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Dietary carbohydrates with different rates of fermentation affect fermentation end-product profiles in different sites of gastro-intestinal tract of weaning piglet

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Abstract

An in vivo *experiment was conducted to examine changes in fermentation end-products in the gastro-intestinal tract (GIT) of weaning piglets by the inclusion of fermentable carbohydrates in the diet. The experiment was repeated in three replicates of 36 piglets. Piglets were raised free of antibiotics and creep feeding prior to weaning at 4 weeks of age. Each replicate was conducted over a period of 10 days. The piglets were offered one of two dietary treatments: control diet (CON), and fermentable carbohydrate enriched diet (CHO); and were subjected to one of the two fasting treatments (i) fasting for 2 days in the beginning of the experimental period and (ii) non-fasting. Piglets were slaughtered on the 1st, 4th and 10th day of each period. Digesta samples were collected from: first half of small intestine, second half of small intestine, caecum, and colon. The dry matter, volatile fatty acid (VFA) profile, and ammonia concentrations were analysed. Food intake, growth and food conversion ratio were also recorded. There were no differences in production performances such as growth and food conversion ratio (FCR) between the treatment groups. Concentrations of VFA were significantly higher, while ammonia concentration was significantly lower in the CHO group compared to the CON group in different fermentation sites within the GIT ($P < 0{\cdot}001$), and on different slaughtering days ($P < 0{\cdot}05$). Fasting had no effect on fermentation end-products. This study concludes that the addition of fermentable carbohydrates of varying fermentabilities stimulated carbohydrate fermentation, with reduction in protein fermentation along the different parts of GIT studied, in weaning piglets.*

Keywords: *carbohydrates, fermentation, piglets, weaning.*

Introduction

Microbial fermentation in the gastro-intestinal tract (GIT) of monogastric animals has gained interest in recent years as it has been realized that the normal GIT microbiota, can play a significant rôle in GIT health, particularly at times of stress, such as weaning of young animals. The interest has become particularly focused, as alternatives are sought for the use of in food antibiotics as growth promoters.

It is known that the GIT microbial community is largely dependent upon the animal diet as the main source of substrate for its metabolism. Consequently, changes in the dietary composition or nutrient density can have dramatic effects on the intestinal microbial community (Bedford and Apajalahti, 2001). Therefore, manipulation of the diet is considered to be one of the easiest ways to influence the composition and activity of the microflora (Gibson and Roberfroid, 1995). As potential sources of energy for bacteria, fermentable versus digestible carbohydrates, can be effective in terms of influencing the gut microbiota beneficially (Cummings and Englyst, 1987; Gibson and Roberfroid, 1995), particularly in relation to fermentation in the large intestine (Williams *et al*., 2001). For example, the addition of selected fermentable carbohydrates has been shown to increase bacterial diversity and lead to more rapid stabilization of the microbial community (Konstantinov *et al*., 2003) in newly weaned piglets.

At weaning, exposure to a solid diet combined with other stresses, can lead to dramatic and sudden changes in the GIT microbiota, which leaves piglets susceptible to the activity of potentially pathogenic bacteria and thus to the well-described 'post-weaning syndrome' (Hopwood and Hampson, 2003). A 'healthy' GIT microflora on the other hand, (meaning one which is stable and diverse) can promote 'colonization resistance', a concept first coined by Van der Waaij (1989), to describe the ability of the natural microflora to protect the host from potentially pathogenic species in the GIT. For example, it has been reported that diets containing specific fermentable carbohydrates are associated with species antagonism or colonization resistance for *Clostridium* spp. in the ileum of weaning piglets (Konstantinov *et al*., 2004). Hence, given the impending EU ban on the use of antimicrobial growth promoters in animal feeds, there

is now an urgent imperative to examine alternative ways to improve pig health by stimulating the autochthonous gastrointestinal microflora.

