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Influence of feeding and management on gut microbiota and barrier function

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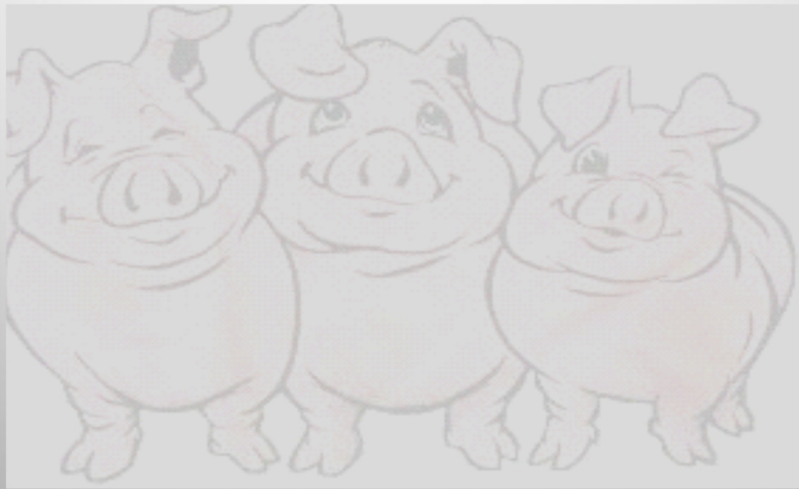
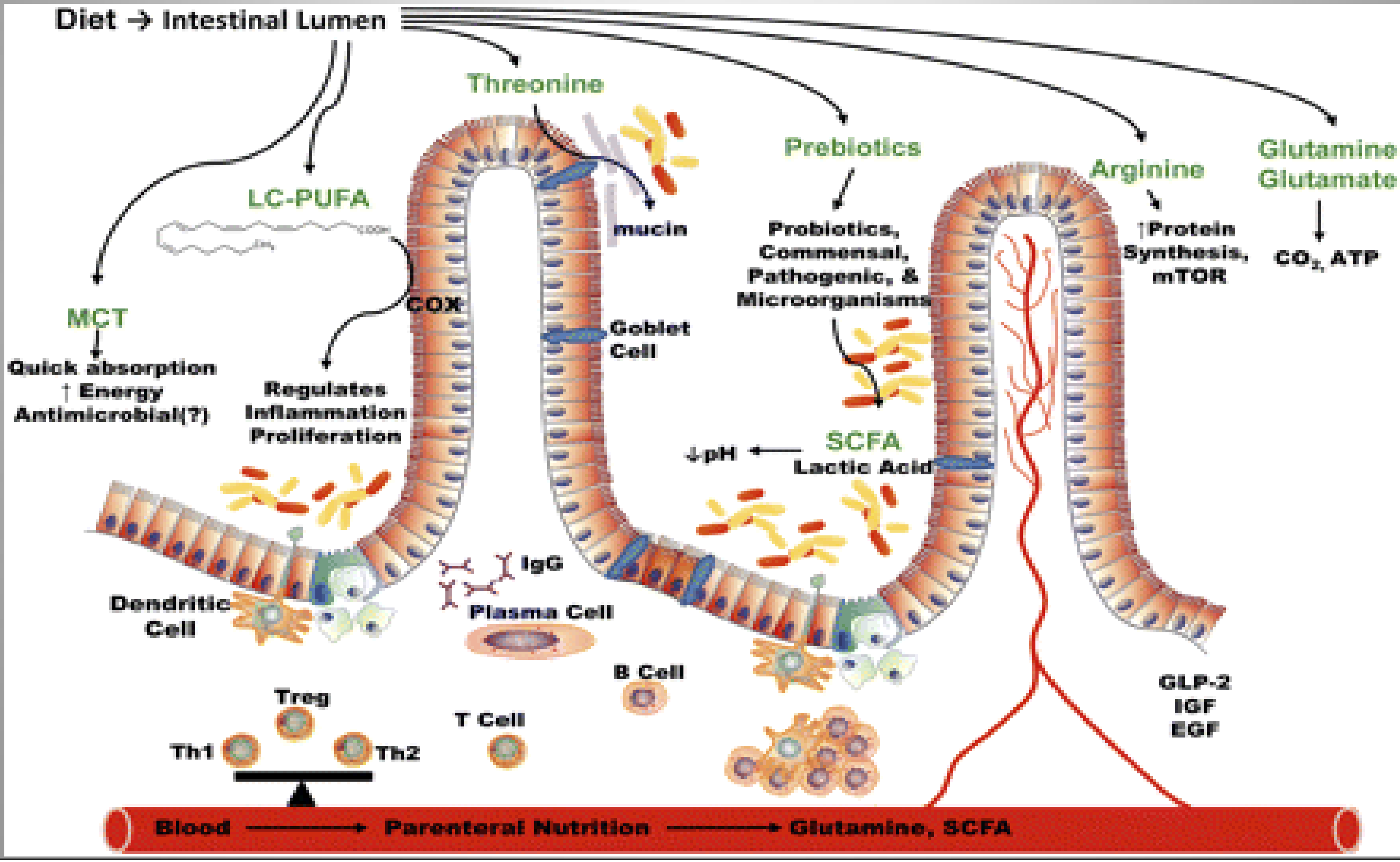
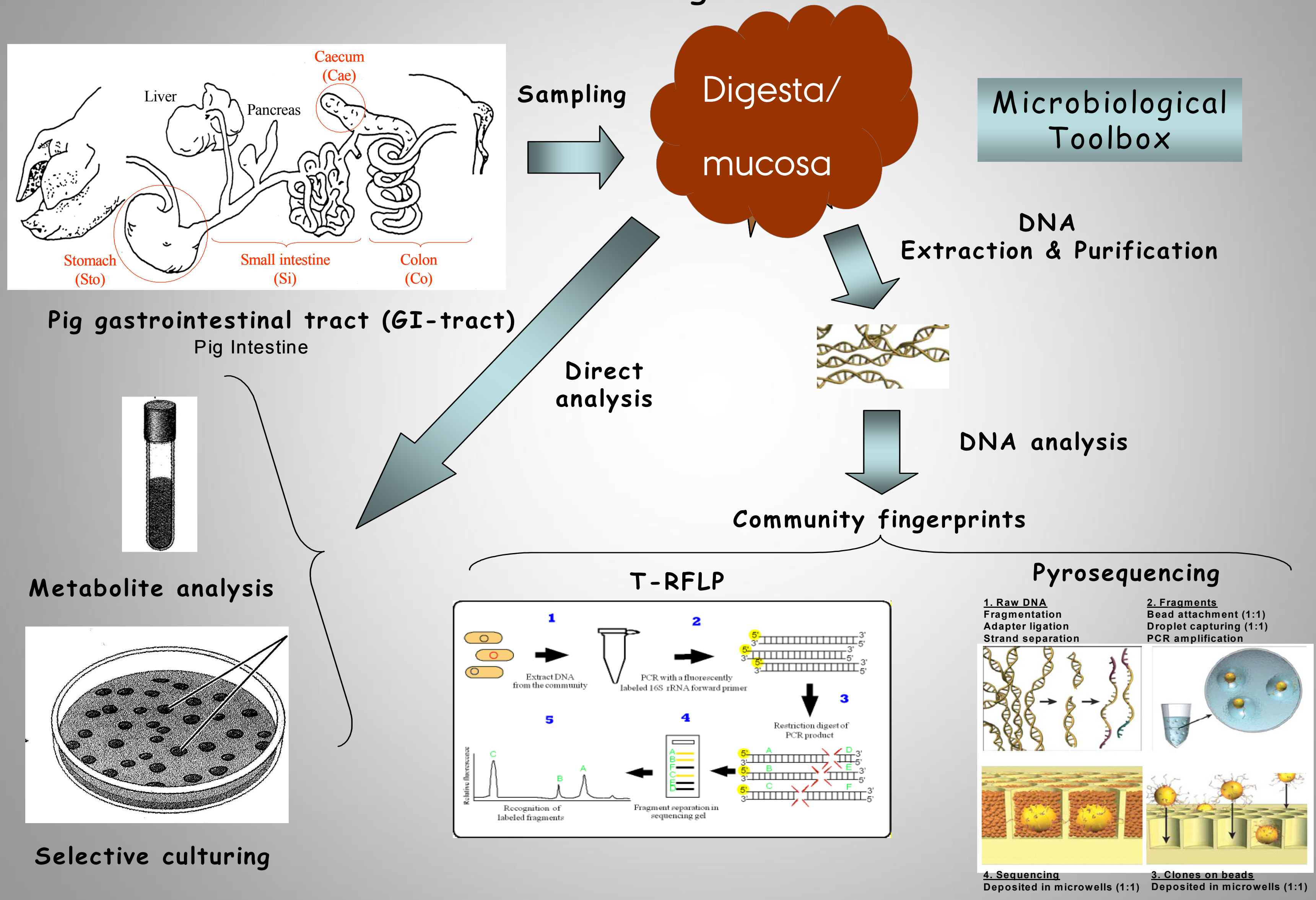


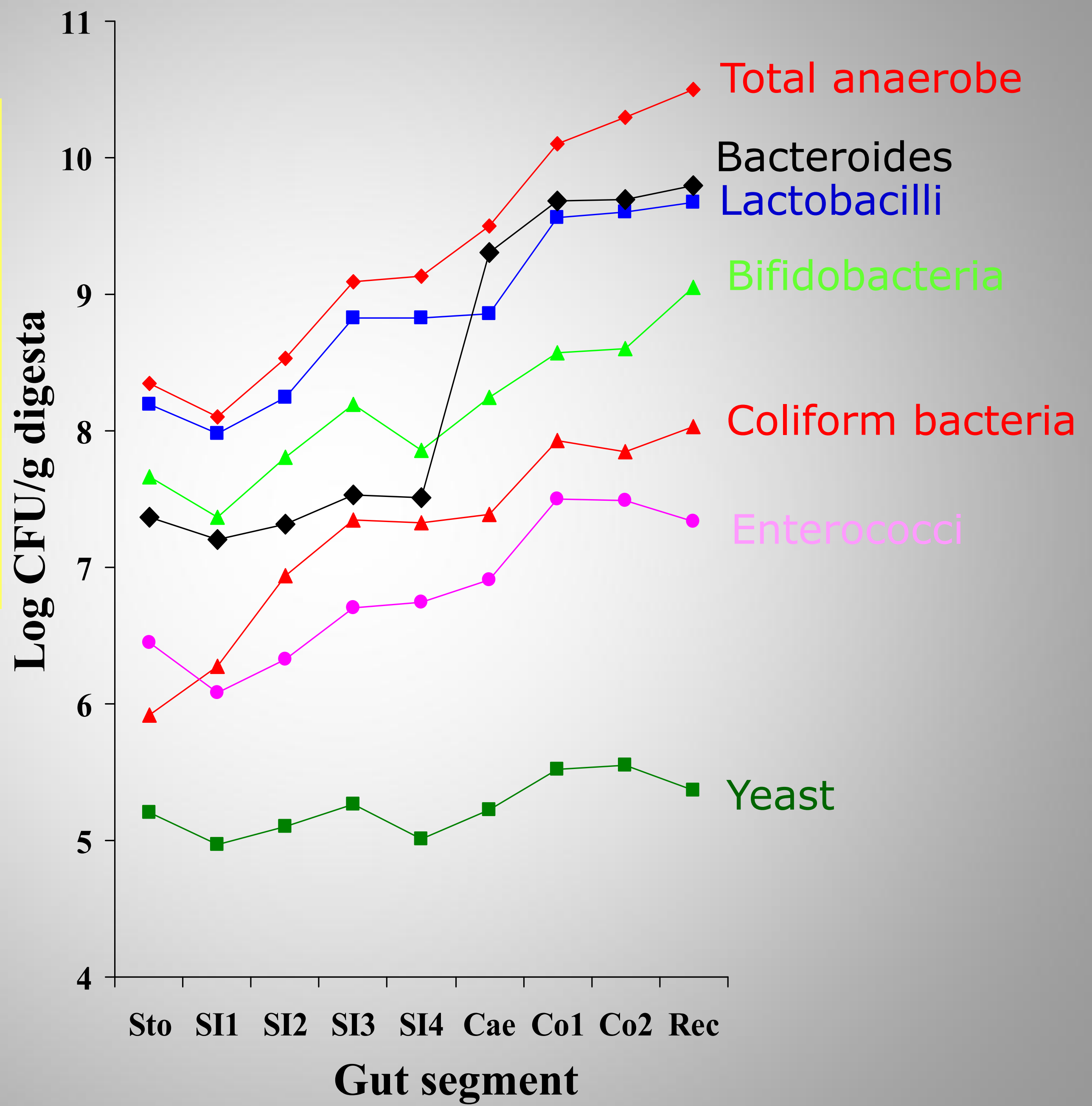
Mechanisms by which diet and feed additives may modulate the intestinal ecosystem and barrier function



Microbiological toolbox



Density of selected groups of cultivable bacteria in various section of the gastrointestinal tract of slaughter pigs



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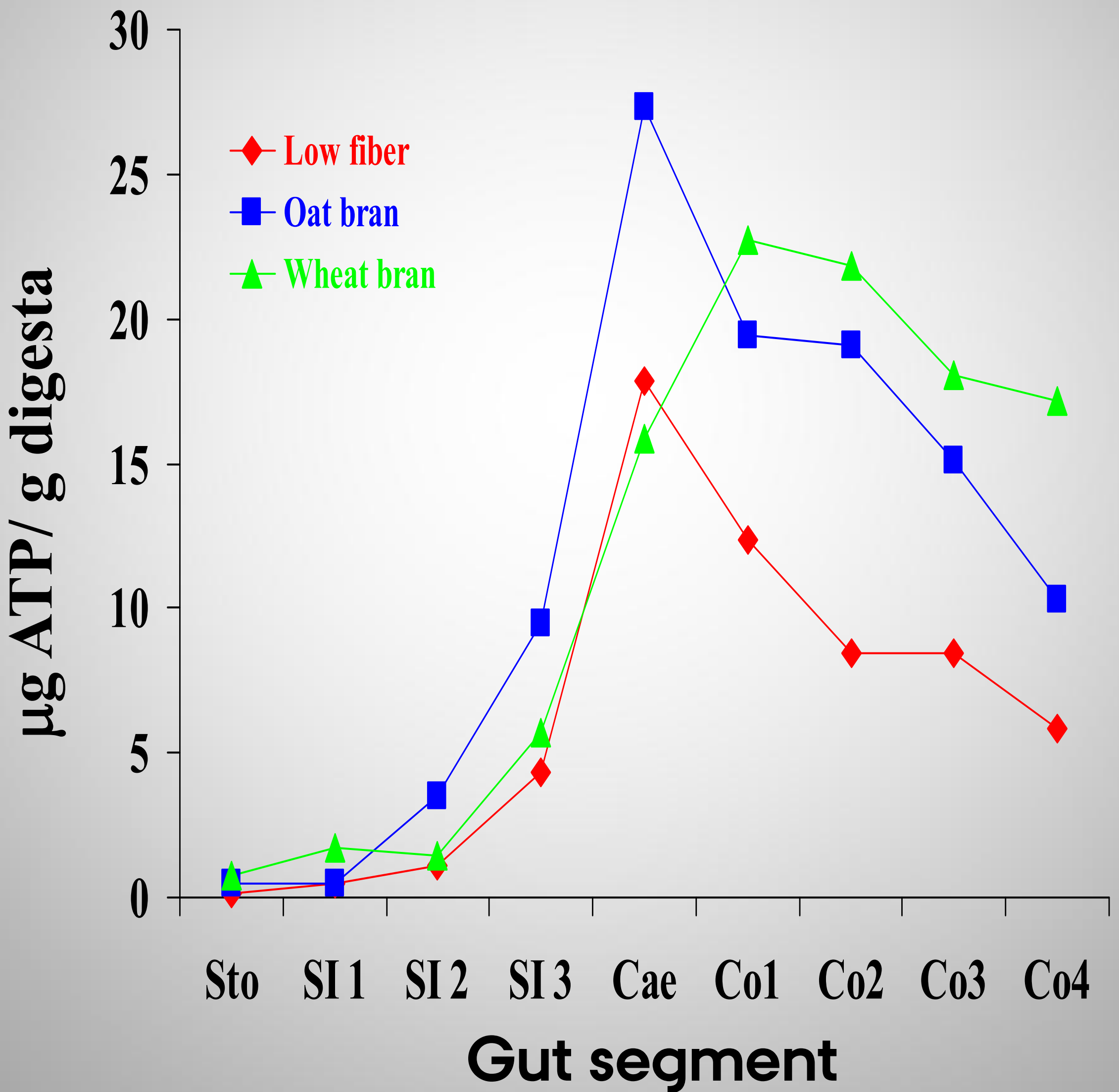
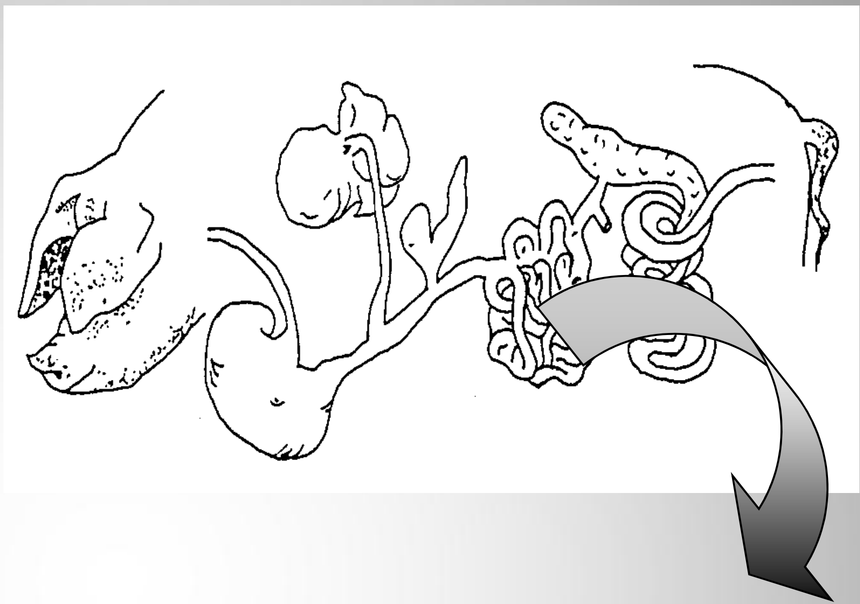
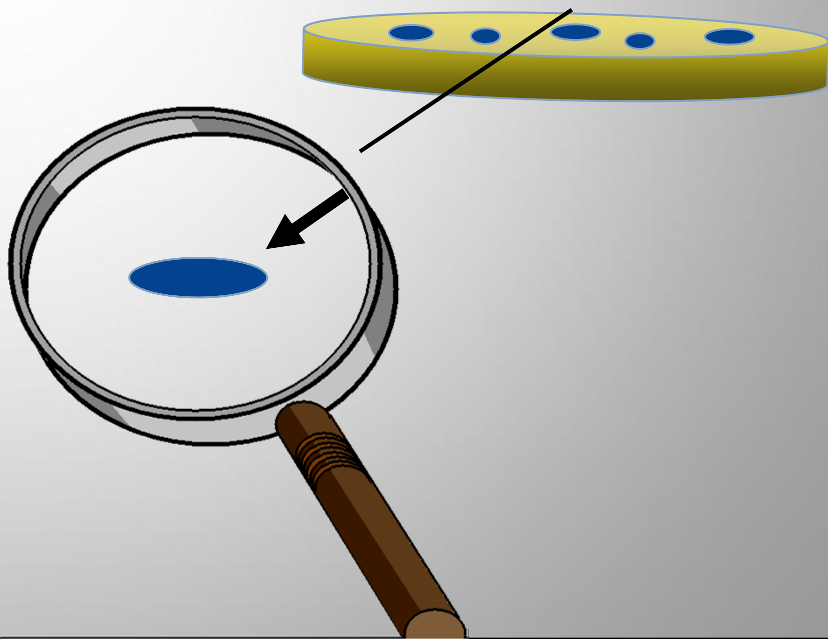
Microbial activity in various regions of the gastrointestinal tract of pigs



