Schothorst Feed Research

Use of prebiotics in the nutrition of weaned piglets

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Introduction

- What are prebiotics?
- Development of microbiota and effect of prebiotics
- Techniques used to quantify effects of prebiotics (GPT, molecular techniques)
- Effect of prebiotic dietary ingredients
- Consequences for growth performance and health
- Discussion and conclusions

What are prebiotics?

A non [enzymatically] digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Gibson and Roberfroid, 1995)

A non [enzymatically] digestible dietary ingredient that beneficially affects the host by stimulating the activity, in terms of fermentation end products and stability of the diverse commensal microbiota in the GIT (Gibson et al. 2004; Awati, 2005)

Importance of commensal microbiota

Stable and diverse commensal microflora is required for

- **colonisation resistance**
  - **competition for nutrients**
  - **competition for epithelial binding sites**
  - **production of antimicrobial factors (LA, bacteriocins)**
- **development of the immune system**
- **VFA production**
- **motility of the gut**
- **fermentation of dietary fibre as energy source**

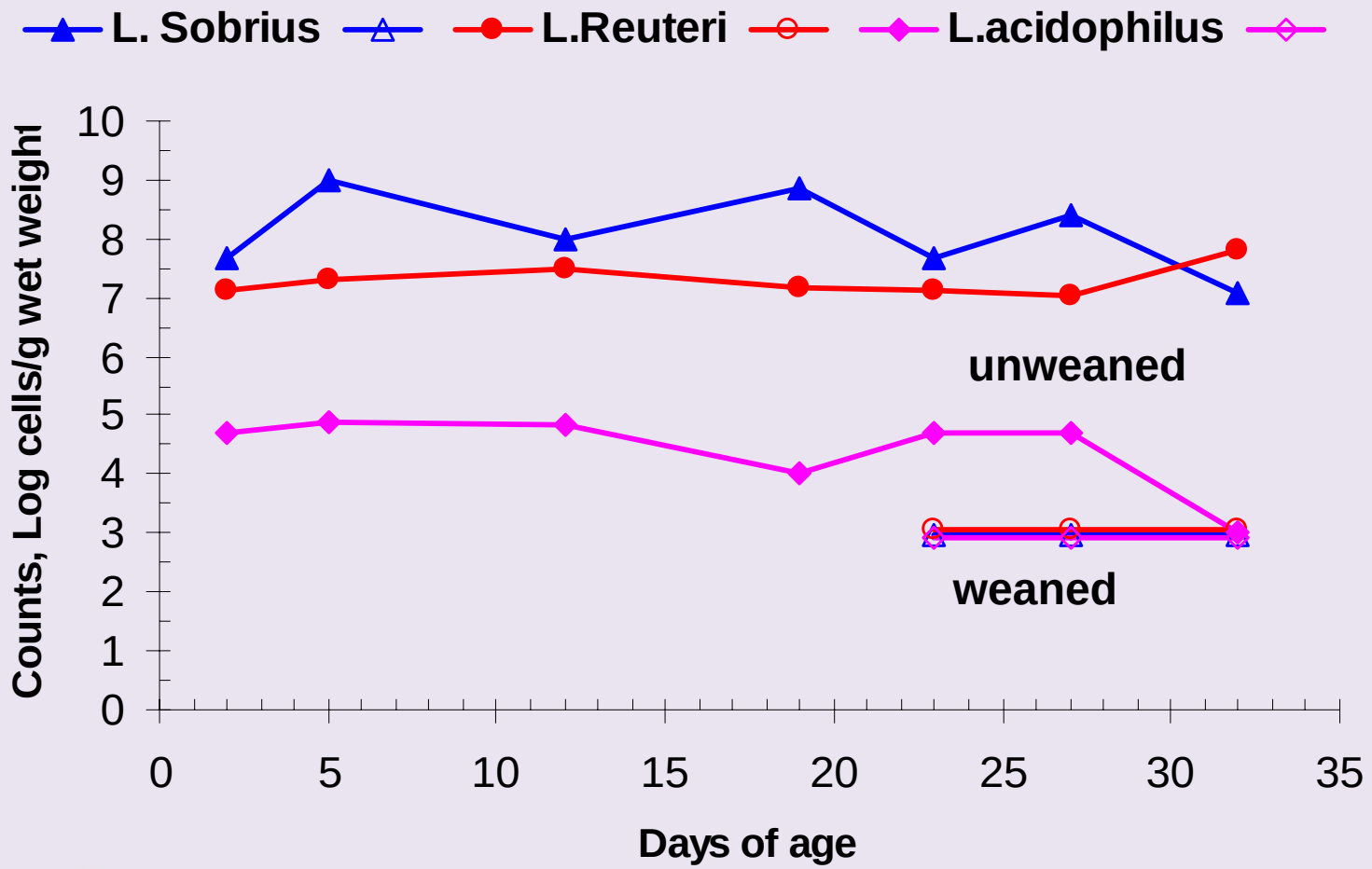
Development of microbiota

Newborn piglets: developing microflora (Inoue et al., 2005; Konstantinov et al, 2006a)

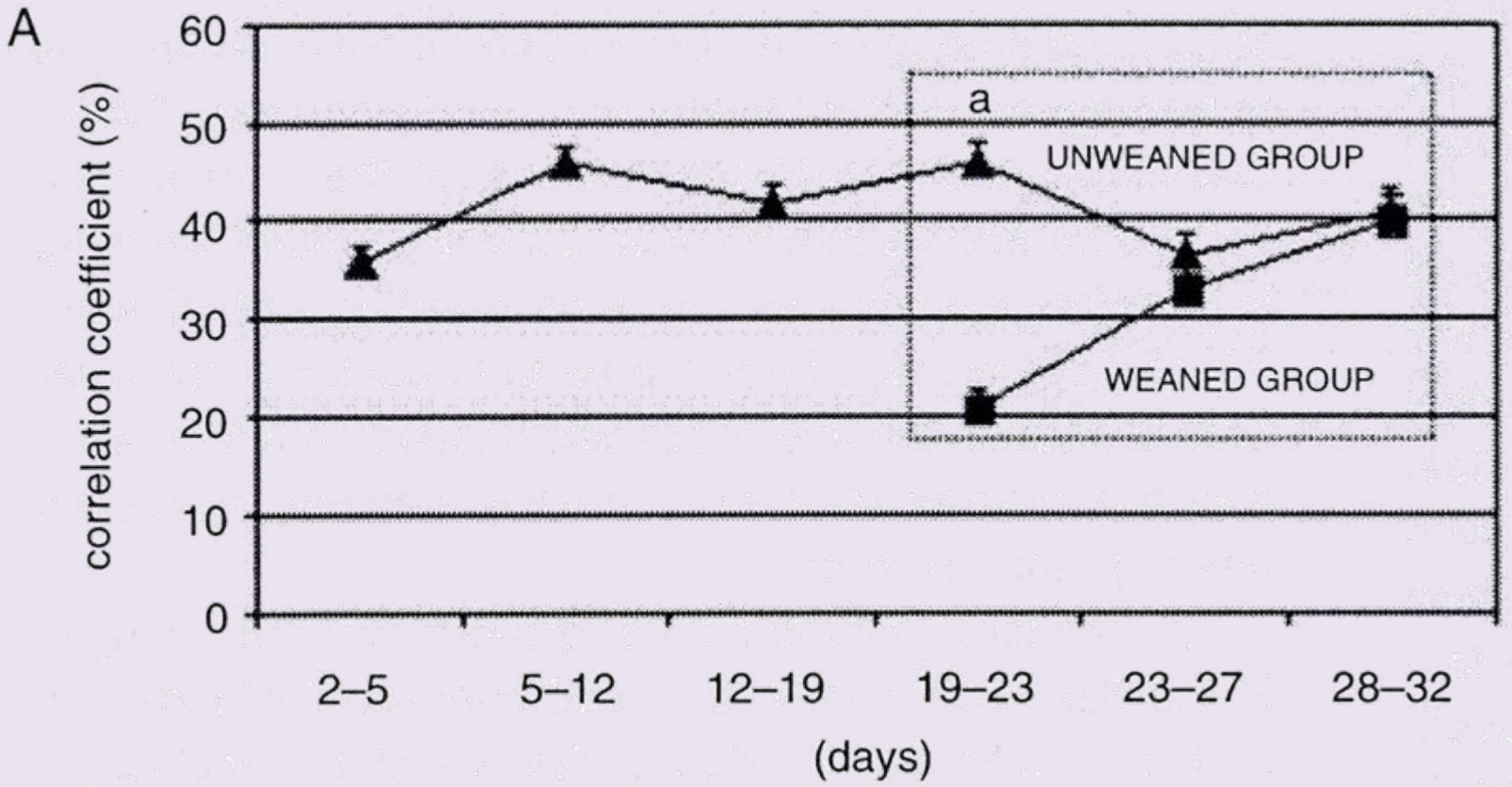
- **Periode around weaning characterised by:**
  - **significant decline in potentially beneficial lactobacilli**
  - **compositional and functional instability of microbiota**
  - **increased numbers of E. coli and Clostridium sp.**
- **Balance between healthy commensal microbiota and pathogens easily disturbed: susceptible to enteric diseases (Hopwood and Hampson, 2003)**

RT-PCR results for LAB in porcine ileum

(Konstantinov et al., 2006)



Stability of ileal microbiota (DGGE-patterns)



Moving window correlation (Konstantinov ea 2006)

Mode of action prebiotics

Influence on composition and functionality of microbiota in GIT; development of stable, diverse microflora

To what extent can prebiotic diet ingredients influence microbial population, health and production performance of weaned piglets.

