# Alternatives to Antibiotics in Livestock Management

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# Why antibiotics & why we need alternatives to antibiotics?

- Antibiotics are common additives used in commercial animal feeds, except for European Union (banned for the risk of more bacteria resistance in human antibiotic therapy)

- Better growth in general explained by their decreasing action on the number of competitive pathogenic bacteria in the gut

- Thus reduced bacterial load would decrease immune stimulation associated with impaired performance

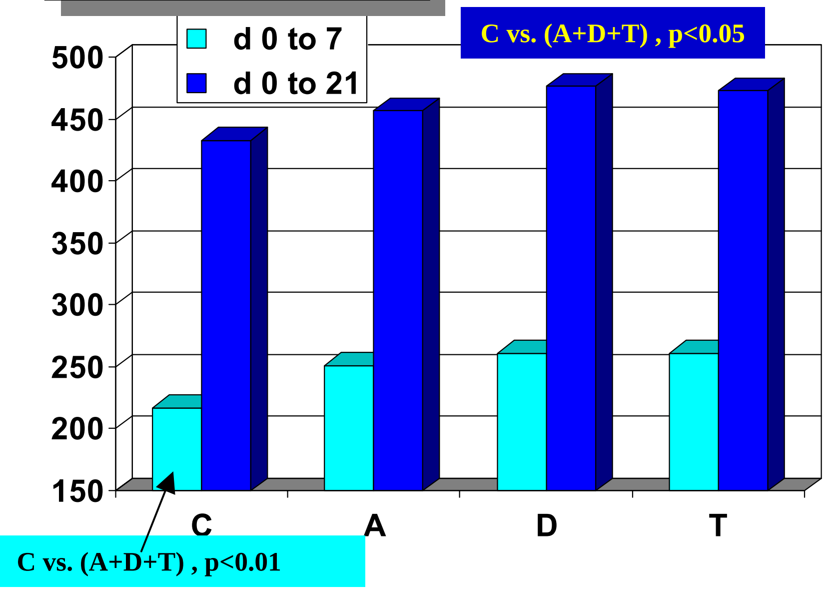
# Why antibiotics & why we need alternatives to antibiotics?

- Growth promoting effect (and > returns) seen in apparently healthy animals
- Their main use not for therapy
- Not all their effects are explained by their direct effect on single bacteria categories

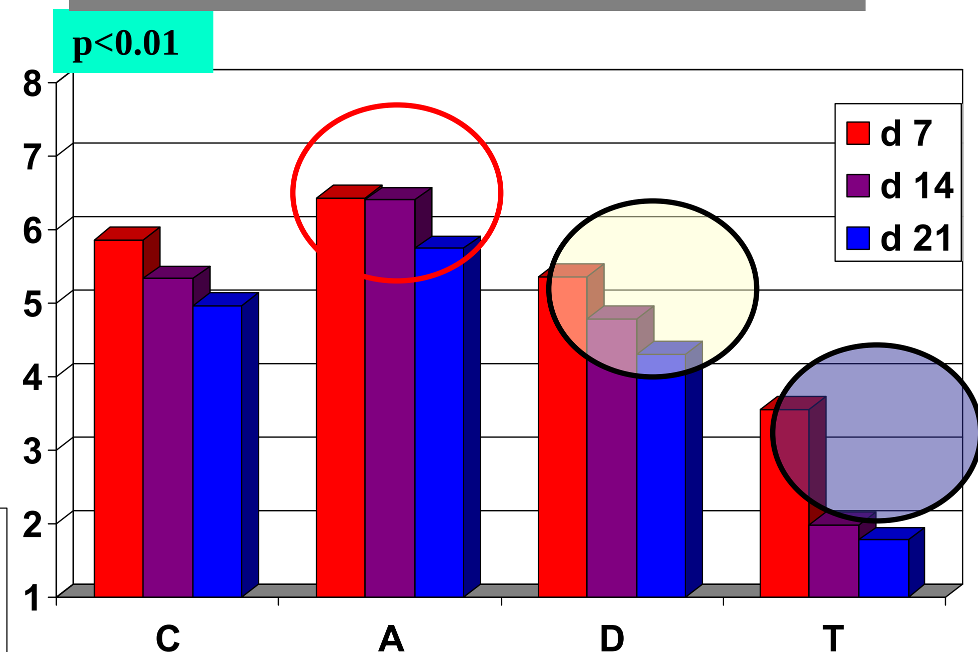
  (a review *Niewold T 2007. Poultry Sci. 86: 605-609*)
- ….

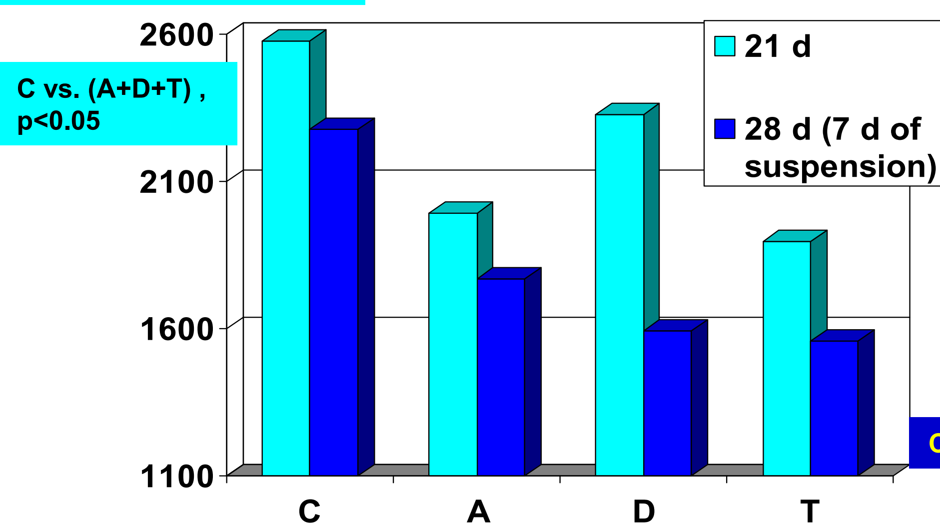
# Different classes of antibiotic improved feed intake (& growth) and reduced the activation of the immune response in healthy weaned pigs but had differing effects on gut microbiota (C=control, A=amoxicillin , D=doxycycline, T=tilmicosin )

Feed intake, g/d




Enterobacteria faecal counts, log10 CFU/g




Blood IgM

C vs. (A+D+T) , p=0.08

# Why antibiotics & why we need alternatives to antibiotics?

- Growth promoting effect …..
- … main use is not for therapy
- Not all their effects are explained by their direct effect on single bacteria categories …..

- Need of alternatives to maintain performances

# Solutions that will be discussed

- Prerequisite nutritional strategies to reduce the need of in feed antibiotics
- Feeds & additives mainly aimed to act directly on microflora
- Nutrients & additives that control or potentiate the gut barrier

# Prerequisite nutritional strategies to reduce the need of in feed antibiotics

- Undigested feed protein may stimulate the overgrowth of undesired bacteria,
- and accelerate the production of toxic nitrogenous compounds harmful to intestinal health

- The low protein diets should be fortified with crystalline essential amino acids

# The dietary protein % affects inflammatory markers after post-weaning colibacillosis in pig. Same SID AAcid's % *(Opapeju et al., 2010).*

Low CP (17.6%) Vs. high CP diet (22.5%)

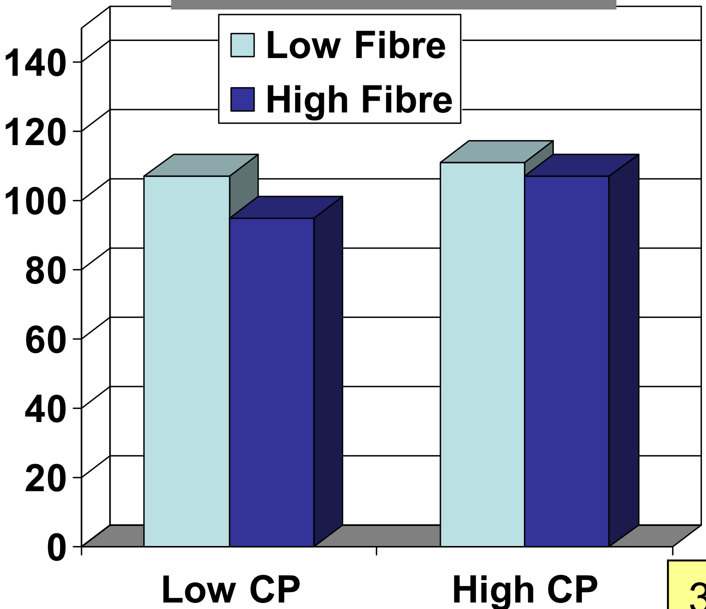
| Item, in blood | 24 h before challenge | 8 h post | 72 h post |
|---|---|---|---|
| TNF-$\alpha$ | ~↓ | ↓ | = |
| IL-1$\beta$ | = | ↓↓ | = |
| Haptoglo-bulin | = | ↓ | = |

# Prerequisite nutritional strategies to reduce the need of in feed antibiotics

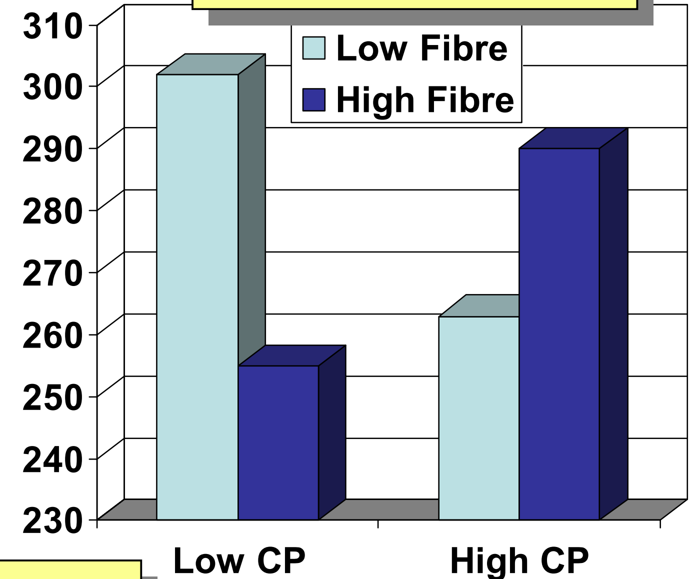
- Quality of dietary polysaccharides affects the development and the variability of gut microbiota
- 2 apparently opposite strategies
- A) supply carbohydrates with very high digestibility → less substrates for gut microbes and colonization by pathogens
- B) Use feed grains or by-products containing specific fibers with prebiotic effects in the hind gut
- CP % should be tuned to the quality of CHO

# Tuning the quality of carbohydrates with the CP %: average daily gain of weaning pigs (Bikker et al., 2006)

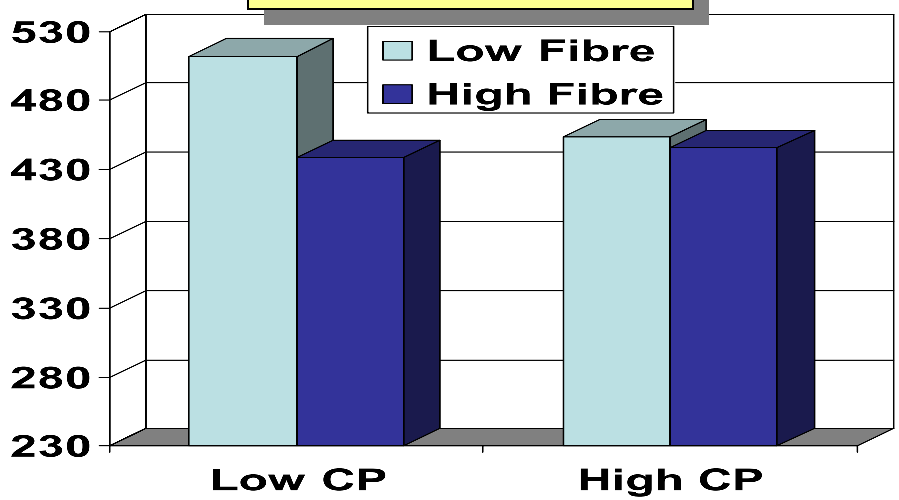
1st wk post-weaning




2nd wk post-weaning




3d wk post-weaning



# Feeds & additives mainly aimed to act directly on microbiota

- Organic acids
- Essential oils & extracts of herbs
- Spray dried plasma (and other products containing Immunoglobulins)

