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Effects of a dietary organic acid mixture and of dietary fibre levels on ileal and faecal nutrient apparent digestibility, bacterial nitrogen flow, microbial metabolite concentrations and rate of passage in the digestive tract of pigs

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Six 34-kg barrows were fitted with a post-valve T-caecum cannula and assigned to six dietary treatments according to a 6 × 5 change-over design to study how a mixture of formic acid, sorbate, and benzoate (0 or 8.4 g/kg feed) influences apparent ileal and faecal digestibility coefficients, bacterial nitrogen (N) flow, microbial metabolite concentrations, and passage rate in pigs fed isoenergetic diets with medium, high, or very high fibre content (neutral-detergent fibre (NDF): 199, 224, and 248 g/kg dry matter, respectively). These barley and soya-bean meal based diets contained 0, 75, and 150 g/kg barley fibre (NDF: 577 g/kg) and 0, 8, and 16 g/kg rapeseed oil, respectively. The dietary organic acid mixture improved the apparent ileal digestibility of 14 of the 17 amino acids analysed ($P < 0.05$). Increasing levels of dietary fibre linearly decreased the apparent ileal digestibility of six of the 17 amino acids analysed ($P < 0.05$). Ileal flows of bacterial N and amino acids as assessed on the basis of purine flow were decreased by the dietary organic acid mixture ($P < 0.05$) but were not affected by dietary fibre level ($P > 0.05$). As assessed on the basis of diaminopimelic acid flow, bacterial N flow was increased by both the dietary organic acid mixture and increased dietary fibre levels ($P < 0.05$). The dietary organic acid mixture reduced the concentration of lactic acid and increased that of acetic acid in ileal digesta ($P < 0.05$), while dietary fibre levels had a quadratic effect on concentrations of acetic, propionic, and butyric acid ($P < 0.05$). The mean retention time of Co (solute marker) and Yb (particle marker) in the large intestine decreased in a linear manner by increasing dietary fibre levels ($P < 0.05$) but was not affected by the dietary organic acid mixture ($P > 0.05$). The results show that a dietary organic acid mixture has a positive effect on the apparent ileal digestibility of most amino acids irrespective of dietary fibre levels. This could be at least partly related to changes in bacterial N flow in the ileum. However, different bacterial markers showed opposite effects on bacterial N flow, which makes it questionable to use a constant bacterial marker / bacterial N ratio to estimate bacterial N flow. Increasing levels of dietary fibre had negative effects on the apparent ileal amino acid digestibilities and shortened the mean retention time of digesta in the large intestine.

Keywords: bacterial protein, digestibility, organic acids, pigs, retention time

Introduction

Dietary acidification with organic acids has been shown to improve the growth and feed-to-gain ratio of weaned piglets and growing-finishing pigs, but the mechanisms whereby dietary organic acids enhance pig performance are still poorly understood (Ravindran and Kornegay, 1993; Partanen and Mroz, 1999). Several studies have demonstrated that dietary organic acids improve the ileal apparent digestibility of protein and amino acids in growing-finishing pigs (Mosenthin *et al*., 1992; Kemme *et al*., 1999; Mroz *et al*., 2000; Partanen *et al*., 2001). A common hypothesis has been that dietary acidification improves protein digestibility by lowering gastric pH, which consequently increases pepsin activity. This should, in theory, improve both the ileal apparent and true digestibility of amino acids. However, dietary formic acid did not affect the true ileal digestibility of lysine in soya-bean meal as determined with the homoarginine technique, although the ileal apparent digestibility of dietary lysine was improved (Partanen *et al*., 2001).

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Ileal digesta contain considerable amounts of protein and amino acids from various endogenous secretions and protein synthesised by microbes of the digestive tract. The estimated amounts of bacterial nitrogen (N) in ileal digesta have ranged greatly from 0.14 up to 0.74 of total ileal N (Wünsche *et al.*, 1991; Schulze *et al.*, 1994; Bartelt *et al.*, 1999; Huang *et al.*, 2001). Because organic acids are antimicrobial agents, their effects on ileal apparent amino acid digestibilities can be mediated via microbes of the digestive tract. Several authors have reported changes in bacterial counts and microbial metabolite concentrations in gastric and intestinal digesta of pigs fed diets supplemented with formic acid (Roth *et al.*, 1992; Canibe *et al.*, 2005). Reduced bacterial protein synthesis may contribute to the improved ileal apparent protein and amino acid digestibilities seen with the diets supplemented with organic acids (Partanen *et al.*, 2001).

The length of time that digesta remain in the digestive tract of the pig and are exposed to digestive enzymes and microbial degradation largely influences the extent to which the feeds are digested. In general, highly soluble dietary fibres such as pectins reduce the rate of passage in the stomach and small intestine of pigs, while cellulose and insoluble lignified dietary fibre reduces transit time in the lower part of the digestive tract (Wenk, 2001). Ravindran and Kornegay (1993) suggested that dietary acidification may reduce the gastric emptying rate, but the effect of dietary organic acid supplementation on the passage rate of digesta through the digestive tract of pigs has not been investigated so far.

The objective of this study was to determine how an organic acid mixture based on formic acid used in diets with increasing fibre content levels influences the ileal and faecal apparent digestibility of nutrients, particularly amino acids, and bacterial N flow, microbial metabolite concentrations in the ileum, and the rate of passage in the upper and lower part of the digestive tract of pigs.

Material and methods

The experimental protocol was reviewed and approved by the Animal Care Committee of MTT Agrifood Research Finland.

Animals and surgery

Seven 34-kg barrows (Yorkshire × Finnish Landrace) were surgically fitted with a T-shaped silicone cannula at the caecum according to the post-valve T-caecum (PVTC) cannulation method (Van Leeuwen *et al.*, 1991). Before surgery, the pigs were fasted for 36 h and given no access to water for 12 h. After pre-medication with Stresnil (8 mg/kg i.m., Orion, Finland) and atropine (0.05 mg/kg i.m., Leiras, Finland), anaesthesia was induced by i.v. injection of pentothal (Natrium Abbott) and maintained by inhalation of halothane (1 to 4% as necessary). To minimise pain, the pigs were injected with Finadyne (2.2 mg/kg i.m., Orion, Finland) immediately after surgery, after which they were given Finadyne (250 mg/day, Orion, Finland) with feed for 3 days. To prevent infections, the pigs were injected with Tribrissen (20 mg/kg i.m., Pitman-Moore GmbH, Germany) immediately after surgery, after which they were given Oriprim (10 g/day, Orion, Finland) with feed for 7 days. The pigs were allowed a 16-day recovery period, during which their daily allowance was gradually increased until the pre-surgical level of feed intake was achieved. Access to water was *ad libitum*. The pigs were housed in metabolic pens measuring 1.43 m × 1.23 m with a slatted plastic floor and transparent plastic sides at an ambient temperature of 20 to 23 °C.

