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IMPACT OF BIOACTIVE SUBSTANCES ON THE GASTROINTESTINAL TRACT AND PERFORMANCE OF WEANED PIGS

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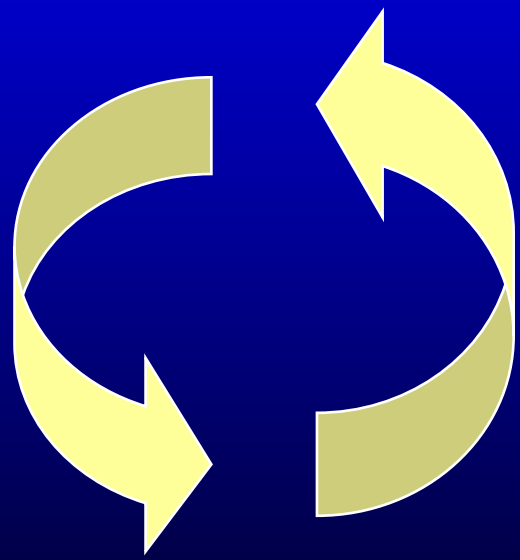
Outline

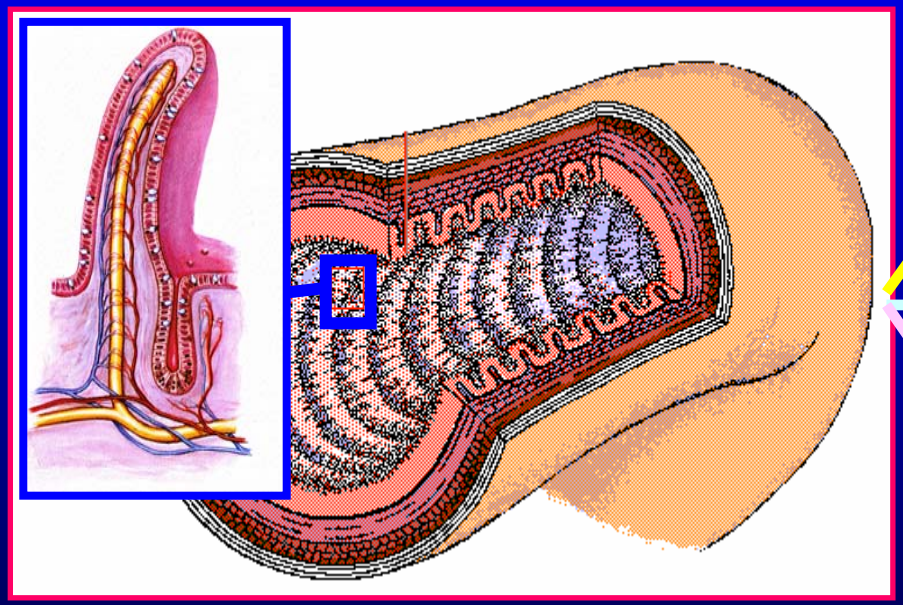
- **Background**
  - → the weaning process
  - → changes in gut architecture and function post-weaning
- **Organic acids**
  - → organic acids
  - → Na-butyrate
  - → specific amino acids
- **Animal and plant protein sources**
  - → spray dried plasma
  - → bovine colostrums
  - → plant proteins
- **Plant extracts and natural substances**
- **Conclusions and perspectives**

Introduction

- **Weaning transition = ACCUMULATION OF STRESS**
  - abrupt separation from the sow
  - mixing, new environment
  - change from the milk to less digestible and more complex (dry) diets
- **Consequence 1 = UNDER-NUTRITION**
  - growth check
  - feed (and water) intake low and highly variable
- **Consequence 2 = DIGESTIVE DISORDERS**
  - architecture and functions of the small intestine
  - gut microflora
  - local immune system



Functions of the intestine



PHYSIOLOGY

- to absorb nutrients, electrolytes & water
- to secrete electrolytes and 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

ORGANIC ACIDS

Organic acids

From the meta-analysis conducted by Partanen (2001):

- **OA improve growth, feed intake and feed to gain ratio**
- **Performance response to OA greater in younger pigs**
- **OA improve nutrient digestibility by reducing microbial activity and microbial protein synthesis in the gut, in connexion with reduced pH and antimicrobial activity of OA → nutrients spared from microbes to the animal**
- **Inconsistent effects of OA on incidence of diarrhoea and *E. coli* counts…**
- **OA MAY also influence pancreatic secretion and gut morphology…**

→ Needs for additional investigations in GIT physiology

Organic acids

	Formic ac	Fumaric ac	Citric ac	K di-formate
Exp	6	18	9	3
Obs	10	27	19	13
dose (g/kg feed)	3-18	5-25	5-25	4-24
Feed intake[$]	106[a]**	100[b]	99[b]	108[a]***
Weight gain[$]	110[a]***	104[b]**	104[b]**	112[a]***
Feed : gain[$]	97.5*	97**	96**	96*

[$]Treated/control * 100

a,b: differences between organic acids ($P < 0.05$)

*, **, *** significant effect of OA supplementation ($P < 0.05$, 0.01 and 0.001, respectively)

(Review by Partanen et al 2001)

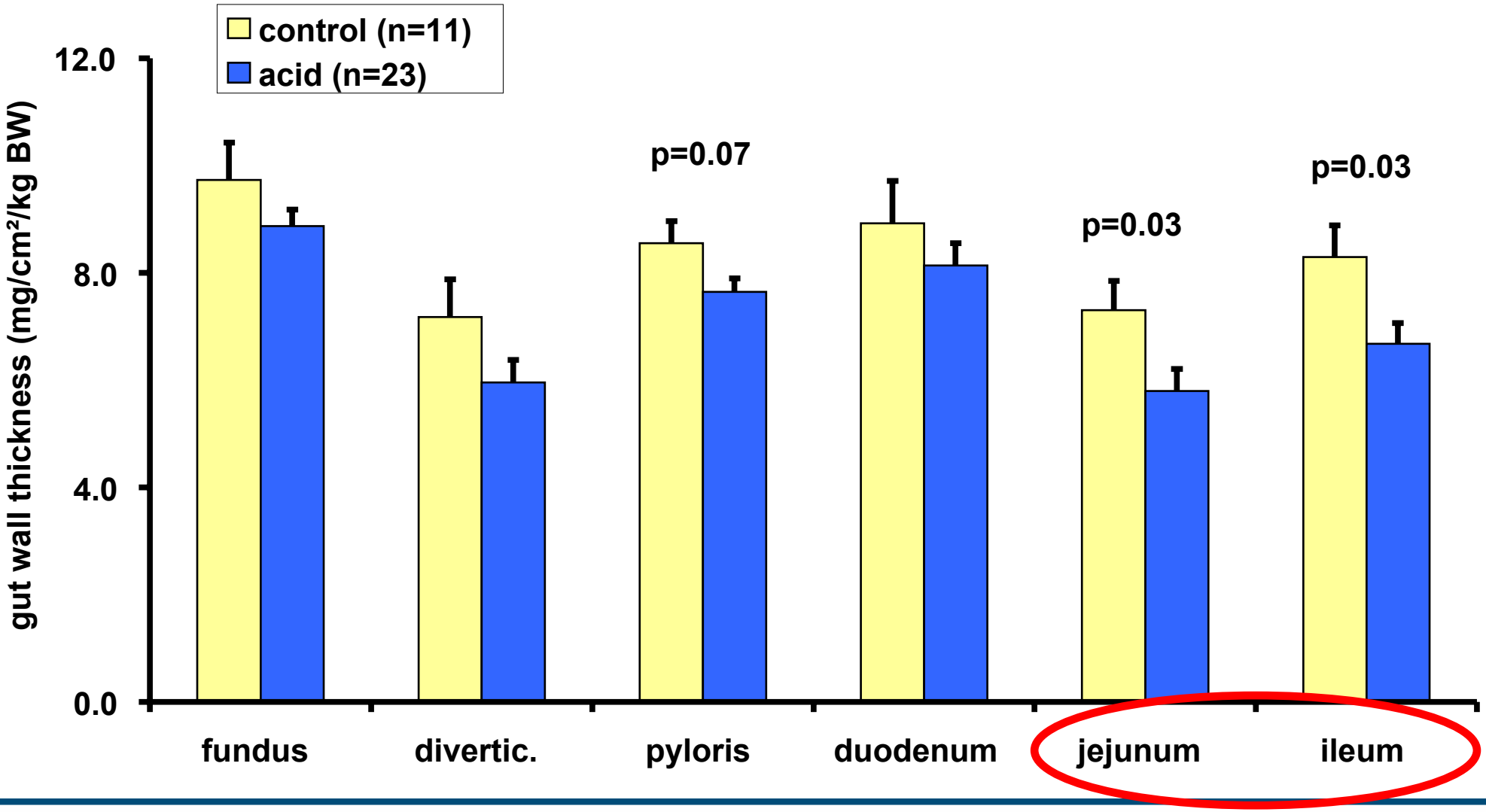
Dietary OA supplements:

- Organic acidifiers:

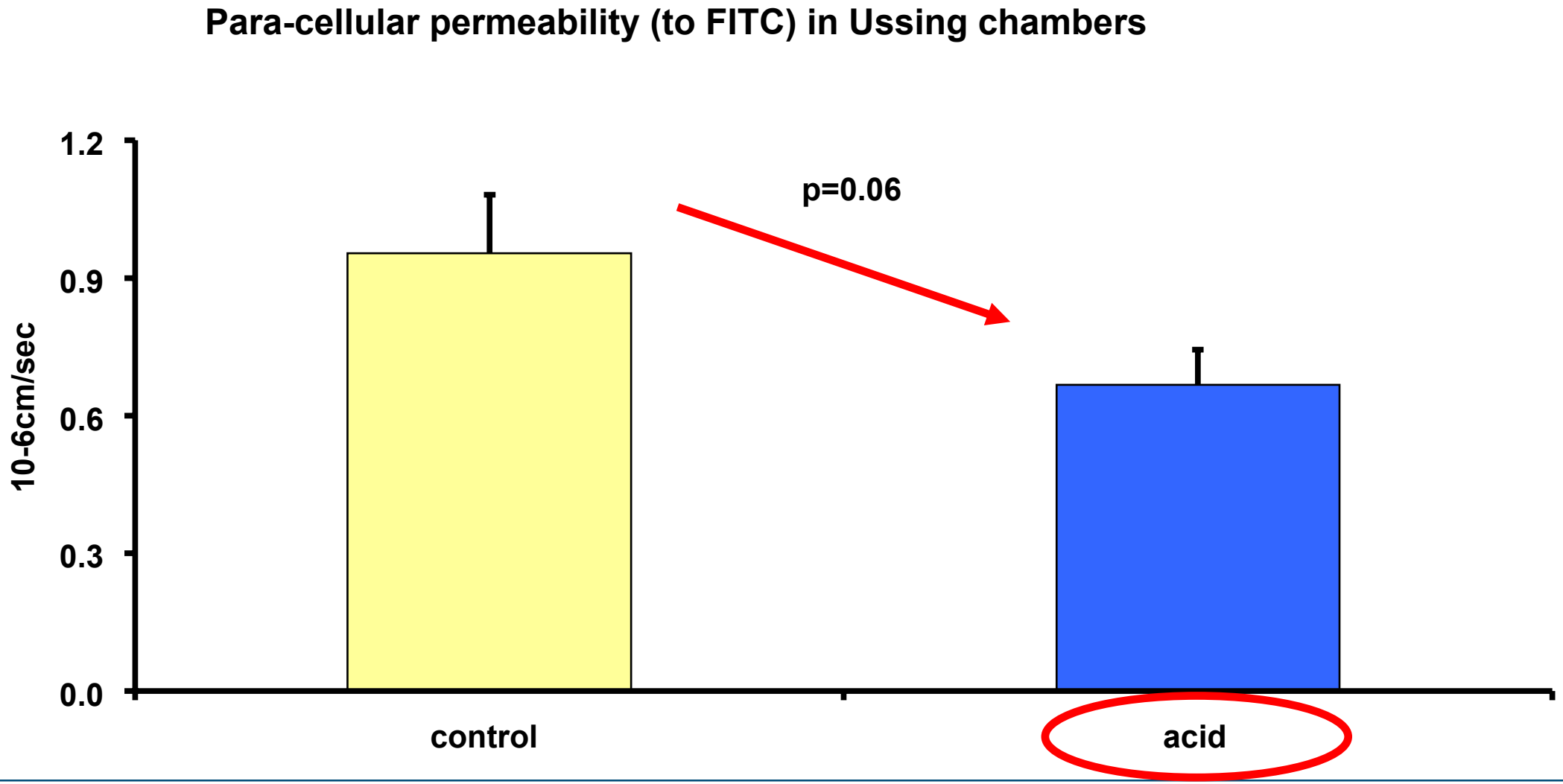
  blend 1: 0.8% of benzoic, phosphoric, citric and formic acids.

  blend 2: 1.2% of benzoic, phosphoric, citric, formic, acetic and lactic acids.

