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Probiotics – Do they have a role in the pig industry? EAAP Congress Dublin 2007





Outline of presentation

- Gut bacteria rôles
- Introduction to probiotics
- Mechanisms of action
- Examples from Feed For Pig Health Project
- Large Scale trials of Probiotics (literature)
- Practical considerations of delivering probiotics to pigs
- Conclusions



An Introduction To Gut Bacteria

10¹³ bacteria in the intestine

500 - 1000 species of bacteria

100 X more genes than in the human genome

- Hydrolytic enzymes (e.g. glycosidases)
- Vitamin production
- Detoxification of harmful substances
- Exclusion of dangerous pathogens (active or passive)
- Reduce ammonia/amine production

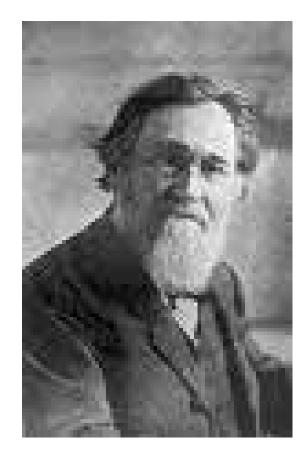
Mice with no gut bacteria have **60% less body fat** but **increased** food consumption – Gut bacteria facilitate more efficient use of food.



Probiotics - History

- "The Prolongation of Life"
 (London, William Heinemann
 1907)
- Bulgarians eating lots of yogurt have longer lives
- Due to modulation of bacterial communities in the colon

 Also proposed removal of colon as a good thing !



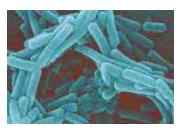
Elie Metchnikoff (1845-1916)



Probiotics – what are they?

"Live microbial cultures given to animals with the intention of improving health or production parameters."

Bacteria e.g.

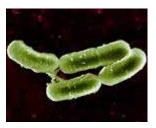


Lactic acid bacteria (e.g. Lactobacillus spp.)

Bacillus

Enterococcus





Yeast - Saccharomyces





If probiotics work – how do they work?

Mechanisms are varied (probably synergistic):

- Enhance host defences against pathogens innate and adaptive immune system, improve gut barrier function
- Microbe-microbe interactions

specific biocidal products (bacteriocins) or alteration of environment (lactic acid, local pH) may reduce levels of undesirable bacteria

➤"competitive exclusion"

In the second digestibility of feed – healthier animals grow faster, more resistant to disease



Problems using live organisms (not chemicals)

- Storage/treatment sensitive
- May change with time (gene switching)
- Similar strains may have very significantly different characteristics

Benefits

7

- "Natural"
- Safe
- Multiple benefits (health, nutrition, meat quality??)



Isolating/Assessing Potential Probiotics

- Vlasta Demeckova and Peter Brooks (University of Plymouth, UK) have isolated and characterised many lactic acid bacteria from pig faeces. Ideal organisms for fermented liquid feed applications
- Characterised for ability to:
 - ferment pig feed rapidly
 - produce mainly lactate (little acetate)
 - bind to pig gut epithelium
 - > aggregate E. coli
 - > inhibit adhesion of *E. coli* to gut epithelia



Feed For Pig Health – assessment of probiotics

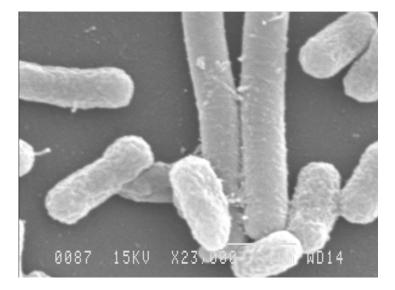
- Lab-based:
 - Able to kill/inhibit pathogens
 - Interact positively with gut wall and immune system
 - Change gut microbial environment
- Whole animal (small scale):
 - No large negative effects on growth
 - Protect against disease challenge
- On-farm Studies:
 - Effect on performance characteristics
 - Different production stages: pregnancy, lactation, weaning, growing



Probiotics – *in vitro* effects on pig pathogens

Ability to kill/inhibit pathogens

in vitro killing of pathogens such as *Salmonella* or *E. coli* can be indicative but *in vivo* situation may be very different – biofilms, cooperation between different gut bacteria.