Experimental diets

The medium-fibre diet was based on barley and soya-bean meal. High-fibre and very-high-fibre diets were obtained by including 75 and 150 g/kg respectively of barley fibre (Tähkä-Ohrarehu 12, Altia Ltd, Koskenkorva, Finland) from integrated starch-ethanol production in the diet based on barley and soya-bean meal. Per kg, the barley fibre used contained 39 g ash, 121 g crude protein, 68 g crude fat, 183 g crude fibre, 577 g neutral-detergent fibre (NDF), 209 g acid-detergent fibre (ADF), and 31 g lignin. The diets were formulated to be isoenergetic by adding rapeseed oil in the high-fibre and very-high-fibre diets. The diets supplied 9.2 MJ of net energy and 6.9 g of ileal apparent digestible lysine per kg. The nutrient contents of the experimental diets were calculated by using tabulated values for different feed ingredients (Tuori *et al.*, 1996). Vitamins and minerals were supplemented to meet or exceed Finnish feeding recommendations according to Tuori *et al.* (1996). For the determination of digestibility coefficients, chromium-mordanted straw prepared according to Udén *et al.* (1980) was added to feeds (1.6 g/kg) as an indigestible marker. The formulations of medium-fibre, high-fibre, and very-high-fibre diets are given in Table 1. These diets were prepared either with or without an organic acid mixture that contained 94% formic acid (BOLIFOR FA 1100L, 76% formic acid, 5.5% ammonium formate, and 18.5% water (v/w); Kemira Chemicals Ltd, Finland), 3% potassium sorbate, and 3% sodium benzoate. This mixture was chosen based on preliminary information from an *in vitro* study which indicated that small amounts of potassium sorbate or sodium benzoate in formic acid could result in greater changes in fermentation patterns in the small intestine than plain formic acid (Partanen and Jalava, 2005). The acid mixture provided 6.1 g formic acid, 0.4 g ammonium formate, 0.2 g potassium sorbate, and 0.2 g sodium benzoate per kg of feed. The diets were pelleted through 4-mm die (Amandus Kahl Nache, Germany) without using steam.

Experimental procedures

The experiment was carried out with six pigs according to a 6 × 5 cyclic change-over design (Davis and Hall, 1969). There were five 14-day experimental periods, and each pig received a different diet in each period. The pigs were

Table 1 *Ingredients and calculated composition of experimental diets with different amounts of fibre*[†]

	Fibre content		
	Medium	High	Very high
Ingredients (g/kg)			
Barley	830.3	743.2	653.9
Barley fibre	–	74.9	149.8
Soya-bean meal	142.2	146.8	152.7
Rapeseed oil	–	7.7	16.4
Mineral and vitamin pre-mix[‡]	13.0	13.0	13.0
Limestone	7.7	7.7	7.7
Monocalcium phosphate	3.9	3.8	3.7
L-lysine HCl	1.3	1.3	1.2
Chromium mordanted straw	1.6	1.6	1.6
Calculated composition			
Net energy (MJ/kg)	9.2	9.2	9.2
Crude protein (g/kg)	158	158	159
Ileal apparent digestible amino acids (g/kg)			
Lysine	6.9	6.9	6.9
Methionine and cystine	4.3	4.3	4.3
Threonine	4.2	4.2	4.2
Calcium (g/kg)	6.9	6.9	6.9
Phosphorus (g/kg)	5.2	5.2	5.2

[†] Diets were prepared either with or without 8.4 g/kg of an organic acid mixture that provided 6.4 g formic acid, 0.4 g ammonium formate, 0.2 g potassium sorbate, and 0.2 g sodium benzoate per kg of feed.

[‡] The pre-mix supplied (g/kg): calcium, 2.3; phosphorus, 0.8; magnesium, 0.5; sodium chloride, 3.3; (mg/kg) iron, 103; copper, 22; zinc, 91; manganese, 23; selenium, 0.28; iodine, 0.28; retinol, 1.6; α-tocopherol, 50 mg; thiamine, 2; riboflavin, 5; pyridoxine, 3; biotin, 0.2; pantothenic acid, 14; nicotinic acid, 20; folic acid, 2; menadione, 2; (μg/kg) cholecalciferol, 13; and cyanocobalmin, 20.

weighed at the beginning of each period. All pigs were given the same amount of feed, i.e. 100 g per kg of mean metabolic body weight ($M^{0.75}$) of the pigs. The feed allowance was kept constant for the whole 14-day experimental period. The pigs were fed twice daily, at 0600 and 1800 h. Feed was mixed with water (2 l/kg feed) before feeding. The pigs consumed their meals rapidly, generally in less than 30 min.

Each experimental period was started with six days of adaptation. At 1200 h on day 7, *ca* 100-ml spot samples were taken from ileal digesta for the determination of pH, microbial metabolites, and microbial activity (ATP concentration). The chosen sampling time was based on the results of our previous study (Partanen *et al.*, 2001). Samples for the determination of microbial metabolite concentrations were prepared as described by Partanen *et al.* (2001) and stored at −18 °C until analysed. For the determination of ATP concentration, 5 g of freshly collected digesta were mixed with 10 ml of cold PCA-EDTA solution (2 mol/l perchloric acid with 10 mmol/l EDTA) and frozen immediately at −80°C until analysed.

The rate of passage was determined by means of a single pulse dose using cobalt (Co) and ytterbium (Yb) as solute and particle markers, respectively. During the feeding at 0600 h on day 8, the pigs received 27 g of Yb-labelled barley fibre (7.5 mg of Yb per g) prepared according to Beauchemin and Buchanan-Smith (1989) and 20 ml of lithium-Co-EDTA solution (3.4 mg of Co per ml) prepared according to Udén *et al.* (1980) per kg of feed. Spot samples of ileal digesta consisting of about 50 ml were collected 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, 22, 24, and 30 h after dosing the markers. Faeces were collected quantitatively from 0600 h on day 8 to 0600 h on day 12 for the total of 96 h according to Van Kleef *et al.* (1994). The faecal bags were emptied 6, 12, 18, 24, 28, 32, 36, 40, 48, 54, 60, 72, 84, and 96 h after dosing the markers. All faeces passed between emptying times were pooled, mixed, and subsampled for the analysis of Co and Yb, taking the midpoint of the collection period as the time of defaecation. The rest of the faeces were pooled for the analyses of Cr and nutrients.

To determine the ileal apparent digestibility coefficients, ileal digesta were collected for a total of 12 h on days 12 and 14 as follows: 0600 to 0800 h, 1000 to 1200 h, and 1400 to 1600 h (on day 12) and 0800 to 1000, 1200 to 1400, and 1600 to 1800 h (on day 14). Digesta samples were collected directly into a plastic bag attached to the cannula. The plastic bags were removed every 15 min, weighed, and frozen instantly at -20 °C. The collected digesta were pooled for analyses of Cr and nutrients.

Separation of bacterial fraction

The bacterial fraction of the ileal digesta was isolated from digesta collected after the fifth experimental period. The pigs received the same experimental diets as during the fifth period for an extra 4 days before digesta were collected. To obtain a sufficient amount of bacteria for the analyses, the collected digesta were pooled across fibre levels to two 1000-ml samples, one for diets without and one for diets with the organic acid mixture. Bacteria were isolated from the digesta by a differential centrifugation procedure adapted from Ahvenjärvi *et al.* (2000). The collected digesta, which were stored at 39 °C during the collection, were first divided into liquid and solid phases by straining through two layers of cheesecloth. The solid phase remaining on the cheesecloth was washed twice with 250 ml of 0.9% NaCl solution (39 °C) and strained through two layers of cheesecloth. The liquid phases were pooled and centrifuged first at 1000 **g** for 10 min to remove feed particles. The collected supernatant was then centrifuged at 10 000 **g** for 30 min to sediment bacteria. To remove bacteria from the feed particles, the solid phase that had remained on the cheesecloth was mixed with 800 ml of a solution containing 0.9% of NaCl and 0.1% of carboxymethyl cellulose. The mixture was first kept for 15 min at 39 °C and then overnight at 4 °C. The following day, it was strained through two layers of cheesecloth, and the bacteria were isolated from the liquid phase by differential centrifugation as described above. The bacterial pellets were pooled to obtain one sample for diets without and one for diets with the organic acid mixture. They were diluted with distilled water, freeze-dried and analysed for dry matter (DM), N, amino acids, total purines, and diaminopimelic acid (DAPA).