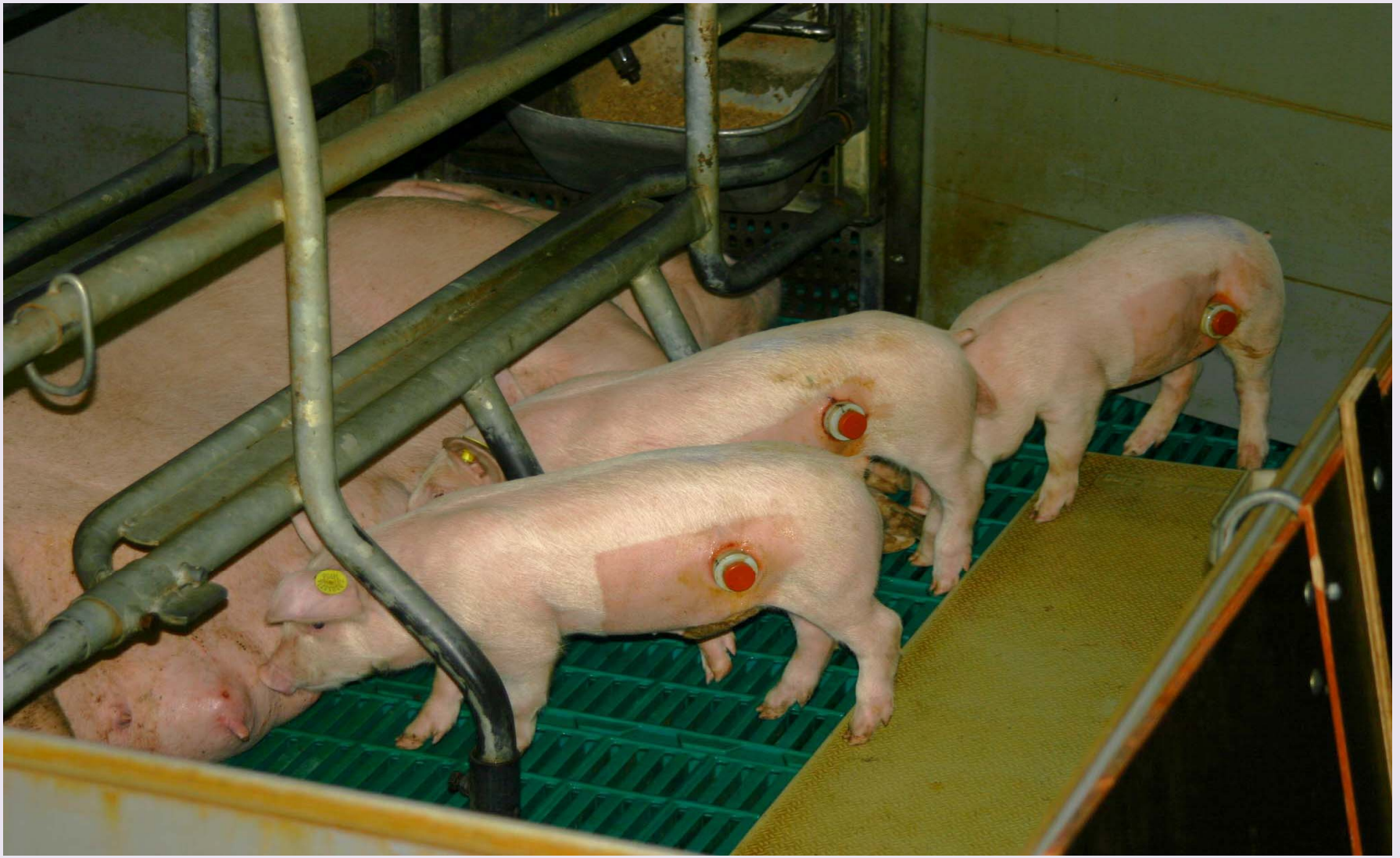
Methods

In-vitro

- Gas Production Technique (GPT)
  - With and without enzymatic pre-digestion

In-vivo

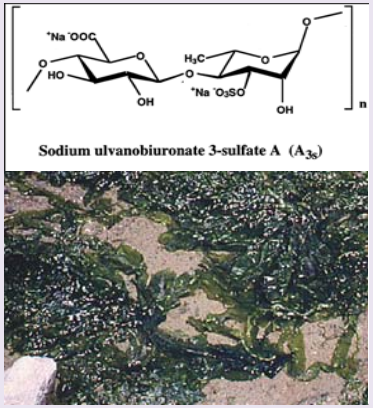
- Sampling of faeces
- Serial slaughter of piglets, sampling of GIT
- Serial sampling of ileally cannulated piglets (no data yet)
- Growth performance studies

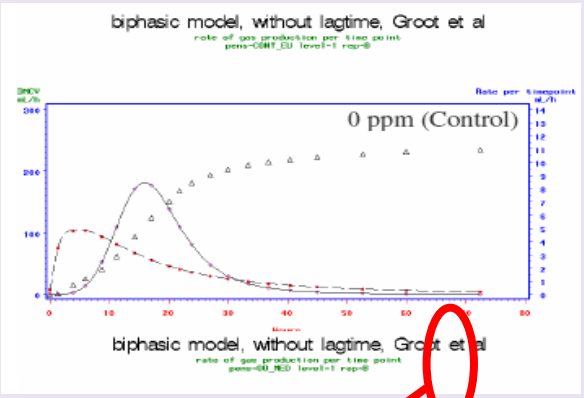


In vitro gas production technique

Substrate + Inoculum → Gas production (OMCV in mL)

Seaweed



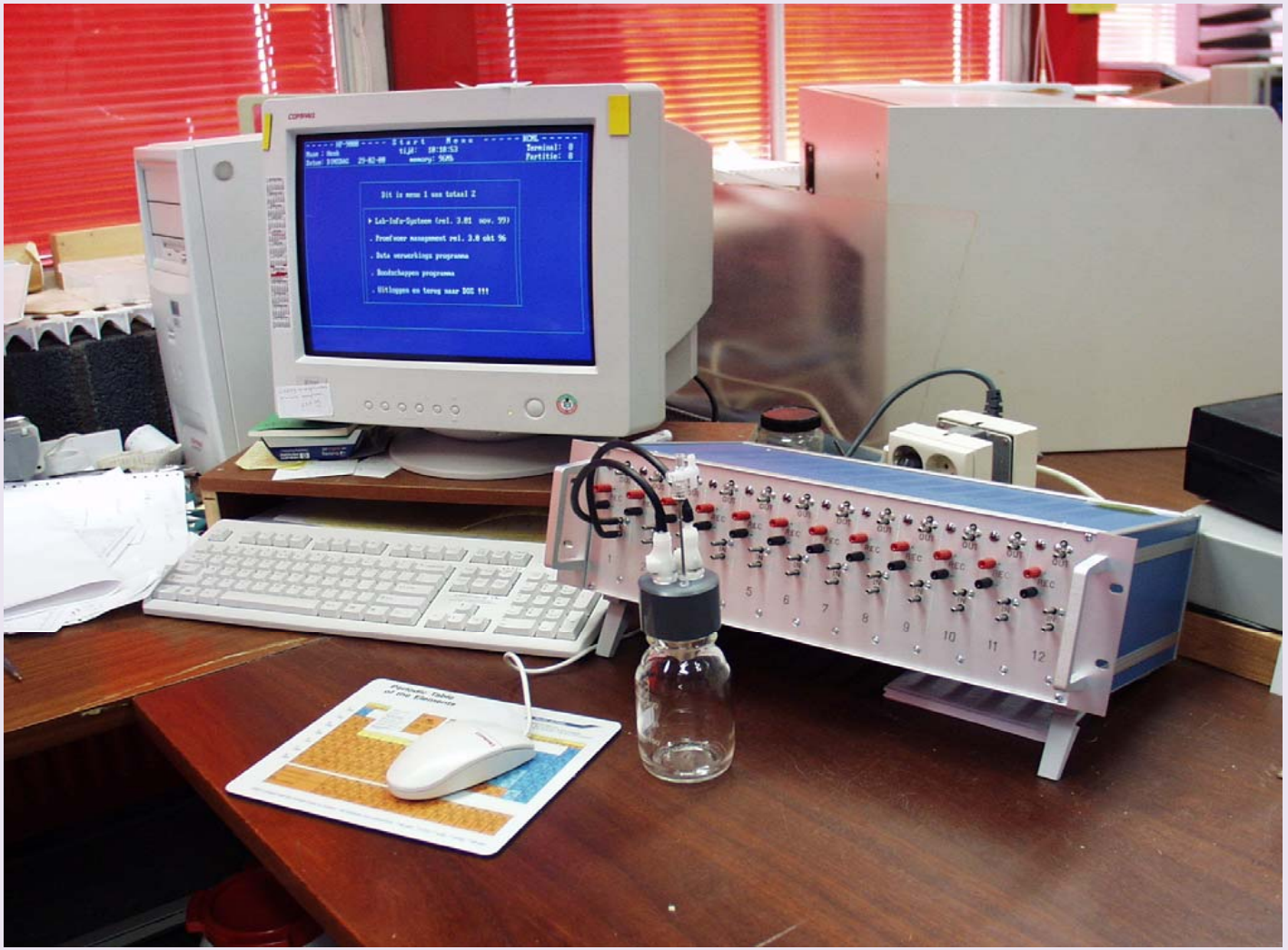


Fermentation metabolites in fluids

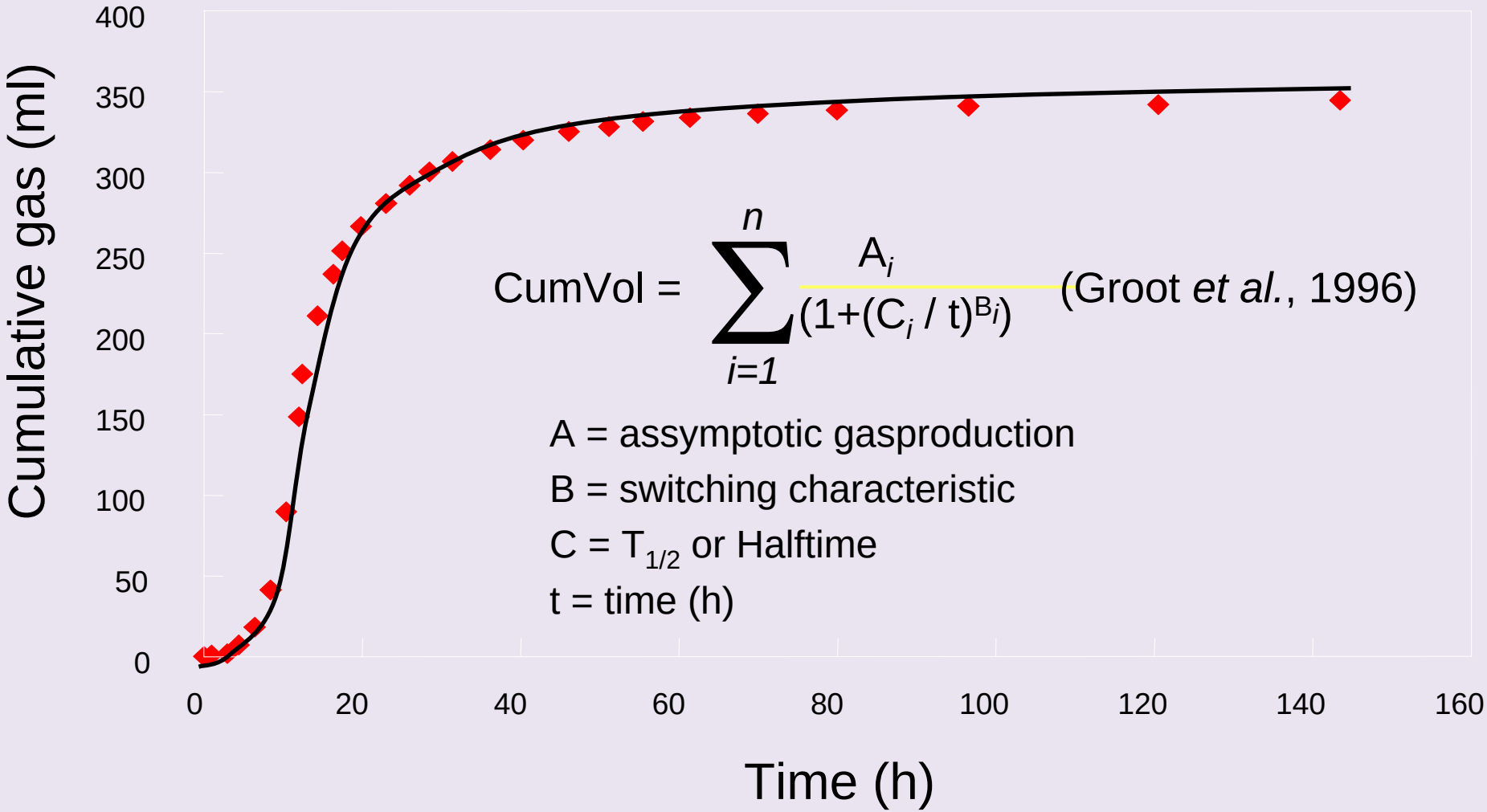
Nutrient residue
VFA, Lactate
Ammonia
Microbiota

