Asian-Aust. J. Anim. Sci.
Vol. 20, No. 9 : 1444 - 1452
September 2007

www.ajas.info

# Differences in Microbial Activities of Faeces from Weaned and Unweaned Pigs in Relation to *In vitro* Fermentation of Different Sources of Inulin-type Oligofructose and Pig Feed Ingredients

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**ABSTRACT :** An *in vitro* experiment was conducted to evaluate the differences in microbial activity of five faecal inocula from weaned pigs and one faecal inoculum from unweaned pigs in combination with 6 substrates. The substrates tested were negative control diet, corn, soybean meal, oligofructose (OF), ground chicory roots and a mixture (60% chicory pulp and 40% OF). The inocula used were derived from pigs fed either a corn-soy based diet without antibiotics (NCON), the NCON diet supplemented with oligofructose (OF), a mixture of chicory pulp (40%) and OF (60%) (MIX), ground chicory roots (CHR) or the NCON diet supplemented with antibiotics (PCON). The cumulative gas production measured fermentation kinetics and end products, such as total gas production, ammonia and volatile fatty acids, were also determined. Both the substrate and the inoculum significantly affected the fermentation characteristics. The cumulative gas production curve showed that different substrates caused more differences in traits of fermentation kinetics than the different inocula. Inocula of weaned pigs gave a significantly higher VFA production compared to the inoculum from unweaned animals, whilst the rate of fermentation and the total gas produced did not differ. OF showed the highest fermentation kinetics and the lowest $NH_3$, pH and OM loss compared to other substrates. It was concluded that the microbial activity was significantly affected by substrate and inoculum. Inoculum from weaned pigs had more potential for microbial fermentation of the carbohydrate ingredients and oligofructose than that of unweaned pigs. A combination of high and low polymer inulin may be more beneficial to the gut ecosystem than using high- or low-polymer inulin alone. (**Key Words :** Oligofructose, Fermentation, Pig, Faeces, *In vitro*)

## INTRODUCTION

Oligofructoses (OF) are non-digestible oligosaccharides and regarded as prebiotics which are not metabolized by endogenous enzymes in the gastrointestinal tract (GIT) of human and monogastric animals. It can be fermented by beneficial bacteria such as bifidobacteria and lactobacilli in the large intestine (Tomomatsu, 1994; Gibson and Roberfroid, 1995; Metzler et al., 2005). However, Houdijk et al. (1999) reported that oligofructose may be partly or completely fermented in the distal part of the small intestine in pigs. The site in the gut of pigs where the fermentation of OF occurs may depend on the molecular structure (chain length) of the non-digestible carbohydrates which can have a major impact on fermentation processes (Roberfroid et al., 1998).

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Received February 21, 2006; Accepted April 3, 2007

The principal energy sources for microbial fermentation are dietary carbohydrates which are not digested in the small intestine (Pluske et al., 1999). The major end products of carbohydrates fermentation in the large intestine are volatile fatty acids (VFA), $CH_4$, $H_2$, $CO_2$ and $NH_3$. Oligofructose influences the microbiota composition and activity, and by the production of VFA it may beneficially affect the gut ecology and health (Houdijk et al., 1997; Estrada et al., 2001; He et al., 2002; Xu et al., 2002). Porcine faecal, colonic- and caecal digesta have shown to differ for *in vitro* fermentation characteristics (Houdijk et al., 1997; Williams et al., 1998). The knowledge on the site of fermentation in the GIT of different sources of OF is important because it may differently affect the intestinal ecology and health of pigs.

The cumulative gas production technique can be used to study the effect of inocula (as source of microflora) and substrates on *in vitro* fermentation characteristics between inocula and substrate (Williams et al., 2001). Bauer et al. (2004) reported that faecal inocula can be used for *in vitro* assessment of large intestinal fermentation in pigs. The

better understanding on the site of fermentation by different OF sources will give opportunities to manipulate the gut ecology and health in young pigs. Differences in the rate of fermentation between OF sources as measured *in vitro* may be indicative of the site in the GIT where the fermentation of these carbohydrates occurs. In addition, the major end products of fermentation (VFA and $NH_3$) measured *in vitro* may indicate into which direction fermentation in the GIT will be directed (Williams et al., 2005).

The aim of this study was to determine the differences in *in vitro* fermentation characteristics when inocula of faeces from weaned and from unweaned pigs and different sources (they differed in their degree of polymerization) of inulin derived fructans (OF, chicory roots and chicory pulp) and pig feed ingredients as substrates.