# Organic acids

- Overt antimicrobial activity, related to their penetration into the microbes (Cherrington et al. 1991), differing from the action of HCl

- Improve growth, feed intake and feed to gain (Meta-analysis by Partanen, 2001), particularly in younger pigs

- In the gut reduce microbial protein synthesis and improvement of digestibility → more nutrients available for the animal

# OA in pig feed (Treated/Control, Partanen, 2001)

| | 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 | 106a** | 100b | 99b | 108a*** |
| ADG | 110a*** | 104b** | 104b** | 112a*** |
| Feed:gain | 97.5* | 97** | 96** | 96* |

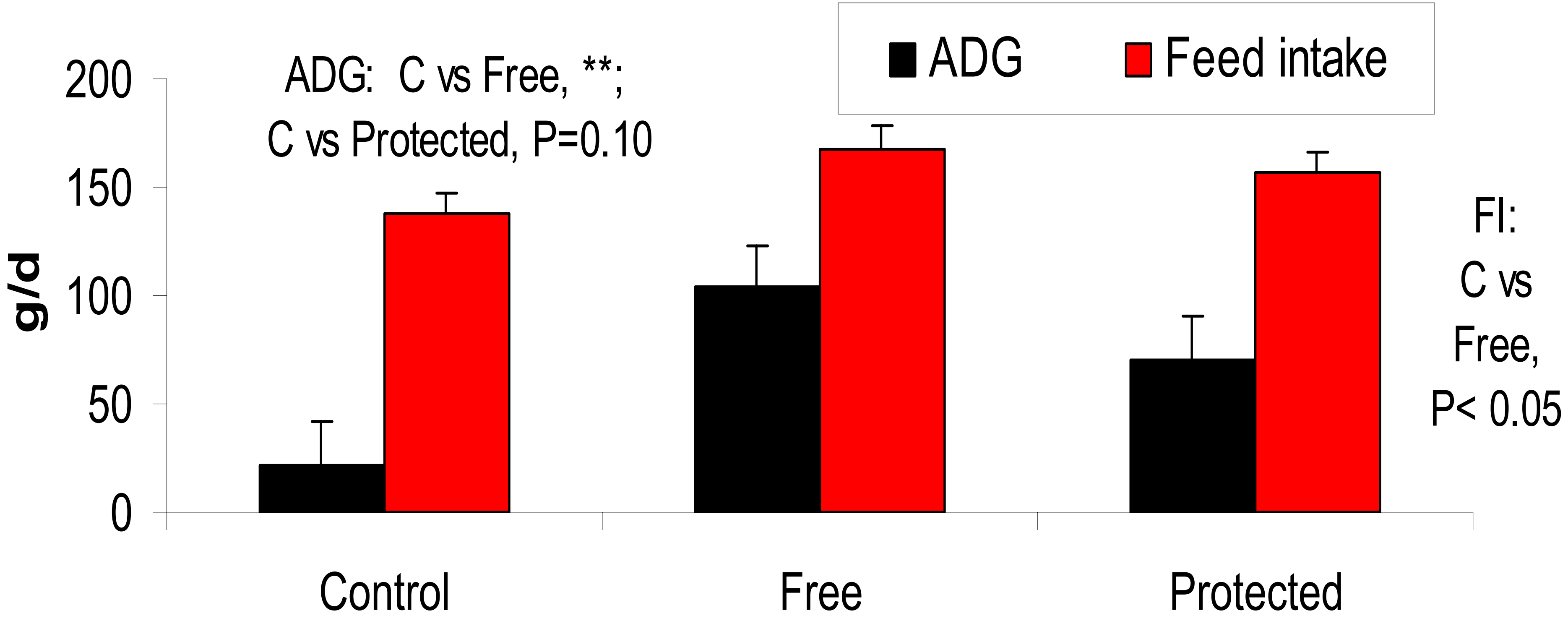
# The effect of OA is less consistent that antibiotics

Efficacy affected by

- Feeds with high buffering capacity (protein source and some minerals)
- Fermentability of the feeds by acid-producing bacteria (Milk whey and lactic acid bacteria..)
- Variability of the basic microbiota
- Adaptation of the stomach to an acidic diet → pay attention to the passage to a diet without OA

Gastroprotection?

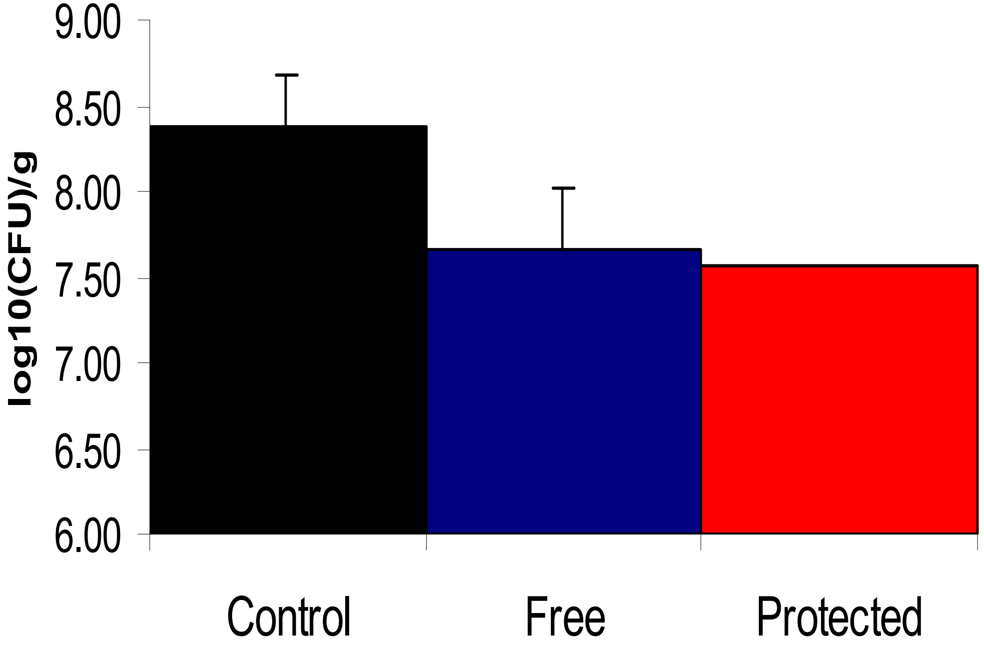
# Effect of Calcium Formate free and fat-protected on growth and feed intake of E.coli K88 challenged pigs *(Bosi et al., 2007)*



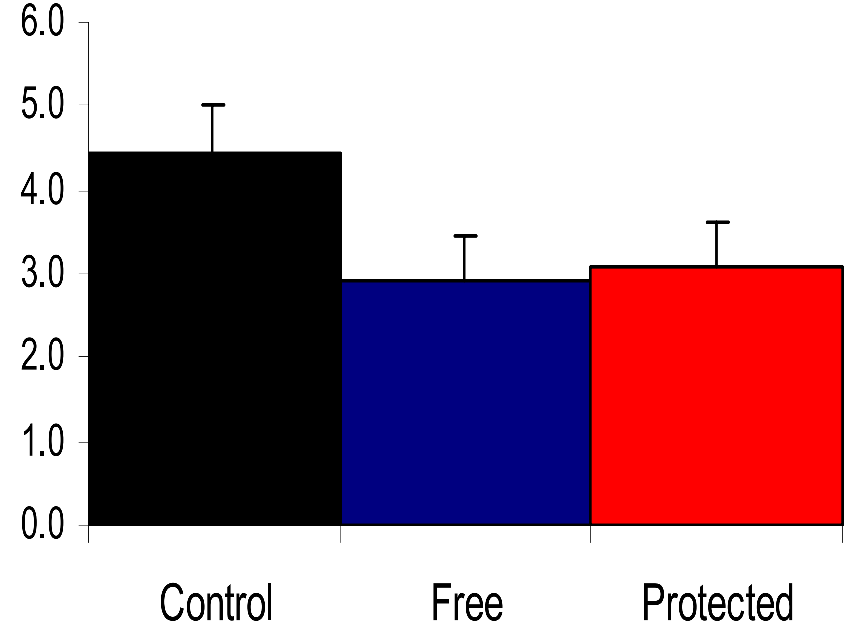

**Also less deaths for colibacillosis (5% vs. 1.6% vs 3.3%)**

# Calcium Formate free and fat-protected reduced total E.coli faecal excretion and days in diarrhoea of E.coli K88 challenged pigs (P<0.05, *Bosi et al., 2007*)

