Assessment of intestinal health in swine (and poultry)

ECVCN Residency class 2016

Edgar Garcia Manzanilla, DVM, PhD, MPVM, Dipl. ECPHM
Pig Development Department, TEAGASC Moorepark, Ireland

Teagasc
Agriculture and Food Development Authority
The Irish Agriculture and Food Development Authority

Objective

Company…………research………………EFSA………user

What is intestinal (or gut) health?

Bischoff *BMC Medicine* 2011, 9:24
http://www.biomedcentral.com/1741-7015/9/24

BMC Medicine

DEBATE Open Access

'Gut health': a new objective in medicine?

Stephan C Bischoff

'Gut health' is a term increasingly used in the medical literature and by the **food industry.**

From a scientific point of view, however, it is still extremely unclear exactly what gut health is, how it can be defined and how it can be measured.

What is intestinal (or gut) health?

Table 1 Gut health and gastrointestinal health[a]

Five major criteria for a healthy GI system	Specific signs of GI health
Effective digestion and absorption of food	Normal nutritional status and effective absorption of food, water and minerals Regular bowel movement, normal transit time and no abdominal pain Normal stool consistency and rare nausea, vomiting, diarrhoea, constipation and bloating
Absence of GI illness	No acid peptic disease, gastroesophageal reflux disease or other gastric inflammatory disease No enzyme deficiencies or carbohydrate intolerances No IBD, coeliac disease or other inflammatory state No colorectal or other GI cancer
Normal and stable intestinal microbiota	No bacterial overgrowth Normal composition and vitality of the gut microbiome No GI infections or antibiotic-associated diarrhoea
Effective immune status	Effective GI barrier function, normal mucus production and no enhanced bacterial translocation Normal levels of IgA, normal numbers and normal activity of immune cells Immune tolerance and no allergy or mucosal hypersensitivity
Status of well-being	Normal quality of life 'Qi (ch'i)', or positive gut feeling Balanced serotonin production and normal function of the enteric nervous system

[a]GI, gastrointestinal; IBD, inflammatory bowel disease; IgA, immunoglobulin A.

(((gut health[Title]) OR intestinal health[Title])) AND pig* 27 results PUBMED

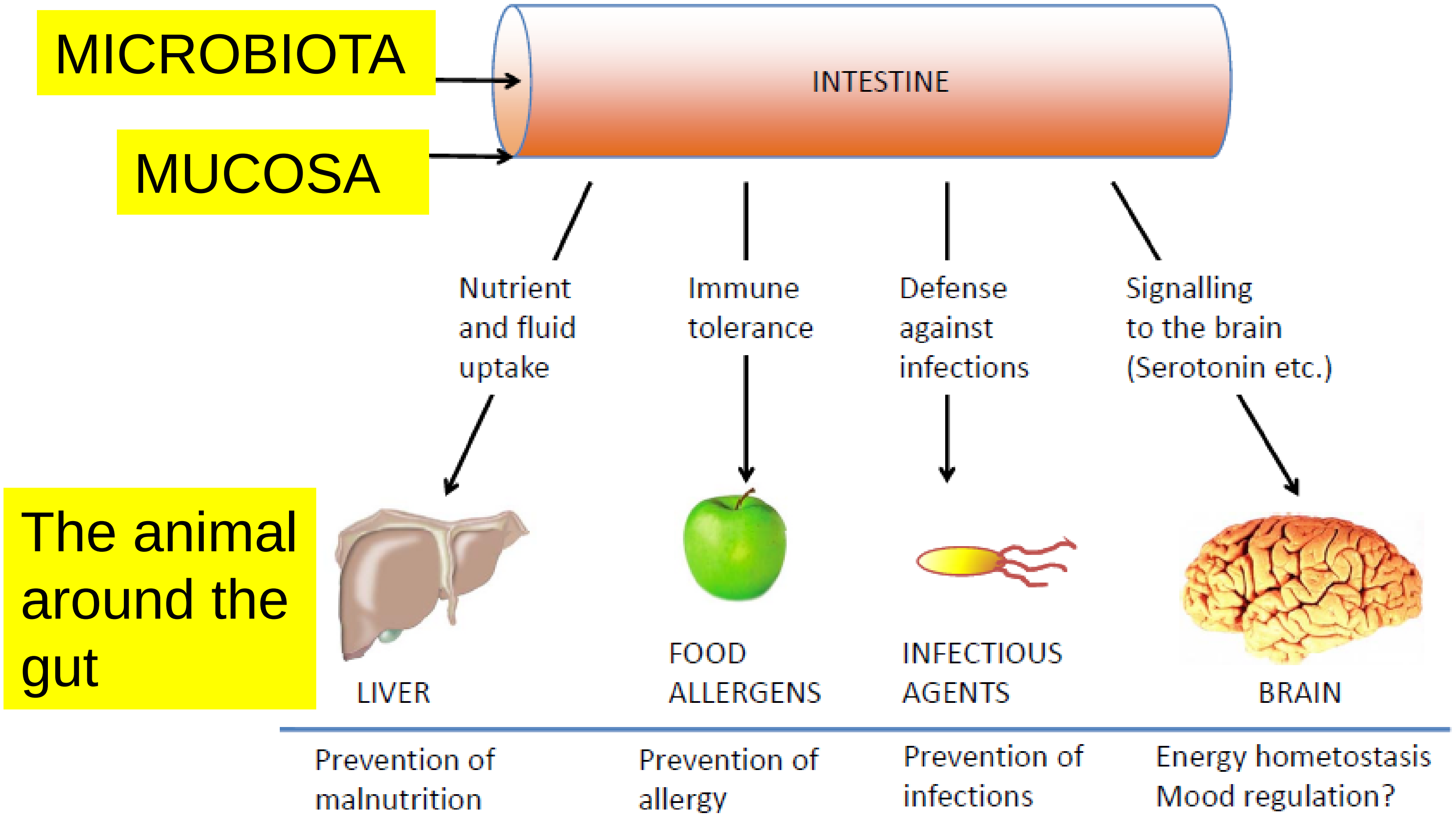
FACTOR STUDIED:

Algae, Seaweed, Laminarin
Cysteamine
Fatty acids
Deoxynivalenol
Fiber, Prebiotic
Monosodium Glutamate, Glutamine
N-acetylcysteine
Enzyme complex
Plant extracts, Essential oils
Amino acids, L-methionine
Dietary protein, Different cereals
Antimicrobial peptide
Wheat bran
Zinc oxide
Probiotic, Bacillus spp, Lactobacillus
…

DEPENDENT VARIABLE:

Gut health
Intestinal health
Growth performance
Nutrient digestibility
Peptide transporters
Immune status/response
Blood profile
Inflamation
Experimental infection/Challenge
Fermentation
Intestinal population/microbiota
Mucin gene expression
Cytokine gene expression
In vitro model
…

Components of gut health



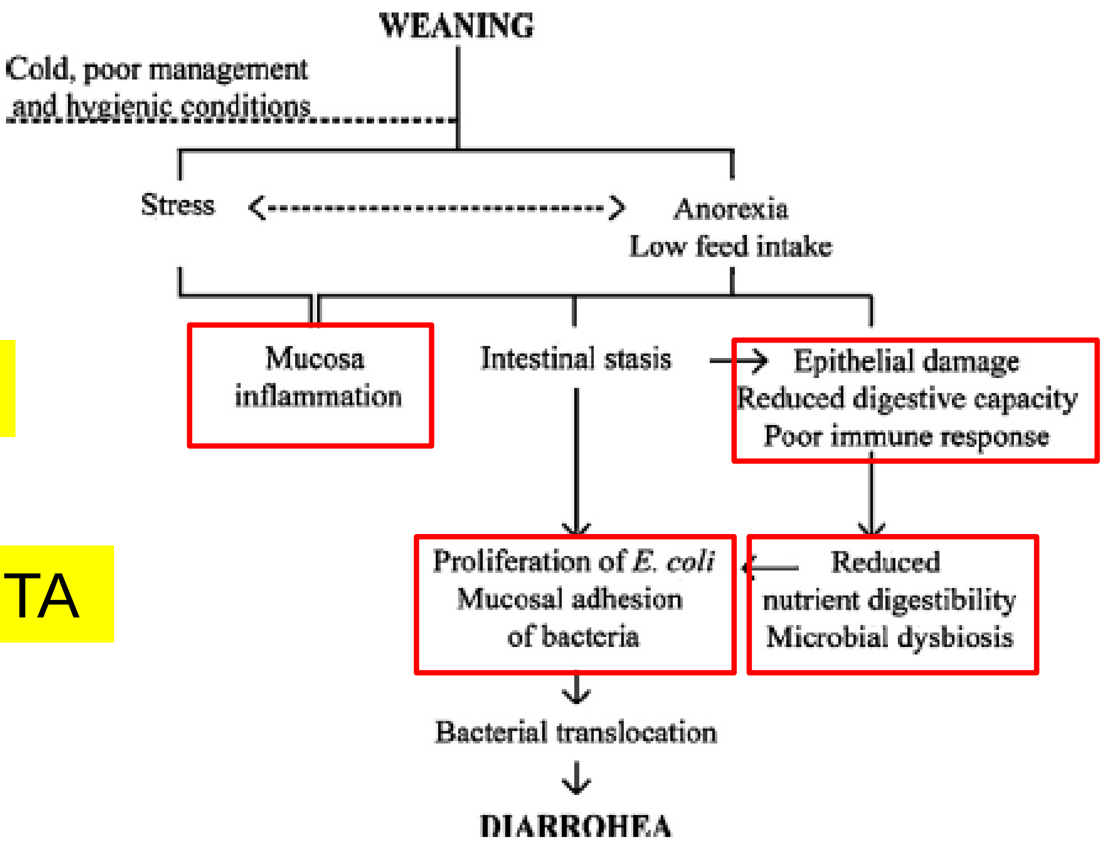
What about the pig?