Pellikaan, 2006

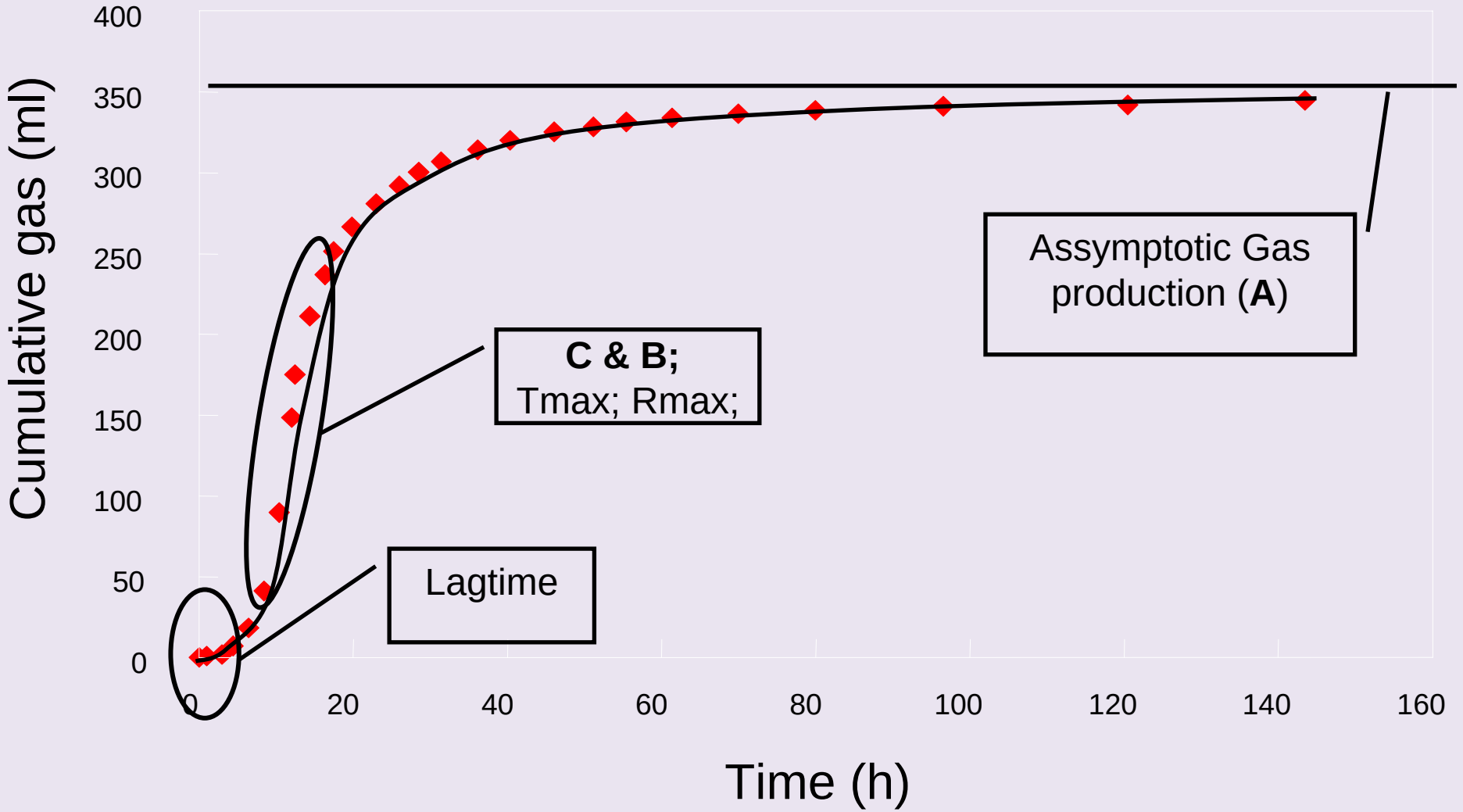
Gas Production Test



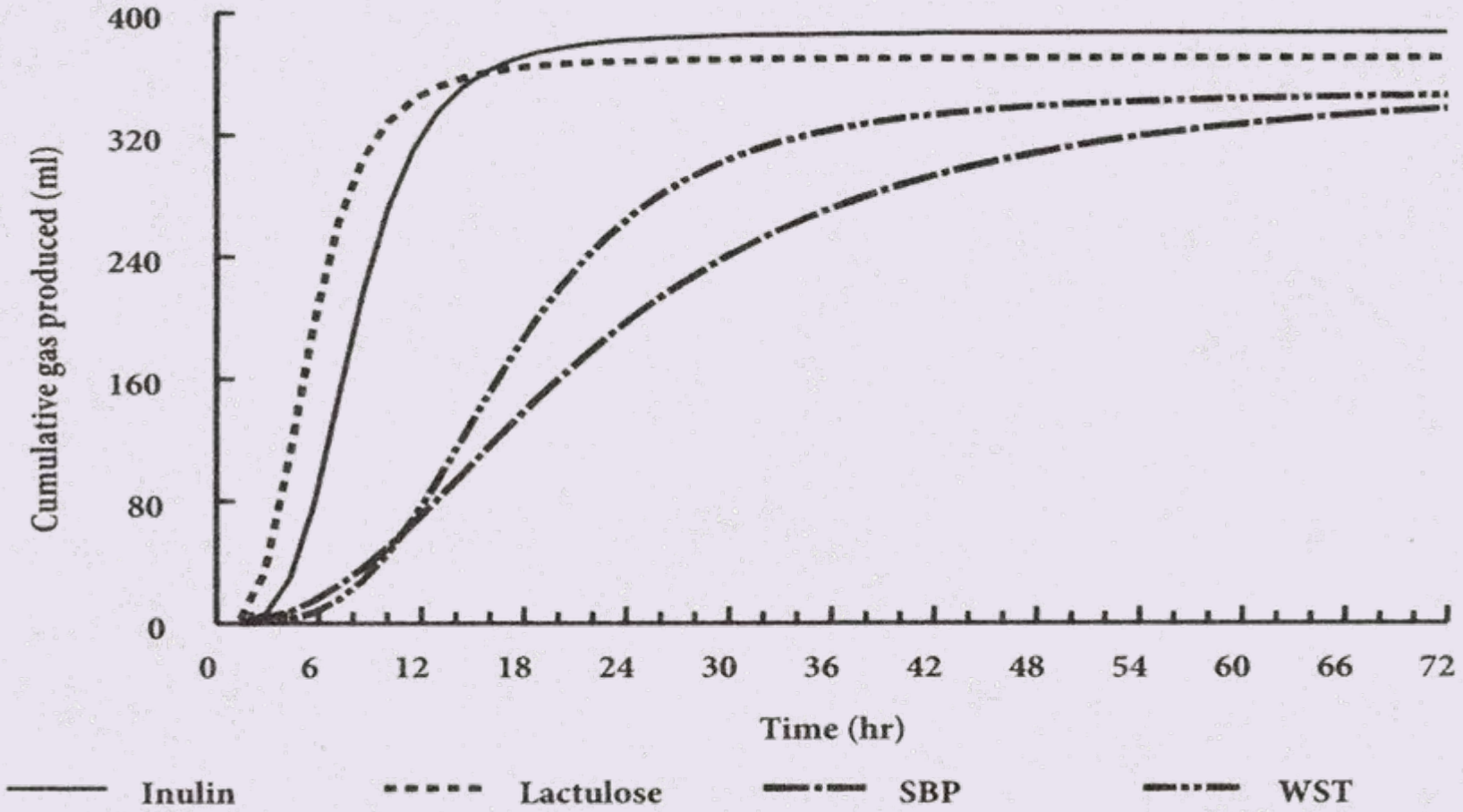
Fermentation Kinetics



Fermentation Kinetics

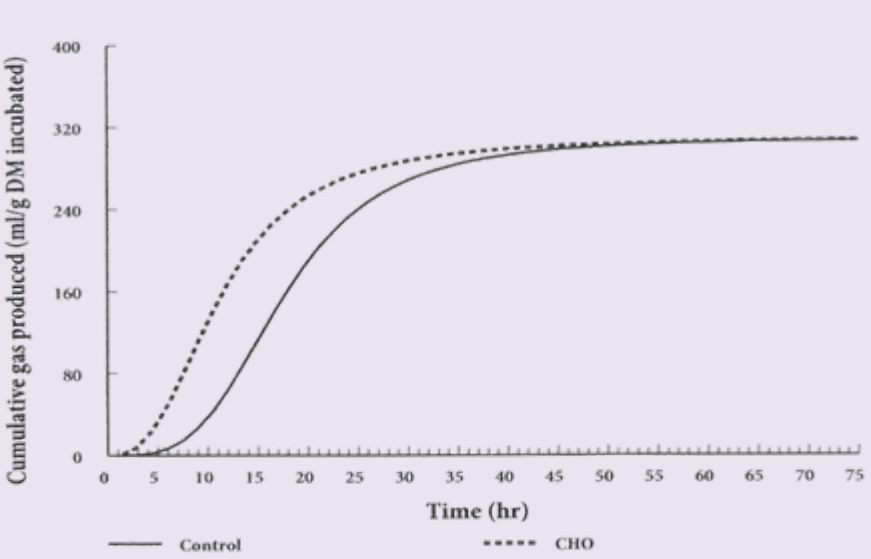


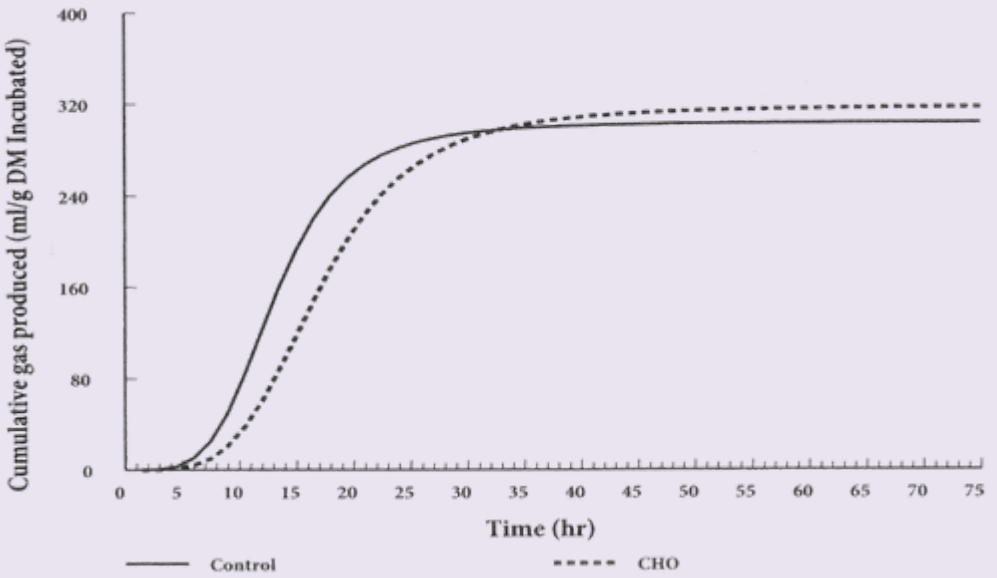
GPT



Fermentation of carbohydrates by faecal inoculation of suckling piglets (Awati et al., 2006) (2nd study ileal inoculation)

