EAAP Meeting, Nantes, August 29, 2013
Interplay EU Project – Workshop 2

Effect of sow antibiotic treatment and offspring diet on microbiota and gut barrier throughout life

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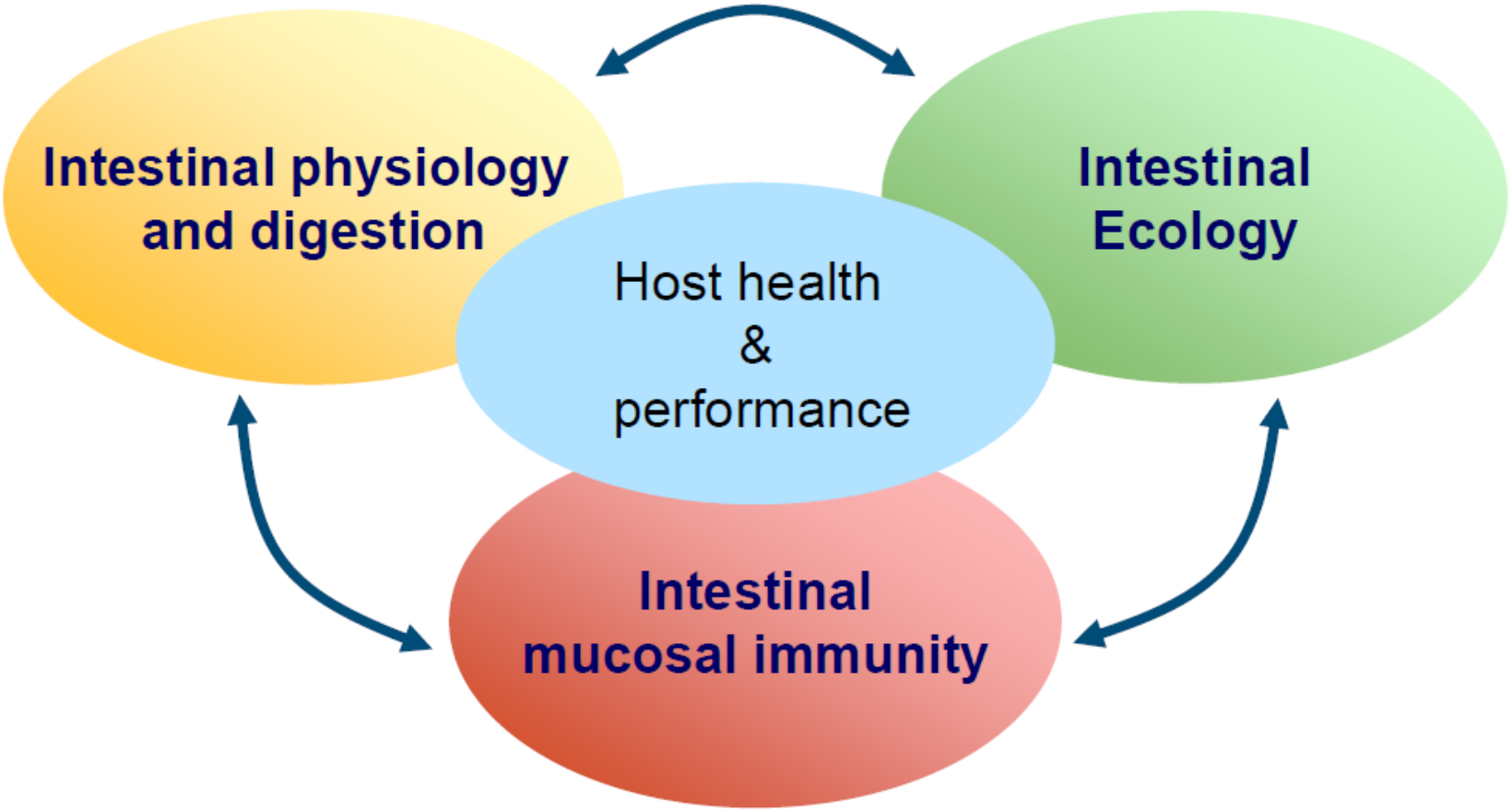
BACKGROUND:

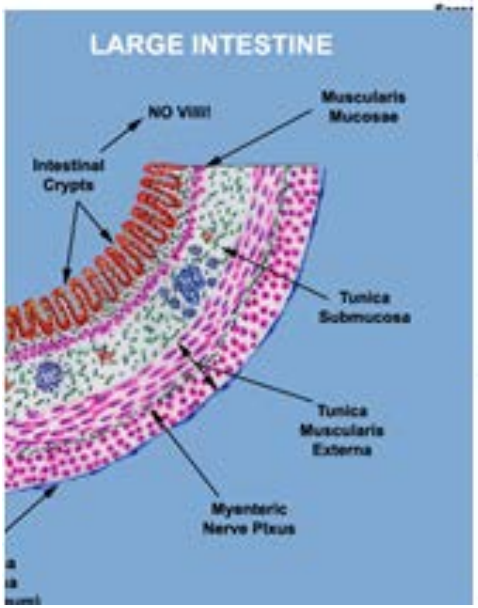
- intestinal function
- intestine and body health

The ‘golden triangle’ of intestinal interactions



(Smidt, 2012. DPP, Keystone, CO, USA)

Functions of the intestine



PHYSIOLOGY

- to absorb nutrients, electrolytes, water
- to secrete electrolytes & water
- to act as a **selective barrier** (control of harmful antigens & pathogens entry)

MICROBIOLOGY

- to harbour & feed commensal bacteria
- to limit pathogen growth/effects (colonisation resistance)

IMMUNOLOGY

- to tolerate self- and harmless antigens (food, commensal bacteria)
- to mount appropriate immune responses to pathogens and harmful antigens

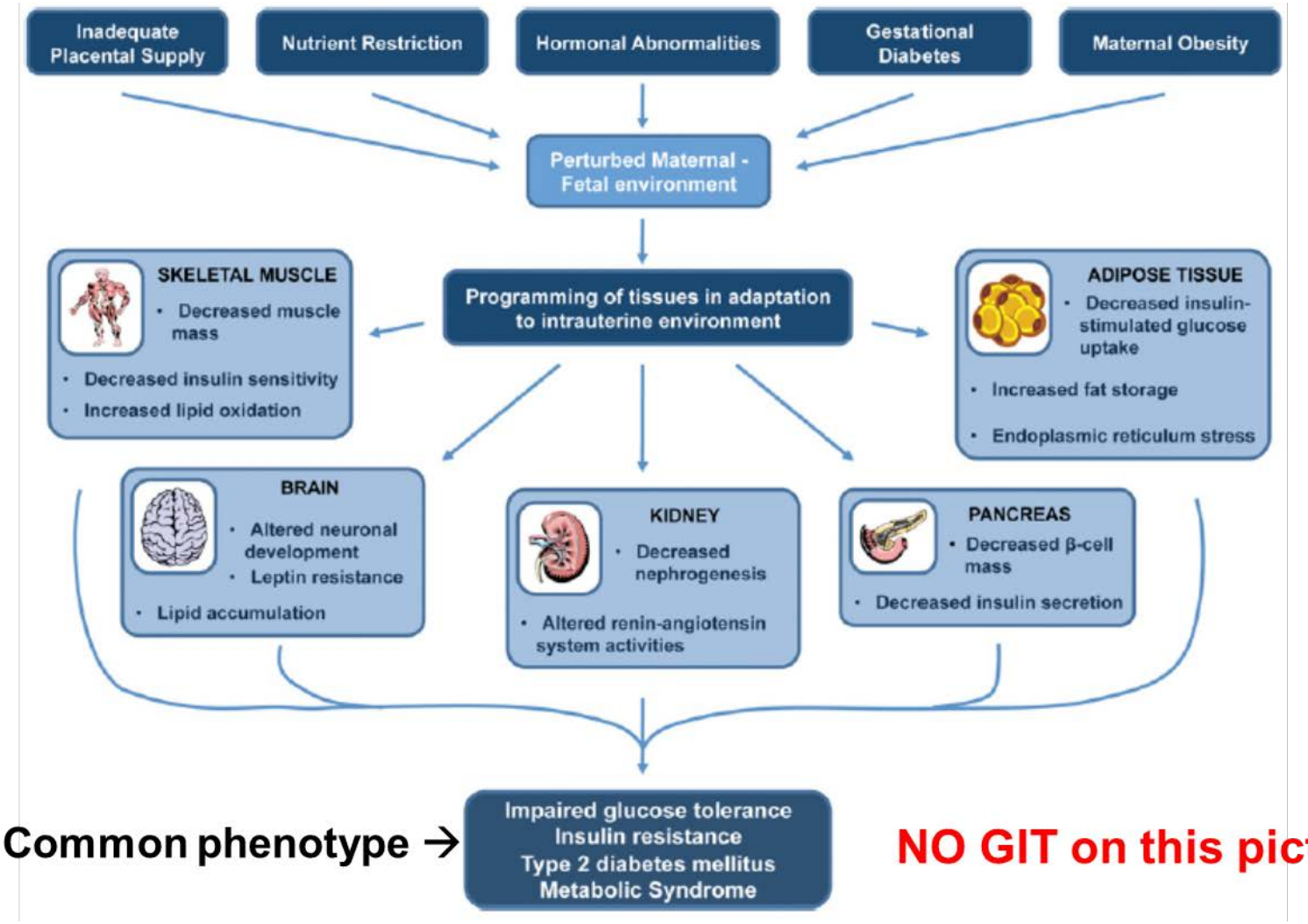
(http://www.vetmed.vt.edu/education/curriculum/vm8054/Labs/Lab19/Lab19.htm)

Importance and complexity of gut barrier function

Mucus Defensins Immunoglobulin A (IgA) Digestive enzymes and peptides	Secreted into the lumen
Epithelium - **enterocytes/colonocytes** - **tight junctions/proteins** (e.g. occludin, claudins, zonula occludens) - **cell protection systems** (e.g. HSP)	**Epithelial barrier**
Immune cell network (intra-epithelial lymphocytes, dendritic cells, T and B lymphocytes, mast cells, etc.)	Lamina propria