Lactobacillus aggregating E. coli



Probiotics – *in vitro* effects on pig pathogens

Interaction with pig gut wall

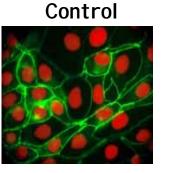
- Cell cultures of gut epithelial cells provide an excellent model system. Pig gut epithelial cell lines are available.
- Enterotoxigenic *E. coli* (ETEC) causes disruption of cell layer integrity (analogous to gut situation). Addition of Lactic Acid Bacteria (LAB) can prevent this process.

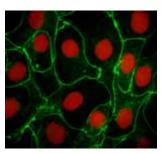


Protective effect of Lactobacillus sobrius

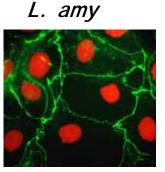
LABs protect tight junction integrity

Occludin immunofluorescence





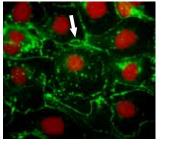
L. sobrius

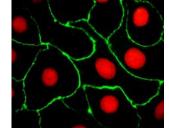


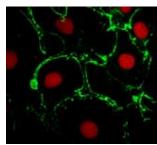
ETEC (E)

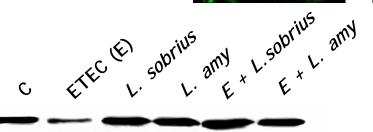
E + *L. sobrius*

E + *L. amy*









Occludin





Western blot analysis

Probiotics and post-weaning colibacillosis

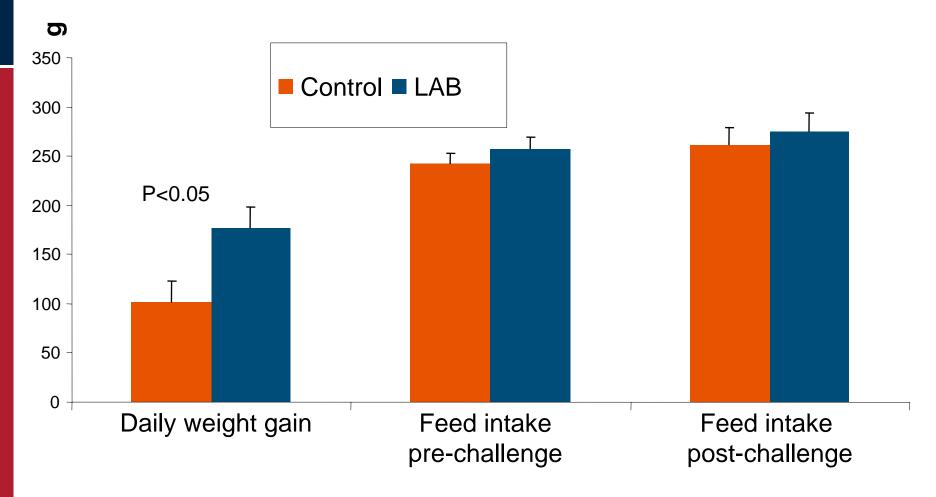
Pigs fed *Lactobacillus sobrius* ¹ (LAB - 10¹⁰ bacteria per day) for seven days post-weaning then challenged with ETEC.

	Control (cfu/ml)	LAB diet (cfu/ml)
Total Bacteria	10 ¹⁰	5 ×10 ⁹
L. sobrius	10 ⁴	10 ⁸ *
ETEC	10 ⁶	10 ⁴ *

¹L. sobrius appears to be an important member of the gut community of healthy young pigs!



Effect of *L. sobrius* on growth and feed intake of *ETEC* challenged pigs (7 days post-weaning)



Post-weaning growth check reduced



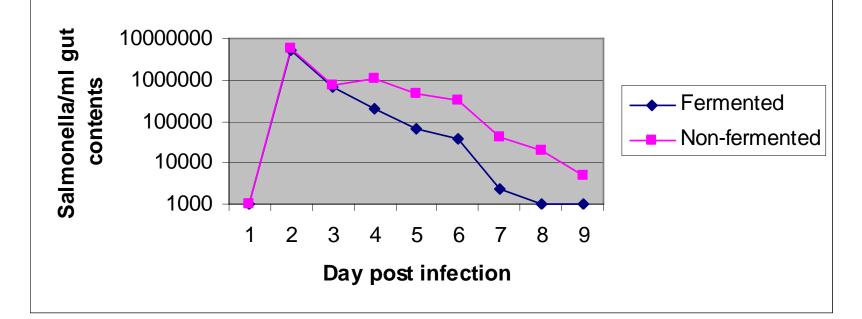
Fermented liquid feed

- Natural or using a "starter"
- Ferment total mix or component (cereal)
- Good Points
 - Lactic acid
 - Bioavailability
 - Bactericidal (pathogens)
- ? Bad Points
 - Reproducibility
 - "Bad" fermentation (ethanol, aversion)
 - Lysine reduced



Do probiotics affect Salmonella carriage?