Chemical analyses

The experimental feeds were sampled in each period, analysed for DM and pH, and the samples were pooled across periods for further analyses. The spot samples of ileal digesta and faeces collected for the determination of passage rate were dried at 60 °C for 24 h, whereas the ileal digesta and faeces collected for the determination of digestibility coefficients were freeze-dried. The samples were ground to pass through a 1-mm sieve before the analyses.

DM content was determined by drying at 103 °C for 16 h. The ash, ether extract (after acid hydrolysis), and crude fibre contents were determined using standard methods (Association of Official Analytical Chemists (1990), methods 942.05, 962.09, and 920.39). NDF and ADF were determined according to Van Soest *et al.* (1991) and Robertson and Van Soest (1981), respectively. N content was determined by the Dumas method with a Leco FP 428 N analyser (Leco Corp., St Joseph, USA). The dietary pH of the pelleted feed was measured after this was mixed with water (1:5) and stirred with a magnetic stirrer for 2 min. The contents of DAPA and other amino acids were analysed according to the official method of the European Commission (1998) using a Biochrom 20 amino acid analyser (Biochrom Ltd, Cambridge, UK). The concentrations of purines were determined according to Zinn and Owens (1986), and those of Cr, Co, and Yb by atomic absorption spectrophotometry according to Williams *et al.* (1962). Formic acid was analysed with the UV method using a commercial kit (cat. no. 0 979 732, Boehringer Mannheim, R-Biopharm GmbH, Darmstadt, Germany) and the selective clinical chemistry analyser Pro 981489 (KONE Instruments, Espoo, Finland). To obtain supernatant for the formic acid analysis, 10 g of pelleted feed were mixed with 290 g of H_2O, stirred twice for 5 min with 30 min of cooling (at −18 °C) in between, and then centrifuged at 2000 **g** for 10 min. Lactic acid was analysed according to Haacker *et al.* (1983), short chain fatty acids according to Huhtanen *et al.* (1998), ammonia according to McCullough (1967), and ATP according to Jensen and Jørgensen (1994).

Calculations and statistical methods

The apparent digestibility coefficients were calculated by using Cr as an indigestible marker as follows:

$$\text{ileal or faecal apparent digestibility coefficient} = 1 - (\mathrm{Cr_F}/\mathrm{Cr_D}) \times (\mathrm{N_D}/\mathrm{N_F}),$$

where $\mathrm{Cr_F}$ = the content of Cr in feed, mg/kg DM, $\mathrm{Cr_D}$ = the content of Cr in digesta or faeces, mg/kg DM, $\mathrm{N_D}$ = the content of nutrient in digesta or faeces, g/kg DM, and $\mathrm{N_F}$ = the content of nutrient in feed, g/kg DM.

The flow of bacterial markers (BM), i.e. purines and DAPA, in the ileum was calculated as follows:

$$\text{BM flow (g/kg DM intake)} = (\mathrm{Cr_F}/\mathrm{Cr_D}) \times \mathrm{BM_D},$$

where $\mathrm{Cr_F}$ and $\mathrm{Cr_D}$ are as mentioned above and $\mathrm{BM_D}$ is the content of purines or DAPA in digesta, g/kg DM. Bacterial N flow was calculated as follows:

$$\text{bacterial N flow (g/kg DM)} = \text{BM flow}/(\mathrm{BM/N})_\mathrm{B},$$

where $(\mathrm{BM/N})_\mathrm{B}$ = the ratio of purines of DAPA to N in bacteria isolated from the ileal digesta. Bacterial amino acid flows and contributions were calculated by using the determined amino acids profile of isolated bacteria.

The mean retention time (MRT) of digesta in the terminal ileum and in the whole digestive tract was calculated from the concentration of solute (Co) and particle markers (Yb) in the digesta and faeces according to Faichney (1993) as follows:

$$t = \Sigma C'_i t'_i \Delta t_i / \Sigma C'_i \Delta t_i,$$

where C_i is the marker concentration at time t_i after dosing so that $C'_i = (C_i + C_{i-1})/2$, $t'_i = (t_i + t_{i-1})/2$, and $\Delta t_i = t_i - t_{i-1}$. The MRT of digesta in the large intestine was calculated as the difference between the MRT in the whole digestive tract and in the terminal ileum.

The statistical analysis of data was carried out using the GLM procedure of Statistical Analysis Systems Institute (1999) and the following model (Davis and Hall, 1969):

$$Y_{ijk} = \mu + A_i + P_j + O_k + F_l + (O \times F)_{kl} + e_{ijkl},$$

where A_i is the effect of animal i ($i = 1-6$), P_j is the effect of experimental period j ($j = 1-5$), O_k is the effect of dietary organic acid mixture k ($k = 1-2$), F_l is the effect of dietary fibre content l ($l = 1-3$), and $(O \times F)_{kl}$ is the interaction between dietary organic acid mixture and fibre content. It was assumed that no animal × period interactions existed. Residuals were checked for normality and plotted against fitted values. The effect of dietary fibre level was tested with orthogonal polynomials (linear and quadratic effect). Whenever the probability for an organic acid mixture × fibre level interaction was $P < 0.10$, the differences between the treatments were detected by using the Tukey test (accepted significance level was < 0.05).

Results

Chemical composition of diets

The dietary NDF contents averaged 199, 224, and 248 g/kg DM for medium-fibre, high-fibre, and very-high-fibre diets, respectively (Table 2). The crude protein and amino acid contents were similar in the six experimental diets. The pH of non-acidified diets decreased from 5.54 to 5.33 with increasing barley fibre inclusions. The dietary organic acid mixture lowered dietary pH on average to 4.55 irrespective of dietary fibre content, whereas the analysed formic acid contents decreased slightly with increasing fibre content.

Table 2 *Analysed composition of experimental diets with different amounts of fibre content and supplemented with 0 or 8.4 g/kg of a mixture of formic acid, sorbate and benzoate*

	Fibre content					
	Medium		High		Very high	
Organic acid mixture	−	+	−	+	−	+
Dry matter (g/kg)	885	885	890	888	890	889
Composition (g/kg dry matter)						
Ash	48	48	48	47	50	47
Crude protein	178	183	179	180	183	170
Ether extract	30	32	39	40	50	51
Crude fibre	48	54	56	58	69	67
Neutral-detergent fibre	198	200	227	220	245	250
Acid-detergent fibre	59	61	68	68	77	80
Alanine	7.2	7.3	7.2	7.3	7.6	7.2
Arginine	9.9	10.1	10.3	10.1	10.4	10.0
Aspartic acid	13.4	14.0	13.6	13.7	14.2	13.6
Cystine	3.4	3.5	3.6	3.6	3.5	3.2
Glutamic acid	31.4	31.8	31.3	31.1	33.2	31.8
Glycine	7.2	7.1	7.2	7.2	7.4	7.1
Histidine	4.1	4.2	4.2	4.3	4.4	4.2
Isoleucine	7.1	7.0	7.1	7.1	6.9	6.8
Leucine	12.2	12.5	12.2	12.1	12.5	11.9
Lysine	8.7	9.0	8.9	9.0	9.1	8.6
Methionine	2.8	2.9	2.8	2.8	2.9	2.7
Phenylalanine	8.7	8.7	8.6	8.7	8.5	8.8
Proline	14.2	13.7	13.5	12.9	13.4	12.5
Serine	8.0	8.1	7.9	8.1	8.2	8.0
Threonine	6.4	6.5	6.3	6.4	6.5	6.3
Tyrosine	6.0	5.9	6.0	5.9	5.9	6.0
Valine	11.6	11.4	11.5	11.5	10.6	10.8
Formic acid	0.1	7.3	0.0	6.8	0.0	5.9
pH	5.54	4.58	5.42	4.53	5.33	4.55
Purines (g/kg dry matter)	7.6	7.2	7.6	7.5	7.6	7.7

Health of pigs

The pigs remained healthy and consumed their feed allowances throughout the experiment. They grew at an average rate of 921 g/day. In the fourth period, the cannula of one pig came off. The pig was tranquillised, and the cannula was reintroduced into the fistula. The pig recovered well, and it was subjected to the experiment procedures as planned, except that ileal digesta samples were not taken for the determination of passage rate. Post-mortem examinations carried out at the conclusion of the experiment revealed no abnormalities or adhesions due to the cannulation, implying that the digestive tract of each pig functioned normally.