Substrate adaptation

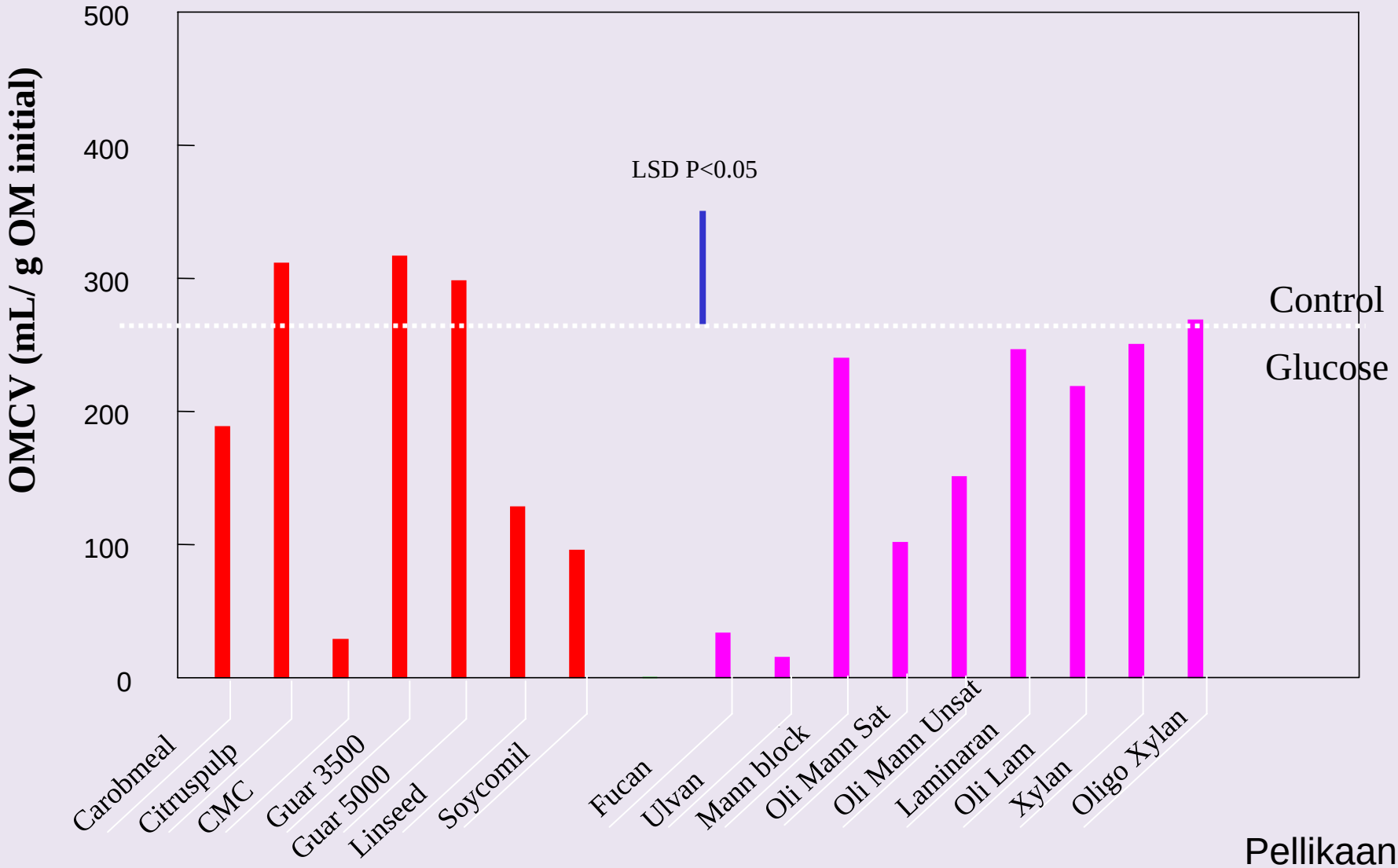




Gas production after 72 h fermentation of SBP (left) and WST (right) by faecal inoculation of piglets fed a CON or CHO diet (Awati et al., 2006)

GPT, gasprod.

