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# The effect of high and low dietary crude protein and inulin supplementation on nutrient digestibility, nitrogen excretion, intestinal microflora and manure ammonia emissions from finisher pigs

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*A 2 × 2 factorial experiment was performed to investigate the interaction between a high- and low-crude-protein (CP) diet (200* v. *140 g/kg) and inulin supplementation (0* v. *12.5 g/kg) on nutrient digestibility, nitrogen (N) excretion, intestinal microflora, volatile fatty acid (VFA) concentration and manure ammonia emissions from 24 boars* (n = *6, 74.0 kg live weight). The diets were formulated to contain similar concentrations of digestible energy and lysine. Pigs offered the high-CP diets had a higher excretion of urinary N* ($P < 0.001$)*, faecal N* ($P < 0.01$) *and total N* ($P < 0.001$) *than the pigs offered the low-CP diets. Inulin supplementation increased faecal N excretion* ($P < 0.05$) *and decreased the urine N : faeces N ratio* ($P < 0.05$) *compared with the inulin-free diets. There was no effect* ($P > 0.05$) *of dietary treatment on N retention. There was an interaction* ($P < 0.05$) *between dietary CP concentration and inulin supplementation on caecal* Enterobacteria *spp. Pigs offered the diet containing 200 g/kg of CP plus inulin decreased the population of* Enterobacteria *spp. compared to those with the inulin-supplemented 140 g/kg CP diet. However, CP level had no significant effect on the population of* Enterobacteria *spp. in the unsupplemented diets. Inulin supplementation increased caecal Bifidobacteria* ($P < 0.01$) *compared with the inulin-free diets. There was no effect of inulin supplementation on VFA concentration or intestinal pH* ($P > 0.05$)*. Pigs offered the 200 g/kg CP diets had higher* ($P < 0.05$) *manure ammonia emissions from 0 to 240 h of storage than pigs offered the 140 g/kg CP. In conclusion, inulin supplementation resulted in an increase in Bifidobacteria concentration and a reduction in* Enterobacteria *spp. at the high CP level indicating that inulin has the ability to beneficially manipulate gut microflora in a proteolytic environment.*

**Keywords:** inulin, microflora, pigs, protein

## Introduction

The formulation of commercial diets supplies excess dietary protein in order to satisfy the needs for the first limiting amino acid(s) (Lenis, 1989). As a result, incomplete digestion and consumption of excess amino acids are largely responsible for unnecessary nitrogen (N) excretion and half of ingested N is excreted as urea in urine (Jongbloed and Lenis, 1992). The urea is then rapidly converted into ammonia by the urease enzyme present in faeces, whereas faecal N in the form of bacterial protein degrades gradually (Van der Peet-Schwering *et al*., 1999). More importantly, the end products of proteolytic fermentation are potentially harmful to performance and are involved in the clinical expression of diarrhoea (Macfarlane *et al*., 1992; Aumaitre *et al*., 1995), whereas branched-chain fatty acids (BCFA) such as isobutyric and isovaleric acid are major odour-causing compounds (Mackie *et al*., 1998). It is well documented that reductions in total N excretion, ammonia emissions, offensive volatile fatty acids (VFA) and other odorous compounds are achievable (Sutton *et al*., 1996; Hayes *et al*., 2004) by lowering dietary crude protein (CP).

Physiologically, fructo-oligosaccharides, like inulin, are classified as dietary fibre (Flamm *et al*., 2001) resistant to complete enzymatic degradation in the small intestine. Inulin is selectively fermented by *Bifidobacteria* and *Lactobacilli* to short-chain fatty acids (SCFA), lactate and gas (Roberfroid *et al*., 1998). In a high proteolytic environment, inulin supplementation may regulate metabolic activity, decreasing the protein : carbohydrate ratio in the hindgut. As a result, carbohydrate fermentation may suppress the formation of BCFAs and ammonia, which are produced from

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protein fermentation (Macfarlane and Macfarlane, 2003), while stimulating SCFAs and beneficial bacteria. By increasing the carbohydrate : protein ratio the partitioning of N excretion can be manipulated to reduce the amount of excess urinary N excreted by the pig, and therefore improving nutrient management (Canh *et al.*, 1997; Mroz *et al.*, 2000).

It is our hypothesis that inulin supplementation in a high-CP diet will reduce urinary N excretion, enhance the proliferation of lactic acid-producing bacteria, reduce BCFAs and ammonia emissions compared with an unsupplemented diet. The objective of the experiment is to compare the effects of two levels of CP in diets (200 and 140 g/kg) and inulin inclusion (0 and 12.5 g/kg) on nutrient digestibility, N excretion, large intestinal microflora, VFA concentration and manure ammonia emissions from finisher boars.

## Material and methods

All procedures described in this experiment were conducted under experimental licence from the Irish Department of Health in accordance with the Cruelty to Animals Act 1876 and the European Communities (Amendments of the Cruelty to Animals Act 1976) Regulations, 1994.

