microbiota in chickens

2.2. Effect of **genetics** on digestive microbiota

2.2.3. Assessment of **present knowledge** and **hypotheses** on this relationship

Present knowledge Studies in mammals, fish and birds



Effect of host **genetics** on digestive microbiota

A part of individual variability



Hypotheses on genetic effect

Selective pressure within the **digestive tract habitat**

Secretions (antimicrobial compounds : HCl, bile acids, defensins ...)

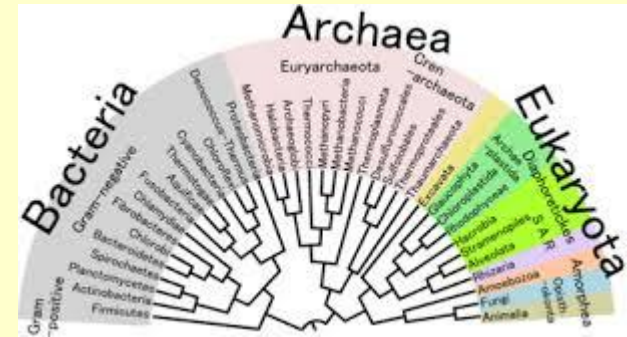
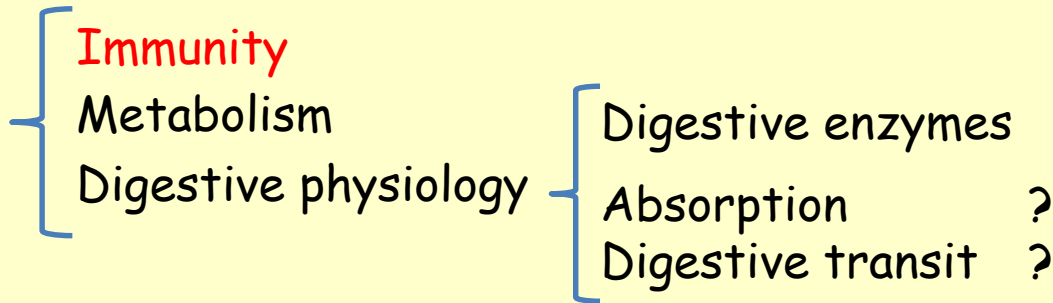
Digestion → Undigested compounds = Substrates for microbiota

Digestive tract motility

Epithelial cell surfaces



Potential implied genes



Results of adaptation during host speciation and the evolution of pluricellular eukaryotes to their surrounding prokaryotes



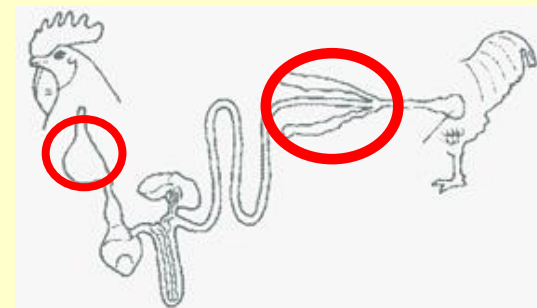
Stepwise changes of host and its microbiota Selective pressure

→ Mutualistic relationship between the host and the colonizers

Promotion of beneficial microbes



→ Highly specialized digestive organs (e.g. crop and ceca in poultry)



→ Complex sets of loci

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2. Genetic control of digestive efficiency and digestive microbiota