Apparent ileal digestibility coefficients

Table 3 shows that the dietary organic acid mixture improved the ileal apparent digestibility coefficients of crude protein and all amino acids ($P < 0.05$) except for methionine, proline, and valine ($P > 0.05$). The ileal apparent digestibility coefficients of crude protein, cystine, glycine, methionine, proline, threonine, and valine decreased ($P < 0.05$), whereas that of ether extract increased ($P < 0.05$) in a linear manner with increasing dietary fibre levels. The quadratic effect of fibre level was not significant. A tendency for an organic acid mixture × fibre level interaction ($P = 0.06$) was found for histidine. The dietary organic acid mixture improved the ileal apparent digestibility of histidine in the high-fibre diet ($P < 0.05$) but not in the medium-fibre or very-high-fibre diets ($P > 0.05$). There was a moderate organic acid mixture × fibre level interaction ($P = 0.07$) in the ileal digestibility of NDF too. In non-acidified diets, the fibre addition decreased the NDF digestibility in a quadratic manner ($P < 0.05$) but in acidified diets, the effect of fibre level was not significant.

Faecal apparent digestibility coefficients

A significant ($P < 0.05$) or moderate ($P < 0.10$) organic acid mixture × fibre level interaction was observed for the faecal digestibility coefficients of all the nutrients except for ADF and hemicellulose (Table 3). The dietary organic acid mixture improved the faecal digestibility of ash and ether extract in medium- and high-fibre diets ($P < 0.05$) but not in the very-high-fibre diet ($P > 0.05$). The faecal digestibility of ether extract increased linearly with increasing dietary fibre levels ($P < 0.05$) in non-acidified diets. The quadratic effect of fibre level was not significant. In diets supplemented with organic acid, the faecal digestibilities of ash, crude protein, NDF, and hemicellulose were lower in the very-high-fibre diet than in the high- and medium-fibre diets ($P < 0.05$), whereas the differences between the latter two were not significant. The faecal apparent digestibility of ether extract was lower in the medium-fibre than in the high-fibre and very-high-fibre diets ($P < 0.05$). The daily excretion of faeces and faecal water content increased linearly with increasing dietary fibre levels ($P < 0.05$) but it was not influenced by the dietary organic acid mixture (results not shown). The recovery of Cr in faeces averaged 0.99 ± 0.08 of intake.

Bacterial nitrogen flow at the ileal and faecal level

The experimental feeds contained on average 7.5 ± 0.18 g purines per kg of DM (Table 2) but no DAPA was found. The purine/bacterial N ratio was 1110 and 980 mg/g and the DAPA/bacterial N ratio was 34 and 40 mg/g in bacteria isolated from the ileal digesta of pigs fed diets with 0 or 8.4 g/kg of the organic acid mixture, respectively. The amino acid composition of these bacteria averaged as follows: alanine 8.6, arginine 4.4, aspartic acid 9.2, cystine 1.4, glutamic acid 12.4, glycine 4.8, histidine 2.1, isoleucine 4.6, leucine 7.0, lysine 4.6, methionine 1.5, phenylalanine 4.9, proline 5.0, serine 4.3, threonine 4.3, valine 5.2, and tyrosine 3.7 g per 16 g N. The differences between the two samples were small, ranging from 0 to 0.3 g per 16 g N, i.e. less than 7% of the mean.

The concentration of purines in ileal digesta (Table 4) was decreased by the dietary organic acid mixture ($P < 0.05$), whereas the DAPA concentration changed in the opposite direction ($P < 0.01$). Increasing dietary fibre

Table 3 *Apparent ileal and faecal digestibility coefficients of nutrients in experimental diets with different amounts of fibre content and supplemented with 0 or 8.4 g/kg of a mixture of formic acid, sorbate and benzoate*

	Fibre content							Significance		
	Medium		High		Very high					
Organic acid mixture	−	+	−	+	−	+	s.e.	Acid	Fibre	Acid × fibre
Ileal apparent digestibility										
Dry matter[†]	0.724	0.722	0.679	0.700	0.667	0.676	0.0074		***	
Crude protein[‡]	0.788	0.803	0.770	0.799	0.775	0.779	0.0060	**	*	
Ether extract[†]	0.680	0.686	0.707	0.736	0.760	0.765	0.0089	¶	***	
Neutral-detergent fibre[‖]	0.489^{b}	0.391^{ab}	0.344^{a}	0.367^{a}	0.355^{a}	0.355^{a}	0.0250		**	¶
Amino acids										
Alanine	0.736	0.754	0.718	0.759	0.724	0.745	0.0077	***		
Arginine	0.853	0.863	0.847	0.863	0.848	0.857	0.0044	**		
Aspartic acid	0.786	0.808	0.777	0.807	0.780	0.801	0.0055	***		
Cystine[§]	0.817	0.832	0.817	0.828	0.806	0.814	0.0062	*	¶	
Glutamic acid	0.839	0.855	0.832	0.845	0.842	0.849	0.0051	**		
Glycine[‡]	0.734	0.749	0.711	0.742	0.714	0.728	0.0067	**	*	
Histidine	0.824^{abc}	0.834^{bc}	0.807^{a}	0.839^{c}	0.817^{ab}	0.828^{abc}	0.0048	***		¶
Isoleucine	0.837	0.834	0.820	0.843	0.817	0.833	0.0058	*		
Leucine	0.826	0.836	0.815	0.833	0.815	0.829	0.0052	**		
Lysine	0.825	0.841	0.815	0.847	0.819	0.834	0.0059	***		
Methionine[§]	0.825	0.840	0.810	0.831	0.821	0.813	0.0074			
Phenylalanine	0.809	0.811	0.791	0.813	0.787	0.811	0.0074	*		
Proline[†]	0.845	0.837	0.819	0.817	0.814	0.802	0.0062		***	
Serine	0.801	0.818	0.787	0.815	0.788	0.809	0.0058	***		
Threonine[§]	0.768	0.786	0.751	0.782	0.748	0.775	0.0064	***	¶	
Tyrosine	0.794	0.793	0.778	0.794	0.771	0.800	0.0071	*		
Valine[‡]	0.844	0.846	0.826	0.842	0.817	0.827	0.0065	¶	**	
Faecal apparent digestibility										
Dry matter	0.832^{c}	0.839^{c}	0.812^{b}	0.831^{c}	0.786^{a}	0.789^{a}	0.0035	**	***	¶
Ash	0.510^{a}	0.556^{b}	0.505^{a}	0.560^{b}	0.490^{a}	0.493^{a}	0.0084	***	***	*
Crude protein	0.810^{abc}	0.834^{c}	0.801^{ab}	0.825^{bc}	0.796^{ab}	0.785^{a}	0.0071	*	**	*
Ether extract	0.591^{a}	0.633^{b}	0.656^{b}	0.704^{c}	0.709^{c}	0.717^{c}	0.0070	***	***	*
Neutral-detergent fibre	0.613^{c}	0.566^{bc}	0.531^{ab}	0.560^{bc}	0.485^{a}	0.480^{a}	0.0160		***	¶
Acid-detergent fibre[†]	0.393^{b}	0.404^{b}	0.306^{ab}	0.385^{b}	0.274^{a}	0.303^{ab}	0.0239	¶	***	
Hemicellulose	0.694^{c}	0.638^{bc}	0.627^{ab}	0.638^{bc}	0.581^{ab}	0.564^{a}	0.0145		***	¶

[a,b,c] Within a row means that have different superscripts differ significantly ($P < 0.05$).