### *Experimental diets*

The experiment was designed as a 2 × 2 factorial experiment comprising of four dietary treatments. All diets were formulated to have identical digestible energy (DE; 13.7 MJ/kg) (Sauvant *et al.*, 2004) and total lysine (10.0 g/kg). The amino acid requirements were met relative to lysine (Close, 1994). The experimental treatments were as follows: (1) 200 g/kg CP, (2) 200 g/kg CP plus 12.5 g/kg inulin, (3) 140 g/kg CP and (4) 140 g/kg CP plus 12.5 g/kg inulin. The inulin was substituted for wheat on a weight for weight basis as previous work with inulin had shown it to have a similar DE to that of wheat (Pierce *et al.*, 2005a). Dietary analysis indicates an average CP content of 148.2 g/kg and 202.4 g/kg for the low- and high-CP diets, respectively. The 140 g/kg CP diet was formulated by decreasing the soya-bean meal content from 265 to 112.5 g/kg and supplementing with synthetic amino acids as follows: lysine HCl 4.9 g/kg, DL-methionine 0.5 g/kg and L-threonine 2.1 g/kg. The inulin (Raftiline ST®) was manufactured by Orafti S. A., Tienen, Belgium. All diets were fed in meal form. The dietary composition and analysis is presented in Table 1.

**Table 1** *Composition and analysis of experimental diets (as-fed basis)*

| | Treatment | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Ingredients (g/kg) | | | | |
| Wheat | 704.3 | 691.8 | 855.0 | 842.5 |
| Soya-bean meal | 265 | 265 | 112.5 | 112.5 |
| Soya oil | 5.7 | 5.7 | 0 | 0 |
| Lysine HCl | 0 | 0 | 4.9 | 4.9 |
| DL-methionine | 0 | 0 | 0.5 | 0.5 |
| L-threonine | 0 | 0 | 2.1 | 2.1 |
| Dicalcium phosphate | 7.5 | 7.5 | 7.5 | 7.5 |
| Salt | 5.0 | 5.0 | 5.0 | 5.0 |
| Limestone | 10.0 | 10.0 | 10.0 | 10.0 |
| Mineral and vitamin† | 2.5 | 2.5 | 2.5 | 2.5 |
| Chicory inulin | 0 | 12.5 | 0 | 12.5 |
| Analysed composition (g/kg) | | | | |
| Dry matter | 872.0 | 870.0 | 866.0 | 873.0 |
| Crude protein (N × 6.25) | 202.9 | 201.8 | 151.7 | 144.7 |
| Neutral-detergent fibre | 128.9 | 126.6 | 107.7 | 98.4 |
| Acid-detergent fibre | 45.2 | 49.5 | 55.8 | 51.5 |
| Hemicellulose | 83.7 | 77.1 | 51.9 | 46.9 |
| Crude ash | 42.0 | 42.7 | 38.7 | 40.9 |
| Crude oil | 20.0 | 20.0 | 14.5 | 14.3 |
| Gross energy (MJ/kg) | 16.1 | 16.1 | 15.8 | 15.6 |
| Lysine | 9.9 | 10.4 | 9.9 | 9.8 |
| Methionine and cysteine | 6.0 | 6.1 | 5.4 | 5.4 |
| Threonine | 7.0 | 7.0 | 6.4 | 6.4 |
| Tryptophan | 1.9 | 1.9 | 1.8 | 1.8 |
| Calculated composition (g/kg) | | | | |
| Starch‡ | 426.0 | 418.0 | 517.0 | 509 |
| Sugar‡ | 41.0 | 41.0 | 30.8 | 30.5 |
| Digestible energy‡ | 13.75 | 13.75 | 13.70 | 13.70 |
| Dietary electrolyte balance (meq/kg)§ | 198.0 | 197.0 | 128.0 | 127.0 |
| Non-starch polysaccharides¶ | 109.8 | 121.3 | 91.6 | 103.3 |

†Provided per kg of complete diet: 3 mg retinol, 0.05 mg cholecalciferol, 40 mg α-tocopherol, 90 mg copper as copper II sulphate, 100 mg iron as iron II sulphate, 100 mg zinc as zinc oxide, 0.3 mg selenium as sodium selenite, 25 mg manganese as manganous oxide and 0.2 mg iodine as calcium iodate on a calcium sulphate/ calcium carbonate carrier.
‡Sauvant *et al.* (2004).
§Calculated as $(K^+ + Na^+ - Cl^-)$.
¶NSP calculated as (organic matter − (crude fat + crude protein + starch + sugar)) (Canh *et al.*, 1998b).

### *Animals and management*

Twenty-four finishing boars (progeny of meat-line boars × (Large White × Landrace sow)) with an initial live weight of 74 (s.d. 2.6) kg were used in this experiment. The pigs were blocked on the basis of live weight and within each block were randomly allocated to one of four dietary treatments. The pigs were allowed a 14-day dietary adaptation period after which time they were weighed. Sixteen pigs were selected according to a uniform weight and transferred to individual metabolism crates. The pigs were given a further 5 days to adapt to the metabolism crates before collections begun. The collection period was subdivided into two parts to facilitate studies on ammonia emission (days 1 and 2) and apparent digestibility and N balance (days 3 to 7). The daily feed allowance (DE intake (MJ/day) = 3.44 × (live weight)$^{0.54}$ (Close, 1994) was divided over two meals. Water was provided with meals in a 1 : 1 ratio. Between meals, fresh water was provided *ad libitum* from a nipple drinker. The metabolism crates were



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located in a temperature-controlled room, maintained at a constant temperature of 22°C (±1.5°C).