## Total E.coli faecal excretion

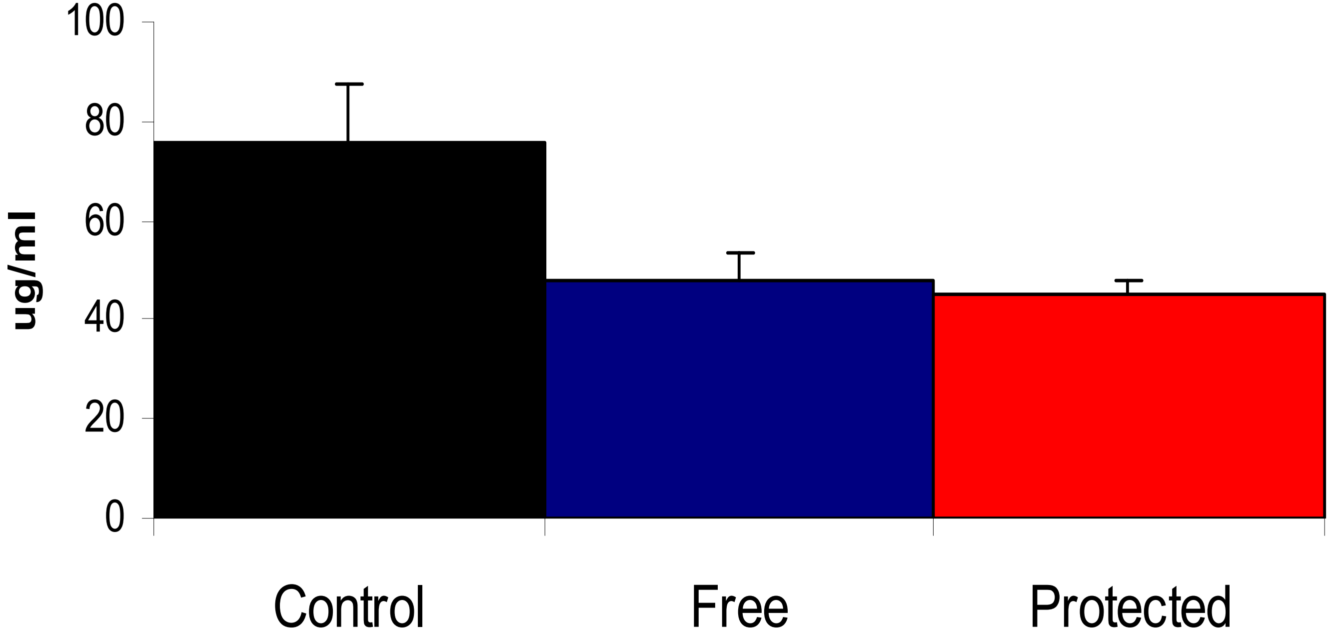



## Days in diarrhoea

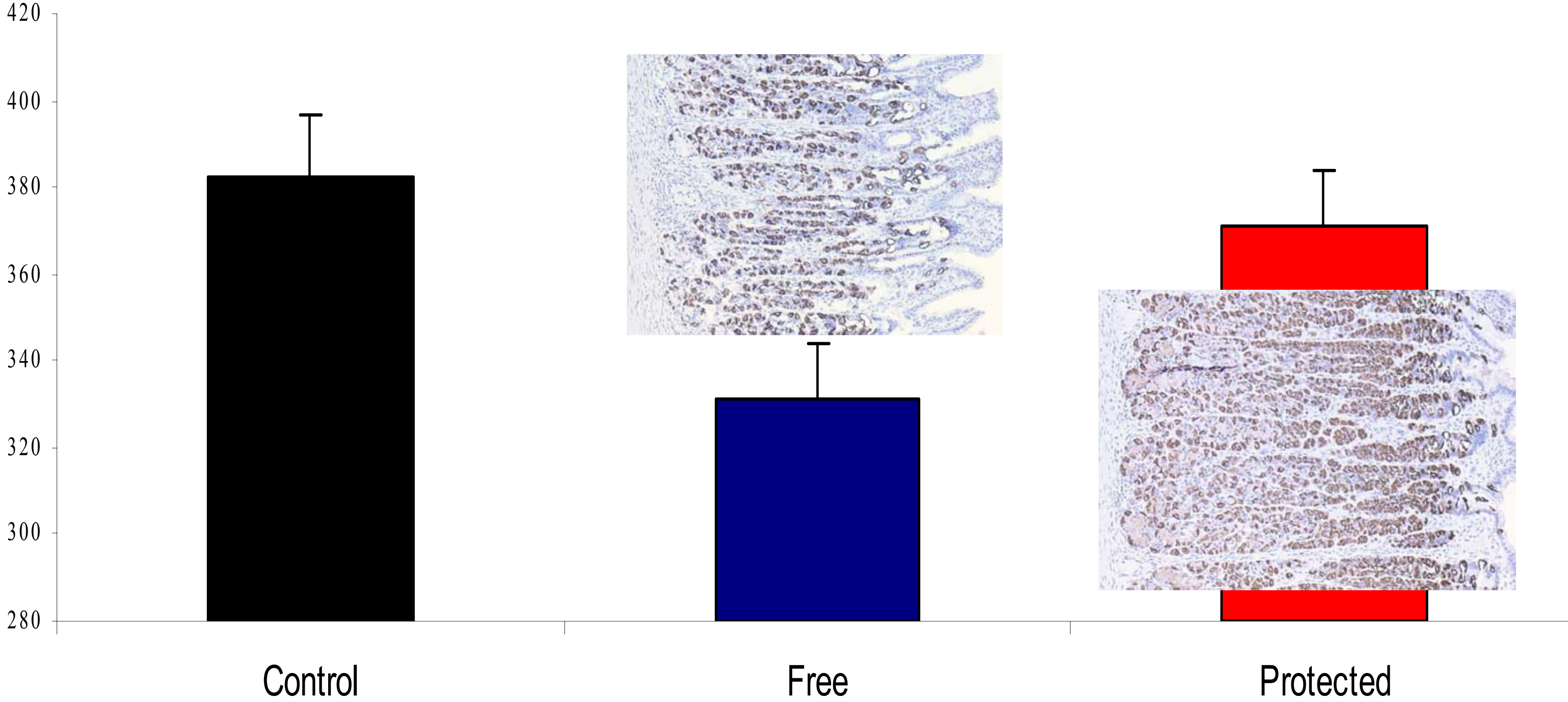



**No reduction of E.coli K88 excretion**

Calcium Formate free and fat-protected reduced total IgA in saliva ($P<0.05$), but not in b**lood serum and jejunum secretum** , of E. coli K88 challenged pigs *(Bosi et al., 2007)*



# The fat-protection of Calcium formate prevents the reduction of the number of HCl secreting parietal cells in the gastric fundic mucosa of piglets *(Bosi et al., 2006)*



# Essential oils & extracts of herbs

- Antimicrobial properties of various extracts of herbs and spice preparations reported since old ages
- Essential oil of the plant more frequently the biologically active
- Efficacy depends to variation in oil composition, changes in lipid solubility at the surface of the bacteria & the disintegration of the outer membrane of certain pathogens

# Antimicrobial activity of selected essential oils *in vitro* (Lallès et al., 2009)

| | Carvacrol | Cinnamon oil | Cinnam-aldehyde | Eugenol | Thymol | Oregano oil | Thyme oil | Clove oil | Reference |
|---|---|---|---|---|---|---|---|---|---|
| MBC[a] | | $3\text{-}10\ 10^3$ | | | | | | 10,500 | Cava *et al.*, 2007 |
| | 100-283 | 100-133 | | 300-466 | 100-233 | | | | Si *et al*., 2006a, b |
| | | | | | | 156-625 | 156-2,500 | | Burt *et al*., 2003 |
| | 3.12 - 50 | | | | | 3.12 - 50 | | | Karioti *et al*., 2006 |
| | | | | | | 1 - 200 | | | Sivropoulou *et al*., 1996 |
| MIC[b] | | 500-3,000 | | | | | | $3\text{-}4\ 10^3$ | Cava *et al.*, 2007 |
| | 3.12 - 50 | | | | | 3.12 - 50 | | | Karioti *et al*., 2006 |
| | 55.5 - 274.5 | 500 | | 410 | | | 500 | 500 | Dušan *et al*., 2006 |
| | | | | | | 2,500-40,000 | 5,000-40,000 | | Peñalver *et al*. 2005 |
| | | | | | | 1,200-20,000 | 300-20,000 | 1,200-25,000 | Hammer *et al*., 1999 |
| | | 800-3,200 | | | | | | 1,600-6,400 | Prabuseenivasan *et al*., 2006 |
| | | 75-600 | 75-600 | | | | | | Ooi *et al*., 2006 |
| MEC[c] | 103-286 | | 83-176 | 207-496 | 117-323 | | | | Michiels *et al*., 2007 |

[a]MBC (μg $mL^{-1}$): minimal bactericidal concentration
[b]MIC (μg $mL^{-1}$): minimal inhibitory concentration
[c]MEC (μg $mL^{-1}$): minimal effect concentration

**Bacterial species inhibited in *in vitro* studies when plant essential oils or pure oil components were added to growth medium (Lallès et al., 2009)**

| Bacterial species | Essential oil source or pure component | References |
|---|---|---|
| *Acinetobacter baumanii* | *Oregano vulgare, Syzygium aromaticum, Thymus vulgaris* | Hammer *et al*., 1999 |
| *Acinetobacter calcoacetica* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Aeromonas hydrophila* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Aeromonas sobria* | *O. vulgare, S. aromaticum, T. vulgaris* | Hammer *et al*., 1999 |
| *Agrobacterium tumefaciens* | carvacrol | Karioti *et al*., 2006 |
| *Alcaligenes faecalis* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Bacillus amyloliquefaciens* | *O. vulgare, Thymbra sintensi* (thyme) | Özcan *et al*., 2006 |
| *B. brevis* | *O. vulgare, T. sintensi* | Özcan *et al*., 2006 |
| *B. cereus* | *O. vulgare, T. sintensi,* carvacrol | Karioti *et al*., 2006; Özcan *et al*., 2006 |
| *B. megaterium* | *O. vulgare, T. sintensi* | Özcan *et al*., 2006 |
| *B. subtilis* | *Cinnamonum zeylanicum, Eugenia caryophyllus* (clove), *O. vulgare, T. sintensi, T. vulgare, S. aromaticum,* carvacrol, eugenol, thymol | Sivropoulou *et al*., 1996; Dorman and Deans, 2000; Bozin *et al*., 2006; Özcan *et al*., 2006; Prabuseenivasan *et al*., 2006 |
| *B. subtilis var. niger* | *O. vulgare, T. sintensi* | Özcan *et al*., 2006 |
| *Beneckea natriegens* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Bifidobacterium longum* | thymol | Si *et al*., 2006a |
| *B. breve* | thymol | Si *et al*., 2006a |
| *Brevibacterium linens* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Brocothris thermosphacta* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Citrobacter freundii* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Clostridium sporogenes* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Enterococcus faecalis* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Hammer *et al*., 1999; Dorman and Deans, 2000 |
| *Enterobacter aerogenes* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Erwinia carotovora* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Escherichia coli (several strains, including K88, O157:H7, ETEC)* | *C. zeylanicum, E. caryophyllus, O. vulgare, S. aromaticum, Thymus mastichina, T. vulgaris, Thymus zygis,* carvacrol, cinnamon oil, clove oil, eugenol, thymol | Sivropoulou et al., 1996; Dorman and Deans, 2000; Bozin et al., 2006; Özcan et al., 2006; Prabuseenivasan et al., 2006 |
| *Flavobacterium suaveolens* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |

| Bacterial species | Essential oil source or pure component | References |
|---|---|---|
| *Klebsiella pneumoniae* | *C. zeylanicum, E. caryophyllus, O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Hammer *et al*., 1999; Dorman and Deans, 2000; Prabuseenivasan *et al*., 2006; |
| *Lactobacillus acidophilus* | carvacrol, cinnamon oil, thymol | Si *et al*., 2006a |
| *L. plantarum* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol, thymol | Dorman and Deans, 2000; Si *et al*., 2006a |
| *Leuconostoc cremonis* | *O. vulgare, S. aromaticum, T. vulgaris* | Dorman and Deans, 2000 |
| *Listeria monocytogenes* | *O. floribundum, O. glandulosum, Thymus guyonii, Thymus munbyanus, Thymus numidicus, Thymus pallescens* | Hazzit *et al*., 2006 |
| *Micrococcus flavus* | *O. vulgare, T. vulgaris* | Bozin *et al*., 2006 |
| *Micrococcus luteus* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000; Karioti *et al*., 2006 |
| *Moraxella sp.* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *Proteus mirabilis* | carvacrol | Karioti *et al*., 2006 |
| *Proteus vulgaris* | *C. zeylanicum, E. caryophyllus, O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000; Prabuseenivasan *et al*., 2006 |
| *Pseudomonas aeruginosa* | *C. zeylanicum, E. caryophyllus, O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol, thymol | Sivropoulou *et al*., 1996; Hammer *et al*., 1999; Dorman and Deans, 2000; Bozin et al., 2006; Karioti et al., 2006; |
| *P. talassi* | carvacrol | Karioti *et al*., 2006 |
| *Rhizobium leguminosarum* | *O. vulgare,* carvacrol, thymol | Sivropoulou *et al*., 1996 |
| *Salmonella choleraesuis* | *O. vulgare, T. mastichina, T. zygis* | Peñalver *et al*., 2005 |
| *S. enteritidis* | *O. vulgare, T. mastichina,T. vulgaris, T. zygis,* carvacrol | Peñalver *et al*., 2005; Bozin *et al*., 2006; Karioti *et al*., 2006; |
| *S. essen* | *O. vulgare, T. mastichina, T. zygis* | Peñalver *et al*., 2005 |
| *S. pullorum* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |
| *S. typhi* | *O. vulgare, T. vulgaris* | Bozin *et al*., 2006 |
| *S. typhimurium* | *O. vulgare, S. aromaticum, T. mastichina, T. vulgaris, T. zygis,* carvacrol, cinnamaldehyde, cinnamon oil, clove oil, eugenol, thymol | Sivropoulou *et al*., 1996; Hammer *et al*., 1999; Peñalver *et al*., 2005; Si *et al*., 2006a,b; |
| *Sarcina lutea* | *O. vulgare, T. vulgaris,* carvacrol | Bozin *et al*., 2006; Karioti *et al*., 2006 |
| *Serratia marcescens* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Hammer *et al*., 1999; Dorman and Deans, 2000; |
| *Shigella sonei* | *O. vulgare, T. vulgaris* | Bozin *et al*., 2006 |
| *Staphylococcus aureus* | *C. zeylanicum, E. caryophyllus, O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol, thymol | Sivropoulou *et al*., 1996; Dorman and Deans, 2000; Hammer *et al*., 2000 ; Bozin *et al*., 2006; Karioti *et al*., 2006; Prabuseenivasan *et al*., 2006 |
| *S. epidermidis* | *O. vulgare, T. vulgaris* | Bozin *et al*., 2006 |
| *Yersinia enterocolitica* | *O. vulgare, S. aromaticum, T. vulgaris,* carvacrol, eugenol | Dorman and Deans, 2000 |

# The case of thymol

- **Principal essential oil of the Thyme herb**
- **Phenolic compound with *In vitro* demonstrate antibacterial effect (Jugl-Chizzola et al. 2005; Si et al. 2006)**
- **The *In vivo* antimicrobial activity of the Thyme herb/Thymol is not well demonstrated (Hagmüller et al. 2006; Michiels, 2009)**
- **Antimicrobial activity of the Thymol is abolished when mixed with the diet. 1-3% Thymol in the diet to have the same effects obtained in *in vitro* test (Si et al. 2006)**
- **Reduced palatability of the diet also at low doses (Jugl-Chizzola et al. 2006)**