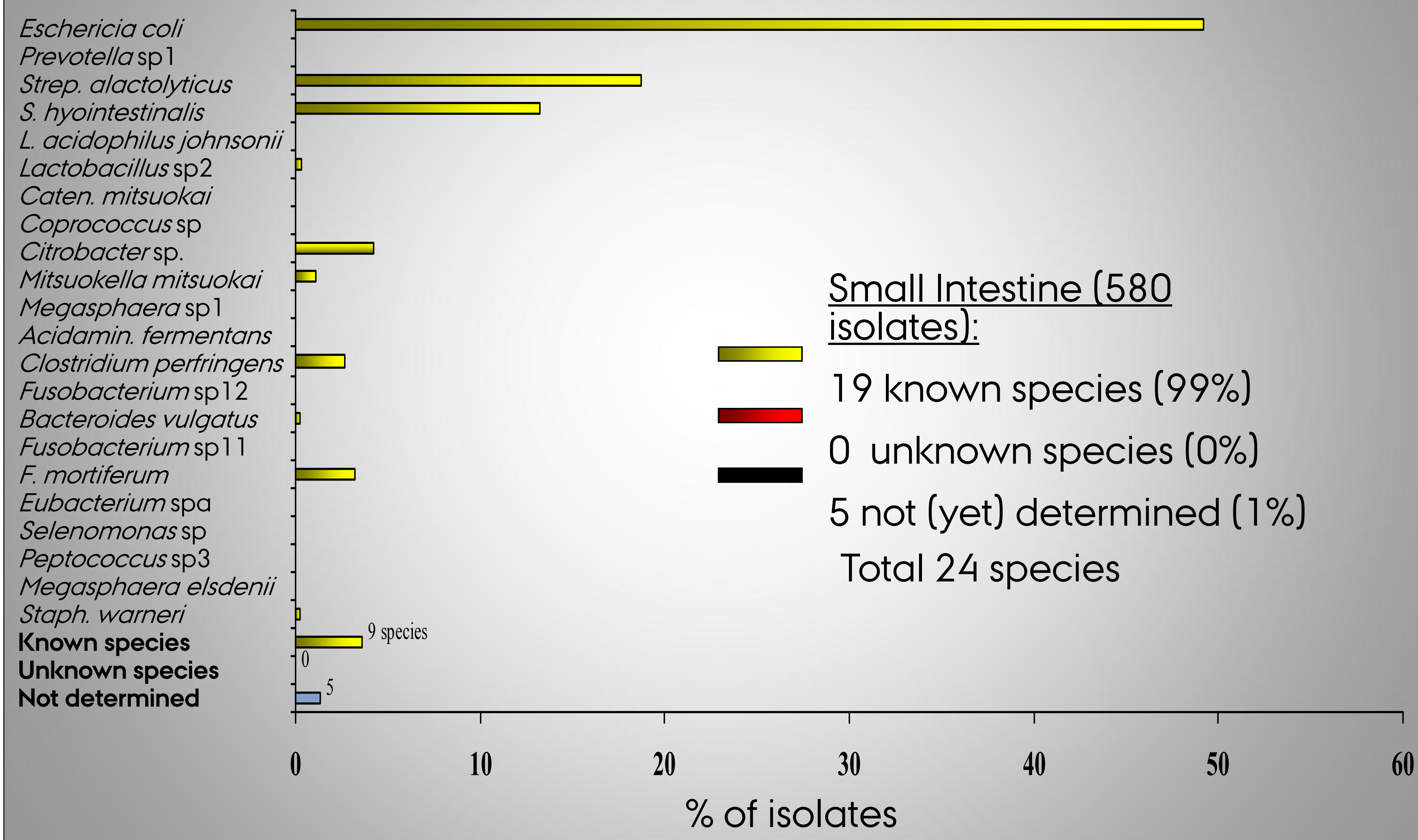
Characterise the culturable bacteria in the gastrointestinal tract of pigs



Are we able
to cultivate gut bacteria ?



Relative distribution of isolates on species



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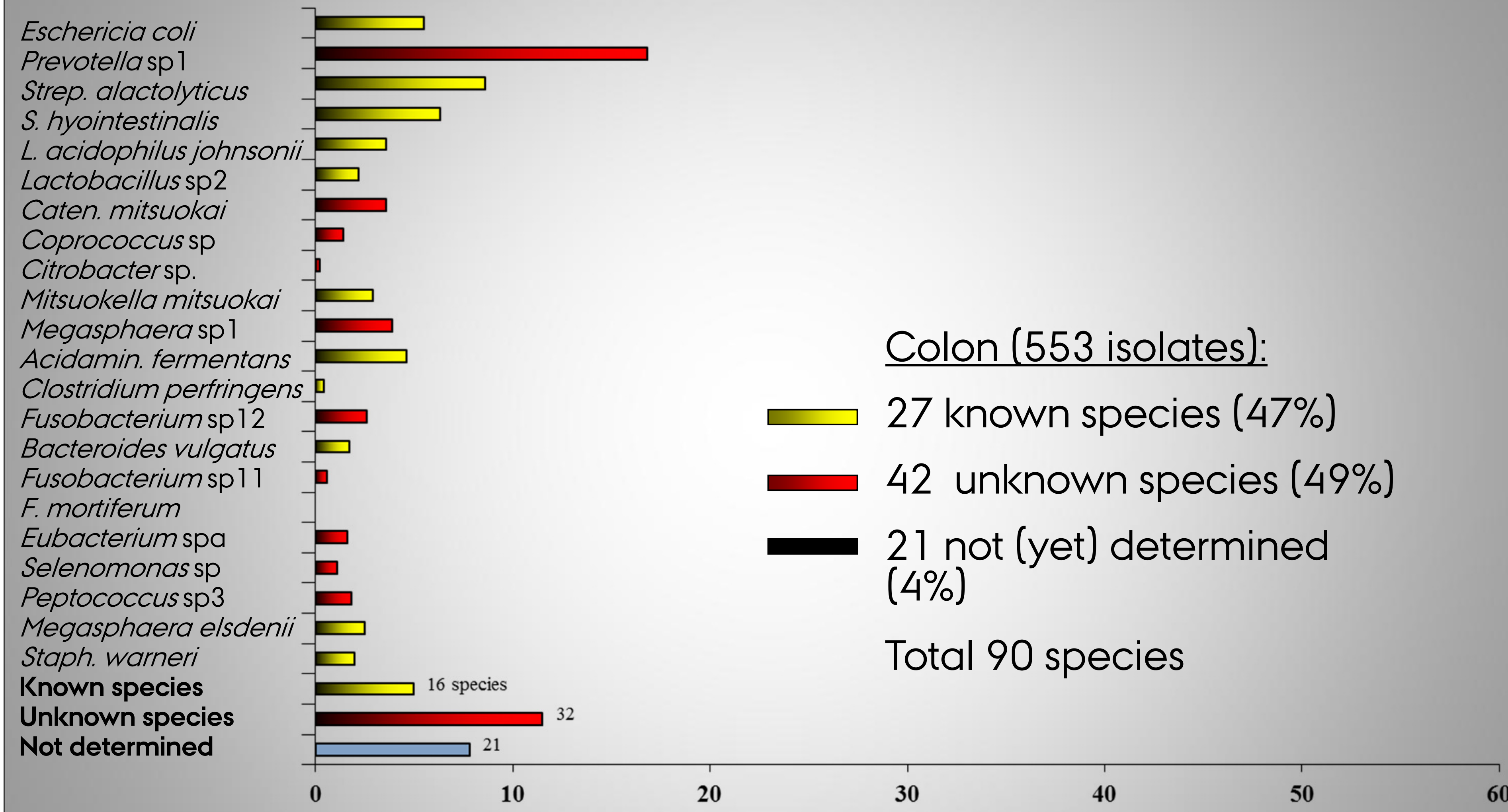

Colon (553 isolates):

27 known species (47%)

42 unknown species (49%)

21 not (yet) determined (4%)

Total 90 species

% of isolates

16S rDNA match of predominant colon isolates

Isolate/species	Proportion of isolates (%)	GenBank 16S rDNA match	Similarity (%)
Prevotella sp1	16.8	OTU 16	98,8
Streptococcus alactolyticus	8.6	*Streptococcus alactolyticus*	100
Streptococcus hyointestinalis	6.3	*Streptococcus hyointestinalis*	99.8
Escherichia coli	5.5	*Escherichia coli*	99.8
Acidaminococcus fermentans	4.6	*Acidaminococcus fermentans*	99.4
Megasphaera sp1	3.9	*Megasphaera elsdenii*	96.0
Lactobacillus acid. johnsonii	3.6	*Lactobacillus johnsonii*	98.7
Selenomonas sp1	3.6	URB 4C28d-14	98.1
Mitsuokella multiacidus	2.9	*Mitsuokella multiacidus*	98.7
Prevotella sp22	2.6	OTU 18	97.2
	58.4		

OTU: Uncultured Bacterium (Pig) **URB: Uncultured Rumen Bacterium**

Comparison between isolates and most dominant clones*

Clones (9270)			Isolates** (791)
Rank	Closest related species (similarity)	% of clones	% of isolates
1	Streptococcus alactolyticus (99.7)	7.9	22.8
2	Escherichia coli (100.0)	4.8	12.2
3	Lactobacillus sorbius (99.2)	4.5	12.0
4	Faecalibacteria prausnitzii (92.6)	4.0	<0.1
5	Lactobacillus johnsonii (99.8)	3.2	5.3
6	Faecalibacteria prausnitzii (97.6)	2.9	<0.1
7	Sporobacter termitidis (90.7)	2.4	
8	Gemella haemolysans (87.1)	2.1	
9	Lactobacillus reuterii (99.5)	1.9	4.7
10	Eubacterium rectale (92.6)	1.6	

* Data from Leser et al. 2002, ** Similarity to clones >97%

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Bacteroides-Prevotella cluster

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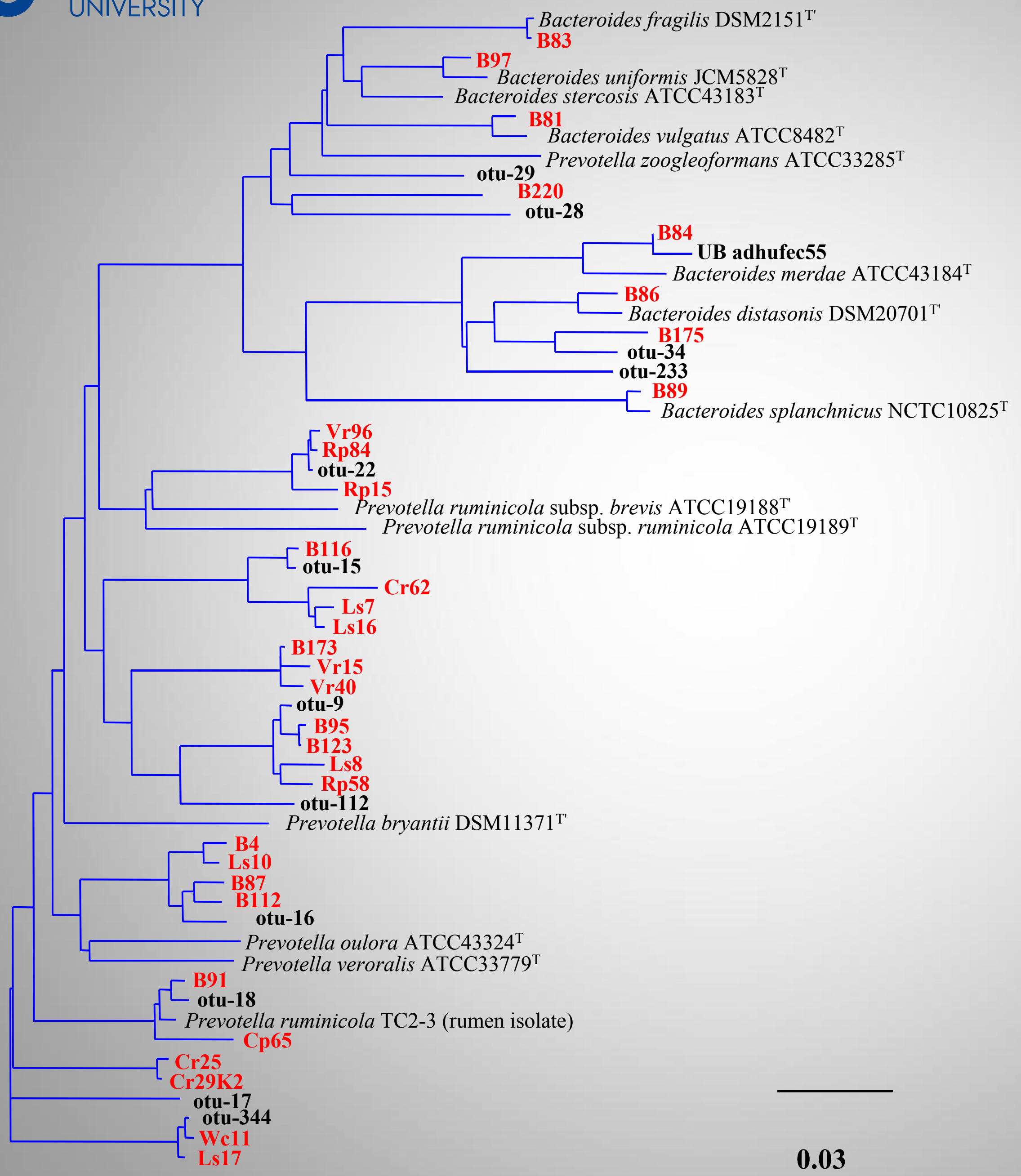

Isolates

Type strains (Strains)

Uncultured bacteria clones

Leser et al. 2002 (**otu**)
Suau et al. 1999 (**adhufec**)

(Algorithm: Neighbor-Joining)

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Bacteroides-Prevotella cluster

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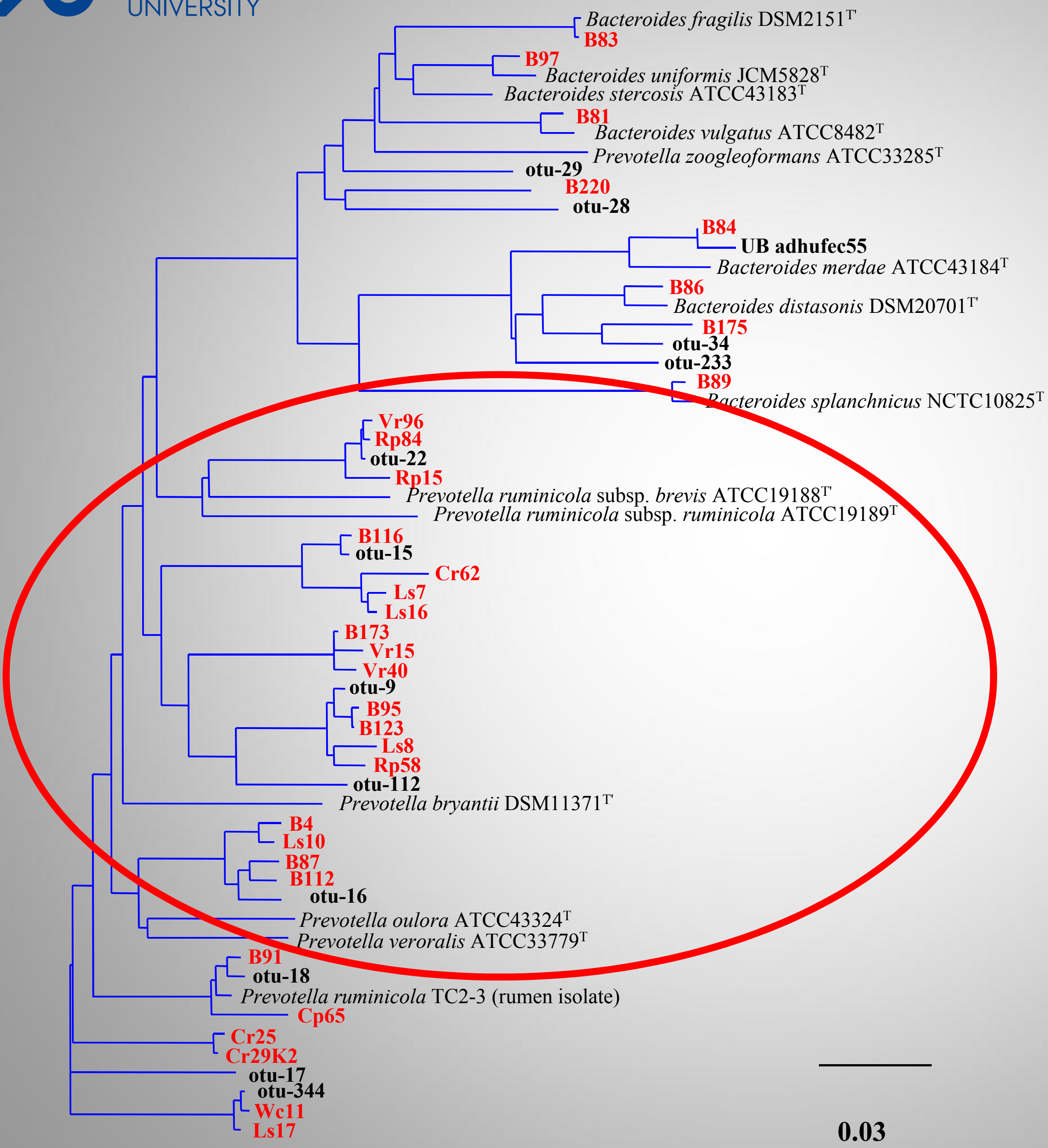

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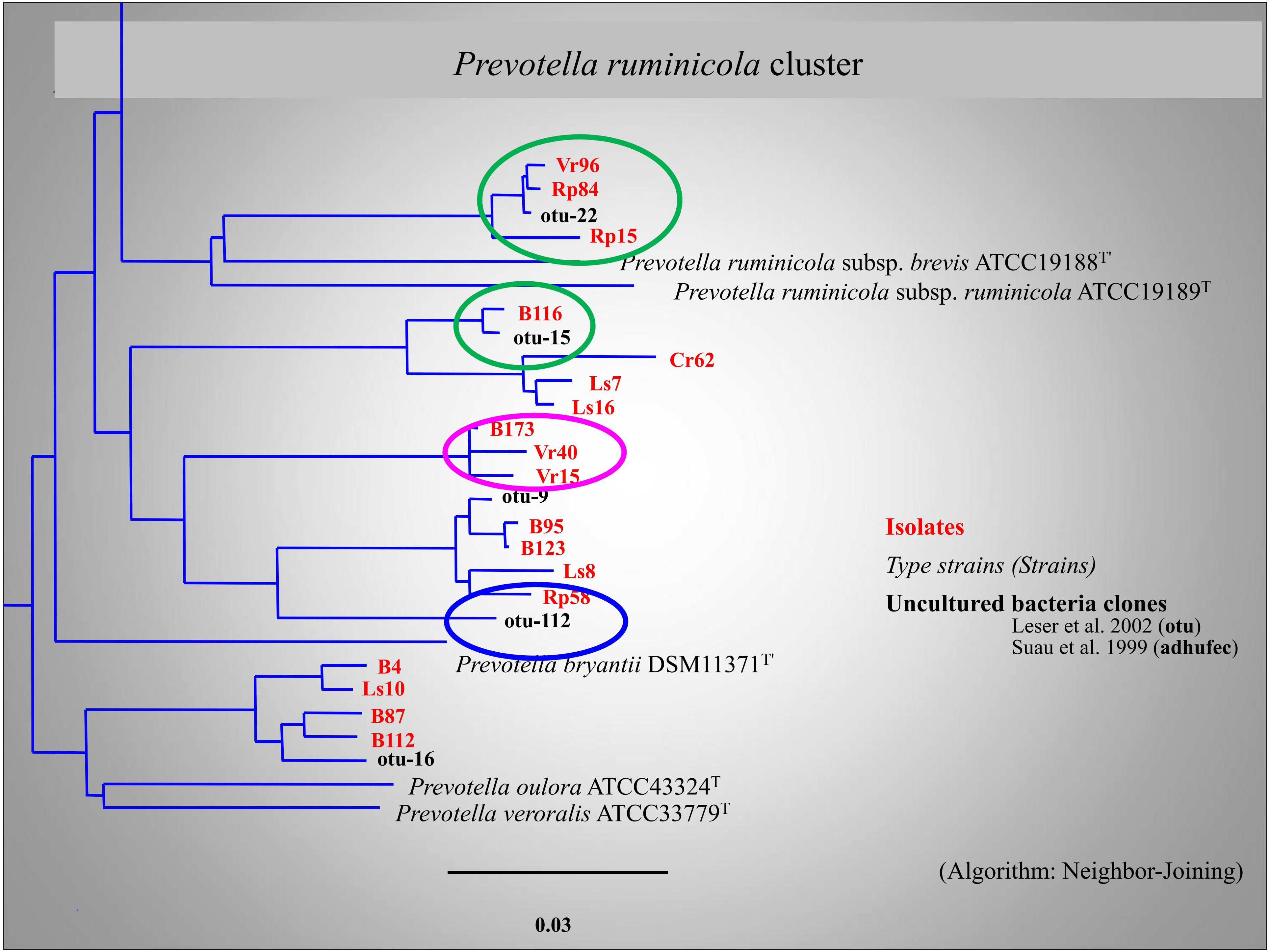
Prevotella ruminicola cluster
Vr96
Rp84
otu-22
Rp15
Prevotella ruminicola subsp. brevis ATCC19188T
Prevotella ruminicola subsp. ruminicola ATCC19189T
B116
otu-15
Cr62
Ls7
Ls16
B173
Vr40
Vr15
otu-9
B95
B123
Ls8
Rp58
otu-112
Prevotella bryantii DSM11371T
B4
Ls10
B87
B112
otu-16
Prevotella oulora ATCC43324T
Prevotella veroralis ATCC33779T
Isolates
Type strains (Strains)
Uncultured bacteria clones
Leser et al. 2002 (otu)
Suau et al. 1999 (adhufec)
(Algorithm: Neighbor-Joining)
0.03