Several published studies have shown the effect of an individual source of fermentable dietary fibre on fermentation in the large intestine. However, it has been shown that fermentation takes place in both the small and large intestines of pigs, (Drochner, 1991; Jensen and Jorgensen, 1994). Therefore, to stimulate colonization resistance along the entire tract, it is potentially important to stimulate carbohydrate fermentation in the small intestine as well. However, using *in vitro* techniques, Bauer *et al.* (2001) and Williams *et al.* (2005) have shown that not all carbohydrates ferment in the same way. There is considerable variation both in rates of fermentation, and the end-products produced. In order to stimulate fermentation along the entire tract, it would therefore be necessary to select ingredients with a range of fermentation rates, as the more rapidly fermented ingredients would be more likely to be fermented in the small intestine, and the more slowly, in the large intestine.

As part of the stress syndrome associated with the post-weaning period in piglets temporary anorexia is frequently reported. At first, some piglets avoid eating at all, and may then suddenly eat a large amount of food between 24 and 72 h after removal from the sow. This may lead to severe upsets of digestive function and to diarrhoea (Hopwood and Hampson, 2003).

The present study was designed with the following aims: (i) to determine whether addition of fermentable carbohydrates of varying fermentation rates in the weaning diet, could stimulate positive fermentation, as measured by concentrations of fermentation end-products along four different areas of the GIT, (ii) to determine whether enforced fasting at the beginning of weaning had an effect on fermentation end-products, and whether there was a diet/fasting interaction. If fermentation could be stimulated significantly, this would have implications for GIT health during the weaning period.

Material and methods

An *in vivo* experiment was designed as a split plot design. The experiment was conducted in three identical replicates. Each replicate was conducted over a period of 10 days. For each replicate, four litters of nine piglets were used (in total 36 piglets per period – 108 piglets in total). At the start of each period, one piglet from each litter was killed upon removal from the sow. This was called day 1. These piglets were not subjected to any treatment. The remaining eight piglets from each litter were divided into four treatment combinations. One piglet from each treatment combination from every litter (4 treatment combinations $\times$ 4 litters $=$ 16 piglets) were sacrificed on day 4 and the remaining piglets on day 10 post weaning. Digesta samples from four areas of the tract were collected and analysed for volatile fatty acid (VFA) and ammonia concentrations. Digesta pH was also recorded. All the procedures involving animals were conducted in accordance with the Dutch law on experimental animals and had been approved by the Wageningen University Animal Experimental Committee (Dier Experimenten Commissie).

Animals and housing

The 108 crossbred piglets were taken away from the sows at 4 weeks of age and transported to the experimental facility. The piglets had received only sow milk during the pre-weaning period, having neither exposure to creep food nor any antibiotic treatment. During the experimental period of 10 days, the piglets had free access to their diet (except the fasted piglets for the first 48 h) and clean drinking water. Food intake was measured per piglet during the experiment. Piglets from the same litter were kept in adjacent pens separated by a wire mesh, so that they could have visual contact with their littermates but no interference in dietary or stress treatment. This arrangement was to prevent cross-contamination between litters but the continued contact with littermates was designed to reduce stress.

Experimental design

Newly weaned piglets were offered diets that were either rapidly digested, or diets that contained complex carbohydrates and hence encouraged fermentation. Some piglets were offered these diets on the day of weaning while others were offered the diets after a 2-day fast. Piglets were killed at 1, 4 and 10 days after weaning and samples taken from both the small and large intestine to determine the concentrations of fermentation end-products such as VFAs, lactic acid and ammonia to estimate the places in the GIT where fermentation was most active.