# The case of thymol

- **Our *In vitro* results showed that Thymol had an antimicrobial activity 100-700 fold less than the antibiotics (depending by the antibiotics molecule) →= 1-4% of the diet!**

- **Short and long term effects of a high dose of Thymol (1% diet), added in the weaning diet of piglets challenged or not with Salmonella Typhimurium (Trevisi et al., 2007)**

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## Effect of 1% thymol on growth performance (pooling pigs with or whithout Salmonella challenge)

| | Diet | |
|---|---|---|
| | Control | Thymol |
| **Body weight** before challenge, kg | 7.08 | 6.58 |
| **ADG** before challenge, g | 19 A | -48 B |
| **ADG** 0d to 20d after challenge, g | 342 | 316 |
| **Daily feed intake, g** | | |
| before challenge | 127 A | 89 B |
| 0 d to 20d after challenge | 474 a | 413 b |
| **Feed to gain** to 20 d after challenge | 1.40 a | 1.31 b |

**A,B: p<0.05; A,B: p<0.01.**

**No effect of Salmonella challenge**

# Thymol and Salmonella infection

Thymol alleviated some effects of the Salmonella infection, reducing:

- reducing inflammatory cytokine TNF-alpha mRNA

- preventing the raise of body temperature after 24h from the Salmonella infection observed in unsupplemented pigs

However

- Did not reduce the translocation of Salmonella to mesenteric lymph nodes on 2 d after infection (all challenged pigs were positive)

# The limits of oral supply of some EO

- **Thymol, Carvacrol, Eugenol, E-cynnamaldeide rapidly disappear from the stomach, are absorbed, & recovered in blood and in the urine** *(Kohlert et al., 2002; Bhattaram et al., 2002, including pigs Michiels et al., 2008, Bosi et al. unpublished)*

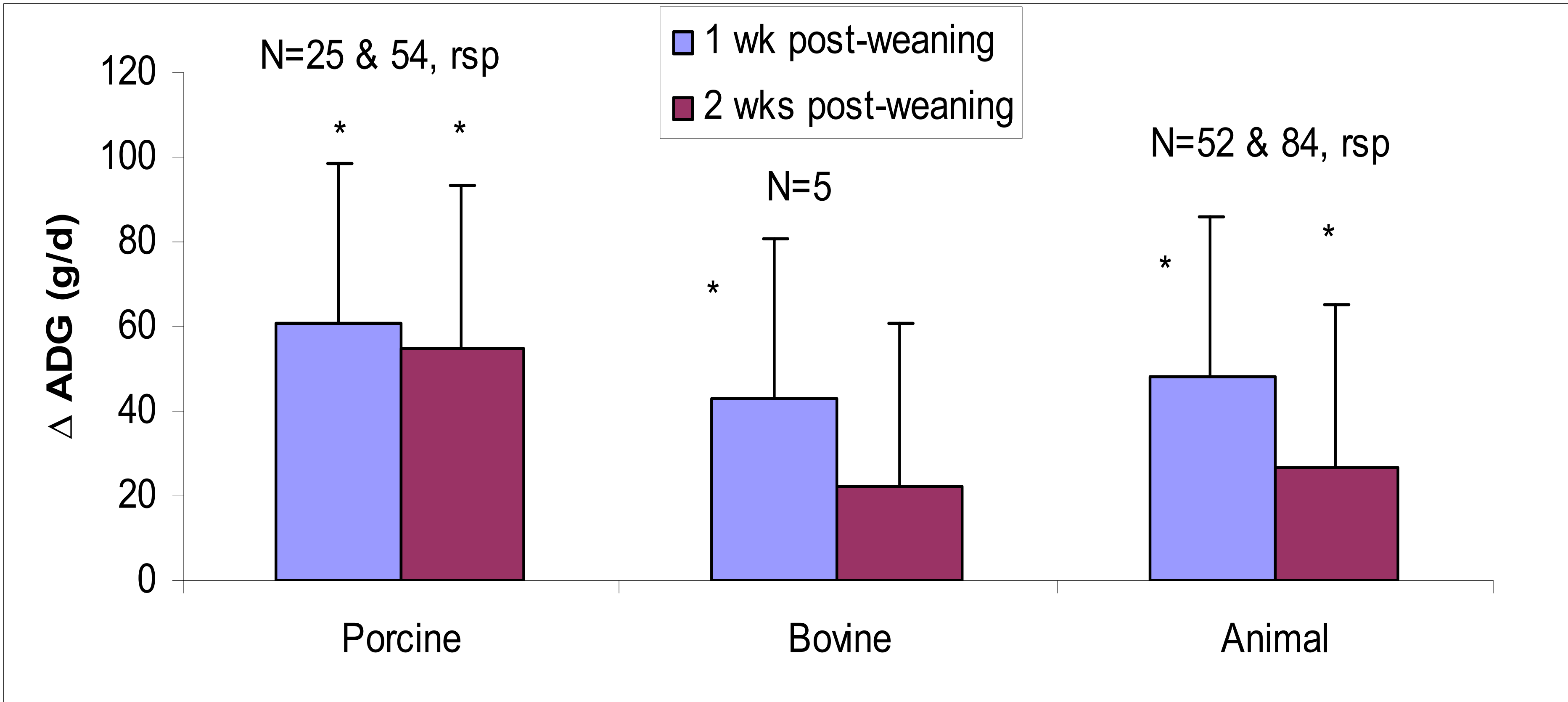
- **Systemic effects to be more studied**

**-Protected products commercially available and under development**

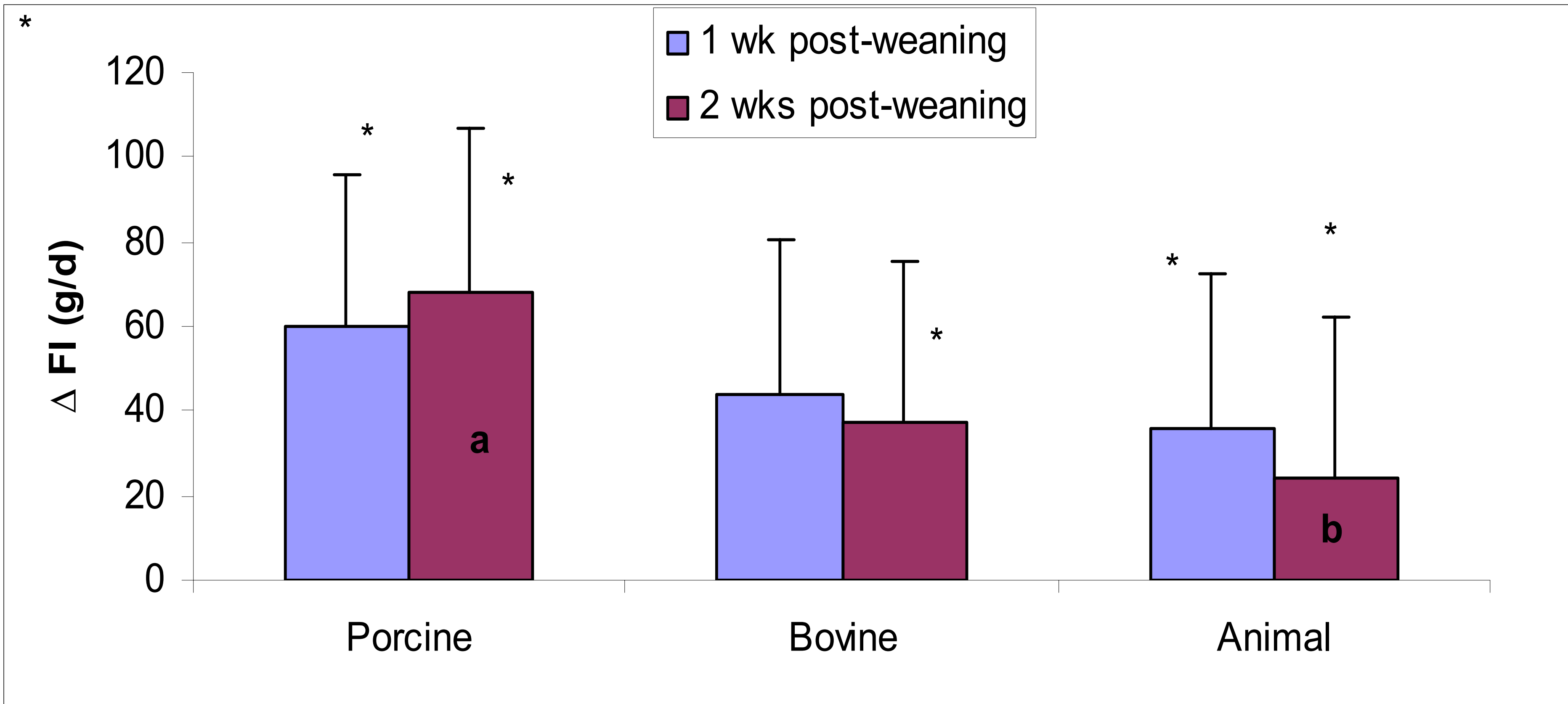
# Spray dried plasma

- <u>porcine</u>, bovine, animal (various sources)
- SDP improves growth and feed intake
- Ig G fraction mostly responsible *(Pierce et al 2005)*
- SDP with higher IgG conc. → more protective *(Bosi et al 2001, 2004; Conde 2005)*
- protective effects and lower diarrhoea 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)*

# ADG improvement (± SD) with different sources of SDP (reviewed by Torrallardona, 2010)



# Daily feed intake improvement (± pooled SD) with different sources of SDP (reviewed by Torrallardona, 2010)



# 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**

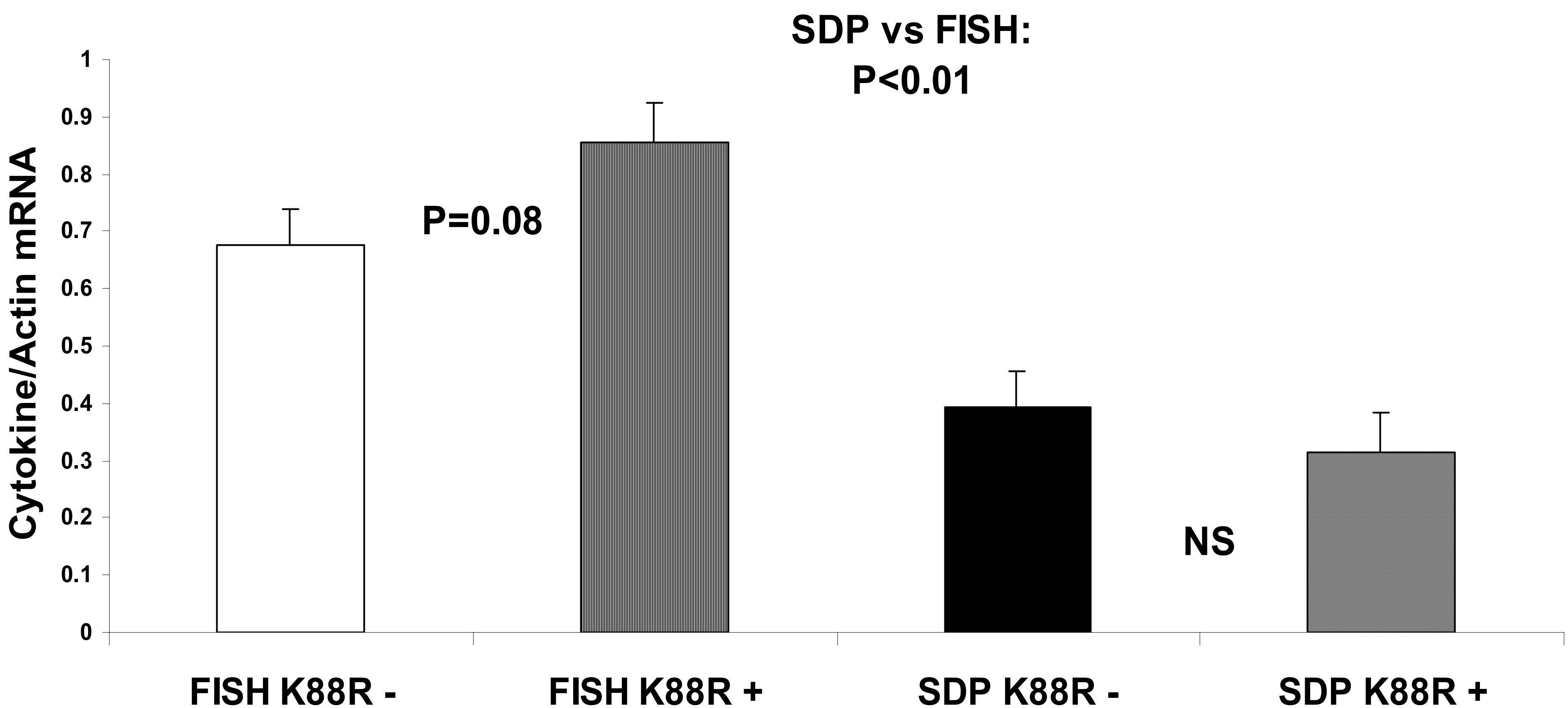
- **SDP increases villous height and intestinal counts of lactobacilli BUT NOT ALWAYS** *(Conde 2005; Torrallardona et al 2003, 2007)*