*Ammonia emissions*

Four separate collections of total faeces and urine were taken at 12-h intervals during collection days 1 and 2. Urine was collected in a plastic container, via a funnel below the crate. Faeces were collected in a tray directly underneath the metabolism crate. Following collection, the excreta were stored separately in sealed containers at 4°C. After the last collection, the urine and faeces samples were mixed together (w/w) according to the original excretion ratio. Samples (2 kg) of the manure homogenate from each pig were placed in duplicate, in containers within a climate-controlled room maintained at 20°C. Ammonia emission from the manure was measured over 240 h from the first container, in a laboratory-scale set-up according to the method of Derikx and Aarnink (1993). The equipment consisted of a sealed vessel containing 2 kg slurry, vacuum pump and three impingers in series per sample. The first two impingers contained 1 mol/l nitric acid and the third impinger contained water. The ventilation rate in the container was 4.2 l/min. The first impinger was replaced at 48, 96 and 144 h and the second impinger was replaced at 96 h. Samples were taken from all three impingers at 240 h. The concentration of ammonia-nitrogen ($NH_3$-N) in the impingers was determined by the microdiffusion technique of Conway (1957). Ammonia production (g/day) from manure is compared between the different dietary treatments using the quantity volatilised from 0 to 240 h. The sample in the second ventilated container was used to conduct pH analysis of the slurry whenever the first impinger was replaced.

*Apparent digestibility and nitrogen balance study*

During collections, urine was collected in a plastic container, via a funnel below the crate, containing 20 ml of sulphuric acid (25% $H_2SO_4$). To avoid N volatilisation, the funnel was sprayed four times daily with dilute sulphuric acid (2% $H_2SO_4$) solution. The urine volume was recorded daily and a 50-ml sample was collected and frozen for laboratory analysis. Total faeces weight was recorded daily and oven dried at 100°C. A sample of freshly voided faeces was collected twice daily and frozen for N analysis. At the end of the collection period, the faeces samples were pooled and a subsample retained for laboratory analysis. Feed samples were collected each day and retained for chemical analysis.

*Microbiology*

All 24 pigs remained on their respective dietary treatments until slaughter. Digesta samples (approximately 10 ± 1 g) were aseptically removed in aerobic conditions from the caecum and colon of each animal immediately after slaughter, stored in sterile containers (Sarstedt, Wexford, Ireland) on ice and transported to the laboratory within 7 h. *Bifidobacteria* spp., *Lactobacillus* spp. and *Enterobacteria* spp. were isolated and counted according to the method described by O'Connell *et al*. (2005). *Lactobaccilus* spp. were chosen because of their health-promoting properties (Gibson and Roberfroid, 1995) while *Enterobacteria* spp. were chosen because of the harmful effects of some species in the gastro-intestinal tract (Gibson and Roberfroid, 1995).

*pH measurements*

Samples of digesta from the caecum and proximal colon were taken and placed in universal containers. The pH of the digesta was taken on site, immediately after collection. All pH measurements were made on a Mettler Toledo MP 220 pH meter, which was calibrated with certified pH 4 and pH 7 buffer solutions. Distilled water was added to some very viscous samples to enable their pH to be read.

*Volatile fatty acid analysis and sampling*

Samples of digesta from the caecum and the colon of individual pigs ($n = 24$) were taken for VFA analysis. VFA concentrations in the digesta were determined using a modified method of Porter and Murray (2001). First, 1 g of sample was diluted with distilled water (2.5 × weight of sample) and centrifuged at 1400 × **g** for 4 min (Sorvall GLC – 2B laboratory centrifuge). Then, 1 ml of the subsequent supernatant and 1 ml of internal standard (0.5 g 3-methyl-*n*-valeric acid in 1 l of 0.15 mol/l oxalic acid) were mixed with 3 ml of distilled water. Following centrifugation to remove the precipitate, the sample was filtered through Whatman 0.45-μm polyethersulphone membrane filters into a chromatographic sample vial. Finally, 1 μl of sample was injected into a model 3800 Varian gas chromatograph with a 25 m × 0.53 mm i.d. megabore column (coating CP-Wax 58 (FFAP) – CB (no. CP7614)) (Varian, Middelburg, The Netherlands).

*Laboratory analysis*

Proximate analysis of diets for dry matter (DM) and ash was carried out according to the Association of Analytical Chemists (1995). The DM of the food and faeces was determined after drying for 24 h at 103°C. Ash was determined after ignition of a known weight of concentrates or faeces in a muffle furnace (Nabertherm, Bremen, Germany) at 500°C for 4 h. The gross energy of feed and faeces samples was measured using an adiabatic bomb calorimeter (Parr Instruments, IL, USA). The neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) content of feed and faeces was determined using a Fibertec Extraction Unit (Tecator, Sweden) according to the method of Van Soest *et al*. (1991). The N content of feed and urine was determined using the LECO FP 528 instrument (Leco Instruments (UK) Ltd). The dietary concentrations of lysine, threonine, tryptophan, methionine and cysteine were determined by high-performance liquid chromatography (Iwaki *et al*., 1987). The N content of fresh faeces was analysed by the



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macro-Kjeldahl technique using a Buchi digestion and distillation apparatus.