Conclusion

- Defined >120 species from 2200 isolates
  > 60% no match to any known species

- Despite high diversity, 75% of the isolates were covered by eighteen OTUs (<1% of isolates each)

- Good agreement between our isolates and OTUs obtained from pig clone library

however some dominant pig clone library OTUs were not between our isolates

and some of our isolates could not be related to any of the OTUs in the pig clone library

Most pig gastrointestinal bacteria are cultivable

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Local Strain Collection of Gastrointestinal Bacteria

127 'Speci... perational Taxonomic Units (OTU)'

...dy known species

...ecies

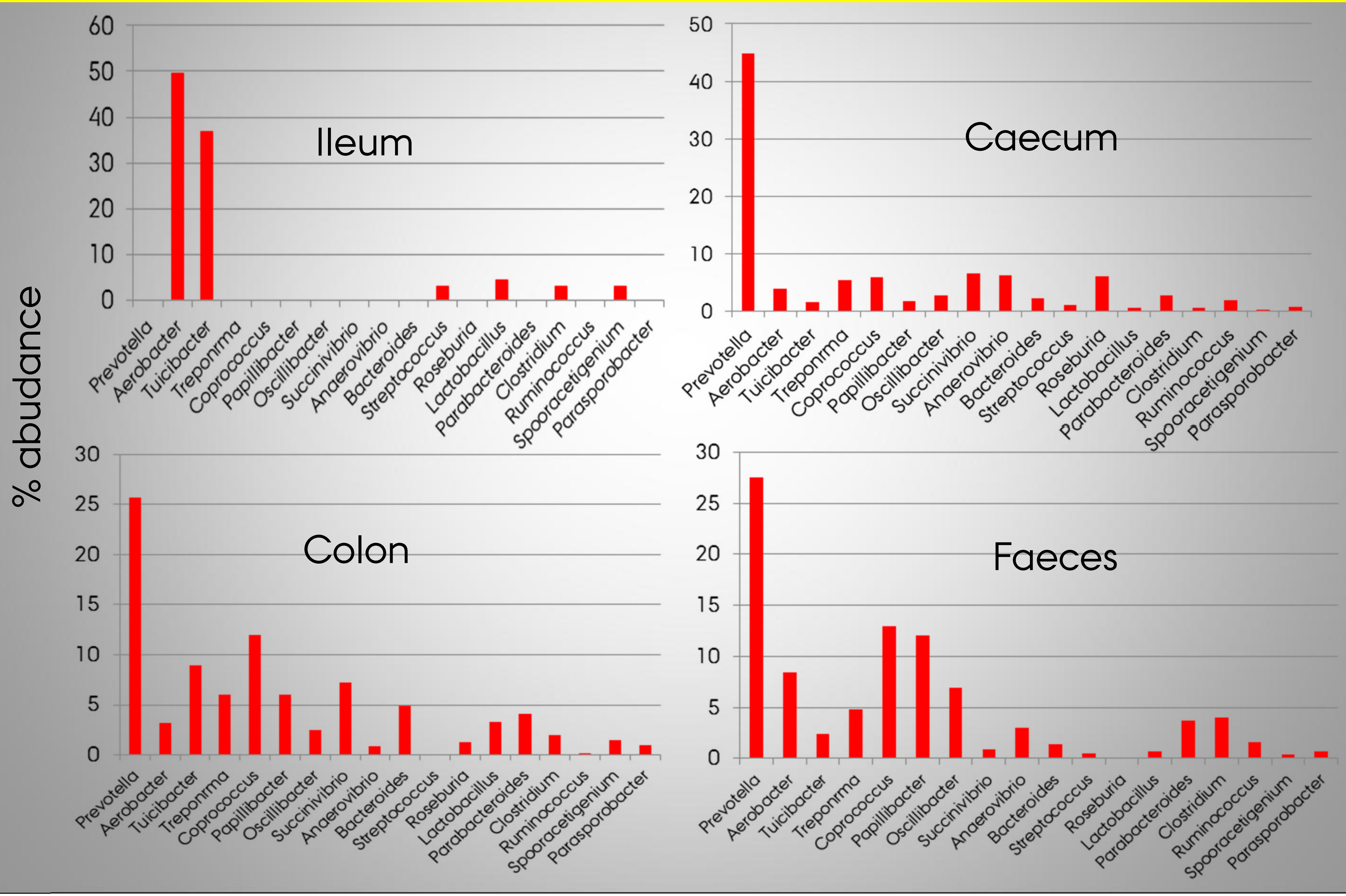
22 yet ...

Potential for studies on e.g.:

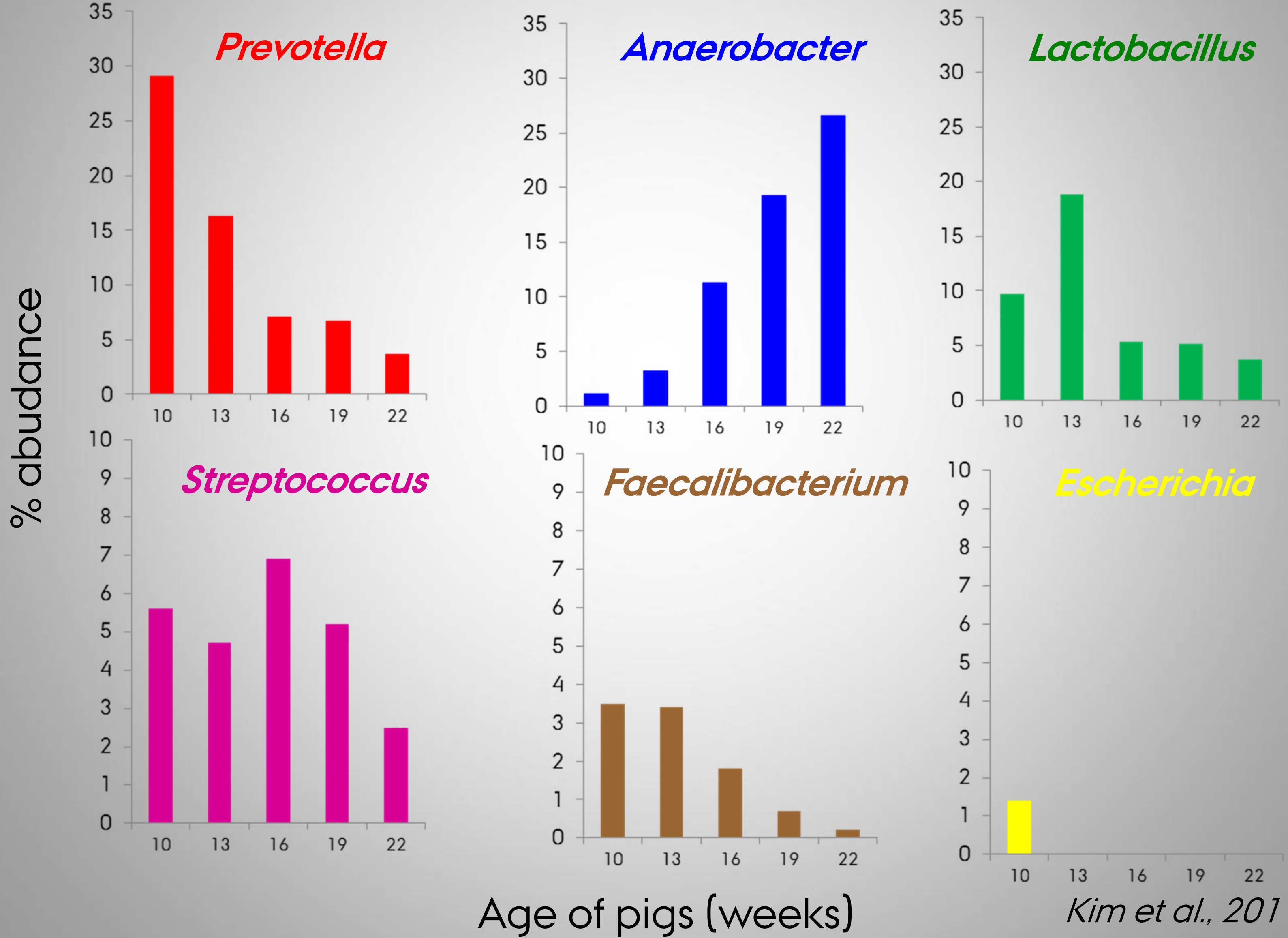
Probitotics

Host-microbe interactions

Abudance in most dominating bacteria genera in various sections of the gastrointestinal tract of pigs using pyrosequensing (Looft et al., 2014)



Age related change in bacterial populations in faeces of pigs



Kim et al., 2011

Important differences between the microbial ecosystem in the stomach/small intestine and the caecum/colon

Stomach and small intestine

- Few bacterial species (20-30)
- Low density/high activity
- Compete with the host for digestible components (use around 6% of the energy)
- Produce toxic compounds
- Prevent growth of pathogenic bacteria

Caecum and colon

- Many bacterial species (>400)
- High density/low activity
- Support the host with energy (around 16%)
- Produce toxic compounds
- May support growth of pathogenic bacteria

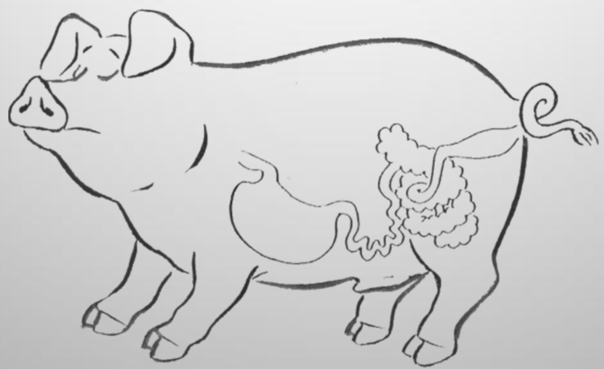
Factors that modulate the microbiota in the gastrointestinal tract

Feed

- Diet composition
- Feed processing
- Fermented liquid feed
- Prebiotics
- Low protein diets

Additives

- In-feed antibiotics
- zinc oxide
- organic acids
- probiotics
- Plant extracts/species



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Feed additives
Results from Danish weaner trails

Product type	Number of trails	Trails with significance	ADG (% increase)	FCR (% increase)
Antibiotics	13	8	+ 8,9	- 3,5
Acids/salts	53	16	+ 5,1	- 1,2
Acid/salts with significant effect	*16*	*16*	*+ 9,4*	*- 3,8*
Probiotics	17	1	+ 2,2	- 1,1
Aroma compounds	27	2	+ 2,2	-1,1
Enzymes	9	0	+ 2,1	0,0
Oligosaccharides	7	1	+ 2,3	- 1,2

Kjeldsen, 2008

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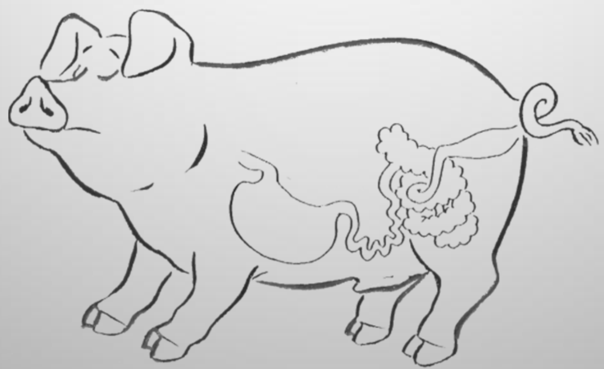
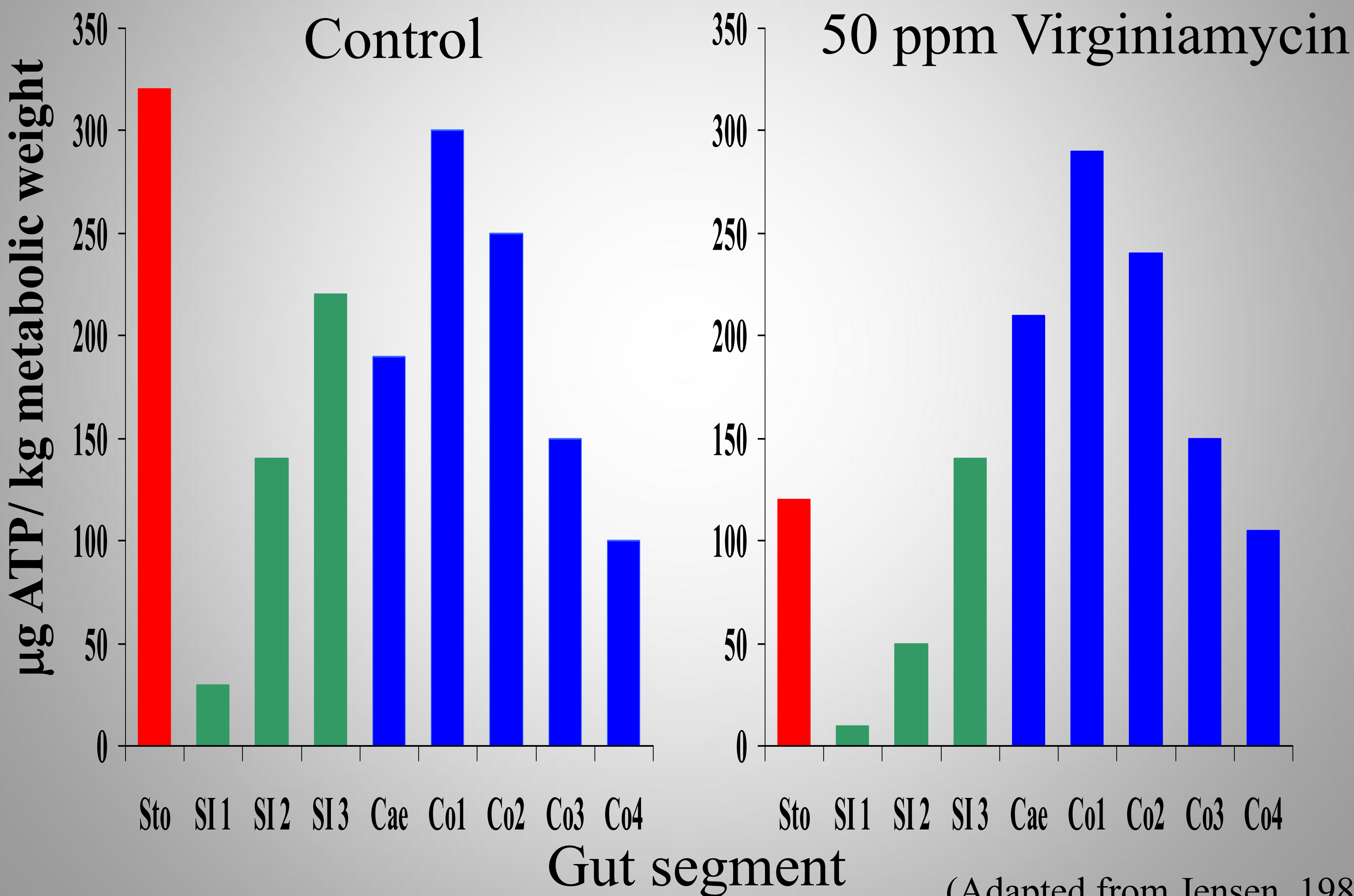
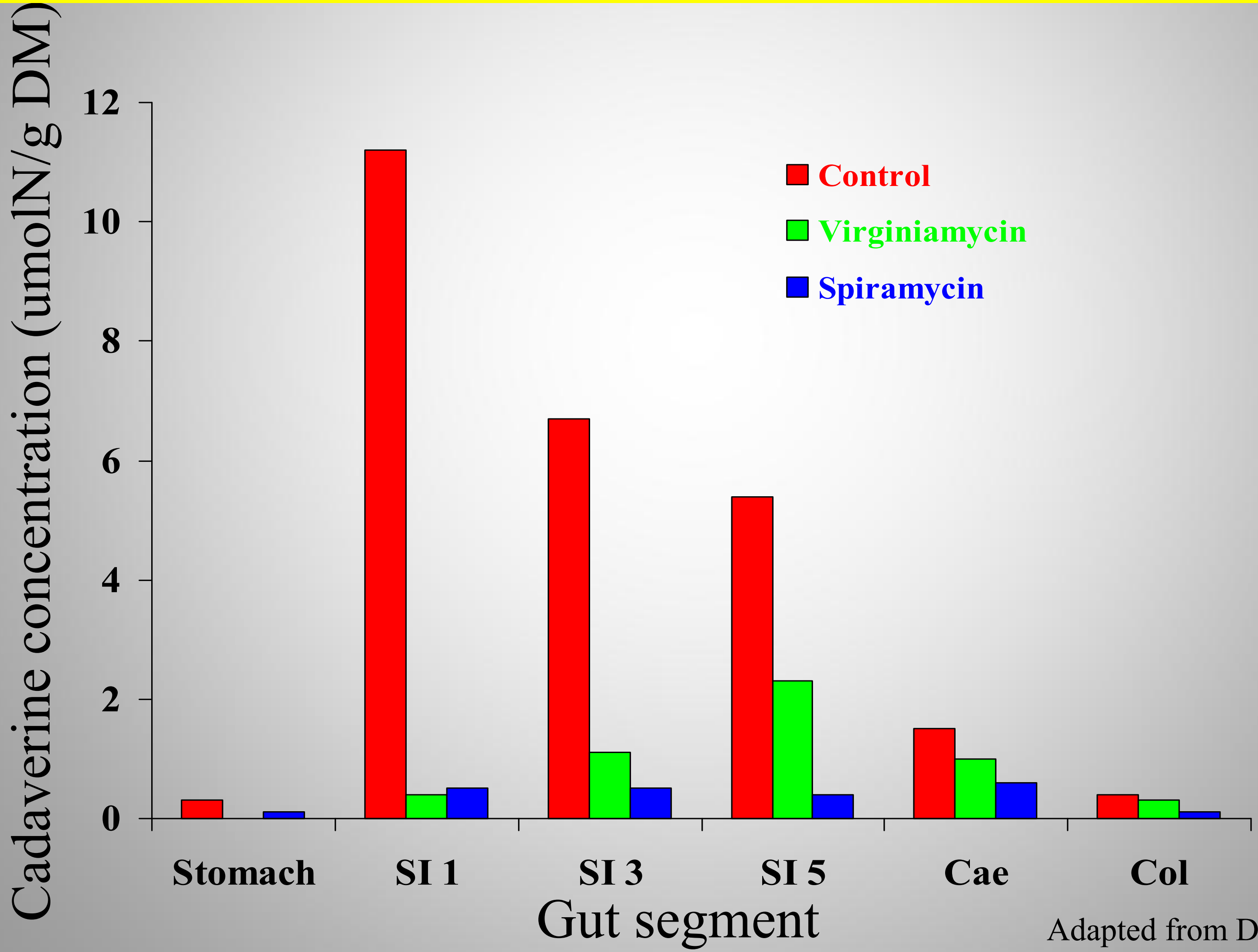


Effect of Virginiamycin on the microbial activity in various regions of the gastrointestinal tract of pigs