- **anti-inflammatory effects of SDP on intestine after *E. coli* challenge** *(Touchette et al 2002; Bosi et al 2004)*

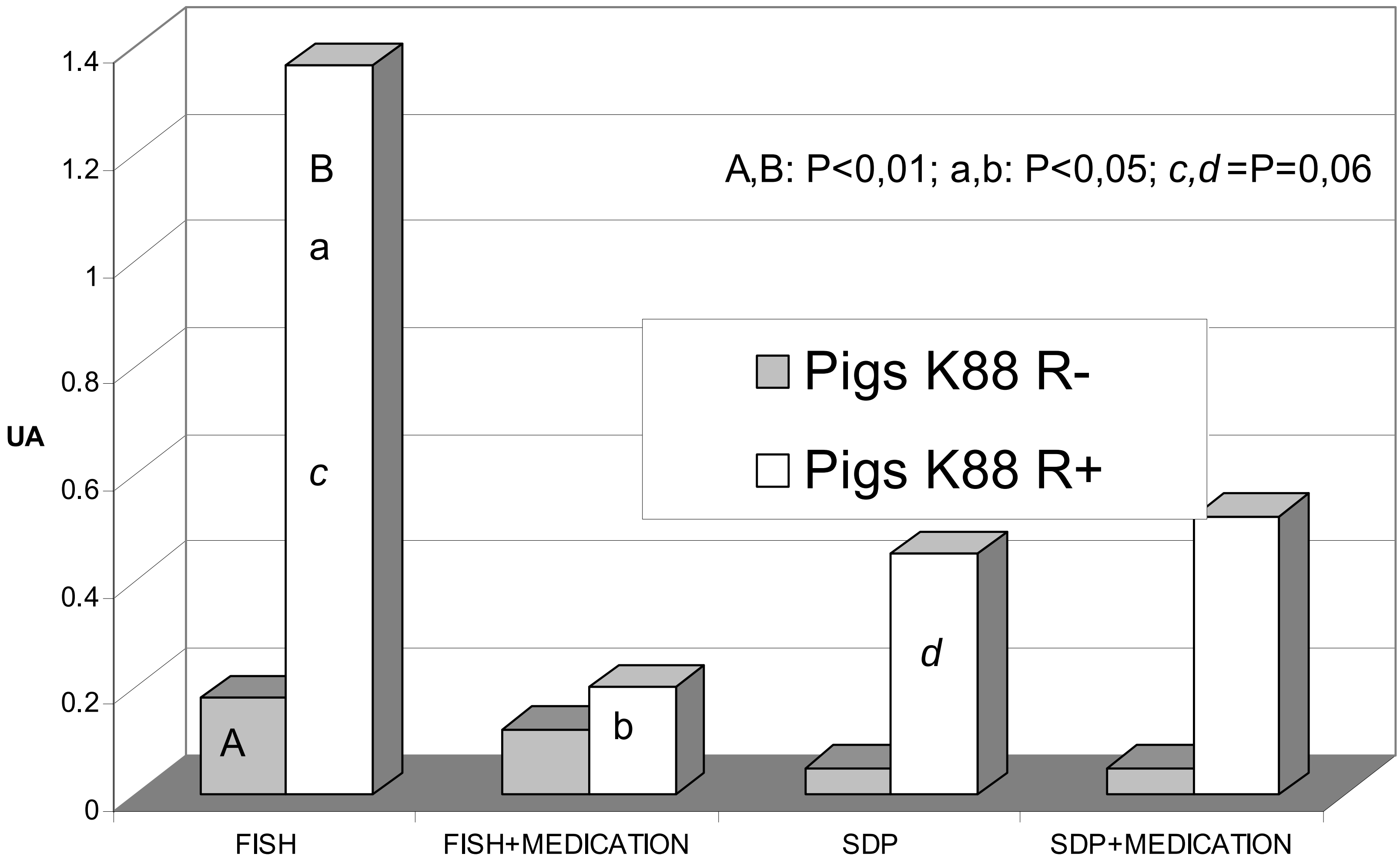
- **However SDP obtained from infected pigs can veiculate porcine circovirus type 2** *(Patterson et al., 2010)*

- **Performance improvements related to animal genotype (susceptibility to E. coli K88)**

# SDP (but not fish protein) prevents the production of inflammatory IL-8 mRNA in weaned piglets orally challenged with *E. coli* K88 in susceptible pigs (Bosi et al., 2004).



# SDP and Medication reduce the need plasma IgA specific activity (units/ml) in weaned piglets orally challenged with *E. coli* K88 in susceptible pigs (Bosi et al., 2004).



# Other protein sources

- **Skimmed milk protein**

  → **reduced intestinal villous atrophy PW as compared to feather meal,** *(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)*

# Nutrients & additives that can control or potentiate the gut barrier

- Probiotics
- Amino acids
- Zn
- Lectins & other competitors of bacterial adhesion

# Probiotics

- “Live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO)
- Slow diffusion in feed compounds

# Why probiotics?

- Need of a rapid maturation of the gut mucosa and of associated lymphatic tissue,

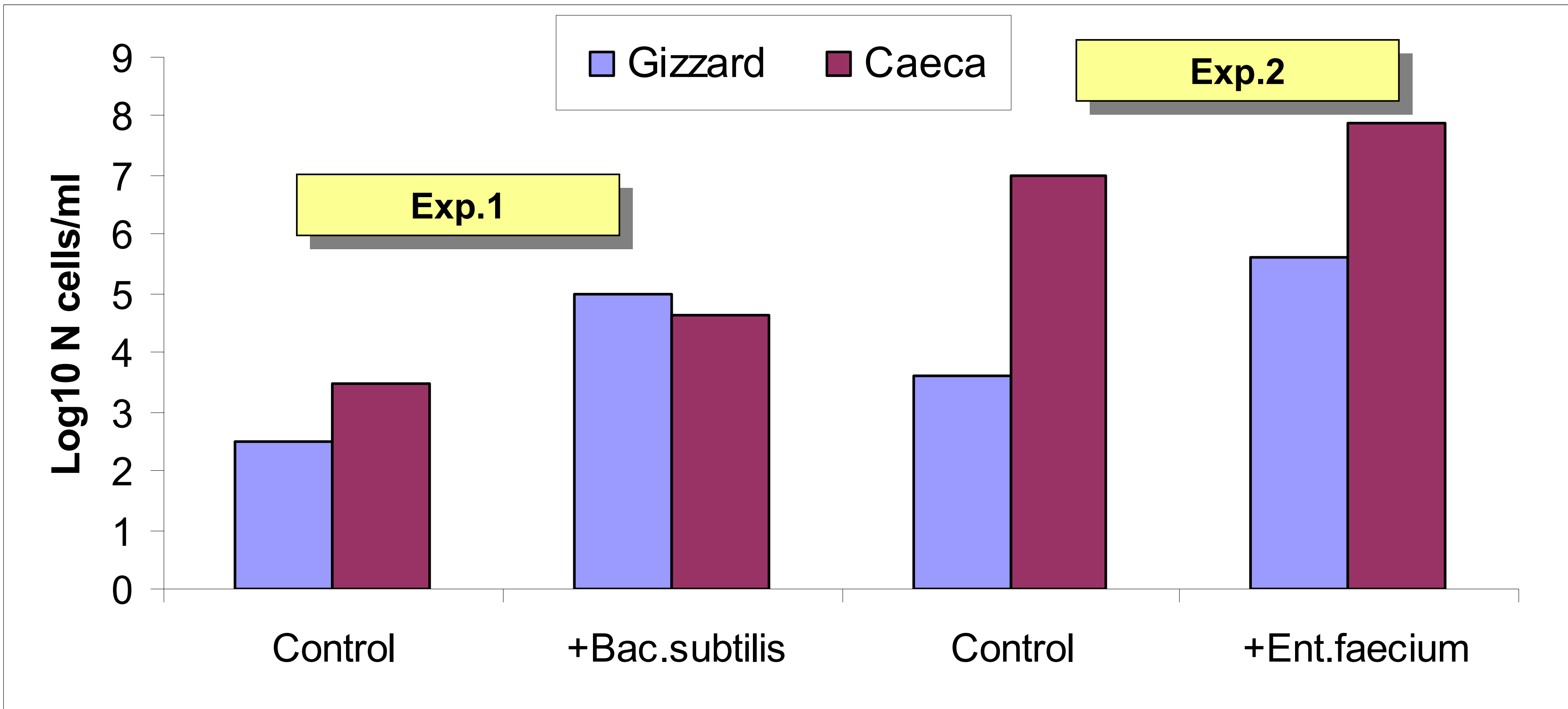
- and of a stable and complex bacterial community in the digestive tracts

- Genotype, suckling, mother environment shape the neonate gut microbiota

# 1st goal: manipulate the gut microbiota of the newborn

- Direct supply at birth→ time consuming, often not accepted by the breeder
- In poultry: establishing beneficial bacteria in the chicken gut prior to hatching
- …

# Presence of bacteria (qPCR) at 48 h after inoculation in the embryo's gizzard content with *Bacillus subtilis* or *Enteroc. faecium* ,or at hatch (96 h post inoculation) in the caeca content (de Oliveira et al., 2010)



# 1st goal: manipulate the gut microbiota of the newborn

- …supply at birth…
- In poultry, … bacteria prior to hatching…
- Manipulating newborn environment by affecting the mother microbiota

→ *Enterococcus faecium* NCIMB 10415 and *Bacillus cereus*, var. toyoi fed to gestating-lactating sows & piglets

→some reduction of piglets diarrhoea, but also indication of a more tolerogenic condition of the immune system

(Maaroufi&Le Dividich, 2006 , Scharek et al. 2005 , Toras et al., 2006 , Scharek et al., 2007 , Taras et al., 2005 , Stamati et al., 2006)

# Comments

- Few results in scientific literature
- Chicks inoculated pre-hatching are less susceptible to pathogenic bacteria?