F. Molist et al. / Animal Feed Science and Technology 189 (2014) 1–10

The animal around the gut

MUCOSA

MICROBIOTA

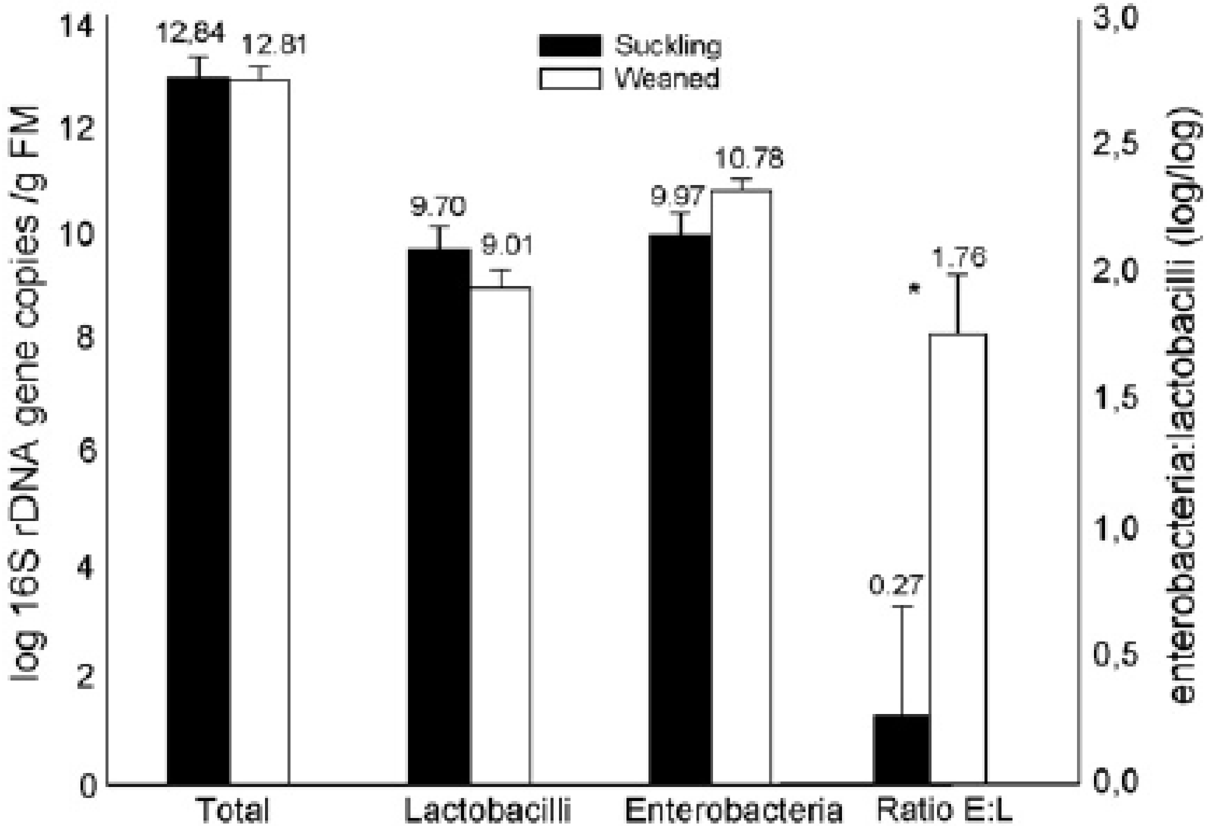


Changes in physiology and structure with solid diet: Suckling vs. 7d after weaning

	Suckling	Weaned	*P*-value
Empty weight (g)			
Stomach	37.4 ± 7.54	38.7 ± 4.88	0.737
Small intestine	330 ± 56.4	370 ± 98.1	0.393
Large intestine	120 ± 31.2	195 ± 98.1	0.027
pH			
Stomach	3.2 ± 0.84	4.4 ± 0.67	0.019
Ileum	7.0 ± 0.44	6.7 ± 0.37	0.371
Cecum	6.3 ± 0.13	5.8 ± 0.12	<0.0001
Colon	6.9 ± 0.29	6.03 ± 0.18	<0.0001

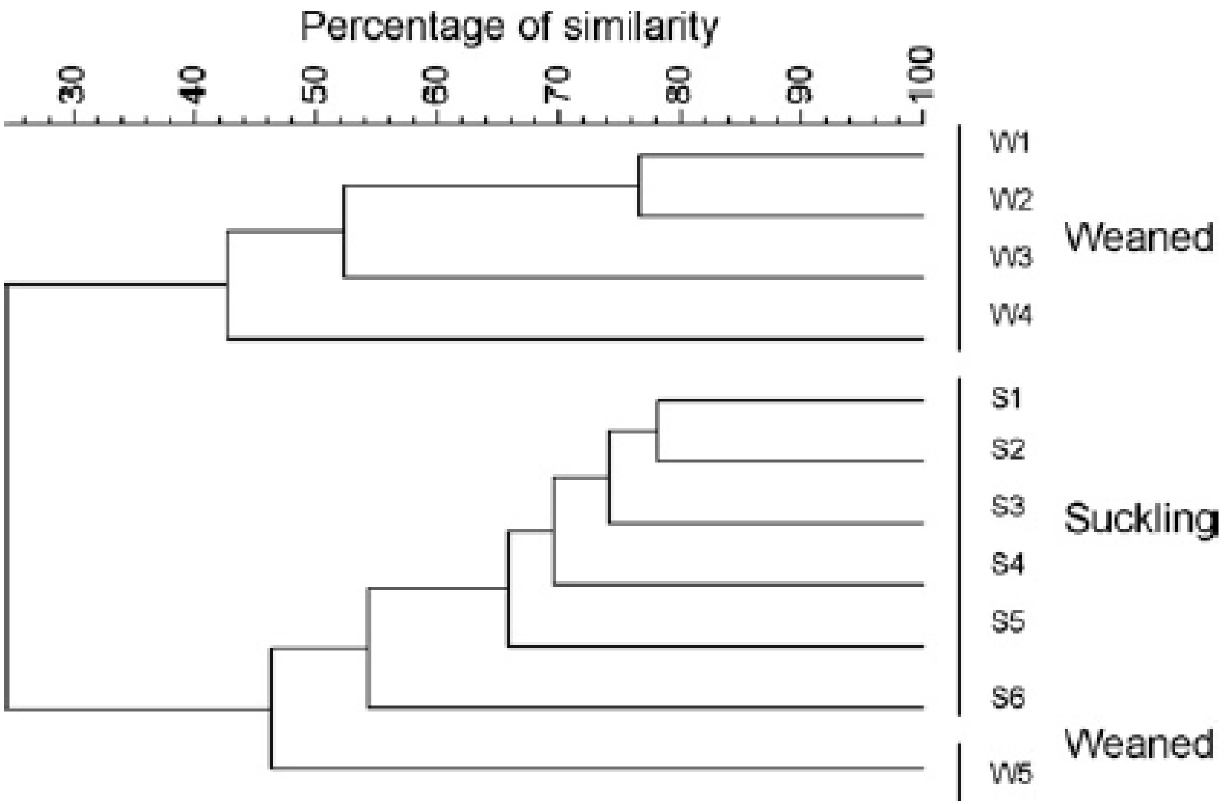
M. Castillo et al. / Veterinary Microbiology 124 (2007) 239–247

Microbiota: suckling vs. 7d after weaning



M. Castillo et al. / Veterinary Microbiology 124 (2007) 239–247

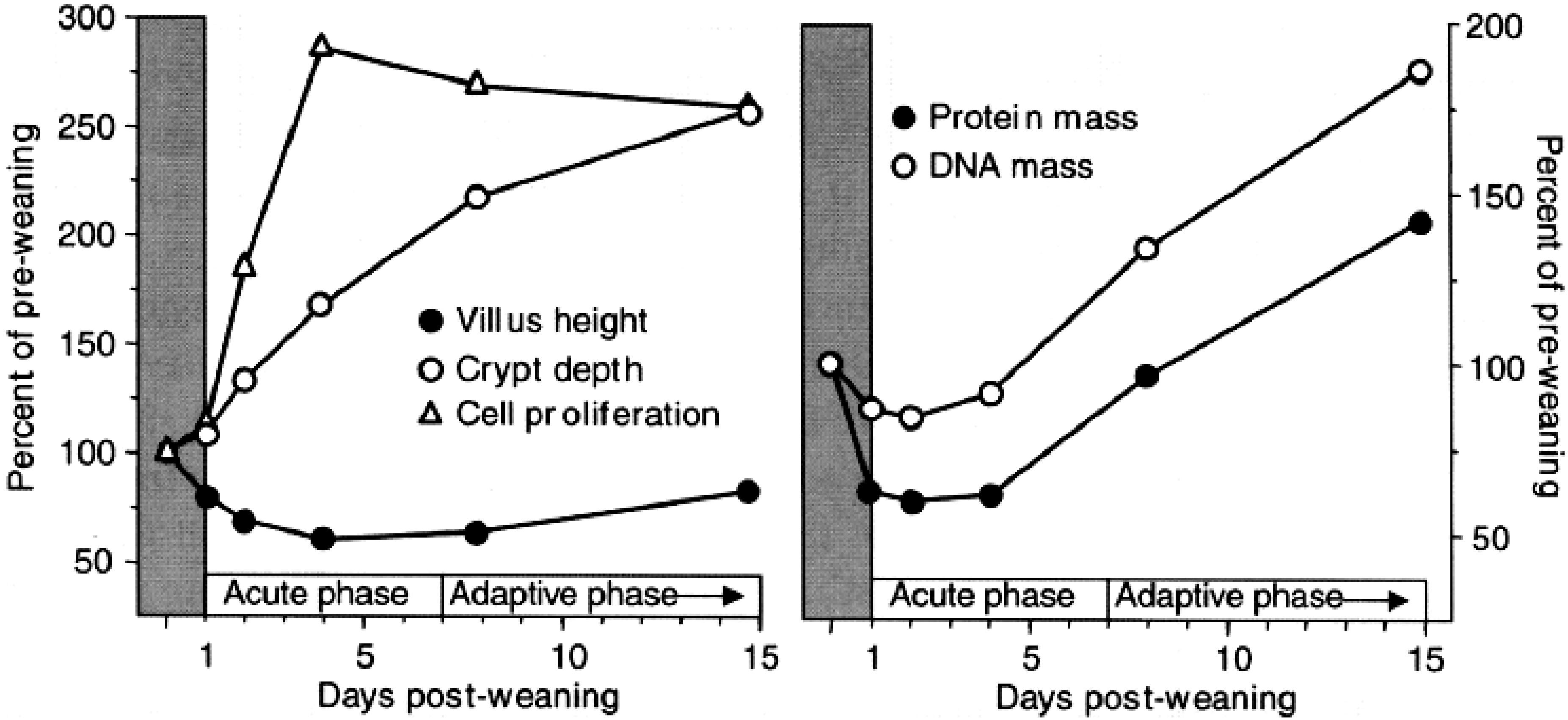
Microbiota: suckling vs. 7d after weaning



Those animals which microbiota does not adapt to the solid diet will have diarrhea

M. Castillo et al. / Veterinary Microbiology 124 (2007) 239–247

Intestinal Mucosa: Evolution after weaning



Pluske *Journal of Animal Science and Biotechnology* 2013, **4**:1

Intestinal Mucosa: suckling vs. 7d after weaning

	Suckling	Weaned	*P*-value
Caecum histological study			
Crypt depth (μm)	373 ± 48.6	359 ± 30.6	0.566
Crypt density[a]	12.4 ± 0.36	10.5 ± 0.25	<0.001
Mitoses/100 cells	1.0 ± 0.47	1.8 ± 0.47	0.021
Goblet cells/100 cells	25.5 ± 3.48	19.4 ± 3.30	0.011
Intraepithelial lymphocytes/100 cells	1.4 ± 0.67	2.6 ± 0.91	0.024
Lymphocyte density[b]	1.7 ± 0.45	2.3 ± 0.40	0.045
Total cell density[b]	8.7 ± 0.90	8.9 ± 0.47	0.665
Lymphocyte/total cells	0.2 ± 0.04	0.3 ± 0.07	0.109
Mucosal thickness (μm)	470 ± 74.8	528 ± 70.8	0.243
Muscular layer thickness (μm)	370 ± 49.5	328 ± 37.0	0.111
Total intestinal wall thickness (μm)	848 ± 98.1	856 ± 95.1	0.899

M. Castillo et al. / Veterinary Microbiology 124 (2007) 239–247

Solution: AGP, profilaxis, metafilaxis…

EFSA (food)

Antibiotic growth promoters

Fact: No prescription

Theory:
Same antibiotics, lower dose
Reduces microbial population, less inflamation, more nutrients available… pathology???