*Statistical analysis*

The data were analysed as a 2 × 2 factorial using the GLM procedure of the Statistical Analysis Systems Institute (SAS; 1985). The model used included the effect of protein level and inulin supplementation and the associated two-way interaction. Starting metabolic live weight (live weight$^{0.75}$) were included as covariates in the model. The manure pH data, measured over 10 days, were analysed by the repeated measures procedure using the Proc Mixed procedure of SAS 6.14 (Littell *et al.*, 1996). The individual pig was the experimental unit. The data in the tables are presented as least-square means (LSM) ± s.e.

## Results

*Coefficient of total tract apparent digestibility and nitrogen balance study*

The effect of dietary treatment on the coefficient of total tract apparent digestibility and N balance data are presented in Table 2.

Inulin supplementation had a significant effect on the apparent digestibility of NDF, hemicellulose and N. Pigs offered inulin-supplemented diets had a decreased NDF (0.59 *v.* 0.65; s.e. 0.018; $P < 0.05$), hemicellulose (0.59 *v.* 0.65; s.e. 0.020; $P < 0.05$) and N digestibility (0.89 *v.* 0.92; s.e. 0.005; $P < 0.01$) compared to those with unsupplemented diets.

Pigs offered high-CP diets had an increased apparent digestibility of NDF (0.66 *v.* 0.58; s.e. 0.018; $P < 0.01$) and hemicellulose (0.74 *v.* 0.51; s.e. 0.019; $P < 0.001$) compared to those with the low-CP diets.

There was a significant interaction ($P < 0.05$) between dietary CP and inulin supplementation on the apparent digestibility of ADF. Pigs offered the unsupplemented 140 g/kg CP diet had a significantly higher ADF digestibility compared to those with the inulin-supplemented 140 g/kg CP diet. However, there was no significant effect of inulin supplementation in the high-CP diet.

The excretion of faecal N, and the ratio of urine N : faeces N were significantly affected by the addition of inulin to the diets. Pigs offered inulin-supplemented diets had a higher excretion of faecal N (7.98 *v.* 6.22 g/day; s.e. 0.463; $P < 0.05$), and a lower ratio of urine N : faeces N (3.55 *v.* 4.75; s.e. 0.422; $P < 0.05$) compared to those with inulin-free diets.

**Table 2** *The effect of dietary crude protein and inulin inclusion on apparent nutrient digestibility and nitrogen balance (least-square means with s.e.)*

| | Crude protein level (g/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 200 | | 140 | | | Significance | | |
| Inulin supplementation | − | + | − | + | s.e. | Protein | Inulin | Protein × inulin |
| *n* | 4 | 4 | 4 | 4 | | | | |
| Weight (kg) | 74.0 | 73.5 | 74.5 | 74 | 2.60 | ns | ns | ns |
| Dry-matter intake (kg/day) | 2.07 | 2.05 | 2.07 | 2.09 | 0.046 | ns | ns | ns |
| Nitrogen intake (g/day) | 67.16 | 66.12 | 52.30 | 48.31 | 1.461 | *** | ns | ns |
| Digestibility coefficients | | | | | | | | |
| Dry matter | 0.897 | 0.888 | 0.908 | 0.897 | 0.006 | ns | ns | ns |
| Organic matter | 0.911 | 0.903 | 0.921 | 0.911 | 0.005 | ns | ns | ns |
| Neutral-detergent fibre | 0.675 | 0.657 | 0.629 | 0.530 | 0.024 | ** | * | ns |
| Acid-detergent fibre | 0.561 | 0.580 | 0.674 | 0.590 | 0.027 | * | ns | * |
| Hemicellulose | 0.744 | 0.730 | 0.564 | 0.465 | 0.025 | *** | * | ns |
| Nitrogen | 0.918 | 0.888 | 0.924 | 0.900 | 0.007 | ns | ** | ns |
| Gross energy | 0.892 | 0.885 | 0.902 | 0.890 | 0.006 | ns | ns | ns |
| Faeces dry matter (g/kg) | 0.306 | 0.268 | 0.299 | 0.301 | 1.849 | ns | ns | ns |
| Fresh faeces output (kg/day) | 0.741 | 0.963 | 0.667 | 0.765 | 0.093 | ns | ns | ns |
| Urine output (kg/day) | 3.474 | 3.260 | 2.255 | 2.303 | 0.250 | ** | ns | ns |
| Nitrogen (N) balance | | | | | | | | |
| Faecal N excretion (g/day) | 7.26 | 9.64 | 5.19 | 6.32 | 0.644 | ** | * | ns |
| Urinary N excretion (g/day) | 36.39 | 34.75 | 22.50 | 20.47 | 1.390 | *** | ns | ns |
| Total N excretion (g/day) | 43.65 | 44.39 | 27.68 | 26.79 | 1.380 | *** | ns | ns |
| N retention (g/day) | 23.51 | 21.73 | 24.62 | 21.51 | 1.421 | ns | ns | ns |
| N retention/intake | 0.35 | 0.32 | 0.47 | 0.45 | 0.024 | *** | ns | ns |
| Urine N : faeces N ratio | 5.14 | 3.87 | 4.35 | 3.23 | 0.532 | ns | * | ns |

Abbreviations are: s.e. = standard error, ns = non-significant ($P > 0.05$).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.