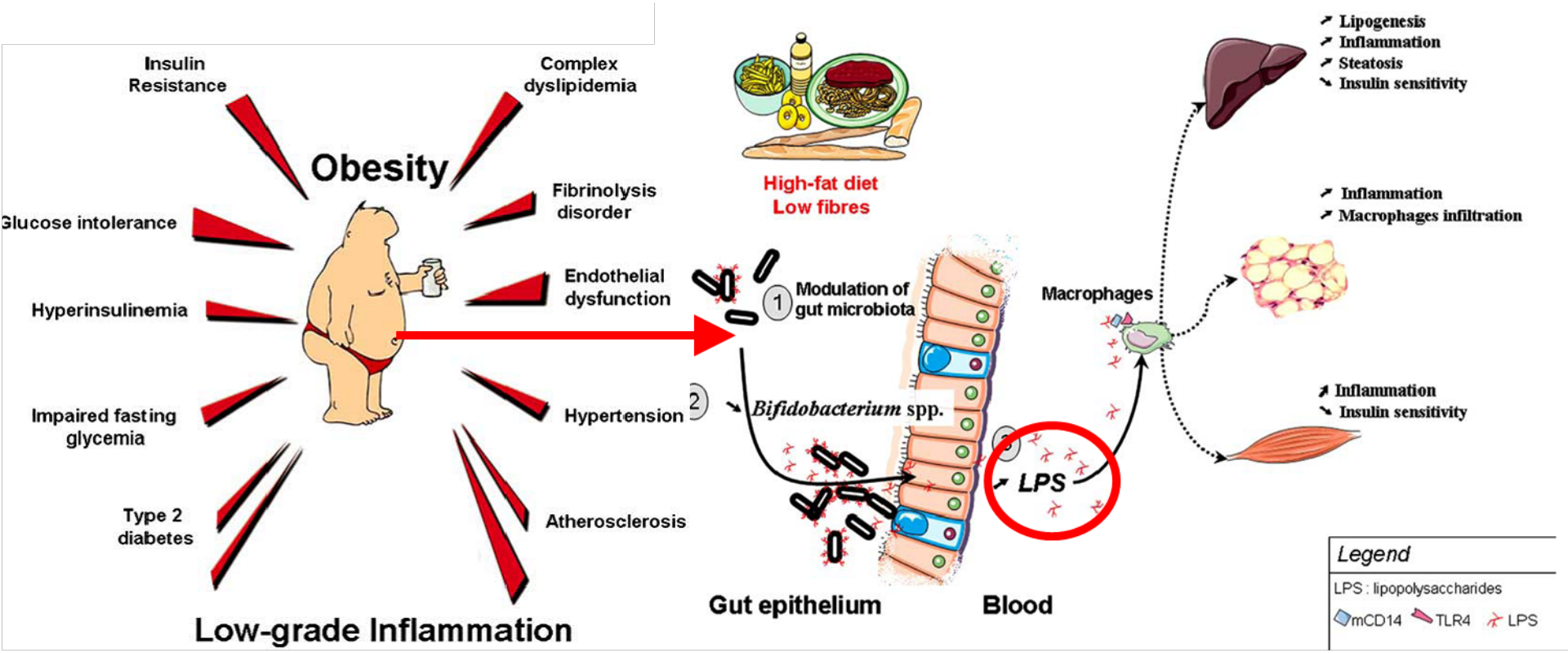
(Lallès 2010. In Dynamics in Animal Nutrition, Wageningen Academic Publishers, pp 31-51)

Developmental Origin of Health & Disease (DOHaD)



(Warner & Ozanne, 2010. Biochem J 427:333-347)

Role of GIT in food-induced metabolic disorders and obesity



→**Critical role of the GIT in the onset and development of metabolic diseases and obesity!**

(Cani et al. 2009. Curr Pharm Design 15:1546-1558)

Questions

What are the physiological consequences of neonatal disturbances in gut colonization in pigs:

- **Before and after weaning (short-term)?**
- **In adulthood (long-term; model for humans)?**
  - **With a balanced diet**
  - **With an unbalanced diet (e.g. high fat)?**

Neonatal antibiotics and gut physiology

Neonatal antibiotic treatment alters gastrointestinal tract developmental gene expression and intestinal barrier transcriptome **2005. Physiol Genomics *23*: 235-45.**

Alexandra Schumann,[1,3] Sophie Nutten,[1] Dominique Donnicola,[1] Elena M. Comelli,[1] Robert Mansourian, Christine Cherbut,[1] Irène Corthesy-Theulaz,[1] and Clara Garcia-Rodenas[1]

Departments of [1]Nutrition and Health and [2]Bioanalytical Science, Nestle Research Center, Vers-chez-les-Blanc, Lausanne; and [3]Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

Microbial manipulation of the rat dam changes bacterial colonization and alters properties of the gut in her offspring **2008. Am J Physiol *294*: 148-154.**

Frida Fåk,[1] Siv Ahrné,[2] Göran Molin,[2] Bengt Jeppsson,[3] and Björn Weström[1]

[1]Department of Cell and Organism Biology, and [2]Food Hygiene, Department of Food Technology, Engineering and Nutrition Lund University, Lund, and [3]Department of Surgery, Malmö University Hospital, Malmö, Sweden

Antibiotic Administration Early in Life Impairs Specific Humoral Responses to an Oral Antigen and Increases Intestinal Mast Cell Numbers and Mediator Concentrations[▽]

S. Nutten,*† A. Schumann,† D. Donnicola, A. Mercenier, S. Rami, and C. L. Garcia-Rodenas

Nestlé Research Center, Lausanne, Switzerland **2007. Clin Vaccin Immunol *14*: 190-197.**

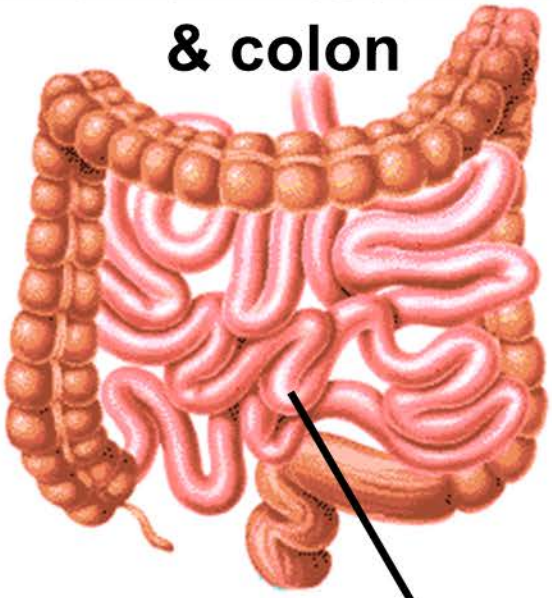
→ **Indication from rodent studies of short-term alterations in barrier function**
→ **Very little data on long-term consequences of early disturbances (imprinting)**

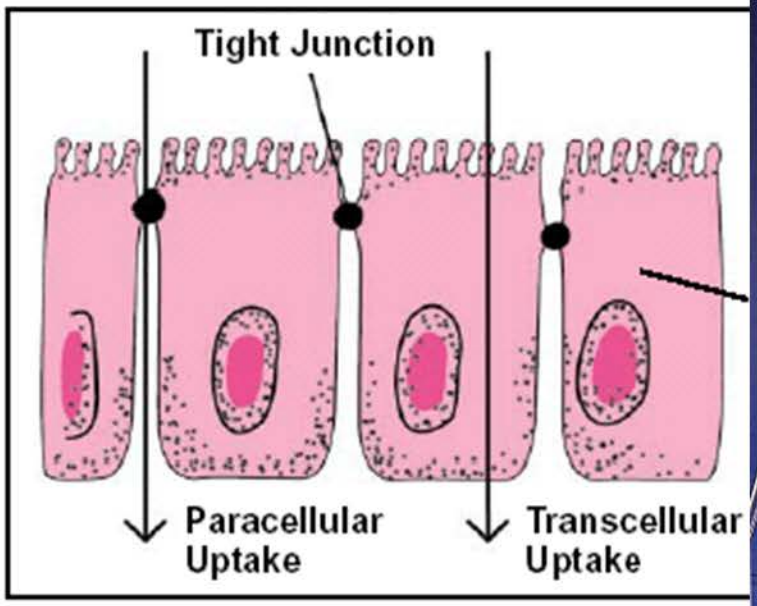
Intestinal and colonic physiology

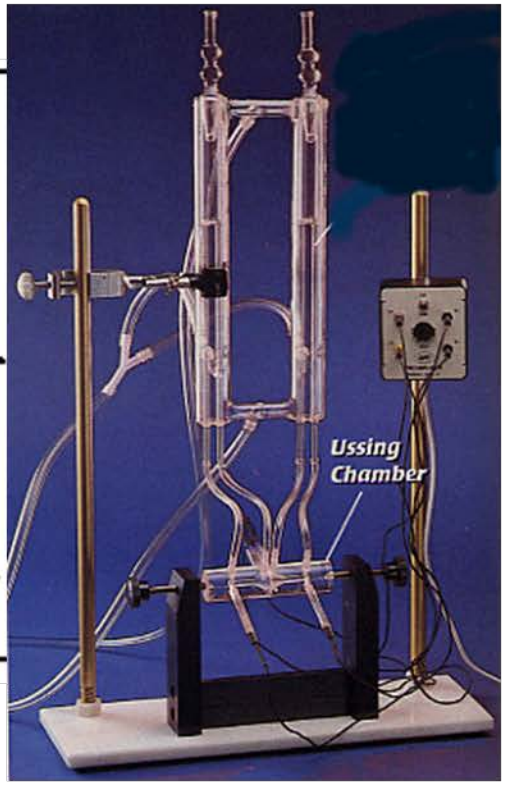
Permeability (para- and trans-cellular)