EMA (medicine)

Therapeutic use of antibiotics

Fact: Prescription

Theory:
Same antibiotics, high dose
Fights a particular pathogen

Methods: The world after PCR

Meet Kary Mullis, inventor of the PCR

"Sometimes a good idea comes to you when you are not looking for it. Through an improbable combination of coincidences, naïveté, and luck mistakes, such a revelation came to me one Friday night in April, 1983, as I gripped the steering wheel of my car and snaked along a moonlit mountain road into northern California's redwood country." *Sci. Am.* 1990 *262*:56-61, 64-5.

Methods: Microbiota

Culture-based methods → Molecular methods

T-RFLP
DGGE
Microarrays
(90s)

Next Generation Sequencing
(2005)

Third Generation Sequencing
(recently)

Cheaper and faster

Methods: Microbiota

Culture-based methods → **Molecular methods**

Culture-based methods

PROS

- -Cheap
- -Live bacteria
- -Physiology and biochemistry

CONS

- -Time consuming
- -Processed immediately
- -Unculturable bacteria

Molecular methods

PROS

- -All bacteria present
- -Fast and samples can be frozen

CONS

- -Expensive
- -Inconsistency on sample processing and data analysis
- -Not well developed for function

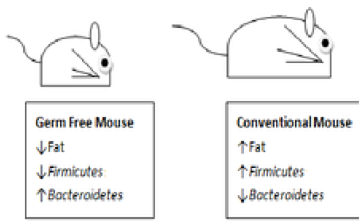
We should go beyond: “Firmicutes and bacteroidetes are the main phyla and such and such genera are different”.

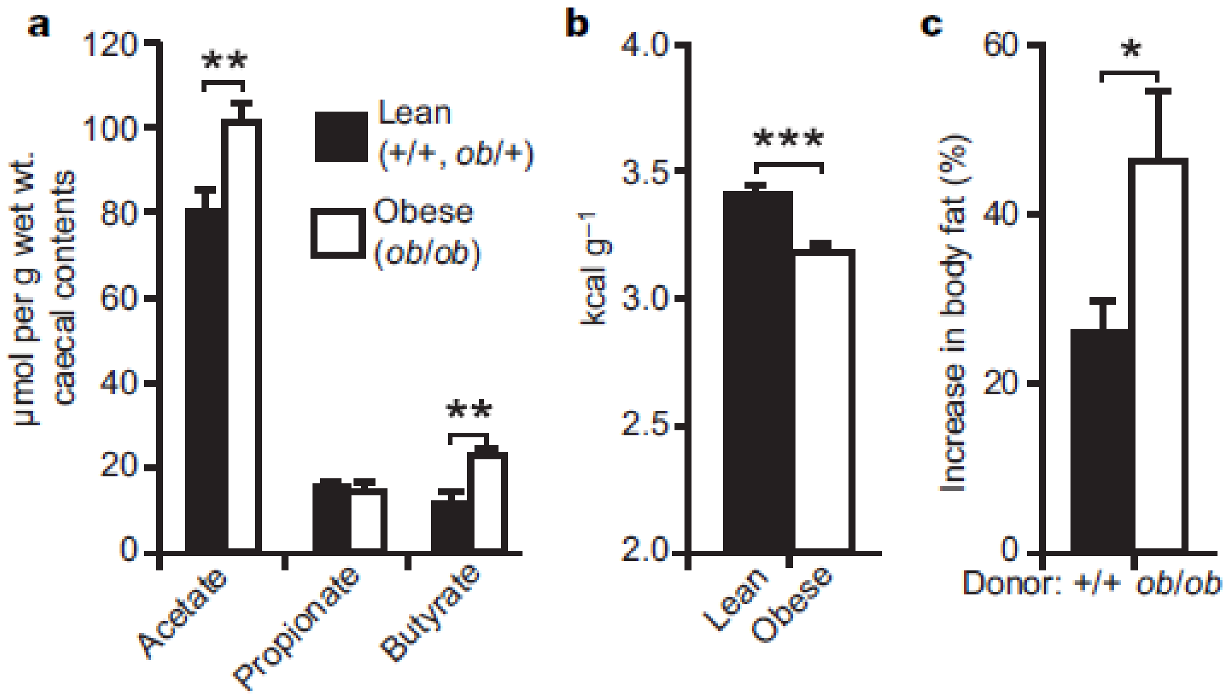
Methods: Microbiota functional measure

Volatile Fatty Acids

Acetic
Propionic
Butyric
Valeric
Iso forms

Lactic etc…



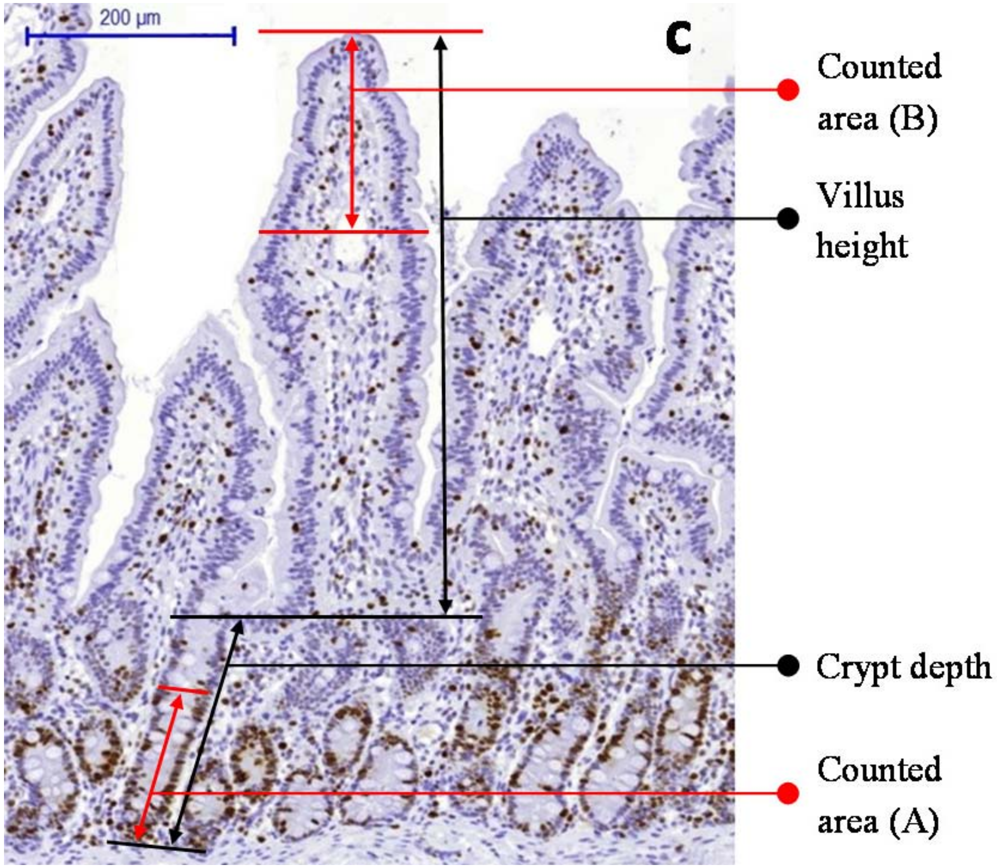


An obesity-associated gut microbiome with increased capacity for energy harvest

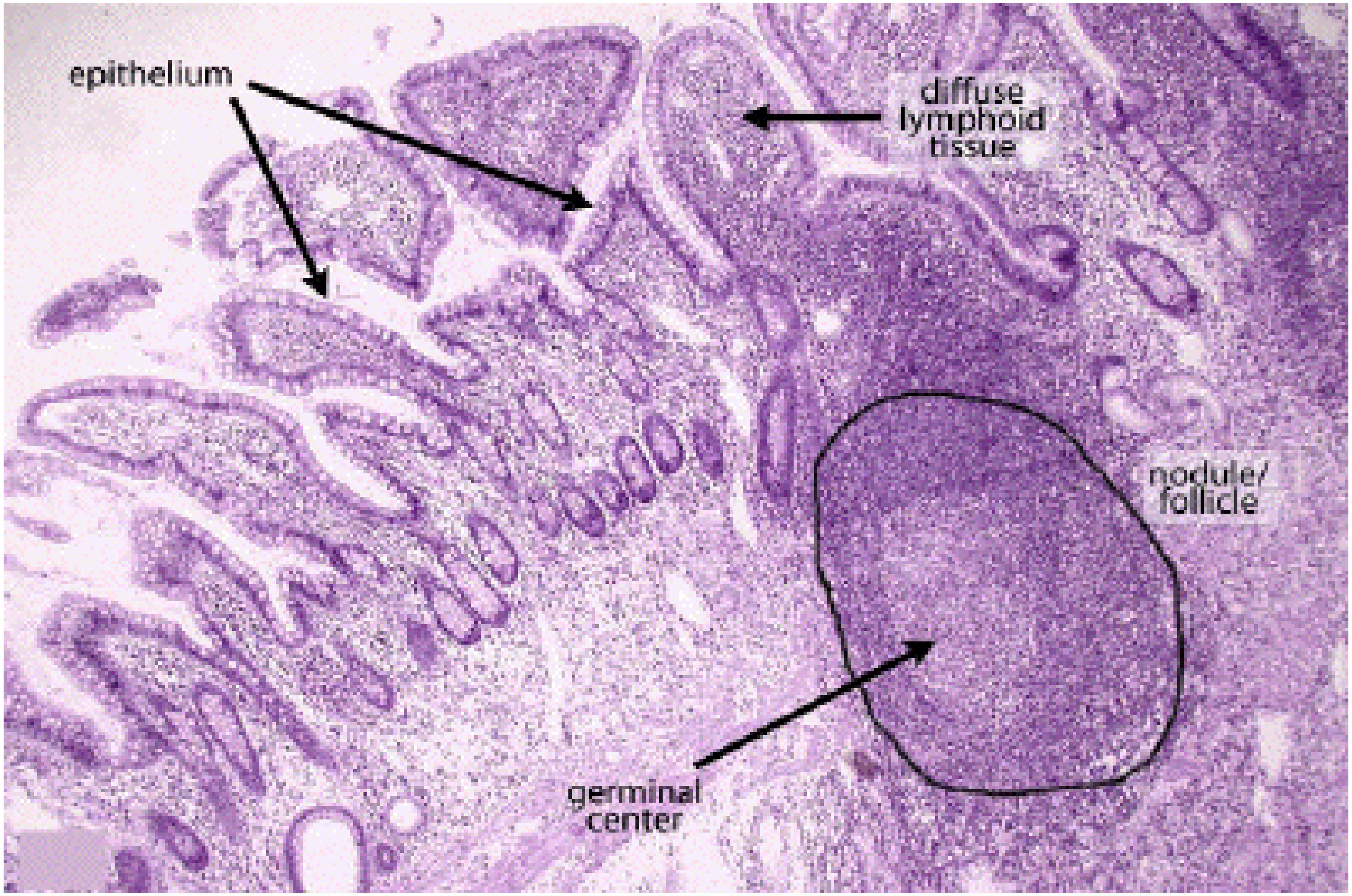
Peter J. Turnbaugh[1], Ruth E. Ley[1], Michael A. Mahowald[1], Vincent Magrini[2], Elaine R. Mardis[1,2] & Jeffrey I. Gordon[1]

