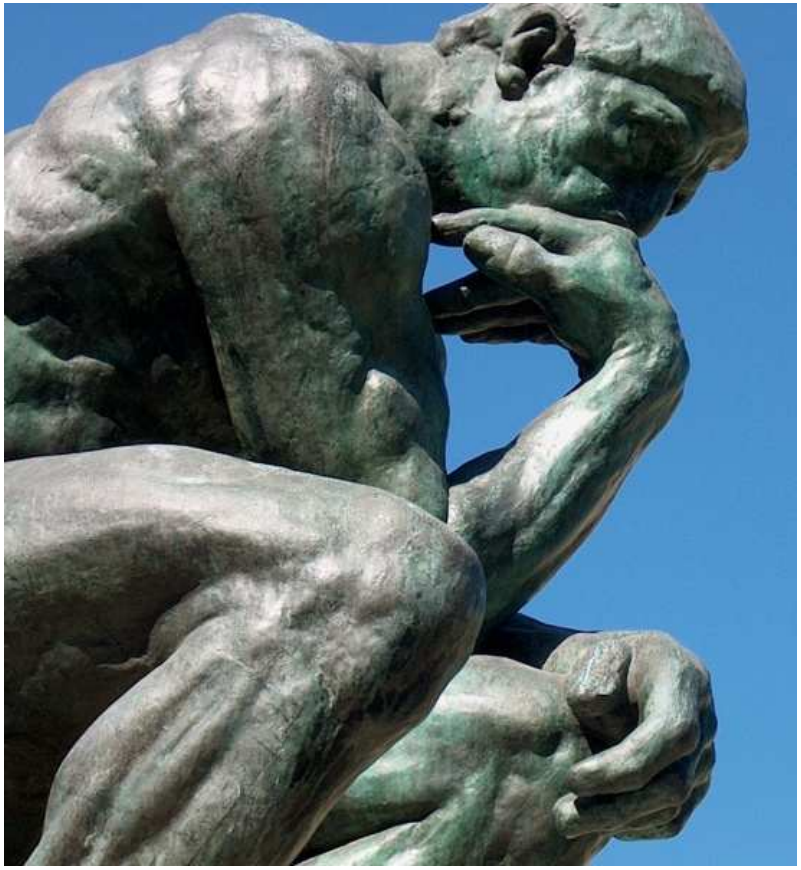
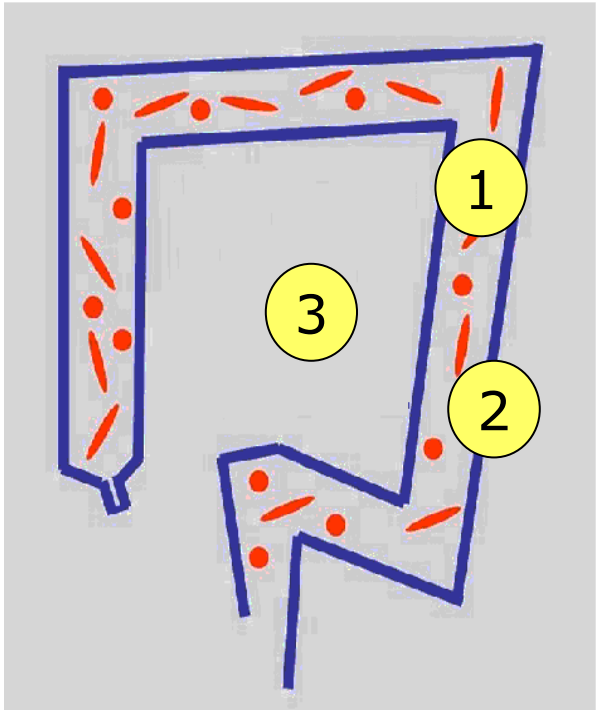

9th Annual
GutDay
2007



- www.gutflora.org Actual and independent information on probiotics
- probiotica.pagina.nl Link analysis of our Dutch link web page
- Organise scientific workshops
- Develop accredited lectures or trainings for health professionals
- Sponsor and facilitate the Dutch Darmendag
- (Modest) sponsor PhD Thesis printing costs, provided contribution to the website



1
Jeroen v.d. Bovenkamp, Wageningen (#3)
Evan Abrahamse, Wageningen (#1)
Odette Pérez, Wageningen (#5)
Saskia van As, Hilversum (#2)

2
Petra Roubos, Wageningen (#7)
Wendy Rodenburg, Wageningen (#6)
Steven Vanhoutvin, Maastricht (#10)
Julia Volman, Maastricht (#11)

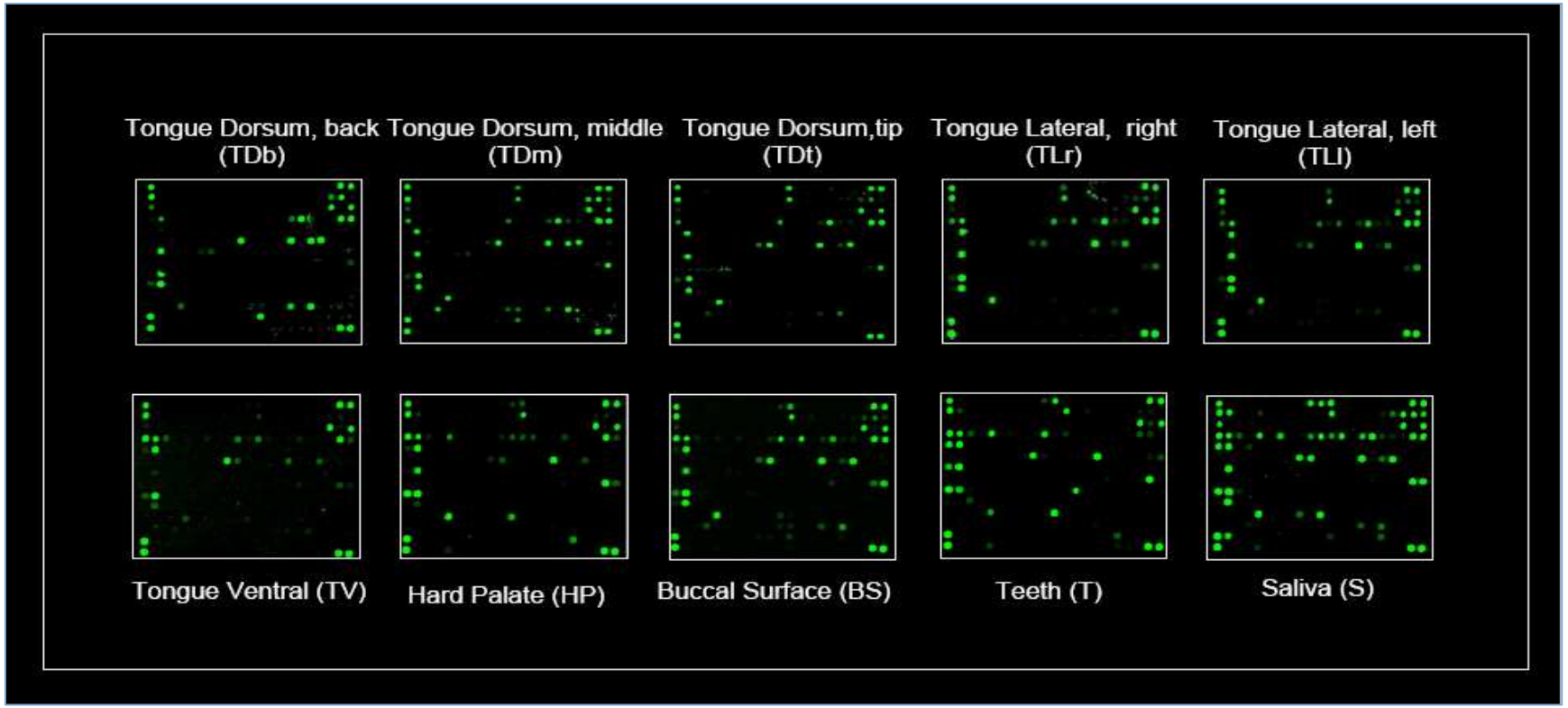
3
Willem Swinkels, Lelystad (#8)
M.I. Gracia/Arno van der Aa, Spain & Lelystad (#4)

Site-specific detection of oral microbiota by the Human Oral Microbe Identification Microarray (HOMIM)

Jeroen van de Bovenkamp[1,2], Marijke Beenes[1,2], Susan Boches[3], Bruce Paster[3,4], Raymond Schipper[1,2]

[1]Top Institute Food and Nutrition, and [2]Wageningen University, Laboratory of Food Chemistry, Wageningen, The Netherlands

[3]The Forsyth Institute, Department of Molecular Genetics, and [4]Harvard School of Dental Medicine, Department of Oral Biology, Boston, MA, USA



In total 69 phylotypes or species were identified:

tongue dorsum 45; lateral 40; ventral 23; hard palate 38; buccal 44; teeth 35; saliva 56