## MATERIALS AND METHODS

### Collection and preparation of inocula

Faecal samples were collected from five treatment groups of 6-8 (8 week-old) pigs. The treatments consisted of pigs fed different sources of OF in their diet. The pigs were weaned at 4 weeks of age and had a body weight ranging between 14-15 kg. A minimum of 6 pigs per treatment were randomly selected from the pens for faecal sample collection, in order to obtain sufficient amounts of inocula for each treatment group. In case of unweaned piglets, all the animals from two litters of suckling piglets (14-16 days old and 4.0-4.5 kg) were used to collect faecal material. The weaned pigs were fed commercial creep feed before weaning. The selected litters of unweaned piglets did not receive any creep feed. Weaned pigs were fed the experimental diets for 28 days after weaning (and had creep feed before weaning) according to the treatments. The faeces were collected directly from the rectum of each pig with a gloved finger and immediately placed in $CO_2$ filled containers to maintain an anaerobic condition, and transported in an insulated container on ice to the lab within 1.5 h of sample collections. Equal amounts of faeces from each animal per treatment were pooled and diluted with an anaerobic sterile saline, pre-warmed to 39°C. The faecal samples were diluted 1:2. Unweaned piglets yielded fewer faeces so the dilution for unweaned pigs is 1:25. Samples were then homogenized for one minute using a hand-blender. The mixture was filtered through two layers of sterile cheese cloth, and the resultant filtrate used as inoculum. All these procedures were carried out under a continuous flow of $CO_2$ to maintain strictly anaerobic conditions (Awati et al., 2005).

### Diets

Weaned pigs were fed either a basal corn-soy diet supplemented with either corn starch (NCON), oligofructose (OF; Raftifeed IPE, Orafti, Tienen, Belgium), grinded chicory roots (CHR), a mixture of chicory pulp and oligofructose (MIX) or corn starch and antibiotics (PCON). The NCON diet did not include antibiotics and inulin type oligofructose. The main ingredients (g/kg) of the basal diet were corn (600.0), soy flakes (160.0), tapioca (73.2), potato protein (46.9), whey powder (40.0) and molasses (35.0). In addition, vitamins, minerals and amino acids were supplemented to meet or exceed the requirements of weaner pigs (NRC, 1998).

### Substrates

Six substrates were tested: four substrates were chosen from experimental treatments, the basal diet (Negative control), a blend of high- and low-polymer inulin (Raftifeed IPE, average DP = 10, Orafti, Tienen, Belgium), grinded chicory roots, and a blend of chicory pulp and oligofructose (60:40). Two major feed ingredients, corn and soybean meal, were also chosen as substrates. All the inulin-type fructans were obtained from Orafti (Tienen, Belgium). The air dried substrates were ground to pass a 1 mm sieve. The basal diet (negative control) and the mixture (60% chicory pulp+40% oligofructose) were also used as substrates in combination with the inoculum of unweaned pigs.

### Cumulative gas production and experimental design

Cumulative gas production by the pressure-volume measurements was carried out manually according to a modified *in vitro* fermentation method of Theodorou et al. (1994). 0.5 g of the test substrate was weighed into fermentation bottles (100 ml serum bottles), and pre-warmed (39°C) semi-defined anaerobic medium (82 ml; Lowe et al., 1985) was added. Five ml of the filtrate (inoculum) was injected into each bottle within 1.5 h of faeces collection from the pigs. The bottles were then incubated at 39°C and incubation was terminated at 48 h.

In total five inocula from the different dietary treatments were used in combination with six substrates, resulting in a 5×6 factorial design. To compare the fermentation characteristics of unweaned animals with those of weaned animals a 2×2 factorial design was used with inocula from the NCON and unweaned animals in combination with two substrates (basal diet and MIX). Per inoculum-substrate combination four replicate bottles were used.

### Analyses

Following *in vitro* fermentation, samples of fermentation fluid were collected for analyses of the content of volatile fatty acids (VFA) and ammonia ($NH_3$). VFA were analyzed by gas chromatography (GC; Fisons HRGC Mega 2, CE Instruments, Milan, Italy) using a glass column fitted (6 ft×2 mm) fitted with Chromosorb 101 (80-100 mesh), as carrier gas $N_2$ saturated with formic acid, at