nature
NATURE | Vol 444 | 21/28 December 2006

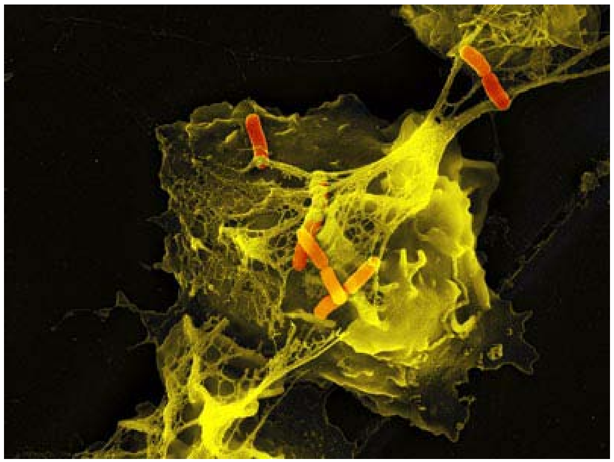
Methods: Mucosa

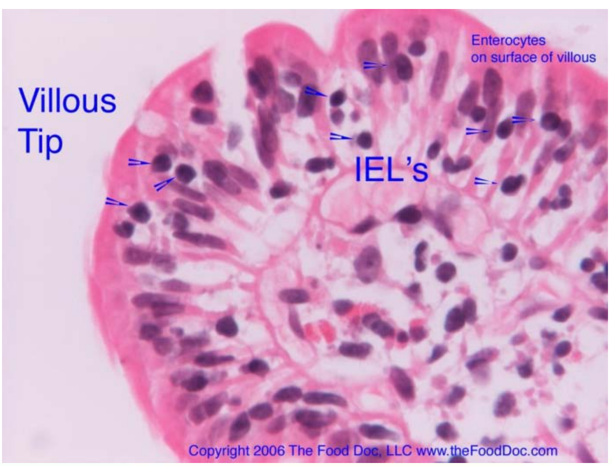


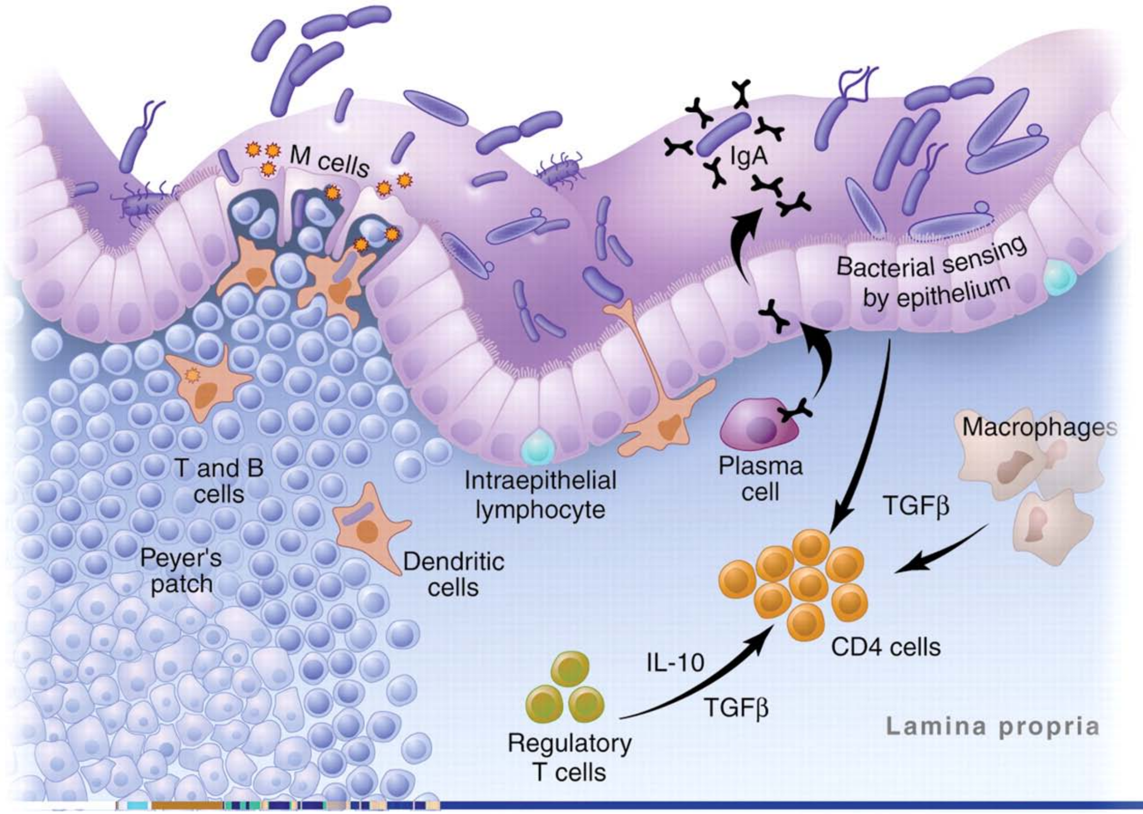
Methods: GALT, Gut Associated Lymphoid Tissue



Methods: GALT







-In situ methods
-Cytometry
-Molecular (–omics) methods

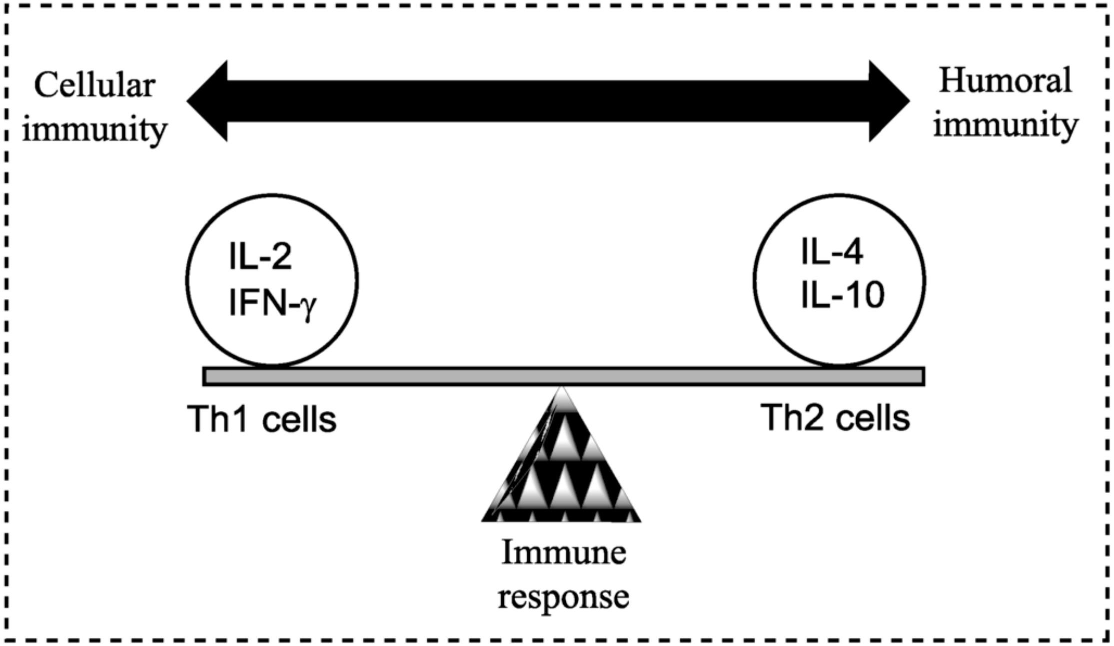
Methods: GALT, Gut Associated Lymphoid Tissue

Table 6. Leukocyte cell subsets (flow cytometry analysis) in ileocolic lymph node and ileal Peyer's patches of pigs fed the experimental diets[1]

	Lymph node				Ileal Peyer's patches			
Item	CT[2]	SDPP[2]	XT[2]	SEM	CT[2]	SDPP[2]	XT[2]	SEM
$SWC3^+$, %	15.6	12.6*	15.7	0.9	18.4	10.5*	21.6	2.4
$CD21^+$, %	41.6	33.2*	32*	2.6	34.6	38.2	37.2	6.4
$\gamma\delta TCR^+$, %	16.5	12.9**	17.1	1	3	3.6	3.2	0.3
$CD4^+$, %	28.1	27.4	26.5	1.5	2.2	2.1	2.1	0.2
$CD8^+$, %	24.5	25.9	24.8	2.2	3.1	3.3	3	0.3

Methods: The animal surrounding the gut

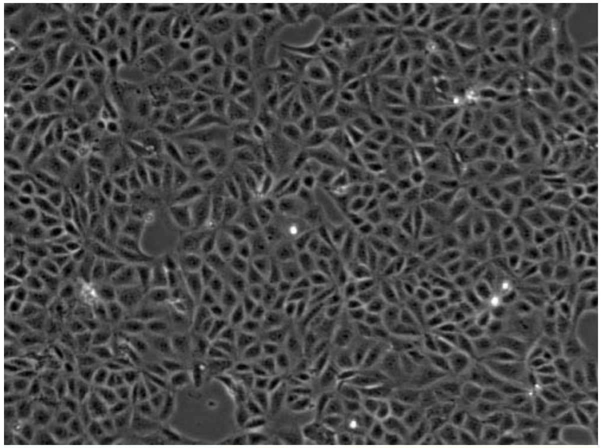
There is no win-win situation with immune function. Moving towards one type will reduce the other.

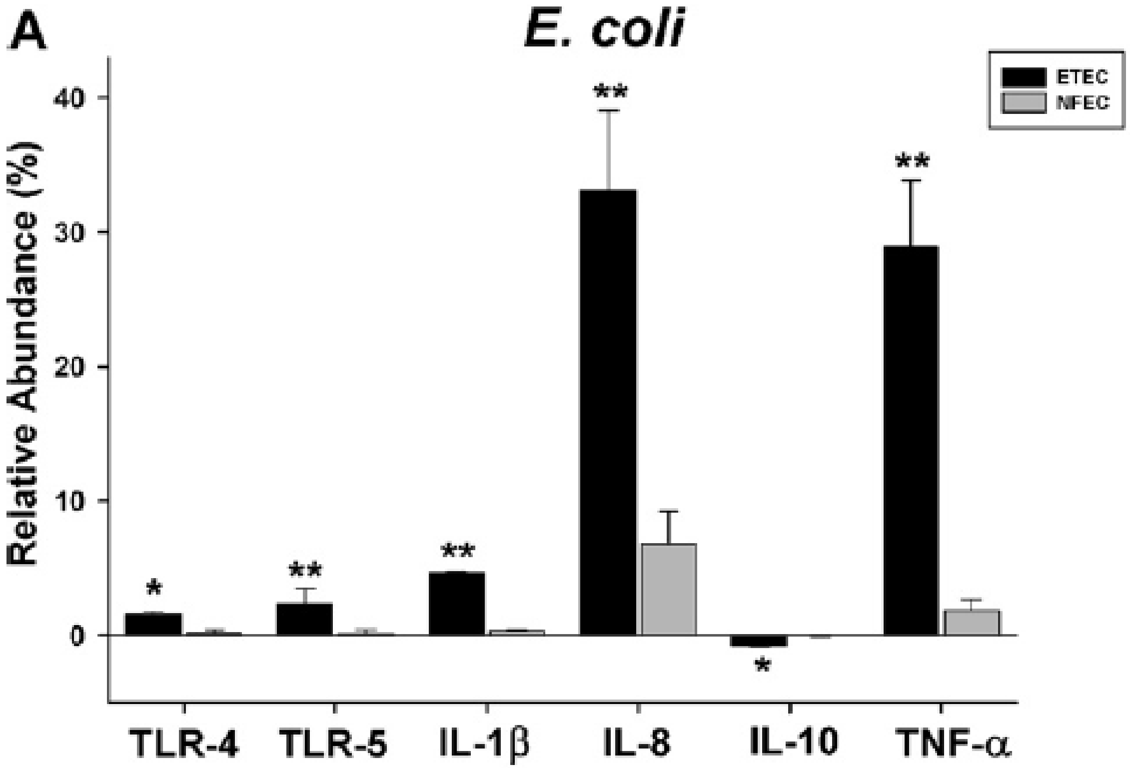


In vitro methods & Experimental infections

In vitro methods are very useful for screening

Experimental infections will show differences that are not seen in physiological conditions