WAGENINGEN UNIVERSITY
WAGENINGEN UR

The Forsyth Institute

HARVARD SCHOOL OF DENTAL MEDICINE

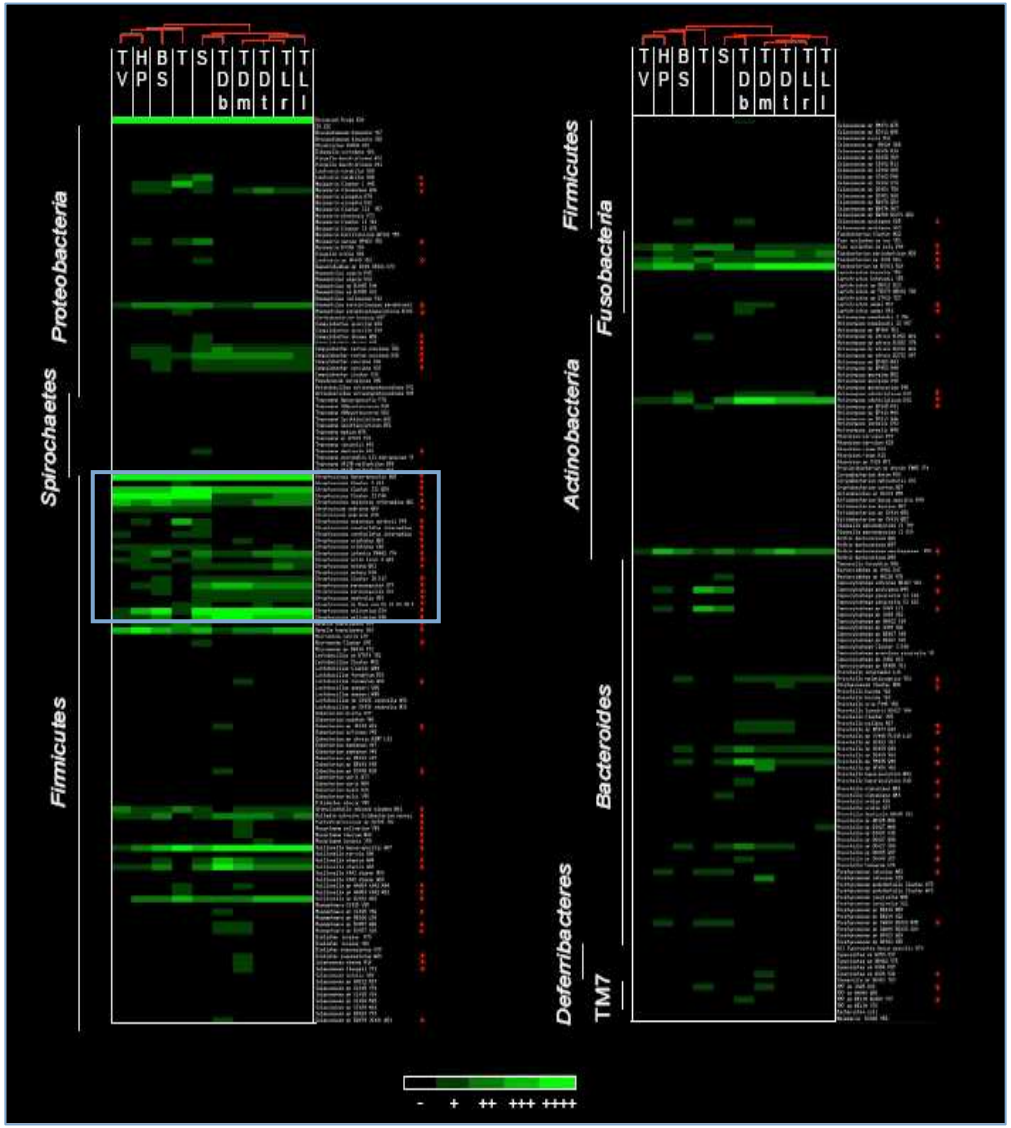


Figure 1. Cluster analysis

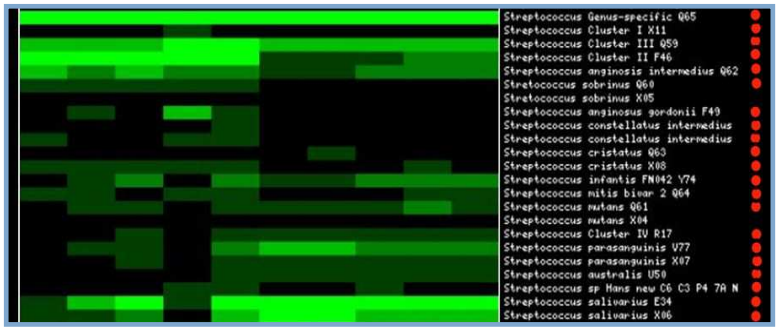


Figure 2. Detail Cluster analysis

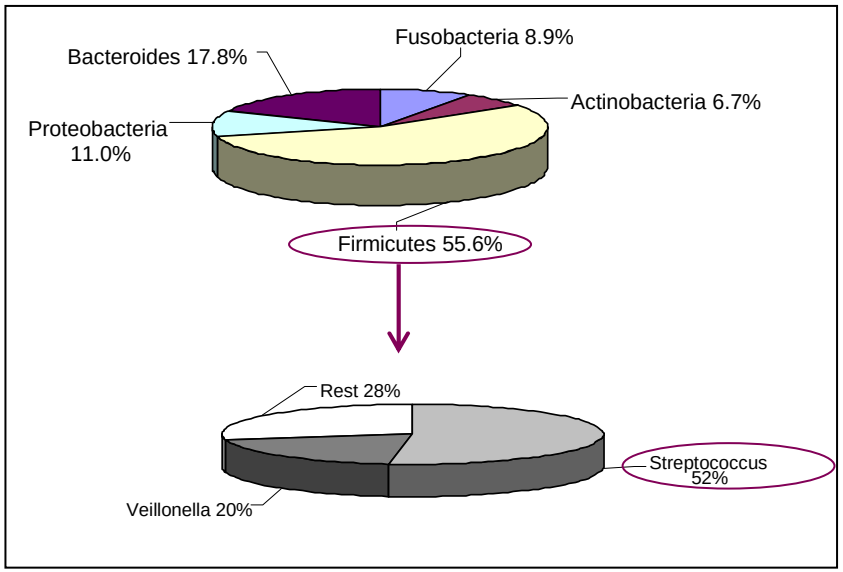
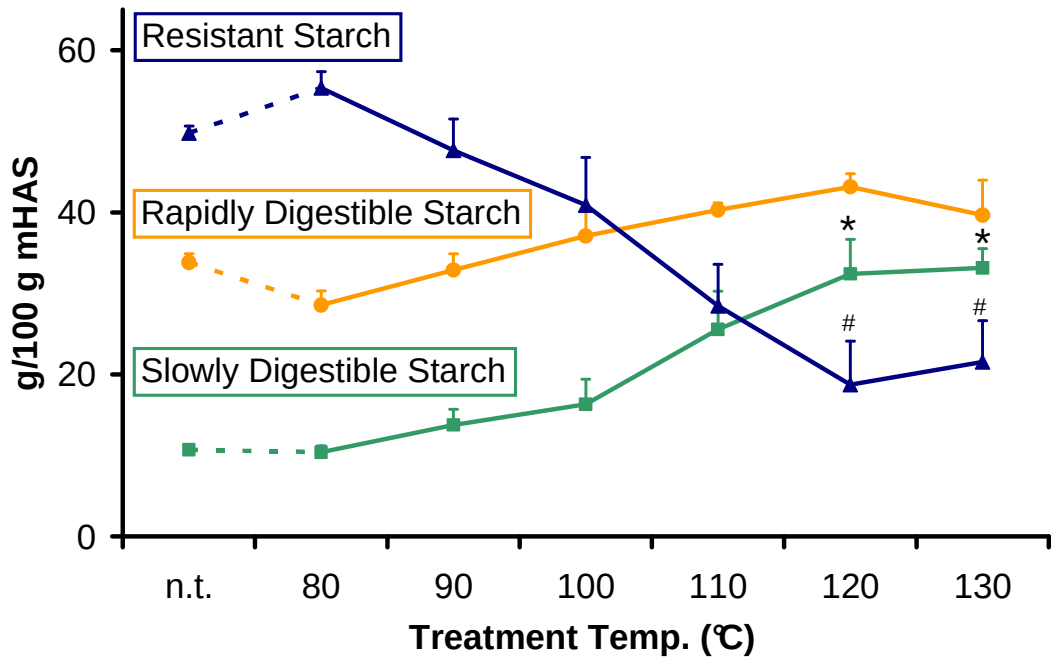


Figure 3. phylotypes and species present on the tongue dorsum

- HOMIM allows the identification of 250 species simultaneously
- microbiota on the tongue dorsum and lateral sites are very similar
- bacteria in saliva probably originate from the tongue dorsum

Heating in a Liquid decreases the Resistant Starch Fraction and increases the Slowly Digestible Starch Fraction of a Specific Starch