[†] The effect of fibre level is linear ($P < 0.001$); [‡]($P < 0.01$); [§]($P < 0.05$).

[‖] The effect of fibre level is quadratic in non-acidified diets ($P < 0.05$).

[¶] Approaching significance ($P \leq 0.1$).

levels linearly decreased purine concentration in the ileal digesta ($P < 0.01$) but did not affect DAPA concentration. The ratios of DAPA / total N, purines / total N and DAPA/purines were larger in pigs fed the diets supplemented with organic acid than those fed non-acidified diets ($P < 0.01$). The DAPA/purine ratio also increased linearly with increasing levels of dietary fibre ($P < 0.05$) but the quadratic effect of fibre level was not significant.

The flow of N in the ileum was decreased by the dietary organic acid mixture ($P < 0.01$) and it increased linearly with increasing levels of dietary fibre ($P < 0.001$). The quadratic effect of fibre level was not significant. The ileal purine flow was decreased by the dietary organic acid mixture ($P < 0.01$) but it was not influenced by dietary fibre levels ($P > 0.05$). The flow of bacterial N as assessed by purine flow and the proportion of bacterial N were decreased by the dietary organic acid mixture ($P < 0.01$) but they were not influenced by dietary fibre levels ($P > 0.05$). Contrary to the situation with purines, the flow of DAPA and bacterial N as assessed by DAPA flow were increased by the dietary organic acid mixture ($P < 0.05$) and in a linear manner with increasing levels of dietary fibre ($P < 0.01$). The quadratic effect of fibre level was not significant. Based on DAPA, the proportion of bacterial N was increased by the dietary organic acid mixture ($P < 0.01$) but was not affected by dietary fibre levels ($P > 0.05$).

The dietary organic acid mixture did not affect the faecal purine concentration ($P > 0.05$), which decreased linearly with increasing levels of dietary fibre ($P < 0.05$). The quadratic effect of fibre level was not significant. The faecal flow of total N and purines was decreased by the dietary organic acid mixture ($P < 0.05$) but the organic

Table 4 *Mean amounts of nitrogen (N), diaminopimelic acid (DAPA), total purines, and bacterial N flow in the ileum and faeces of pigs fed diets with different amounts of fibre content and supplemented with 0 or 8.4 g/kg of a mixture of formic acid, sorbate and benzoate*

	Fibre content									
	Medium		High		Very high			Significance		
Organic acid mixture	−	+	−	+	−	+	s.e.	Acid	Fibre	Acid × fibre
Dry matter (DM) intake (kg/day)	1.93	1.93	1.94	1.94	1.94	1.94	0.002			
Bacterial markers in ileal digesta (g/kg DM)										
Purines[‡]	10.6	9.7	9.9	8.7	9.2	8.0	0.42	**	**	
Purines to total N (g/g)	0.483	0.465	0.482	0.447	0.466	0.435	0.0138	*		
DAPA	0.34	0.40	0.33	0.38	0.34	0.38	0.024	*		
DAPA to total N (g/g)	0.016	0.019	0.016	0.020	0.017	0.020	0.0011	**		
DAPA to purines (g/g)[§]	0.032	0.042	0.034	0.044	0.038	0.047	0.0023	***	‖	
Ileal flow (g/kg DM intake)										
Total N[†]	6.27	5.88	6.43	6.07	7.07	6.51	0.176	**	**	
Purines	3.02	2.75	3.10	2.71	3.28	2.83	0.147	**		
DAPA[‡]	0.096	0.113	0.103	0.118	0.123	0.132	0.0067	*	**	
Bacterial N										
Purines as bacterial marker	2.90	2.63	2.97	2.60	3.14	2.72	0.140	**		
DAPA as bacterial marker[‡]	2.60	3.06	2.79	3.19	3.32	3.58	0.182	*	**	
Proportion of bacterial N (g/total N)										
Purines as bacterial marker	0.46	0.45	0.46	0.43	0.45	0.42	0.013	*		
DAPA as bacterial marker	0.42	0.51	0.43	0.53	0.47	0.55	0.030	**		
Purines in faeces (g/kg DM)[§]	14.4	12.8	13.9	12.8	12.0	12.2	0.67		‖	
Faecal flow (g/kg DM intake)										
Total N[‡]	5.44	4.85	5.70	5.02	5.97	5.84	0.204	**	**	
Purines	2.44	2.07	2.62	2.16	2.55	2.58	0.148	*		
Bacterial N[§]	2.20	2.13	2.37	2.21	2.31	2.62	0.139			
Proportion of bacterial N (g/total N)	0.40	0.44	0.41	0.44	0.38	0.45	0.013	***		

[†] The effect of fibre level is linear ($P < 0.001$); [‡] ($P < 0.01$); [§] ($P < 0.05$).
[‖] Approaching significance ($P < 0.1$).

acid mixture did not affect bacterial N flow ($P > 0.05$). Faecal total N flow increased linearly with increasing dietary fibre levels, whereas purine and bacterial N flows did not change ($P > 0.05$). Because no bacteria were isolated from the faeces, the determined ileal purine / bacterial N ratios were used in calculations. The proportion of bacterial N in the faeces was higher in diets with than without the organic acid mixture ($P < 0.001$) but it was not influenced by dietary fibre levels ($P > 0.05$).

When using purines as the bacterial marker, the dietary organic acid mixture decreased the proportion of bacterial amino acids in ileal digesta ($P < 0.05$) but this proportion was not influenced by dietary fibre levels ($P > 0.05$) (results not shown). The decreasing effect of dietary organic acid mixture ($P < 0.05$) on the total and bacterial flow of alanine, isoleucine, leucine, and lysine in the ileum as assessed by purine flow is presented in Figure 1. The share of these amino acids produced by bacteria represented over 0.60 of the total amount of amino acids flowing in the ileum. The opposite observation was made with DAPA as the bacterial marker, for the dietary organic acid mixture increased the proportion of bacterial amino acids in the ileal digesta ($P < 0.05$), except in the case of cysteine and proline (results not shown). In addition, increasing levels of dietary fibre increased the proportions of bacterial arginine, histidine, isoleucine, leucine, lysine, and tyrosine in a linear manner ($P < 0.05$).

Microbial metabolites and activity

The pH of ileal digesta was not influenced by the dietary treatments ($P > 0.05$; Table 5). The dietary organic acid mixture lowered ileal concentration of lactic acid ($P < 0.05$) and increased that of acetic acid ($P < 0.001$). Concentrations of other microbial metabolites were not influenced by the dietary organic acid mixture ($P > 0.05$). Dietary fibre levels had a quadratic effect on ileal concentrations of acetic, propionic, butyric, and isovaleric acid ($P < 0.01$), the concentrations being lowest in the high-fibre diet. The molar proportions of volatile fatty acids did not differ between the dietary treatments ($P > 0.05$). The ATP content of the ileal digesta was not influenced by the dietary organic acid mixture ($P > 0.05$) but it decreased linearly with increased dietary fibre level ($P < 0.05$). The quadratic effect of fibre level was not significant.