R.G. Hermes et al. / Comparative Immunology, Microbiology and Infectious Diseases 34 (2011) 479–488

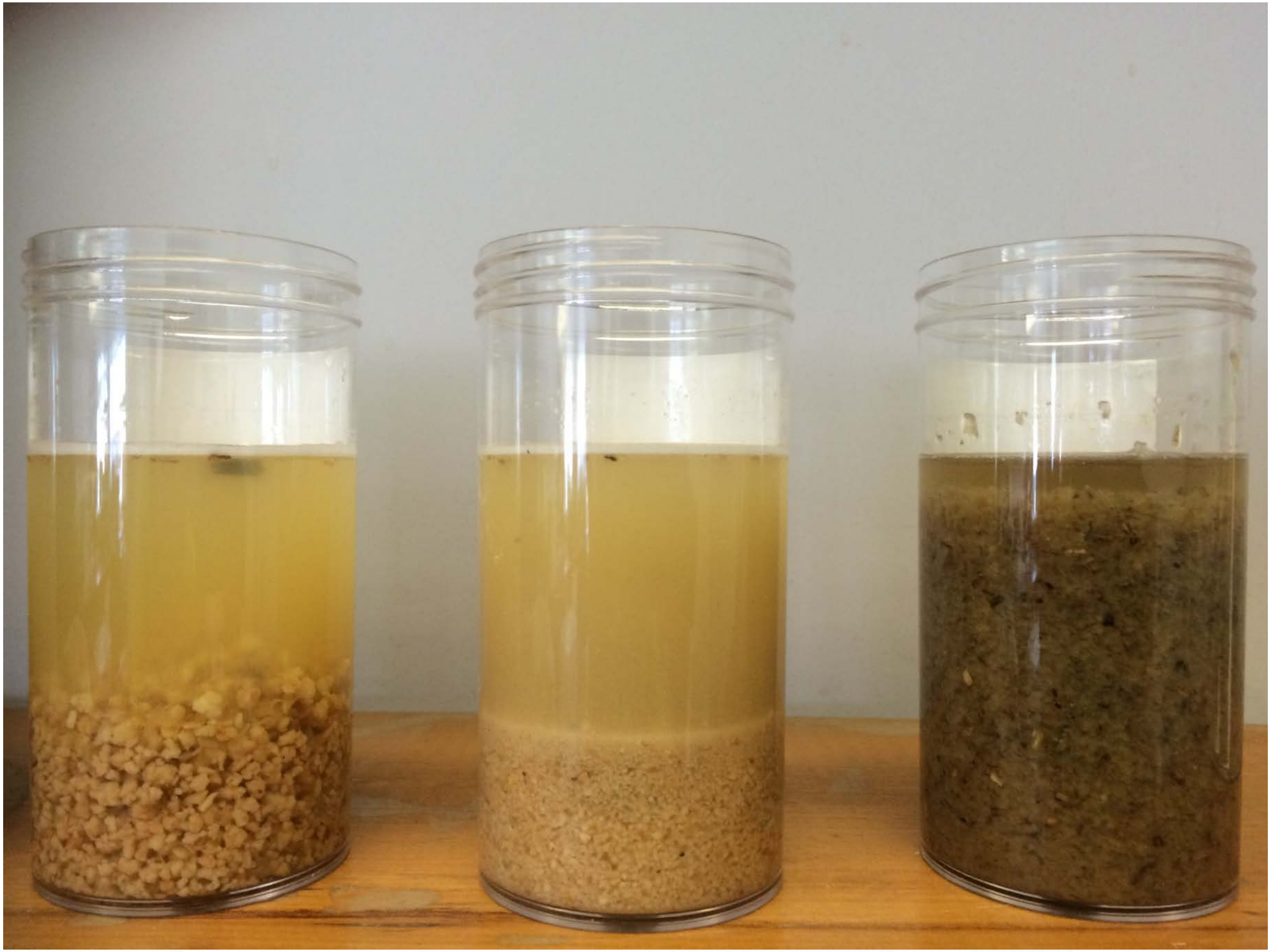
Effect of wheat bran on piglet gut health after weaning

My first approach to fiber

Table 1. Ingredients of the experimental diets (as-fed basis)[1]

Ingredient, %	ST	CC	BP	WB	HF
Barley	15.00	15.00	15.00	15.00	15.00
Soybean meal, 44% CP	27.70	27.70	27.70	27.70	27.70
Corn	54.02	54.02	45.27	42.31	35.55
Soybean oil	0.70	0.70	1.60	2.70	1.60
Sugar beet pulp	—	—	8.00	—	8.00
Wheat bran	—	—	—	10.00	10.00
Calcium carbonate	0.81	0.81	0.61	1.08	0.80
Dicalcium phosphate	0.76	0.76	0.85	0.27	0.50
Sodium chloride	0.25	0.25	0.22	0.25	0.23
Vitamin/mineral premix[2]	0.40	0.40	0.40	0.40	0.40
L-Lysine HCl	0.17	0.17	0.15	0.12	0.07
Threonine	0.04	0.04	0.03	0.02	—
Methionine	—	—	0.02	—	—

[1]Experimental diets: ST = standard corn diet, CC = coarse ground corn diet, BP = sugar beet pulp diet, WB = wheat bran diet, and HF = high-fiber diet.



My first approach to fiber

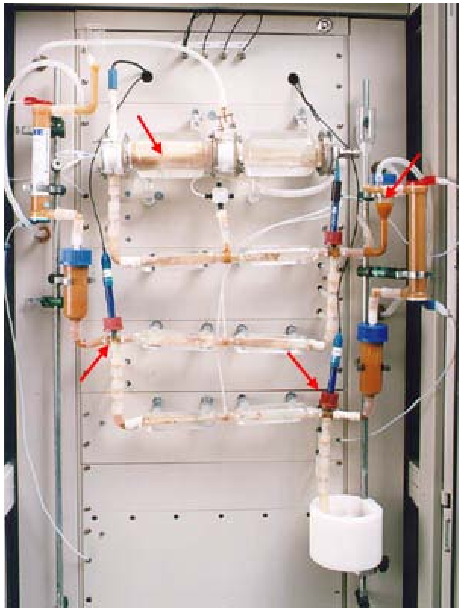
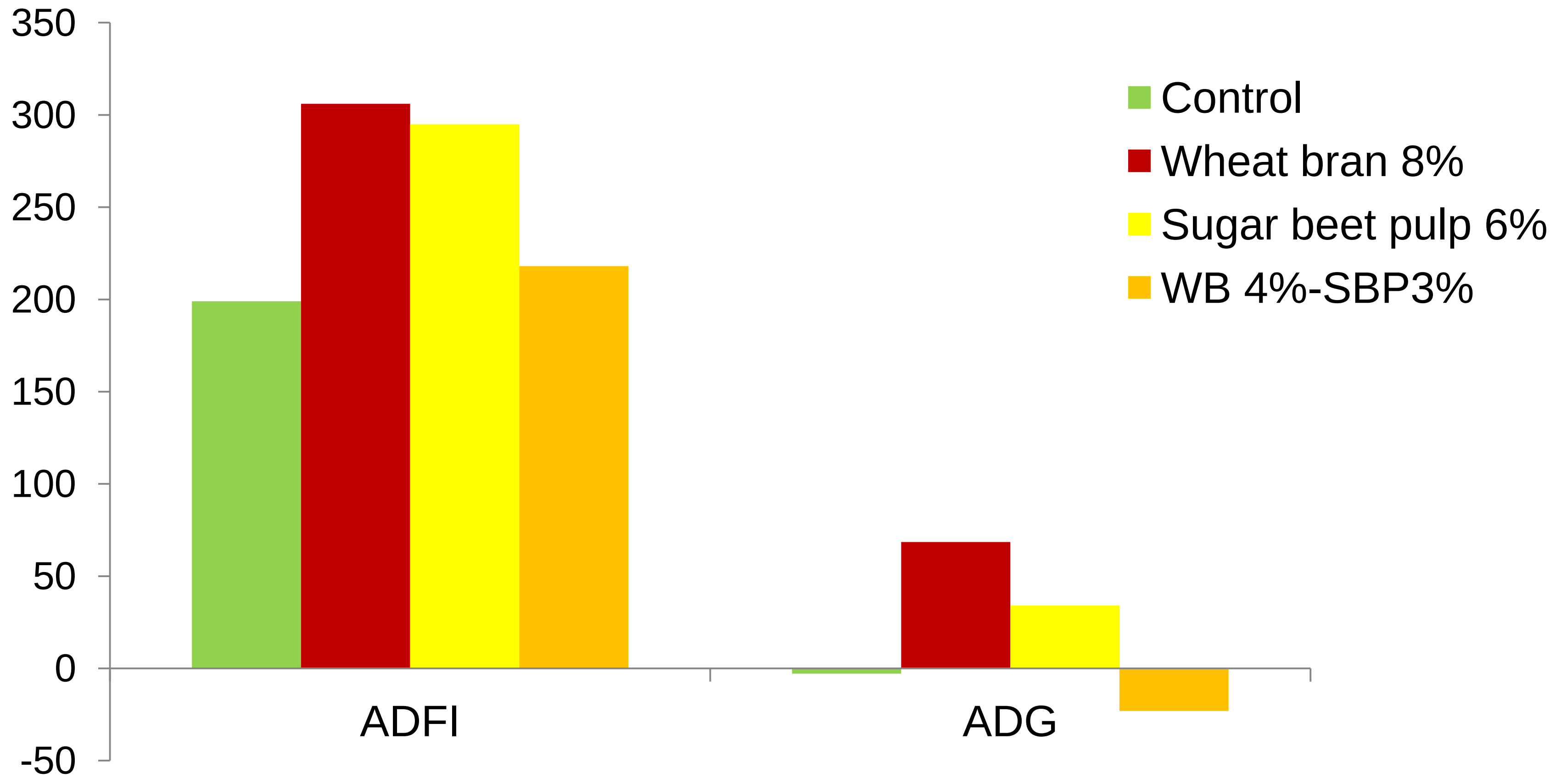


Table 3. Digestibility of OM, CP, and starch in the T-cannulated growing pigs (in vivo), in the Toegepast-Natuurwetenschappelijk Onderzoek dynamic in vitro model (TIM), or using the in vitro static method[1]

Digestibility, %	ST	CC	BP	WB	HF	SEM
OM						
In vivo	70.8^{a}	65.0^{b}	68.1^{ab}	69.7^{ab}	65.1^{b}	1.10
TIM 6 h	61.0^{a}	49.4^{ab}	24.4^{bc}	40.4^{abc}	16.5^{c}	5.09
TIM 8 h	74.9^{a}	61.7^{ab}	33.3^{c}	48.5^{abc}	24.0^{c}	4.84
Static method	82.5^{a}	81.0^{ab}	78.4^{b}	81.3^{a}	74.0^{c}	0.58
CP						
In vivo	76.6^{a}	72.8^{ab}	65.9^{b}	73.3^{ab}	70.9^{ab}	2.42
TIM 6 h	81.8^{a}	75.4^{ab}	43.0^{c}	64.9^{abc}	56.0^{bc}	3.16
TIM 8 h	91.9^{a}	87.7^{ab}	50.1^{d}	76.6^{bc}	68.6^{c}	1.67
Starch						
In vivo	89.3	88.9	89.5	87.7	89.8	1.79
TIM 6 h	68.6	61.1	52.2	68.8	50.2	3.76
TIM 8 h	75.0	67.4	56.3	81.2	64.7	3.83
Static method	83.6^{a}	70.4^{b}	83.0^{a}	80.2^{ab}	83.4^{a}	2.40

[a–d]Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]Experimental diets: ST = standard corn diet, CC = coarse ground corn diet, BP = sugar beet pulp diet, WB = wheat bran diet, and HF = high-fiber diet.