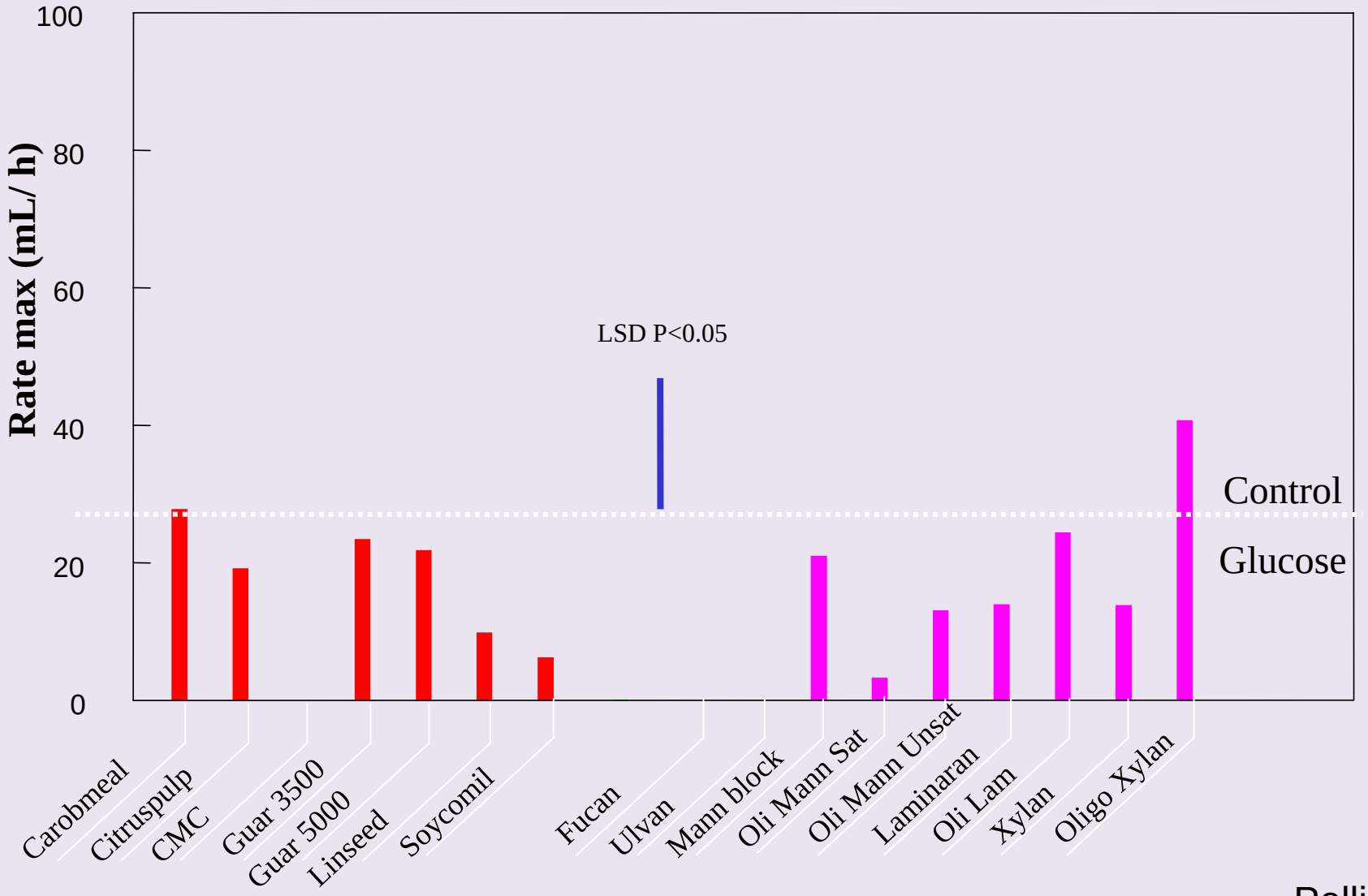
Inoculum; Faeces



Pellikaan, 2007

GPT, max. rate of gasproduction

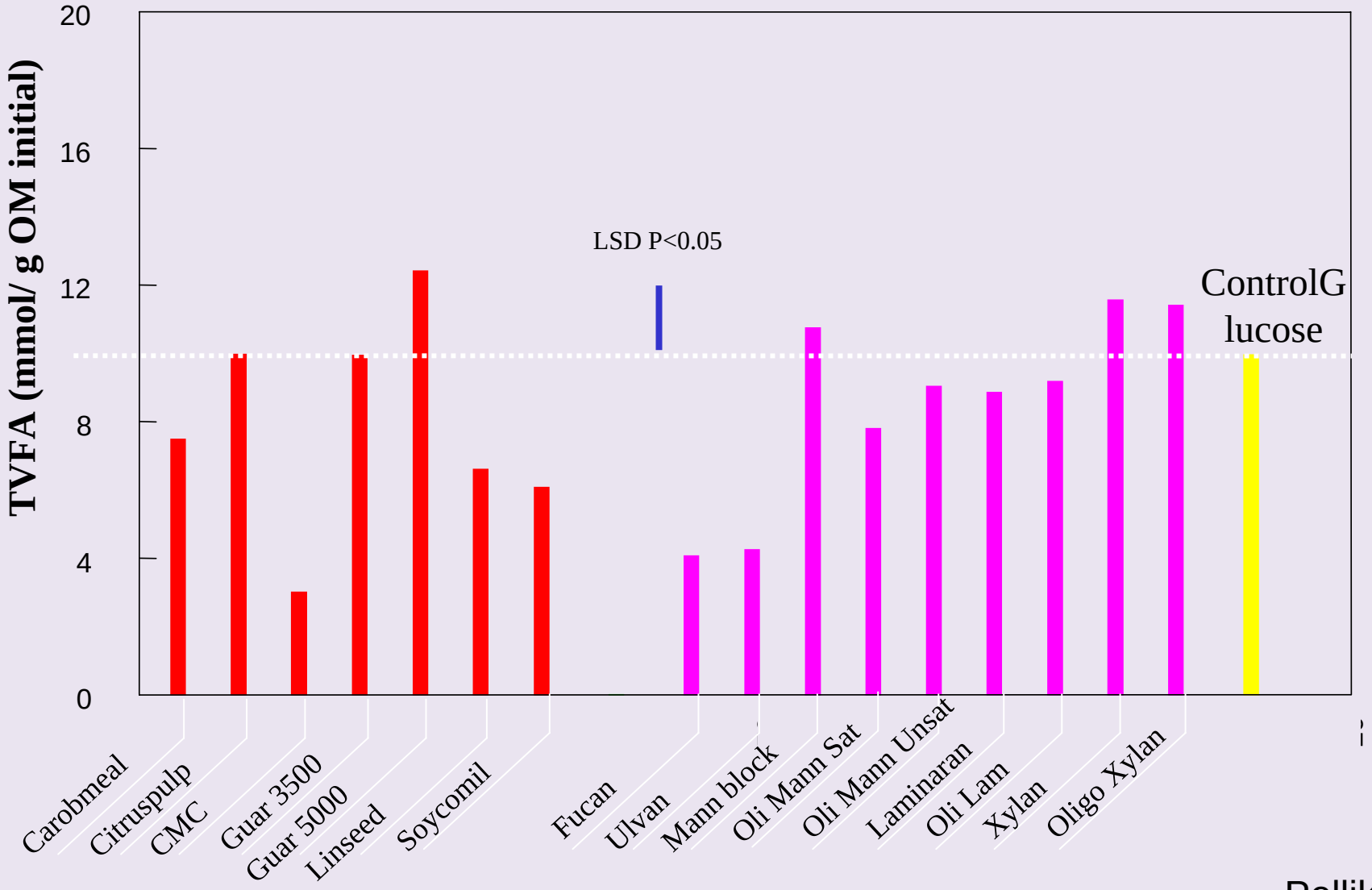
Inoculum; Faeces



Pellikaan, 2007

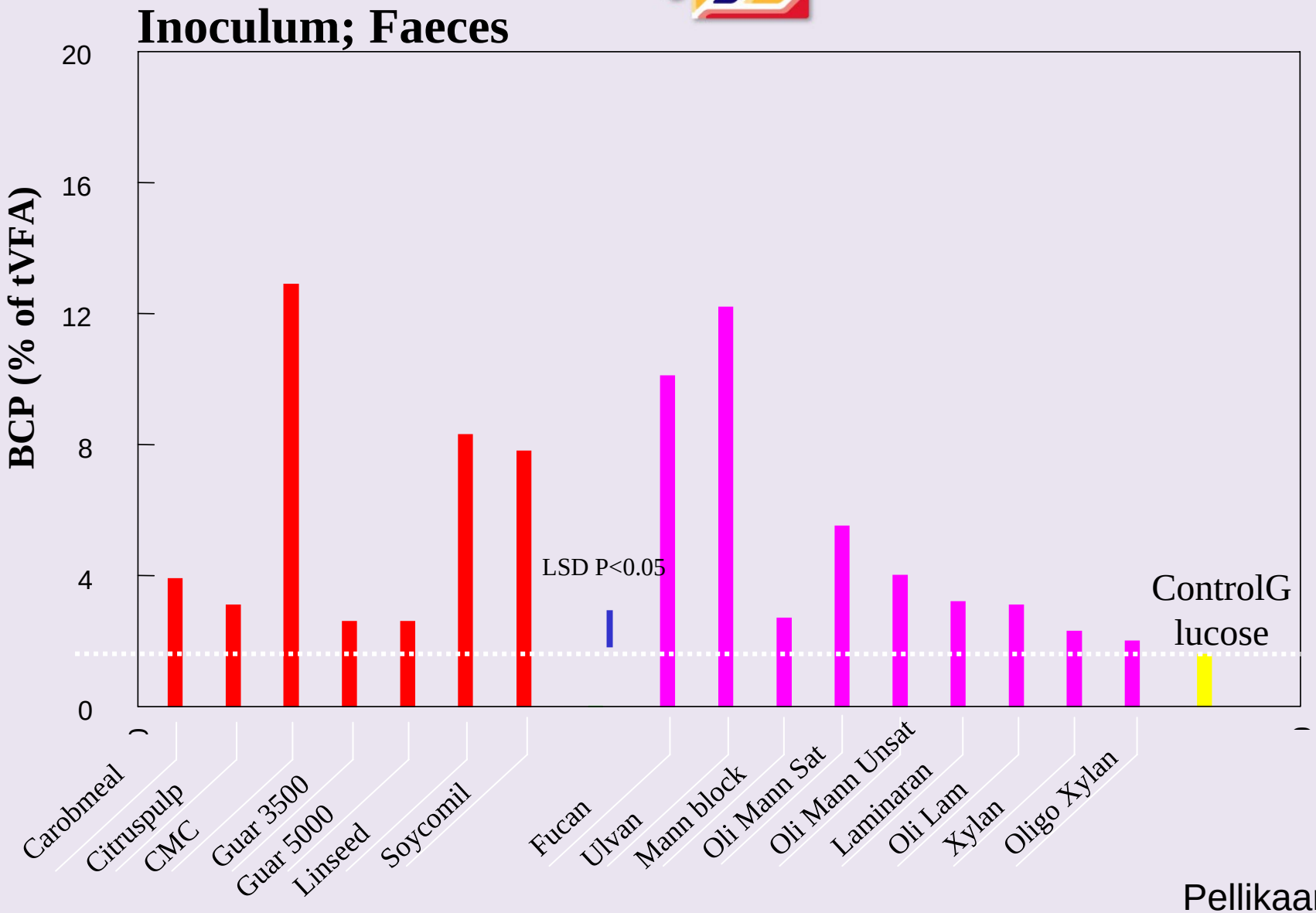
GPT, Total VFA

Inoculum; Faeces



Pellikaan, 2007

GPT, BCP%



Pellikaan, 2007

Selected results

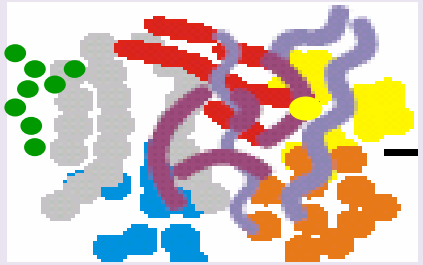
Testing PENS for their fermentability:

- Well - highly fermentable PENS
  - Carob pulp
  - Oligo-laminaran
  - Laminaran
  - Mannuronic block
  - Xylan
  - Oligo-xylan
  - Guar5000
  - Citrus pulp
  - Guar3500
- Gas production characteristics:
  - OMCV → 184 - 313 mL/g OM
  - Rmax → 12 – 41 mL/h
- Fermentation end-products:
  - Total VFA → 7.5 – 12.4 mmol/g OM
  - Ammonia → 2.9 – 4.2 mmol/g OM
  - BCP → < 4 % of Total VFA

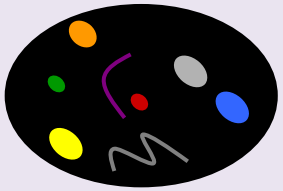
Pellikaan, 2007

Molecular techniques

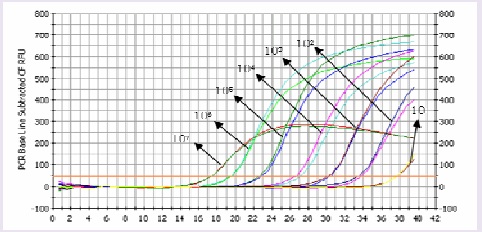
Sample



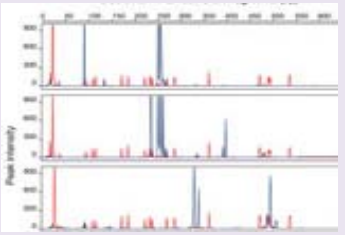
Amplification nucleic acids (PCR)