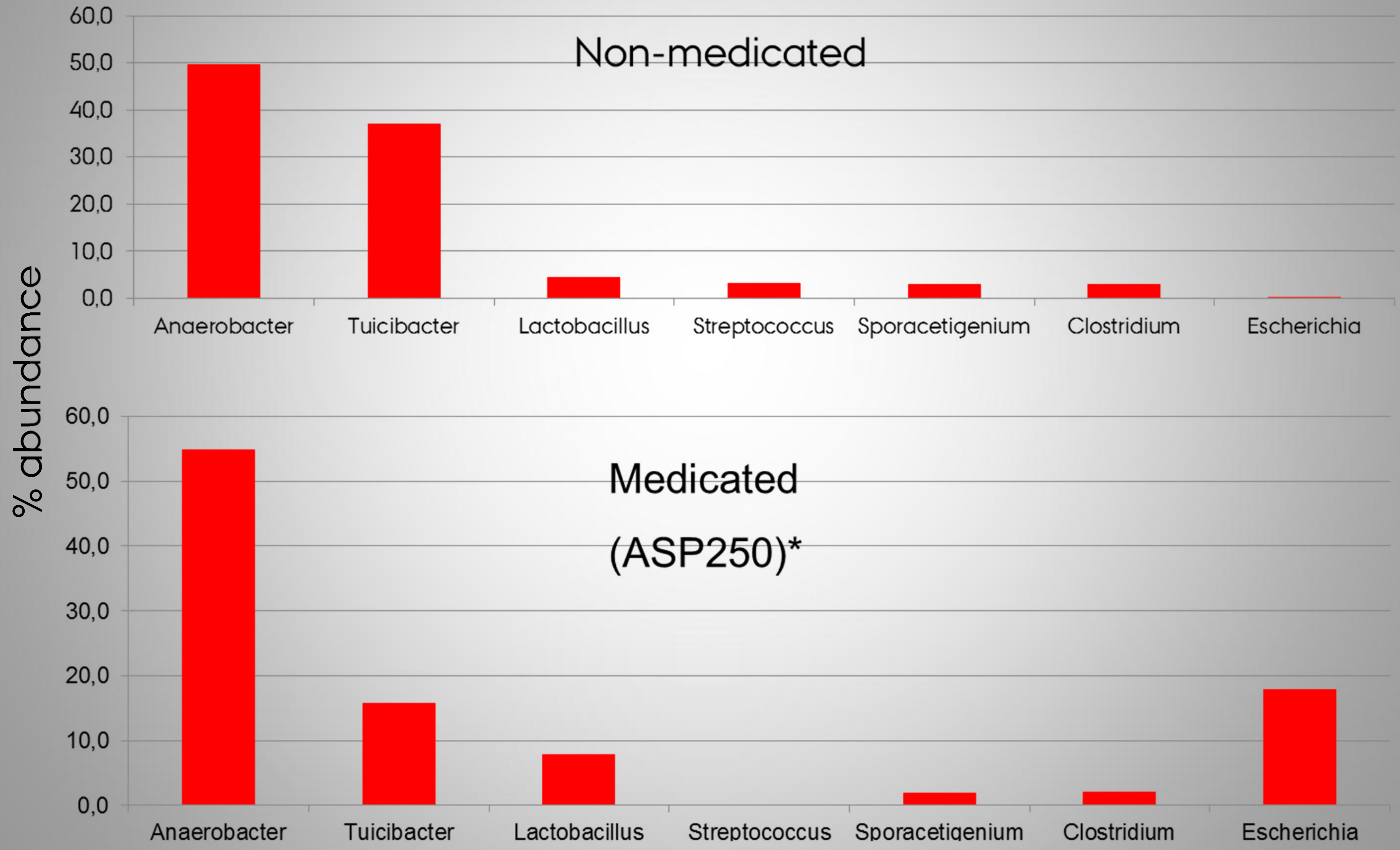
(Adapted from Jensen, 1988)

Effect of Virginiamycin (20 ppm) and Spiramycin (20 ppm)on cadaverin concentration in various regions of the gastrointestinal tract of piglets



Adapted from Dierick et al., 1985

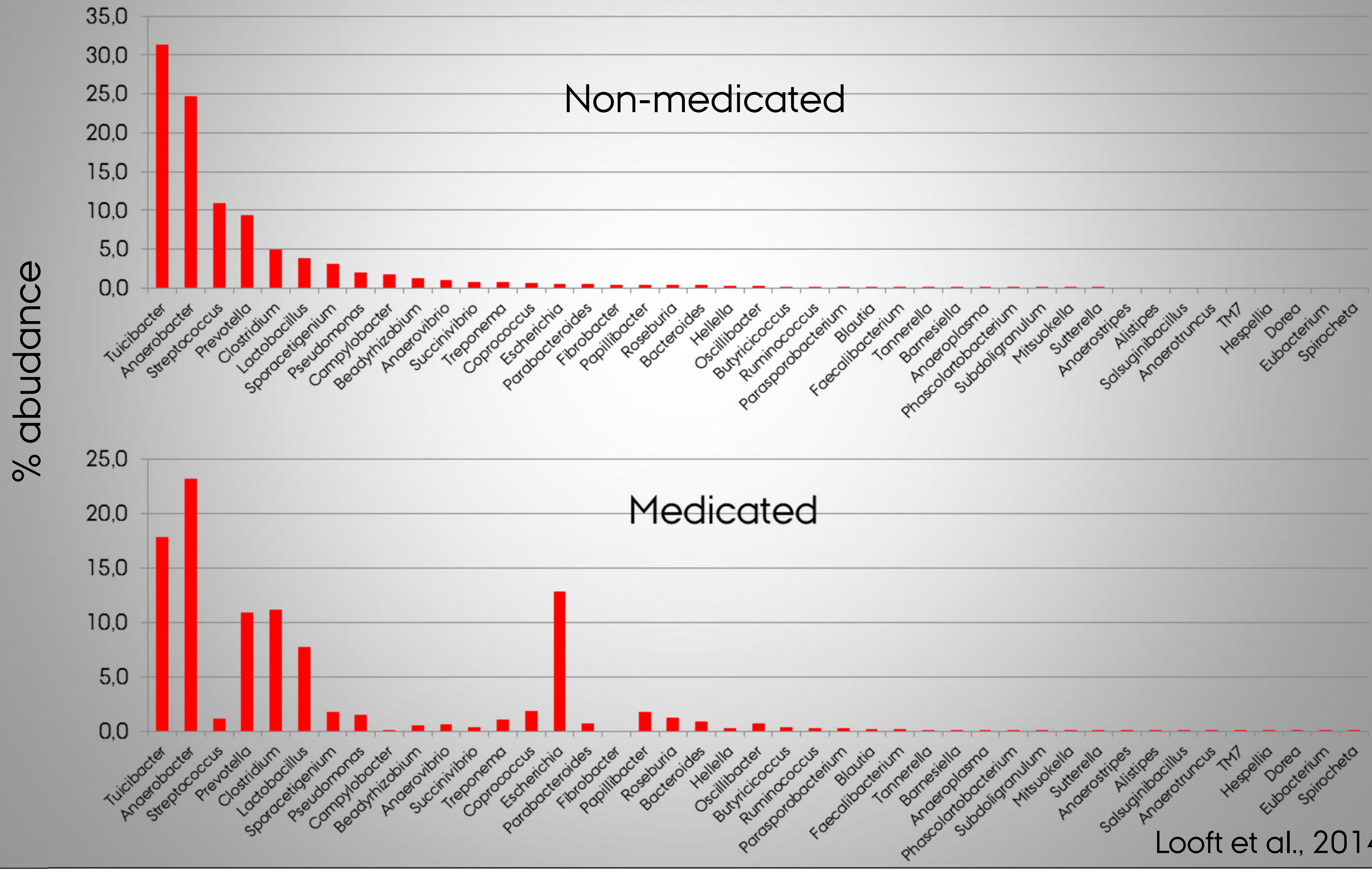
Effect of in-feed antibiotics on abundance of most dominating bacteria in ileal lumen samples



*100 ppm chloreteracyclin, 100 ppm sulfamethazine, 50 ppm penicillin)

Looft et al., 2014

Effect of in-feed antibiotics on most dominating bacteria in ileal mucosa samples



Looft et al., 2014

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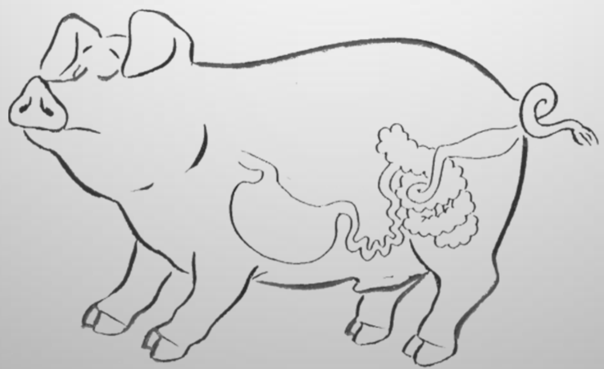
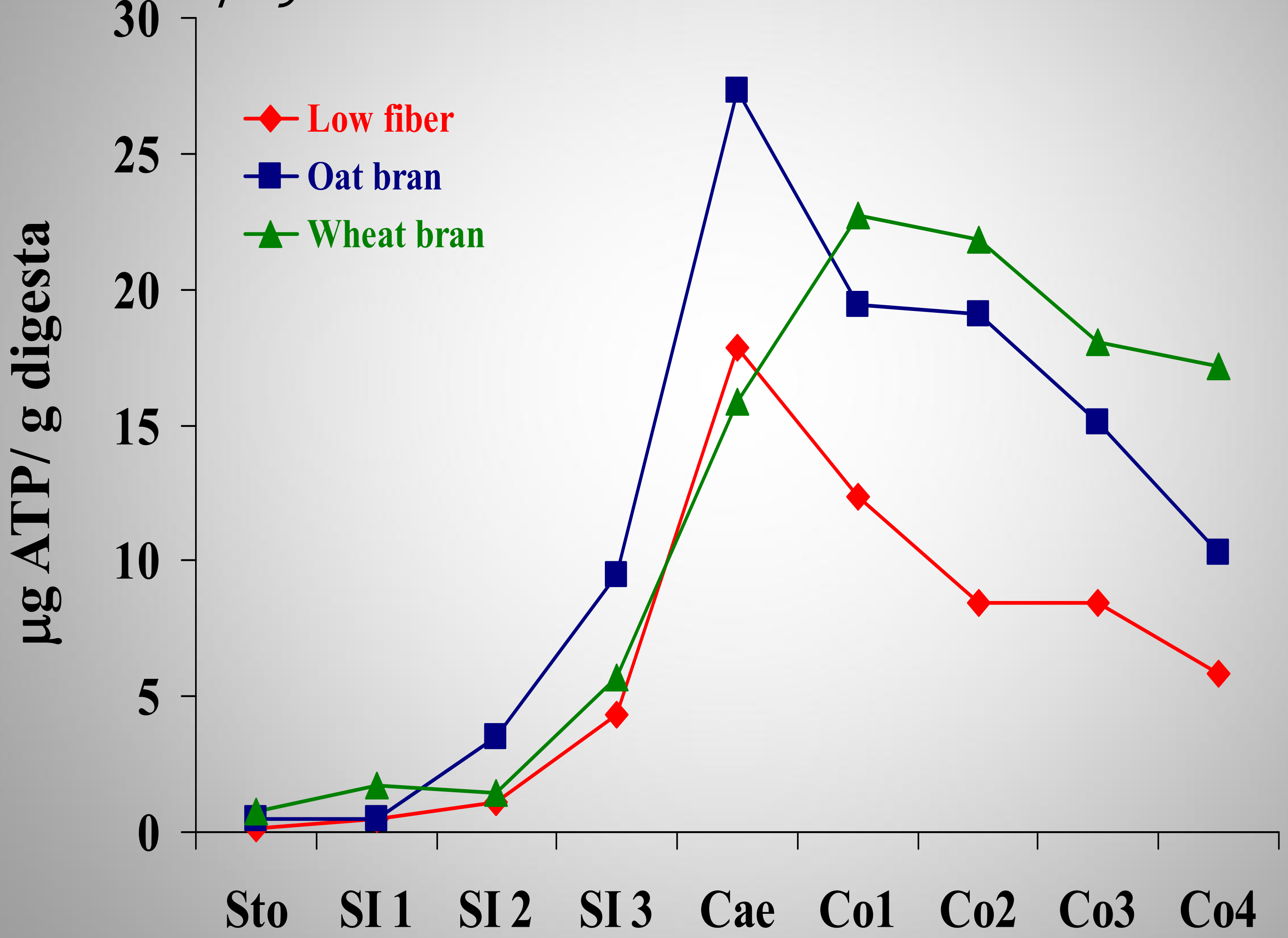
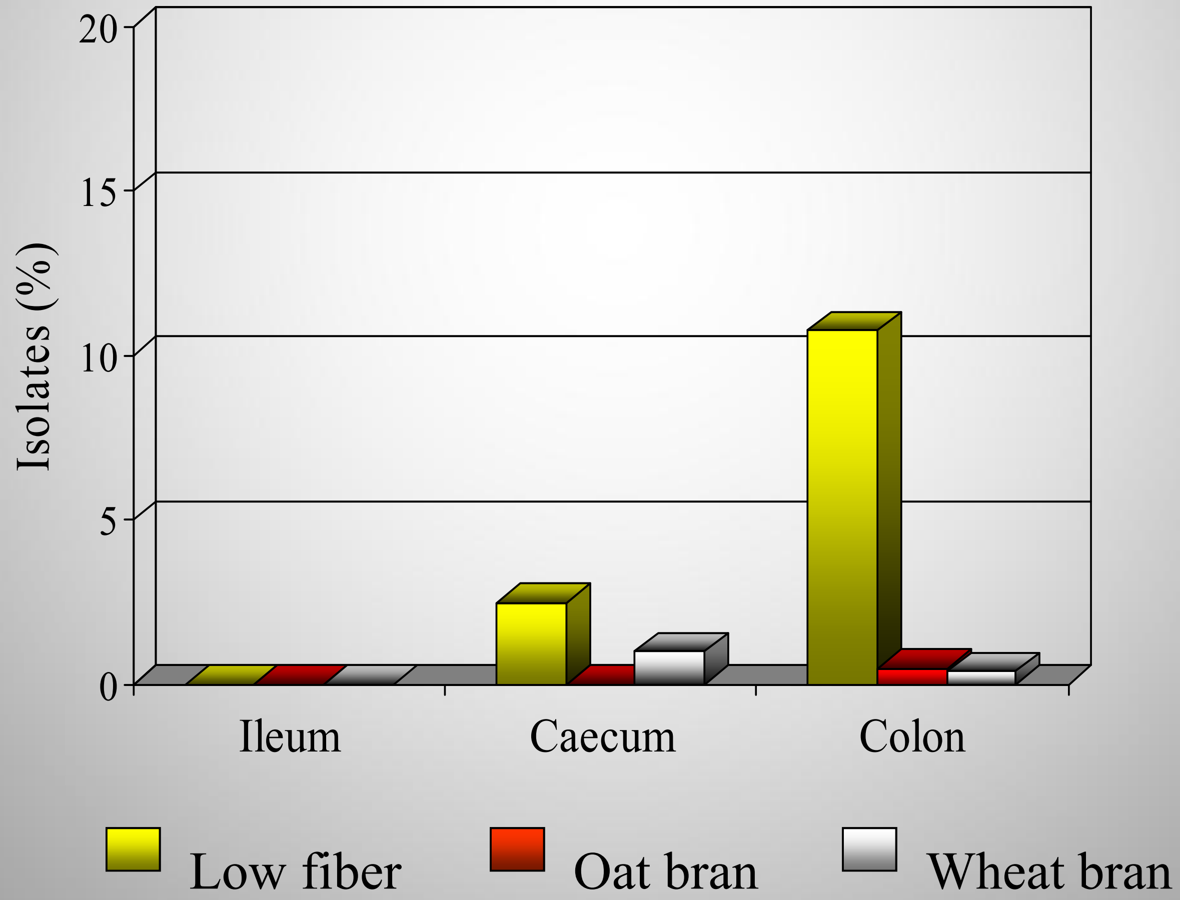


Microbial activity in various regions of the gastrointestinal tract of pigs



Relative distribution (diets and segments)
Acidaminococcus fermentans



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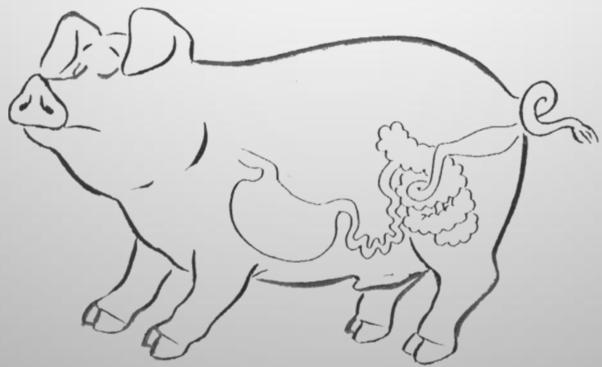
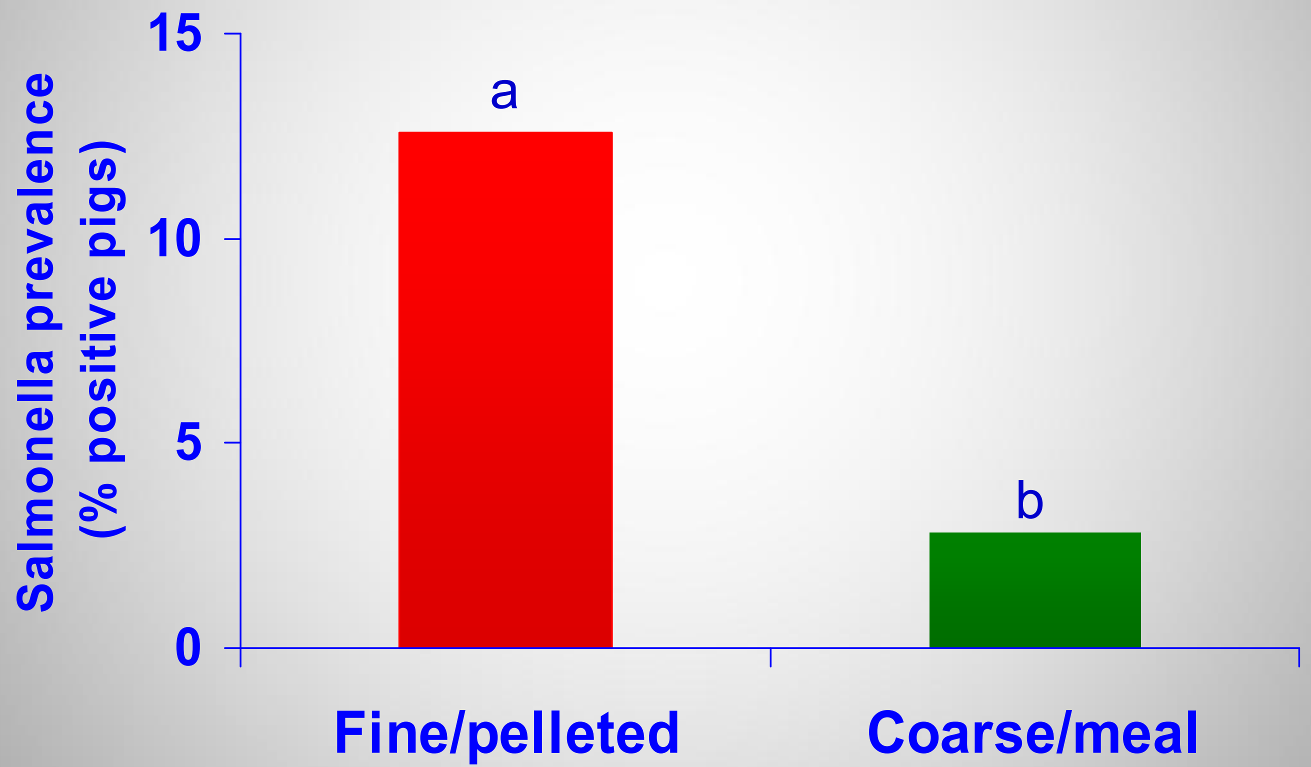
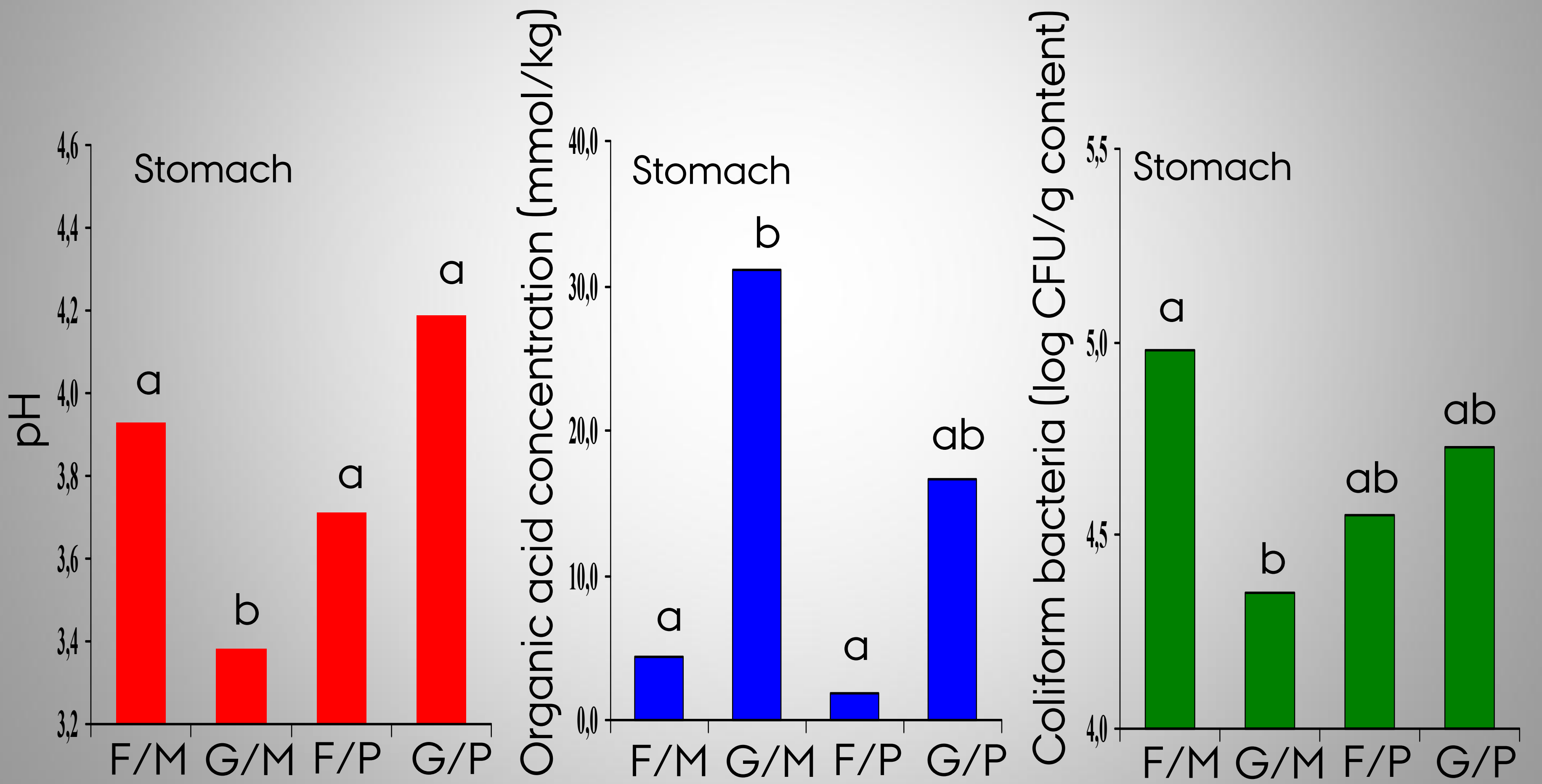