Evan Ambrahamse, Numico



Englyst method, n.t. = no treatment

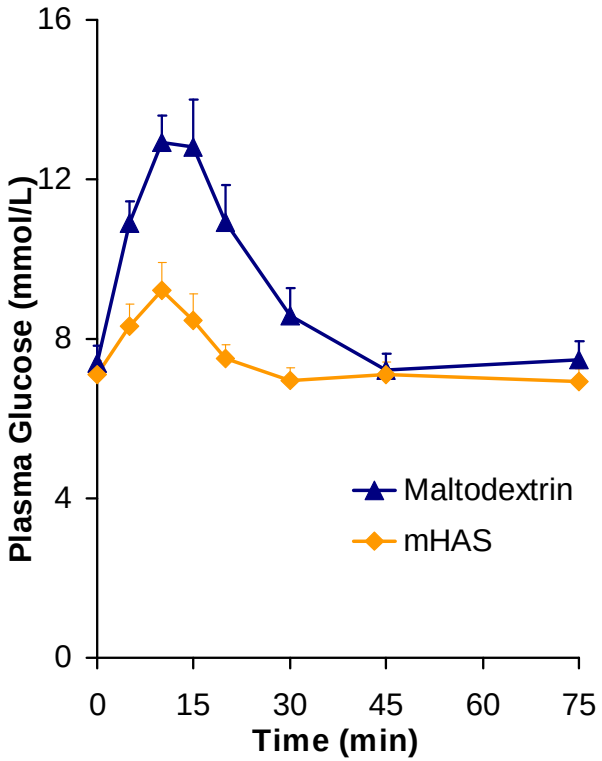
means ± SEM (n=3)

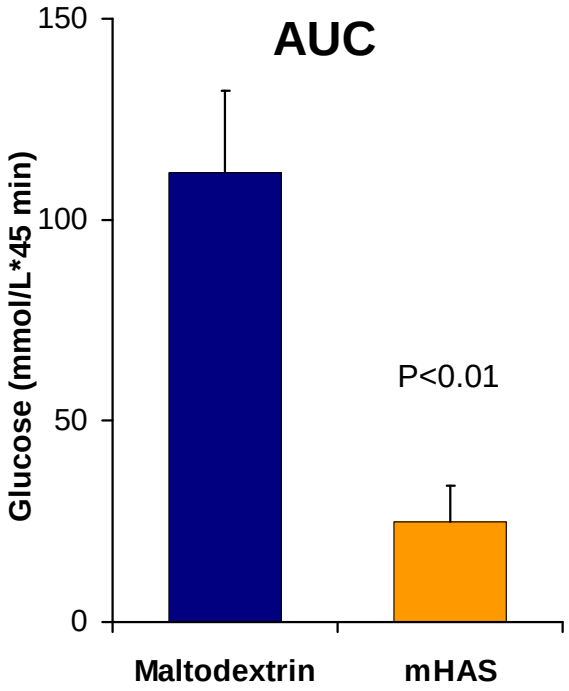
 # RS different from n.t. and heated at 80, 90 and 100 ºC, P<0.05

** SDS different from n.t and heated at 80, 90 and 100 ºC, P<0.05*

Starch Fraction	***Available Glucose ?***	***Physiological effect***
Resistant	no	Colonic Fermentation
Rapidly Digestible	yes	Determines glycemic response
Slowly Digestible	yes	Limited influence on glycemic response

Significantly lower glucose levels in blood after administration





2.0 g available glucose / kg BW, means ± SEM (n=9).

Insulin response of the sterilised specific starch was also significantly lower

The sterilised specific starch may provide a suitable low glycemic carbohydrate source for liquid products

Could be useful for diabetics

DIGESTION AND FERMENTATION OF ^{13}C LABELLED BARLEY: A CURVE FITTING APPROACH

Wang Hongwei, et al. **Center for Medical Biomics, University Medical Center Groningen**

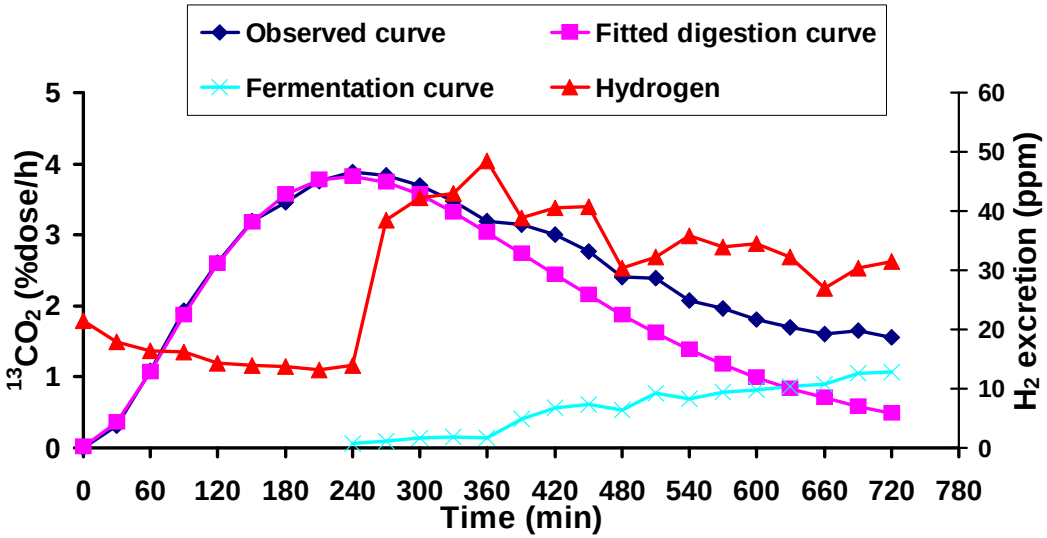


Fig 1. Hydrogen and $^{13}CO_2$ response and the fitted digestion and fermentation curves of 17 healthy subjects after ingestion of 86 g (dry weight) boiled naturally ^{13}C-labelled barley kernel.

1, Hydrogen and $^{13}CO_2$ in breath air were measured. Four hours after intake of the test meal, hydrogen increased significantly and stayed increased during the experiment.

2, This time point was regarded as the start of the fermentation process and the digestion curve was fitted using $^{13}CO_2$ data of the first 4 hours.

3, The difference between the observed curve and the fitted digestion curve represented the fermentation curve.

Result 2

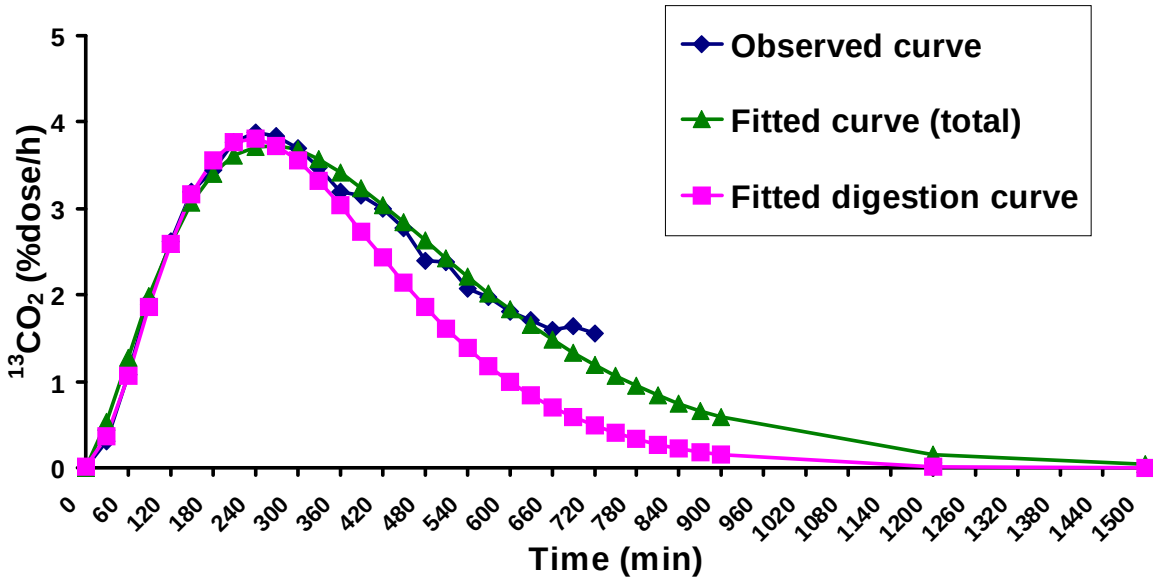


Fig 2. Curves of extrapolated $^{13}CO_2$ data of 17 healthy subjects after ingestion of 86 g (dry weight) boiled naturally ^{13}C-labelled barley kernel.

4, With the observed 12-h $^{13}CO_2$ data, a curve describing the total recovered ^{13}C was fitted. This curve and the fitted digestion curve were extrapolated to baseline. This showed, that about 20 hours after the intake of the test meal, breath $^{13}CO_2$ will return to baseline and that about 14.3% of recovered ^{13}C will then be from colon fermentation.

5, Of the 86 g barley, 50 g is available carbohydrate, 17.6 g is dietary fiber and resistant starch. The energy content of dietary fiber and resistant starch accounts for 14.8% of total energy.