Absorptive & secretory capacities

Small intestine & colon





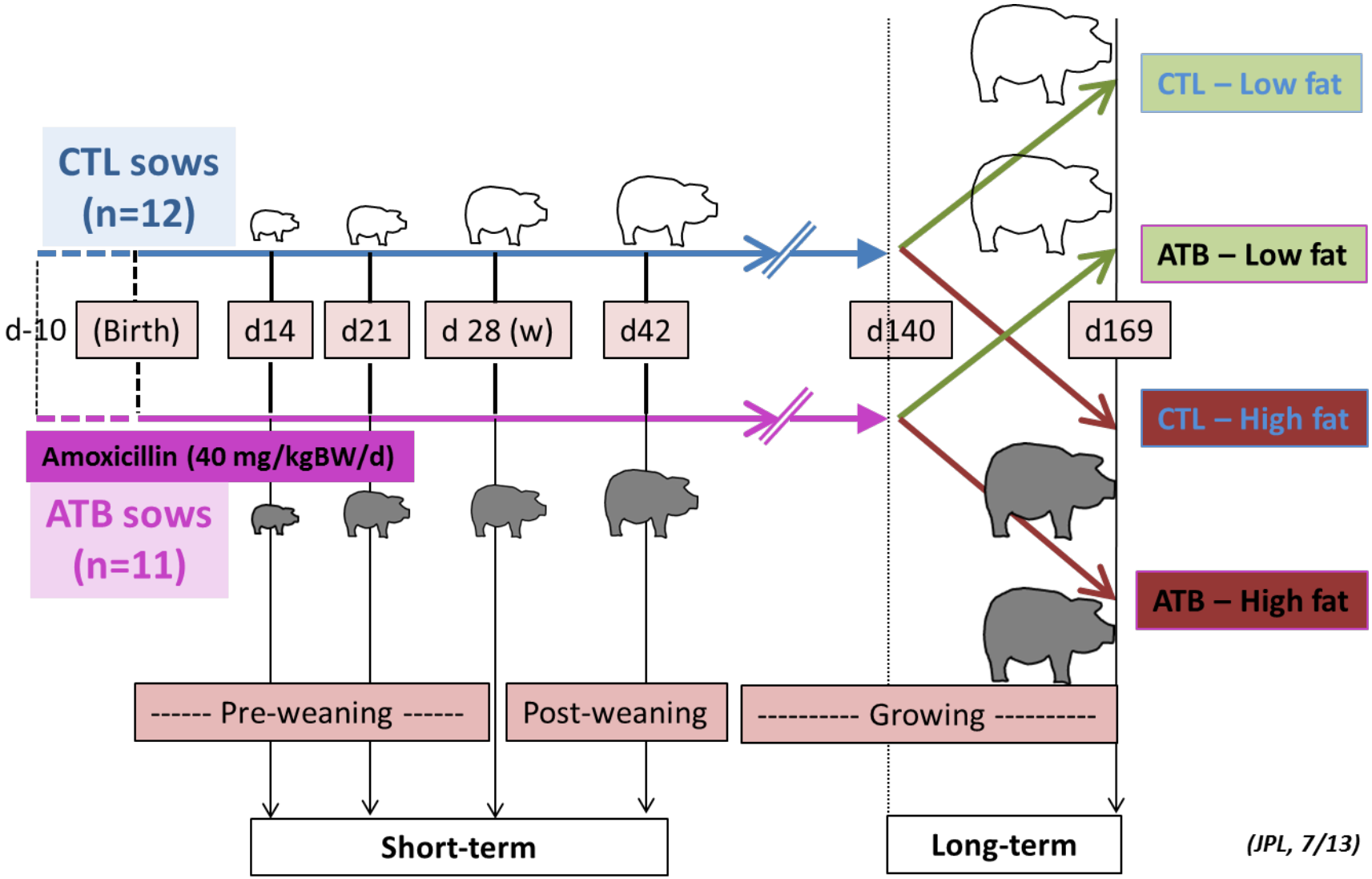


Ussing chambers

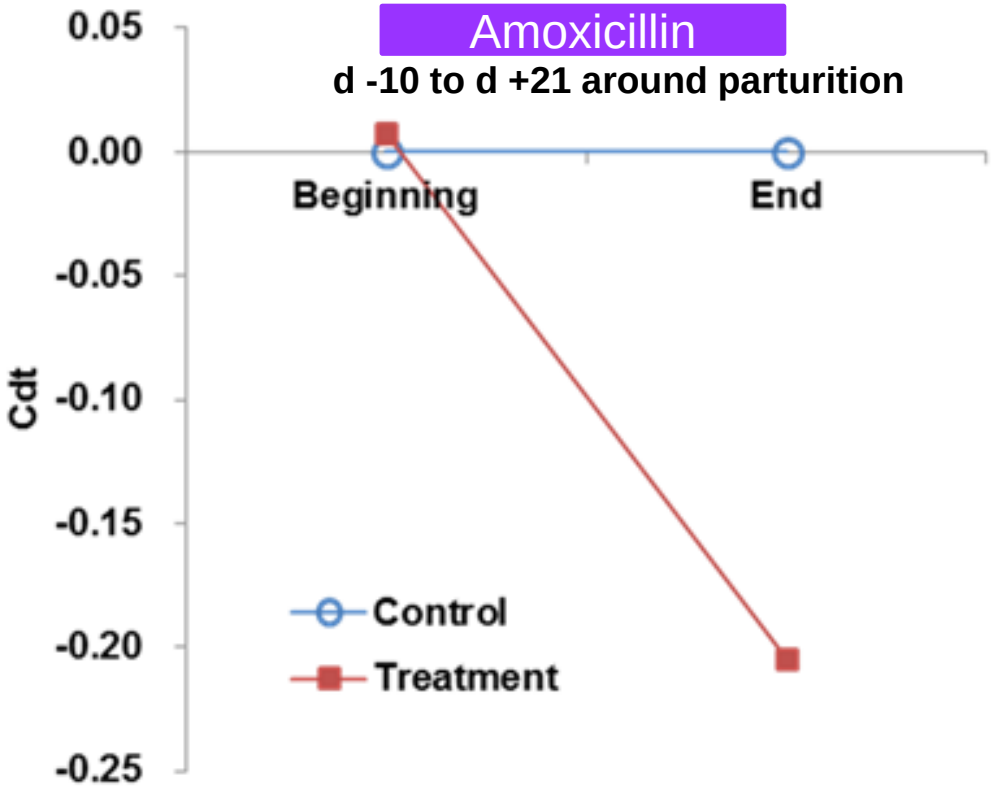
Key-enzyme activities

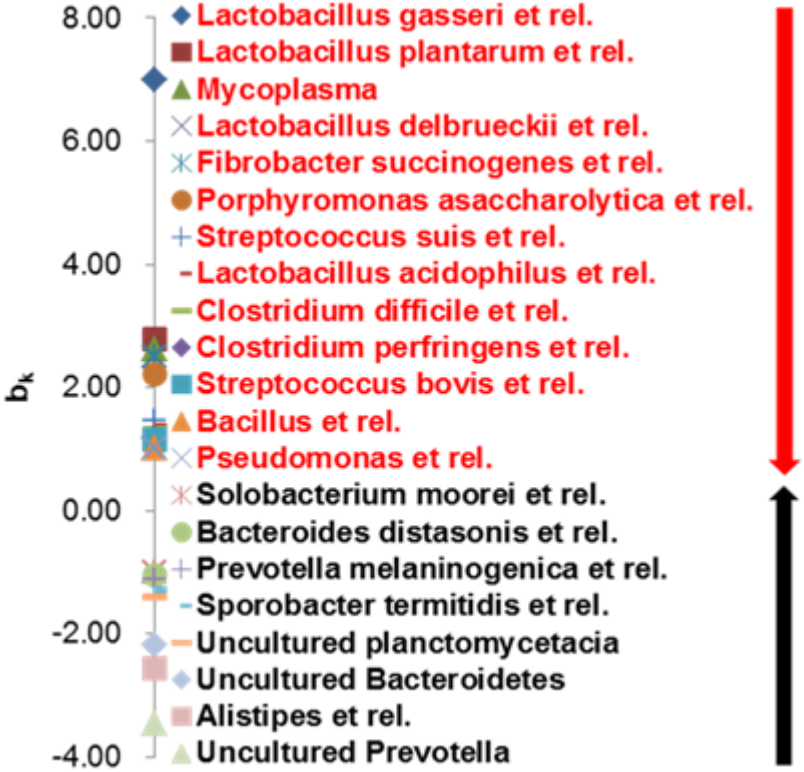
THE SWINE MODEL OF ANTIBIOTIC-INDUCED GUT DISTURBANCES

Protocol for short-term and long-term effects of perinatal antibiotic treatment in pigs



Antibiotic treatment affects sows' faecal microbiota composition





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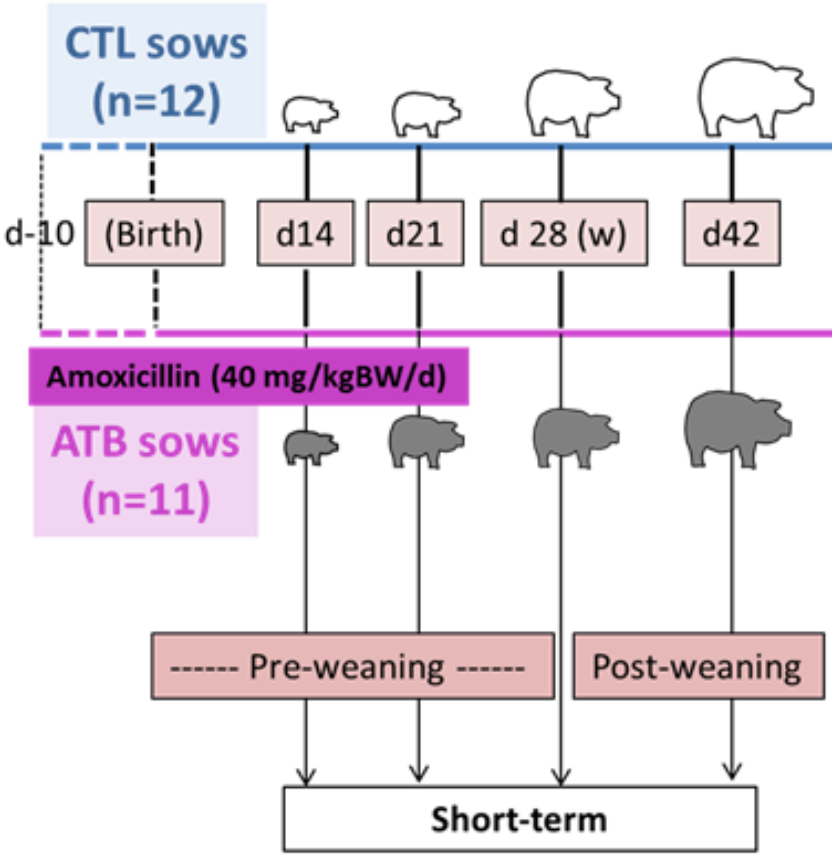
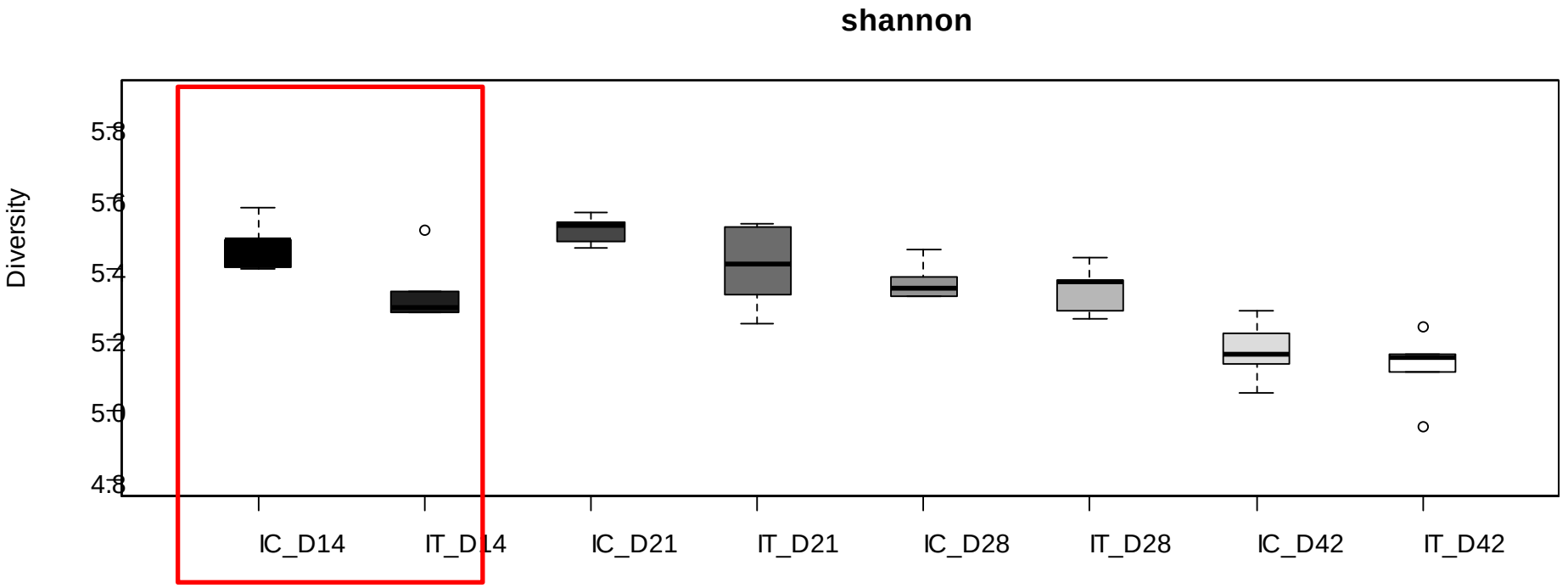
SHORT-TERM EFFECTS OF MATERNAL ANTIBIOTIC TREATMENT ON GUT FUNCTION



DISTAL ILEUM

Maternal antibiotic treatment reduces microbial diversity of offspring ileum transiently



→ Microbial diversity in ileum of ATB offspring is lower at day 14 ($P<0.05$)