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A reduction in dietary CP level had a strong impact on the N balance data. Pigs offered the high-CP diets had an increased excretion of faecal N (8.45 *v.* 5.75 g/day; s.e. 0.472; $P < 0.01$), urinary N (35.57 *v.* 21.49 g/day; s.e. 0.999; $P < 0.001$), total N excretion (44.02 *v.* 27.23 g/day; s.e. 0.989; $P < 0.001$) and urinary output (3.37 *v.* 2.28 kg/day; s.e. 0.180; $P < 0.01$) compared to those with low-CP diets. Pigs offered the low-CP diets had an increased apparent N absorption coefficient (0.45 *v.* 0.33; s.e. 0.026; $P < 0.001$) compared to those with the high-CP diets.

*Microbiology study*

The effect of dietary treatment on selected microbial populations in the caecum and colon is presented in Table 3.

There was a significant interaction between dietary CP and inulin supplementation on the population of *Enterobacteria* spp. ($P < 0.05$) and *Lactobacilli* spp. in the caecum digesta ($P < 0.1$). Pigs offered the diet containing 200 g/kg CP plus inulin had a decreased population of *Enterobacteria* spp. compared to those with the unsupplemented 200 g/kg protein diet. However, pigs offered the inulin-supplemented 140 g/kg CP diet had an increased population of *Enterobacteria* spp. compared to those with the unsupplemented 140 g/kg CP diet. Pigs offered the inulin-supplemented 200 g/kg CP diet had a higher population of *Lactobacilli* compared to those with the unsupplemented 200 g/kg CP diet. However, there was no effect of inulin supplementation in the 140 g/kg CP diets.

Pigs offered inulin-supplemented diets had a significantly higher population of *Bifidobacteria* in the caecum than inulin-free diets (8.63 *v.* 8.25 log 10 c.f.u./g digesta; s.e. 0.095; $P < 0.01$).

The population of *Bifidobacteria* in the colon were significantly affected by dietary CP. Pigs offered diets containing 140 g/kg CP had a significantly higher population of *Bifidobacteria* in the colon than the 200 g/kg CP diet (8.82 *v.* 8.59 log 10 c.f.u./g digesta; s.e. 0.085; $P < 0.05$).

*Ammonia emission study*

The effect of dietary treatment on manure ammonia emissions and slurry pH during storage are presented in Table 4.

Pigs offered diets containing 140 g/kg CP had significantly lower ammonia emissions from 0 to 96 h (1.43 *v.* 2.37 g/day; s.e. 0.229; $P < 0.01$), 96 to 240 h (3.16 *v.* 5.31 g/day; s.e. 0.293; $P < 0.01$) and from 0 to 240 h (4.59 *v.* 7.68 g/day; s.e. 0.405; $P < 0.001$) than those offered the 200 g/kg CP diets. This equates to a 40% reduction in ammonia emissions over 10 days of storage by reducing the CP content by 60 g/kg.

There was no interaction ($P > 0.05$) between treatment and time on slurry pH over 240 h of storage. There was a significant effect of dietary CP on urine pH and slurry pH. Pigs offered diets containing 140 g/kg CP had a significantly lower slurry pH (8.92 *v.* 9.09; s.e. 0.044; $P < 0.05$) than those offered the high-CP diets.

*Volatile fatty acid study*

The effect of dietary treatment on the concentration and profile of caecal and colonic VFA is shown in Table 5.

There was no effect ($P > 0.05$) of dietary treatment on total VFA concentration and molar proportions of VFA in the caecum.

Pigs offered diets containing 140 g/kg CP had a lower proportion of butyric acid in the colon than pigs offered the 200 g/kg CP diets (0.13 *v.* 0.15; s.e. 0.005; $P < 0.05$).

**Table 3** *The effect of dietary crude protein and inulin inclusion on microbial ecology and pH in the caecum and colon (least-square means with s.e.)*

| | Crude protein level (g/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 200 | | 140 | | | Significance | | |
| Inulin supplementation | − | + | − | + | s.e. | Protein | Inulin | Protein × inulin |
| *n* | 6 | 6 | 6 | 6 | | | | |
| Caecum bacterial populations (log 10 c.f.u./g digesta) | | | | | | | | |
| *Enterobacteria* spp. | 7.47 | 6.90 | 7.32 | 8.03 | 0.329 | ns | ns | * |
| *Lactobacilli* spp. | 8.28 | 9.04 | 8.80 | 8.75 | 0.229 | ns | ns | † |
| *Bifidobacteria* spp. | 8.18 | 8.70 | 8.32 | 8.57 | 0.131 | ns | ** | ns |
| Colon bacterial populations (log 10 c.f.u./g digesta) | | | | | | | | |
| *Enterobacteria* spp. | 8.03 | 7.17 | 6.92 | 7.43 | 0.396 | ns | ns | ns |
| *Lactobacilli* spp. | 8.49 | 9.04 | 8.93 | 8.89 | 0.215 | ns | ns | ns |
| *Bifidobacteria* spp. | 8.48 | 8.70 | 8.83 | 8.80 | 0.118 | * | ns | ns |
| pH | | | | | | | | |
| Caecal pH | 5.76 | 5.62 | 5.65 | 5.78 | 0.118 | ns | ns | ns |
| Colonic pH | 5.99 | 5.86 | 5.81 | 5.84 | 0.096 | ns | ns | ns |

Abbreviations are: s.e. = standard error, ns = non-significant ($P > 0.05$).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
† = approaching significance ($P < 0.1$).