2.1. Effect of genetics on digestive efficiency

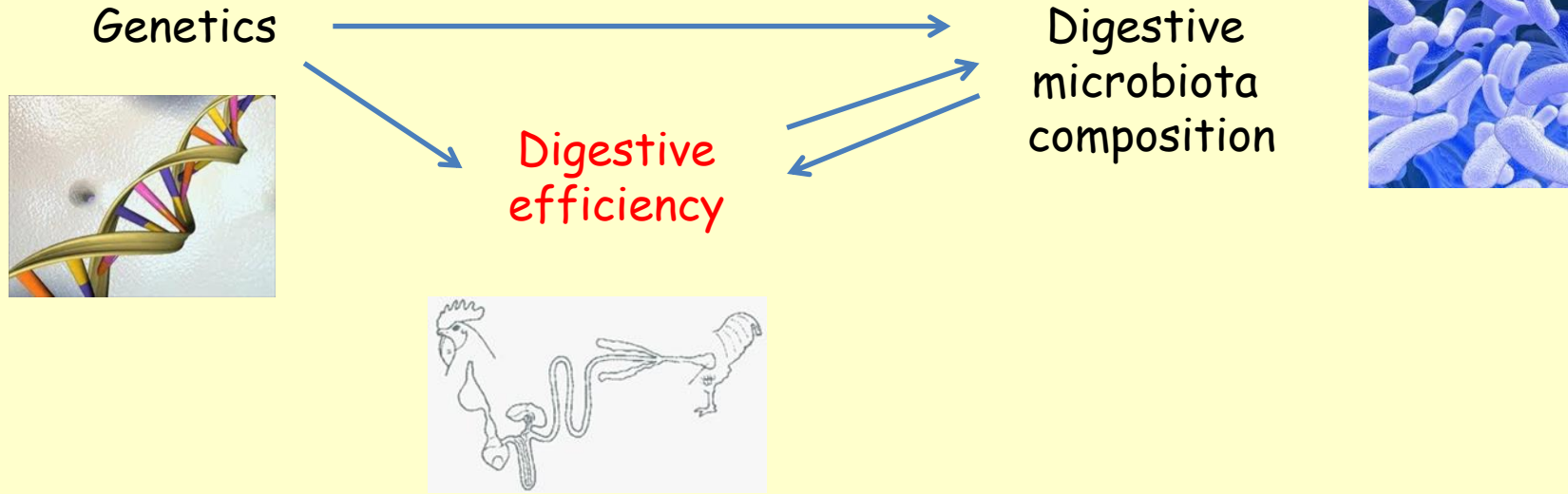
2.2. Effect of genetics on digestive microbiota

2.2.1. In mammals / model animals

2.2.2. In birds

2.2.3. Assessment of present knowledge and hypotheses

3. Conclusion and further knowledge needed



Further **studies** are needed
to **understand** these
interactions

Numerous parameters can influence digestive microbiota



Genetics

Maternal effect (maternal nutrition)

Environmental factors

First days of life from hatcher to farm

Rearing environment

Diet composition and structure

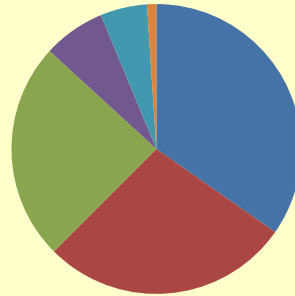


Each step of microbial colonization



Variability between individual birds

Hierarchy in all these factors ?



- Factors 1
- Factors 2
- Factors 3
- Factors 4
- Factors 5
- Factors 6



Methodology → A faithful representation of the reality

Sampling conditions

Steps of sample processing



Standardisation is needed



Digestive microbiota composition and FUNCTIONS



Thorough
composition



Who are they ?

What are
they doing ?

Potentially

Overall
bacterial genes

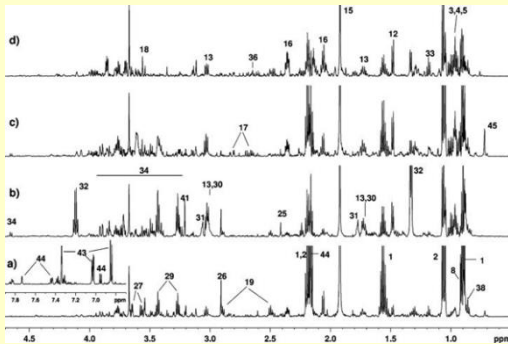
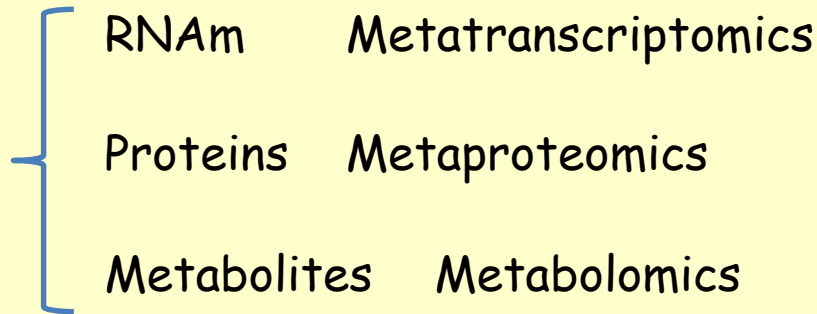
Overall
bacterial products