# 2nd: re-equilibrate the microbiota after weaning

- Transient drop of some favourable bacterial strains

- Re-integrate bacteria that where already present and are typical →e.g. *Lactobacillus sobrius* in pigs (Konstantinov et al., 2006)
- → Martin et al., 2009, tested 19 rod-shaped isolated from sows milk

- Add beneficial bacteria with high settling ability

# Effect of dose of B. animalis (Ra18, the best to settle among 12 strains) on daily live weight gain (2 wks post weaning) and Bifidobacteria counts in cecum

| Dose | ADG, g | Bifidobacteria (Log10 CFU/g) |
|---|---|---|
| 0 | 164 | 4.70 |
| $10^7$ | 168 | 4.88 |
| $10^9$ | 173 | 5.95 |
| $10^{11}$ | 187 | 6.81 |

**P=0.07** **P=0.01**

(Modesto et al., 2009)

# But often reduced efficacy in field

- Already established microbe community
- Insufficient or less efficient bacterial cells added
- Presence of inhibitors
- Fermentable substrate not adequate
- The probiotic replaces other favourable commensal colonies

# 3rd: Growth & Health promoting effect in stressed animals

- In feed antibiotics, effective in more perturbed conditions
- Challenge models are useful

# 1.Probiotic species-resident in pig

***Lactobacillus sobrius*** (Isolated in healthy piglets with an excellent microbiota variability)

- Contrasted growth reduction induced by E.coli K88 challenge in piglets
- Reduced the E. coli K88 quantified by RT-PCR in ileum ,
- Improved the anti-k88 IgA content in blood serum (not in saliva and intestinal secretum)

(Konstantinov et al., 2008)

# Bifidobacteria beneficial in healthy piglets (Modesto et al., 2009), then tested in Challenge models

No positive effect on

- growth, immunity, pathogen excretion ( E. coli k88 challenge)

- growth, immunity, Salmonella translocation to mesenteric lymph nodes (S. Thyphimurium challenge)

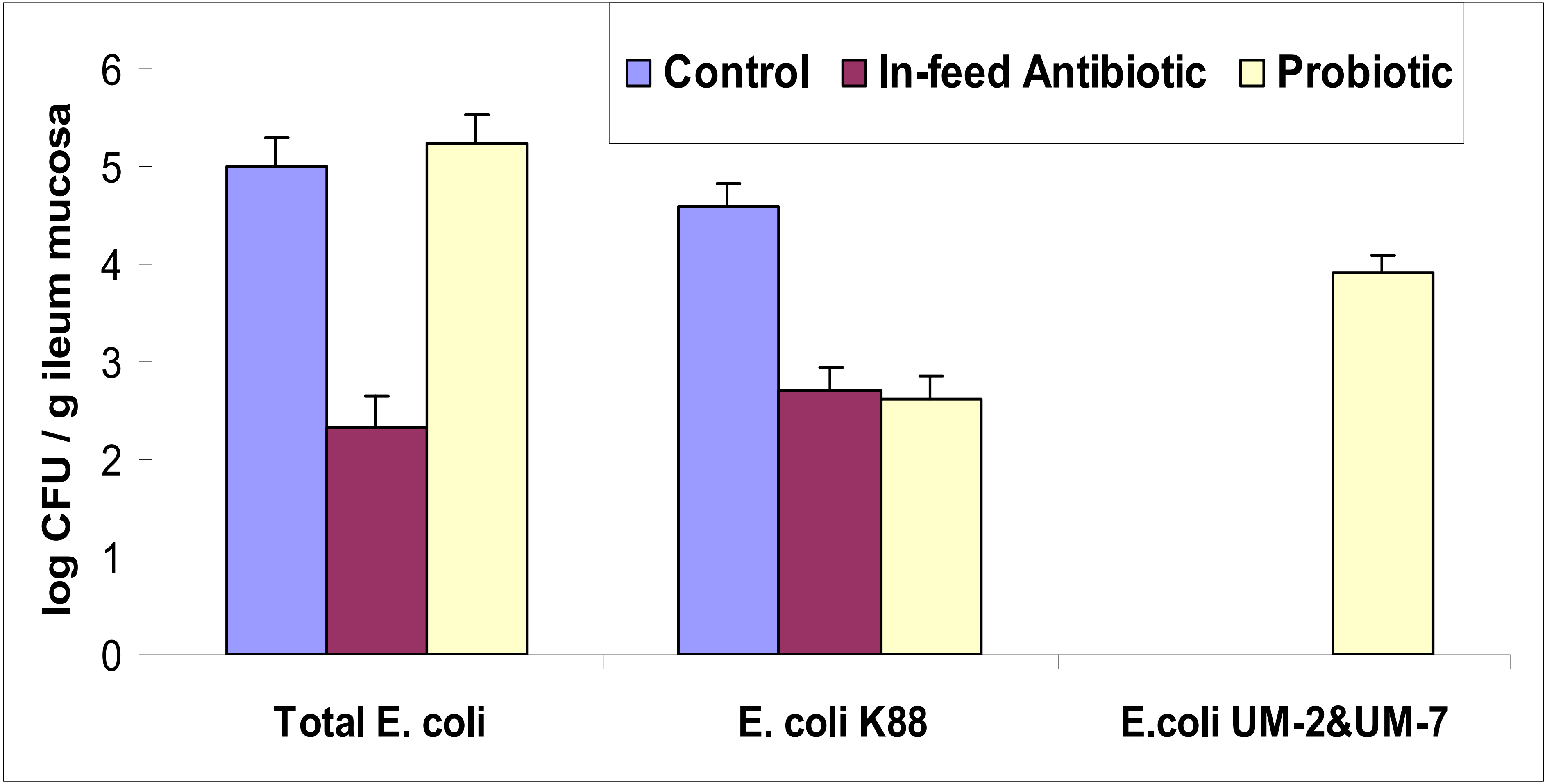
# Enterococcus faecium (NCIMB 10415)

- After challenge with S. Thyphimurium:
- \+ Salmonella  in tonsil, colon, faeces,
- \+ raise of body T°
- ~ + diarrhoea (Szabo et al., 2009)
- Less CD8+ lymphocytes → impairment of the cellular immunity against Salmonella?

- Conversely: less pigs naturally infected by Clamidia (Pollman et al., 2005)

## 2.Production of antibacterial substances

After the procedure of screening on 463 *E.coli* isolates (Setia et al., 2009), colicinogenic *E. coli* reduced *E.coli* counts from ileum mucosa of early-weaned pigs challenged with ETEC (Bhandar et al., 2009) (17% + 22% CP diets)



# 3.Interplay with the host and activation of barrier mechanism

- At the lumen-epithelium interface

→ Adhesion depending on glycosylation pattern of the intestinal cell layer

→ Activation of receptors by different surface motifs of bacteria

- Sampling by M cells & dendritic cells
- Various demonstrations ((*L.plantarum* 299, Gross et al., 2008; *E.coli* Nissle 1917, Sonnenborn & Schulze, 2009; Remer et al, 2009)

# 4.A not species-resident Probiotic

## *Lactobacillus rhamnosus* GG 53103

- Largely tested in other species
- Tested for adhesion inhibition to jejeunal pig mucus of some pathogenic strains (Collado et al., 2007)

- 2 trials with piglets challenged with E.coli K88

→ Zhang et al., 2010, 6 pigs/diet, $10^{11}$CFU LGG/pig/day

→ Bosi et al., 18 pigs/diet, $10^{10}$CFU LGG/pig/d

- Opposite results!

# Zhang et al., 2010, LGG vs C

- Less pigs & pig days with diarrhoea
- Coliforms, log10(CFU/g faeces),3rd d post-challenge: 7.7 vs. 9.1
- Jejunum, more total sIgA
- Less serum IL-6 & TNFα, at 6-12 h post-challenge, IL-1β =

# Bosi et al., LGG vs C

- Reduced growth, feed intake & villous height (in 3 points of SI)
- More diarrhoea, on d4-d6, and more faecal excretion of E. coli K88
- Total IgA in serum, trend of reduction before, and reduction after challenge
- Anti K88 IgA scarcely affected in blood & intestinal secretion

# Summary on Probiotics

- ≠ strategies to affect the settling of beneficial microbiobes and/or  stabilize microbiota
- More knowledge required on interplay mechanisms between commensal microflora and gut mucosa in healthy and stressed animals
- Induction of tolerance may be favourable in healthy animals, but not desired tool under enteropathogen challenge
- Animal genetic variability on the response to beneficial microbes?

# Amino acids

- Some AA have specific roles in the metabolism
- Short term/long term regulation
- Stress period can give more evidence to peculiar metabolic channeling of AA’s

# Gut health and Threonine

- Uses by the gut wall

✓ 60-50% of the digestible Thr intake is consumed by the gut itself (mucosal cells) (Stall et al., 1998)

- Around 20% of gut digestive secretion is made of mucins

✓ Thr is the first AA of mucins (Han and Lee, 2000)

- Impact of a Thr deficiency on the gut development and mucin content (Law et al., 2007)
- Thr is the 1st essential amino acid in immunoglobulins

- Check carefully the Thr dietary levels

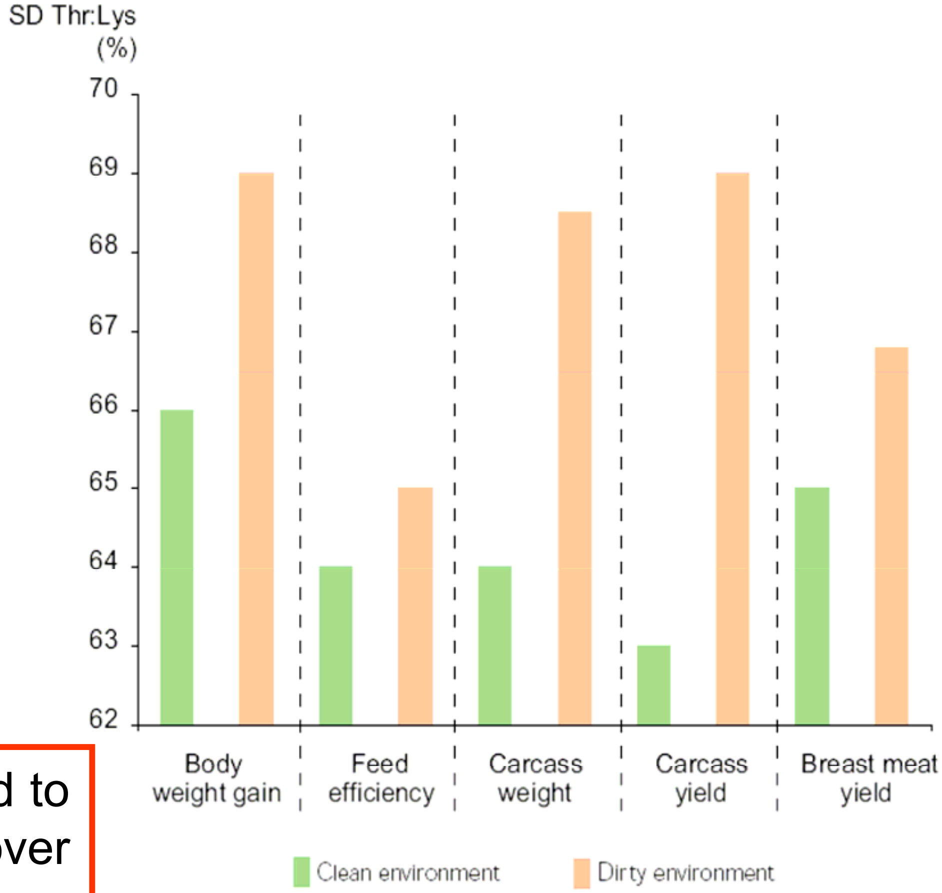
# Threonine contribution to the immune function

## Impact of sanitary condition on threonine requirement in broilers:

**Results:**

- **In the clean environment, body weight gain was optimised for a SD Thr:Lys ratio at 66%.**

- **Optimal SD Thr:Lys ratio was always higher in dirty conditions than in clean conditions**




Poor sanitary environmental conditions lead to a higher SD Thr:Lys requirement to cover higher maintenance needs.