Effect of different fiber sources on productive performance of piglets 10d after weaning (Molist et al., 2009)



Effect of wheat bran in piglets challenged with E coli K88+

Item	Diets[a] NC	PC	WBf	WBc	SEM[b]	P - value
E. coli K88 determination	4.7^{x}	4.7^{xy}	2.2^{xy}	0.7^{y}	2.66	0.021
E. coli population	6.3^{x}	6.3^{xy}	4.9^{y}	4.1^{y}	2.11	0.014
Faecal score[c]						
6 h post-challenge	1.5	0.6	1.0	0.5	0.93	0.157
24 h post-challenge	1.4	0.6	1.0	0.5	0.75	0.066
48 h post-challenge	1.5^{x}	0.6^{xy}	1.1^{xy}	0.5^{y}	0.71	0.025
72 h post-challenge	1.5^{x}	0.5^{y}	1.1^{xy}	0.5^{y}	0.70	0.014
Overall	1.3^{x}	0.5^{y}	1.0^{xy}	0.5^{y}	0.66	0.020
Richness						
Chao2	431.8^{x}	195.1^{y}	313.0^{xy}	230.1^{xy}	35.24	0.050
ICE	192.9^{x}	133.5^{y}	190.2^{x}	158.5^{xy}	21.29	0.001
MM mean	237.3^{x}	170.6^{z}	245.1^{x}	188.4^{y}	8.80	0.001

NC - neg control

PC - Antibiotics

WBf - Wheat bran fine

WBc - Wheat bran coarse

F. Molist et al. / Livestock Science 133 (2010) 214–217

Effect of wheat bran in piglets challenged with E coli K88+

Item	Diets NC	PC	WBf	WBc	SEM	*P*
Faecal score*	1·5[a]	0·5[b]	1·1[a,b]	0·6[b]	0·13	0·001
E. coli (log cfu/g digesta)	7·7	7·2	7·5	5·3	2·04	0·150
VFA concentration (mg/l)						
Total	255[b]	300[b]	253[b]	351[a]	76·5	0·041
Acetic acid	155[b]	156[b]	135[b]	193[a]	26·2	0·002
Propionic acid	62	70	63	81	18·1	0·222
Butyric acid	33	35	28	35	11·2	0·654
Isoacids	5·0	4·0	6·0	7·0	2·6	0·145

Taxon	Microbial level (%) NC	PC	WBf	WBc	SEM
Day 9					
Phylum Firmicutes	59·1	66·3	67·9	70·1	2·38
Class Clostridia	56·8	64·4	65·6	67·3	2·32
Phylum Bacteroidetes	34·7[a]	27·2[a,b]	25·5[a,b]	23·0[b]	2·52
Class Bacteroidetes	34·3[a]	26·9[a,b]	25·0[a,b]	22·6[b]	2·52

F. Molist *et al.* *British Journal of Nutrition* (2012), **108**, 9–15

E. coli Attachment to epithelial cells

Influence of dietary ingredients on *in vitro* inflammatory response of intestinal porcine epithelial cells challenged by an enterotoxigenic *Escherichia coli* (K88)

Rafael G. Hermes[a,*], Edgar G. Manzanilla[a], Susana M. Martín-Orúe[a], José F. Pérez[a], Kirk C. Klasing[b]

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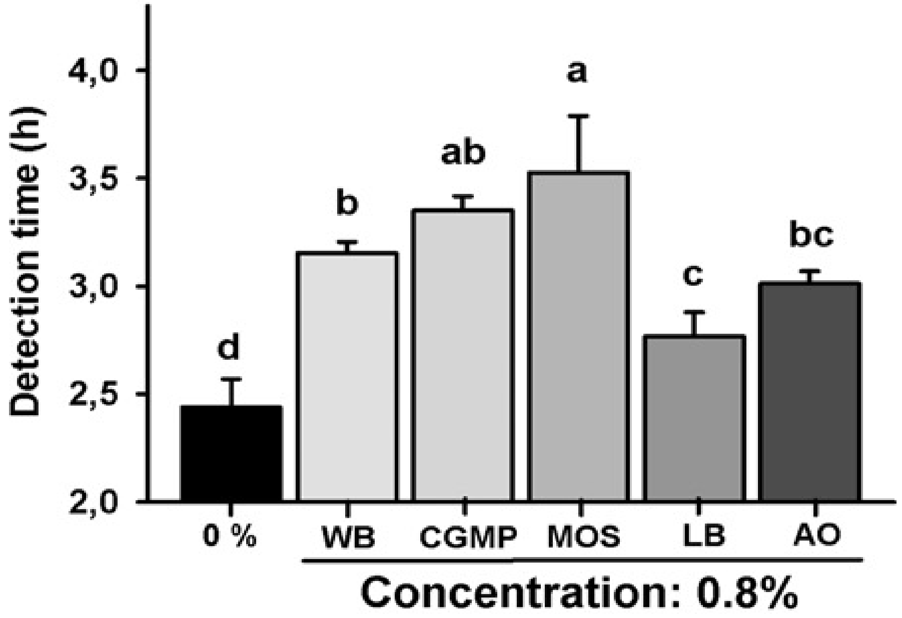
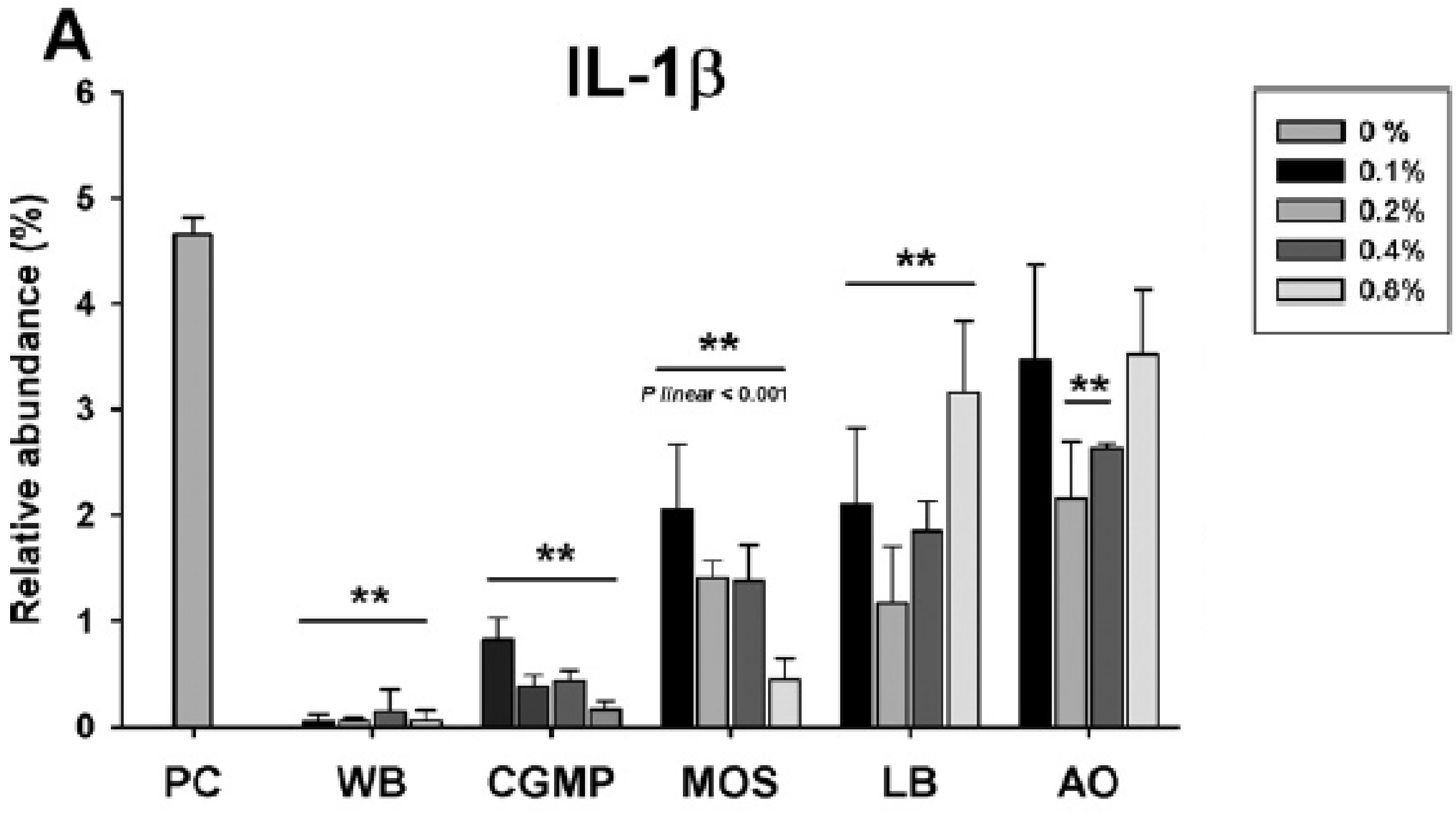


Wheat Bran
Casein GlycoMacroPeptide
MannanOligoSaccharides
Locust Bean
Arpergillus Oryzae

R.G. Hermes et al. / Comparative Immunology, Microbiology and Infectious Diseases 34 (2011) 479–488

Effect of different fibers in cells challenged with E coli K88+



Final application

PLOS | ONE

A Proteinaceous Fraction of Wheat Bran May Interfere in the Attachment of Enterotoxigenic *E. Coli* K88 (F4+) to Porcine Epithelial Cells

Gemma González-Ortiz, Sílvia Bronsoms, H. C. Quarles Van Ufford, S. Bart A. Halkes, Ritva Virkola, Rob M. J. Liskamp, Cees J. Beukelman, Roland J. Pieters, José Francisco Pérez, Susana María Martín-Orúe

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Research article

New properties of wheat bran: anti-biofilm activity and interference with bacteria quorum-sensing systems

Gemma González-Ortiz, H. C. Quarles Van Ufford, S. Bart A. Halkes, Marta Cerdà-Cuéllar, Cees J. Beukelman, Roland J. Pieters, Rob M. J. Liskamp, José F. Pérez, Susana M. Martín-Orue

Patents | English | French

Wheat bran soluble extract as anti-biofilm agent

WO 2015071413 A1

ABSTRACT

The present invention relates to products, compositions, methods and uses thereof which are useful in relation to the prevention and treatment of pathogen infections, preferably bacterial infections. More specifically, the invention relates to the use of wheat bran soluble extract as bacterial anti-biofilm agent.