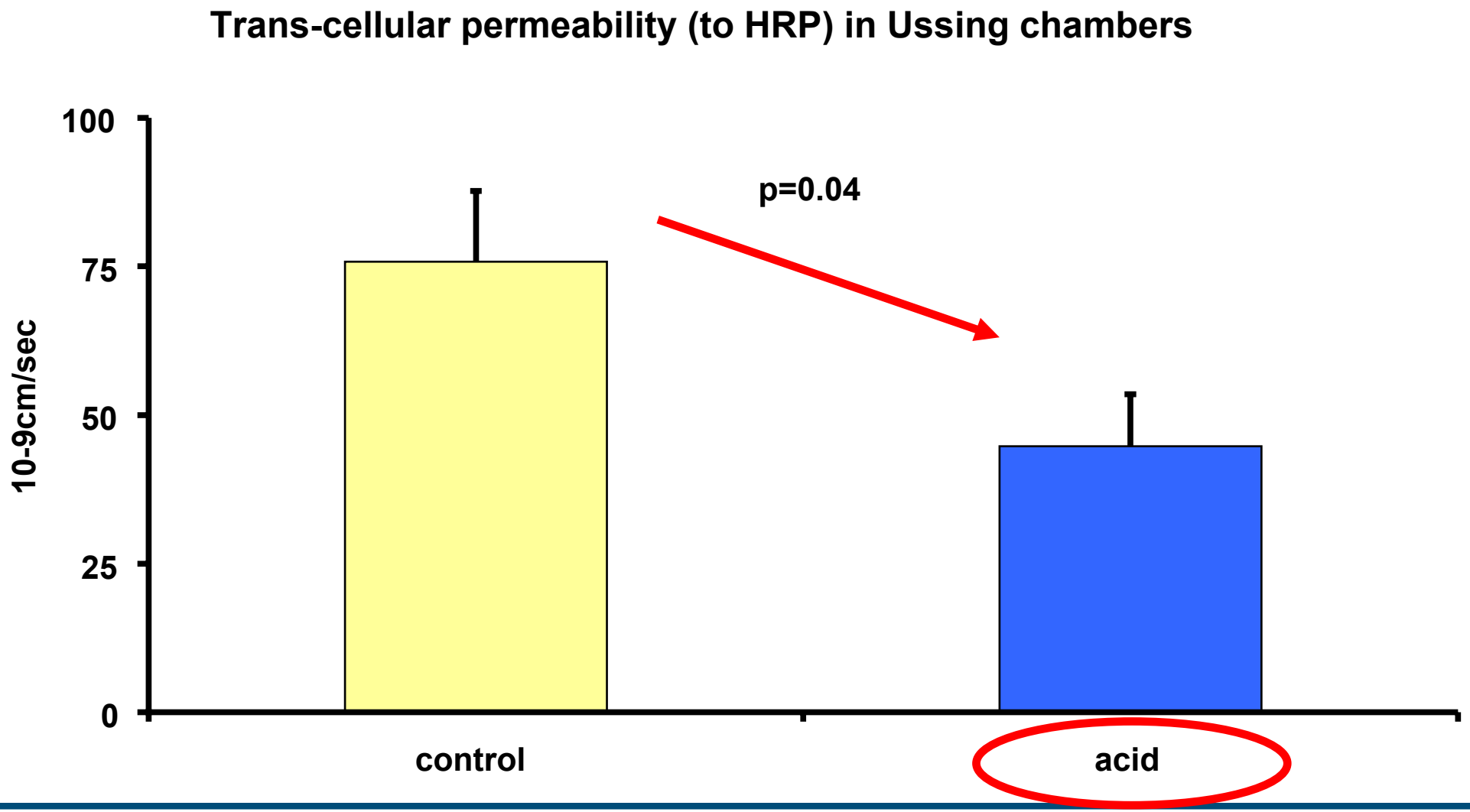
Organic acids & gut wall thickness of small intestine



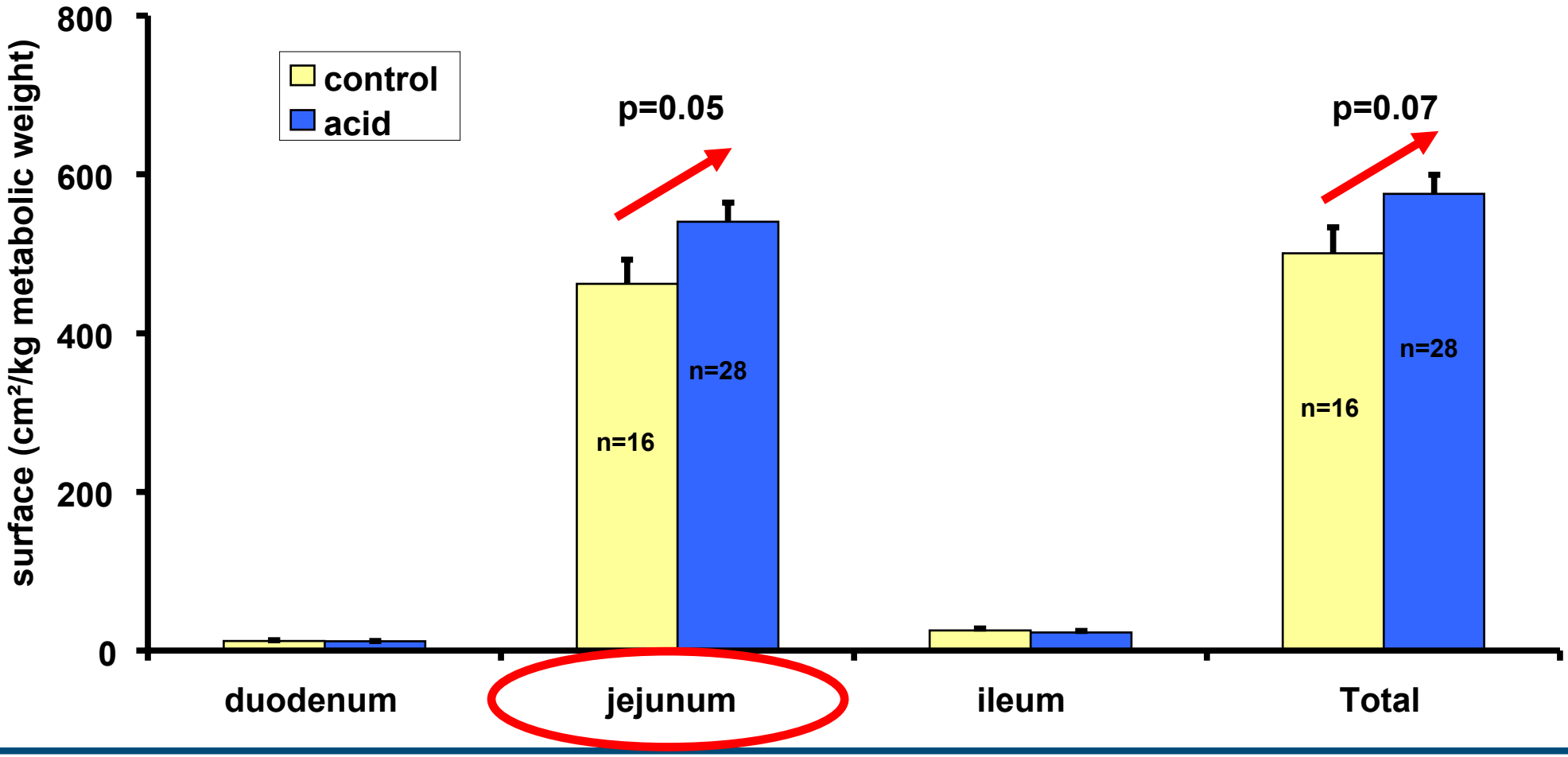
Organic acids and jejunal wall permeability



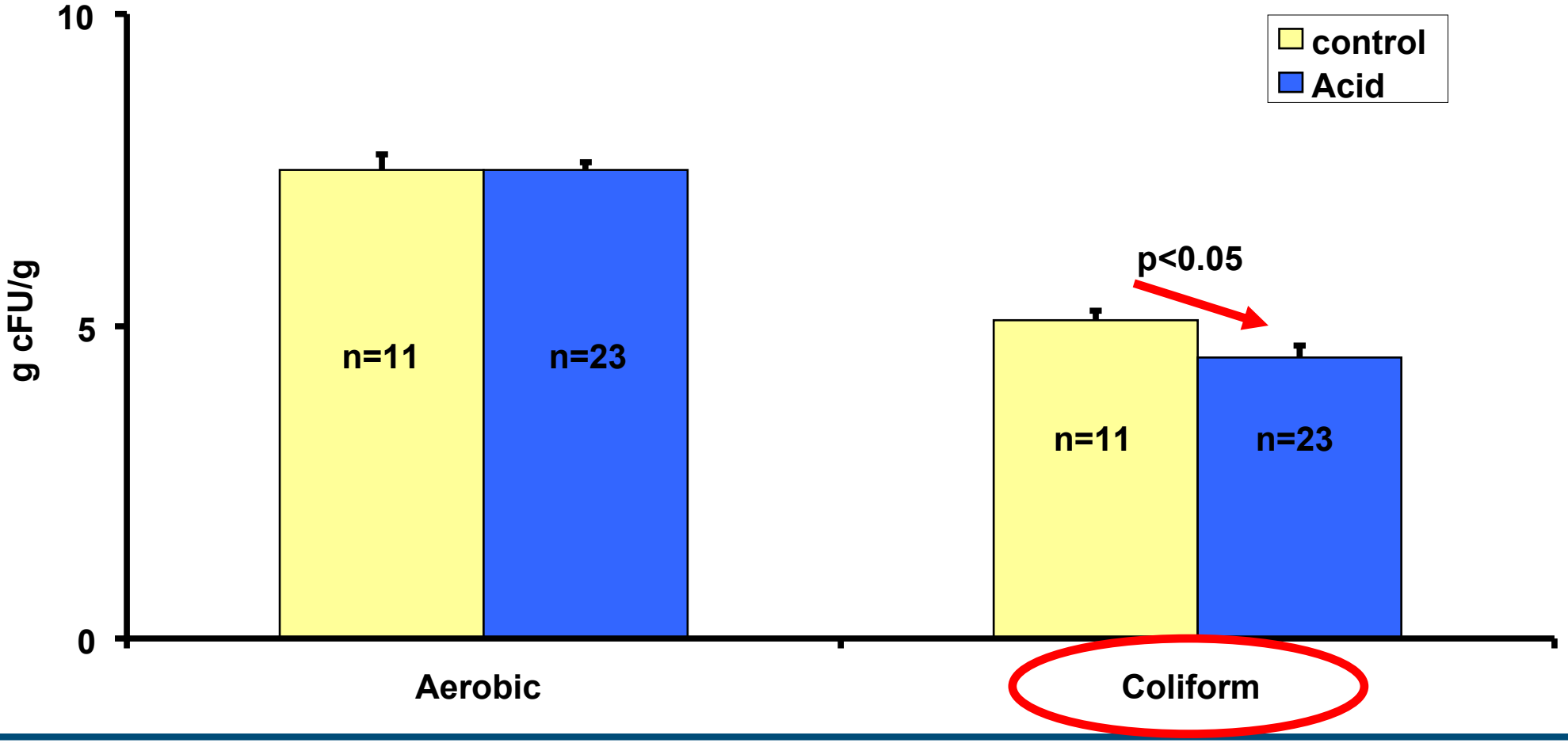
Organic acids & jejunal wall permeability



Organic acids & gut surface area



Organic acids & bacterial counts in faeces



Summary on organic acids

- Dietary organic acid supplementation showed:
  - No taste-aversion starting from day 1 post-weaning.
  - Increase in external surface area and decrease in wall thickness of the **jejunum**.
  - Decrease in jejunal permeability to macromolecules.
  - Decrease in faecal counts of coliform bacteria.

➔ This suggests that organic acidifiers 1) boost performance after weaning and 2) increase the barrier function of the gut wall possibly by inhibiting bacterial pressure (*E. coli*) on it.

SODIUM BUTYRATE

Na-butyrate, background

- **Short chain fatty acid (SCFA)**
- **Product of fermentation in the large intestine**
- Energy substrate for the colonocyte
- **Properties of butyrate:**
    - trophic effect on the colonic mucosa
    - maintenance of colonic barrier integrity
    - immuno-modulating properties
- **Effects on the small intestine known very little**
- butyrate → pre-gastric hydrolysis of milk fats in suckling mammal

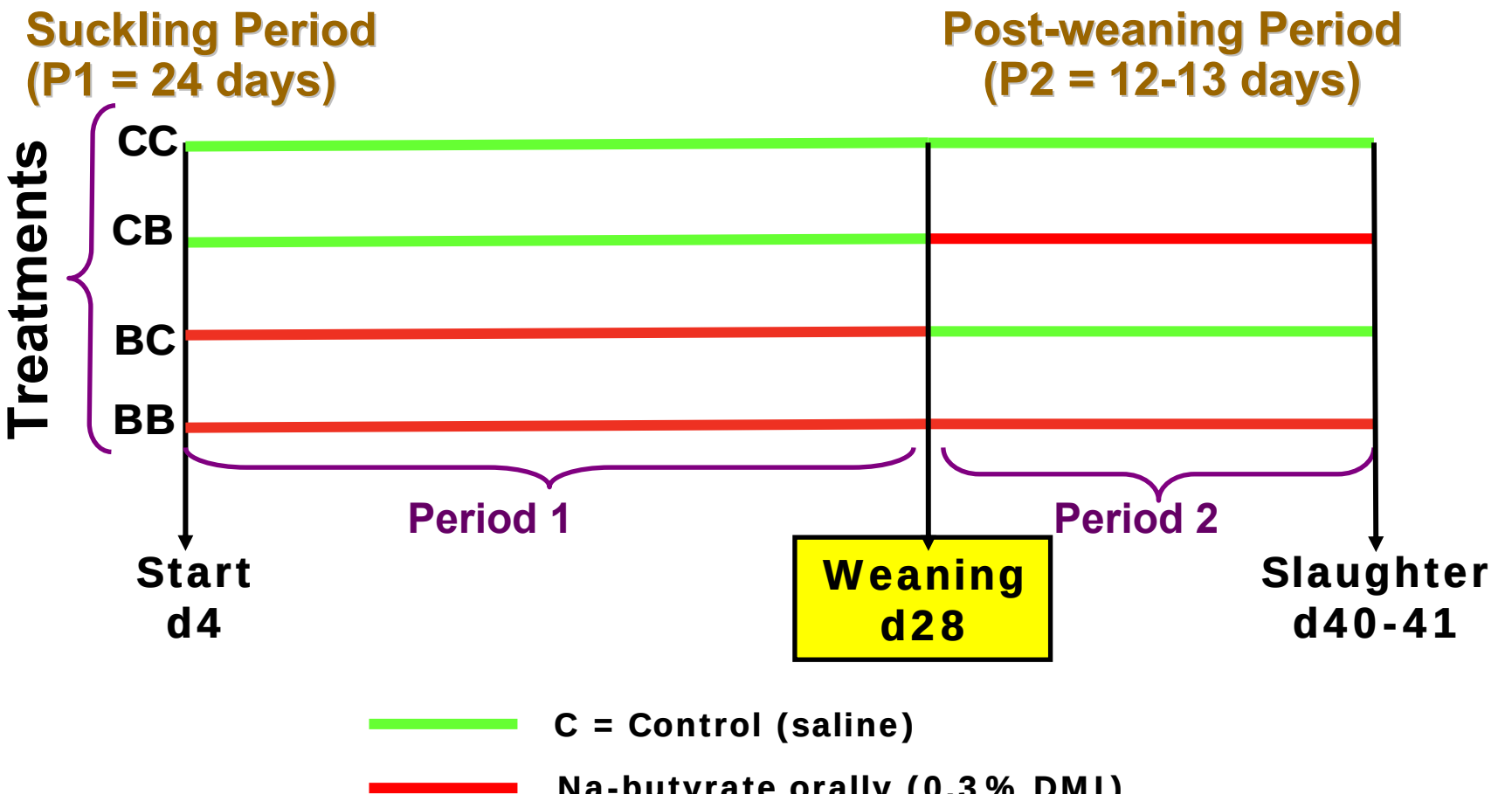
Some studies in farm animals (baby calves & pigs):

- improved growth performance and feed efficiency
- decreased frequency of diarrhea
- Increased enzyme activities (pancreas, intestine)