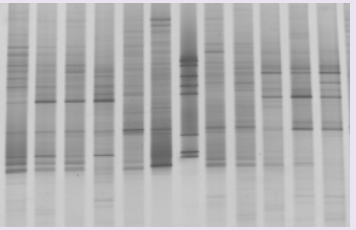
Q-PCR



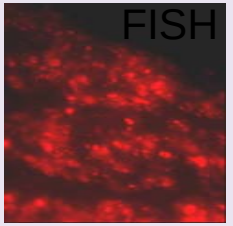
T-RFLP



DGGE



Fixation of bacteria



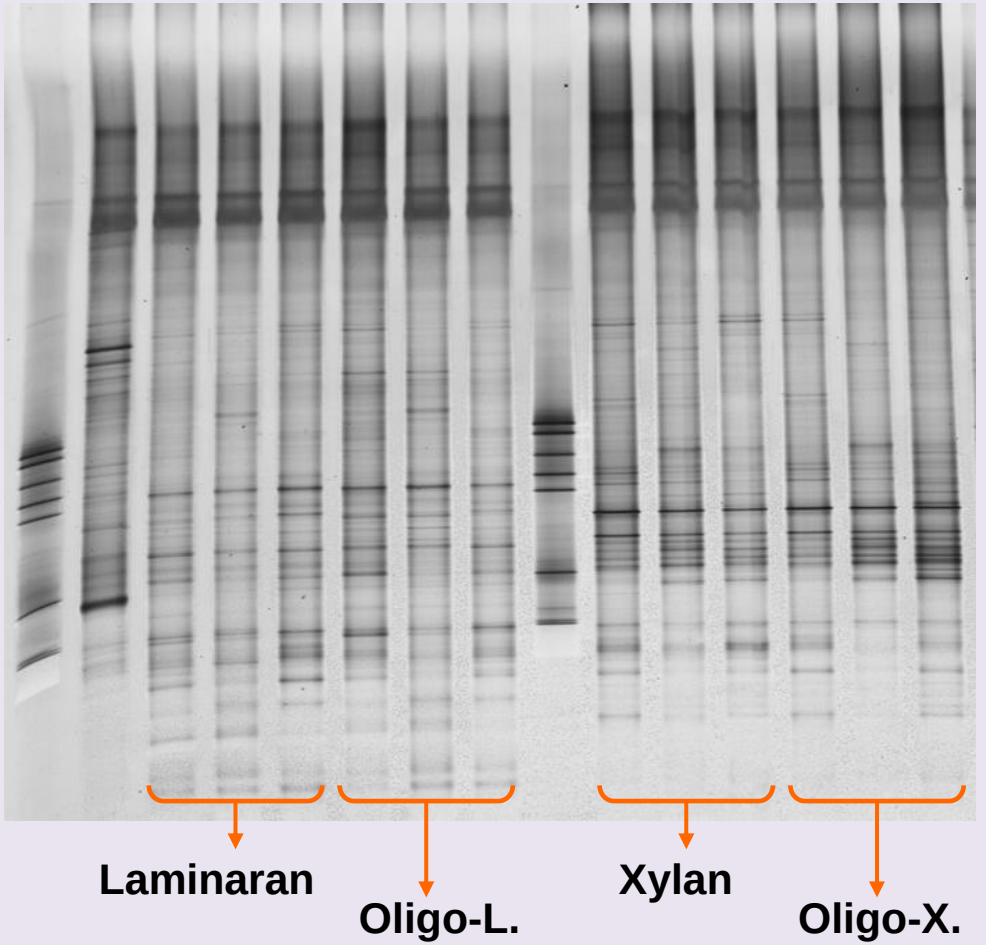
Probe design

PFGE

Smidt et al., 2006

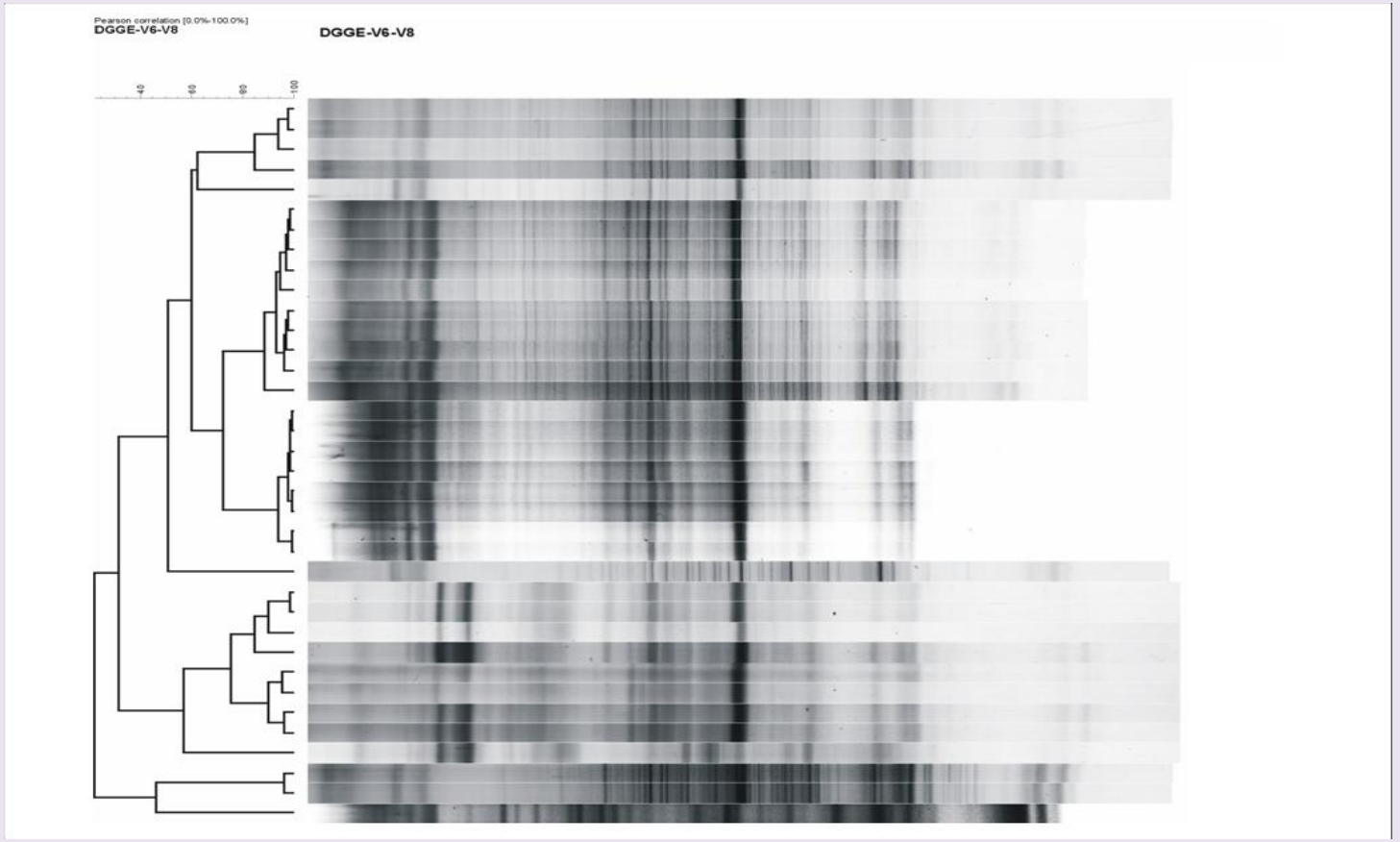
Results, example

- Specific community profiles with different fermentable substrates
- Only minor differences between mono- and oligomers



Results overview

Total microbiota



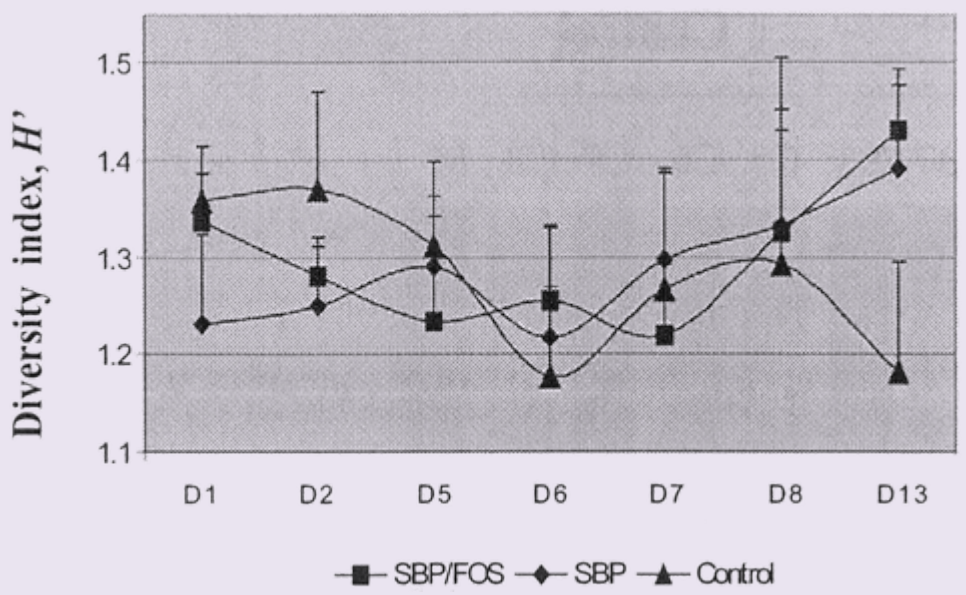
- No specific community structure with different PENS

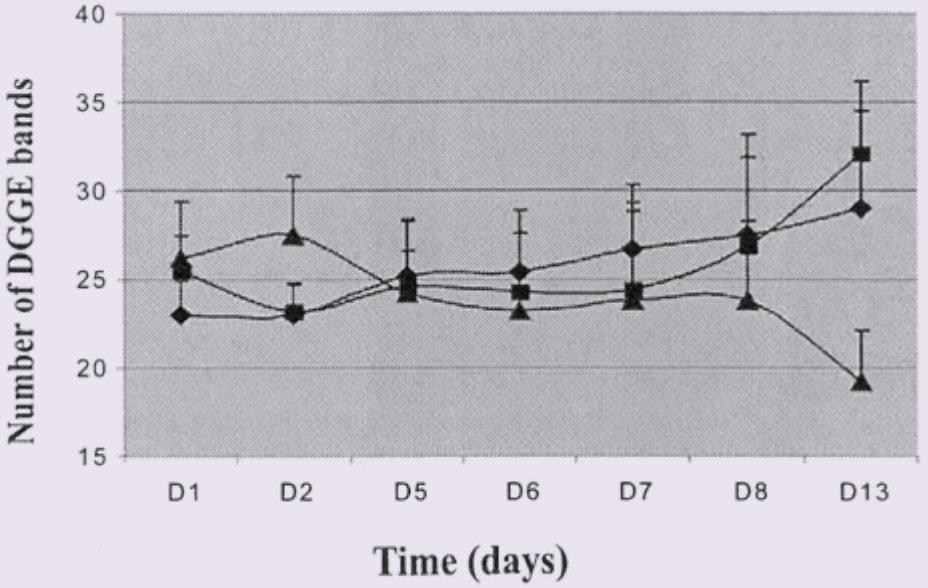
Results, overview

Testing PENS as additives (compared to a control):

- Addition of small amounts of PENS to culture bottles seem to be of minor influence on fermentation characteristics of substrate
- Largest effects appear to occur in the first phase, but are non-significant
- Interesting effects of oregano oil and sanguinarine

Effects of prebiotic dietary ingredients on microbiota development





Influence of weaning diet on DGGE banding patterns in faeces of piglets (Konstantinov et al., 2003)

Prebiotic effects in ileum and colon
(Konstantinov, 2004)

High fibre diet (SBP, In, Lac, WS) vs low fibre diet,

during 10 d post weaning

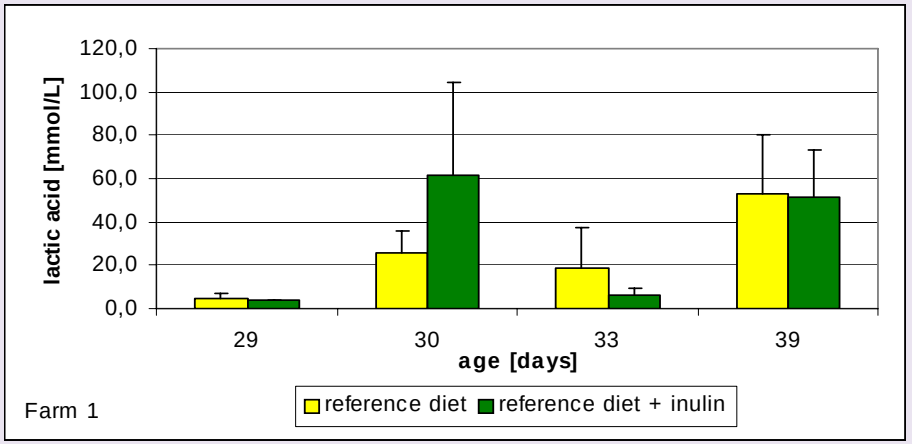
- **Higher bacterial diversity in the colon (DGGE bands, shannon index of diversity) after 10 d.**
- **Higher similarity index of ileal and colon samples, d 10**
- **Increased diversity of Lactobacillus, specific growth of L. amylovorus and L. reuteri like phylotypes**
- **Higher lactic acid concentration in ileal luminal samples**