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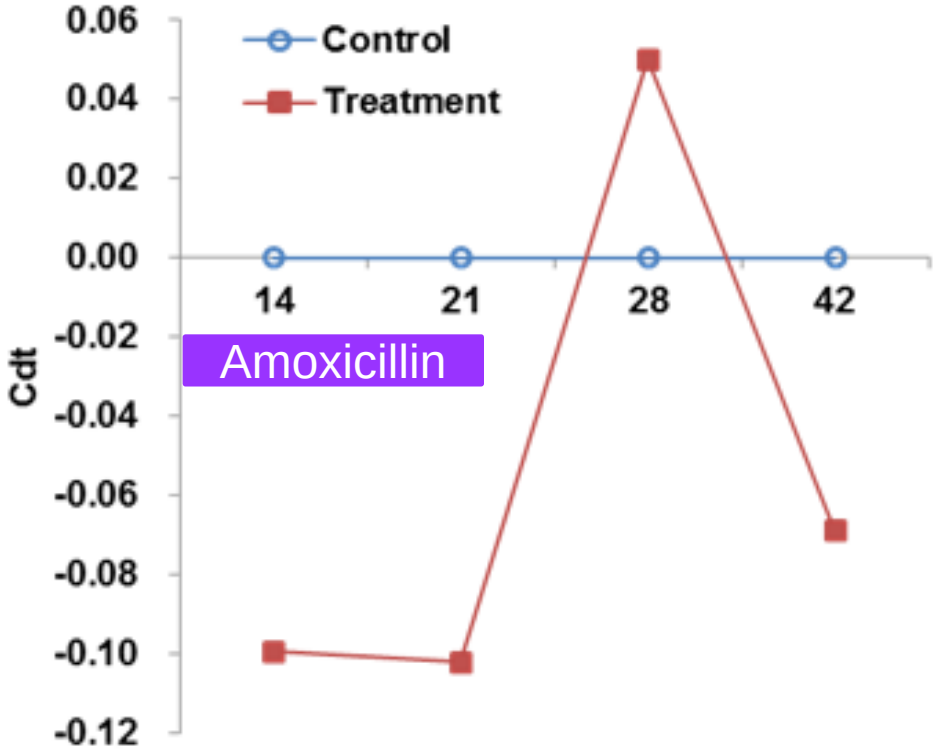
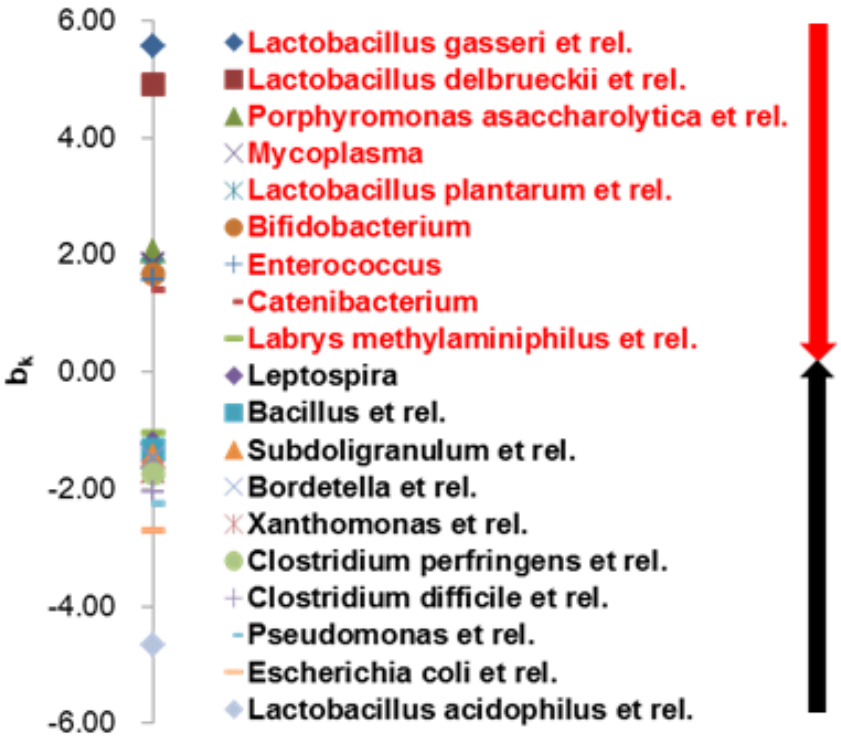
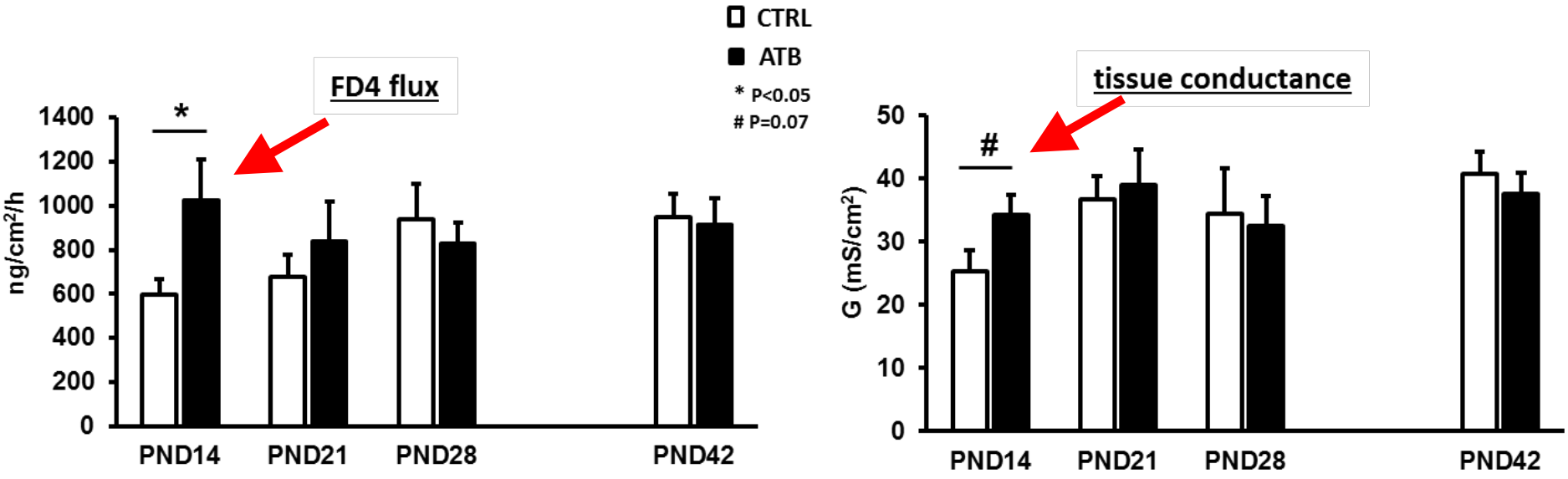
Maternal antibiotic treatment impacts ileal microbiota composition of offspring





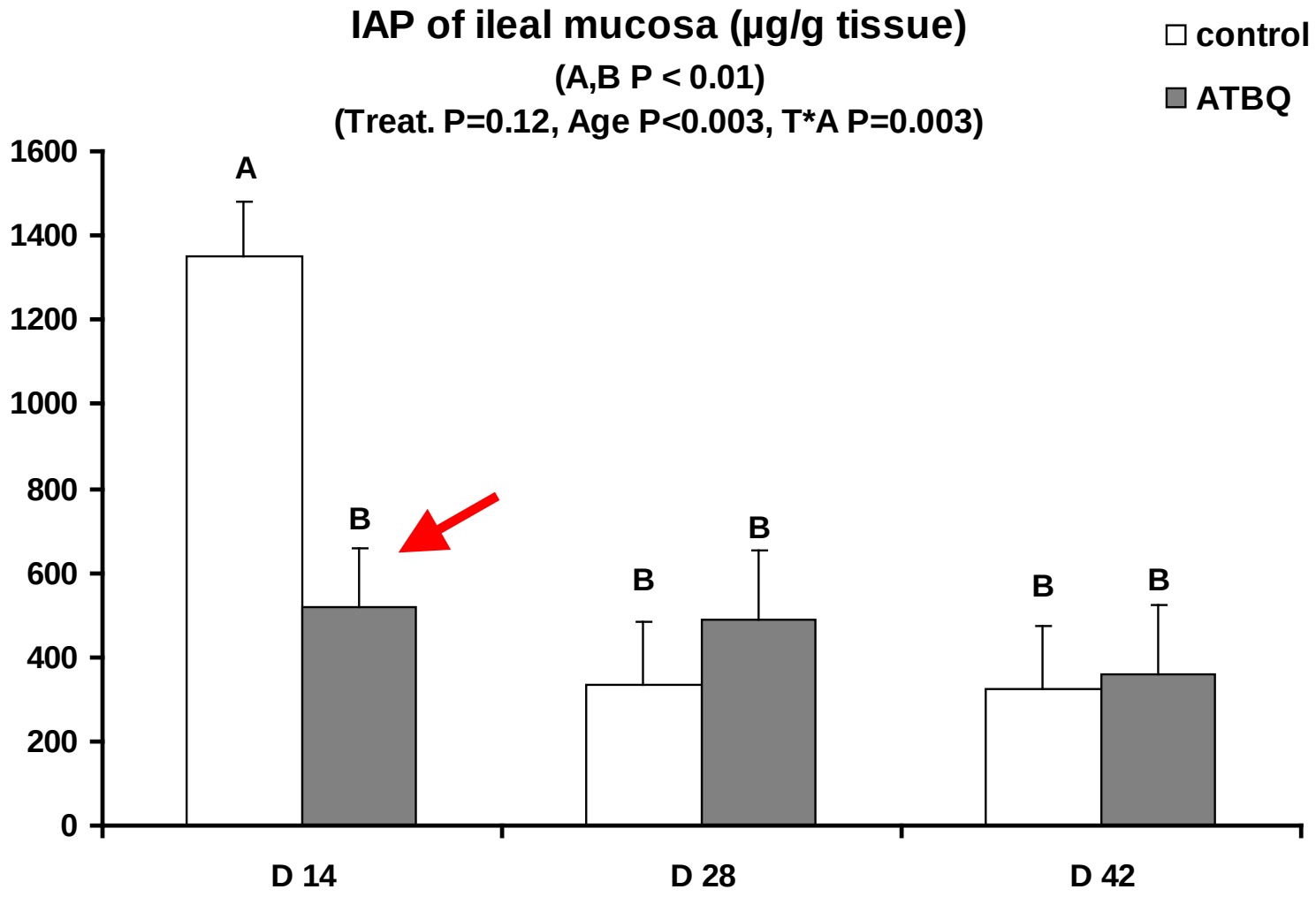
→Microbial composition in ileum of ATB offspring is affected

Ileal paracellular permeability is transiently increased in offspring born to antibiotic-treated sows