=> Na-butyrate: « Growth Promoter »

Na-butyrate experiment

Hypothesis: Na-butyrate provided early after birth is able to speed up the maturation of the GIT and help overcome the critical period of weaning

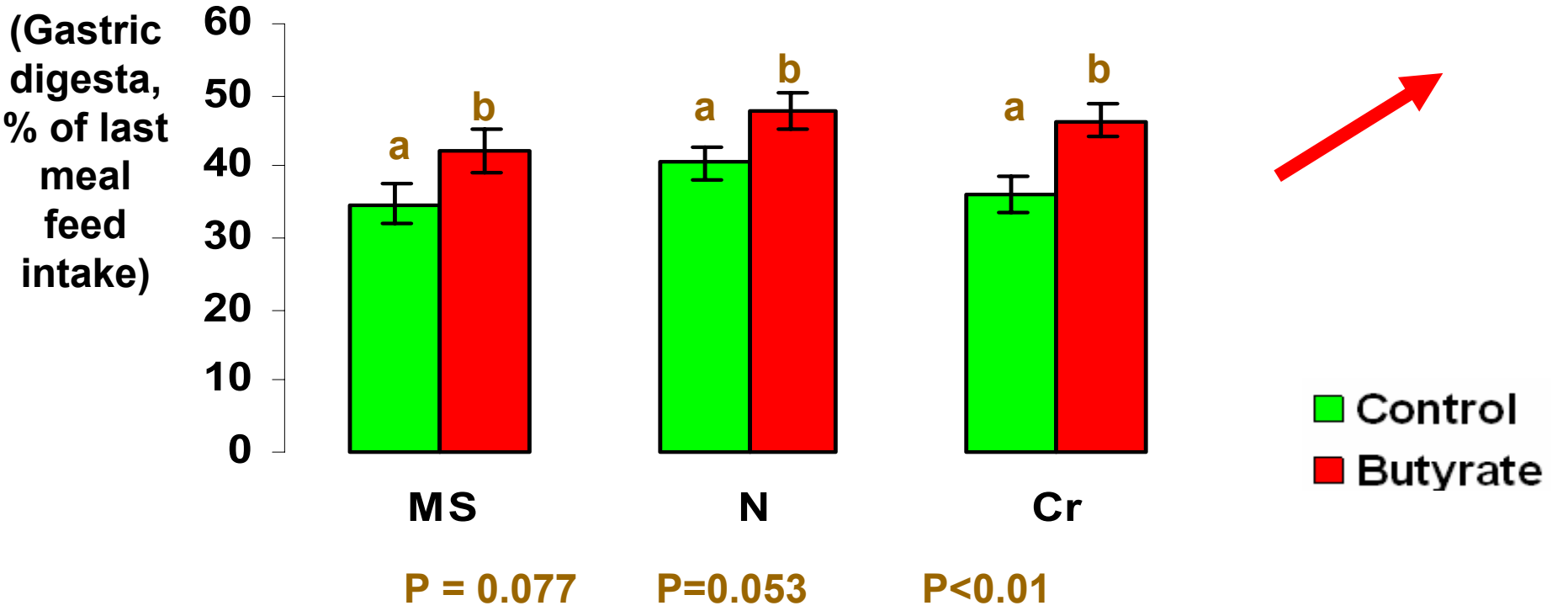


Growth, feed intake and digestibility are increased when butyrate is fed during suckling

	CC	CB	BC	BB	SEM	Statistics		
						Before	*After*	*Interaction*
Initial BW, kg	2.6	2.6	2.5	2.7	0.1	-	-	NS
ADG, g								
4 to 28 d	**270**	**271**	**290**	**306**	**17**	0.18	-	-
28 to 40 d, g	**133^{A}**	**174^{B}**	**195^{B}**	**191^{B}**	**11**	**0.01**	0.15	0.10
4 to 40 d, g	**225^{A}**	**240^{B}**	**260^{B}**	**270^{B}**	**11**	**0.02**	0.32	0.83
ADFI, g	**293^{A}**	**336^{B}**	**353^{B}**	**360^{B}**	**10**	**0.01**	**0.04**	0.13
Digestibility, %								
DM	84.2	82.2	86.0	83.8	0.9	0.08	**0.03**	0.88
OM	86.0	83.8	87.5	85.4	0.9	0.09	**0.02**	0.97
N	80.7	77.2	83.9	79.3	2.0	0.20	0.06	0.79

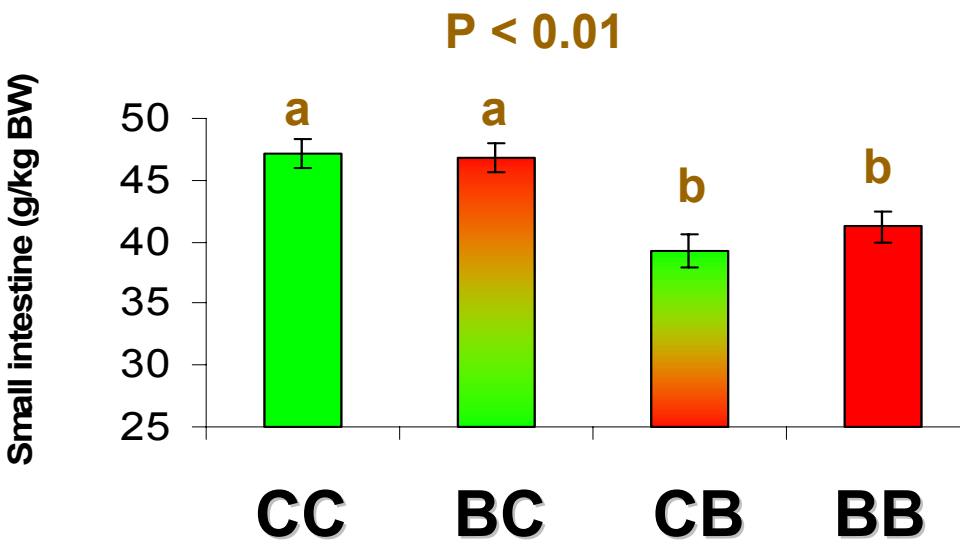
Le Gall et al, unpublished, 2006

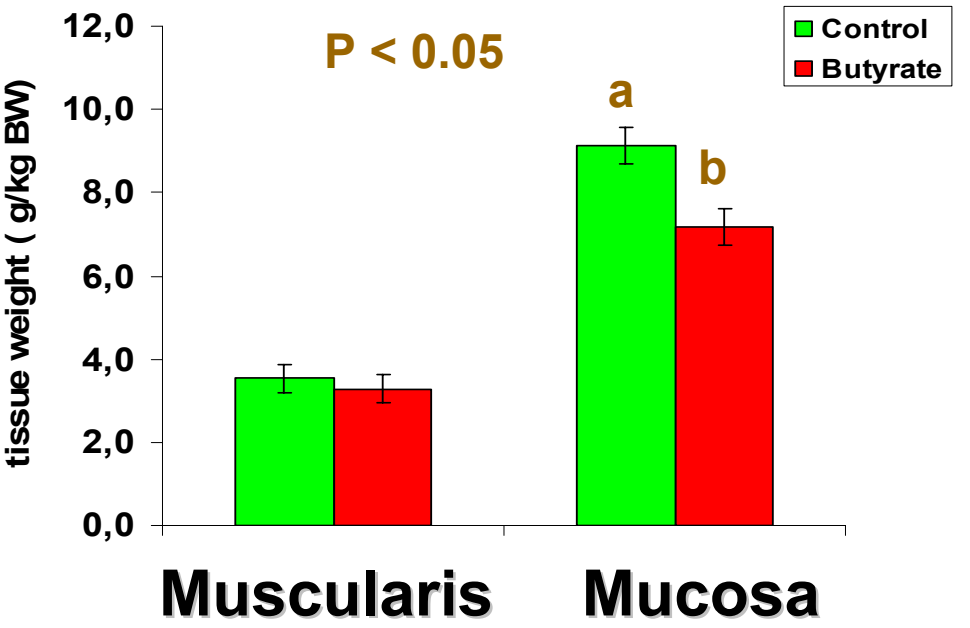
Gastric retention is higher when butyrate is fed during the suckling period



Le Gall et al, unpublished, 2006

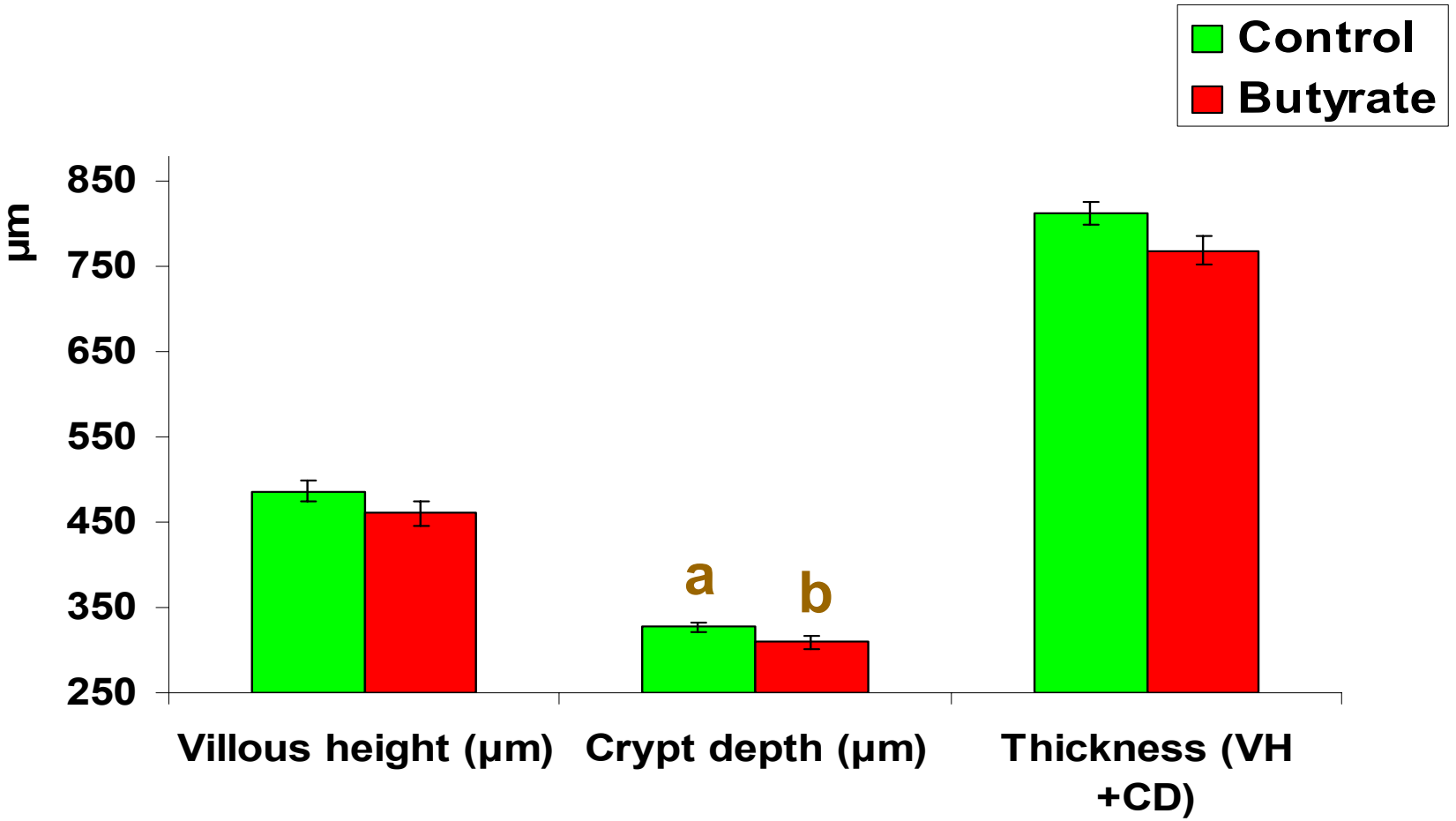
Small intestine and its mucosa are lighter when butyrate fed during suckling period