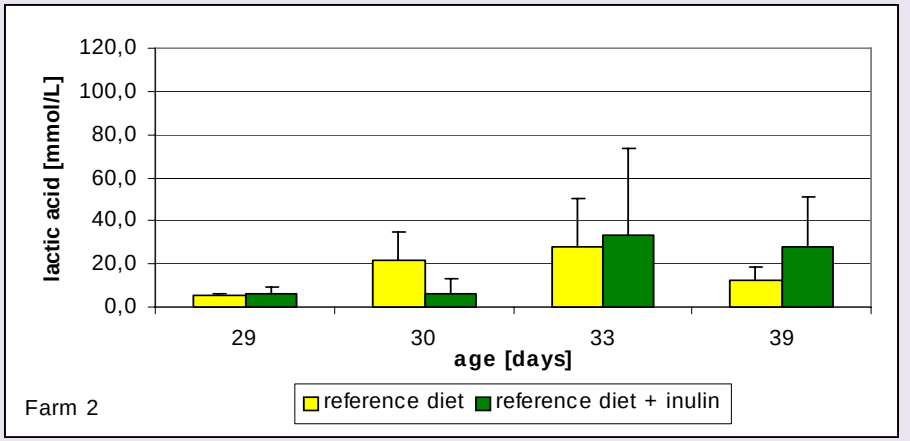
Reference diet + inulin / carob

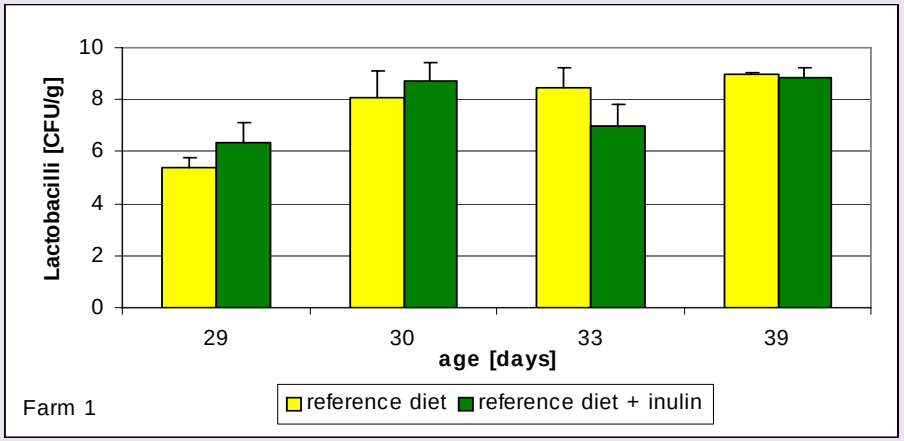
Ingredients	*%*
Barley meal	30.0
Wheat meal	29.7
Peas (44% starch)	5.0
Whey powder	8.0
Wheat bran	2.5
Soy concentrate	4.0
Maize starch	4.0 / 2.5 / 1.0
Inulin (Raftiline HP)	1.5
Carob pulp	3.0
Potato protein	5.0
Maize gluten meal	4.0
Sunflower meal	2.5
Vitamins and minerals	to 100.0

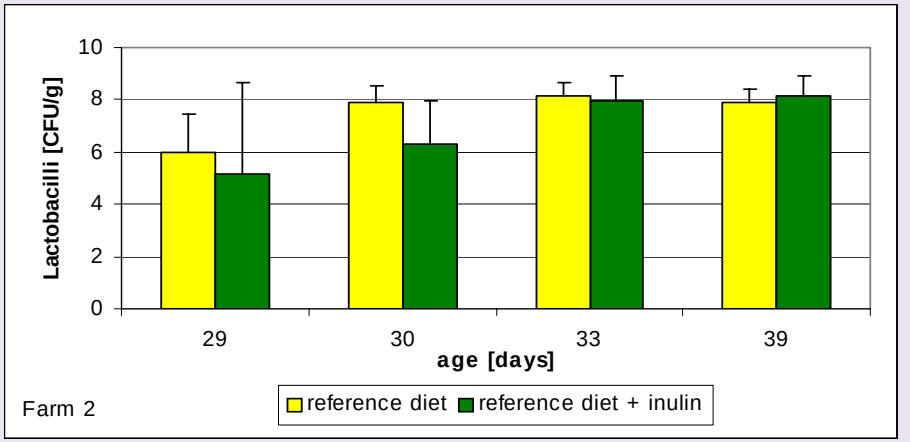
Effect of 1,5% inulin
(Janczyk ea, unpublished)

Lactic acid and lactobacilli in distal jejunum of piglets







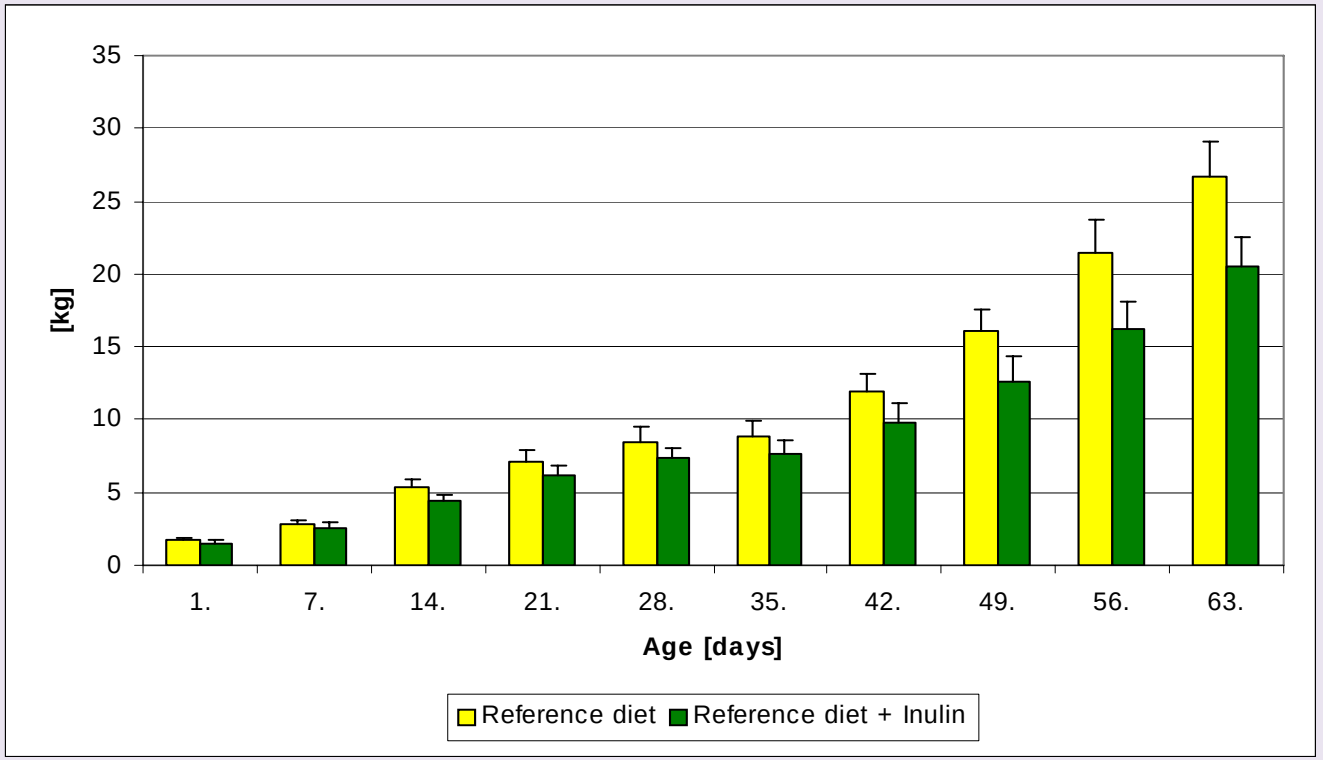


Effect of 1,5% inulin
(Janczyk ea, unpublished)