Dietary treatments

The control diet (CON) was semi-purified, given that the fermentable carbohydrate content had to be minimized and most normal pig diet ingredients do contain a significant fermentable fraction. The test diet with added fermentable carbohydrates (CHO) was based on this same diet but had added carbohydrates in the form of unmolassed sugar-beet pulp (SBP), wheat starch (WST), lactulose and inulin. These ingredients had been chosen following testing for their fermentability using an *in vitro* technique which measures cumulative gas production as an indicator of rate of fermentation, and SCFA and ammonia as end-products (Williams *et al.*, 2005). Both diets contained neither antibiotics nor added copper or zinc. The ingredients were chosen according to both rate and end-products of fermentation, assuming that the more rapidly fermented ingredients would be fermented primarily in the small intestine and the more slowly fermented ingredients in the large intestine. Lactulose and inulin were more rapidly fermentable compared with wheat starch and SBP. For both diets, the main source of starch used was native corn starch, with an ileal digestibility of $\sim$97% (Martinez-Puig *et al.*, 2003), to have a better contrast in amounts of fermentable substrates reaching large intestine in two diet groups. The diets were composed in such a way that total energy and protein contents were comparable. The composition of the diets is shown in Table 1.

Fasting treatment
The animals with enforced fasting (fasting) were not offered any food for 48 h from the moment of arrival at the experimental facility. The non-fasted animals (non-fasting) on the other hand, had free access to their diet from the moment of arrival at the facility. All piglets had free access to water at all times.

Slaughter of piglets and sampling
Piglets were slaughtered on day 1, day 4, and day 10 post weaning. The piglets were killed as follows: Injectable Ketamin was used as a pre-anaesthetic, and after 30 min, the piglet was euthanased by intra-cardiac injection of T61 (Hoechst Roussel Vet). Following an incision into the abdomen, the GIT was tied off at regular intervals using plastic strips to minimize mixing of digesta. The entire tract (from pyloric sphincter of stomach to anus) was then lifted from the abdominal cavity and taken to the laboratory. At the laboratory, the GIT was divided into four parts: first half of small intestine (SI1), second half of small intestine (SI2), caecum (CE) and colon (CO) and the digesta emptied by gentle expression into clean glass beakers. Necessary care was taken to avoid intestinal mucus being admixed with digesta. After proper mixing of digesta, the pH (pH meter – Hanna Instruments) was recorded. Samples were collected for dry matter (DM), VFA and ammonia analysis. Additional samples were collected from SI1 and SI2, for lactic acid analysis. All the samples were frozen pending analysis, except for DM/ash which was performed immediately. At the time of analyses, samples were defrosted and centrifuged. The resultant supernatant was then used for the analyses.

Table 1 *Composition of the diets (g/kg)*

	Diets	
	CON	CHO
Ingredients		
Maize starch	504·8	368·1
Sugar-beet pulp	–	50·0
Inulin	–	7·5
Lactulose (~50% DM)	–	20·0
Wheat starch	–	50·0
Fish meal	200·0	200·0
Soya isolate	50·0	45·0
Dextrose	150·0	150·0
Soya oil	15·0	30·0
Cellulose (Arbocel)	50·0	50·0
Pre-mix	10·0	10·0
Calcium carbonate	2·5	1·5
Monocalcium phosphate	1·5	1·5
$KHCO_3$	12·0	12·0
L-lysine HCl	0·6	0·7
DL-methionine	2·0	2·0
L-threonine	1·0	1·1
L-tryptophan	0·6	0·6
Calculated analysis		
Dry matter	916	911
Ash	43	46
Crude protein	179	180
Net energy contents (MJ/kg)	11	11

Analyses
DM samples were collected into pre-weighed and labelled vials, and the samples weighed at the moment of collection. DM was then determined by drying to a constant weight at 103°C (International Standards Organization (ISO), 1999) and ash by combustion at 550°C (ISO, 1978).

VFA samples were collected into vials containing phosphoric acid. VFA concentrations in the fermentation liquids were analysed by gas chromatography (Fisons HRGC Mega 2, CE Instruments, Milan, Italy), using a glass column fitted with Chromosorb 101, N_2 saturated with methanoic acid was used as carrier gas, at 190°C and using *iso*-caproic acid as an internal standard.

Ammonia was determined according to the method described by Searle (1984). In short, the supernatant was deproteinized using 10% trichloro-acetic acid. Ammonia and phenol were oxidized by sodium hypochlorite in the presence of sodium nitroprusside to form a blue complex. The intensity was measured colorimetrically at a wavelength of 623 nm. Intensity of the blue colour is proportional to the concentration of ammonia present in the sample.