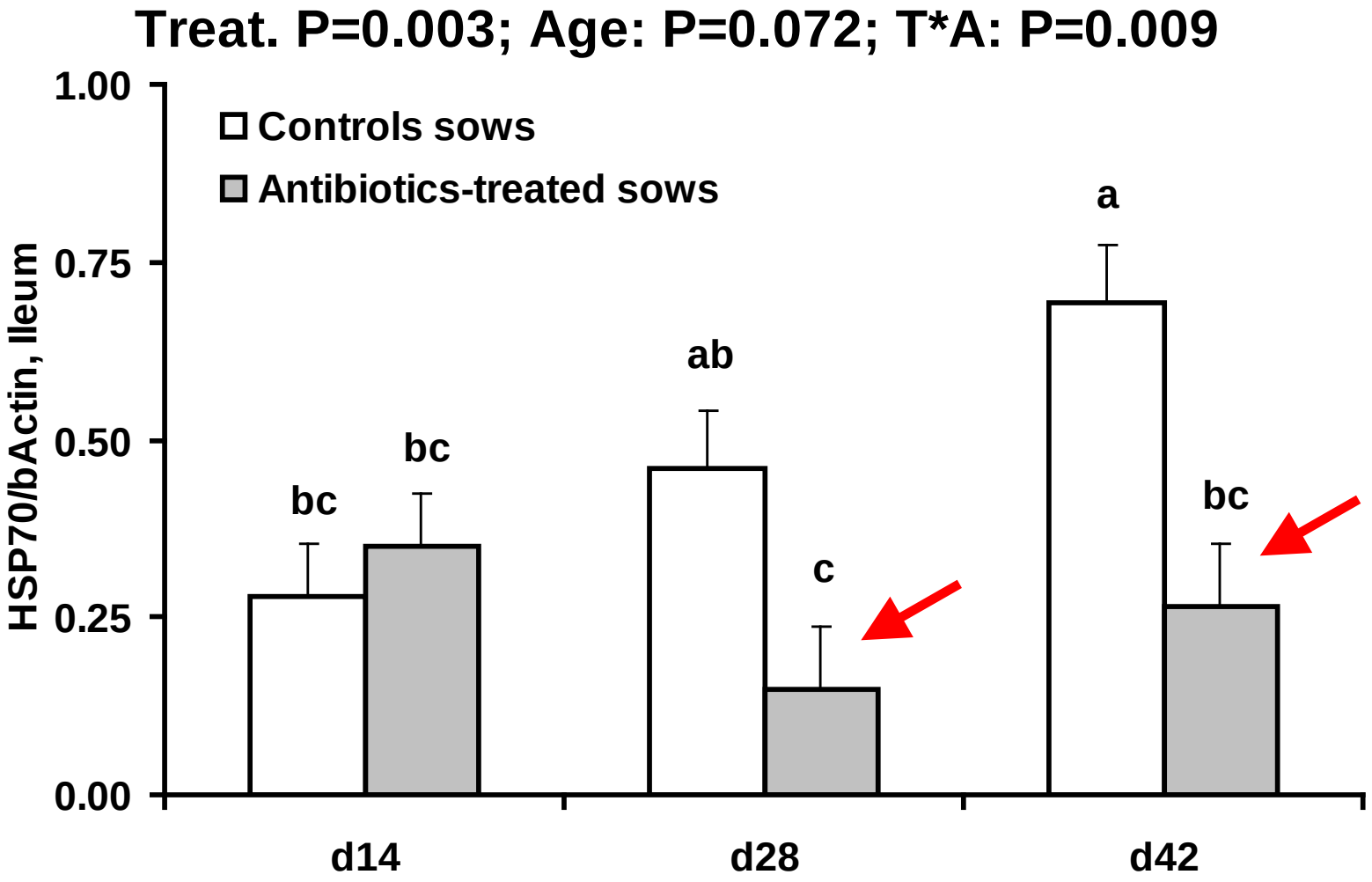


- Effect of peripartum antibiotic previously studied in a rat model: a mixture of antibiotics (mainly against Gram-negative bacteria and anaerobes) given to dams during late gestation and lactation increases in vivo permeability to macromolecules in rats pups aged 14d (Fak et al., AJP 2008)

Ileal IAP is transiently decreased in offspring born to antibiotic-treated sows



Ileal HSP70 is transiently decreased in offspring born to antibiotic-treated sows



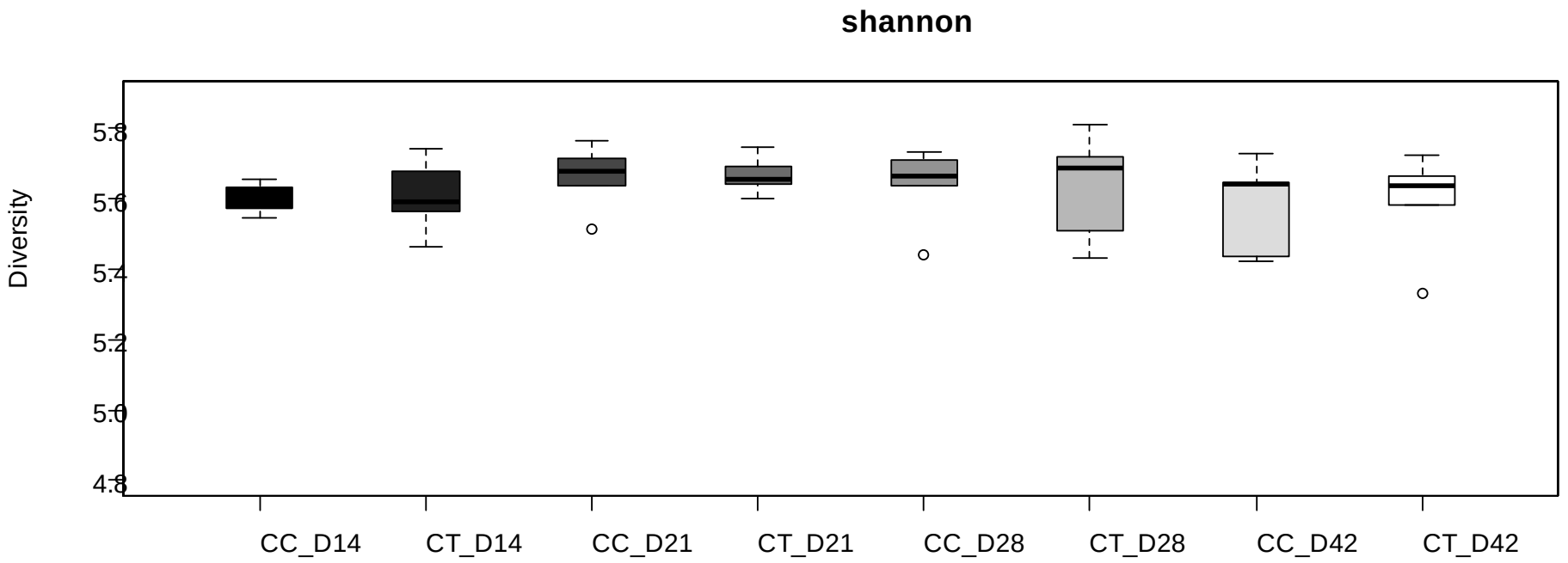
Intestinal immune responses and transcriptomics in offspring born to antibiotic-treated sows

- Gut-associated lymphoid tissue (GALT) in ATB offspring is influenced by maternal antibiotic treatment (at d21)
- Genes are differentially expressed in ileal tissue of ATB offspring (d21 & d42)

→ See oral presentation by Dr S. Ferret-Bernard in this session

PROXIMAL COLON

Maternal antibiotics does not affect microbial diversity of offspring colon



→Microbial diversity in colon of ATB offspring is NOT affected

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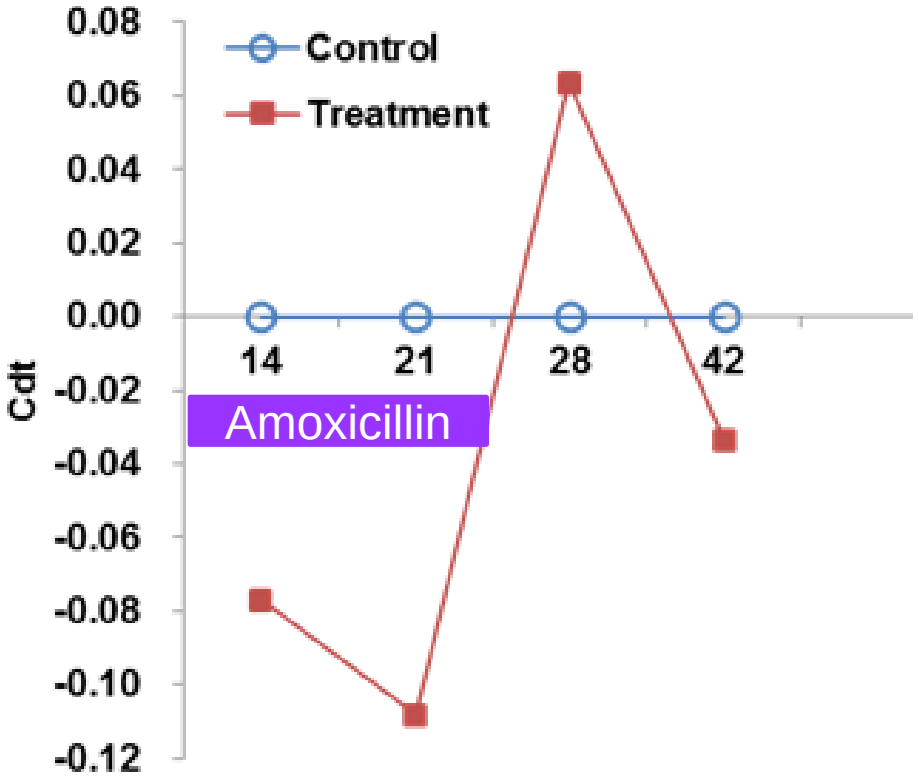
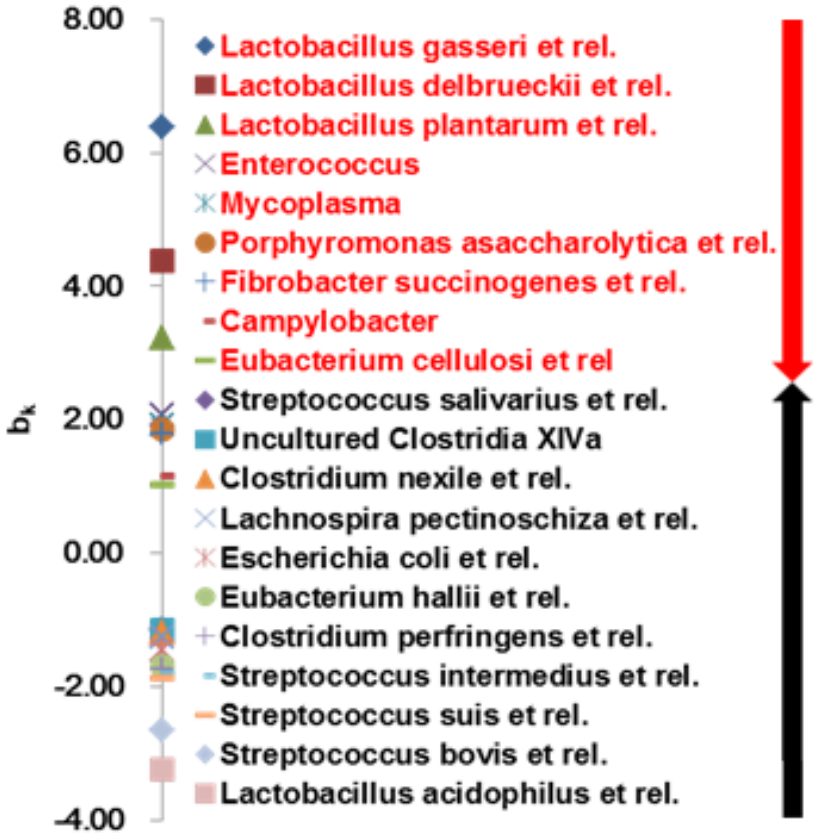
Maternal antibiotic treatment impacts colonic microbiota composition in offspring (ST)