Publication number	WO2015071413 A1
Publication type	Application
Application number	PCT/EP2014/074608
Publication date	May 21, 2015
Filing date	Nov 14, 2014
Priority date	Nov 15, 2013
Inventors	ORÚE Susana María MARTÍN, HERNÁNDEZ Jose Francisco PÉREZ, ORTÍZ Gemma GONZÁLEZ, Less «
Applicant	Universitat Autonoma De Barcelona
Export Citation	BiBTeX, EndNote, RefMan

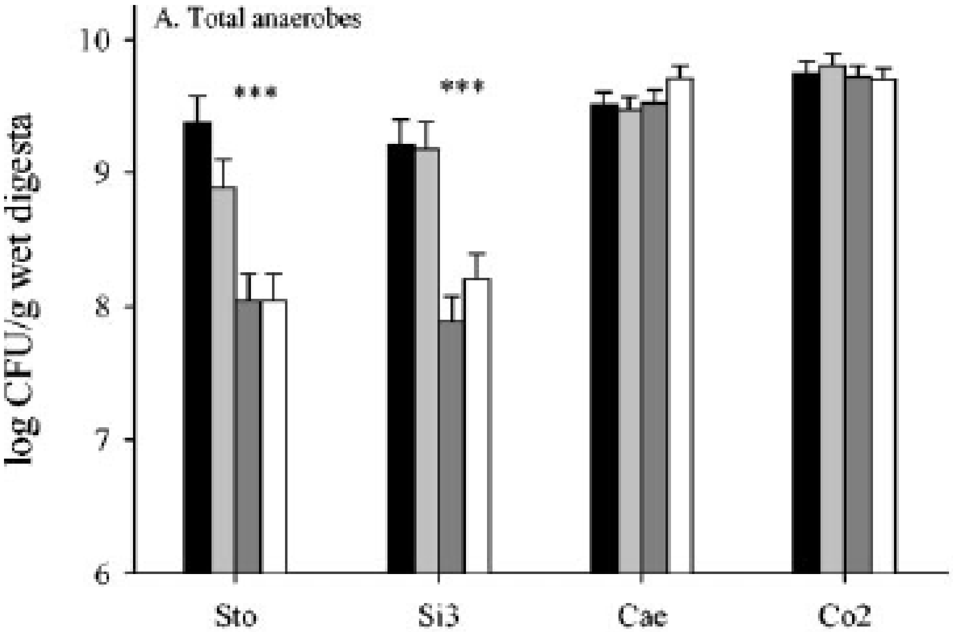
Statistical considerations in studies on gut health

Correlation vs. mechanistic approach (causation)

Influence of Dietary Zinc Oxide and Copper Sulfate on the Gastrointestinal Ecosystem in Newly Weaned Piglets

Ole Højberg,* Nuria Canibe, Hanne Damgaard Poulsen, Mette Skou Hedemann, and Bent Borg Jensen

Applied and Environmental Microbiology, May 2005, p. 2267–2277



Better growth
Less diarrhea

Decrease in lactobacilli
Increase in coliforms
Increase in enterococci

Correlation vs. mechanistic approach (causation)

CLINICAL MANAGEMENT OF ACUTE DIARRHOEA

World Health Organization

unicef

- Development of an improved formula for ORS solution with reduced levels of glucose and salt, which shortens the duration of diarrhoea and the need for unscheduled intravenous fluids[1]
- Demonstration that zinc supplements given during an episode of acute diarrhoea reduce the duration and severity of the episode[2], and
- Findings that zinc supplementation given for 10–14 days lowers the incidence of diarrhoea in the following 2–3 months[3]

Table 2 Zinc, iron and copper concentrations (mg/L) in plasma and blood cells

	Unweaned	Weaned	Weaned + ZnO	SEM*	p value
Plasma					
Zn	1.10^{a}	0.76^{b}	1.32^{a}	0.099	0.001

Table 3 Zinc, iron and copper concentrations (mg/kg) in liver and zinc concentration in pancreas and spleen

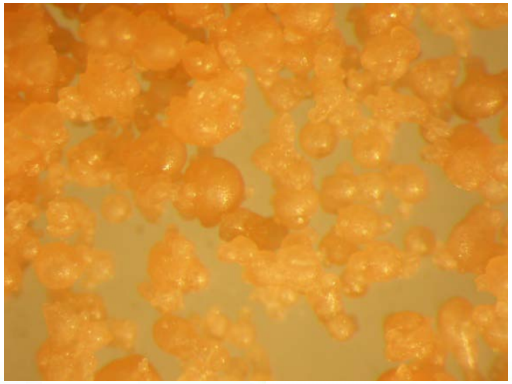
	Unweaned	Weaned	Weaned + ZnO	SEM*	p value
Liver					
Zn	67.0^{b}	69.5^{b}	330.8^{a}	18.4	<0.001
Fe	153	173	156	35.7	0.896
Cu	50.6	48.5	40.1	7.79	0.545
Pancreas Zn	48.7^{b}	35.2^{b}	130.9^{a}	15.54	<0.001
Spleen Zn	22.1	22.7	22.7	0.738	0.801

Journal of Animal Physiology and Animal Nutrition **97** (2013) 6–12

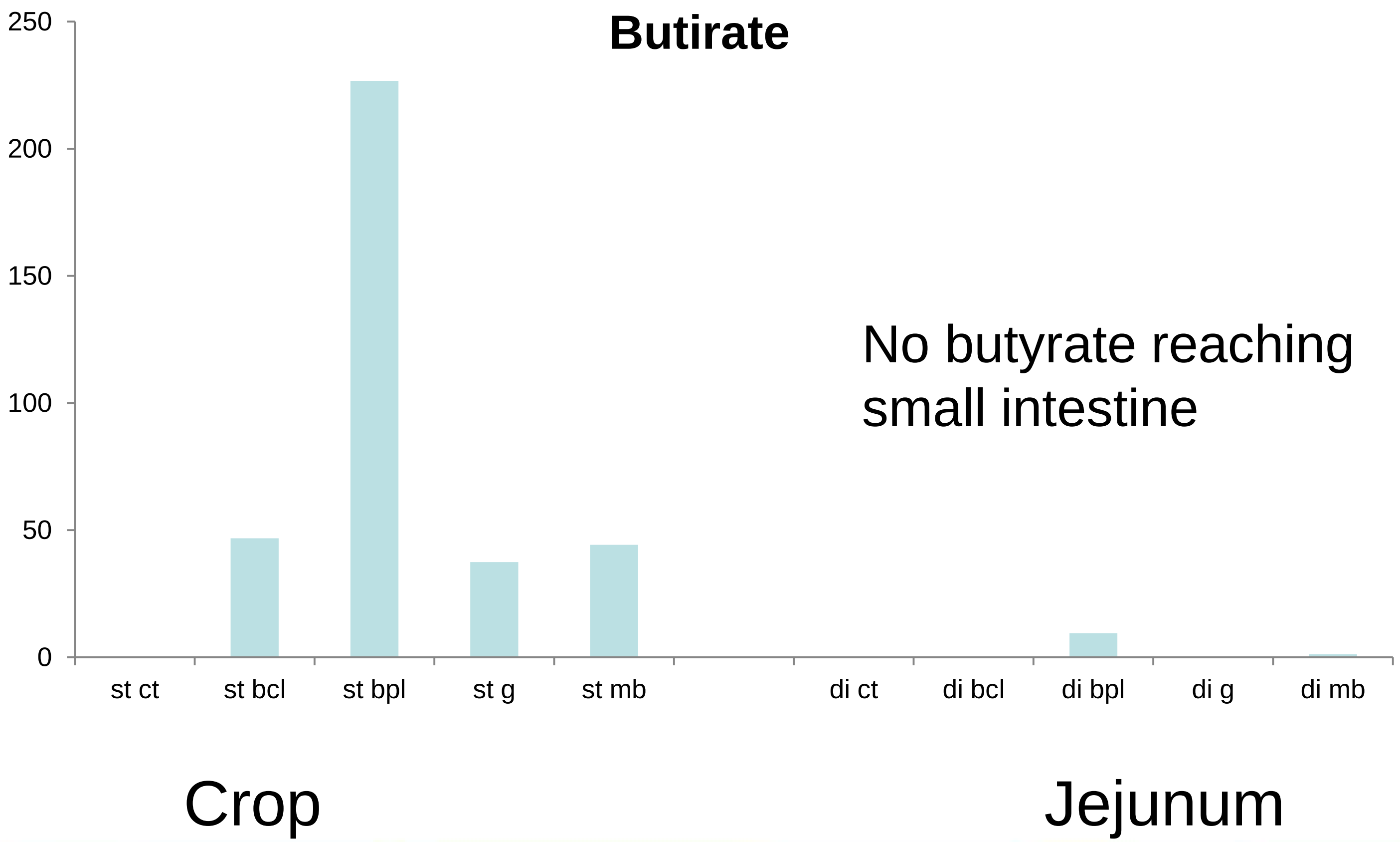
Correlation vs. mechanistic approach (causation)