**Table 1.** Characteristics of fresh faecal inocula from weaned and unweaned pigs[1]

| Inoculum[2] | DM (g/kg) | Ash (g/kg) | pH | Total VFA (mmol/L) | $NH_3$ (mg/L) | BCR |
|---|---|---|---|---|---|---|
| Rectal faeces of pigs fed: | | | | | | |
| NCON | 48.5[a] | 17.3[a] | 6.49[a] | 56.40[a] | 162.6[a] | 0.037[a] |
| OF | 60.4[b] | 20.4[a] | 6.45[a] | 50.47[a] | 208.5[a] | 0.046[ab] |
| CHR | 45.6[a] | 16.2[b] | 6.60[a] | 51.20[a] | 171.7[a] | 0.037[a] |
| MIX | 58.7[b] | 19.8[a] | 6.50[a] | 40.78[a] | 200.0[a] | 0.061[b] |
| PCON | 58.9[b] | 20.5[a] | 6.46[a] | 48.24[a] | 210.6[a] | 0.052[ab] |
| Rectal faeces of suckling pigs: | | | | | | |
| Suckling piglets | ND | 9.7[c] | 5.78[b] | 1.89[b] | 39.2[b] | 0.114[c] |
| SEM | 2.0 | 1.1 | 0.12 | 5.54 | 26.5 | 0.008 |

[1] The values for DM, ash, pH, VFA, $NH_3$ and BCR (branched-chain ratio calculated as total straight chain fatty acids to branched-chain fatty acids) of inocula after dilution.

[2] NCON = Negative control; OF = Oligofructose (Raftifeed IPE, Orafti, Belgium); CHR = grinded chicory roots; MIX = 60% chicory pulp+40% Raftifeed IPE; PCON = Positive control.

ND = Value not determined.

[a, b, c] Different superscripts indicate significant difference ($p<0.05$).

190°C with *iso*-caproic acid as the internal standard.

Ammonia was determined according to a method described by Houdijk et al. (2002b). Supernatants were deproteinized using 10% trichloroacetic acid. $NH_3$ forms a blue complex with phenol and hypochlorite in an alkaline environment, which was measured colorimetrically at 623 nm using a UV spectrophotometer (Beckman-Coulter DU 64, Fullerton, USA). Diet and faeces were analyzed for dry matter (DM; ISO 6496) and inorganic matter (IM; ISO 5984). The pH was measured at the end of fermentation process. The pH of faecal samples was measured by direct insertion of the pH electrode (Hanna instruments) into the bottle of samples.

**Statistics**

The cumulative gas production (ml of gas accumulated $g^{-1}$ OM) was measured manually and a monophasic model as described by Groot et al. (1996; Eqn. 1) was fitted to the data using the nonlinear least squares regression procedure PROC NLIN (SAS Inst. Inc., Cary, NC).

$$G = \frac{A}{(1+(C/t)^B)} \quad (1)$$

Where G = total gas, A = asymptotic gas production, B = switching characteristic of the curve, C = time at which half of the asymptotic has been reached ($T_{1/2}$), t = time.

The maximum rate of gas production ($R_{MAX}$) and the time at which it occurred ($T_{MAX}$) were calculated according to the following equations (Eqn. 2 and 3; Bauer et al., 2001):

$$R_{MAX} = \frac{\{A\times(C^B)\times B\times(T_{MAX}{}^{(-B-1)}\}}{\{1+(C^B)\times(T_{MAX}{}^{(-B)})\}^2} \quad (2)$$

$$T_{MAX} = C\times\left\{\frac{(B-1)}{(B+1)^{1/B}}\right\} \quad (3)$$

A normal mixed model analysis was used to analyses the data of the present study (SAS, 1989). All parameters were tested for significance by analysis of variance using the Tukey multiple range test.

$$Y = \mu+I_i+S_j+(I\times S)_{ij}+\varepsilon_{ijk} \quad (4)$$

Where Y is the dependent variable, μ represents the mean, $I_i$ is the effect of inocula (I = 5), $S_k$ is the effect of substrate j (j = 6), $(I\times S)_{ij}$ is the interaction between substrate and inoculum, and $\varepsilon_{ijk}$ denotes the error term. All statistical analyses were performed using the GLM procedure of SAS version 8.1.

## RESULTS

Table 1 summarizes some characteristics of the faecal inocula from the five dietary treatment groups. Feacal inocula from suckling piglets had a significantly lower concentration of total VFA and $NH_3$, whilst the BCR was a 2 to 3-fold higher compared to the other inocula. The latter suggests that more proteolytic activity occurs in the inoculum of suckling piglets.

**Fermentation kinetics**

Table 2 shows the mean values for parameters of fermentation kinetics. A significant interaction between substrate and inoculum was found for most parameters except pH and $NH_3$. The fermentation characteristics of different substrates were significantly different ($p<0.001$). Both fermentation kinetics and end products are affected more by substrates than by different inocula.