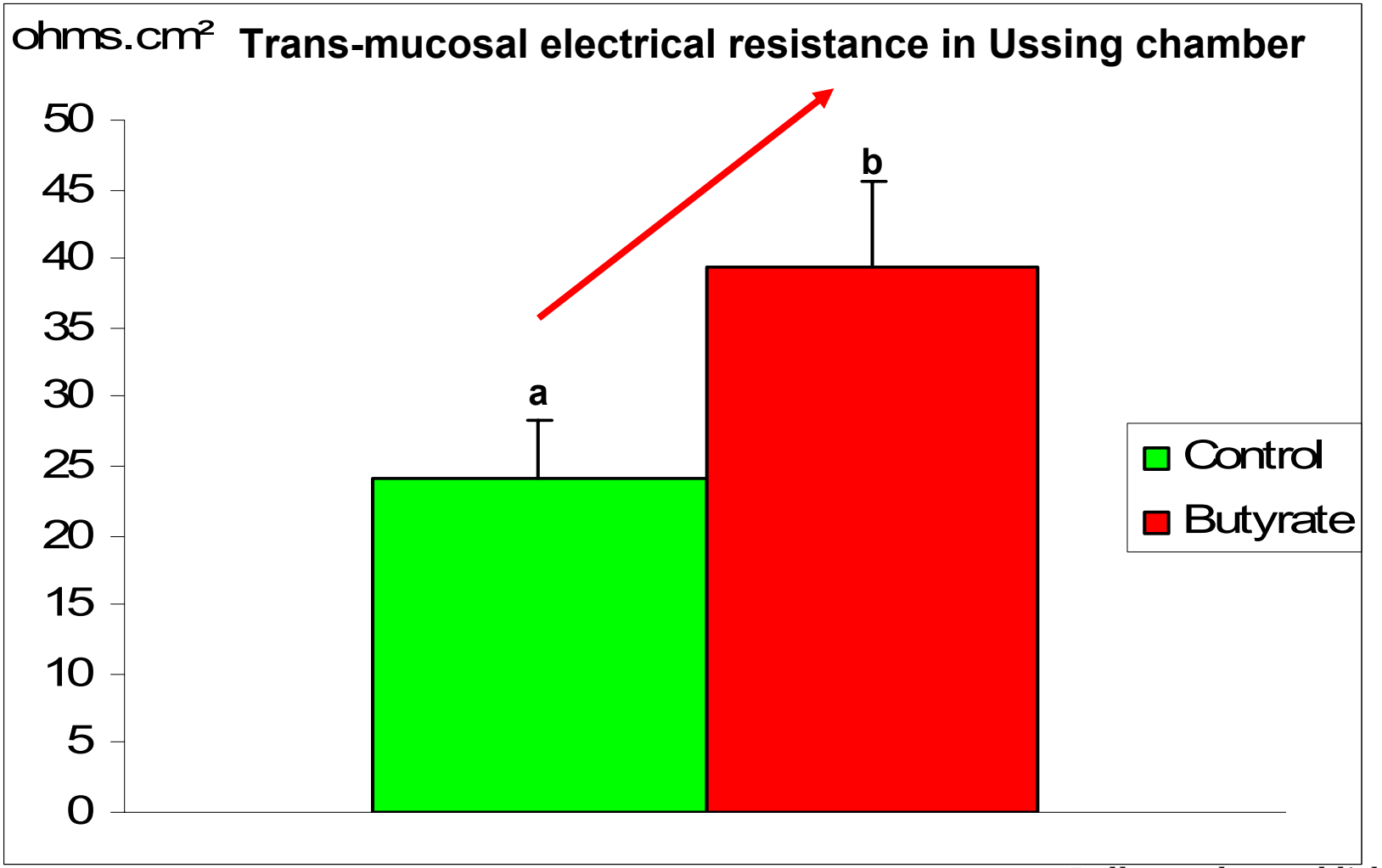
Le Gall et al, unpublished, 2006

Jejunal crypts are shorter when butyrate is fed during the suckling period



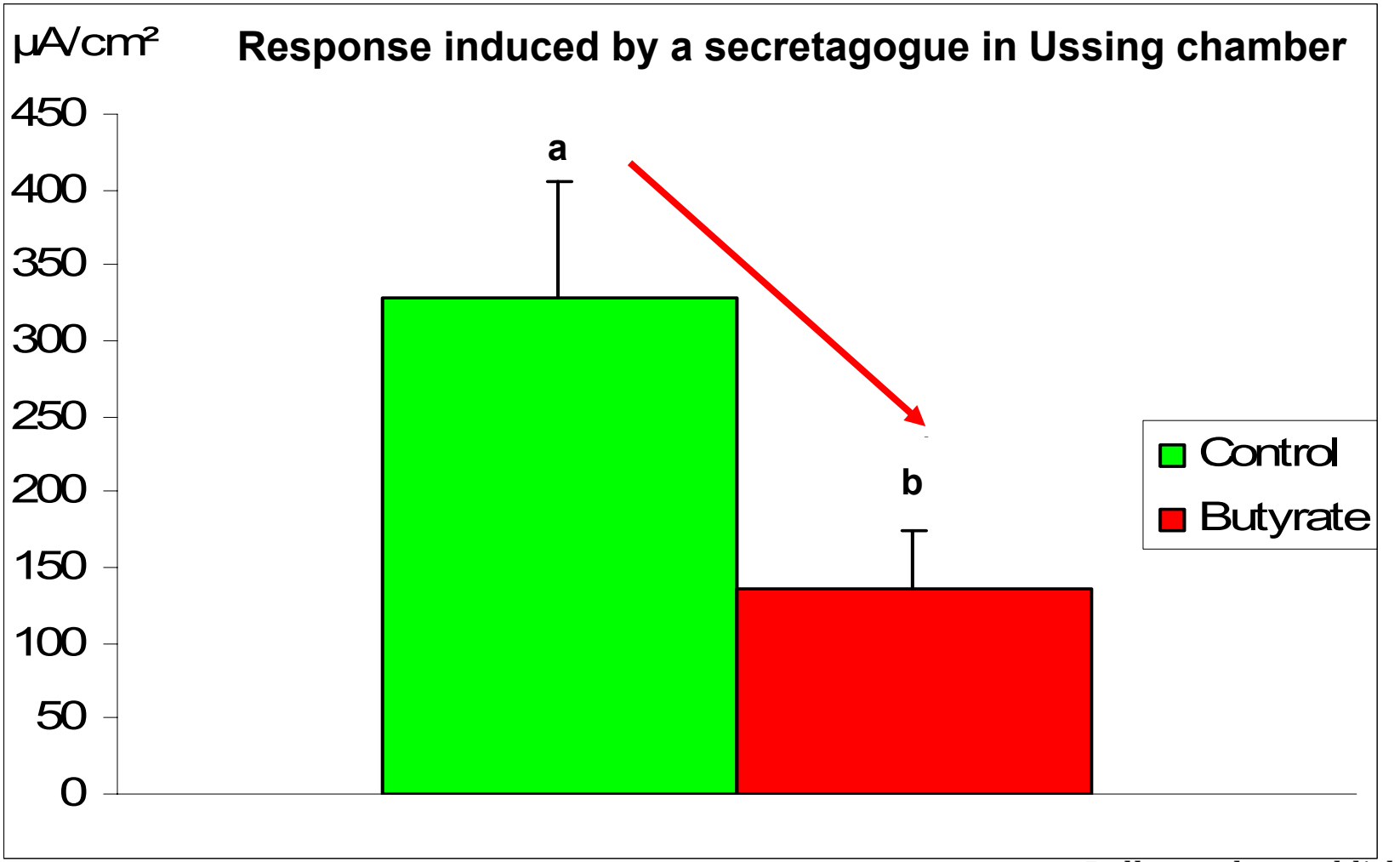
Le Gall et al, unpublished, 2006

Jejunal resistance after weaning is increased in pigs fed butyrate during the suckling the period



Pellet et al, unpublished, 2007

Jejunal secretory capacity after weaning is lower in pigs fed butyrate during suckling



Pellet et al, unpublished, 2007

Summary on Na-butyrate

* Positive effect of oral Na-butyrate on growth performance, and on feed intake after weaning

* Major post-weaning effect of Na-butyrate when delivered during the suckling period

* OM digestibility PW: increased when butyrate fed during suckling period BUT DECREASED when butyrate fed after weaning

* Major target = small intestinal mucosa which is lighter, more resistant and less secretory → possibly less metabolically active due to less bacterial stress on it → more AA available for growth

→ "growth promoter" effect of Na-butyrate

SPECIFIC AMINO ACIDS

Specific amino acids

AA:
- → **bricks** for protein synthesis (all AA)
- → **fuel** for specific cells (e.g. Gln and enterocyte)
- → **functional** roles (much less known)

Gln / Glu:
- → dispensable AA for cell cytoprotection systems (HSPs)
- → improves intestinal mucosa in piglets after weaning *(many publications)*
- → decreases apoptosis:mitosis ratio of intestinal and mucosal immune cells *(Domeneghini et al. 2004)*
- → stimulates both innate and acquired immunity *(Domeneghini et al. 2004)*
- → improves resistance to disease / challenge with ***E. coli*** K88 *(Yi et al 2005)*
- → decreases blood cortisol concentration post-weaning *(Zhou et al 2006)*

Trp:
- → stimulates CCK secretion and pancreatic enzyme production
- → precursor of serotonin → stimulates GIT motility
- → reduces intestinal villous atrophy *(Koopmans et al 2006)*
- → decreases plasma cortisol *(Koopmans et al 2006)* **(→ Gln and Arg spared)**

Thr:
- → requirements increase in disease *(Lobley, Lapierre 2003; Bannink 2006)*
- → major component of intestinal mucin *(Allen 1981)*

Small intestine and glutamine

(ileum, n=4 per treatment)	Control	GLN	Nucl.	GLN + Nucl.	sem
Villus height (µm)	148^{A}	200^{B}	189^{C}	215^{D}	17
Crypt depth (µm)	80^{A}	152^{B}	139^{C}	180^{D}	16
Enterocytes (p. 100 cells)					
* apoptosis	2.79^{A}	2.67^{B}	2.64^{B}	2.43^{C}	0.03
* mitosis	38.6^{a}	40.2^{b}	39.8^{b}	41.3^{b}	0.56
Lymphocytes (p. 100 cells)					
* apoptosis	5.2^{A}	3.3^{B}	3.8^{B}	3.4^{B}	0.14
* mitosis	10.7	11.1	11.3	11.1	0.19
Macrophages (p. 100 cells)	4.1^{A}	4.6^{B}	4.5^{B}	4.7^{B}	0.07
IEL (p. 100 cells)	3.4^{A}	6.4^{B}	5.3^{C}	6.7^{B}	0.1

A, B, C, D: P < 0.01 a, b, c, d: P < 0.05

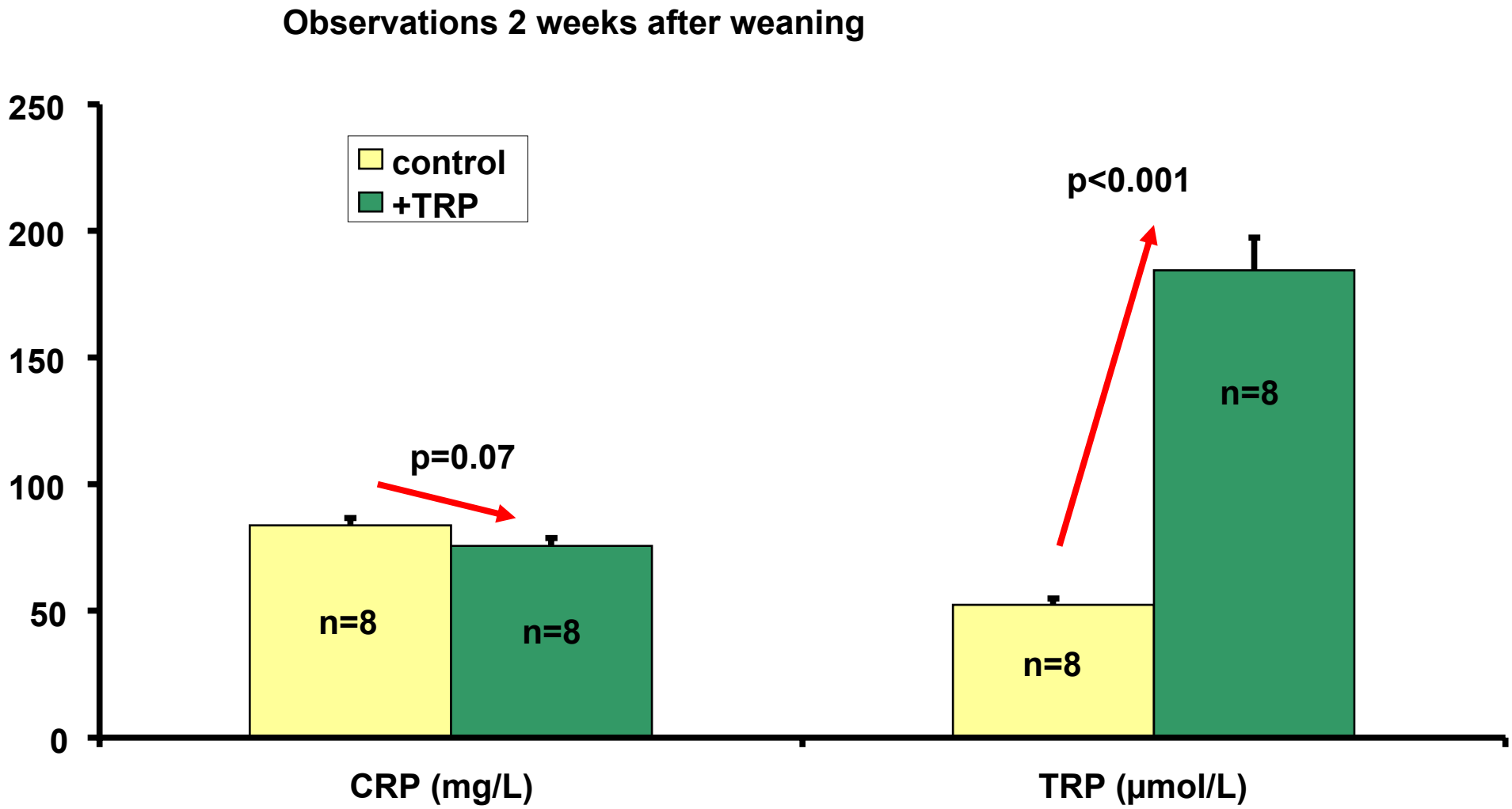
Domeneghini et al. (2004)

Dietary tryptophan supplements:

- Tryptophan (2.1 and 7.1 g/kg)
- Tryptophan (1.8, 2.1 and 7.1 g/kg).

Koopmans et al. unpublished, 2006-2007

Tryptophan, plasma C-reactive protein and plasma TRP



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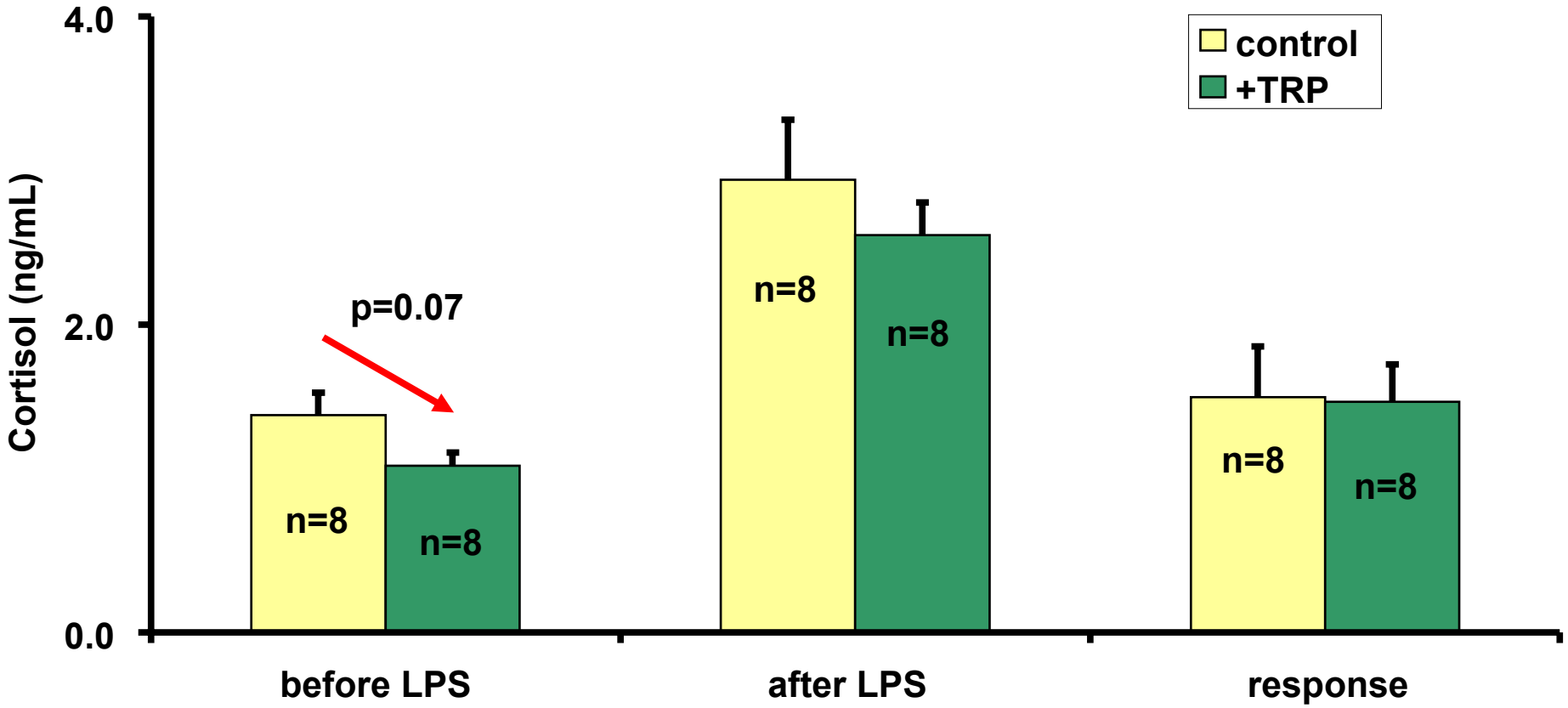
Koopmans et al. unpublished, 2006-2007