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**Table 4** *The effect of dietary crude protein and inulin inclusion on ammonia production and slurry pH (least-square means with s.e.)*

| | Crude protein level (g/kg) | | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | 200 | | 140 | | | | | |
| Inulin supplementation | − | + | − | + | s.e. | Protein | Inulin | Protein × inulin |
| *n* | 4 | 4 | 4 | 4 | | | | |
| Manure volume (kg/day) | 3.98 | 3.98 | 2.85 | 2.64 | 0.366 | ** | ns | ns |
| Faeces : urine ratio (w/w fresh) | 0.238 | 0.396 | 0.355 | 0.277 | 0.049 | ns | ns | * |
| Ammonia (g/day) | | | | | | | | |
| 0–96 h | 2.16 | 2.57 | 1.63 | 1.23 | 0.324 | ** | ns | ns |
| 96–240 h | 5.17 | 5.44 | 3.36 | 2.95 | 0.414 | *** | ns | ns |
| 0–240 h | 7.33 | 8.02 | 4.99 | 4.19 | 0.572 | *** | ns | ns |
| Slurry pH (0-240 h) | 9.15 | 9.02 | 8.91 | 8.93 | 0.06 | * | ns | ns |

Abbreviations are: s.e. = standard error, ns = non-significant ($P > 0.05$).
*$P < 0.05$), **$P < 0.01$, ***$P < 0.001$.

**Table 5** *The effect of dietary crude protein and inulin inclusion on total volatile fatty acids (VFA) concentration in digesta, molar proportions of VFA and pH in the caecum and colon (least-square means with s.e.)*

| | Crude protein level (g/kg) | | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | 200 | | 140 | | | | | |
| Inulin supplementation | − | + | − | + | s.e. | Protein | Inulin | Protein × inulin |
| *n* | 6 | 6 | 6 | 6 | | | | |
| Caecum | | | | | | | | |
| Total VFA (mmol/l digesta water) | 209.21 | 229.02 | 194.32 | 208.38 | 13.45 | ns | ns | ns |
| Acetic acid | 0.592 | 0.606 | 0.614 | 0.608 | 0.009 | ns | ns | ns |
| Propionic acid | 0.229 | 0.220 | 0.223 | 0.218 | 0.008 | ns | ns | ns |
| Isobutyric acid | 0.011 | 0.008 | 0.009 | 0.011 | 0.002 | ns | ns | ns |
| Butyric acid | 0.133 | 0.137 | 0.121 | 0.128 | 0.007 | ns | ns | ns |
| Isovaleric acid | 0.018 | 0.012 | 0.015 | 0.019 | 0.003 | ns | ns | ns |
| Valeric acid | 0.017 | 0.017 | 0.018 | 0.017 | 0.002 | ns | ns | ns |
| Acetic : propionic acid ratio | 2.60 | 2.79 | 2.76 | 2.82 | 0.120 | ns | ns | ns |
| Colon | | | | | | | | |
| Total VFA (mmol/l digesta water) | 220.37 | 243.55 | 220.87 | 228.52 | 11.24 | ns | ns | ns |
| Acetic acid | 0.580 | 0.577 | 0.594 | 0.597 | 0.009 | ns | ns | ns |
| Propionic acid | 0.214 | 0.207 | 0.223 | 0.209 | 0.006 | ns | * | ns |
| Isobutyric acid | 0.014 | 0.013 | 0.012 | 0.014 | 0.002 | ns | ns | ns |
| Butyric acid | 0.144 | 0.157 | 0.130 | 0.138 | 0.007 | * | ns | ns |
| Isovaleric acid | 0.026 | 0.025 | 0.022 | 0.024 | 0.003 | ns | ns | ns |
| Valeric acid | 0.021 | 0.021 | 0.020 | 0.018 | 0.002 | ns | ns | ns |
| Acetic : propionic acid ratio | 2.73 | 2.80 | 2.67 | 2.88 | 0.100 | ns | ns | ns |

Abbreviations are: s.e. = standard error, ns = non-significant ($P > 0.05$).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Pigs offered inulin-supplemented diets had a significantly lower proportion of propionic acid in the colon than inulin-free diets (0.21 *v.* 0.22; s.e. 0.004; $P < 0.05$).

## Discussion

The objective of the current experiment was to investigate the effect of dietary CP and inulin supplementation on nutrient digestibility, N excretion, intestinal microflora, VFA concentration and manure ammonia emissions. The hypothesis was that inulin supplementation of a high-CP diet would reduce urinary N excretion, enhance the proliferation of lactic acid-producing bacteria and reduce BCFAs and ammonia emissions compared with an unsupplemented high-CP diet. The presence of an interaction between dietary CP and inulin supplementation on the population of *Enterobacteria* spp. in the caecum, a positive



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effect of inulin supplementation on the population of *Bifidobacteria* would support the hypothesis that inulin supplementation can manipulate gut microflora in high-CP diets.