Effect of feed structure on Salmonella prevalence



Jørgensen et al., (1999)

Effect of grinding and pelleting on pH, organic acid concentration and the density of coliform bacteria in stomach content from slaughter pigs



Bacteria genera (T-RFs) in the stomach of pigs. Number of pigs in which the bacteria were found (out of 6)

bp	Tentative identifiction	Fine	Coarse
206	*Unknown*	3	5
223	*L. delbreuckii subsp delbreueckii*	0	6
253	*L. delbreuckii subsp bulgaricus*	0	4
267	*L. mucosae*	0	6
390	*M. jalaludinii/multiacidia*	0	6
406	*L. reuteri*	0	6
574	Unknown	0	6
581	S. alactolyticus/hyointestinalis	2	4
589	*S. ruminantum*	0	5
591	*M. elsdenii*	0	5
595	*L. sorbius*	2	5
611	*Weisella sp.*	0	6
605	L. curvatus	0	5

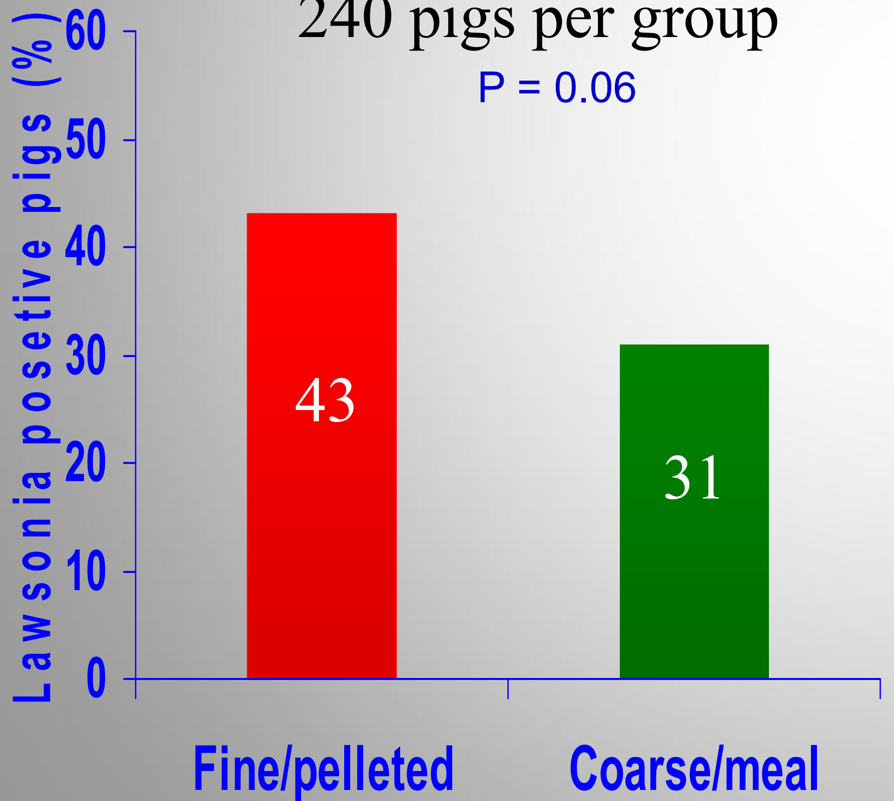
Data from Mikkelsen et al., 2005 and Canibe et al., 2005

Effekt af feedstructur and tylosin on infection with *Lawsonia intracellularis*

Feed structure

Johansen et al., (2003)

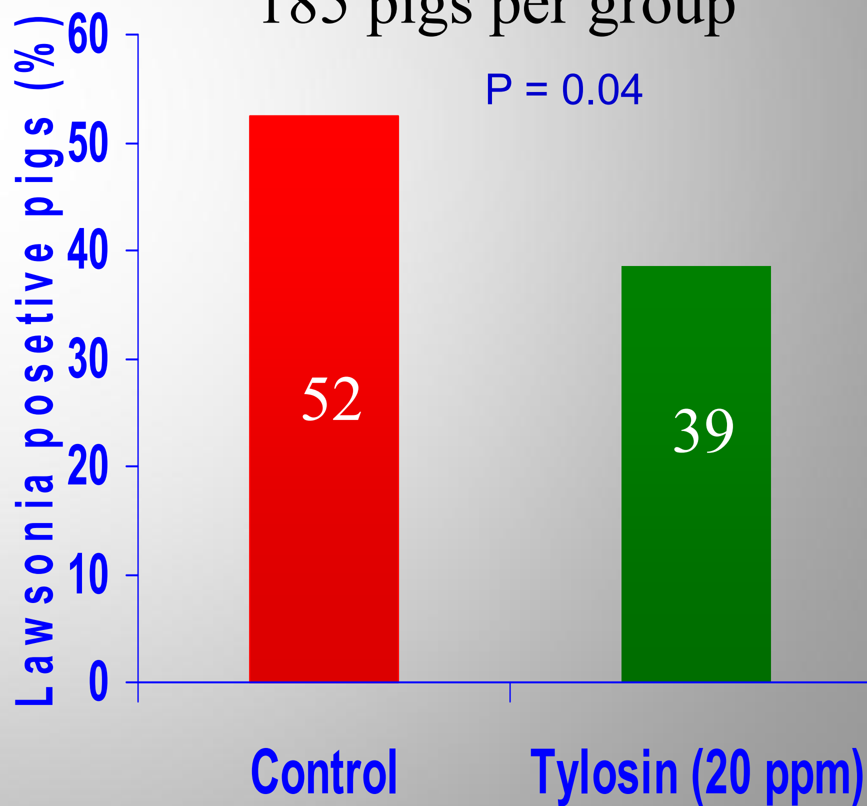
240 pigs per group



Tylosin

Pedersen et al., (2003)

185 pigs per group



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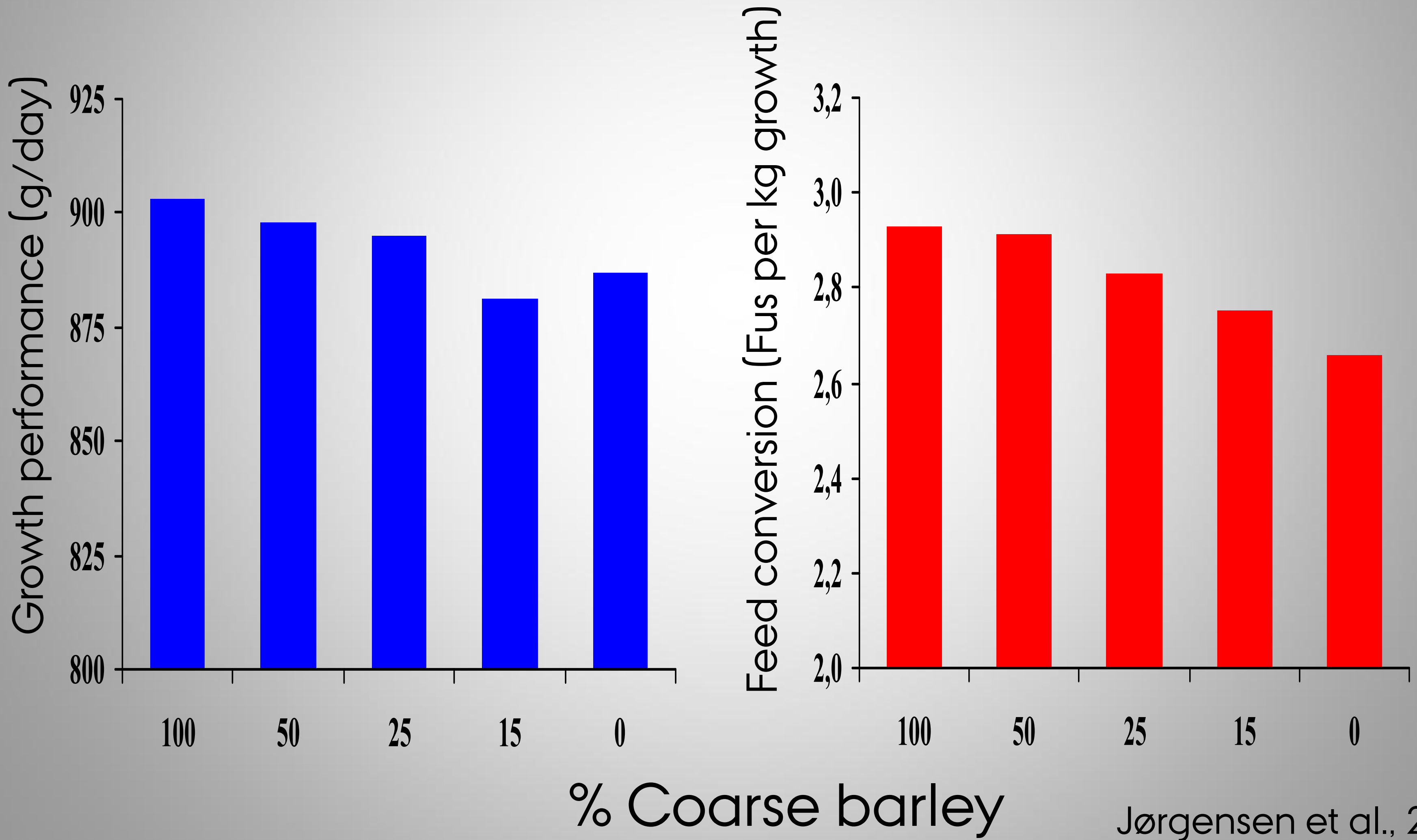
Effect of particle composition (fine vs. coarse barley) on growth performance and feed conversion.



Jørgensen et al., 2002

The stomach as a barrier

Increased microbial activity in the stomach

↓

Increased production of organic acids → decrease pH

↓

Kill enterobacteria entering the stomach

↓

Lower proliferation of enterobacteria in the small intestine

↓

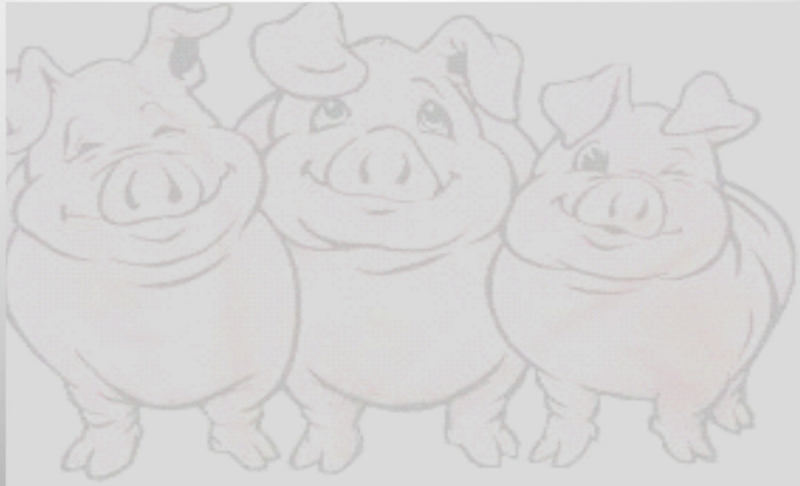
Fewer enterobacteria excreted with faeces

↓

Breaking the vicious circle where pigs in problematic herds infect each other and are re-infected through consumption of faeces

Three ways to reduce pH and increase the concentrations in stomach content:

- Addition of organic acids to the feed
- Use coarse grounded feed
- Use of fermented liquid feed



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Additives

- In-feed antibiotics
- zinc oxide
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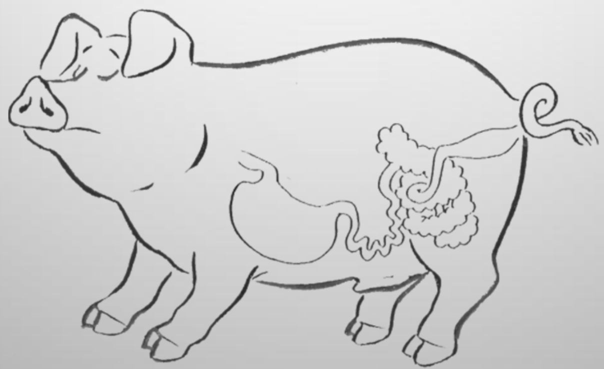
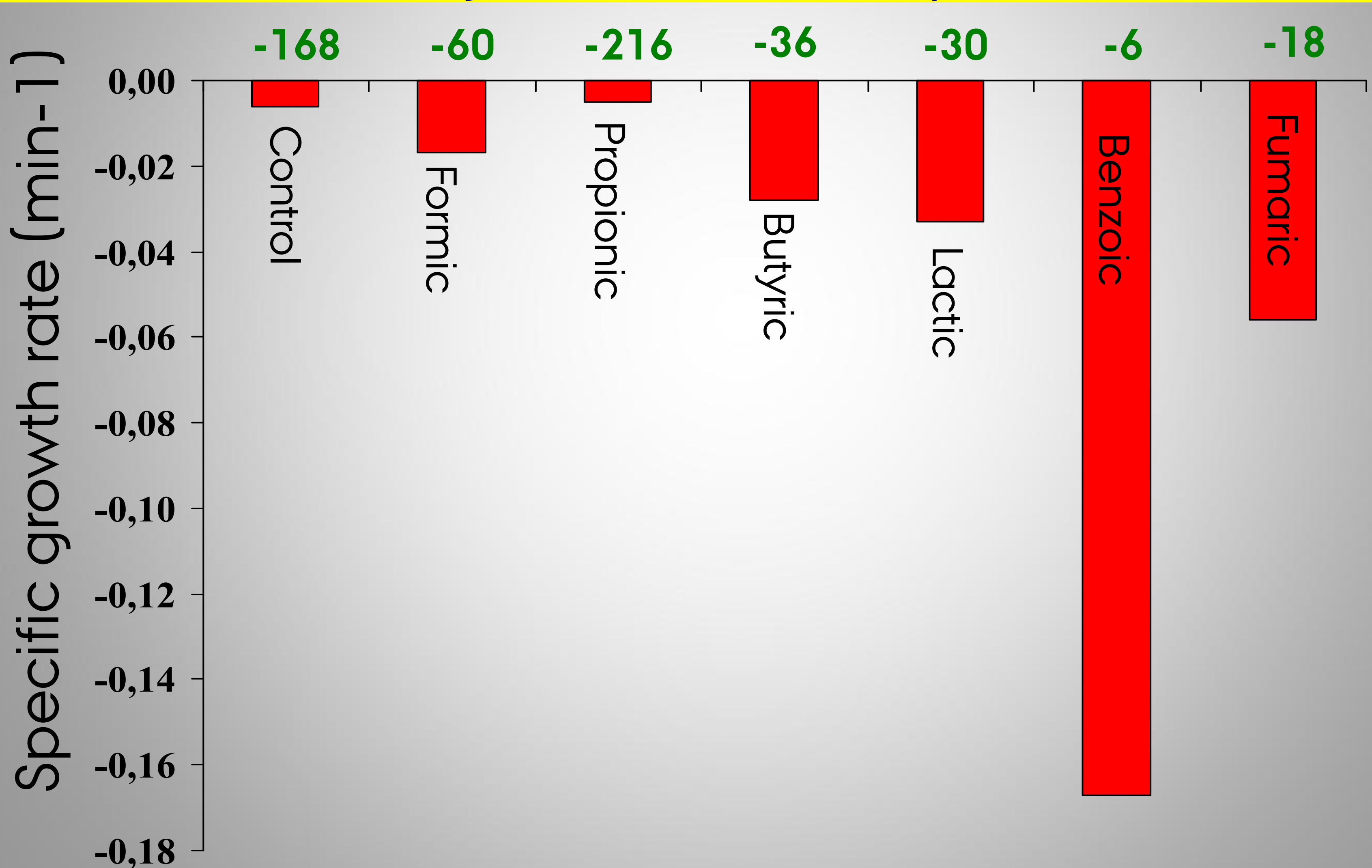
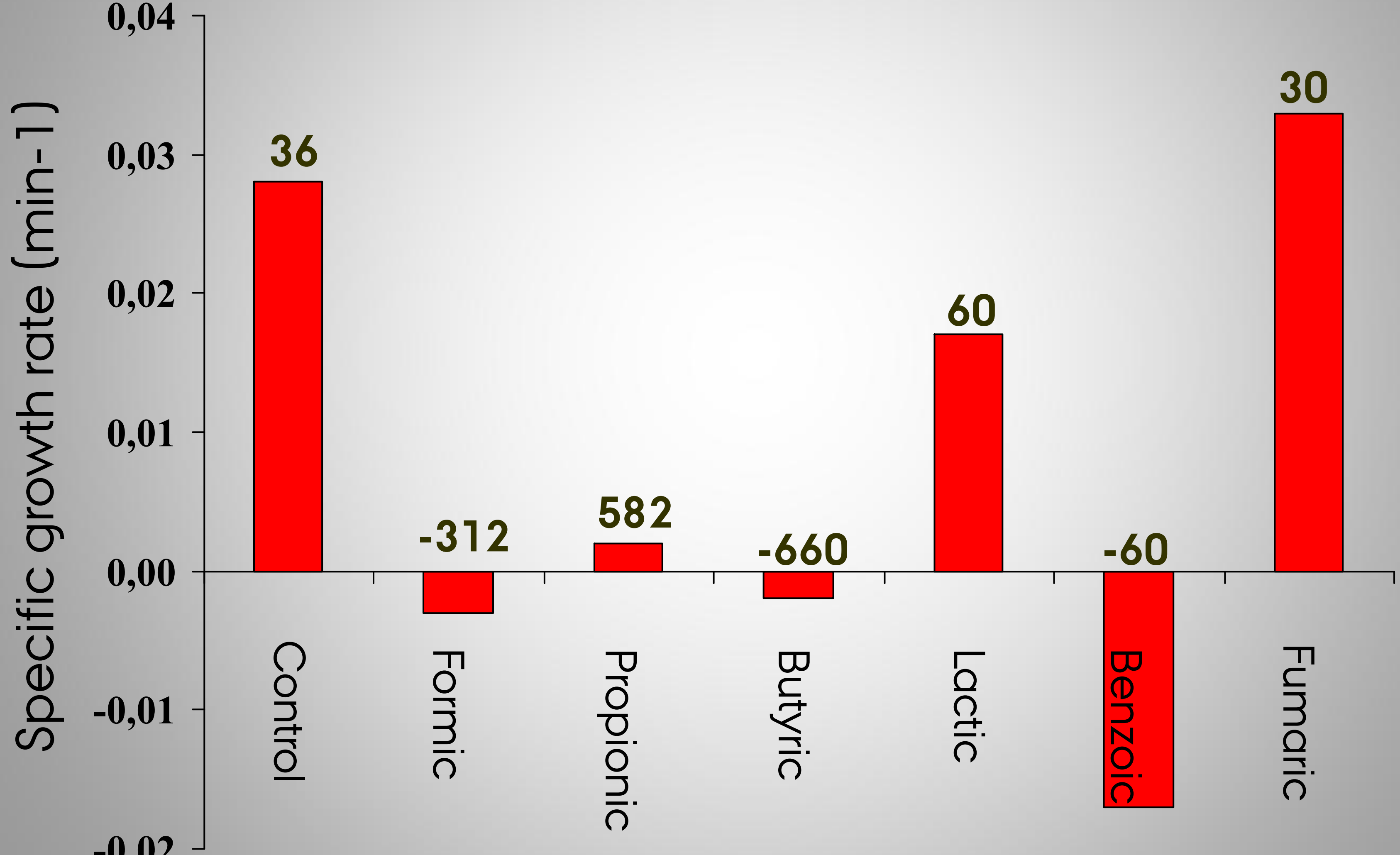
- probiotics
- Plant extracts/species