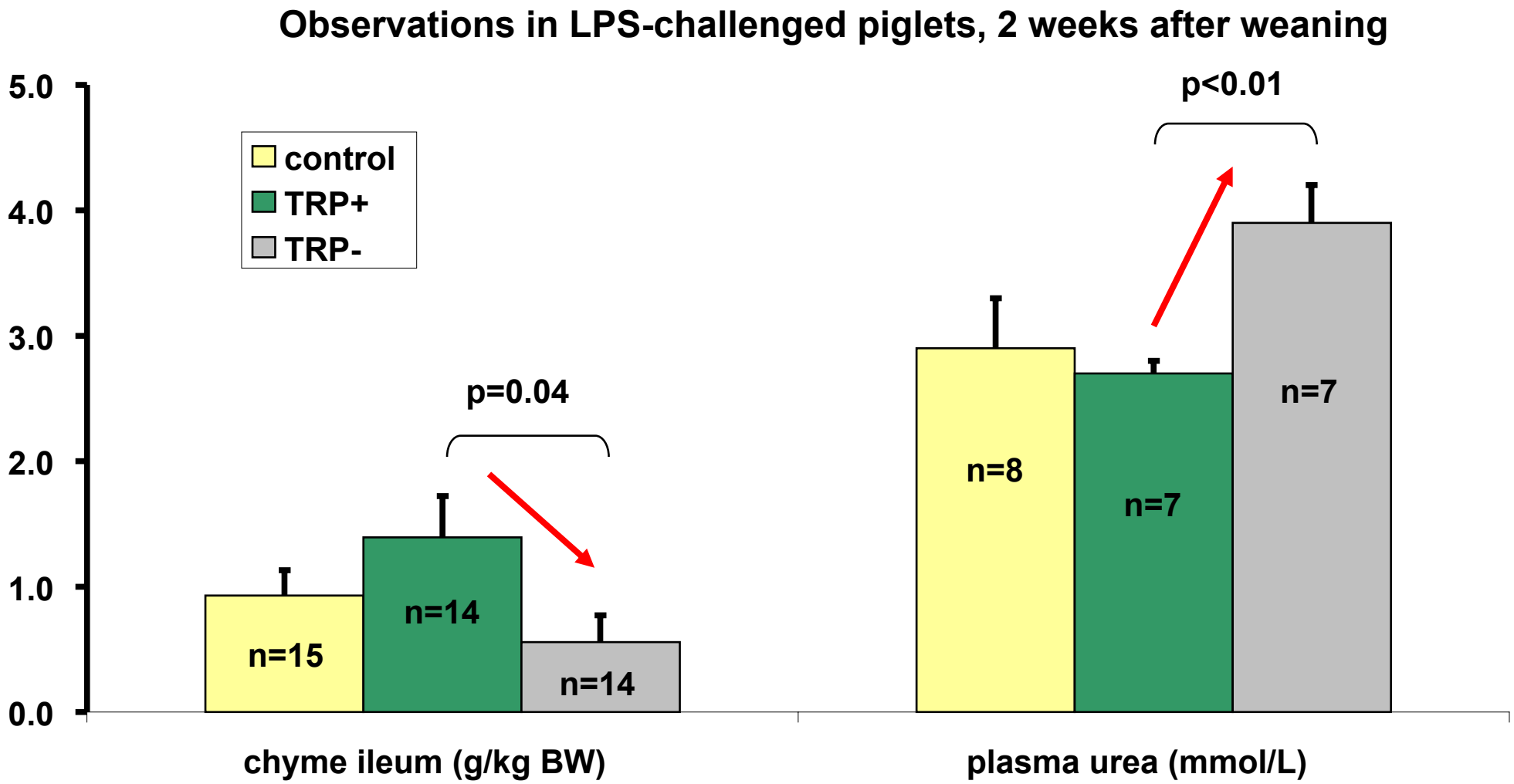
Tryptophan & salivary cortisol in LPS-challenged piglets

Observations before, and 1.5 hour after LPS administration in piglets



Koopmans et al. unpublished, 2006-2007

Dietary TRP, ileal digesta and plasma urea in LPS-challenged piglets



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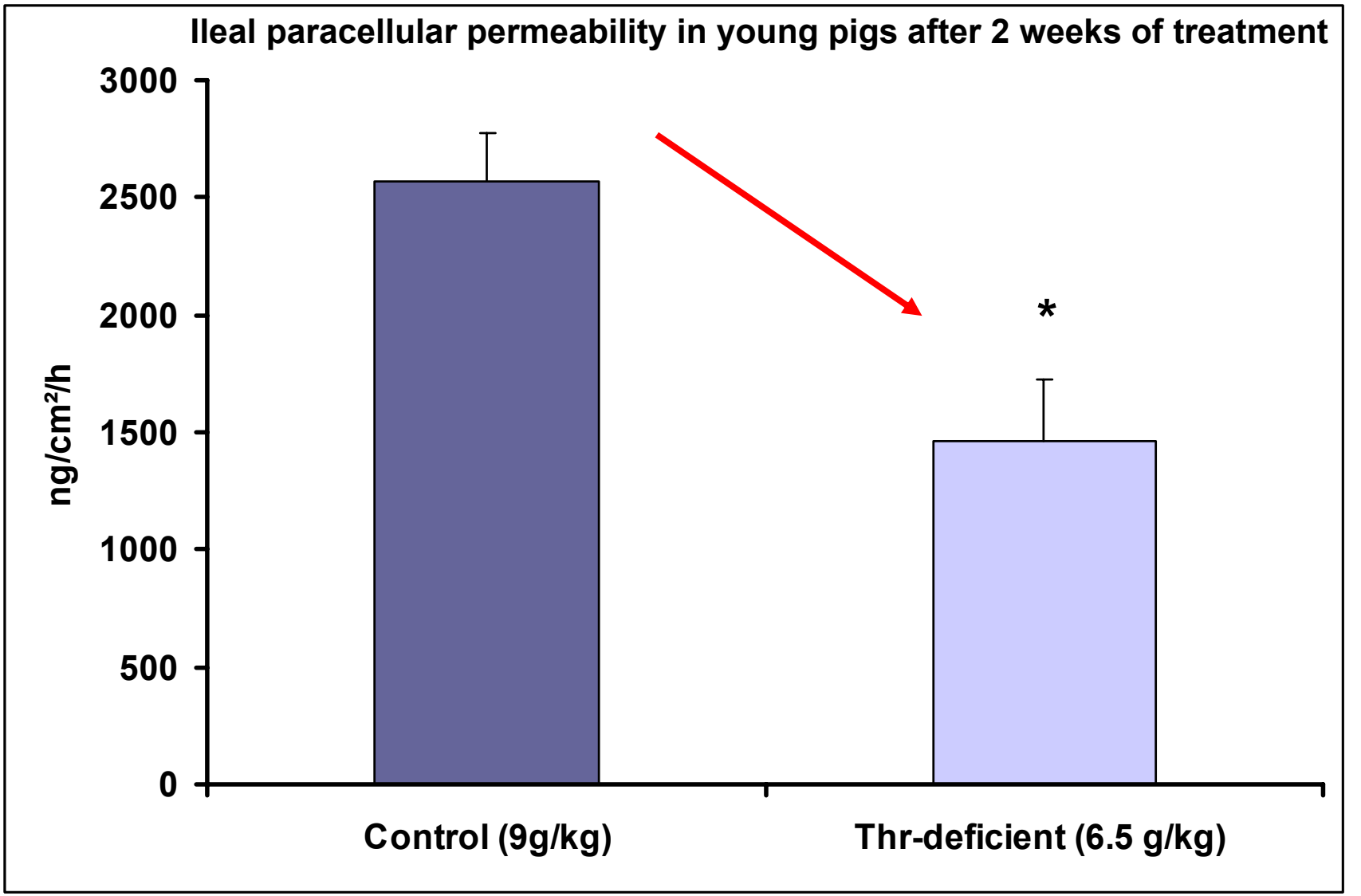
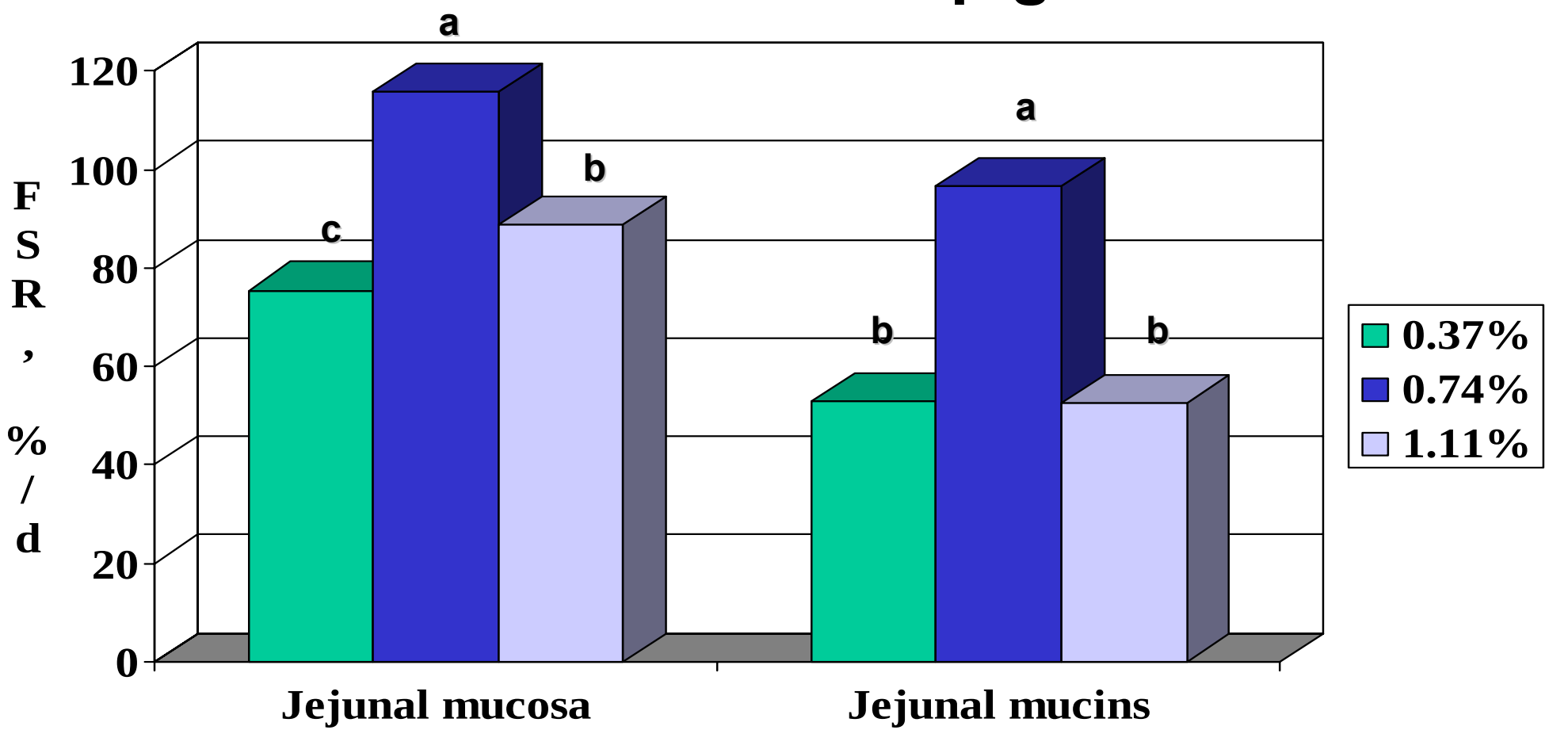
Koopmans et al. unpublished, 2006-2007

Dietary threonine and intestinal barrier



(Hamard et al 2007)

Deficient or excess values of dietary true ileal digestible threonine negatively affect fractional synthesis rates of protein in jejunal mucosa and mucin of weaned pigs



(Wang et al., 2007)

Summary on amino acids

- Glutamine:
  - stimulates intestinal proliferation and limits intestinal apoptosis
  - acts on intestinal epithelial cells AND immune cells
  - stimulates both innate and acquired immunity

- Tryptophan:
  - Decrease ($P<0.1$) in plasma acute phase protein (CRP) and salivary cortisol.
  - Increase in ileal chyme and decrease in plasma urea conc. under LPS-challenge.
  - This suggests that tryptophan decreases inflammation and stress and thereby may affect gut digesta passage time and protein catabolism.