OF (a blend of high- and low polymer inulin) and MIX

**Table 2.** Mean values of the fermentation characteristic parameters according to the effects of substrate and faecal inocula of the weaned pigs[1]

| Factor | OMCV (ml/g OM) | C (h) | $R_{MAX}$ (ml/h) | $T_{MAX}$ (h) | OM loss (%) | pH | $NH_3$ (mg/g OM) |
|---|---|---|---|---|---|---|---|
| Substrate[2] | | | | | | | |
| NCON | 253$^{c}$ | 19.5$^{b}$ | 10.5$^{de}$ | 21.8$^{b}$ | 80.5$^{e}$ | 6.49$^{b}$ | 75.7$^{b}$ |
| Corn | 287$^{b}$ | 22.4$^{a}$ | 12.3$^{d}$ | 18.2$^{a}$ | 84.4$^{d}$ | 6.41$^{c}$ | 63.1$^{cd}$ |
| SBM | 172$^{d}$ | 17.6$^{c}$ | 9.5$^{e}$ | 3.1$^{d}$ | 76.0$^{f}$ | 6.65$^{a}$ | 136.5$^{a}$ |
| OF | 305$^{a}$ | 8.7$^{e}$ | 24.0$^{b}$ | 2.9$^{de}$ | 97.7$^{a}$ | 6.31$^{e}$ | 64.1$^{d}$ |
| CHR | 287$^{b}$ | 5.8$^{f}$ | 32.9$^{a}$ | 2.0$^{e}$ | 95.3$^{b}$ | 6.35$^{d}$ | 67.7$^{c}$ |
| MIX | 298$^{ab}$ | 11.5$^{d}$ | 18.1$^{c}$ | 4.2$^{c}$ | 88.2$^{c}$ | 6.39$^{c}$ | 75.9$^{b}$ |
| SEM | 4.2 | 0.34 | 0.79 | 0.55 | 0.30 | 0.013 | 1.19 |
| Probability | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Inoculum[3] | | | | | | | |
| NCON | 262$^{b}$ | 14.8$^{b}$ | 16.2$^{c}$ | 7.8$^{a}$ | 87.5$^{a}$ | 6.41 | 76.7$^{b}$ |
| OF | 267$^{b}$ | 13.9$^{c}$ | 19.9$^{a}$ | 6.5$^{b}$ | 87.5$^{a}$ | 6.46 | 81.4$^{a}$ |
| CHR | 253$^{b}$ | 16.2$^{a}$ | 16.6$^{bc}$ | 8.0$^{a}$ | 85.9$^{b}$ | 6.42 | 76.3$^{b}$ |
| MIX | 268$^{b}$ | 14.0$^{c}$ | 17.9$^{abc}$ | 7.0$^{b}$ | 88.2$^{a}$ | 6.44 | 82.1$^{a}$ |
| PCON | 285$^{a}$ | 12.4$^{d}$ | 18.9$^{ab}$ | 6.7$^{b}$ | 85.8$^{b}$ | 6.44 | 85.9$^{a}$ |
| SEM | 3.9 | 0.31 | 0.72 | 0.32 | 0.28 | 0.012 | 1.10 |
| Probability | 0.001 | 0.001 | 0.002 | 0.003 | 0.007 | 0.109 | 0.001 |
| Interaction[4] | | | | | | | |
| Sub×inoc | 0.001 | 0.001 | 0.004 | 0.001 | 0.001 | 0.290 | 0.080 |

[1] OMCV = Total gas produced (ml gas/g OM corrected volume); C = time at which half of asymptotic has been reached; $R_{MAX}$ = maximum rate of gas production; $T_{MAX}$ = time at which maximum rate of gas production occurs.

[2] NCON = Negative control; SBM = Soybean meal; OF = Oligofructose (Raftifeed IPE, Orafti, Belgium); CHR = grinded chicory roots; MIX, 60% chicory pulp+40% Raftifeed IPE.

[3] PCON = Positive control. [4] Sub×inoc = substrate×inoculum interaction.

[a, b, c, d, e, f] Means in the same column for substrate or inocula without a common character in the superscript differ significantly ($p<0.05$).