Performance of broilers fed diets containing increasing doses of sodium butyrate

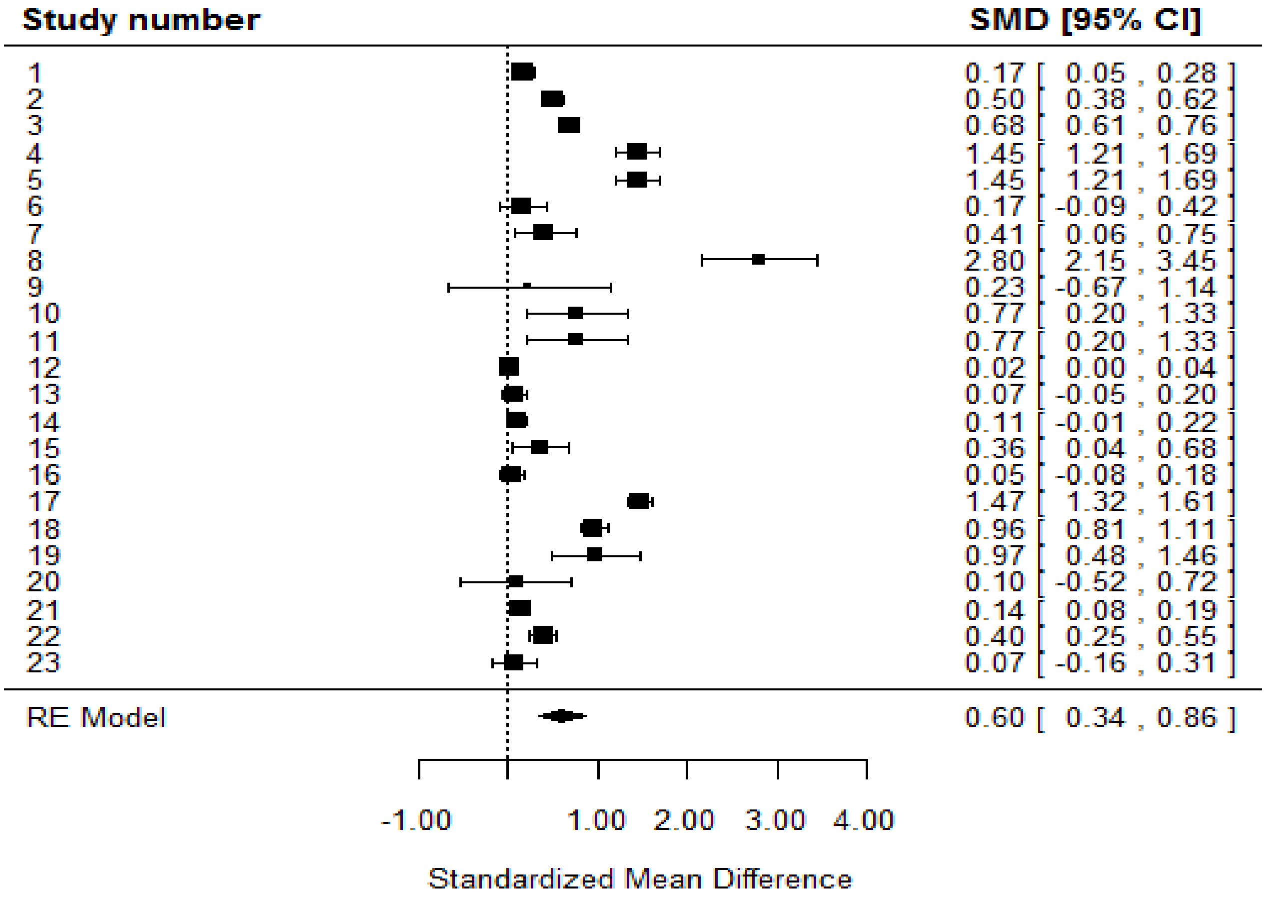
	0.0	0.1	0.3	1.0	SEM	P-value	Lineal P
0-5 d							
ADG	14.4[b]	15.8[ab]	15.7[ab]	16.7[a]	0.51	0.036	0.025
G:F	0.93[b]	0.97[ab]	1.00[ab]	1.03[a]	0.020	0.011	0.009



Correlation vs. mechanistic approach (causation)



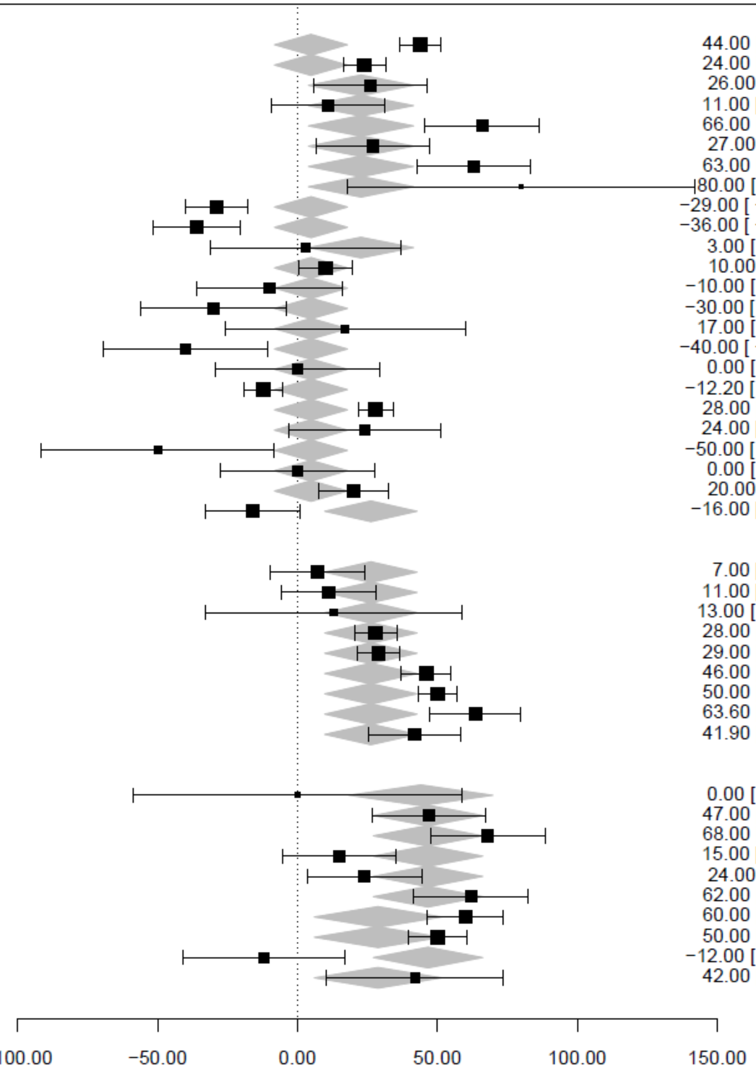
Publication bias: Meta analysis of Tulathromycin



Publication bias

Effect of xylanase on pig growth

Author(s) and Year	C (g/d)	T (g/d)	Mean difference [95% CI]
European diets			
Cadogan et al.,2015	331	375	44.00 [36.58 , 51.42]
Cadogan et al.,2015	306	330	24.00 [16.58 , 31.42]
Ndou et al.,2015	902	928	26.00 [5.62 , 46.38]
Ndou et al.,2015	902	913	11.00 [−9.38 , 31.38]
Ndou et al.,2015	902	968	66.00 [45.62 , 86.38]
Ndou et al.,2015	902	929	27.00 [6.62 , 47.38]
Ndou et al.,2015	902	965	63.00 [42.62 , 83.38]
Nortey et al.,2015	510	590	80.00 [18.02 , 141.98]
L'Anson et al.,2013	402	373	−29.00 [−40.20 , −17.80]
Lyberg et al.,2008	749	713	−36.00 [−51.71 , −20.29]
Woyengo et al.,2008	784	787	3.00 [−30.95 , 36.95]
Kim et al.,2008	920	930	10.00 [0.40 , 19.60]
Widyaratne et al.,2008	920	910	−10.00 [−36.13 , 16.13]
Widyaratne et al.,2008	1020	990	−30.00 [−56.13 , −3.87]
Vahjen et al.,2007	240	257	17.00 [−25.95 , 59.95]
Nortey et al.,2007	950	910	−40.00 [−69.40 , −10.60]
Nortey et al.,2007	990	990	0.00 [−29.40 , 29.40]
Olukosi et al.,2007	736.4	724.2	−12.20 [−19.00 , −5.40]
Kim et al.,2004	393	421	28.00 [21.86 , 34.14]
Park et al.,2002	1075	1099	24.00 [−3.27 , 51.27]
Mavromichalis et al.,2000	880	830	−50.00 [−91.58 , −8.42]
Mavromichalis et al.,2000	910	910	0.00 [−27.72 , 27.72]
Mavromichalis et al.,2000	450	470	20.00 [7.60 , 32.40]
L'Anson et al.,2014	346	330	−16.00 [−32.88 , 0.88]
By−products diets			
L'Anson et al.,2014	346	353	7.00 [−9.88 , 23.88]
L'Anson et al.,2014	346	357	11.00 [−5.88 , 27.88]
O'Shea et al.,2014	918	931	13.00 [−32.87 , 58.87]
Lipinski et al.,2013	949	977	28.00 [20.31 , 35.69]
Lipinski et al.,2013	949	978	29.00 [21.31 , 36.69]
Hanczakowska et al.,2012	894	940	46.00 [37.18 , 54.82]
Hanczakowska et al.,2012	705	755	50.00 [43.14 , 56.86]
Tapingkae et al,2008	335.6	399.2	63.60 [47.32 , 79.88]
Tapingkae et al,2008	335.6	377.5	41.90 [25.62 , 58.18]
American diets			
Olukosi et al.,2007	510	510	0.00 [−58.80 , 58.80]
Ndou et al.,2015	877	924	47.00 [26.62 , 67.38]
Ndou et al.,2015	877	945	68.00 [47.62 , 88.38]
Ndou et al.,2015	877	892	15.00 [−5.38 , 35.38]
Ndou et al.,2015	877	901	24.00 [3.62 , 44.38]
Ndou et al.,2015	877	939	62.00 [41.62 , 82.38]
Fang et al.,2008	786	846	60.00 [46.38 , 73.62]
Fang et al.,2007	828	878	50.00 [39.61 , 60.39]
Owusu−Asiedu et al.,2002	532	520	−12.00 [−40.93 , 16.93]
Owusu−Asiedu et al.,2002	247	289	42.00 [10.45 , 73.55]



The problem with multiple comparisons

Table 3. Intestinal histology of pigs fed the experimental diets[1]

Item	FM18[2] 0	FM18[2] 200	SBM18[2] 0	SBM18[2] 200	SBM20[2] 0	SBM20[2] 200	SEM	XT	Diet	INT[3]
Proximal jejunum										
Villi:crypt, μm:μm	1.86[y]	2.04	2.32[x]	2.19	1.96[y]	1.91	0.094	0.971	0.003	0.275
Mitoses, n/100 cells	1.09[y]	1.17	1.15[y]	1.05	1.32[x]	1.37	0.092	0.870	0.037	0.608
IEL, n/100 cells	31.7	25.1	34.6	28.8	27.7	31.0	2.33	0.137	0.293	0.094
Lamina propria cells, n/1,000 μm²	9.25[y]	9.75	9.35[y]	9.30	10.70[x]	11.9	0.57	0.249	0.004	0.559
Distal jejunum										
Villi:crypt, μm:μm	1.60	1.62	1.64	1.54	1.56	1.79	0.136	0.679	0.819	0.495
Mitoses, n/100 cells	4.31[y]	6.79	2.56[z]	3.99	5.96[x]	5.53	0.540	0.015	<0.001	0.037
IEL, n/100 cells	32.5[x]	29.6	27.8[y]	29.9	32.8[x]	29.0	0.62	0.140	0.185	0.066
Lamina propria cells, n/1,000 μm²	8.60[y]	10.80	10.45[x]	10.55	10.50[x]	9.30	0.866	0.185	0.115	0.009

Table 5. Volatile fatty acid concentration and profile in cecum and colon of pigs fed the experimental diets[1]

Item	FM18[2] 0	FM18[2] 200	SBM18[2] 0	SBM18[2] 200	SBM20[2] 0	SBM20[2] 200	SEM	XT (P-value)	Diet (P-value)	INT[3] (P-value)
Total VFA, μmol/g of fresh matter	233.1	187.1	204.2	181.3	188.4	184.2	13.87	0.045	0.236	0.346
Acetic acid, %	53.1[y]	51.7	53.1[y]	46.6	55.3[x]	54.7	1.54	0.033	0.008	0.147
Propionic acid, %	31.0	30.0	29.5	30.4	28.2	29.8	1.26	0.636	0.502	0.555
Butyric acid, %	13.0	16.2	13.9	16.6	13.6	12.8	1.03	0.050	0.153	0.120
N-Valeric acid, %	1.8[y]	2.7	2.6[x]	4.7	2.1[y]	2.0	0.51	0.027	0.009	0.124
Branched VFA, %	0.76	0.69	0.51	0.45	0.74	0.66	0.116	0.490	0.094	0.996

The problem with multiple comparisons

Increasing the number of comparisons (p-value)

Microarrays, NGS… can provide thousands of variables

Low sample sizes

Hundreds of false positives

Genomes not well annotated


THANKS!!!
GO RAIBH MAITH AGAIBH!!!