- Threonine:
  - Important contribution to intestinal barrier integrity (mucin)
  - Dietary concentration critical for intestinal homeostasis

ANIMAL PROTEIN SOURCES

Spray dried plasma

- **SDP: porcine, bovine, animal (= mixture of unknown composition)**
- **Transient ban on SDP → porcine SDP now allowed in pigs in the EU**
- **Importance of HYGIENE in collecting and processing blood for ensuring good quality of SDP** *(DeRouchey et al 2004)*
- **Transmissible diseases (e.g. pseudorabies, PRRS virus) inactivated by spray drying** *(Polo et al 2005)*

Effects of spray dried plasma

- **SDP improves growth and VFI** *(many references)* **but positive effects are largely independent of feed intake = specific biological properties** *(Jiang et al 2000)*
- **Ig G fraction mostly responsible for this improvement** *(Pierce et al 2005)*
- **SDP with higher IgG conc. = more protective** *(Bosi et al 2001, 2004; Conde 2005)*
- **lower diarrhoea and protective effects of SDP after disease (e.g. challenge with *E. coli* K88)** *(Owusu-Asiedu et al 2003; Conde 2005)*
- **larger effects of SDP in early-weaned pigs** *(Torrallardona et al 2002)* **and in poor environment** *(Coffey & Cromwell 1995)*

NB: little advantage of hyper-immune porcine SDP over conventional SDP in protecting pigs challenged with the same pathogen as used for immunisation (Conde 2005; Niewold et al 2006)

META-ANALYSIS

Use of SDAP in newly weaned piglets

43 publications (75 trials and 12,000 piglets)

Response relative to Control(s) without SDAP

- Mean values of treatments considered
- ADG, ADI, FGR (0-7 and 0-14 days PW)

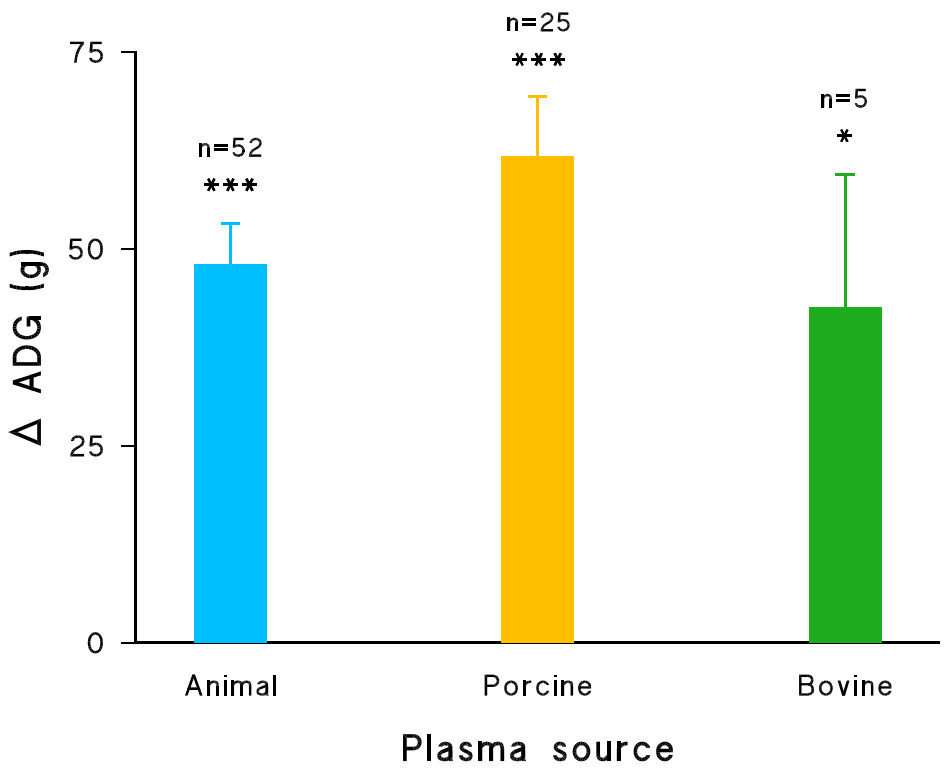
Independent analysis of different factors

- Source of plasma
- Protein source being replaced
- Dose of inclusion
- Age and weight of piglets at weaning
- Infectious challenge and medication of feed

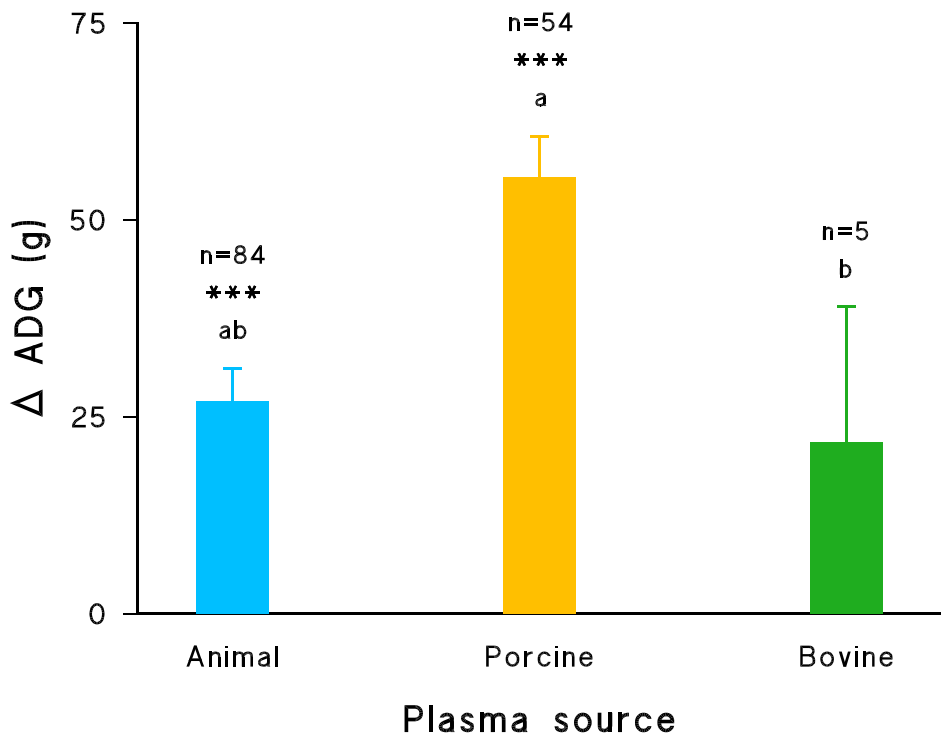
Meta-analysis by Torrallardona (unpublished)

ADG improvement in response to DIFFERENT SOURCES OF SDAP

0-7 days post-weaning



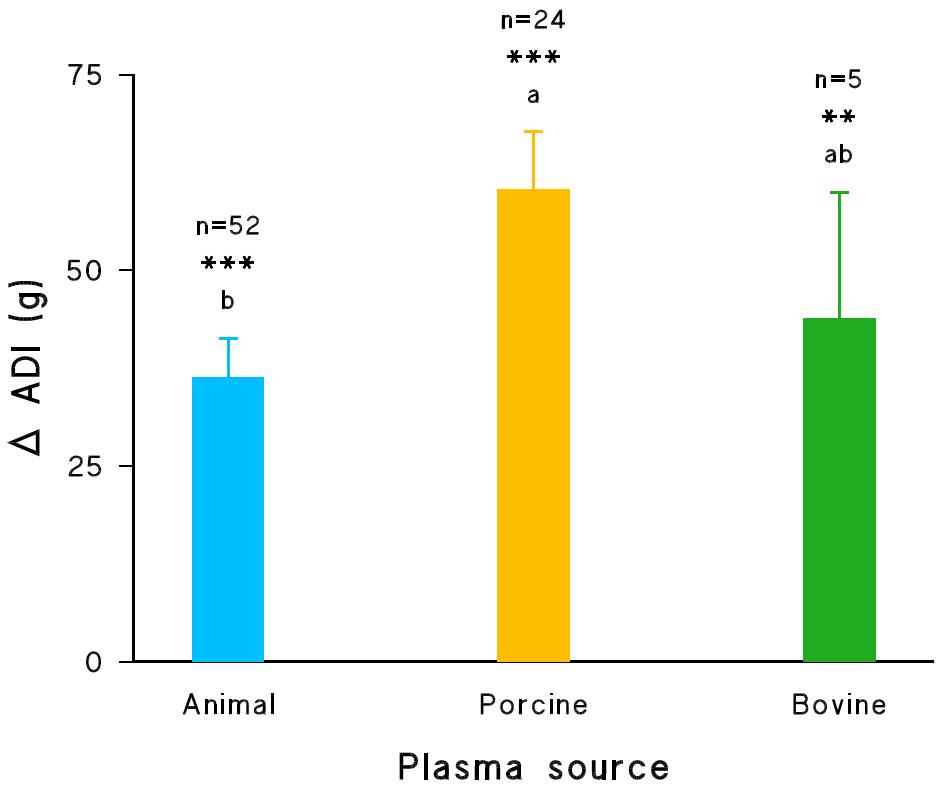
0-14 days post-weaning



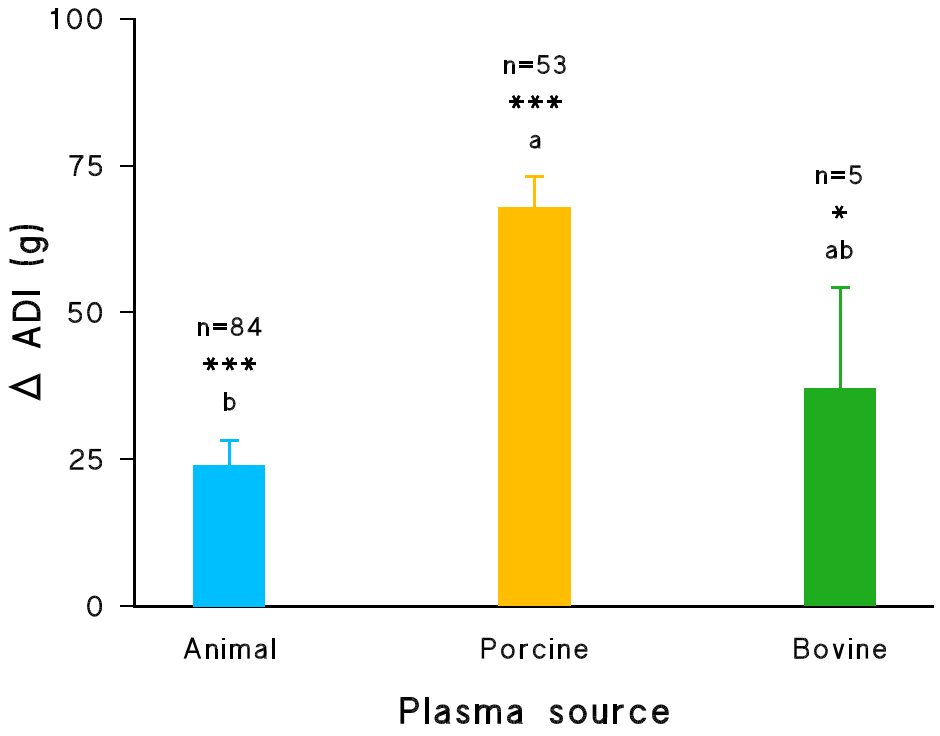
Meta-analysis by Torrallardona (unpublished)

ADFI improvement in response to DIFFERENT SOURCES OF SDAP

0-7 days post-weaning



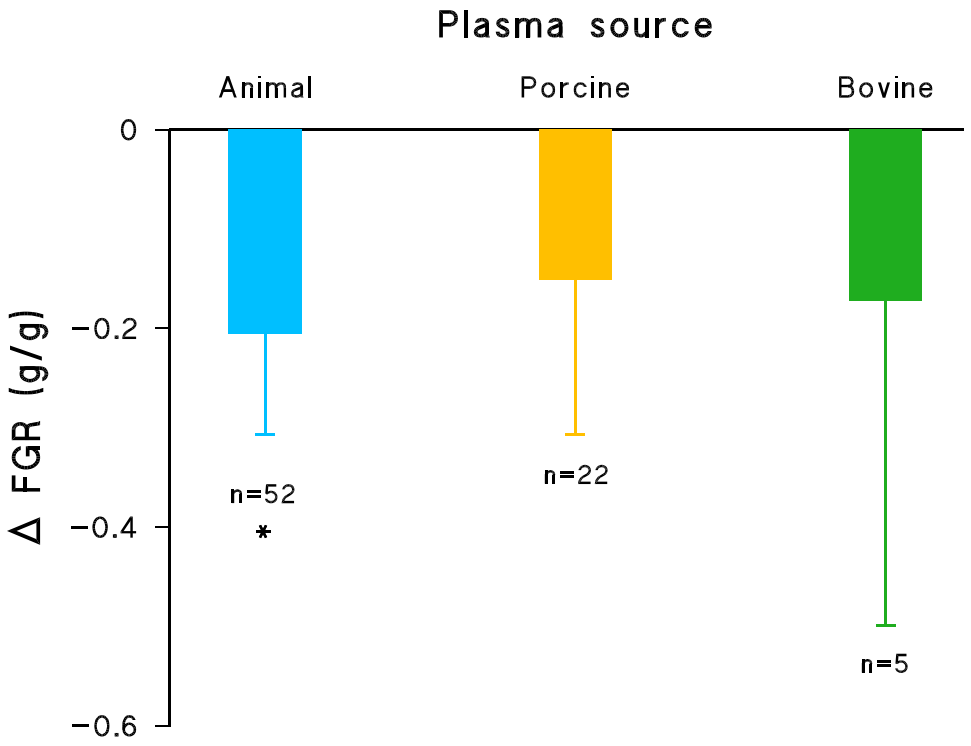
0-14 days post-weaning



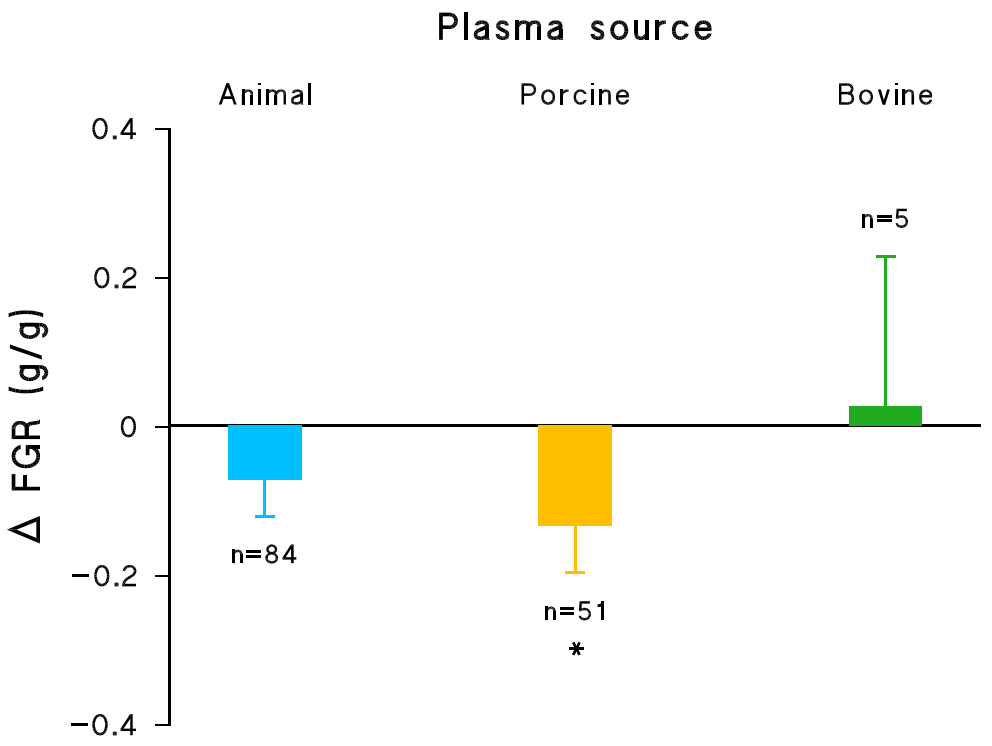
Meta-analysis by Torrallardona (unpublished)