The lactic acid concentration of the digesta was analysed according to the method described by Voragen *et al.* (1986) using a Jasco HPLC unit fitted with a Supelcogel high-performance liquid chromatography column (C-610 H, 30 cm × 7·8 mm ID).

Statistics
Differences between the dietary and fasting treatments and the interactions between them were tested for significance by ANOVA using the repeated measurement procedure with the following model;

$$Y_{ijklm} = \mu + D_i + F_j + S_k + (D \times F)_{ij} + (D \times S)_{ik} + \varepsilon 1_{ijkl} + G_m + (D \times G)_{im} + (S \times G)_{km} + \varepsilon 2_{ijklm}$$

Where Y is the parameter to be tested, μ is the overall mean, D_i effect of the diet i; F_j effect of the fasting stress j; S_k effect of slaughter day k; $(D \times F)_{ij}$, $(D \times S)_{ik}$, $\varepsilon 1_{ijkl}$ is the error term 1, which represents the random effect of animal within diet i, fasting stress j, slaughter day k level; G_m effect of site of GIT m; $(D \times G)_{im}$, $(S \times G)_{km}$ denotes the respective interactions and $\varepsilon 2_{ijklm}$ is the error term 2, which represents the overall error including the GIT sites within the animal. The non-significant interactions were removed from the model. The effect of replicate and litter was tested separately, and was not significant for any of the parameters. It was therefore removed from the statistical model. Differences between treatment least square means were evaluated using Tukey test of multiple comparisons. Differences were considered significant, when $P < 0{\cdot}05$.

The observations for day 1 could not be included in the statistical analysis as those piglets had not received any of the experimental treatments as such. However, the means of the observations on day 1 are presented separately in Table 3.

All statistical analyses were performed using the PROC GLM procedure of the statistical program Statistical Analysis Systems Institute (1990). The data was subjected to the test of normality before parametric analysis.

Results

During the experimental periods, none of the piglets showed any signs of diarrhoea or any other illness.

Animal performance

The mean values for total intake, growth and FCR by day 10 are shown in Table 2. There was no effect of diet, or fasting, or any interaction between diet and stress for any of the parameters (Table 2).

Fermentation end-products

The ls means calculated for the main factors and the probabilities of significance, for the different variables and their interactions, are shown in Tables 3 and 4.

Slaughter day had a significant effect for all the parameters (except pH) shown in Table 4. Total VFA, acetic, propionic, butyric and ammonia concentrations all increased with time, while DM, and lactic acid concentration decreased.

Differences between the GIT sites were highly significant for the parameters shown in Table 4. VFA and ammonia concentrations were higher in the large intestine, especially the caecum. Lactic acid was significantly higher for SI2 compared with SI1. The DM was much higher for the colon contents compared with other GIT sites. SI1 had a significantly lower pH, compared with the other sites. However, after SI1 the pH was lowest in the caecum.

Diet had a significant effect on most of the parameters shown in Table 4, except for butyric acid. It was observed that for the CHO diet, VFA and lactic acid concentrations were higher, while DM, pH, and ammonia concentration were lower, compared with CON.

Fasting seems to have had no effect on the pH, DM, VFA or lactic acid concentrations. However, there was a lower ammonia concentration for the non- fasting compared with fasting animals.

Table 2 *Total food intake, growth and food conversion ratio of the piglets slaughtered on day 10*

Diet[†]	Stress	Total intake (g)	Growth (g)	FCR
CON	Fasting	2099	1348	1·663
	Non-fasting	2177	1301	1·870
CHO	Fasting	2091	1446	1·494
	Non-fasting	2251	1520	1·606
Main effects				
Diet		0·7851	0·2286	0·1729
Stress		0·3306	0·9147	0·3096
Diet × stress		0·7360	0·6422	0·7603
Mean s.e.		120·7	129·6	0·15

[†] CON = control diet and CHO = fermentable carbohydrate enriched diet.