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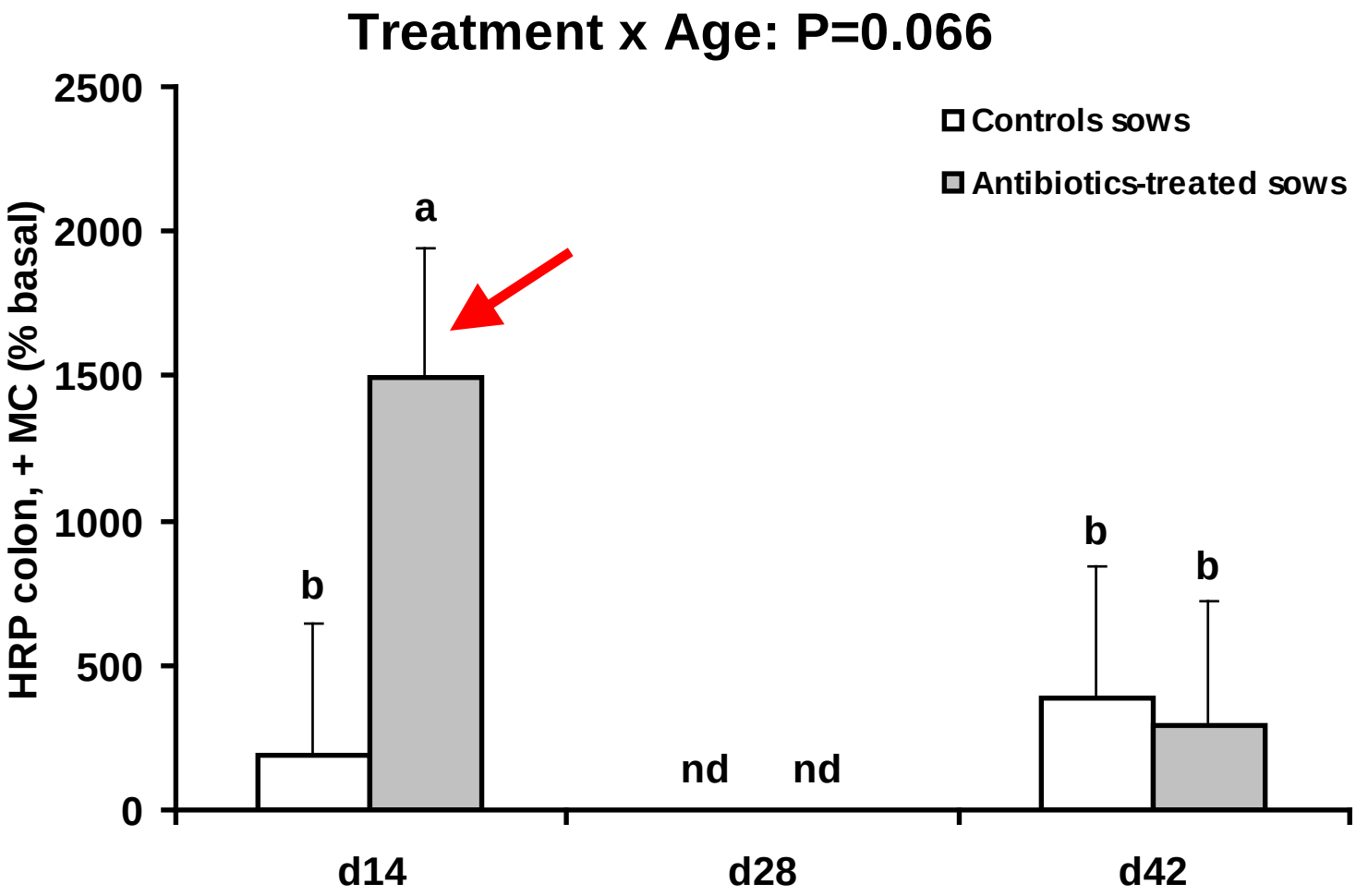
Under oxidative stress, colonic transcellular permeability is transiently increased in ATB offspring



Colonic HSP70 is increased in offspring born to antibiotic-treated sows

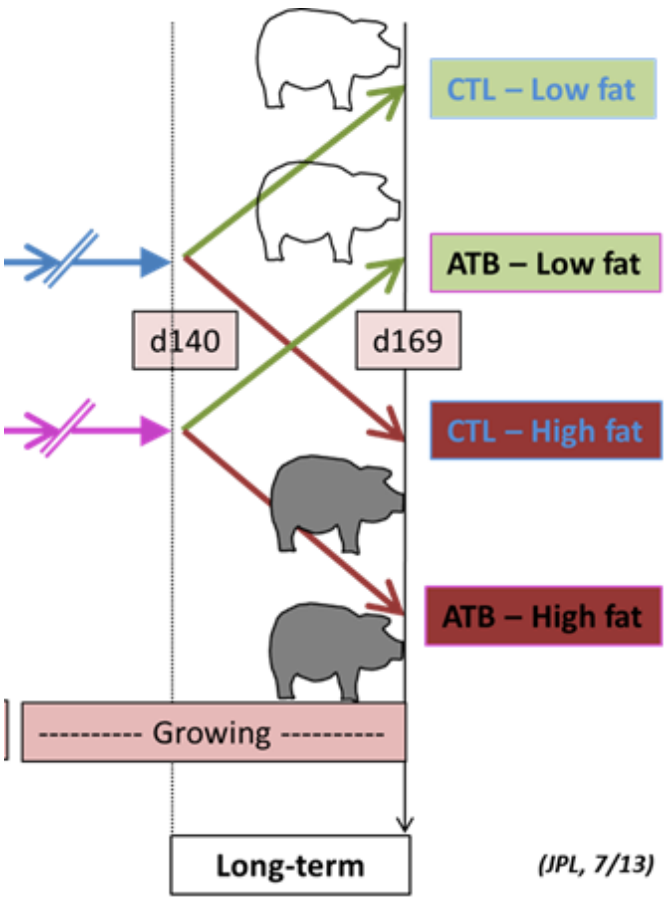
Same results for inducible HSP27

Conclusions on short-term effects

Transient and successive modifications in gut physiology :

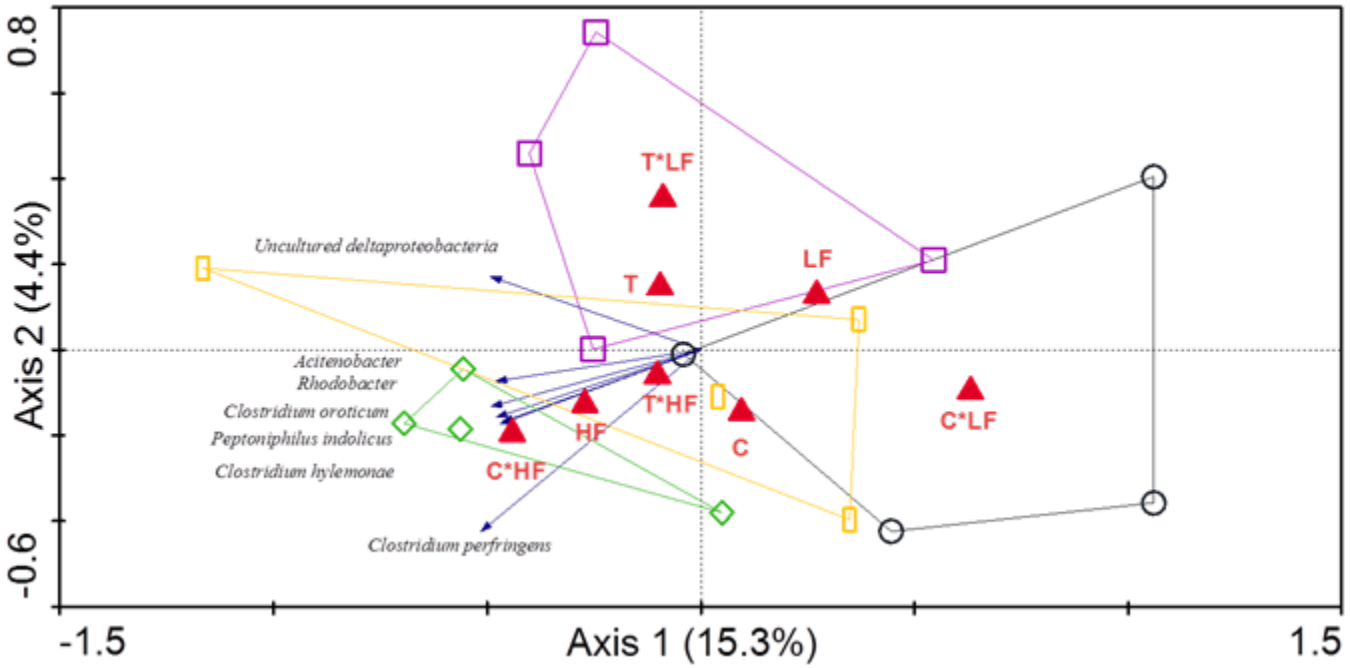
- **Possibly detrimental early in life (d14)**
- **Oriented towards lower (bacterial) stress on the ileum**
- **Oriented towards higher (bacterial) stress on the colon**

LONG-TERM EFFECTS OF MATERNAL ANTIBIOTIC TREATMENT ON GUT FUNCTION



Maternal antibiotic treatment has little effects on ileal and colonic microbiota in adult offspring

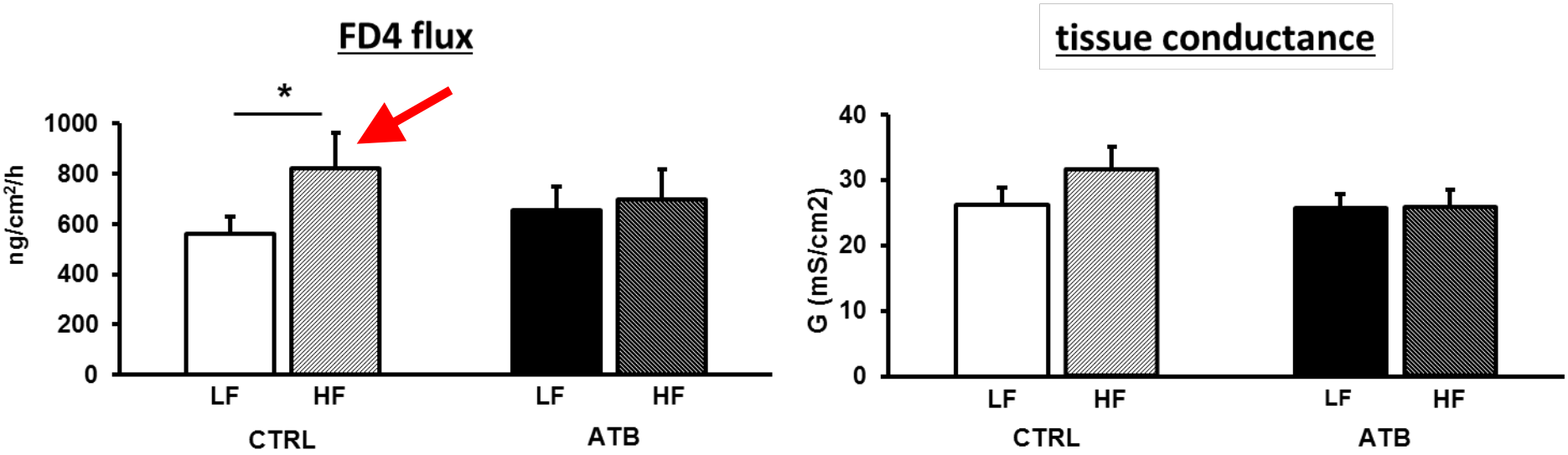
→ Tendency for an interaction between ATB treatment and offspring adult diet (P=0.065):