FGR improvement in response to DIFFERENT SOURCES OF SDAP

0-7 days post-weaning



0-14 days post-weaning



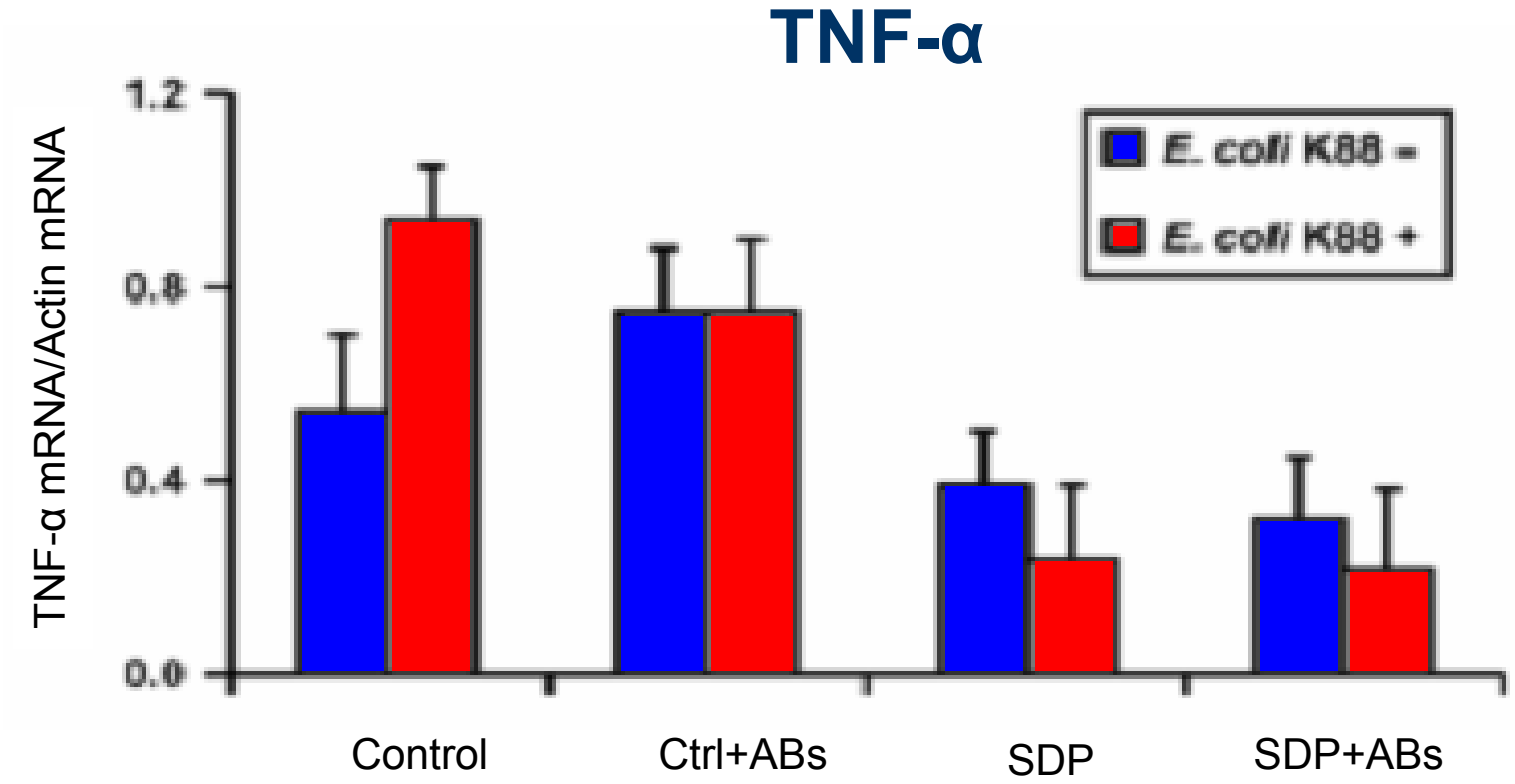
Meta-analysis by Torrallardona (unpublished)

Effects of spray dried plasma (cont.)

- **no or little interaction between SDP and growth-promoter antibiotics** *(Torrallardona et al 2002, 2003; Bikker et al 2004; Bosi et al 2004)* → **different mechanisms of action**
- **SDP reduces intestinal mass and cellularity of the lamina propria** *(Jiang et al 2000)* → **improvement in protein utilisation**
- **SDP increases villous height and intestinal counts of lactobacilli BUT NOT ALWAYS** *(Conde 2005; Torrallardona et al 2003, 2007)*
- **anti-inflammatory effects of SDP on intestine after *E. coli* challenge** *(Touchette et al 2002; Bosi et al 2004)*

Proinflammatory cytokines in jejunum of challenged piglets

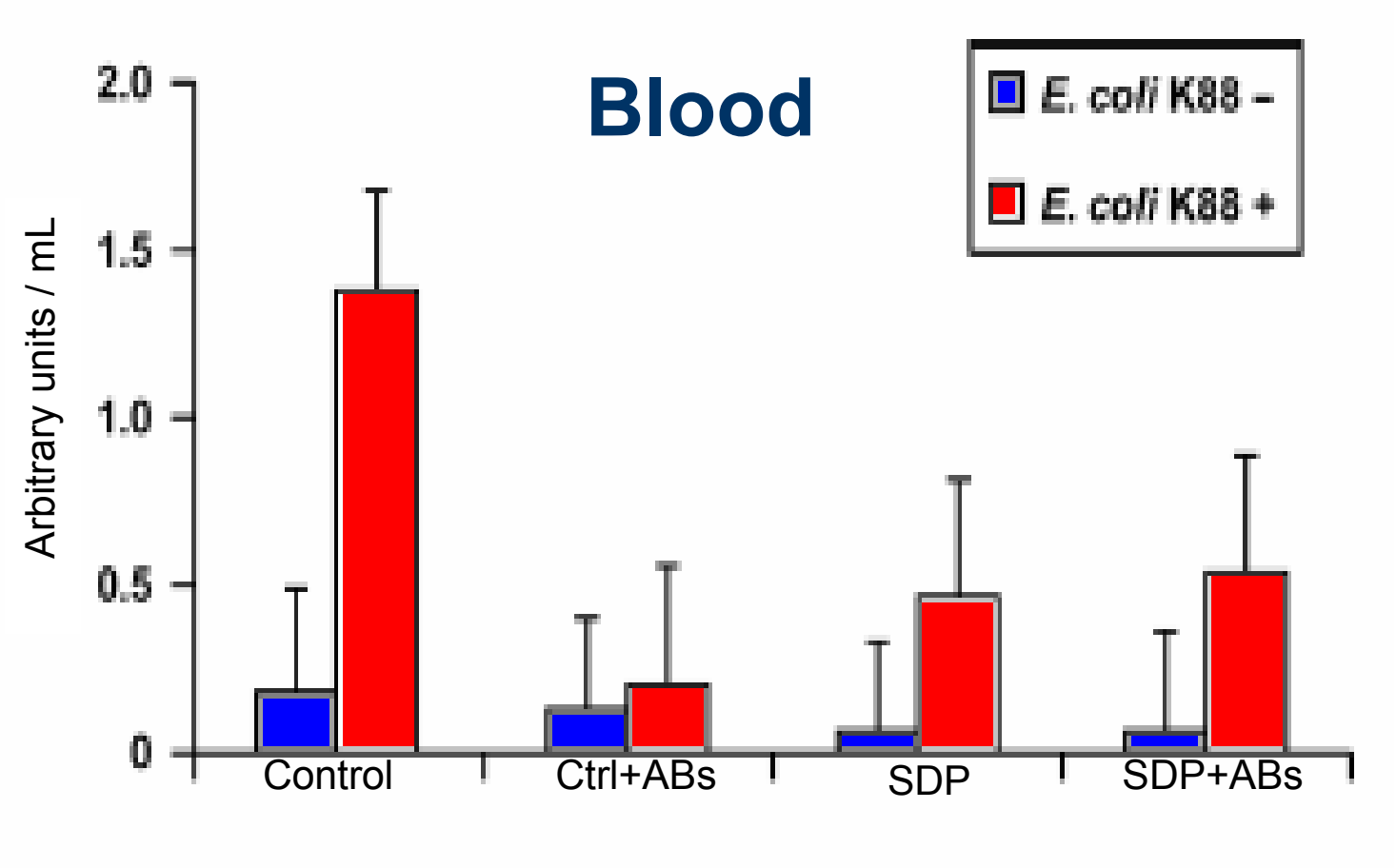
Bosi et al. (2004)



Statistics: SDP (P<0.01)

K88-specific IgA production in E.coli K88 challenged piglets

Bosi et al. (2004)



Statistics: SDP×AB×K88-susceptibility (P<0.05)

Summary on spray dried plasma

SDP (porcine) = protein source of high interest for pre-starter diets for piglets.

Besides its clear positive effects on growth, feed intake and feed efficiency, evidence support that SDP prevents pathogen binding in GIT and reduces PW diarrhoea mainly through action of IgG fraction.

Other protein sources

- **Skimmed milk protein and protein hydrolysis**

  → **SMP reduces intestinal villous atrophy PW as compared to feather meal, but protein hydrolysis has no effect** *(Vente-Spreeuwenberg et al 2004a,b)*

- **Bovine colostrums:**

  → **improves growth performance & feed intake** *(Pluske et al 1999; Luron et al 2004)*

  → **in pair-fed pigs, colostrums improve GIT 'health' by decreasing gastric pH and increasing lactobacilli to coliforms ratio in duodenum, with little effects on other variables of intestinal biology** *(Huguet el al 2006)*

- **Plant proteins:**

  → **plant proteins: reduce improvement of SDP (+ dairy protein)** *(van Dijk et al 2001)* **but no detrimental effect when added to specific protective diets (cooked rice)** *(Montagne et al 2004)*

  → **pea protein isolate: more diarrhoea, higher mortality, increased ETEC colonisation and proliferation in small intestine and shortened villi** *(Owusu-Asiedu et al 2003)*

ESSENTIAL OILS / PLANT EXTRACTS

Essential oils: occurrence and composition

Origanum vulgare

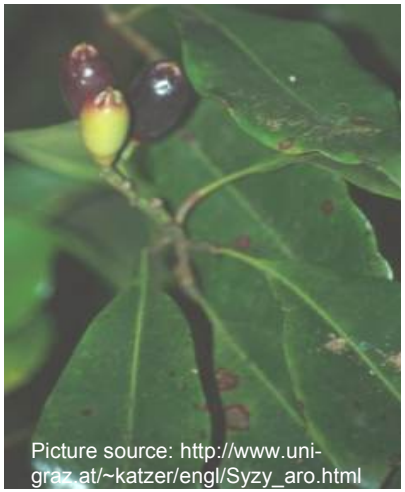
Oregano oil:
Carvacrol – 55 - 80%
Thymol – 2.5 - 14%

Bozin et al 2006; Penalver et al 2005)

Thymus vulgaris

Thymol oil:
Carvacrol – app. 6%
Thymol – 24 - 48%

Bozin et al 2006; Penalver et al 2005)



Syzygium aromaticum

Clove oil:
Eugenol – 85%

Dusan et al 2006

Cinnamomum cassia

Cinnamon oil:
Cinnamaldehyde – 85%

Ooi et al 2006

Cinnamomum zeylanicum

Cinnamon oil:
Eugenol – 77%

Dusan et al 2006

(Janczyk, unpublished)

Factors of variation of composition of essential oils

Composition of essential oils is EXTREMELY variable
(1.5 to 4.5% of the plant) *(Bozin et al 2006; Hazzit et al 2006; Yang et al 2007)*

Composition of essential oils varies according to:

* **plant species and individual**
* **plant state of development**
* **geographic location**
* **climatic condition**
* **distillation method**

(Janczyk, unpublished)

Antibacterial activity of essential oils and their pure components

Escherichia coli (several strains, including K88, O157:H7, ETEC)

C. zeylanicum, *Eugenia caryophyllus*, *O. vulgare*, *S. aromaticum*, *Thymus mastichina*, *T. vulgaris*, *T. zygis*, carvacrol, cinnamon oil, clove oil, eugenol, thymol

Pseudomonas aeruginosa

C. zeylanicum, *E. caryophyllus*, *O. vulgare*, *S. aromaticum*, *T. vulgaris*, carvacrol, eugenol, thymol

Salmonella typhimurium

O. vulgare, *S. aromaticum*, *T. mastichina*, *T. vulgaris*, *T. zygis*, carvacrol, cinnamaldehyde, cinnamon oil, clove oil, eugenol, thymol

Lactobacillus acidophilus, L. plantarum

O. vulgare, *S. aromaticum*, *T. vulgaris*, carvacrol, cinnamon oil, thymol

(Janczyk, unpublished)

Ranges of antimicrobial dosages (µg mL^{-1}) of selected essential oils *in vitro*

	Carvacrol	Cinnamon oil	Cinnamal-dehyde	Eugenol	Thymol	Oregano oil	Thyme oil	Clove oil
MBC[a]	100-283	100-133		300-466	100-233			
						156-625	156-2,500	
	3.12 - 50					3.12 - 50		
						1 - 200		
MIC[b]	3.12 - 50					3.12 - 50		
	55.5 - 274.5	500		410			500	500
						2,500 – 40,000	5,000 – 40,000	
						1,200-20,000	300-20,000	1,200 – 25,000
		800-3,200						1,600-6,400
		75-600	75-600					
MEC[c]	103-286		83-176	207-496	117-323			

[a]**MBC**: minimal **bactericidal** concentration

[b]**MIC**: minimal **inhibitory** concentration

[c]**MEC**: minimal **effect** concentration

(Janczyk, unpublished)