The rate of passage

The MRT values of Co and Yb in the terminal ileum, in the large intestine, and in the whole digestive tract are presented in Table 6. Neither the dietary organic acid

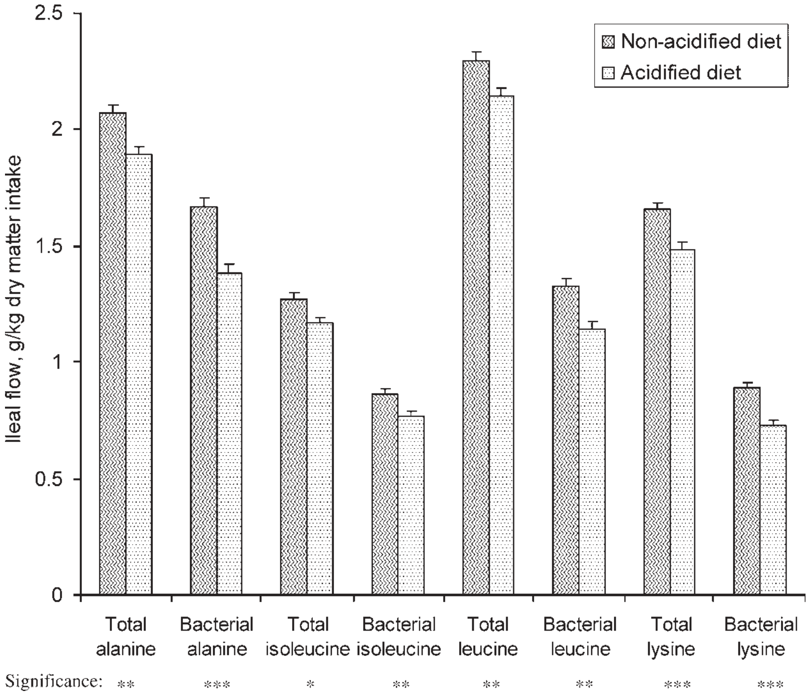

Figure 1 Flows of total and bacterial alanine, isoleucine, leucine, and lysine (g/kg dry matter intake) in the ileum of pigs fed diets with 0 or 8.4 g/kg of a mixture of formic acid, sorbate and benzoate. The bacterial amino acid flows were calculated using total purines as the bacterial marker.

mixture nor fibre levels influenced the MRT of Co and Yb in the terminal ileum ($P > 0.05$). The MRT of Co and Yb in the large intestine and in the whole digestive tract decreased linearly with increasing fibre levels ($P < 0.01$) but the quadratic effect of fibre level was not significant. The MRT of Co and Yb was not affected by the dietary organic acid mixture ($P > 0.05$). The faecal recovery of Co and Yb averaged 0.89 ± 1.13 and 0.78 ± 0.11 of intake, respectively.

Discussion

The diets based on barley and soya-bean meal and with medium, high, or very high fibre levels contained 0, 75, or

Table 5 *Mean values of pH, ammonia, formic acid, lactic acid, volatile fatty acids (VFA), and ATP in the ileal digesta of pigs fed diets with different amounts of fibre content and supplemented with 0 or 8.4 g/kg of a mixture of formic acid, sorbate and benzoate*

	Fibre content									
	Medium		High		Very high			Significance		
Organic acid mixture	−	+	−	+	−	+	s.e.	Acid	Fibre	Acid × fibre
pH	5.7	5.8	5.9	6.0	6.0	6.0	0.16			
Ammonia (mmol/l)	1.94	2.18	2.10	1.98	2.05	1.71	0.168			
Formic acid (mmol/l)	1.14	1.99	5.25	6.61	3.07	3.62	1.901			
Lactic acid (mmol/l)	43.6	26.3	48.0	31.9	35.6	28.0	5.92	*		
VFA (mmol/l)										
Acetic acid[†]	46.3	55.7	30.5	43.3	39.6	52.6	2.98	***	**	
Propionic acid[†]	27.3	26.9	14.7	18.6	23.8	26.0	2.44		**	
Butyric acid[‡]	7.13	10.60	4.68	5.89	8.41	7.86	1.225		*	
Isovaleric acid	2.02	2.55	1.19	1.43	2.89	2.01	0.558			
VFA molar proportions										
Acetic acid	0.58	0.60	0.61	0.61	0.55	0.60	0.025			
Propionic acid	0.32	0.27	0.28	0.28	0.31	0.29	0.015	‖		
Butyric acid	0.08	0.11	0.09	0.09	0.10	0.09	0.011			
Isovaleric acid	0.02	0.02	0.02	0.02	0.04	0.02	0.006			
ATP (mmol/l)[§]	65.1	62.3	60.1	57.2	51.0	50.7	4.52		*	

[†] The effect of fibre level is quadratic ($P < 0.001$); [‡] ($P < 0.01$).
[§] The effect of fibre level is linear ($P < 0.05$).
[‖] Approaching significance ($P \leq 0.1$).

Table 6 *Mean retention time of solute (Co) and particle (Yb) markers in the upper and lower gut and the whole digestive tract of pigs fed diets with different amounts of fibre content and supplemented with 0 or 8.4 g/kg of a mixture of formic acid, sorbate and benzoate*

	Fibre content									
	Medium		High		Very high			Significance		
Organic acid mixture	−	+	−	+	−	+	s.e.	Acid	Fibre	Acid × fibre
Stomach and small intestine										
Co (h)	7.2	7.7	7.8	7.6	7.7	7.9	0.21			
Yb (h)	10.4	11.2	11.0	10.7	10.8	11.2	0.23			‡
Large intestine										
Co (h)[†]	34.1	33.9	32.7	34.1	27.6	30.3	1.32		**	
Yb (h)[†]	33.6	32.9	31.8	32.9	26.9	29.8	1.39		**	
Entire digestive tract										
Co (h)[†]	41.3	41.7	40.5	41.6	35.3	38.2	1.33		**	
Yb (h)[†]	44.0	44.1	42.8	43.6	37.7	41.1	1.38		**	

[†] The effect of fibre level is linear ($P < 0.01$).
[‡] Approaching significance ($P \leq 0.1$).

150 g/kg of barley fibre that was a by-product of integrated starch-ethanol production. Barley fibre consists mainly of endosperm cell-wall material and is rich in both NDF and ADF, whereas the starch content is low. Its crude protein content and the protein's amino acid profile are similar to that of barley (Partanen *et al.*, 1998). Although dietary soya-bean meal levels were increased slightly with increasing barley fibre inclusion levels, the dietary amino acid composition hardly changed. The dietary pH decreased with increasing barley fibre inclusions, which is explained by the lower pH of barley fibre (below 3.5) compared with that of barley (5.8) and soya-bean meal (6.8). The dietary organic acid mixture consisted primarily of formic acid, and it lowered the dietary pH by 0.78 to 0.96 pH units, which is in agreement with previous studies (Partanen *et al.*, 2001; Mroz *et al.*, 2000). The pH-lowering effect of the organic acid mixture was greatest in the medium-fibre diet, and 100% of the added formic acid was recovered in the analyses of this diet. The pH change became smaller with increasing dietary barley fibre content, and 93 and 81% of the added formic acid was recovered from the high-fibre and very-high-fibre diets, respectively. Because no mistakes were noticed in feed mixing, it seems that the barley fibre, which has a good water-holding capacity, may have absorbed and retained formic acid so that it was not released completely when the pelleted feed was mixed with water to measure pH and to extract formic acid for the analyses.