**Table 3.** Mean values of the fermentation characteristic parameters according to the effects of substrate and faecal inocula of weaned and unweaned pigs[1]

| Factor | OMCV (ml/g OM) | C (h) | $R_{MAX}$ (ml/h) | $T_{MAX}$ (h) | OM loss (%) | pH | $NH_3$ (mg/ g OM) |
|---|---|---|---|---|---|---|---|
| Substrates[2] | | | | | | | |
| NCON | 257 | 23.8 | 9.7 | 16.2 | 77.9 | 6.45 | 71.0 |
| MIX | 317 | 14.2 | 16.4 | 7.4 | 88.5 | 6.39 | 68.6 |
| SEM | 7.9 | 0.91 | 0.84 | 0.72 | 0.33 | 0.02 | 1.19 |
| Probability | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.080 | 0.170 |
| Inoculum | | | | | | | |
| NCON | 281 | 16.2 | 13.8 | 8.8 | 85.5 | 6.42 | 72.2 |
| Suckling piglets | 294 | 21.7 | 12.2 | 14.8 | 80.9 | 6.42 | 67.4 |
| SEM | 7.6 | 0.91 | 0.84 | 0.72 | 0.33 | 0.022 | 1.19 |
| Probability | 0.260 | 0.001 | 0.190 | 0.001 | 0.001 | 0.950 | 0.020 |
| Interactions[3] | | | | | | | |
| Sub×inoc | 0.470 | 0.140 | 0.680 | 0.770 | 0.070 | 0.190 | 0.010 |

[1] OMCV = total gas produced (ml gas/g OM corrected volume); C = time at which half of asymptotic has been reached; $R_{MAX}$ = maximum rate of gas production; $T_{MAX}$ = time at which maximum rate of gas production occurs.

[2] NCON = Negative control; MIX = 60% chicory pulp+40% Raftifeed IPE.

[3] Sub×inoc = substrate×inoculum interaction.

had the highest value for total gas production (OMCV), and soybean meal had the lowest gas production ($p<0.001$). All the oligofructose and inulin type substrates had a higher gas production compared to the NCON diet or soybean meal ($p<0.001$). Corn as a substrate had a large gas production comparable to the CHR substrate, but C and $T_{MAX}$ occurred at a significant later time in case of corn. OF and CHR almost completely disappeared with OM losses of 97.7 and 95.3%, respectively. The highest OM loss by fermentation of OF resulted in the lowest pH and $NH_3$ also significantly lowered ($p<0.001$). Soybean meal had the highest $NH_3$ and pH, and also had the lowest OM loss (76.0%). CHR had the highest $R_{MAX}$ and the lowest C and $T_{MAX}$.

The inoculum affected most fermentation characteristics except for pH. Total gas production (OMCV), C, $R_{MAX}$ and $T_{MAX}$ were highest with faeces from PCON, CHR, OF and

**Table 4.** Mean values of VFA and BCR according to the effects of substrate and faecal inocula of the weaned pigs[1]

| Factor | Acetic | Propionic | Butyric | Valeric | Total VFA | BCR |
|---|---|---|---|---|---|---|
| Substrate[2] | | | | | | |
| NCON | 4.34$^{c}$ | 1.97$^{d}$ | 2.09$^{b}$ | 0.42$^{c}$ | 9.23$^{c}$ | 0.045$^{b}$ |
| Corn | 3.90$^{d}$ | 1.96$^{d}$ | 2.28$^{a}$ | 0.36$^{d}$ | 8.87$^{c}$ | 0.041$^{c}$ |
| SBM | 4.49$^{c}$ | 1.88$^{d}$ | 1.39$^{cd}$ | 0.49$^{b}$ | 8.84$^{c}$ | 0.066$^{a}$ |
| OF | 5.30$^{b}$ | 3.53$^{a}$ | 1.47$^{c}$ | 0.55$^{a}$ | 11.06$^{a}$ | 0.020$^{e}$ |
| CHR | 5.49$^{ab}$ | 3.06$^{b}$ | 1.29$^{d}$ | 0.49$^{b}$ | 10.54$^{a}$ | 0.020$^{e}$ |
| MIX | 5.74$^{a}$ | 2.36$^{c}$ | 1.17$^{e}$ | 0.40$^{c}$ | 9.91$^{b}$ | 0.025$^{d}$ |
| SEM | 0.102 | 0.057 | 0.035 | 0.009 | 0.200 | 0.001 |
| Probability | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Inocula-faeces[3] | | | | | | |
| NCON | 4.85$^{a}$ | 1.32$^{a}$ | 1.54$^{ab}$ | 0.47$^{b}$ | 9.96 | 0.035$^{b}$ |
| OF | 4.99$^{a}$ | 1.10$^{bc}$ | 1.68$^{a}$ | 0.54$^{a}$ | 9.85 | 0.035$^{b}$ |
| CHR | 4.52$^{b}$ | 1.06$^{c}$ | 1.53$^{b}$ | 0.41$^{c}$ | 9.18 | 0.034$^{b}$ |
| MIX | 5.07$^{a}$ | 1.16$^{b}$ | 1.68$^{a}$ | 0.45$^{b}$ | 9.75 | 0.036$^{b}$ |
| PCON | 4.96$^{a}$ | 1.21$^{b}$ | 1.65$^{a}$ | 0.39$^{d}$ | 9.93 | 0.040$^{a}$ |
| SEM | 0.095 | 0.001 | 0.032 | 0.008 | 0.185 | 0.001 |
| Probability | 0.003 | 0.030 | 0.004 | 0.001 | 0.060 | 0.001 |
| Interaction[4] | | | | | | |
| Sub×inoc | 0.470 | 0.001 | 0.090 | 0.001 | 0.320 | 0.001 |