Effect of various organic acids (100 mM) on specific growth rate of coliform bacteria in gastric content at pH 4.5

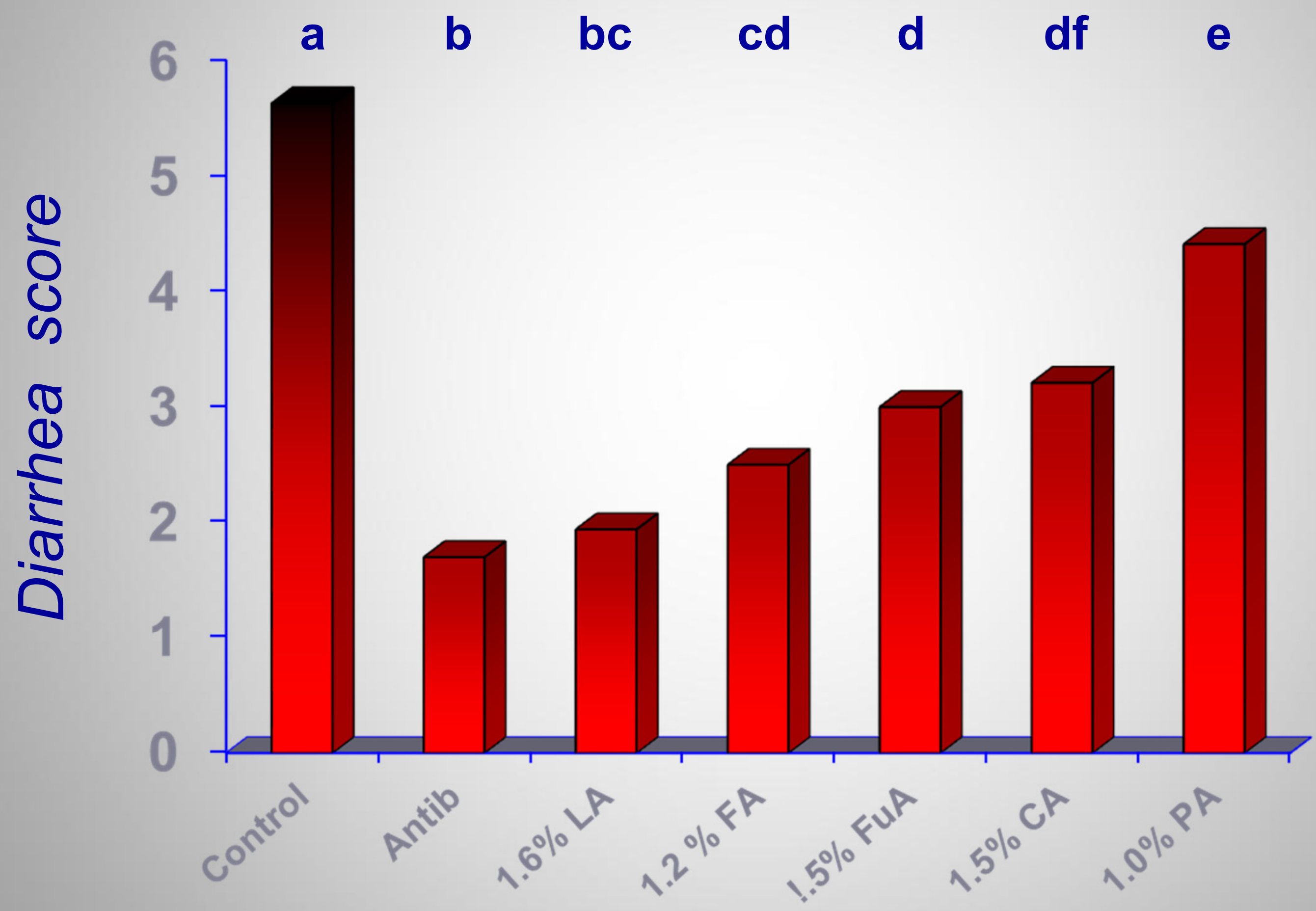


Effect of various organic acids (100 mM) on specific growth rate of coliform bacteria in content from the small intestine at pH 5.5



Effect of organic acid on diarrhea in a herd with ETEC problems

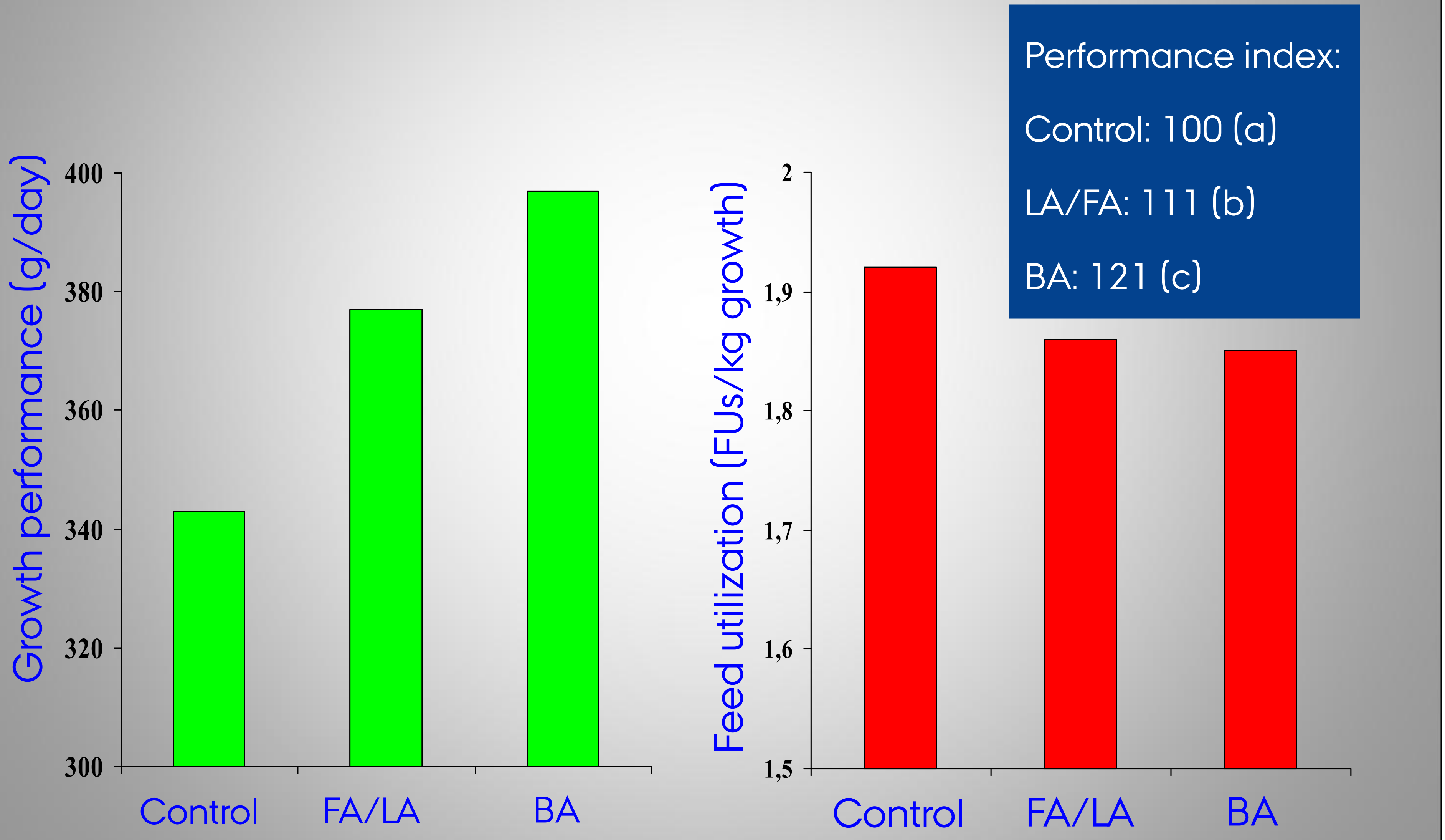
(Weaning at 25 days, 4 weeks in experiment)



N= 4, one replicate is one pen with 12 pigs.
Antibiotics: 40ppm lincomycin og 44ppm spectinomycin

Tsiloyiannis et al., 2001

Effect of 0.7% formic acid /0.7% lactic acid and 2% benzoic acid on growth performance and feed utilisation by piglets after weaning (4-10 weeks)



Effect of organic acids and zinc oxide on diarrhea and killing out range of weanears

	Control	Acid*	Acid + ZnO**
Treatment against diarrhea (days)	8,7 % a	6,9 % b	0,9 % c
Killing out range	2,6 % a	1,9 % ab	1,2 % b

*: 1% lactic acid 1% formic acid og 0,5% benzoic acid

**: 2500 zinc as zinc oxide

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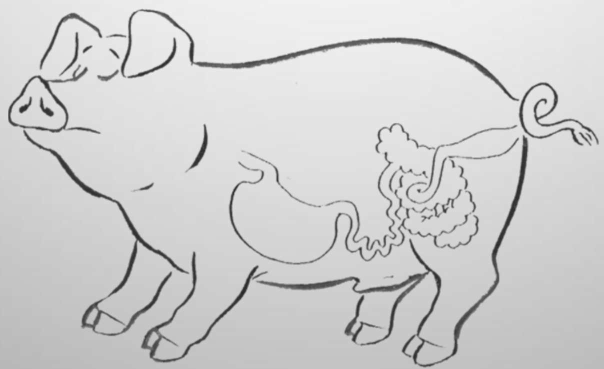

Definitions

Liquid feed (LF): water and feed are mixed shortly before feeding.

Fermented liquid feed (FLF): feed and water are mixed and soaked during a certain period of time, at a certain temperature before feeding.

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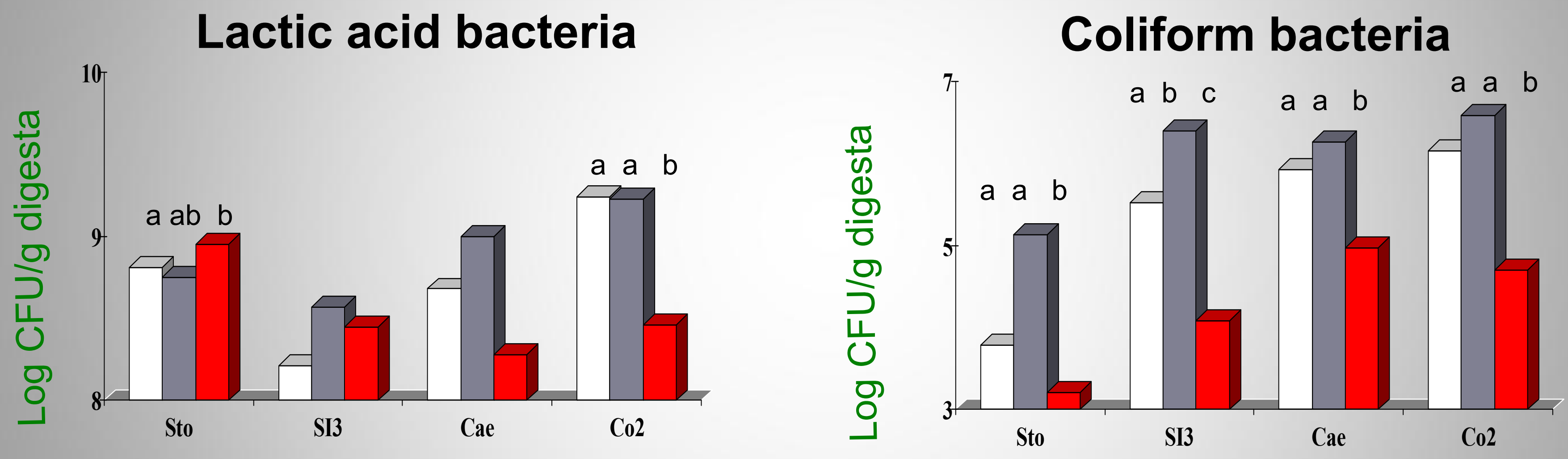
Characteristics of dry feed, LF and FLF

	DF	LF	FLF
DM%	89.3	**27.3**	**24.6**
pH	-	5.9	4.4
Lactic acid bact. (log CFU/g)	<4.3	7.2	9.4
Enterobacteria (log CFU/g)	<4.7	6.2	<3.2
Energy (MJ/kg DM)	18.7	19.0	19.0
Lysine (g/16 g N)	**6.0**	**5.8**	**4.8**
LMW-sugars (% DM)	**3.6**	**2.9**	**0.1**

Canibe and Jensen, 2003

Microbial populations in the GIT



DF NFLF FLF

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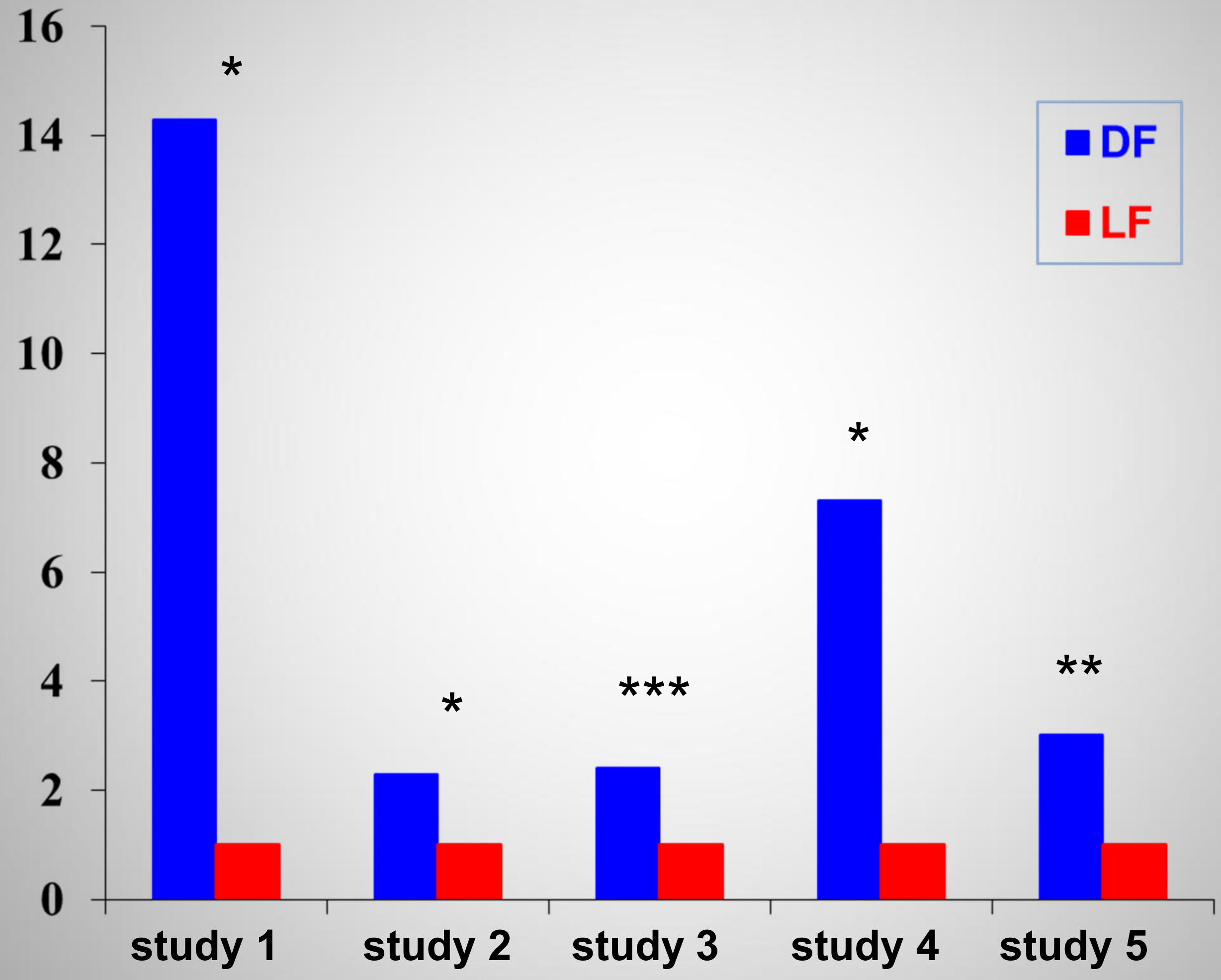
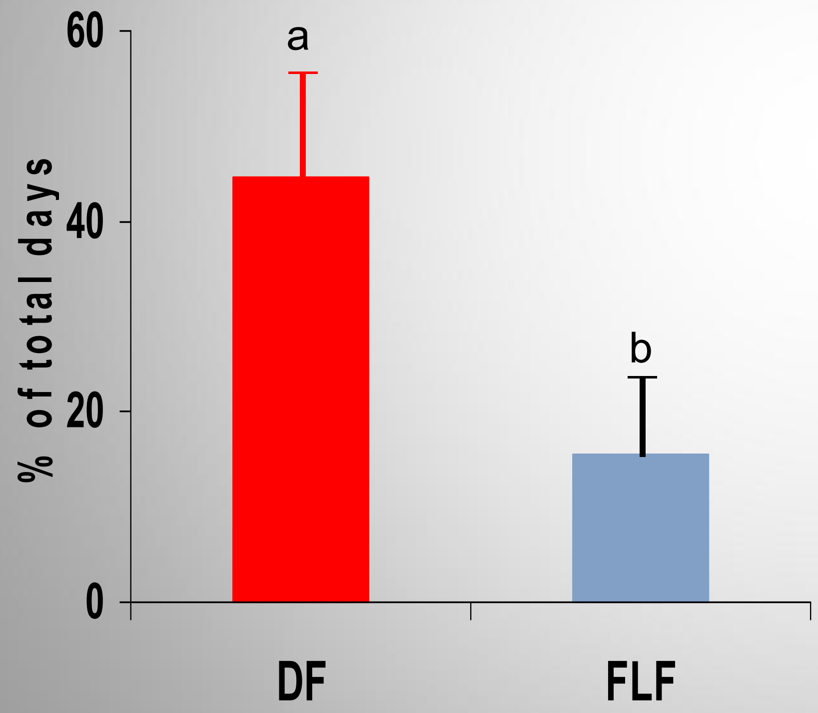
Dry feed as a risk factor for *Salmonella* incidence



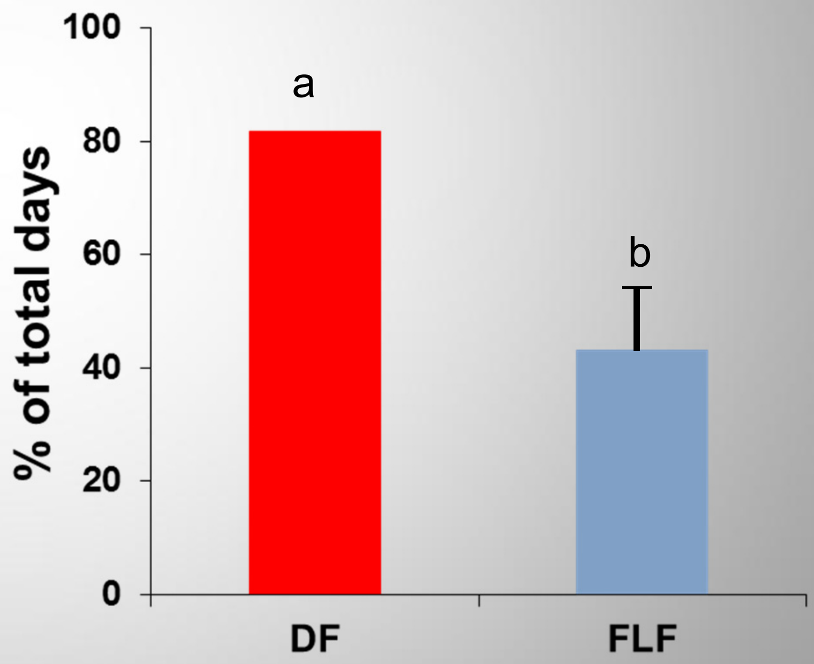
Bager, 1993; Bager & Emborg, 1994; Dahl, 1997, Stege et al., 1997; Dahl (unpubl.)