Really

Animal
phenotype



➔ Bacterial products : RNA, proteins and metabolites



Short chain fatty acids and their derivatives
Carboxylic / Dicarboxylic acids / Alpha-keto acids
Monosaccharides
Secondary amines
Nucleobases
Vitamins
Amino acids and their derivatives

NMR ¹H



CEISAM, CNRS, Nantes

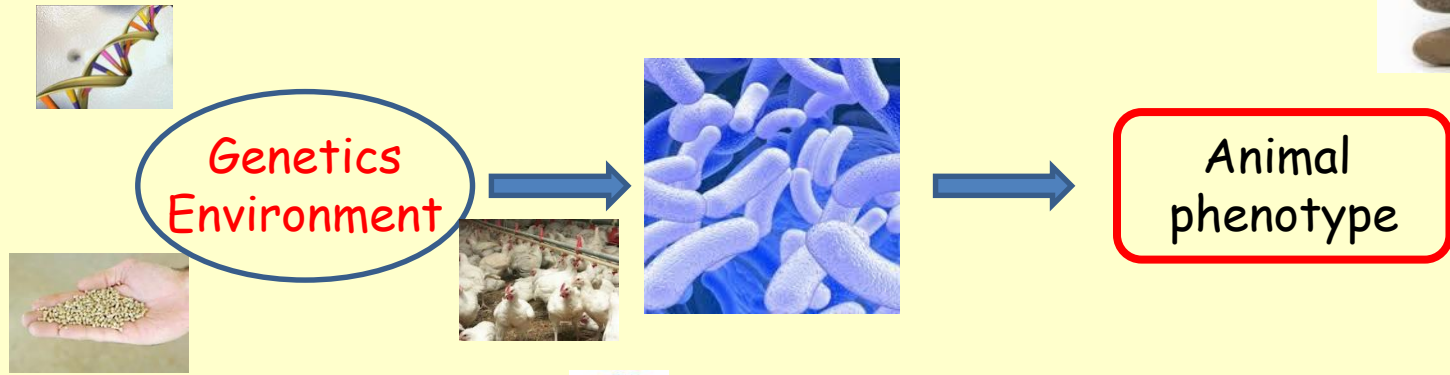


Novel proteins and metabolites

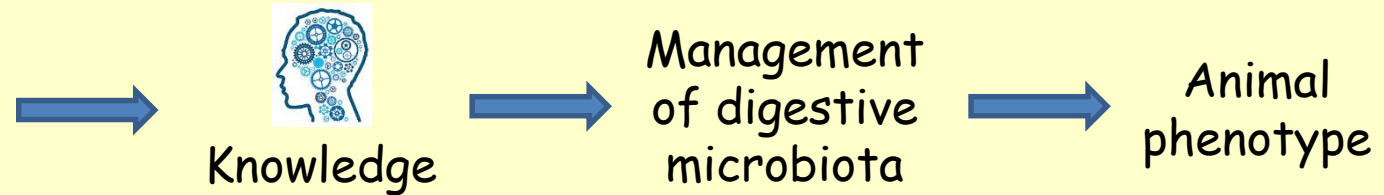
Novel mechanisms involved in host physiology as DE


Take home message

Digestive microbiota : a **complex** dynamic / balance



Global approach
(OMIC methods)



 Without trade-off with other important phenotypic traits

Thank you for your attention