Effect of an oregano oil constituent on the GUT microbiota of weaning piglets

Odette Pérez, S. de Filippi, W. Akkermans-van Vliet, J. Kluess, P. Bikker, J. Fledderus, W. Pellikaan, M. Verstegen, H. Smidt and W. M. de Vos

Weaning of piglets → social structure
Weaning of piglets → environment
Weaning of piglets → diet (milk to solid starter)

- Gastrointestinal disorders
- Intestinal microbiota changes
- Increase in potential pathogens

Dietary intervention strategies ⇨ Changes in microbiota response

Objectives

-Detect, localize, quantify microbial species
- Metabolic activity

Trial

Animals

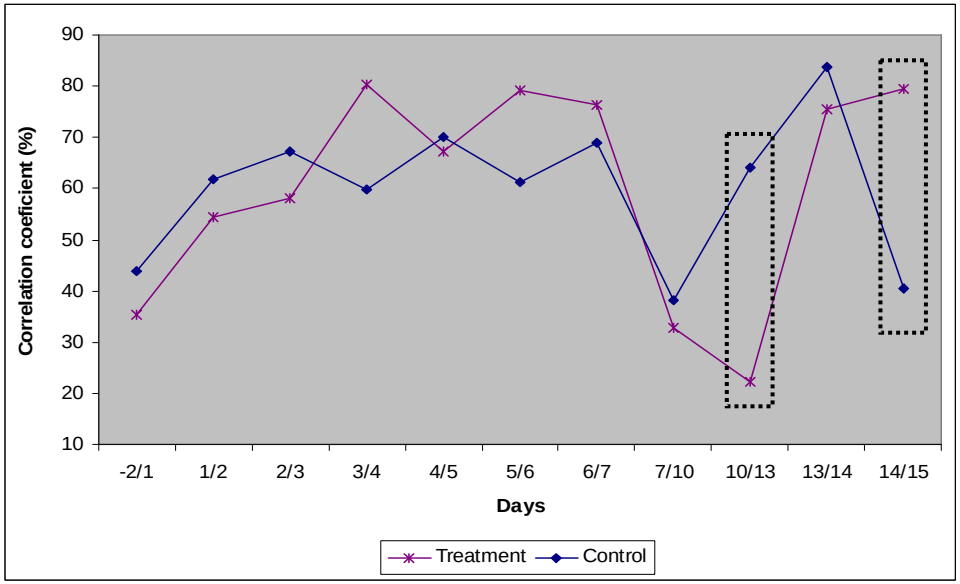
- 12 landrace piglets from 4 litters
- T-cannulation at the terminal ileum between 17 – 18 days of age

Diets

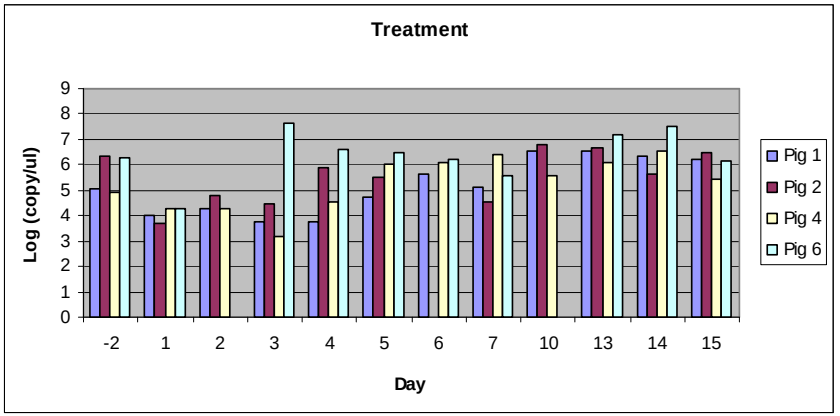
- before weaning piglets are suckled by the sow
- 2 different post weaning starter diets
  - **control** = reference diet
  - **treatment** = oregano oil constituent (150 ppm)

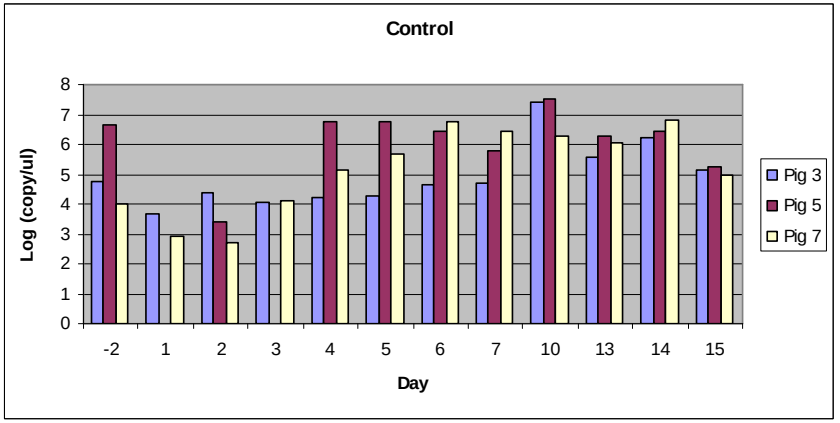
FPH Feed for Pig Health

Moving window correlation for treated and control piglets (variability of general bacterial community)



Lactobacillus quantification





Conclusions

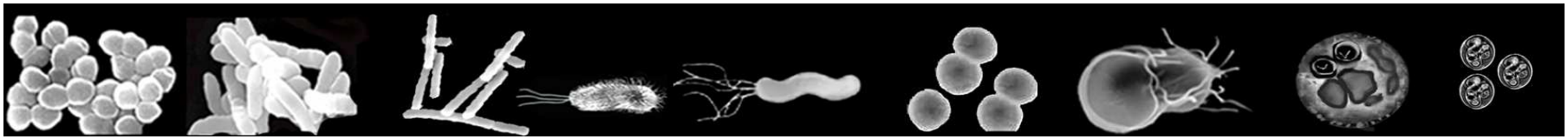
- Preliminary microbiological analyses show that there is not a significant effect of the oregano oil constituent on the total gut microbiota
- After weaning total *Lactobacillus* numbers decrease and slowly increase again in time, for both control and treatment group
- No significant difference in the total bacterial numbers between controls and piglets fed with the additive

Diagnostic Faecal Markers by Intestinal Complaints

MGlab and Advise BV - laboratory for diagnostic faeces analyses

Laboratory: M. Heida, biotechnology Advise: drs. S. van As, MD.

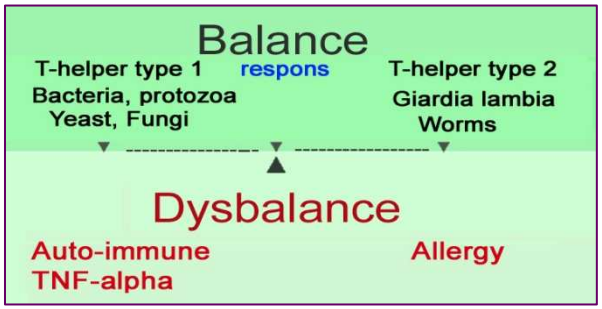
- Analyses of feacal bacteria, fungi, parasites and colon cells.
- Markers for infection, colitis and Crohn's is available
- Certified ELISA's for *Giardia l*., sIgA and markers for celiac disease
- Faeces test used as screening and follow-up
- Macroscopic inspection does not replace faeces analysis



Enterococcen Klebsiella Lactobacillus E. coli Helicobacter Candida alb. Giardia lamblia Dientamoeba f. Cryptosp.

Intestinal Mucosal Response

The effect of bacteria or protozoa is determent by the **interaction** between the host and the guest. Infection is the result of damaged mucosa and receptors, an unbalanced immune response and lack of essential nutrients



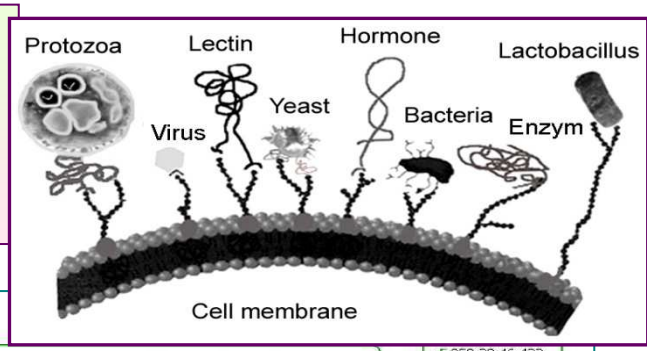
Balance / Dysfunction	
Microflora	High TNF-alpha
sIgA	Obesitas - High Insulin
T-helper type 1 - 2	Western diet
Mucosa	Food allergy
Receptors	Medication

Faecal Markers - MGlab

1. pH, normal pH 6 – 6.8
2. Digestion: Fat, Starch, Fibre
3. Micro-flora: culture and DNA analysis
4. Cytology, colon cells
5. Toxins: Clostridia and Shiga
6. Helicobacter pylori
7. Campylobacter spp.
8. Culture Yeast and Fungi – nystatine
9. Giardia lamblia
10. Cryptosporidium spp.
11. PCR Dientamoeba fragilis
12. sIgA
13. TNF-alpha
14. Specific ELISA's
15. Specific inflammatory factors
16. Screening for celiac disease
17. M2 PK digestive cancer
18. Occult blood

Specific markers for infection
Calprotectine, sIgA,
β-Defensine 2, Lactoferrin,
Anti-Trypsin, TNF-alpha

Questions:
Does an organism bind?
How will the
body respond?