→ No effects on colonic microbiota

DISTAL ILEUM

Ileal paracellular permeability is increased in CTL offspring but not in ATB offspring



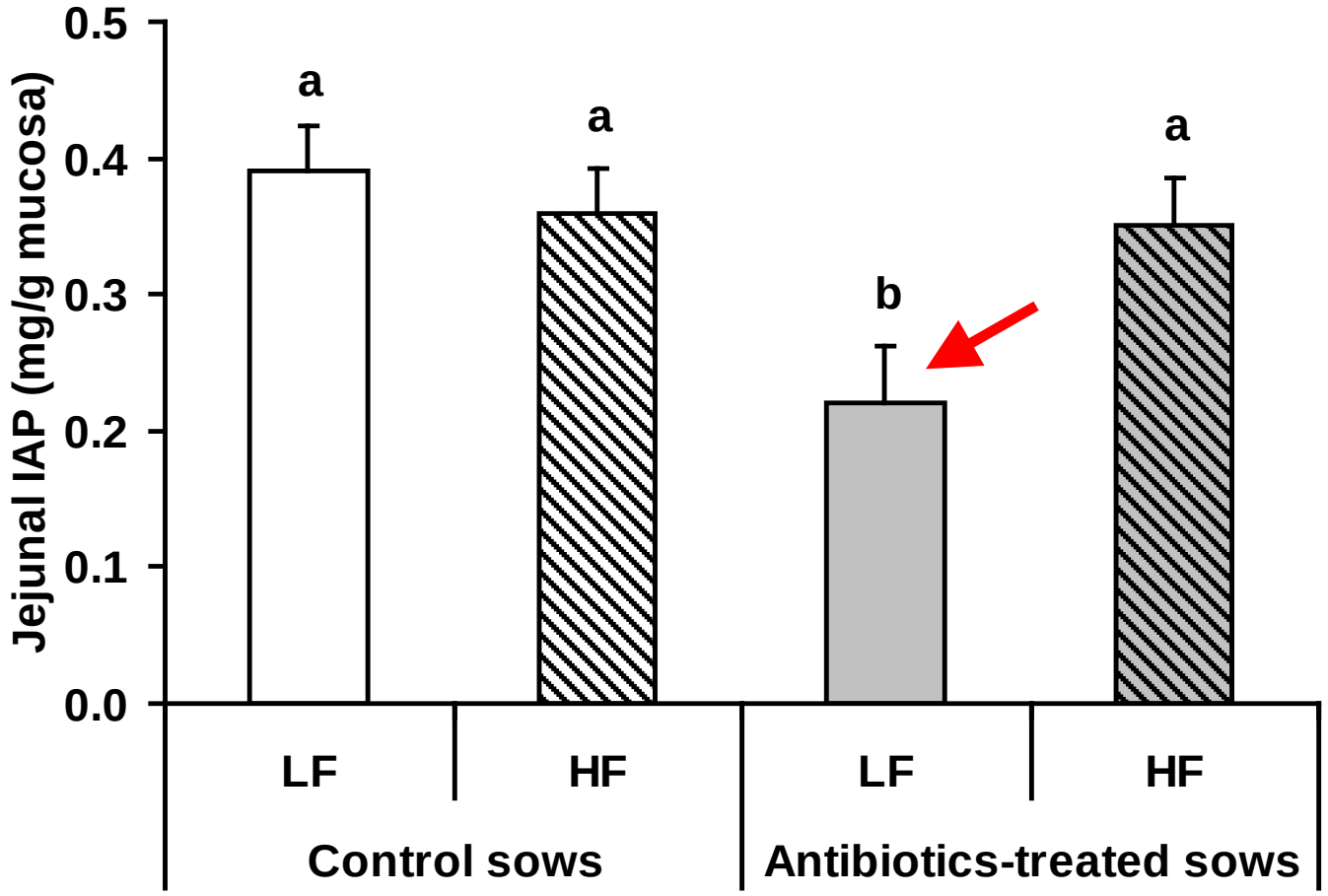
- **Absence of change in gut permeability under a high fat diet linked to differences in gut microbiota**

already demonstrated in rodents:

- increased permeability induced by a HF diet during 4 weeks in mice prevented by the use of broad spectrum antibiotics (Cani et al., Diabetes 2008),
- rats resistant to obesity under 8 weeks of a HF diet characterized by absence of gut barrier defaults and reduced levels of Proteobacteria compared to rats prone to obesity (de La Serre et al., AJP 2010)

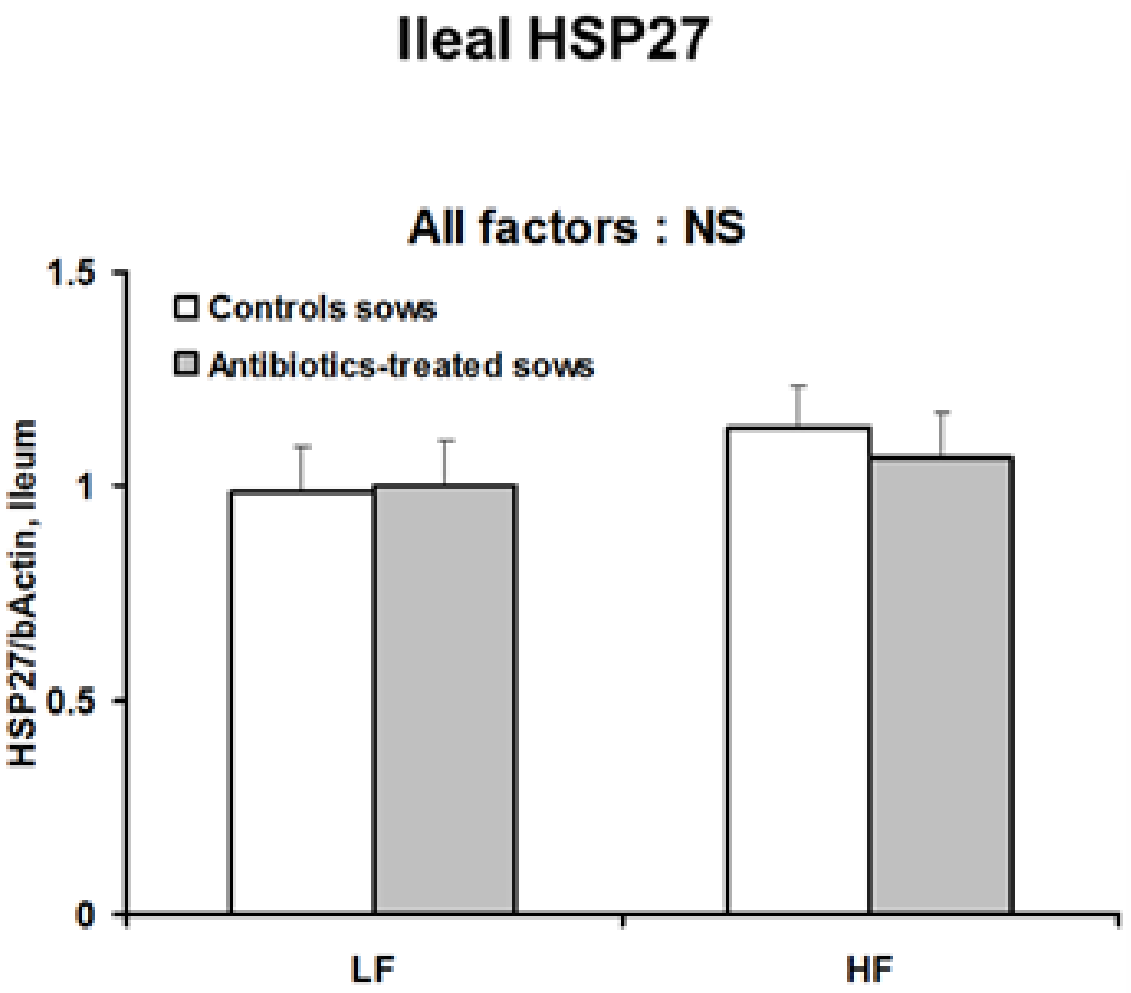
- **Here we show for the first time that early life colonization can also impact the gut barrier function response to a high fat diet in adults**

Jejunal (but not ileal) IAP is lower in adult offspring born to antibiotic-treated



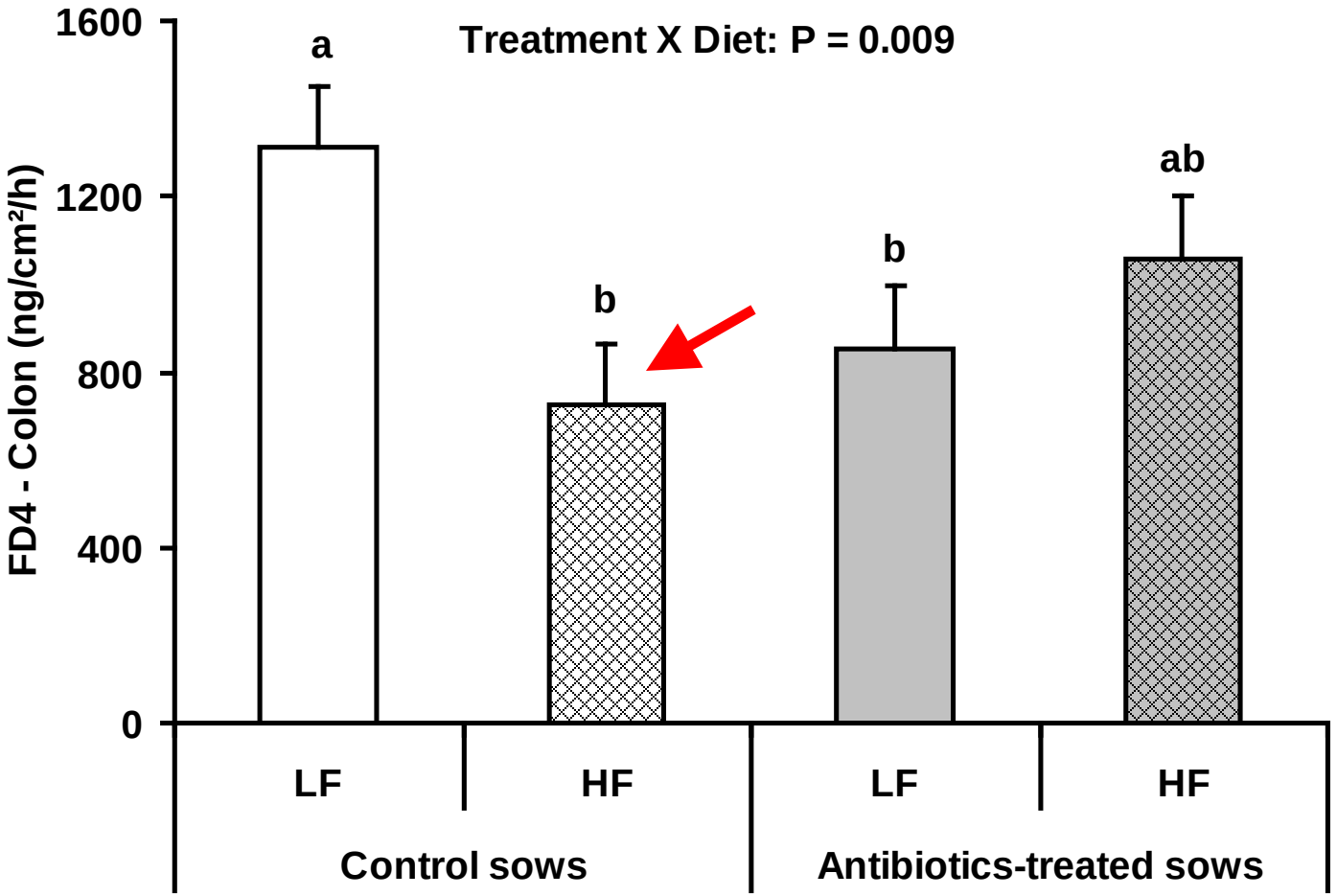
→ Data suggest regional imprinting of intestinal functions (e.g. IAP)