Table 3 *Mean concentrations of fermentation end-products in the gastro-intestinal tract (GIT) of the piglets slaughtered on day 1*

	Fermentation end-products[†]					
	TotVFA	Acet	Prop	But	Lact	Ammo
GIT[‡]						
SI1	4·04	3·75	0·17	0·11	42·21	5·96
SI2	16·80	15·94	0·27	0·21	36·48	8·82
CE	98·70	63·20	21·19	7·38	–	63·58
CO	54·75	33·83	12·92	3·91	–	51·51

[†] TotVFA = total volatile fatty acid concentration (mmol/l digesta water); Acet = acetic acid; Prop = propionic acid; But = butyric acid; Lact = lactic acid (mmol/l digesta water); Ammo = ammonia (mmol/l digesta water).
[‡] SI1 = first half of small intestine; SI2 = second half of small intestine; CE = caecum and CO = colon.

Interaction between GIT site and slaughter day had a significant effect on concentrations of most of the fermentation end-products (see Table 4). There was a significant interaction between diet and GIT site, for propionic acid, DM, pH and ammonia concentrations.

Table 5 shows that GIT site and diet had a significant influence on the proportional production of VFA. For the small intestinal digesta (SI1 and SI2), the acetic acid proportion was significantly higher compared with that of the large intestine, while the propionic and butyric acid proportions were greater for the large intestine compared with the small intestinal digesta. Diet had a significant effect on the acetic acid proportion, while the diet and GIT site interaction had an effect on the acetic and propionic acid proportions.

Discussion

The results of the current study are in agreement with those from previous work (Jensen and Jorgensen, 1994) conducted on older animals in showing a trend for lower overall DM content in digesta from animals fed higher fibre diet with DM content increasing from the beginning of the small intestine to the distal part of the large intestine (the latter presumably resulting from water resorption). Our results also demonstrate significant variation in the DM content of digesta from different segments of the gut according to dietary type. Thus for example the colonic digesta of piglets maintained on the CHO diet had lower DM content than that of piglets on the CON diet, a finding similar to that reported by other workers (Low *et al*., 1978; Knudsen and Hansen, 1991).

The trend of lower pH at the beginning of small intestine with an increase towards the end of the small intestine, was most likely related to the acidity arriving from the stomach, which gradually dissipated as digesta moved along the tract. The slight drop in the caecum, and then rise in the colon, which is in agreement with the study already mentioned (Jensen and Jorgensen, 1994), was most likely related, amongst other factors, to the VFA concentrations. In the present study, the significant effect of interaction between diet and GIT site, revealed a lower pH for the CHO compared to the CON diet for all GIT sites. The study of

Table 4 *Dry matter (DM), pH and end-product profile of digesta of the weaning piglets according to the main effects of diet, stress, slaughter day and gastro-intestinal tract (GIT) area*[†]

	Levels							
	TotVFA	Acet	Prop	But	Lact	Ammo	DM	pH
Diet								
CON	56·79	37·97	10·66	4·94	36·87	24·46	152·35	6·22
CHO	63·43	43·31	12·53	5·28	47·37	16·44	140·66	6·13
Mean s.e.	1·23	0·86	0·25	0·19	1·30	0·549	2·415	0·021
Significance	*	*	***		***	***	‡	‡
Stress								
Fasting	61·99	41·87	11·95	5·15	44·27	21·53	151·97	6·16
Non-fasting	58·23	39·87	11·25	5·07	39·98	19·38	141·05	6·18
Mean s.e.	1·24	0·86	0·25	0·19	1·30	0·549	2·415	0·021
Significance						*	‡	
Slday								
4	55·09	38·27	10·06	4·66	46·63	18·39	163·82	6·22
10	65·13	43·01	13·13	5·56	37·61	22·54	129·19	6·14
Mean s.e.	1·24	0·86	0·25	0·19	1·30	0·549	2·415	0·021
Significance	***	*	***	*	**	***	***	‡
GIT								
SI1	5·02	3·66	0·20	0·41	32·26	4·92	113·87	5·57
SI2	9·79	8·73	0·19	0·46	51·99	4·91	112·02	6·55
CE	118·33	70·47	24·42	9·98	–	35·59	137·23	6·18
CO	107·28	70·46	21·59	9·58	–	36·37	222·92	6·41
Mean s.e.	1·75	1·22	0·36	0·27	1·30	0·77	3·41	0·030
Significance	***	***	***	***	***	***	***	***
Significance of interaction								
Diet × stress								
Diet × Sday								
Diet × GIT			***			***	***	***
GIT × Sday	***	***	***	***	***	***	‡	