MG ADVIES

Faeces micro-flora analysis

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I www.MGlab.nl

Name docter

Date rapport 11 feb 2007

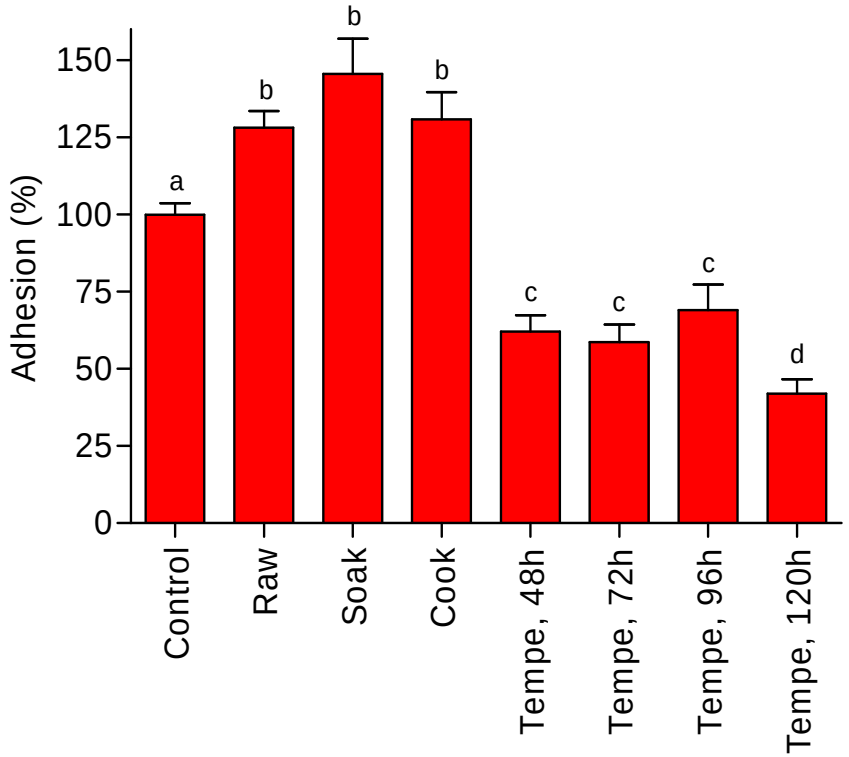
Patient name			Monstercode	
Date			Antibiotica	
Digestive markers				
Test	Normal	Result	Interpretation	Advise
pH	6.0 - 6.8	6	Normal	
Structure		slimy		
Colour		light brown		
Carbohydrate		high		
Plantfiber		normal		
Muscle fiber		not		
Fat	< 3 gr/100 gr	9	Normal	
Aërobic mircoflora		**per gram faeces**		
Enterobacteriaceae	$1.10^5 - 10^7$	$1.0\text{-}10^7$	Elevated in relation to E.coli	
E.coli	$1.10^5 - 10^7$	$1.0\text{-}10^3$	Low	
Enterococcen	$1.10^6 - 10^7$	$2.0\text{-}10^6$	Normal	
Lactobacillen	$1.10^5 - 10^7$	$1.0\text{-}10^3$		
Yeast and Fungi		**per gram faeces**		
Yeast		Candida albicans $2.0\text{-}10^6$	High	
Fungus		NO	Normal	
Anaërobic mircoflora		**per gram faeces**		
Bifidobacterium	$10^8 - 10^9$	$1.6\text{-}10^8$	Normal	
Clostridium	$10^8 - 10^{10}$	$9.6\text{-}10^8$	Normal	
Bacteroides	$10^9 - 5\cdot10^{10}$	$6.4\text{-}10^9$	Normal	
Additional analyses				
Helicobacter pylori			Normal	
Campylobacter		-	Normal	
Clostridium difficile		-	Normal	
Giardia		+	Positive	
Cryptosporidium		Positive	Positive	
Occult bloed		Positive		
Calprotectine	<= 50 mg/kg	5	Normal	
t-Transglutaminase	<= 100 U/l	132	Positive	
M2-PK	<= 6 U/ml	21	Positive	

Pictures S. van As. INFO: www.MGlab.nl

Tempe extracts inhibit the adhesion of ETEC K88 to intestinal cells

Petra Roubos-van den Hil1,M.J.R. Nout, H. Gruppen, R.R. Beumer, J. van der Meulen, M.H. Zwietering.

Tempe a fungal (*Rhizopus spp.*) fermented soya bean product

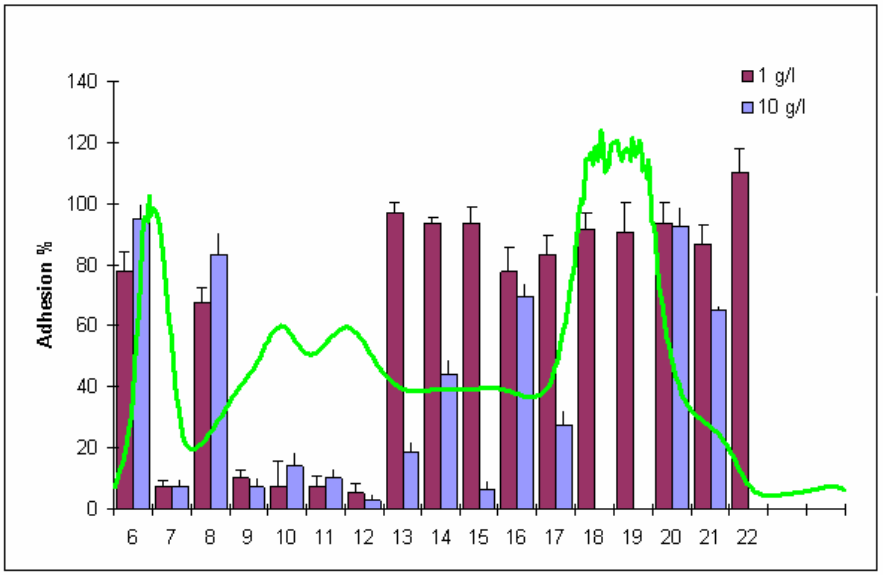


Adhesion of ETEC K88 to Caco-2 epithelial cells that are exposed to tempe extracts

Bacterial adhesion is expressed relative to a control (100%) without added soya bean extract.

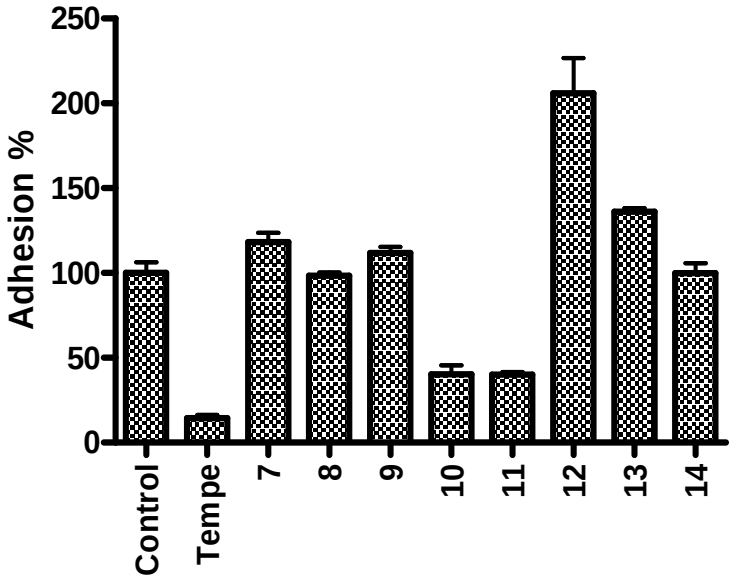
Specific tempe fractions inhibit adhesion of soya bean tempe to intestinal epithelial cells

Microscopic observation of the piglet brush border adhesion test



The green line represents the HPSEC chromatogram of soluble tempe.

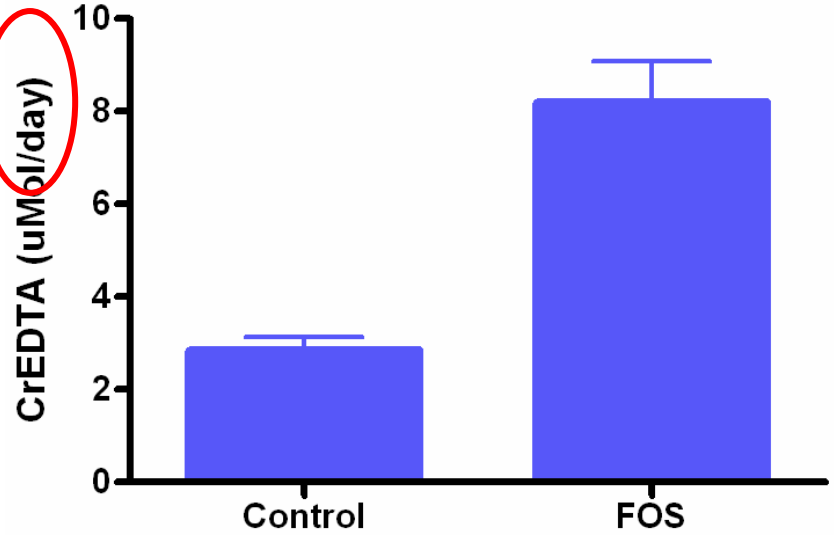
The bars represent the % adhesion of ETEC K88 to brush borders cells