Effect of FLF on swine dysentery

Positive culture from faeces of *B. hyodysenteriae*

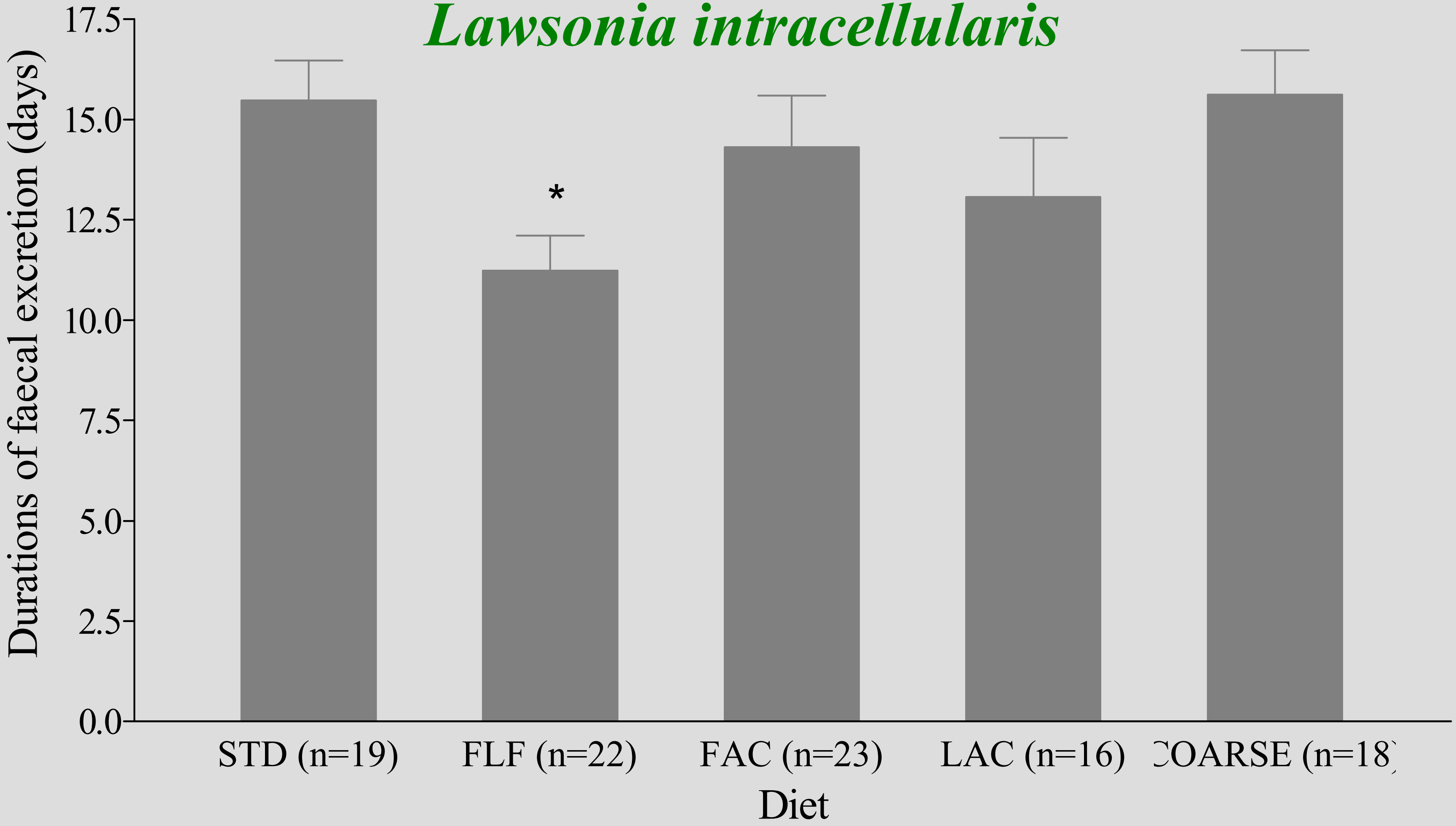


Clinical disease after infection with *B. hyodysenteriae*



Lindecrona *et al.* (2000)

Effect of FLF on infection with *Lawsonia intracellularis*



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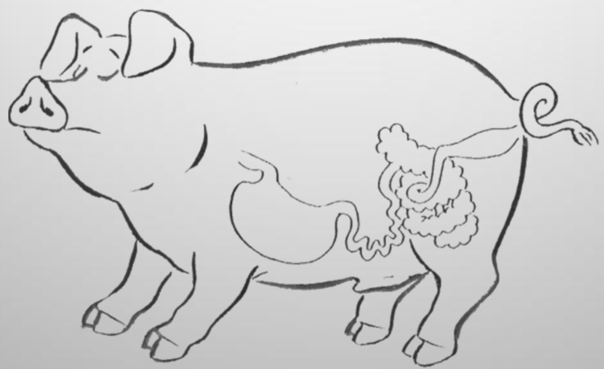
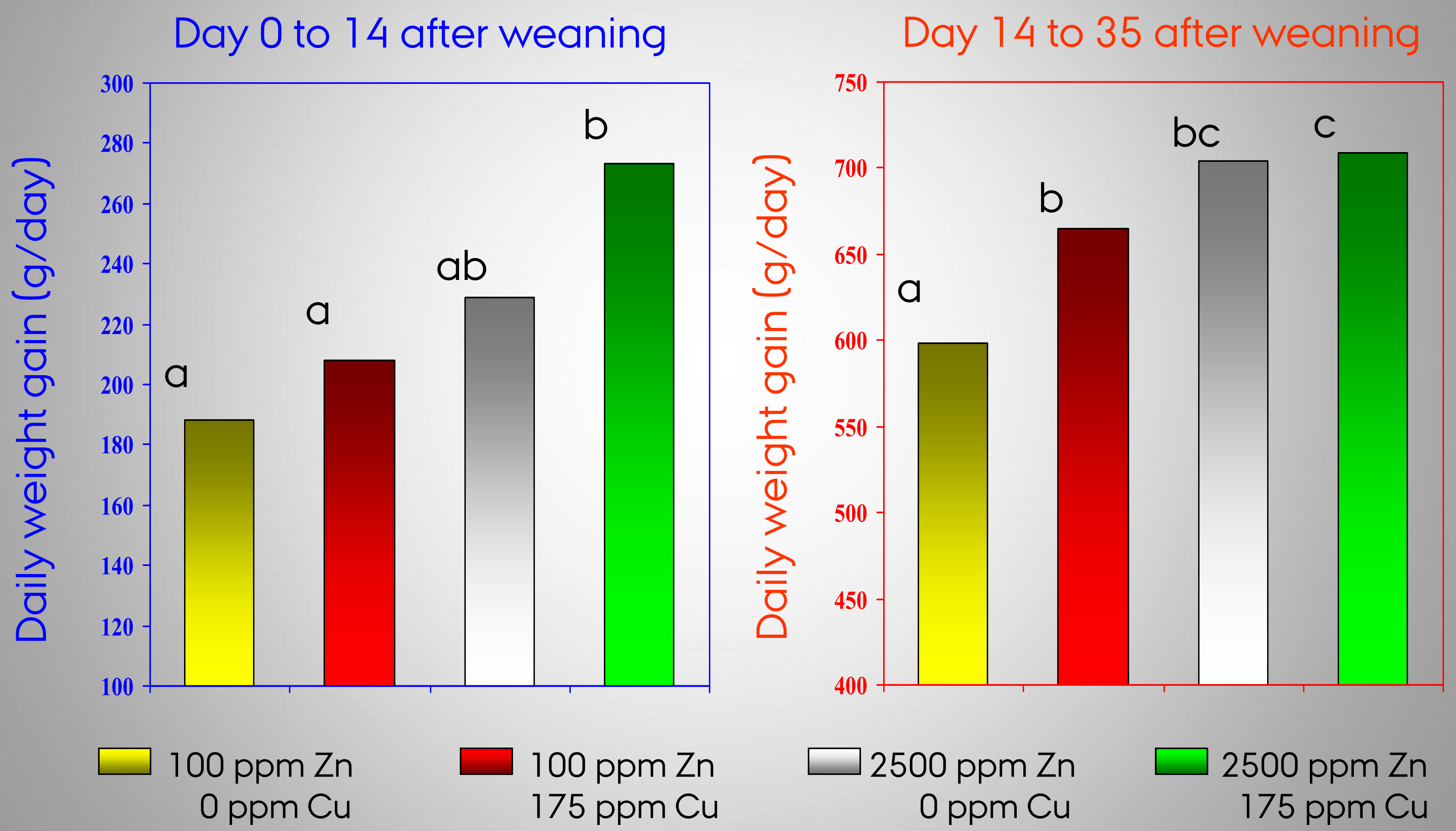


Effect of zinc and copper on daily weight gain in piglets after weaning (at 4 weeks)



Poulsen et al. (2003)

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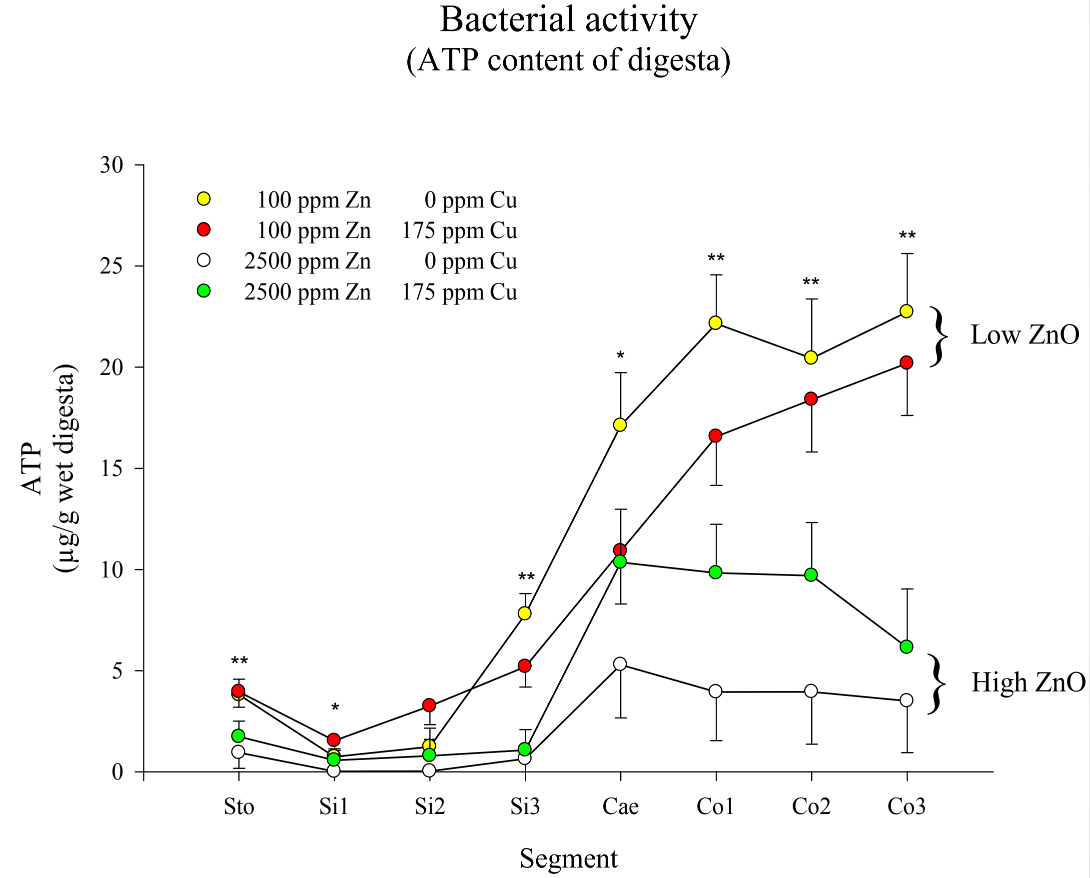
Bacterial activity



Levels of significance: * $P_{Zn} < 0.05$ and ** $P_{Zn} < 0.001$

(Højberg *et al*. 2005, AEM 71:2267)

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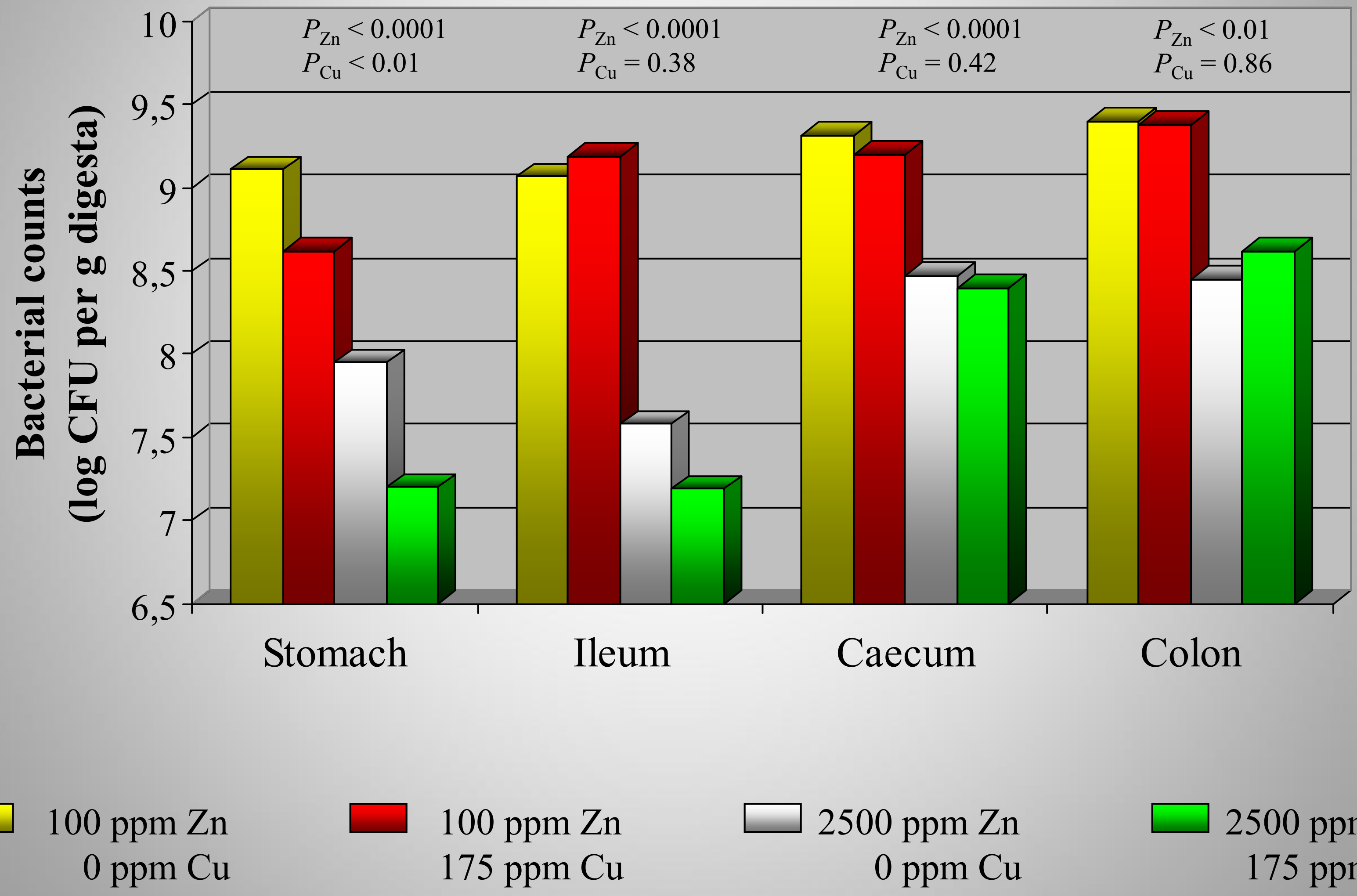
Lactic acid bacteria (MRS agar)



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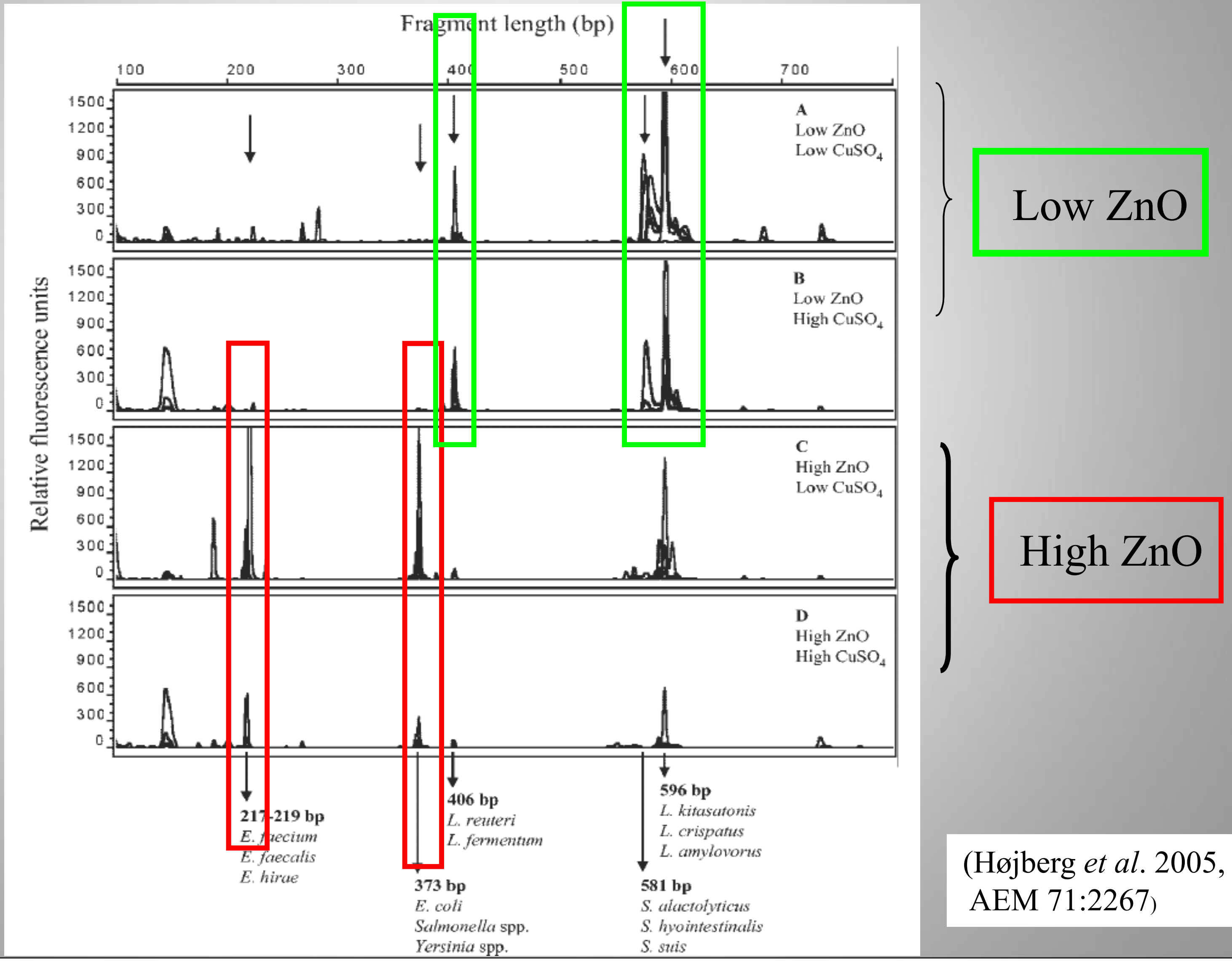
T-RFLP (caecum)