[†] Abbreviations are: TotVFA = total volatile fatty acid concentration (mmol/l digesta water); Acet = acetic acid; Prop = propionic acid; But = butyric acid; Lact = lactic acid (mmol/l digesta water); Ammo = ammonia (mmol/l digesta water); DM = dry matter (g/kg); Slday = slaughtering day; CON = control diet and CHO = fermentable carbohydrate enriched diet; SI1 = first half of small intestine; SI2 = second half of small intestine; CE = caecum; CO = colon.
[‡] Approaching significance ($P < 0·1$).

Jensen and Jorgensen (1994), in contrast, showed a lower pH for the high fibre diet in the large intestine only. In the Danish study (Jensen and Jorgensen, 1994), the high fibre diet contained supplemented pea fibre and pectin along with barley. As these would both be slow fermenting carbohydrate sources (Williams *et al.*, 2005), the effect on pH resulting from the higher VFA concentrations, would only become apparent in the large intestine where the fermentation would take place. In the present study, the CHO diet specifically contained a range of fermentable carbohydrates with variable rates of fermentation, (Williams *et al.*, 2005) which was supposed to stimulate the carbohydrate fermentation along the whole GIT. A decrease in pH in the GIT is suggested to help inhibit the growth of some potential pathogens (Ewing and Cole, 1994).

Both diet and GIT site had a significant effect on fermentation end-products such as total VFA, acetic, propionic, and lactic acids, and ammonia, with a significant interaction for ammonia and propionic acid concentrations. It would seem that the higher contents of fermentable carbohydrates indeed resulted in higher VFA concentrations in the piglets fed the CHO diet compared with the CON diet. The large intestine, probably due to its larger and more diverse microbial population, and the longer transit time of its digesta (Gaskins, 2001), had higher VFA concentrations compared with the small intestine, though the latter also had high concentrations of lactic acid. However, as reviewed by Williams *et al.* (2001), it is also important to note that there is a higher rate of VFA absorption in the colon, associated with water absorption.

In this study, piglets on the CHO diet had higher lactic acid concentrations both in SI1 and SI2 compared with CON piglets, which confirms the more active fermentation occurring with the CHO diet. In this experiment, lactic acid was only measured in the small intestine (SI1 and SI2). SI2 had a significantly higher lactic acid concentration compared to SI1. Apart from the possibility of increased fermentation as such, this GIT effect might also be due to the more rapid flow of digesta in the beginning of the small intestine compared with the end of small intestine (Gaskins, 2001). Such a flow could also lead to the flow of lactic acid from SI1 to SI2, as well as increased production of lactic acid. No information is available quantifying the flow nor absorption of lactic acid in different areas of the small intestine. However, as part of this study using the same pigs, Konstantinov *et al.* (2004) using molecular techniques, showed that the carbohydrate diet, supported the growth of Lactobacillus, a genus known for its production of lactic acid, and considered to be beneficial for GIT health.