[1] Acetic, propionic, butyric and valeric acid expressed in mmol/g OM; Total VFA = acetic+propionic+iso-butyric+butyric+iso-valeric+valeric (in mmol/ g OM); BCR= branched-chain ratio calculated as total straight chain fatty acids to branched-chain fatty acids.

[2] NCON = Negative control; SBM = Soybean meal; OF = Oligofructose (Raftifeed IPE, Orafti, Belgium); CHR = grinded chicory roots; MIX = 60% chicory pulp+40% Raftifeed IPE.

[3] PCON = Positive control.

[4] Sub×inoc = substrate×inoculum interaction.

[a, b, c, d, e, f] Means in the same column for substrate or inocula without a common character in the superscript differ significantly ($p<0.05$).

CHR, as inoculum respectively. OM loss was significantly lower for both PCON and CHR compared to all other inocula ($p<0.007$). pH values after fermentation were similar between the inocula, but ammonia concentrations were lower for CHR and NCON than that of other inocula ($p<0.001$).

The mean values of fermentation characteristics for the faecal inocula of weaned and unweaned pigs and two substrates are shown in Table 3. Total gas production and fermentation kinetics except for $NH_3$ were significantly affected by substrate. The substrate MIX had higher total gas production (OMCV) and $R_{MAX}$, and lower C and $T_{MAX}$ compared to the NCON substrate. The pH was significantly lower in the MIX than that of NCON. Ammonia was numerically in the MIX compared to the NCON. There were significant differences in fermentation characteristics of C, $T_{MAX,}$ ammonia and OM loss between inocula of weaned and unweaned pigs. The inocula of unweaned pigs had higher C, $T_{MAX}$ and lower OM loss and ammonia compared with the inocula of weaned pigs.

**Volatile fatty acids and branched-chain ratio**

The main effects of substrates and fecal inocula on VFA production and BCR are shown in Table 4. The production and proportion of VFA were significantly different according to substrate ($p<0.001$). The oligofructose and inulin substrates produced the highest total VFA, and had the lowest BCR compared to the NCON diet, corn and soybean meal. This is logic because substrates with high protein have mostly more BCR. Corn as a substrate produced the highest butyric acid, and soybean meal produced the highest BCR ($p<0.001$) between substrates. The oligofructose and inulin substrate had significantly lower BCR compared to the grain group substrates (Corn, soybean meal and NCON).

Fermentation characteristics by inocula from CHR had significantly lower acetic acid, and OF inocula produced the highest butyric and valeric acids. The NCON produced the highest propionic acid. Total VFA were not significantly different among the inocula. PCON had the highest BCR between inocula ($p<0.001$). There were significant interaction between substrate and inocula for propionic and valeric acids and BCR ($p<0.001$).

Mean values of VFA and BCR for the substrates and faecal inocula of the weaned pigs and unweaned pigs are shown in Table 5. The substrate MIX had significantly higher acetic acid, and also had significantly lower butyric and BCR compared to the NCON substrate. Fermentation by faecal inocula of weaned pigs produced significantly more VFA, total VFA and BCR compared to the inocula from unweaned pigs. There were significant differences in propionic, butyric acids and BCR between the inocula of weaned and unweaned pigs.

## DISCUSSION

**Differences between substrates**

As it was expected oligofructose and inulin type

**Table 5.** Mean values of VFA and BCR according to the effects of substrate and faecal inocula of the weaned pigs and unweaned pigs[1]

| Factor | Acetic | Propionic | Butyric | Valeric | Total VFA | BCR |
|---|---|---|---|---|---|---|
| Substrate | | | | | | |
| NCON | 4.04 | 1.94 | 1.70 | 0.34 | 8.33 | 0.036 |
| MIX | 5.58 | 2.00 | 1.19 | 0.28 | 9.23 | 0.020 |
| SEM | 0.224 | 0.103 | 0.094 | 0.021 | 0.422 | 0.001 |
| Probability | 0.001 | 0.710 | 0.003 | 0.080 | 0.160 | 0.001 |
| Inoculum[2] | | | | | | |
| NCON | 5.22 | 2.55 | 1.67 | 0.45 | 10.2 | 0.034 |
| Suckling pigs | 4.40 | 1.39 | 1.21 | 0.17 | 7.3 | 0.021 |
| SEM | 0.224 | 0.103 | 0.094 | 0.021 | 0.42 | 0.001 |
| Probability | 0.020 | 0.001 | 0.006 | 0.001 | 0.001 | 0.001 |
| Interaction[3] | | | | | | |
| Sub×inoc | 0.570 | 0.003 | 0.007 | 0.690 | 0.700 | 0.003 |