(Højberg *et al*. 2005, AEM 71:2267)

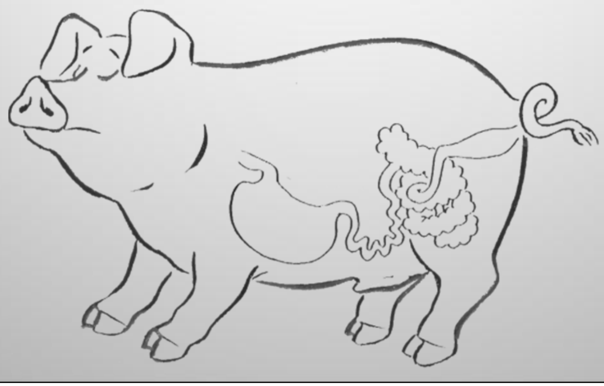
Factors that modulate the microbiota in the gastrointestinal tract

Feed

- Diet composition
- Feed processing
- Fermented liquid feed
- Prebiotics
- Low protein diets

Additives

- In-feed antibiotics
- zinc oxide
- organic acids
- probiotics
- Plant extracts/species



Probiotica

Viable defined microorganism, which alter the microbiota in a way that exert beneficial effects on the host

The three most commonly used organisms as probiotics:
bacillus, **yeast** and **lactic acid producing bacteria** ***(lactobacillus, bifidobacterium and enterococcus)***

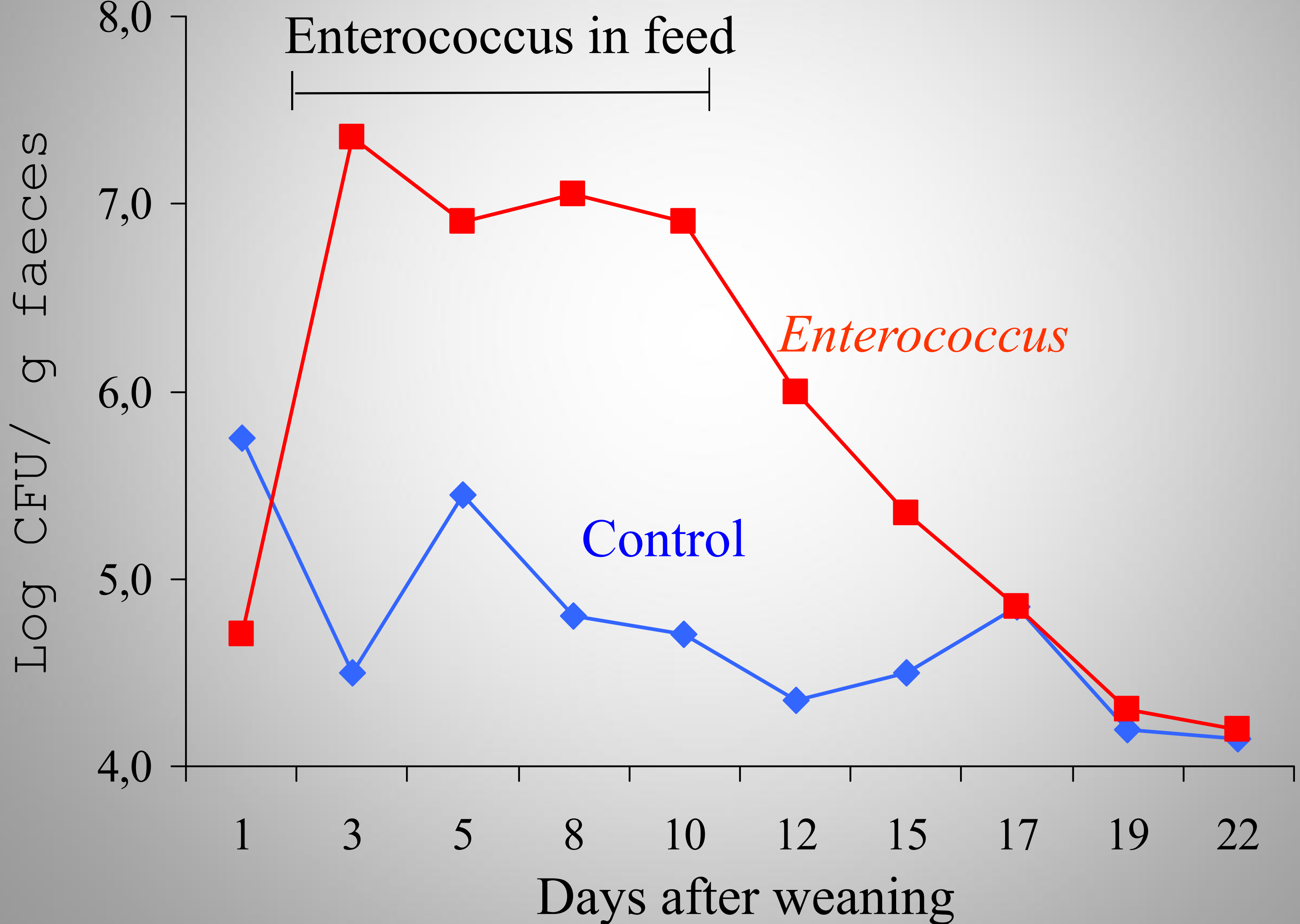
Inconstancy in results properly doe to:
dosage and type of strains, environment and diet type

Needed:
strains as well of doses that gives consistently good results

Mechanism not completely understood, a few have been proposed:

- *inhibit pathogen adhesion by steric hindrance or competitive exclusion*
- *production of products with antibacterial activity (bacteriocins or OA)*
- *modulation of the host immune system*

Development of the population of Enterococci in faeces from piglets fed a control diet or a diet supplemented with 10^9 Enterococcus faecium



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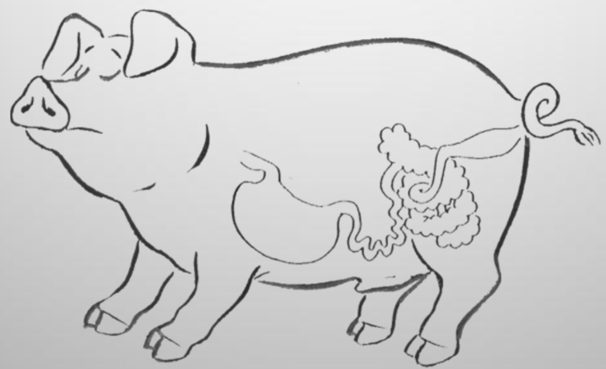
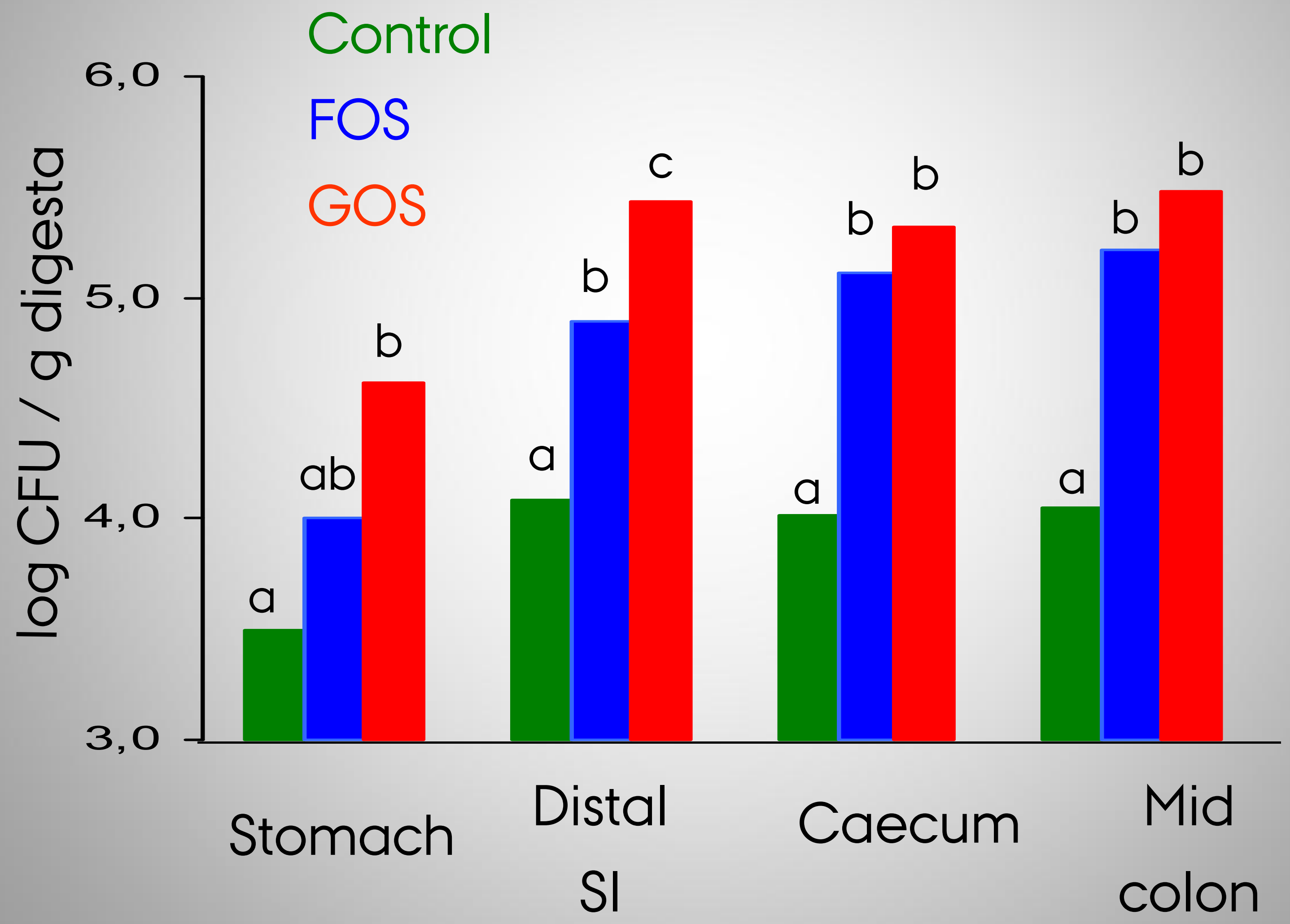


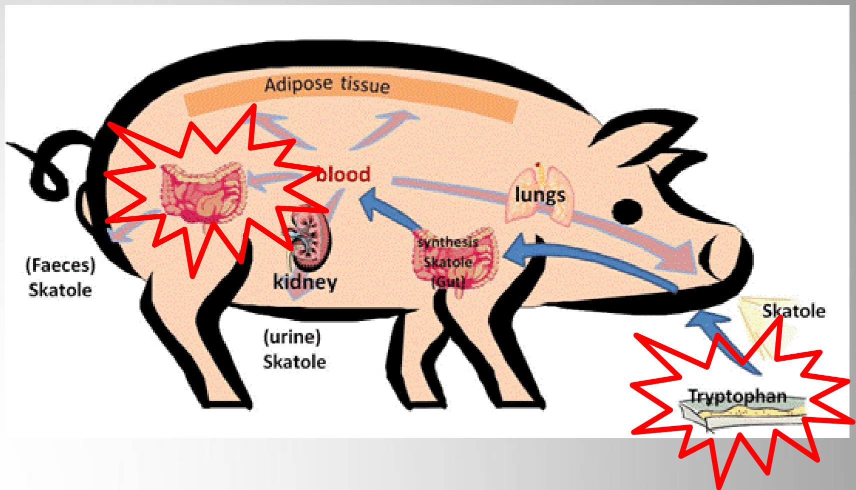
Effect of NDO on yeast



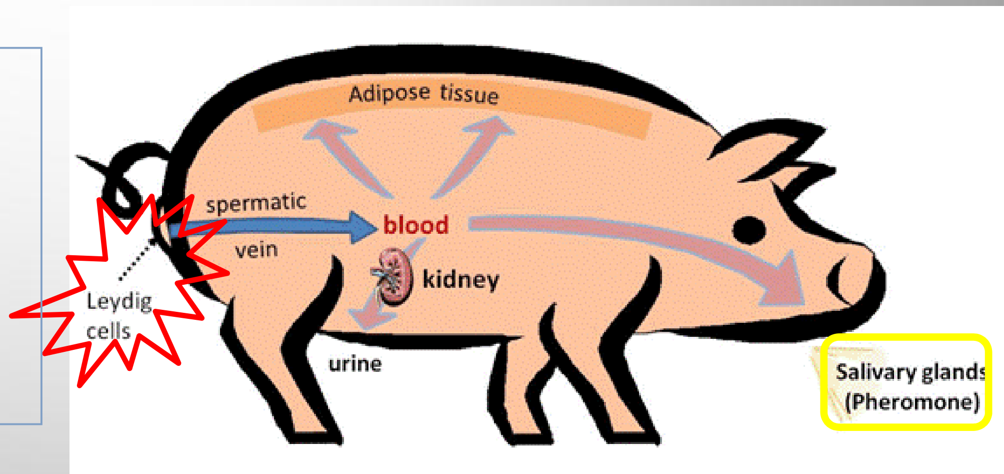
Mikkelsen et al., 2002

Boar taint

- **Skatole**
  - Produced in the large intestine from microbial metabolism of tryptophan
  - Can be reduced by dietary intervention (high fermentable fibre, protein type)



- **Androstenone**
  - Produced in the testicles
  - From there to blood, saliva, and from liver to (bile), urine, addipose tissue.



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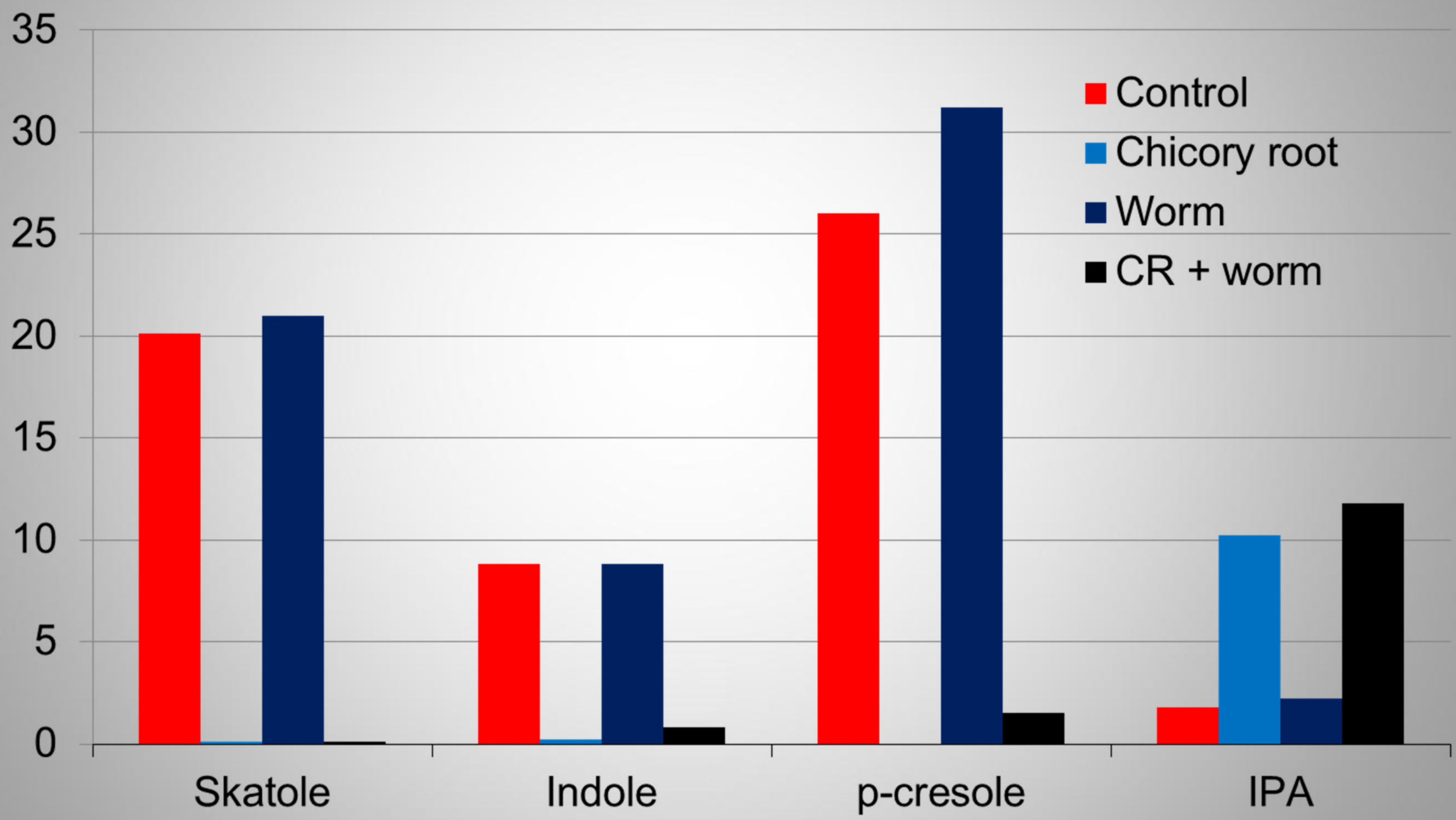
Task 2.6 Experiment with paraxites/chicory root

- Two-factorial experiment(n=72)
- Pigs slaughtered at three times (after 5, 9 and 12 weeks on the experimental diets)

	- Chicory root	+ Chicory root (25%)
- worm	Control (n=3x6)	hickorye (n=3x6)
+ worm	Worm (n=3x6)	Chickory + worm (n=3x6)

Nudelworm

Indols and p-cresol in colon content



Androstenone and skatole in back fat

	Control		Control Worm		Chicory root		Chicory root Worm	
	Male	Female	Male	Female	Male	Female	Male	Female
Androstenone (ng/g)	1,48	0,01	1,21	0,00	1,24	0,01	1,53	0,01
Skatol e (µg/g)	108,7	57,0	93,8	70,1	0,0	0,0	0,0	0,8
Indole (µg/g)	21,4	8,6	14,6	15,2	7,3	1,8	7,4	6,1

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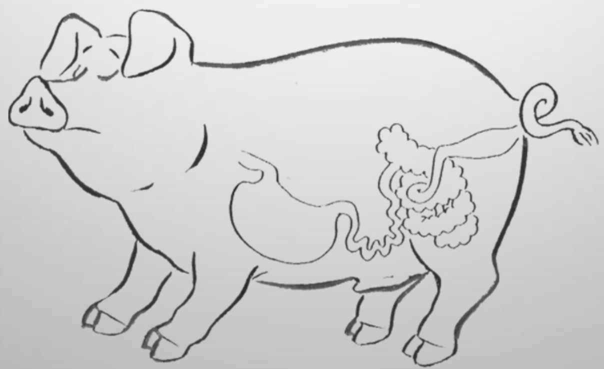

Low protein diets

Source and level known to affect enteric health of weaned pigs

Source:

- animal protein better than plant protein
- more knowledge on microbial composition and activity needed

Level:

- low protein diets supplemented with synthetic AA prevent post weaning diarrhoea without compromising growth performance

Conclusion

Feed

- *FLF:* Increase gut health but reduce growth performance may be a way to make probiotics cost effective.
- *Feed structure:* Increase gut health but reduce growth performance
- *Prebiotics:* Inconsistent results. More research needed. May not be cost effective.

Additives

- *ZnO:* increase growth performance and reduce PWD, but gives environmental problems
- *OA*: right doses and types have beneficial effects on growth performance and health
- *Probiotics*: Inconsistent results. More strains should be tested. May not be cost effective