For the present study during which the piglets had no creep food but only sow's milk during the suckling period, it is important to note the decrease in lactic acid concentration by day 10. Whilst the piglet is being suckled, the small intestinal microbiota is dominated by lactobacilli and streptococci (Hopwood and Hampson, 2003) and the major fermentation

Table 5 *Proportional VFA production in weaning piglets, as expressed by % of the total VFA, according to the main effects of diet, stress, slaughter day and GIT area*

Levels	AP†	PP†	BP†	BCP†
Diet				
CON	73·72	12·37	7·99	2·72
CHO	77·03	11·83	7·28	2·10
MSE§	0·805	0·23	0·52	0·40
Significance	*			
Stress				
Fasting	75·92	12·02	6·86	2·74
Nonfasting	74·84	12·19	8·40	2·07
MSE§	0·81	0·23	0·52	0·40
Significance			‖	
Slday‡				
4	76·48	11·70	7·00	2·70
10	74·28	12·50	8·26	2·12
MSE§	0·81	0·23	0·52	0·40
Significance	‖	*		
GIT				
SI 1	79·82	4·44	7·82	4·55
SI 2	88·70	2·89	5·68	1·20
CE	67·13	20·96	8·24	1·46
CO	65·87	20·12	8·79	2·43
MSE§	1·14	0·33	0·74	0·57
Significance	***	***	*	***
Interaction		Probability		
Diet × stress	0·9170	0·9512	0·6738	0·5055
Diet × Slday	0·8016	0·8327	0·2045	0·3783
Diet × GIT	0·0268	0·0002	0·2624	0·9967
GIT × Slday	0·9068	0·2753	0·2177	0·6985

† AP = acetic acid proportion (%); PP = propionic acid proportion (%); BP = butyric acid proportion (%); BCP = branched chain fatty acid proportion (%).
‡ Slday = slaughtering day.
§ MSE = mean standard error.
‖ Approaching significance ($P < 0·1$)

product is lactic acid. With time, there is development of the small intestinal microbiota both in number and diversity. This might change the end-product profile from lactic acid as the predominant product, to a more diverse VFA profile over time as can be seen in Table 4.

Interestingly, the proportions of VFA also differed significantly according to GIT site (Table 5). The production of different VFA is very well regulated by the availability of substrate to the bacteria, and also to cross-feeding between species. An example of this would be the metabolism of intermediate products such as lactic acid into VFA such as propionic acid (Bernalier *et al.*, 1999; Macfarlane and Macfarlane, 2003). The substrate energy availability plays a major part in determining the proportions of end-products of fermentation (Macfarlane and Macfarlane, 2003). The proportionally higher acetic acid production by the small intestinal microbiota, may be an effect of faster transit of the substrate, as rapid transit may favour bacterial species which are able to ferment rapidly fermentable ingredients most readily. The reverse would then be true for the large intestine, where propionic and butyric acid proportions are higher, resulting from fermentation by bacteria which take longer to ferment more complex substrates.

Conclusion

As an incidental finding, this study found that the forced fasting at the beginning of the weaning period had no effect on end-product profiles post-weaning. However, it could be speculated that post-weaning fasting may be a characteristic of the host, and it would be interesting in a future study to divide the piglets into two groups, based on their own voluntary food intake at the beginning of the weaning period.

In vitro work reported by Bauer (2002), and Williams *et al.* (2005), showed that ingredients commonly used in normal pig diets can vary quite considerably in their fermentability, and that both the rate and end-products of fermentation bore no relation to solubility. 'Fibre' was too vague a concept to determine whether or not an ingredient would be well fermented. Results from the latter paper, were used to design two diets, one of which was highly digestible (and therefore with few fermentable carbohydrates). The other was designed to contain four ingredients with quite variable rates of fermentation, and good production of VFA, to stimulate carbohydrate fermentation along the entire GIT.

The results showed that the addition of carefully selected fermentable carbohydrates, which had been shown to vary in their rates and end-products of fermentation did indeed lead to improved fermentation along the GIT. This was evidenced by the decreased pH, decreased DM in the large intestine, and the generally higher VFA found throughout the tract. All of these factors are considered to be beneficial in terms of maintaining gut health. This will be of particular interest when anti-microbial growth promoters are finally banned in EU from inclusion in animal foods, as an alternative to ease the weaning transition in microbial community, which predisposes piglets to post-weaning diarrhoea.

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