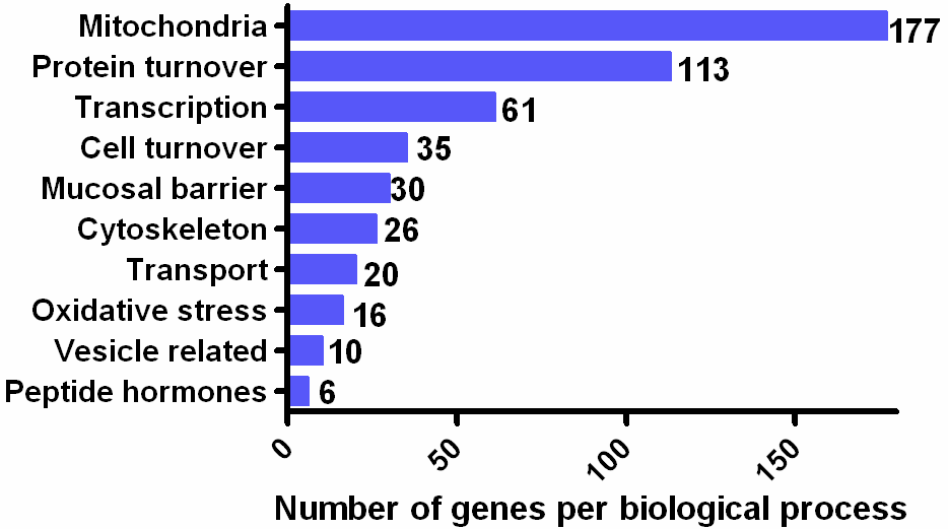


Adhesion inhibition of tempe fractions on Caco-2 cells challenged with ETEC K88. Control without any tempe addition

Fructo-oligosaccharides induced gene expression in rat colon

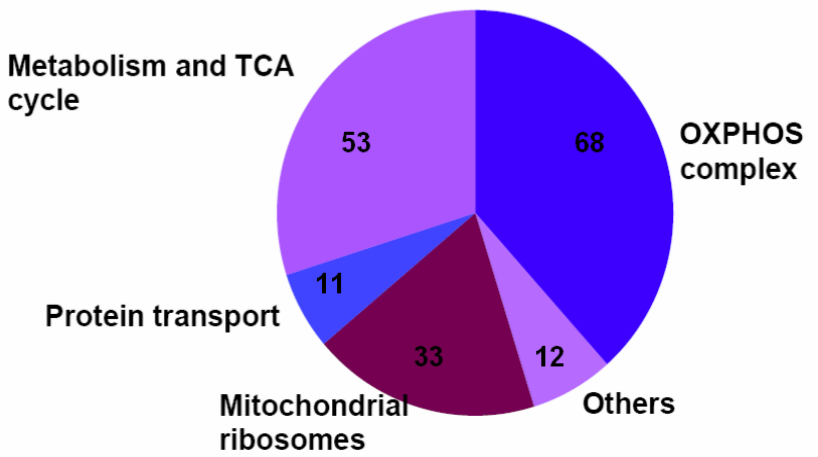
Wendy Rodenburg

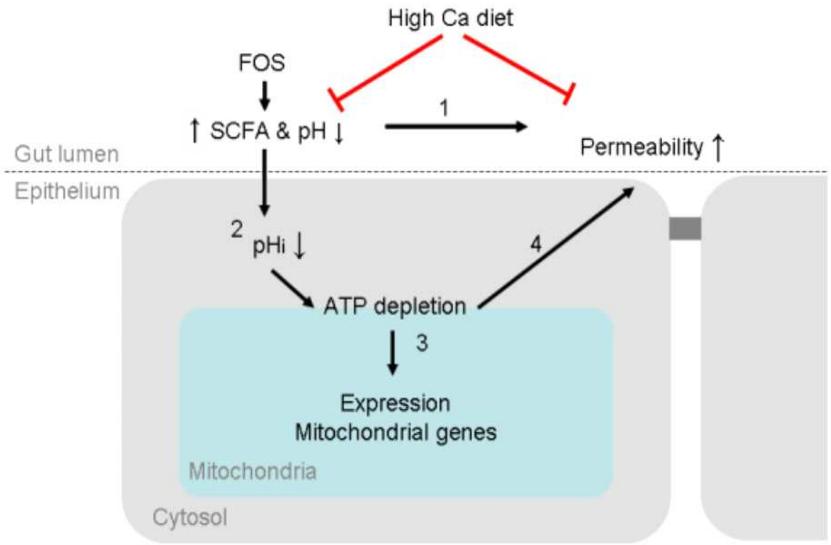




- **Dietary Fructo-oligosaccharides (FOS) increase intestinal permeability in rats**
- **FOS-induced gene expression differences in rat colon, classified into biological processes**

Mitochondrial processes



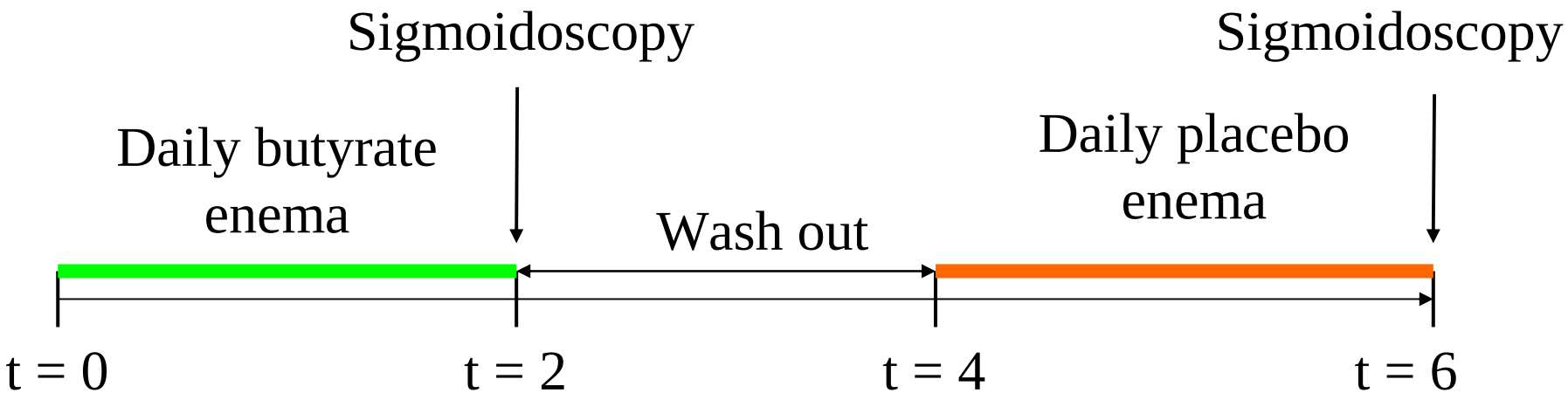


- **Number of genes related to mitochondrial processes affected by dietary FOS in rat colonic mucosa**