[1] Acetic, propionic, butyric and valeric acid expressed in mmol/g OM; Total VFA = acetic+propionic+iso-butyric+butyric+iso-valeric+valeric (in mmol/ g OM); BCR = branched-chain ratio calculated as total straight chain fatty acids to branched-chain fatty acids.

[2] NCON, MIX, 60% chicory pulp+40% Raftifeed IPE.

[3] Sub×Inoc, substrate×inoculum interaction.

substrates produced a higher rate of fermentation kinetics (total gas production) and end product (VFA) than that of the NCON or feed grain substrates. Because the oligofructose and inulin type fructan are known to be highly fermentable carbohydrates. There were six different substrates used in this experiment, including the basal diet (Negative control), OF, grinded chicory roots, and a blend of chicory pulp and oligofructose (60:40), corn and soybean meal.

There were significant differences by the main effects of substrate and inoculum. The different rate of fermentation certainly related to differences in microbial activity and in the chemical structure (degree of polymerization) of substrates (Van Loo, 2004). The production of VFA which are produced as end products of fermentation by microbiota may be beneficial for GIT health (Cummings and Macfarlane, 1991). The individual VFA produced in the large intestine may have a specific role. For instance, acetic acid acts as an energy sources for muscle tissue, and propionic acid is converted in the liver. In addition, butyric acid is the preferred substrate for the colonic epithelial cells (Roediger, 1980), and may act on cell proliferation and differentiation (Metzler et al., 2005). The present results show that type of fermentable carbohydrate is important in determining the amount and profile of VFA in the large intestine.

The fermentation patterns between substrates indicated that both OF (a blend of high and low polymer inulin) and MIX (a mixture of 60% chicory pulp and 40% OF) were the most fermentable in both kinetics and end products between substrates. This result suggests that a mixture of slowly fermentable (high polymer inulin) and rapidly fermentable (low polymer inulin) may produce more total gas production and also may help maintain the fermentation for a longer time (Van Loo, 2004). If fermentation *in vitro* is indicative for *in vivo*, these results mean that this mixture may help maintain the fermentation throughout the large intestine of pigs. Also if continued saccharolytic fermentation occurs due to the presence of fermentable carbohydrates sources, this can reduce the occurrence of proteolytic fermentation in the large intestine, as indicated by a reduction in branched-chain fatty acids and lower concentrations of $NH_3$ (Williams et al., 2001).

Soybean meal was the least fermentable (OMCV) of all substrates, but had nearly double the ammonia than that of all other substrates. There was also high BCR for this substrate. The greater proportion of branched chain fatty acids (BCFA) in soybean meal substrate suggested an increased fermentation of protein during fermentation of soybean meal compared to other substrates. This is because BCFA are mostly produced as end products by the metabolisms of branched-chain amino acids (valine, leucine and iso-leucine). It is thought that this can be the result from a shortage of energy from carbohydrates (Macfarlane et al., 1992). The greater ammonia concentration in soybean meal is most probably related to the relatively high crude protein content of the substrate. Corn as a substrate had greater kinetics and fermented very slowly (greater C and $T_{MAX}$). Corn also produced the highest amount of butyric acid and the smallest amount of acetic acid of all substrates.

Bauer et al. (2001) have shown that feed grain such as maize, barley and wheat had similar VFA concentrations at the end of fermentation for both unweaned and adult pigs. The composition and activity of microflora in the large intestine can be manipulated by use of dietary prebiotics and fermentable carbohydrates (Gibson and Roberfroid, 1995).

When comparing the kinetics and end products of substrates from weaned and suckling pigs, substrate from MIX had greater fermentation, C, OM loss and BCR than that of substrate from NCON. As a result, MIX which consisted of a blend of chicory pulp and oligofructose

(60:40) significantly lowered pH. Also ammonia was numerically lowered compared to the NCON. Most fermentation kinetics and end products parameters of substrates between weaned and unweaned pigs were significantly different.