Corzo *et al.*, 2007

# Threonine contribution to the immune function

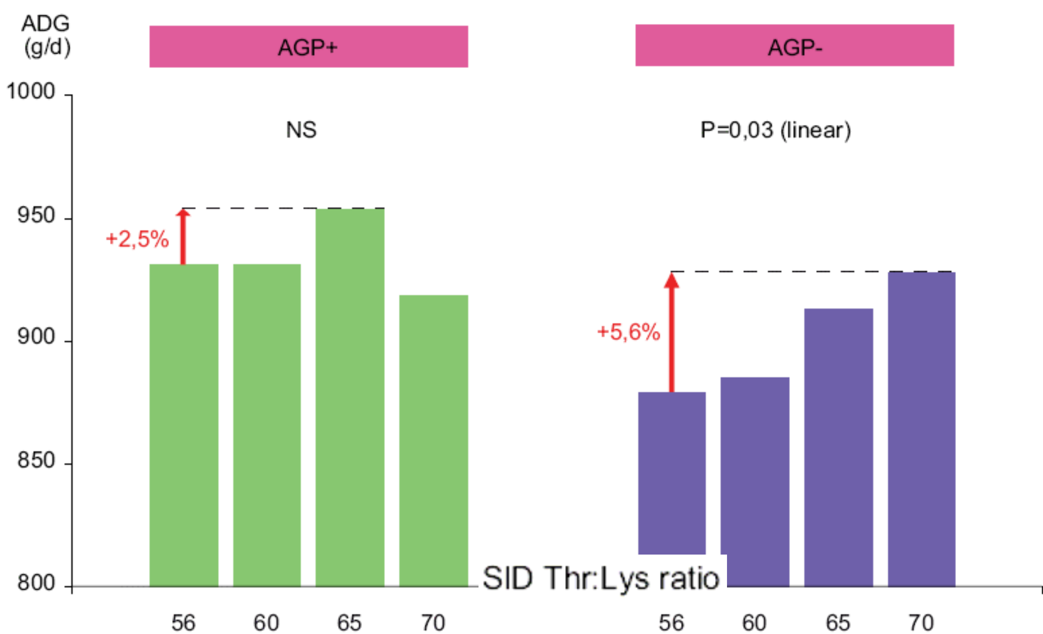
## Impact of the withdrawal of AGP on threonine requirement in growing-finishing pigs:

**Results:**

Inclusion of AGP significantly improved the growth of the pigs (P<0.01)

**When the diet did not contain AGP, dietary Thr**

- **linearly increased daily gain (P=0.03)**
- **and quadratically decreased FCR (P=0.03)**




(Bikker *et al.*, 2006)

# Gut health and other AA’s

**Gln / Glu:**

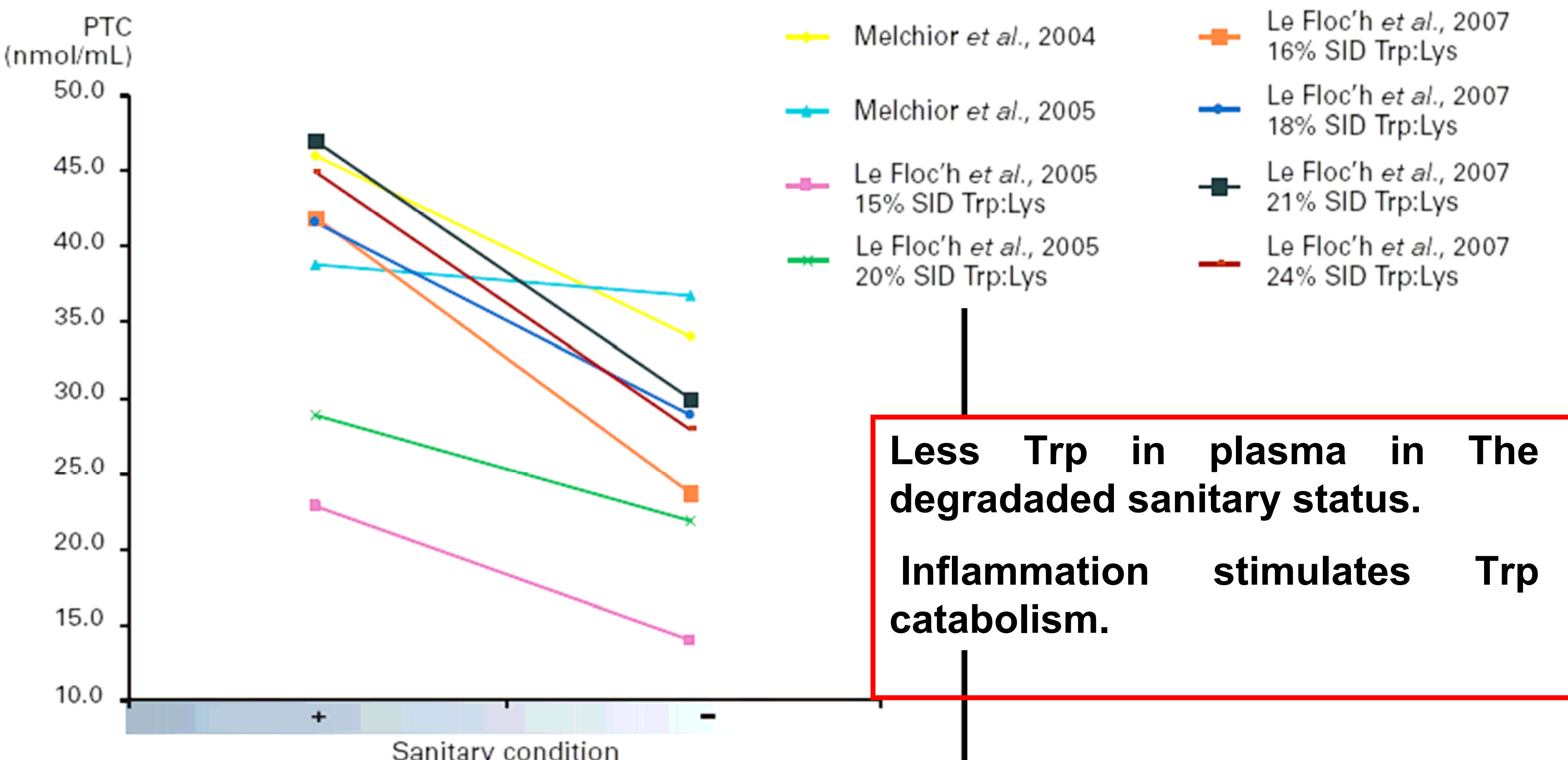
→ **dispensable AA for cell cytoprotection systems (HSPs)**
→ **improves intestinal mucosa in piglets after weaning** *(many publications)*
→ **stimulates both innate and acquired immunity** *(Domeneghini et al. 2004)*
→ **improves resistance to disease / challenge with *E. coli* K88** *(Yi et al 2005)*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

**Arg:**

→ **Dietary supplementation alleviated intestinal mucosal disruption induced by Escherichia coli lipopolysaccharide in weaned pigs***(Liu et al., 2008)*

# Dietary Trpyptophan and the immune function

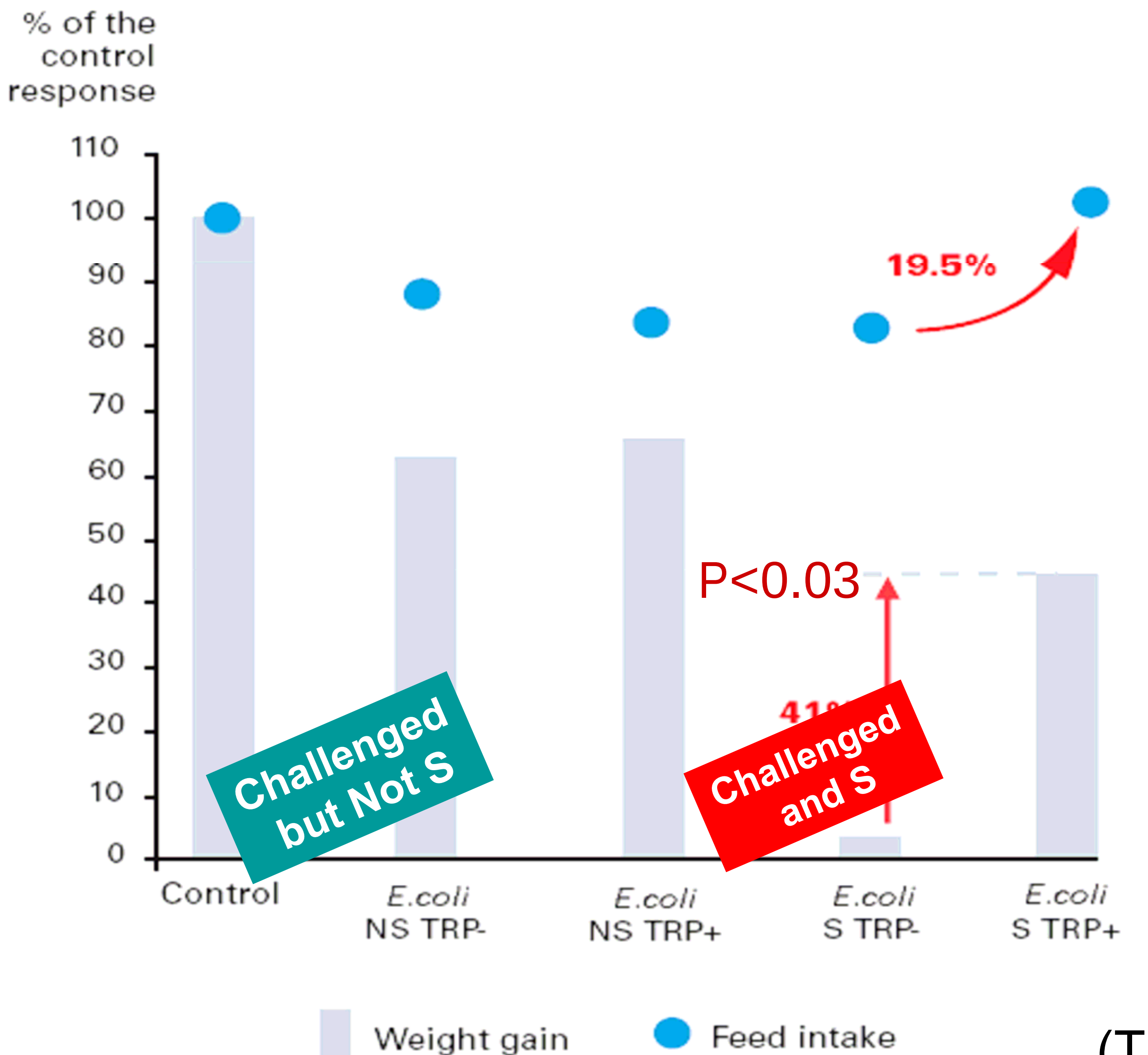
Trp concentration under challenged conditions (adapted from Corrent and Simongiovanni):




**Less Trp in plasma in The degradaded sanitary status.**

**Inflammation stimulates Trp catabolism.**

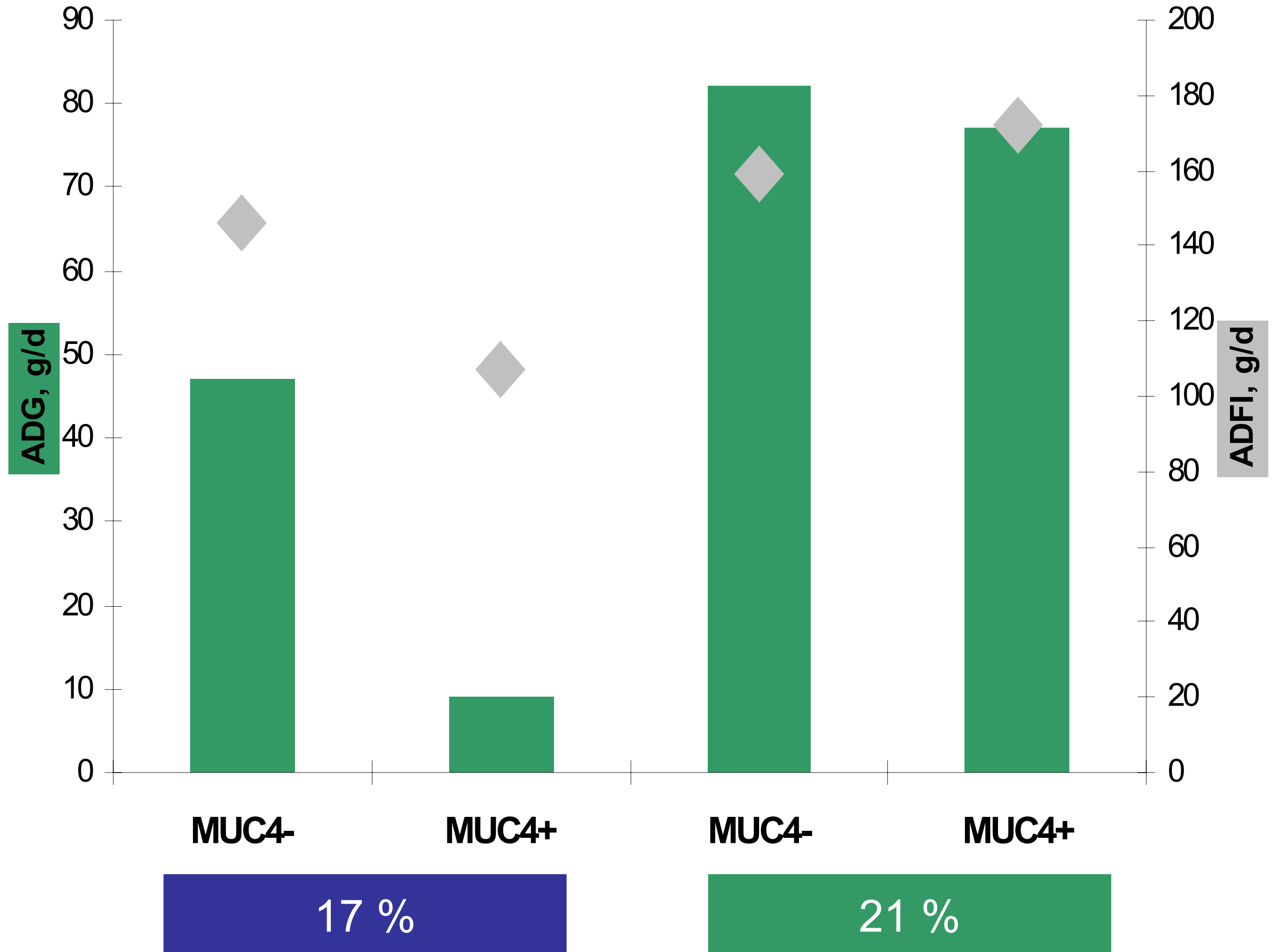
# After challenging piglets with E. coli K88, L-Trp supplementation (S) limits the decrease in feed intake and ADG