The results of the current study indicate a proportional decrease of 0.38 in total daily N excretion as dietary CP was reduced from 202 to 148 g/kg. This decrease in N excretion equates to a proportional reduction of 0.06 in N excretion per 10 g/kg reduction in dietary CP to 148 g/kg. These significant reductions were achieved without a negative effect on N retention, resulting in an increase in N absorption in the low-CP diets. Reductions previously reported in total N, urinary N (Canh *et al.*, 1998a; Carpenter *et al.*, 2004) and faecal N (Lee and Kay, 2003; Portejoie *et al.*, 2004; Leek *et al.*, 2005) are in line with those found in the current study. Carpenter *et al.* (2004) reported a proportional reduction of 0.06 in total daily N excretion per 10 g/kg reduction in dietary CP to 150 g/kg. Kerr and Easter (1995) concluded that for each one-percentage unit reduction in dietary CP combined with amino acid supplementation, total N excretion (faecal plus urinary) could be proportionally reduced by approximately 0.08.

Due to the reduction in faecal and urinary N excretion in this study, there was a significant reduction in pH and manure ammonia emissions. The reduction in manure volume was probably due to a lower water intake in pigs offered the low-CP diet compared to those with the high-CP diet; however, water intake was not measured in the current study. $NH_3$ losses during storage (0 to 240 h) were reduced by 40% by lowering the dietary composition of CP to 140 g/kg. This equates to 6.6% reduction in ammonia emission per day per 10 g/kg reduction in CP. Manure pH is determined by the level of urea hydrolysis, total ammoniacal nitrogen, the dietary electrolyte balance (dEB) and by the VFA concentration of the excreta (Canh *et al.*, 1998b). Only a minor reduction in pH is required to reduce ammonia emissions (O'Connell *et al.*, 2005). At a low pH, ammonia remains stable in the slurry as ammonium. However, at high pH more ammonia will be emitted as observed in the current study. Therefore, the reduction in ammonia concentration and pH of the manure due to reduced dietary CP and dEB resulted in lower ammonia being emitted from low-CP diets compared with high-CP diets. Leek *et al.* (2005) reported a 10.1% reduction in ammonia emissions per 10 g/kg reduction in dietary CP *in vitro* while Hayes *et al.* (2004) achieved an 8.1% reduction per 10 g/kg CP *in vivo*.

Dietary fibres and non-absorbable sugars are known to reduce blood $NH_3$ and serum urea levels (Gibson and Roberfroid, 1995). These effects have been associated with the growth of the colonic biomass and N fixation by colonic bacteria, coupled with colonic acidification and conversion of diffusible $NH_3$ into the less diffusible $NH_4^+$ ion (Gibson and Roberfroid, 1995). The reduction in the ratio of urinary N : faecal N due to inulin supplementation in the current study indicates that a decrease in manure ammonia would be likely. However, there was no response in ammonia emissions to inulin supplementation in the current study. This may be due to a number of reasons. Firstly, there are a number of factors that drive the volatilisation of $NH_3$ such as the equilibrium of ammonia with ammonium, pH, temperature and ammonia concentration (McCrory and Hobbs, 2001). The pH of slurry is of huge relevance to ammonia emissions from pig manure (Sommer and Husted, 1995; O'Connell *et al.*, 2005), with just a minor change having a substantial effect (Canh *et al.*, 1998a). Secondly, there may not have been enough inulin present to bring about a reduction in manure pH and manure ammonia emissions. An inclusion level of 12.5 g/kg was used in this study due to its beneficial effects on piglet health and performance reported in previous studies (Pierce *et al.*, 2005a and 2006a). However, Hansen *et al.* (2007) achieved a 33% reduction in ammonia emissions when inulin was included at a level of 150 g/kg.

Physiologically, fructo-oligosaccharides, like inulin, are classified as dietary fibre (Flamm *et al.*, 2001) resistant to complete enzymatic degradation in the small intestine. In contrast, Houdijk *et al.* (1999) found that fructo-oligosaccharide fermentation is nearly completely precaecal. However, results from the current study indicate that some proportion of inulin is not digested precaecally due to the significant changes in bacteria populations in the caecum and a reduction in the urine N : faeces N ratio. However, it is possible that the *Bifidobacteria* in the caecum could have been washed down from the ileum (Williams *et al.*, 2001). Pierce *et al.* (2005b) concluded that the ileum harbours enough microflora to ferment inulin, which resulted in the absence of an inulin effect on pH and VFA production in the large intestine of piglets. Unfortunately, neither microbial populations nor VFA production were measured from the ileum in the current study.

Inulin supplementation had no effect on total VFA concentration or digesta pH in either the caecum or colon in the current study. Rapid fermentation of fructo-oligosaccharides and inulin by indigenous microflora, specifically *Bifidobacteria*, results in the production of SCFAs, gases and organic acids (Gibson and Roberfroid, 1995). Previous authors have reported that inulin supplementation resulted in a higher capacity for absorption due to an increased proliferation of epithelial mucosa (Sakata, 1987; Howard *et al.*, 1993) and a higher percentage of ileum and caecal goblet cells (Chen *et al.*, 2005). If SCFAs are rapidly absorbed by the intestinal mucosa the concentration remaining in the digesta with potential to reduce pH is limited (Cummings *et al.*, 1987; Alles *et al.*, 1996). Other studies have also found no response in terms of intestinal pH or VFA concentration due to inulin supplementation (Gibson *et al.*, 1995; Kleessen *et al.*, 1997; Houdijk *et al.*, 1997 and 1998).

The depression in digestibility of NDF and hemicelluloses due to the decrease in dietary CP can be explained by differences in the soya-bean meal fraction between the high- and low-CP diets. There is an additional 150 g/kg of wheat in the low-CP diet compared with the high-CP diet.