- **Proposed mechanism of dietary FOS induced intestinal permeability**

Butyrate induces Transcriptional changes in human colonic mucosa

Steven Vanhoutvin



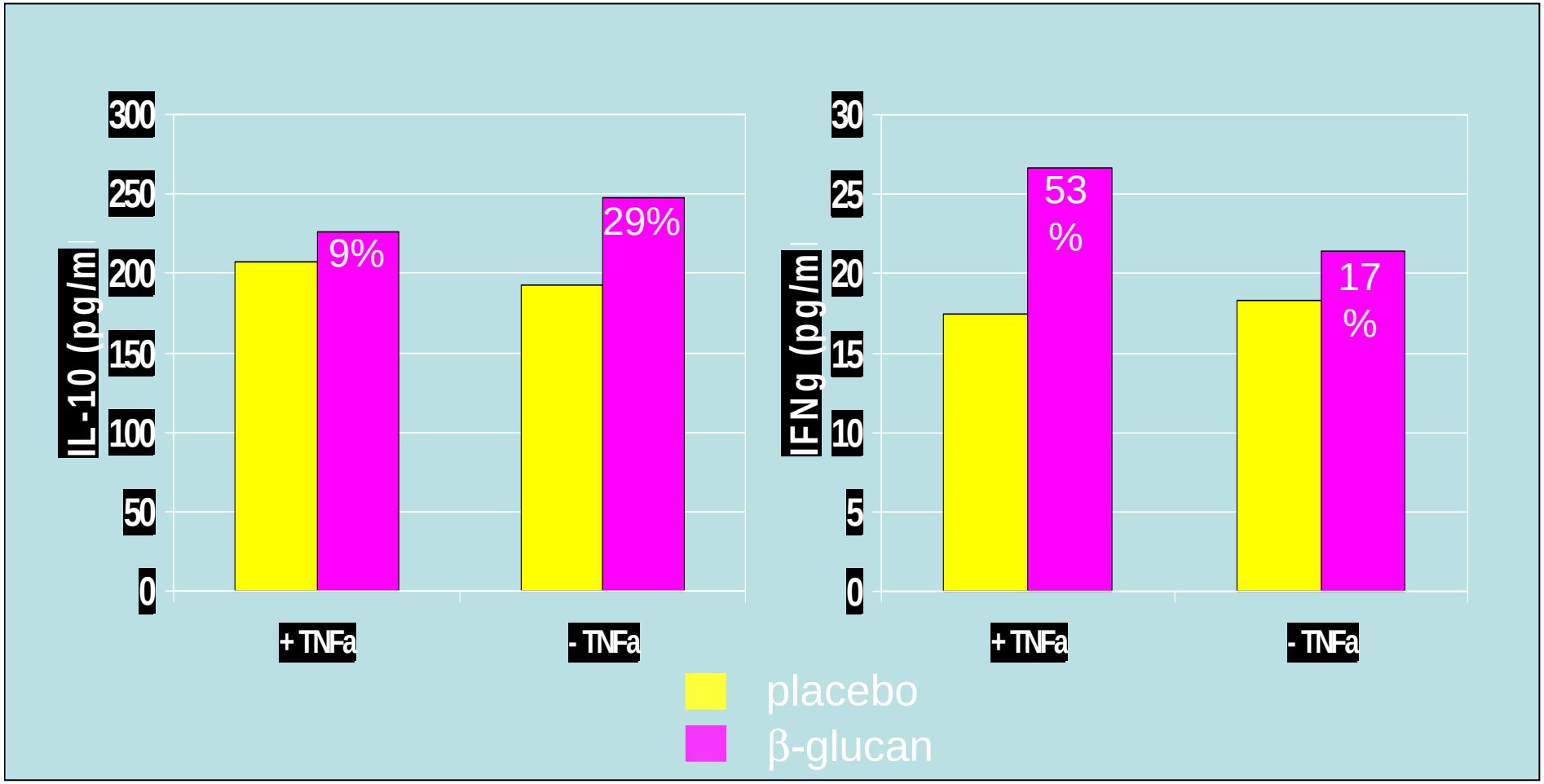
- N=16 (healthy volunteers)
- Randomized
- Double blind
- Cross-over
- 100 mM butyrate daily prior to sleeping

Results:

- Affymetrix whole genome array
- Pathway analysis with Genmapp
- Effect of butyrate on:
  - Fatty acid oxidation
  - TCA cycle
  - Electron transport chain
  - Oxidative stress
  - Glutathione metabolism
  - Epithelial integrity

Within the low range, adding oat β-glucan to enterocytes increased the Th1 response in leukocytes

Julia Volman



What promotes Th1 cytokine production by leukocytes?

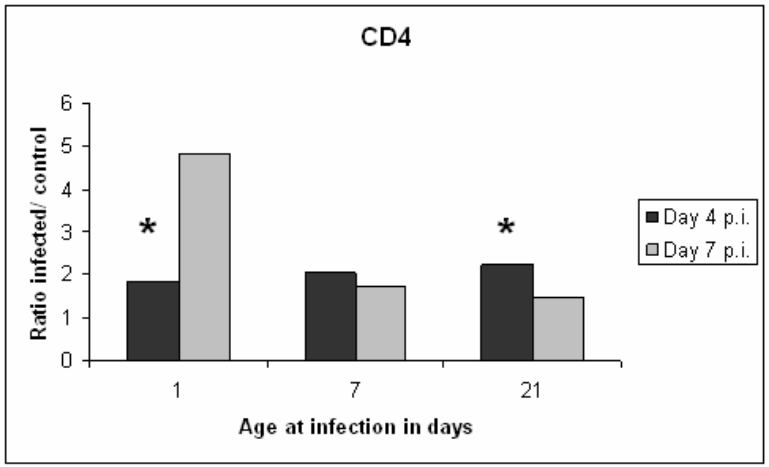
Oat β-glucan decreased TSLP expression

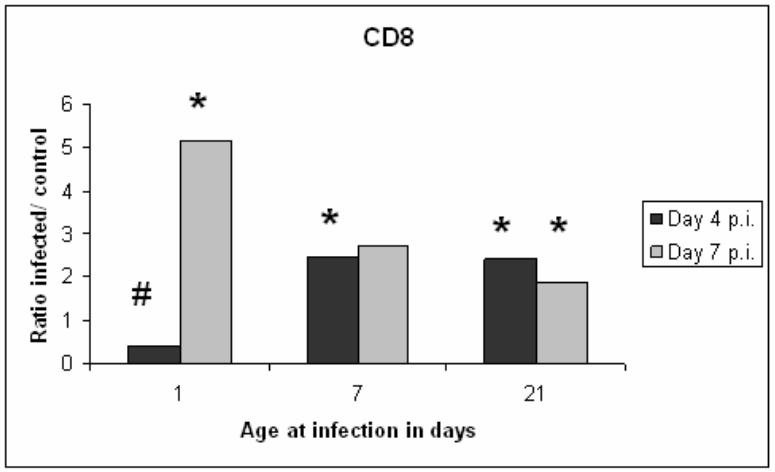
- Isolation of RNA from enterocytes after incubation with fecal water with and without β-glucan ⟶ analyze TSLP mRNA expression

- 5 out of 6 individual subjects showed decreased TSLP mRNA expression after β-glucan exposure

Subject	INT407
1	0.518
2	0.240
3	1.206
4	0.745
5	0.560
6	0.820

DIFFERENCES IN HOST RESPONSE TO EIMERIA INFECTIONS DUE TO MATURATION OF THE INTESTINE

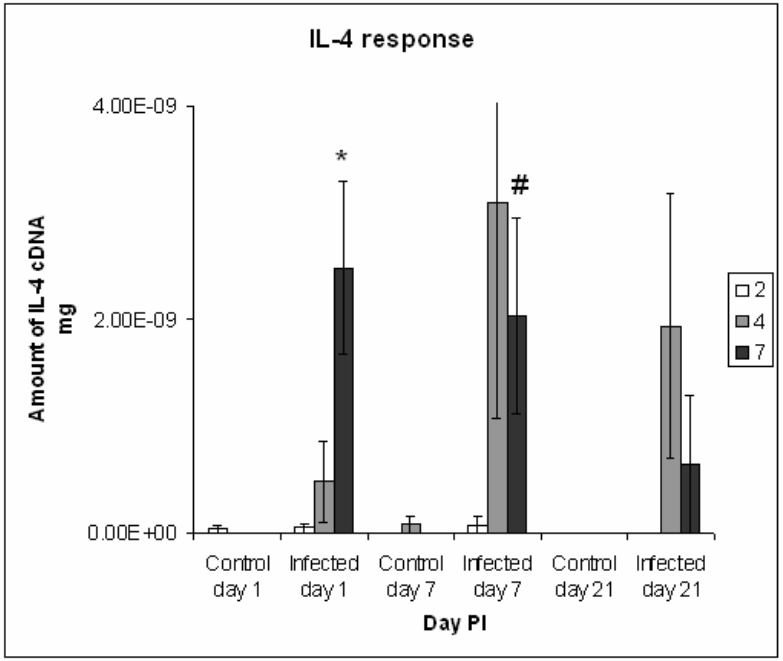


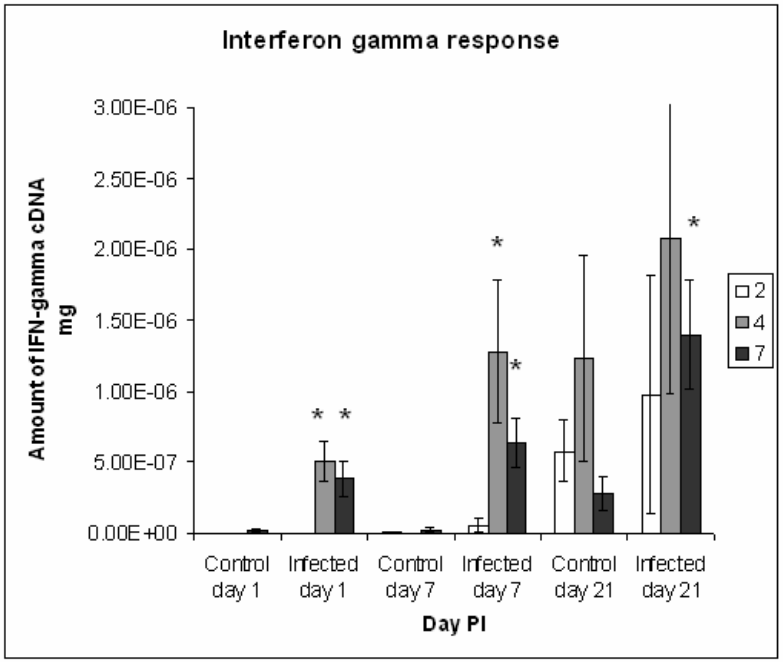


- In general, it was concluded that the strength and kinetics of immune responses to a primary *E.maxima* infection increased with the age of the broilers ($CD8^{+}$).

Division of Infectious Diseases; Lelystad

E-mail: Willem.Swinkels@wur.nl





- One-day-old birds tended to respond in a Th2 mode (IL-4), but as the age of the birds increased the responses to an Eimeria infection became more Th1 biased (IFN-gamma).

- Differences in immune responses had no effect on the amount of parasites in the gut and in excreta.