**Differences between faecal inocula**

There were significant differences in fermentation characteristics (fermentation kinetics and end products) between inoculums, but not for pH. The differences between inocula can be a consequence of differences in microbial composition and their activity.

Unexpectedly, the PCON (with antibiotics) had the highest gas production, $NH_3$ and the maximum rate of gas production. This means that antibiotics may not have affected microbiota with fermentative capacity. Also the BCR was the highest for the PCON and this is a result from fermentation of amino acids. This result is in agreement with the results of a study reported by Houdijk (1998a). They showed that addition of antibiotics (avilamycin) to weaner diets reduced the level of protein fermentation, as indicated by a slight reduction in the concentration of ammonia and lower concentrations of branched chain fatty acids.

Dilution of the faecal samples in weaned pigs was 1:2 and in unweaned pigs was 1:25 because of the differences in the amount of faecal samples. This will probably affect lag time at the beginning of fermentation. Inocula of CHR had the lowest acetic acid and least total VFA compared to other inocula. The total VFA and pH between inocula were not affected.

The OF and MIX had the highest butyric acid between inocula. This agrees with the report of Houdijk (2002b) that substrate from FOS and transgalacto-oligosaccharides produced significant amounts of butyric acid *in vitro*. The butyrate may have been produced from glucose, inulin and a fructose polymer by Clostridia spp. (Schlegel, 1992). Generally, substrate effects tended to be greater than inoculum effects regarding fermentation kinetics and end products. The OM loss of inoculum from PCON and CHR were significantly lower than that of other inocula, and this result suggests that microbial activity of the inocula during fermentation may be, to some extent inhibited by in-feed antibiotics in the inoculum.

The comparison of cumulative gas production curve for different substrates with inocula from weaned and unweaned pigs showed that inocula from weaned pigs fermented both NCON diet and MIX (chicory pulp and OF) much better than the inocula from unweaned pigs. The inocula from weaned pigs gave a significantly higher VFA compared to unweaned piglets, but total gas production and rate of fermentation did not differ. The unweaned pigs were not offered creep feed and only consumed sow's milk. Initial colonization of bacteria in the GIT of young piglet is mainly lactic acid bacteria, and they remain as long as sow's milk is consumed (Mathew et al., 1998). So the ability to ferment by both inocula (from weaned and from suckling pigs) differs. This difference can be related to microbial population itself and to the activity or both. The lower amount of gas production and the slower fermentation that occurred with unweaned inocula does not clarify which is the major reason. The lower gas production and a low rate of fermentation with inocula from unweaned pigs show that the 15 day-old suckling pigs had not yet reached capacity of the microflora of weaned or adult pigs to ferment. Most fermentation kinetics and end products parameters of inoculum between weaned and unweaned pigs differed significantly but some parameters such as OMCV, $R_{MAX}$ and pH were not affected. Generally, inoculum of suckling pigs produced more total gas and used a longer time before reaching the maximum rate of gas production occurs compared to that of weaned pigs.

It was concluded that the different fermentation kinetics ($T_{MAX}$, $R_{MAX}$) from different substrates in the present study show that microbial flora reacts differently to them. Inocula from pigs which had inulin and oligofructose in their diets and used these as substrates produced more gas at a faster rate than with control or feed grain substrates. Oligofructose gave less bacterial proteolytic activity *in vitro*. Both combined oligofructose substrates, OF, and MIX showed the highest fermentation kinetics and the highest total amount of VFA of all substrates. If transferred to *in vivo* this result may help to maintain fermentation also at the distal part of the large intestine and it may last longer. In addition, immediately after weaning pigs eat only a small amount of feed (Dong and Pluske, 2007), and hence, fermentable carbohydrate sources will be limited available at the end of the small intestine and large intestine. It can be argued that at the end of small intestine and the beginning of the large intestine a rapid fermentation of easily fermentable carbohydrates may provide a low pH and this is known to have a strong effect on bacterial survival rate (Metzler et al., 2005). However, if fermentation stops before the end of the large intestine due to lack of saccharolytic sources, then protein may be fermented (used as energy source) with as a consequence an increase of ammonia and nitrogenous products. Therefore, a few days after weaning, when the microbial population starts to develop, the rapid fermentable carbohydrates are less needed and carbohydrate sources with a higher degree of polymerization can be used. The data of inocula from adapted and non-adapted animals support this reasoning. Thus, a combination of slowly fermentable (high polymer fructan) and rapidly fermentable (low polymer fructan) may be more beneficial to the gut

ecosystem and health than single type of OF. A rapid development of a diverse but stable microbial population in the gut of early weanling piglets is of special interest when the use of in-feed antibiotics is prohibited, as is the current situation within the European Union.

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