10 piglets per group, creep feed (RF or RF + inulin) from 14.th day of live

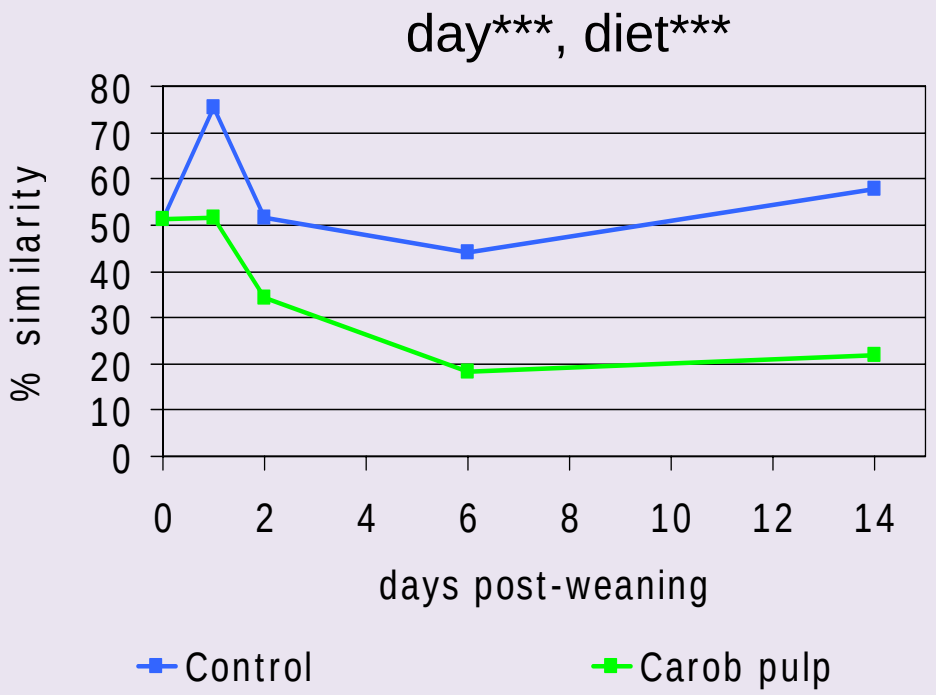
Weaning at 28 days, individual pens

Preliminary results for body weight

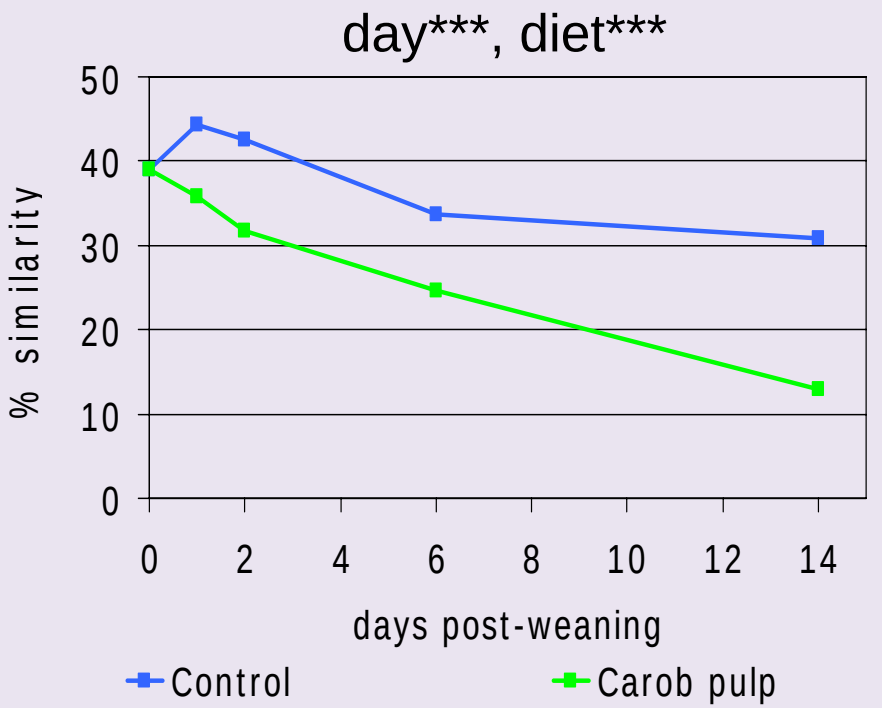


Similarity microbiota

Intra-treatment

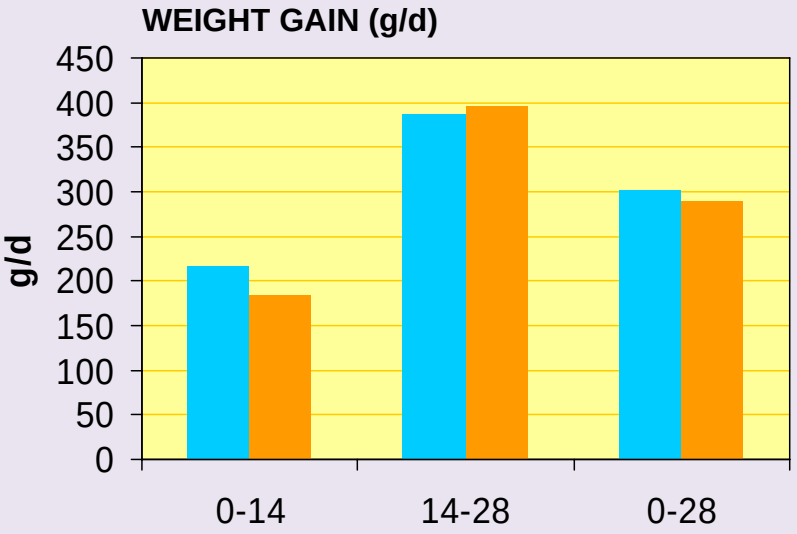


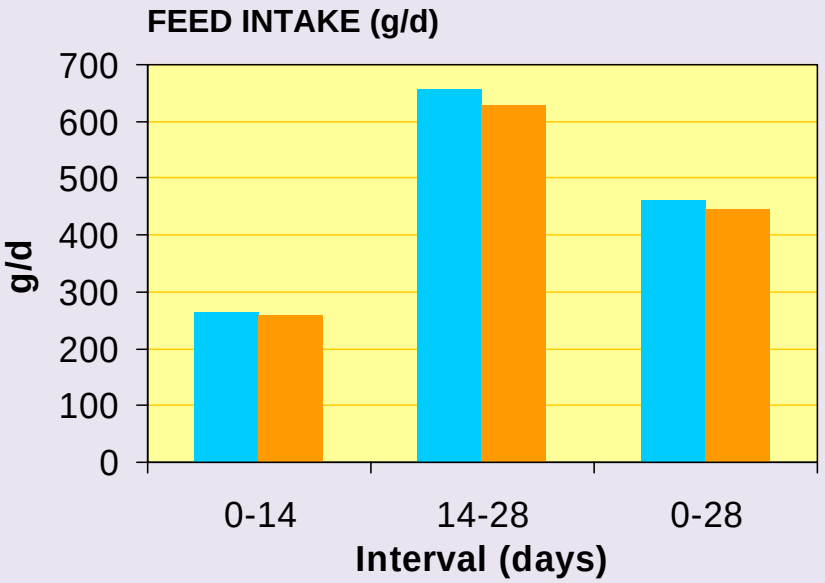
Compared to weaning day

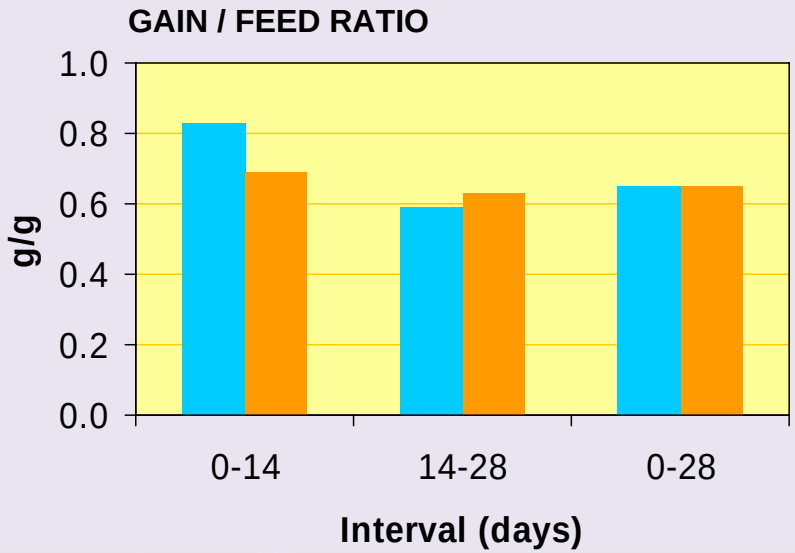


Andrés-Elias et al., 2007; Livest.Sci. 108:280

Influence of 3% carob pulp







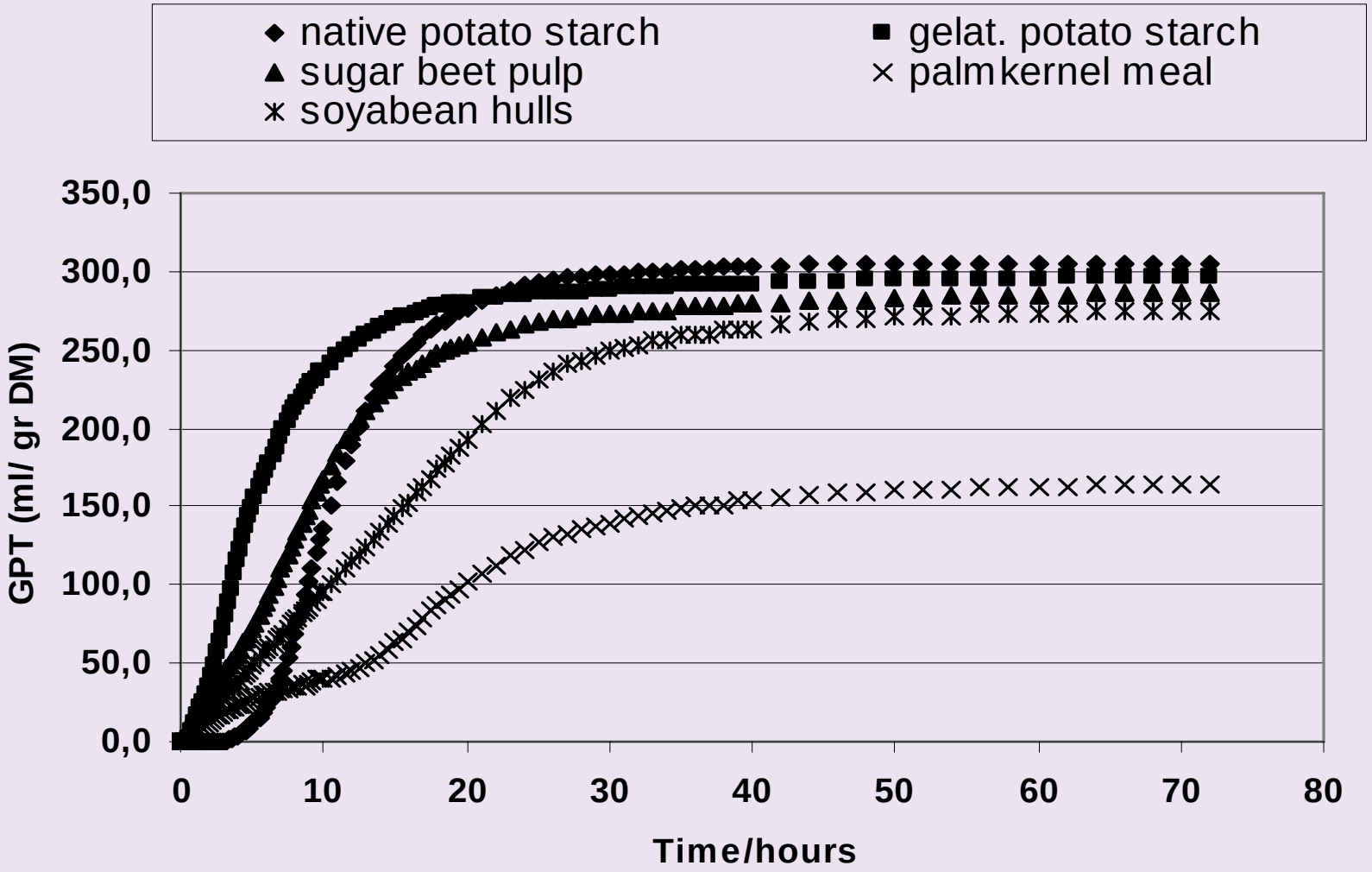
Control Carob Pulp

Andrés-Elias et al., 2007; Livest.Sci. 108:280

Influence of CP (160/220 g/kg) and fCHO (80/130 g/kg) in weaned piglets small intest.

	LP LF	LP HF	HP LF	HP HF	p (CP)	p (NSP)	p (CP*NSP)
Lactobac, cfu	7.9	8.3	7.7	8.6	0.937	0.047	0.354
Coliforms, cfu	7.6	7.1	8.2	6.8	0.82	0.06	0.38
Ammonia	32.7	22.9	73.0	44.1	0.003	0.049	0.315
Lactic acid	12.3	18.3	16.6	26.8	0.160	0.080	0.635
ADFI, wk 1-2	280	263	255	276	0.524	0.850	0.061
ADG, wk 1-2	198	170	182	193	0.652	0.220	0.008

In-vitro fermentation of feed ingredients (LXA-43)



Kinetics of digestion and fermentation

Protein -	slow	slow	fast	fast	P
CHO -	slow	fast	slow	fast	
0 – 7 days post weaning					
Intake, g/d	141	148	146	143	0.52
ADG, g/d	66^{A}	87^{BC}	96^{C}	80^{AB}	0.002
FCR	2.07^{A}	1.67^{BC}	1.56^{C}	1.87^{AB}	0.003
Faecal score	4.59^{A}	4.74^{A}	5.70^{BC}	5.13^{AB}	0.092

Contribution of prebiotics to resistance against bacterial infections (PWC)?

Controversial and insufficient evidence; good (challenge) studies are scarcely available

Circumstantial evidence

- Some indications from the microbiota profiles (e.g. less E.coli)
- High lactose stimulated LAB and reduced E. coli (Pierce, 2006)
- Effect of resistant starch in the diet against salmonella (DK)
- (effect of meal feeding and particle size against salmonella)

But

- Soluble, fermentable NSP may increase haemolytic E.coli in experimentally infected piglets (Hopwood and Hampson, 2003)

Preliminary conclusions

- Prebiotic ingredients can stimulate the post-weaning development of a diverse and stable piglet microbiota.
- Common feed ingredients are the most promising prebiotics and as such need a better characterisation. Specific products may have an additional effect
- Prebiotics may need to be supplied prior to weaning, this application deserves further attention.
- Under hygienic farm conditions prebiotics may not significantly improve growth performance of piglets
- The concept of colonisation resistance and the contribution of prebiotics to it need further validation