The dietary organic acid mixture improved the ileal apparent digestibility of protein and of 14 of the 17 amino acids analysed. Improved ileal amino acid apparent digestibilities have been reported when diets for growing-finishing pigs have been supplemented with propionic (Mosenthin *et al.*, 1992), lactic (Kemme *et al.*, 1999), formic (Mroz *et al.*, 2000; Partanen *et al.*, 2001), butyric, or fumaric acid (Mroz *et al.*, 2000). In the present experiment, the ileal amino acid apparent digestibilities were improved by 0.015 to 0.027. Somewhat larger improvements were reported in the aforementioned studies, which could have been a result of differences in the acid added and the inclusion levels used and diet composition. In this study, the greatest improvements were seen in the ileal apparent digestibility of alanine, threonine, aspartic acid, serine, lysine, and glycine. A tendency for an interaction was found for the apparent ileal digestibility of histidine ($P = 0.06$), which was improved in the medium- and high-fibre diets ($P < 0.05$) but not in the very-high-fibre diet. In the study of Partanen *et al.* (2001), dietary NDF levels of 189 and 219 g/kg DM were obtained by adding 0 or 180 g/kg of wheat bran/middlings to a diet based on barley and soya-bean meal. That fibre addition reduced the ileal amino acid apparent digestibilities more than the barley fibre additions in this study, and the dietary formic acid in that study improved the ileal amino acid apparent digestibilities only in the high-fibre diet.

Increasing dietary fibre levels resulted in a linear decrease in the ileal apparent digestibility of crude protein and of six of the 17 amino acids analysed. Similarly, increasing inclusions (0 to 167.5 g/kg DM) of barley fibre in semipurified barley-distiller's feed diets have decreased the ileal apparent digestibility coefficients of amino acids by 0.005 to 0.008 per each 10-g increase in dietary NDF content (Partanen *et al.*, 1998). In this study, the ileal amino acid apparent digestibilities decreased slightly less. The high-fibre and very-high-fibre diets contained rapeseed oil, which has been shown to improve the ileal apparent digestibility of amino acids (Li and Sauer, 1994). Thus, rapeseed oil additions may have partly diminished the negative effect of barley fibre on ileal amino acid apparent digestibility. In addition to the quantity of dietary fibre, its quality may determine how large an effect fibre has on ileal amino acid apparent digestibilities (Sauer *et al.*, 1991; Mosenthin *et al.*, 1994). Huang *et al.* (2001) found that the ileal apparent digestibilities of several amino acids negatively correlated with the crude protein content associated with NDF in wheat fractions. Poorly digestible cell walls can protect protein from proteolytic enzymes, resulting in poorer amino acid digestibilities.

Amino acids in ileal digesta can originate from undigested feed proteins, various endogenous secretions, sloughed epithelial cells, and microbes. Dietary formic acid has been shown not to influence the secretion of pancreatic juice (Lesniewska *et al.*, 2001), whereas dietary soluble fibres have been shown to increase the secretion of saliva, gastric and pancreatic juices, and bile considerably (Mosenthin *et al.*, 1994). Endogenous secretions provide an easily available N source for bacteria (Bartelt *et al.*, 1999). In addition, the carbohydrate composition of a diet has a distinct influence on the microbial fermentation and the *de novo* synthesis of bacterial amino acids in the small intestine (Wenk, 2001). In this study, bacterial N contributed 0.42 to 0.46 and 0.42 to 0.55 of N in the ileum, as assessed by purine and DAPA flows, respectively. These proportions are within the wide range of values reported in the literature using both bacterial markers (Wünsche *et al.*, 1991; Schulze *et al.*, 1994; Bartelt *et al.*, 1999; Huang *et al.*, 2001; Partanen *et al.*, 2001).

The bacterial marker DAPA is a component of the peptidoglycan layer of the bacterial cell wall; it is not found in animal or plant cells (Czerkawski and Faulds, 1974). Purines from hydrolysed nucleic acids are not as specific to bacteria as DAPA, and those purines found in digesta can originate from microbes, undigested diet, mucosal secretions, and sloughed off cells (Zinn and Owens, 1986). The purine / bacterial N ratio was 1110 and 980 mg/g in the two bacterial isolates. References for purine / bacterial N ratios in ileal and faecal bacteria of pigs were not found in the literature, but the determined ratios are in agreement with values reported for ruminal microbes (Illg and Stern, 1994). The DAPA / bacterial N ratios of 34 and 40 mg/g are among the highest reported in the literature. Bartelt *et al.* (1999) obtained DAPA / bacterial N ratios of 34.4 and 22.3 mg/g for miniature and domestic pigs with re-entrant ileo-caecal cannulae. Köhler *et al.* (1992) reported somewhat lower values for pigs with PVTC cannulae (13.9 to 16.7 mg/g) and ileo-rectal anastomosis (14.3 to 27.5 mg/g). In the study of Wünsche *et al.* (1991), the DAPA / bacterial N ratios were 7.5 to 18.8 mg/g for pigs with re-entrant ileo-caecal cannulae and 10 to 39.6 mg/g for pigs with ileo-rectal anastomoses. In the PVTC cannulation, the caecum is removed, and reflux of digesta from colon to ileum can influence microbial populations in the terminal ileum. According to Wünsche *et al.* (1991), the variation in DAPA / bacterial N ratios is high, the coefficient of variation being over 20%. Based on the observed high DAPA to bacterial N ratio, the contribution of bacterial N to ileal N flow may even be underestimated at 0.42 to 0.55.

The dietary organic acid mixture decreased the ileal flow of purines and bacterial N as assessed by purine flow. These findings are in close agreement with the results of Partanen *et al.* (2001). They support the hypothesis that improved apparent ileal amino acid digestibilities seen in diets supplemented with organic acid arise at least partly from decreased bacterial protein synthesis in the ileum. Over 0.60 of alanine, isoleusine, leucine, and lysine found in the ileal digesta was of bacterial origin as assessed by purine flow, and the improvements seen in the apparent ileal digestibility of these amino acids were primarily a result of decreased bacterial amino acid flows in the ileum. The contribution of bacterial amino acids was the lowest for proline, glycine, and glutamic acid. These are the amino acids that are among the most abundant ones in endogenous protein (Stein *et al.*, 1999).

Increased dietary fibre levels did not affect the bacterial N flow in the ileum as assessed by purine flow, which is in agreement with our previous results (Partanen *et al.*, 2001). In the studies of Schulze *et al.* (1994) and Bartelt *et al.* (1999), increasing fibre inclusions did not affect bacterial N flow as assessed by DAPA flow. In this study, however, increasing levels of dietary fibre increased the flow of DAPA and consequently of bacterial N flow as assessed by DAPA flow. The dietary organic acid mixture increased DAPA flow too. The contradictory results obtained with purines and DAPA indicate that the common assumption of a constant DAPA / bacterial N or purine / bacterial N ratio could be misleading. This is supported by the observation that the DAPA/purine ratio was changed by both the dietary organic acid mixture and increased fibre levels. The DAPA/purine ratio of digesta averaged 0.035 and 0.044 g/g in pigs fed diets without and with organic acid supplementation, and a similar difference was seen in the DAPA / purine ratio of bacteria that were isolated from digesta (0.031 and 0.041 g/g, respectively). According to Dufva *et al.* (1982), the concentration of DAPA can vary greatly among different species of bacteria in the rumen. References for DAPA / bacterial N ratios of bacteria found from the digestive tract of pigs are scarce. Laplace *et al.* (1985) reported a DAPA / bacterial N ratio of 24.8 for a pure *E. coli* culture and values of 29.9 and 19.9 g/g in bacteria isolated from the faeces of pigs fed standard and semi-synthetic diets, respectively. The latter figures support the conclusion that the use of a constant DAPA / bacterial N ratio could be misleading.