Division of Infectious Diseases; Lelystad

E-mail: Willem.Swinkels@wur.nl

CALSPORIN®: Efficacy in broilers (50 ppm)

Arno van der Aa

Meta-analysis of 4 different EU efficacy trials (n= 5524)

- **Trials were carried out at the following institutes:**

Trial	Institute	EU Country	Year	Design	Dose of CALSPORIN®
1	Imasde	Spain	2005	CALSPORIN® vs Control Mash feeds	50 ppm*
2	Coren	Spain	2005	CALSPORIN® vs Control Pelleted feeds	50 ppm*
3	IRTA	Spain	2005/2006	CALSPORIN® vs Control Pelleted feeds	50 ppm*
4	Imasde	Spain	2006	CALSPORIN® vs Control mash feeds	50 ppm*

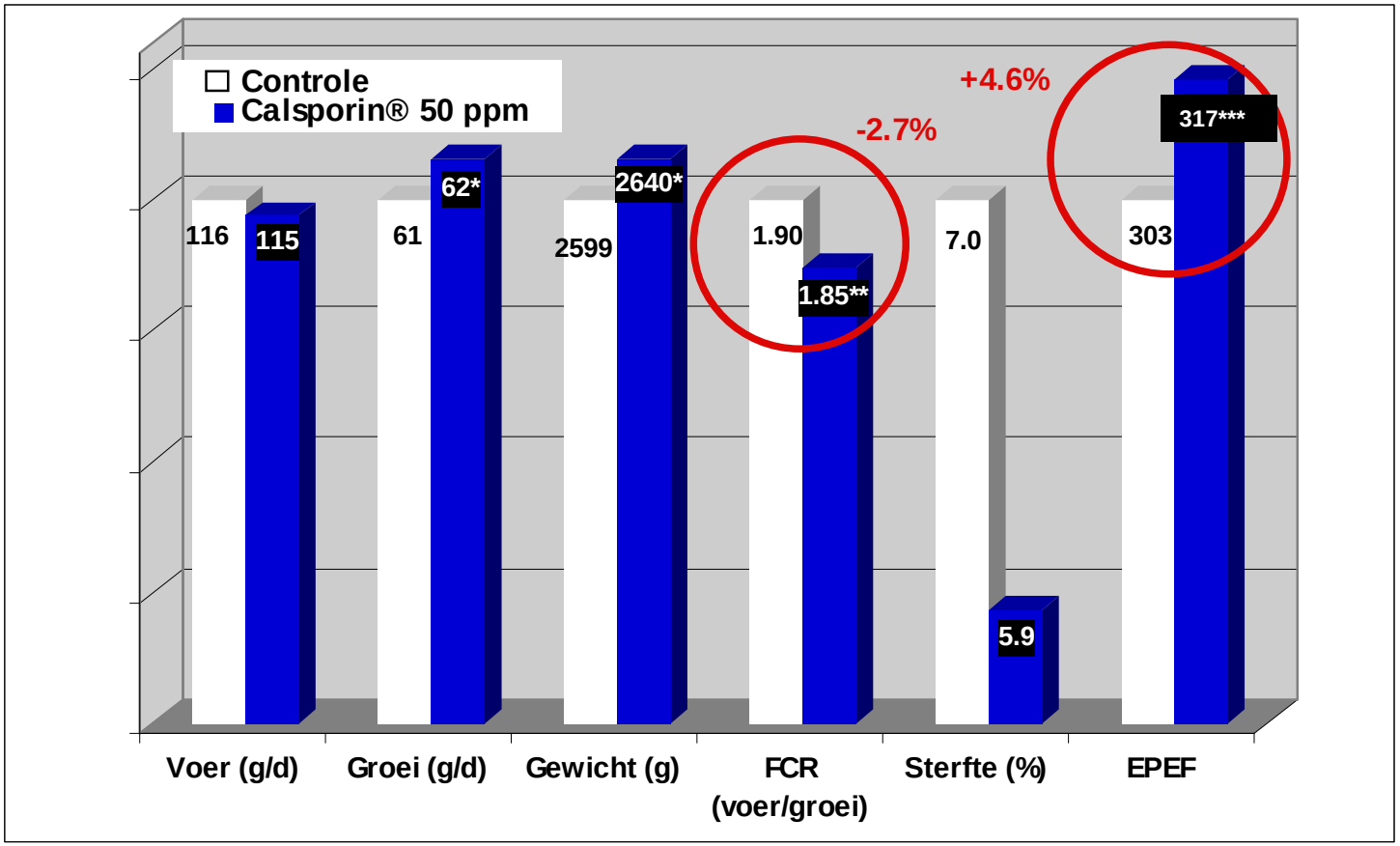
* 50 ppm (50 g/Mt feed)

- **Calsporin is a sporeforming bacillary probiotic: Bacillus subtilis C-3102**
- **The product is highly concentrated: 1.0 x 10^{10} CFU/gram**
- **In these experiment a dosage of 50 ppm was used,**

supplying 5.0 x 10^{5} CFU/gram of final feed to the animal from day 1 to 42

- **Calsporin is heat stable up to 90℃**

Efficacy of Calsporin® (Meta-analysis/EU 50 ppm dossier)

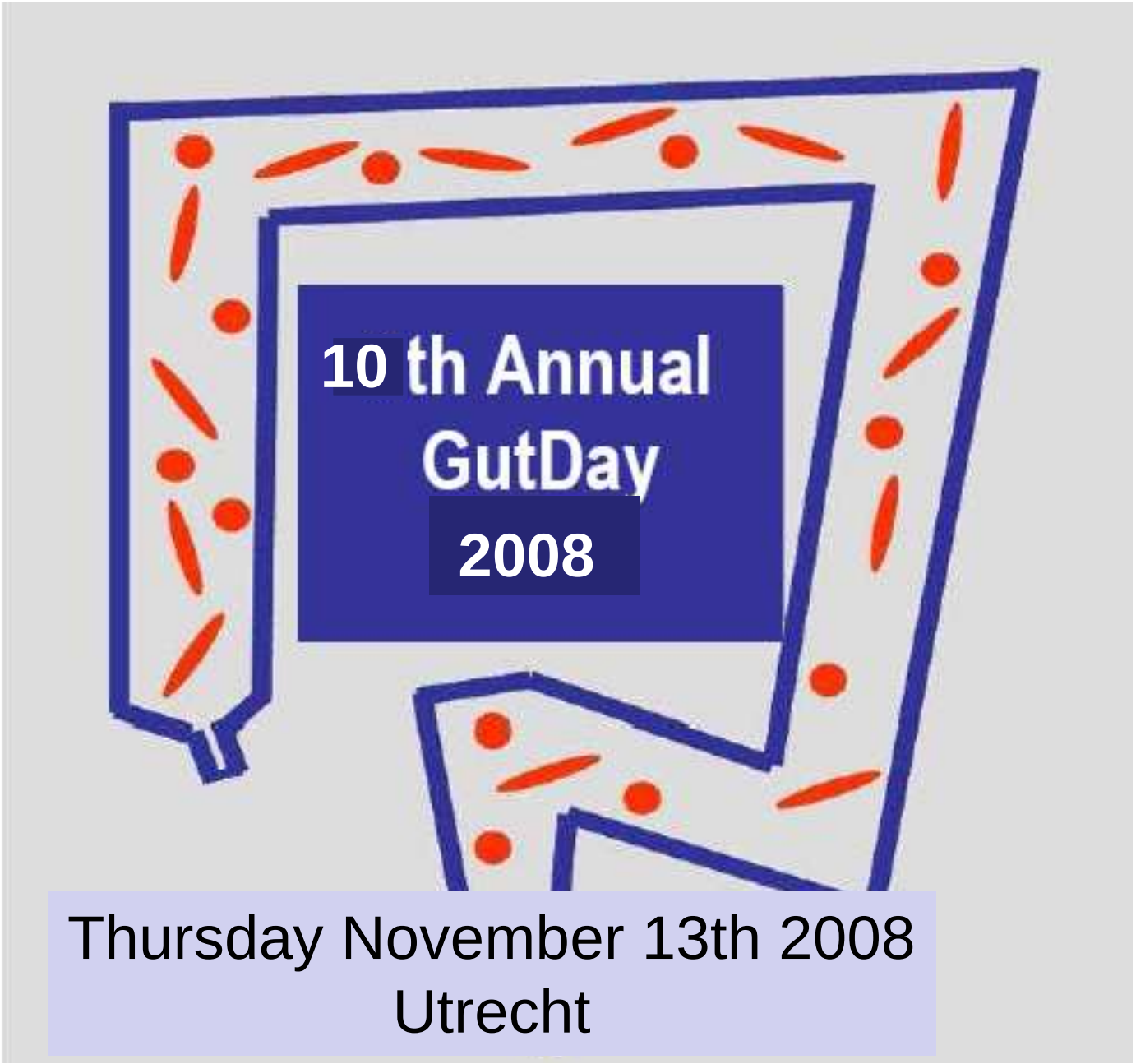


*= Near significant trend: 0.05< P<0.1 **= significant: P< 0,05 ***= significant: P<0,01

EPEF= European Production Efficiency Factor =

[Average Daily Gain x (100-mortality)]

[(10xFeed to gain ratio)]



Thursday November 13th 2008
Utrecht