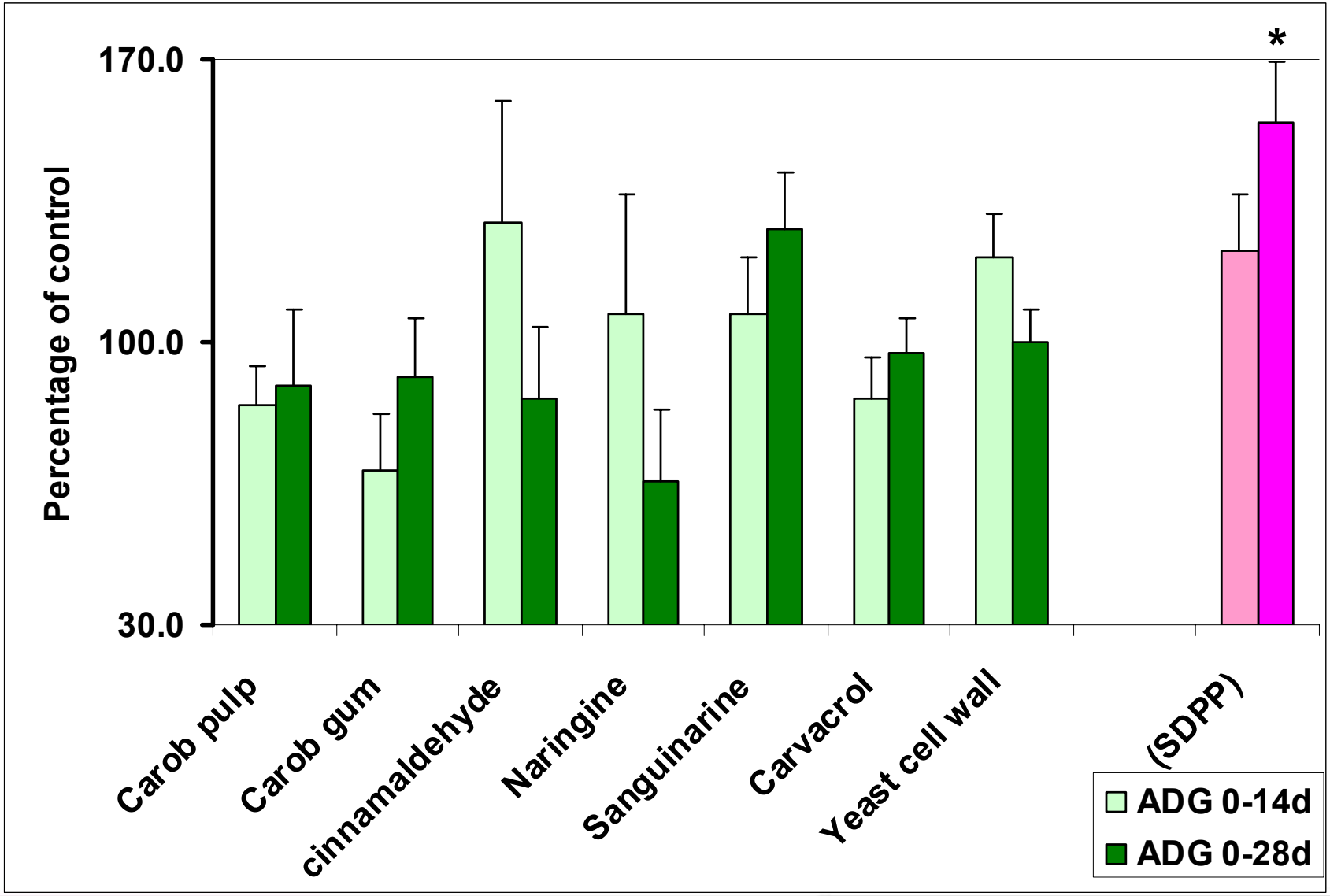
Essential oils and feeding studies in weaning piglets

Supplement, its amount	ADFI (kg)	ADG (kg)	FCR	Weaning age (d)	weaning BW (kg)	Duration (d)	Reference
Control	0.258^{x}	0.187^{x}	1.38	23	7.9	14	Kommera *et al*., 2006
Antibiotic*	0.345^{y}	0.255^{y}	1.35	23	7.9	14	
PEP1000-1^{a}, 1g kg^{-1}	0.279^{x}	0.199^{x}	1.40	23	7.9	14	
Control	0.263^{x}	0.169^{x}	1.56	21	5.4	14	Oetting *et al*., 2006
Antibiotic**	0.316^{y}	0.218^{y}	1.45	21	5.4	14	
Herbal extract^{b}, 0.7g kg^{-1}	0.261^{x}	0.151^{x}	1.73	21	5.4	14	
Herbal extract^{b}, 1.4g kg^{-1}	0.239^{x}	0.151^{x}	1.87	21	5.4	14	
Herbal extract^{b}, 2.1g kg^{-1}	0.282^{x}	0.185^{x}	1.52	21	5.4	14	
Control	0.453^{x}	0.237^{x}	1.91^{x}	28	8.0	21	Molnar and Bilkei, 2005
Oregpig^{c}, 1g kg^{-1}	0.453^{x}	0.242^{x}	1.87^{y}	28	8.1	21	
Oregpig^{c}, 2g kg^{-1}	0.482^{y}	0.258^{y}	1.87^{y}	28	8.2	21	
Oregpig^{c}, 3g kg^{-1}	0.475^{y}	0.258^{y}	1.84^{z}	28	8.1	21	

(Janczyk, unpublished)

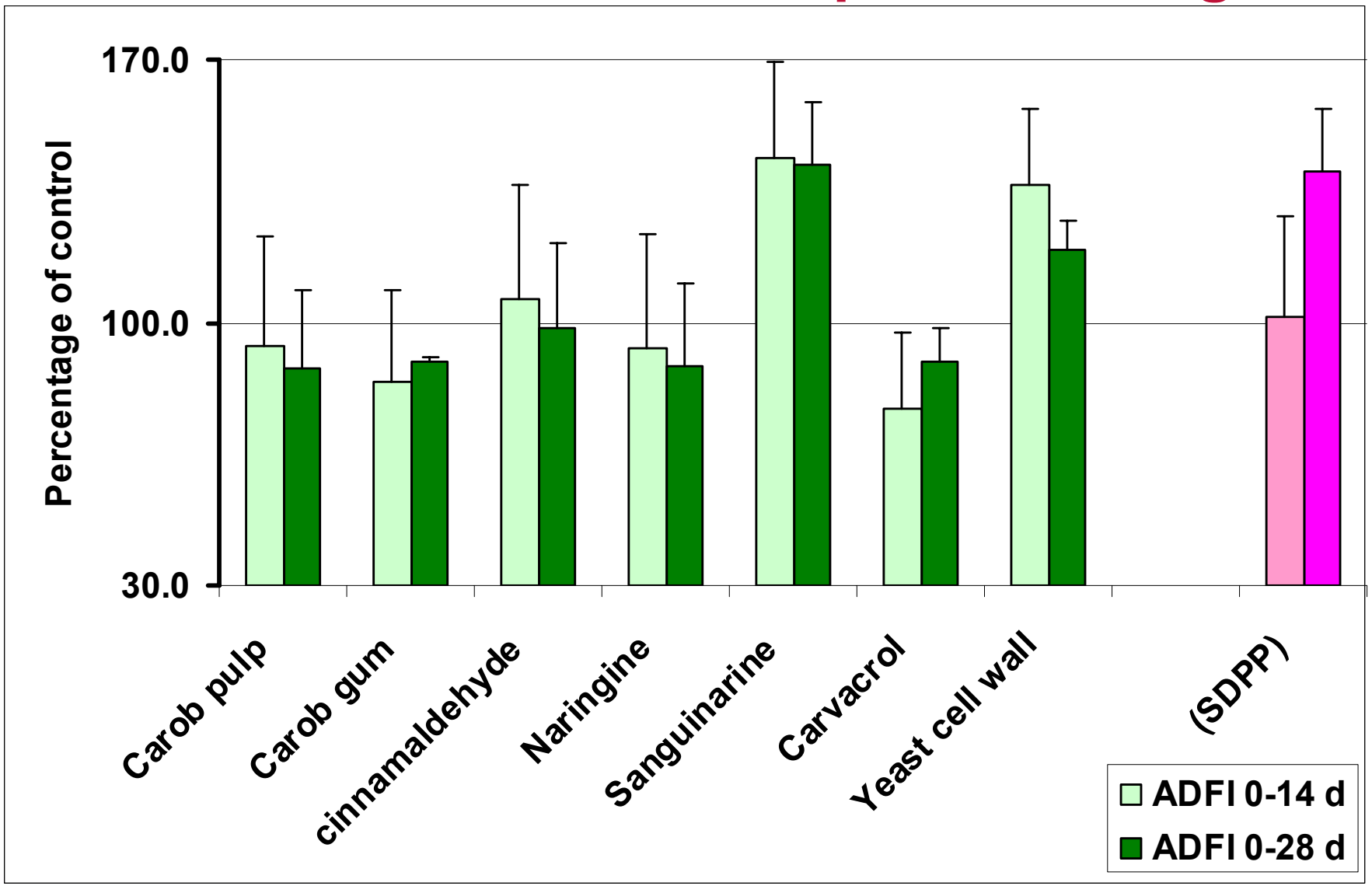
^{a}PEP-1000-1 – essential oil mix containing anis oil, citrus oil, oregano oil, and natural flavours
Biotronic – organic and inorganic acids mix containing phosphoric and lactic acids
^{b}Herbal extract in this study contained 20% of essential oils: 3.3% clove oil, 3.3% eugenol, 3.3% oregano oil, 3.3% carvacrol, 6.7% thyme oil
^{c}Oregpig – 500g oregano oil, 33.4g thymol, dried oregano flowers and leaves ad 1000g. Concentration of carvacrol – 60g kg-1, thymol – 55 g kg-1

Plant extracts and ADG post-weaning



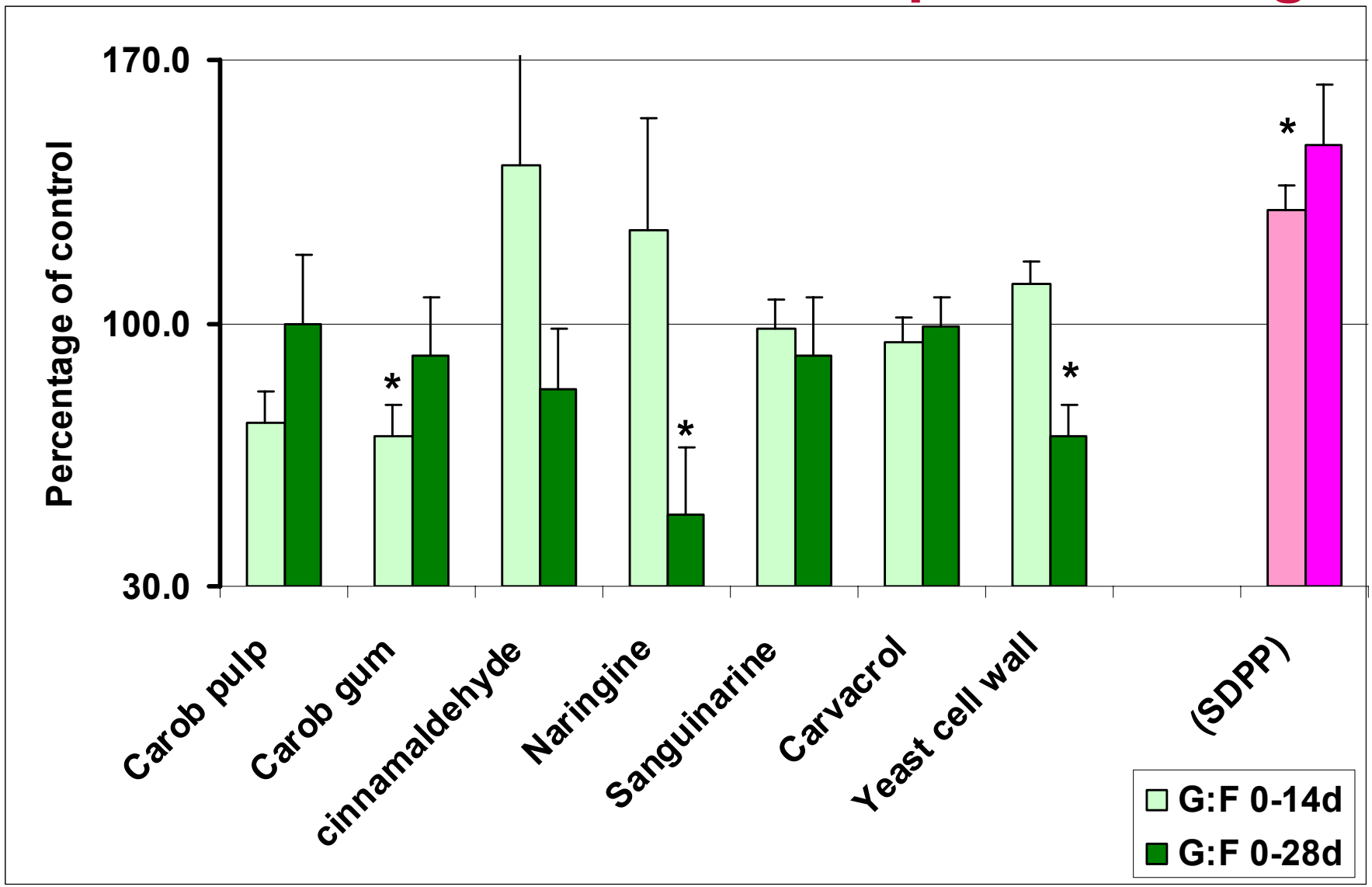
Andrés-Elias et al., unpublished

Plant extracts and ADFI post-weaning



Andrés-Elias et al., unpublished

Plant extracts and G:F ratio post-weaning



Andrés-Elias et al., unpublished

Conclusions on essential oils and plant extracts

* Wide range of bacteria sensitive to the essential oils *in vitro*

* Large variations in the antibacterial activity *in vitro* depending on the essential oil source, active component, tested bacteria

* Dosages of the essential oils used *in vivo* several times higher than in vitro

* Variable and inconsistent results of *in vivo* studies on essential oils

* Variable and inconsistent results of *in vivo* studies on plant products / extracts

General conclusions

- A lot of experimental work done over the recent years for finding alternative substances to in-feed antibiotics and optimising diet formulation
- (some) organic acids and spray dried plasma are among the best alternatives.
- other substances (e.g. butyrate, bovine colostrums) are promising
- new insights on FUNCTIONAL amino acids (e.g. Trp, Thr) are being generated
- in many cases, the modes of actions (when known) are quite different among products
- HOWEVER, data on plant extracts (e.g. essential oils) or components are somewhat disappointing: lack of effect, positive effects BUT high variability, negative effects…

Perspectives

New investigations are warranted on the following topics:

- mechanisms of action for promising compounds (e.g. butyrate, functional amino acids, etc.)
- bioavailability and pharmaco-kinetics of essential oils / plant extracts
- zootechnical and biological effects of BLENDS of alternatives based on the fact that GIT disorders post-weaning display highly complex aetiology and mechanisms
- negative interactions between feed components and added alternatives OR among alternatives must be understood

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