(Trevisi et al., 2007)

# L-Trp alleviates PW growth lag also in healthy piglets if they are genetically susceptible to E. coli K88 (Trevisi et al., 2010)

**1st wk post-weaning, results according to the gene marker MUC4**



# Summary on Trp

- Piglets susceptible to pathogen E. Coli K88 are more sensitive to a TRP dietary deficiency.
- Going from 17% to 21% SID Trp:Lys enhances feed intake and body weight gain of pigs from 21 to 42 days of age.
- In a group of animals, L-Trp supplementation secures the diet and allows to obtain the best performances

# The case of Zinc oxide for piglets

- FARMACOLOGICAL doses of zinc oxide (3g/kg diet) improve growth and/or reduce diarrhea (in most researches)
- Need of alternative similar solutions to overcome the excess of Zn in effluents and in liver
- But explanations are conflict
- May be also other Zn sources (Mavromichalis et al., 2001; Buff et al., 2005) or ZnO preparations (Kim et al., 2010), but currently not diffused

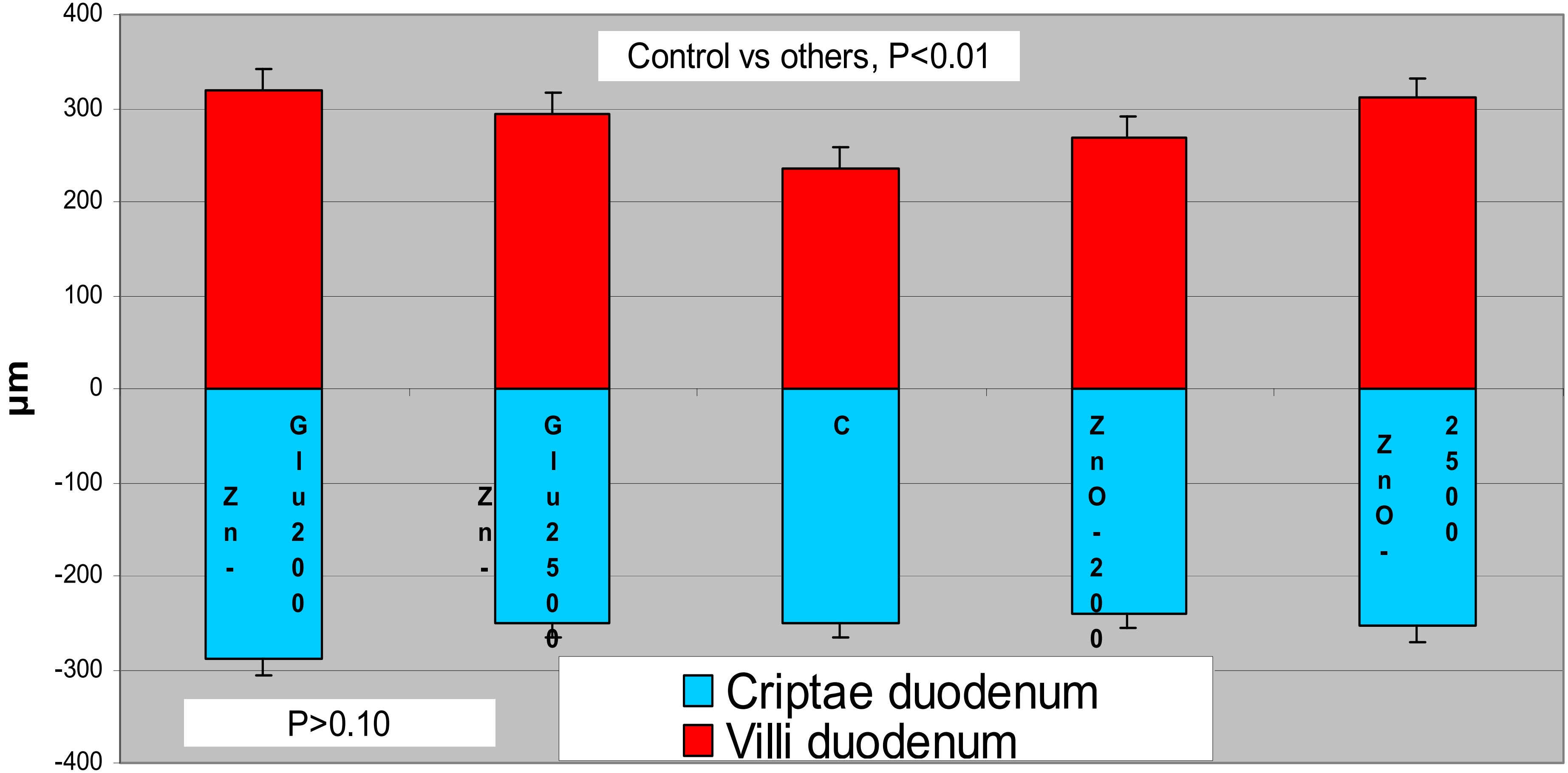
# Possible mechanisms of supra-nutritional ZnO

- Antimicrobial, but higher dose required to protect enterocytes against E.coli K88 *(Roselli et al, 2003)*,
- and effect additive with antibacterial agents carbadox *(Hill et al, 2001)*
- Improves enterocyte resistance to enteropathogens *(Roselli et al, 2003, Zhang et al., 2009)* and abrogates the translocation of lactic acid bacteria*(Broom et al, 2006)*
- Stabilize gut microbiota (*Katouli et al, 1999*)
- Better repair of villi, because Zn stimulates IGF-1 *(Tarnow et al, 1994)* or IGF1R *(Li et al, 200)*
- Less intestinal $Cl^-$ secretion elicited by inflammatory pathway in human entereocytes *(Berni Canani et al. 2010)*
- Reduced expression of genes associated with inflammation *(Sargeant et al, 2010)* (antimicrobial effect?)

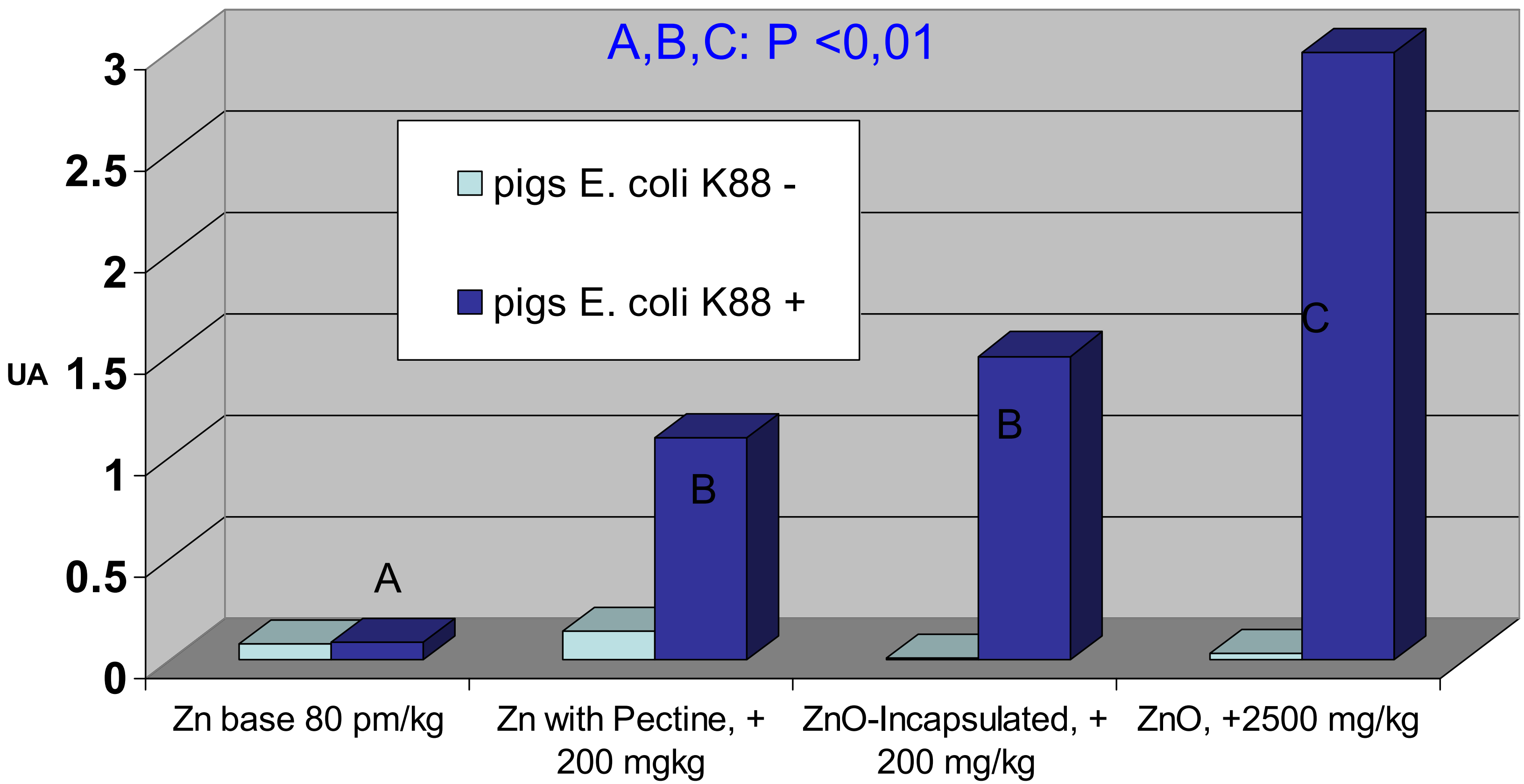
# Dietary addition with different forms (ZnO and zinc-glutamate chelated) and levels (200 & 2500 mg Zn/kg feed) in K88-challenged pigs (Bosi et al., 2003)

- did not affect growth
- combined reduction of faecal excretion and IgA production suggested a direct action of zinc in the gut, and that this action does not depend on Zn source
- Zn liver, strongly increased with Zn addition
- More research required to test preparation efficient at lower doses

# Effect of chemical form (ZnO or Zn-glutamate) and dose (+200 or +2500 mg Zn/kg) of supplemented zinc on measures of duodenal villi and criptae (µm) of E.coli challenged piglets (Mazzoni et al., 2010).



# Effect of the source and level of added zinc, and of susceptibility to intestinal adhesion of E. coli K88 on IgA K88-specific in plasma of pigs weaned and challenged with *E. coli* K88.



# Conclusions

- Worldwide trend to reduce in feed-antibiotic
- More microbial disease pressure on the gut of farm animals.
- More research on dietary tools to manipulate gut microbial colonisation
- and to strengthen the intestinal barrier function, robustness and immunological competence of young animals
- Consider animal genetic variability

# ..and..

- Urgent for animals more exposed to stress, like in early life, weaning and abrupt changes of environment
- No magic bullet, but an integrated approach,combining
- Use of various additives
- Better knowledge on nutrients requirements
- Improved management