Comparisons of DAPA and purines as bacterial markers in pigs are scarce. In dogs, the use of DAPA as a bacterial marker resulted in a smaller standard error for bacterial N than purines (Karr-Lilienthal *et al.*, 2004). In this study, the standard error of the mean for bacterial N flow in the ileum was smaller when bacterial N flow was assessed by means of purines rather than DAPA. This speaks in favour of the use of purines as a bacterial marker to estimate bacterial N flow in the ileum of pigs. However, dietary purines may not be completely digested, and undigested purines as well as purines from endogenous secretions may confound the measurements of purine concentrations in digesta. It would have been better to isolate bacterial fractions from ileal digesta from each pig during each experimental period instead of obtaining only two pooled samples. However, this would have required the collection of considerably larger amounts of digesta because we were able to obtain only about 3.2 g bacterial DM per kg fresh ileal digesta. Furthermore, it seems that both the endogenous and bacterial N flows should have been determined to find out why dietary organic acid mixture improved the ileal apparent digestibility of amino acids.

Estimates of bacterial N contribution in faeces have ranged greatly depending on diet composition and the method of determination (Dierick *et al.*, 1990: Sauer *et al.*, 1991). Dietary fibre generally stimulates microbial activity and bacterial protein synthesis in the large intestine (Wenk, 2001). In this study too, faecal bacterial N flow increased linearly with increasing dietary fibre levels. The dietary organic acid mixture did not affect the faecal bacterial N flow, but it increased the proportion of bacterial N in faeces. This supports the hypothesis that dietary organic acids restrict microbial fermentation in the small intestine, and this restriction could result in increased amounts of carbohydrates being fermented in the large intestine. Because faecal bacteria were not isolated and analysed for bacterial marker, the obtained faecal bacterial N flows should be considered as rough estimates.

Both the ileal and faecal apparent digestibility of ether extract improved with higher dietary fibre levels. The diets were formulated to be isoenergetic, and so rapeseed oil was added to medium- and high-fibre diets. The higher the ether extract content, the higher is the ileal and faecal apparent digestibility of ether extract (Jørgensen *et al.*, 1992). The dietary organic acid mixture improved the faecal digestibility of ether extract, whereas it produced only a tendency towards improvement in ileal apparent digestibility. The positive effect of organic acid on ether extract digestibility is in agreement with previous results (Mroz *et al.*, 2000; Partanen *et al.*, 2001) and it may result from changes in microbial activity in the digestive tract. Microbial deconjugation and dehydroxylation of bile impair lipid absorption by the host animal. Lactobacilli inhabiting the small intestine may be largely responsible for bile salt hydrolysis (Anderson *et al.*, 1999). In this study, ileal lactic acid concentrations were reduced by the dietary organic acid mixture, indicating that the growth of lactic acid bacteria was restricted. Faecal ether extract digestibilities were lower than ileal ones, which is in accordance with our previous results (Partanen *et al.*, 2001) and could have been caused by microbial fat synthesis in the large intestine (Dierick *et al.*, 1990).

Both the dietary organic acid mixture and higher fibre levels resulted in changes in microbial metabolite concentrations in the ileal digesta. The dietary organic acid mixture decreased the lactic acid and increased the acetic acid concentration. Reduced lactic acid concentration in the stomach and small intestine of pigs has been reported when diets have been supplemented with formic acid or formates (Roth *et al.*, 1992; Partanen *et al.*, 2001; Canibe *et al.*, 2005). The decrease in lactic acid concentration may result from a reduction in microbial fermentation of glucose from starch hydrolysis. Bacteria compete with the host for nutrients in the stomach and small intestine. The reduced production of lactic acid may enable the increased absorption of glucose in the small intestine. The increased acetic acid concentrations seen in the diets supplemented with formic acid are also in agreement with previous results (Roth *et al.*, 1992; Partanen *et al.*, 2001). The decreased lactic acid and increased acetic acid production observed indicate that a shift may have occurred in the composition of the small intestine's bacterial flora. Dietary carbohydrate composition has a profound effect on microbial fermentation and metabolite concentrations in the ileum (Dierick *et al.*, 1990). In this study, fibre level had a quadratic effect on concentrations of acetic, propionic, and butyric acid, and the lowest concentrations were observed in the medium-fibre diet. The concentration of ATP is used to estimate microbial activity in the gastrointestinal tract of monogastric animals (Jensen and Jørgensen, 1994). In this study, the ileal ATP concentrations decreased with increasing dietary fibre levels, which indicates that the carbohydrates of high-fibre and very-high-fibre diets were less fermentable and decreased microbial activity in the small intestine compared with barley-based medium-fibre diet.

The MRT of the solute marker Co was *ca* 3.2 h shorter than that of the particle marker Yb in the terminal ileum, whereas the MRT in the large intestine was fairly similar for both markers. Neither dietary organic acid supplementation nor fibre level influenced the MRT of Co and Yb in the terminal ileum, which indicates that improvements seen in the ileal apparent digestibility of amino acids were not caused by changes in digesta passage rate. According to Wenk (2001), soluble fibres can increase the passage rate of digesta in the upper digestive tract, but we did not see such an effect.

Dietary organic acid supplementation did not influence the MRT of Co and Yb in the large intestine or the whole digestive tract. Fibre addition resulted in linearly decreasing MRT in the large intestine and the whole digestive tract, which is in agreement with previous findings (Wenk, 2001). The MRT of markers in the whole digestive tract are similar to those reported for diets based on maize and soya-bean meal by Pond *et al.* (1986). The amount of faeces collected from the pigs during the 4-day period increased linearly from 1.12 to 1.43 kg/day. This was primarily caused by linearly increased excretion of water (from 0.81 to 1.05 kg/day) in faeces, which is in agreement with previous reports (Wenk, 2001). DM excretion increased too, but less (from 0.32 to 0.39 kg/day), which is explained by the poorer digestibility of barley fibre compared with that of barley.

Conclusions

The results show that a dietary mixture of formic acid, sorbate, and benzoate has a positive effect on the ileal apparent digestibility of protein and all amino acids except for methionine, proline, and valine. The faecal digestibility coefficients of crude protein and ether extract improved too, but the effect of the organic acid mixture was dependent on the dietary fibre level. The bacterial markers purines and DAPA showed opposite effects on bacterial N flow in the ileum, which makes the use of a constant bacterial marker / bacterial N ratio questionable for the estimation of bacterial N flow in the ileum. For the same reason, the hypothesis that dietary organic acids decrease the contribution of bacterial protein in the ileal digesta could not be confirmed. The dietary organic acid mixture reduced lactic acid and increased acetic acid concentrations in the ileum, whereas the concentrations of acetic, propionic, and butyric acid were lower in the high-fibre diet than in the medium-fibre and very-high fibre diets. The improved ileal and faecal apparent digestibilities seen in the diets supplemented with organic acid were not explained by MRT in the terminal ileum. Dietary fibre addition shortened the MRT of digesta in the large intestine, and it increased both faecal water and DM excretion.

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