Effect of fermented feed on carriage of Salmonella Typhimurium in piglets



Carriage of Salmonella reduced in FLF group Translocation into lymph nodes reduced in FLF group



Large scale trials – problems of design

Finishing pigs – Fermented vs "control"

- ~1000 pigs kept from 30-100kg
- Liquid diet with cereal fraction fermented in test group
- Fermented with *Pediococcus acidilactici* (commercial starter)
- Control cereal treated under same conditions but no starter
- Feeding effectively ad libitum

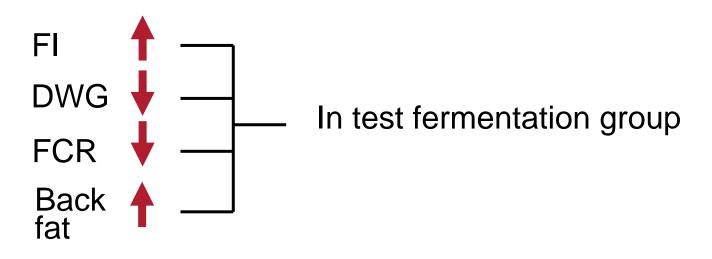
	рН	Lactate (mM)	LAB/ml
Control	4.7	84	2x10 ⁹
Fermented	4.3 (~3)	98 (~200)	3x10 ⁹ (10 ¹¹)

NB - Both feeds fermented – (But not much!!)!

http://www.bpex.org/technical/publications/pdf/FinishingPigs Trial_3_Report.pdf



Large Scale Trial – Finishers - Results



BUT BOTH feeds were fermented!

"Control" naturally fermented

"Test" Starter driven



Large Scale Trial – Finishers - Conclusions

- Does NOT mean that ALL fermented feeds are useless!
- Did not compare non-fermented feed
- One organism (low dose, limited fermentation)
- High health status and welfare environment pigs
- Finishing pigs (what about weaners and farrowing sows?)



Large Scale Trials – Nursery Pigs*

- Comparing the efficacy of probiotics (10¹² cfu Bacillus licheniformis and Bacillus subtilis per tonne of food) with a conventional antibiotic regime (400 ppm neomycin, 1 week, 100ppm neomycin + oxytetracycline, 1 week, 20ppm tylosin 7 weeks) for combating *E. coli* diarrhoea
- ~22,000 piglets
- Study period 20 70 days

* Kritas and Morrison (2005) Vet Record 156:447-448



Large Scale Trials – Nursery Pigs: Results

- Bodyweight, ADG, ADFI and FCR similar in both groups
- Cost of feed per pig and per kg bodyweight gain similar
- Mortality rate and causes similar

Conclusion.

This probiotic mix gives similar results, at no increased cost, to the regime using sub-therapeutic levels of antibiotics. Environmental benefits should encourage further studies

Large Scale Trial – Farrowing

• 109 gilts

Test ~10⁶ spores of *Bacillus licheniformis* and *B. subtilis* per gramme of feed.

Treat <u>sows</u> 14 days prior to farrowing \rightarrow weaning Food consumption postpartum

Weight loss postpartum Piglet diarrhoea Pre-weaning mortality Piglet body weight at weaning



Alexopoulos et al. (2004) J. Animal Phyiol Nutr 88:381-392

Delivery of Probiotics

- Freeze-dried additives
- Heat treated pelletted in feed
- Incorporated into water
- Fermented liquid feed
 - Natural
 - Whole feed or component
 - Starter Batch/Continuous



Conclusions

Choose the right probiotic for right conditions

- A particular probiotic organism may be excellent in a sub-optimal population of pigs but insignificant in a high health status herd
- Probiotics may be excellent in controlling postweaning diarrhoea but may show minimal effects in growing/farrowing/lactating pigs
- Control of storage/fermentation conditions are critical to ensure reproducibility



THANK YOU!

EU Framework 6

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