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Soya-bean meal is a far more digestible ingredient than wheat with regard to the NDF fraction with each having a digestibility of 0.81 and 0.29, respectively (O'Doherty and Dore, 2001). The higher content of insoluble non-starch polysaccharide (NSP) (94 g/kg DM) (Bach Knudsen, 1997) in wheat compared with soya-bean meal (16 g/kg DM; Choct, 1997) accounts for the poor fibre digestibility of wheat. Therefore, as the inclusion level of soya-bean meal decreased and dietary wheat increased from high- to low-CP diets, respectively, it would be expected that the apparent digestibility of fibre fractions would decrease. These results are in agreement with those found by O'Connell *et al*. (2006) who observed a decrease in ADF and hemicellulose digestibility due to a decrease in dietary CP level.

Inulin supplementation caused a reduction in ADF digestibility at low protein levels. Inulin supplementation also caused a reduction in NDF digestibility. This depression in NDF digestibility was most pronounced in the low-protein diets (interaction, $P < 0.1$). It would seem that the reductions encountered in ADF and NDF digestibility with low-protein diets could be due to effects on the gut microflora. This is supported by the increased *Enterobacteria* spp. numbers in the caecum of the low-CP, inulin-supplemented pigs. The increase in the population of *Enterobacteria* spp. may be due to excessive quantities of carbohydrate entering the colon. When excessive quantities of carbohydrate enter the colon, the fermentative capacity of the pig may be exceeded (Soergel, 1994; Williams *et al*., 2001; Pierce *et al*., 2006b). This may be due to differences in diet formulation between the high- and low-CP diets. There is an additional 150 g/kg of wheat in the low-CP diet compared with the high-CP diet. Wheat contains a higher proportion of fermentable NSPs (arabinoxylan 60 g/kg) than soya-bean meal (arabinoxylan 42 g/kg) (Dierick and Decuypere, 1994). Pigs offered the low-CP, inulin-supplemented diets had a potential fermentable NSP (Dierick and Decuypere, 1994) intake of 141 g per pig per day (based on a daily feed intake of 2.09 kg) and pigs offered the low-CP diets had a potential fermentable NSP intake of 116 g/kg (based on a daily feed intake of 2.07 kg). Therefore, the increase in potentially fermentable NSP may have caused an over supply of fermentable substrate in the large intestine, resulting in the proliferation of *Enterobacteria* spp. Similar reductions in fibre digestibility were recorded by Pierce *et al*. (2006b) when excess fermentable carbohydrate (lactose) was offered to finisher pigs.

Also, Brunsgaard (1998) found that pigs offered a wheat-based diet had a greater presence of mannose and galactose residues compared with pigs offered a barley-based diet which are thought to be receptors for *Salmonella* spp. (Giannasca *et al*., 1996). The density of coliform bacteria has been reported to be a reliable indicator of the population of *Salmonella* in pigs (Mikkelsen *et al*., 2004), thus further emphasising the link between high dietary wheat and the occurrence of coliform bacteria in the hindgut.

Saccharolytic species of bacteria such as *Lactobacilli* spp. and *Bifidobacteria* spp. also take part in the breakdown of complex carbohydrates (Saylers, 1979). If carbohydrate fermentation is compromised (O'Doherty *et al*., 2005) *Enterobacteria* spp. may be allowed to proliferate. Unfortunately only *Lactobacilli* spp., *Bifidobacteria* spp. and *Enterobacteria* spp. were measured in the current study.

Pigs offered the diet containing 200 g/kg CP plus inulin had a decreased population of *Enterobacteria* spp. and a higher population of *Lactobacilli* spp. compared to those with the unsupplemented 200 g/kg protein diet. The results indicate that inulin is delivering more nutrients to the large intestine and increasing *Lactobacilli* spp. particularly at high CP concentrations. Lactic acid bacteria are believed to create a barrier against colonisation by coliform bacteria (Stewart *et al*., 1993) and the inclusion of inulin in the current study was seen to result in a proportional decrease in coliform numbers at high CP concentrations. The population of *Bifidobacteria* spp. in the colon were also affected by dietary CP indicating the detrimental effect of the products of protein fermentation on *Bifidobacteria* spp.

The increase in *Bifidobacteria* concentrations in the caecum due to inulin supplementation is an indication of improved gut health. It is well documented that fructo-oligosaccharides and inulin are selectively fermented by most strains of *Bifidobacteria* (Wang and Gibson, 1993; Bunce *et al*., 1995; Houdijk *et al*., 1997) through the production of β-fructosidases as demonstrated in pure culture (Wang, 1993).

### *Conclusions*

In conclusion, supplementary inulin reduced the urine N : faeces N ratio indicating that inulin may have a role to play in reducing excess N excretion. In the inulin-supplemented diets, at the high concentration of CP, *Enterobacteria* was significantly reduced compared with the low level of CP. *Bifidobacteria* concentrations in caecal digesta were significantly increased due to the inclusion of inulin. As a result, we can conclude that inulin can have an impact in high-CP diets to manipulate beneficially hindgut microflora. Reducing dietary CP from 202 to 148 g/kg can reduce excess N excretion by 38% and significantly reduce manure ammonia emissions by 40%.

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