NO long-term effects of maternal antibiotic treatment on offspring ileal HSPs

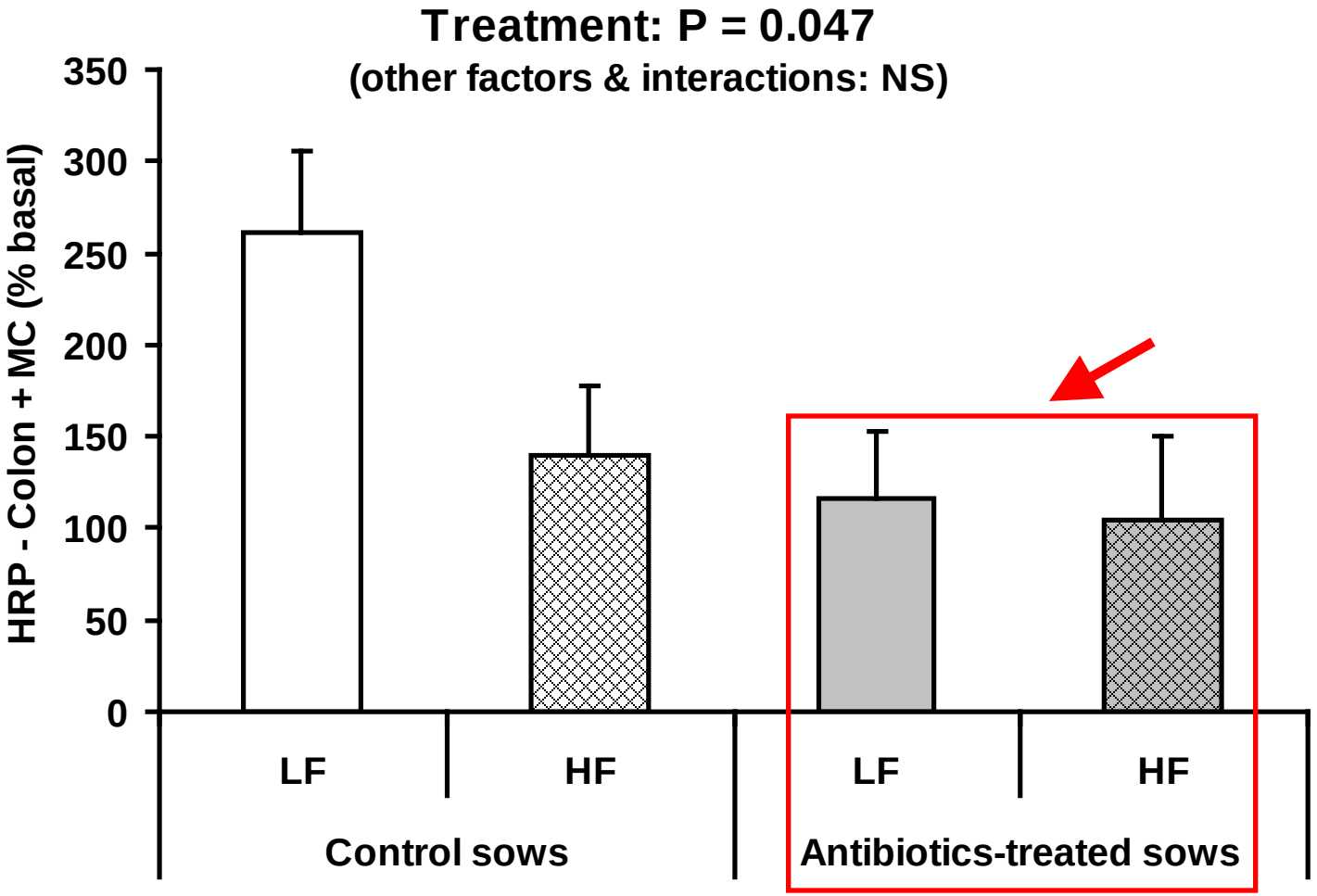


PROXIMAL COLON

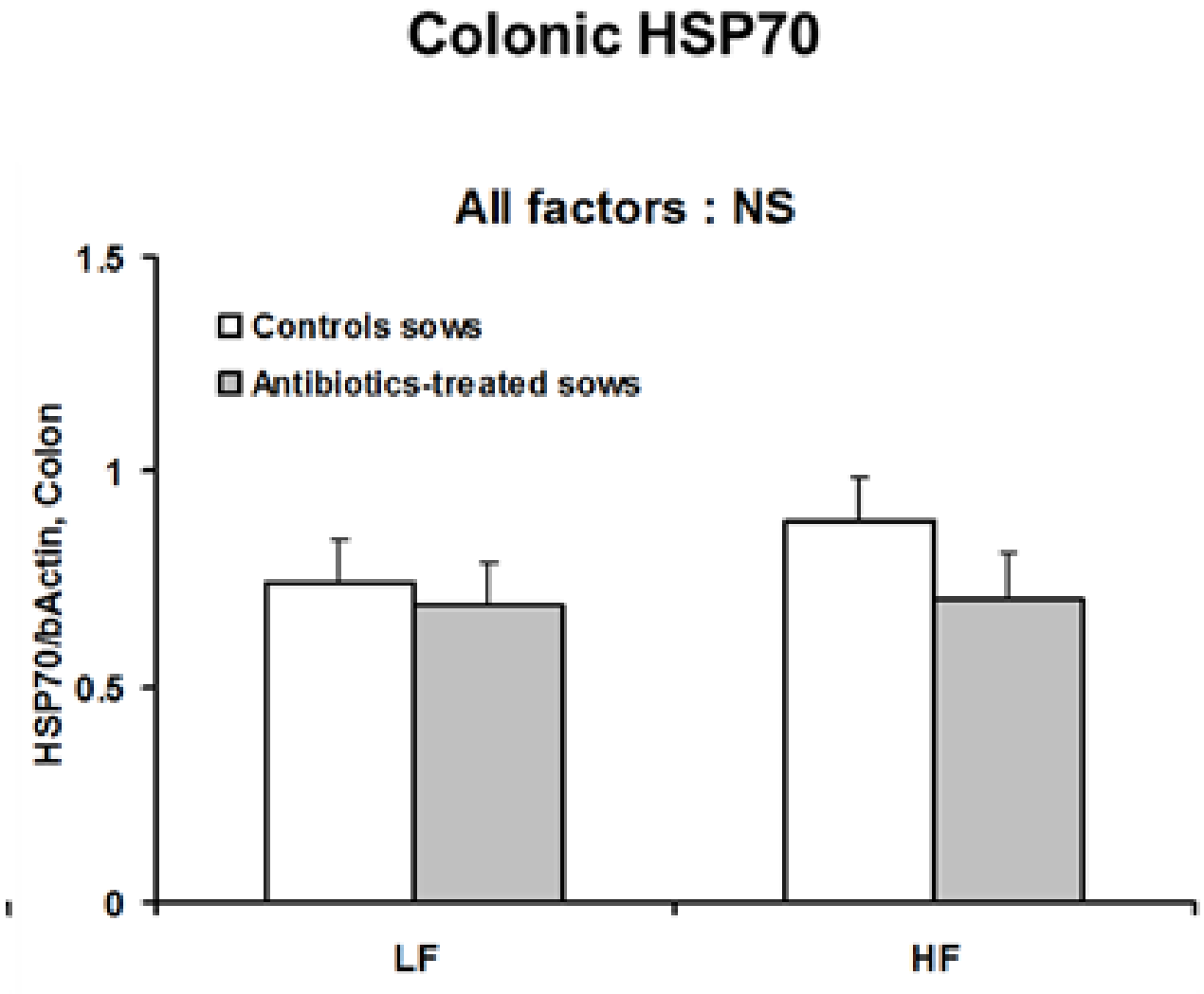
Colonic paracellular permeability is decreased with HF diet in CTL but not ATB offspring



Under oxidative stress, colonic transcellular permeability is lower in ATB offspring



NO long-term effects of maternal antibiotic treatment on offspring colonic HSPs



Conclusions on long-term effects

Variable	Site	Perinatal antibiotics	Adult diet (HF)	ATBQ * AD interaction
Villus-crypt architecture	J,I	no	no	no
Alkaline phosphatase	**J**,I	**YES**	no	**YES**
Aminopeptidase N	J,I	no	no	no (# J)
Dipeptidyl-peptidase IV	**J,I**	**YES**	no	no
Sucrase	**J**,I	no	YES	**YES**
HSP 27 and HSP 70	I	no	no	no
Paracellular perm.	**I,Co**	no	no	**YES**
Paracellular perm/OxS	**I,Co**	no	no	**YES**
Transcellular perm.	I,Co	no	no	no
Transcellular perm./OxS	**Co**	**YES**	no	no
PC/TC perm. Ratio	**Co**	**YES**	no	no
Basal Isc current	**I**	**YES**	no	no
Na^+-Glucose absorpt.	**I**	no	no	**YES**
Carbachol- Cl^- secretion	I	no	no	no

OxS: oxidative stress (monochloramine)

→ Perinatal disturbances in mother's microbiota DO HAVE long-lasting SELECTIVE and REGIONAL effects on key functions of offspring gut

CONCLUSIONS AND PERSPECTIVES

Conclusions

1/ Early disturbances in GIT bacterial colonisation (induced by maternal treatment with antibiotics) have significant consequences on various facets of GIT function during GIT development and also later in life.

2/ The affected functions include:

- **Ileal and colonic permeability**
- **Key-intestinal enzymes (e.g. IAP)**
- **Cytoprotection systems (e.g. HSP; short-term only)**
- **Intestinal immunity & transcriptomics (short-term)**

Perpectives

1/ Correlations between microbiota composition and physiology in small intestine and colon (coll. WUR)

2/ Underlying regulatory pathways

- *** target genes: mRNA levels**
- *** epigenetic modifications (e.g. IAP, Klf4)**

3/ Can probiotics (e.g. *L. amylovorus*) restore normal gut physiology after antibiotic-induced disorders (ongoing Interplay exp. 2)?

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SCIENCE & IMPACT

Thank you for your attention!

Work supported financially by the European Union (Interplay project No 227549).

SEVENTH FRAMEWORK PROGRAMME