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The application of low crude protein wheat-soyabean diets to growing and finishing pigs

2. The effects on nutrient digestibility, nitrogen excretion, faecal volatile fatty acid concentration and ammonia emission from boars

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Diets containing 132, 152, 183 and 206 g/kg crude protein (CP) were fed to growing and finishing boars to evaluate the effect on nutrient digestibility, N balance, faecal volatile fatty acids (VFA) and ammonia-N (NH_3–N) emission. Dietary CP concentration was adjusted by altering the ratio of wheat:soyabean meal. Lysine, threonine, tryptophan and total sulphur-containing amino acids were included in all diets at concentrations equivalent to that in the highest CP diet. All diets were formulated to provide 9.7 MJ/kg of net energy. Urine and faeces were collected from 16 boars (4 boars per treatment) housed in metabolism crates. Collections were performed at 72, 80 and 87 kg live weight. NH_3–N emission was measured over 10 days using a laboratory scale procedure. Reducing the concentration of dietary CP decreased N intake (linear, $P < 0.01$), the excretion of urinary N, ammoniacal N and total N (linear, $P < 0.001$; cubic, $P < 0.001$) and the emission of NH_3–N (linear, $P < 0.001$; cubic, $P < 0.01$). Total N excretion and NH_3–N emission decreased 8.7% and 10.1% per 10 g/kg reduction in dietary CP concentration between 205.6 and 131.9 g/kg, respectively. There was no interaction between dietary CP concentration and collection period. N balance differed between the

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collection periods and less NH_3–N was emitted at 87 kg than at 72 kg. Decreasing dietary CP reduced faecal VFA concentration (linear, $P < 0.05$) and the molar proportions of acetic and butyric acids (quadratic, $P < 0.01$).

Keywords: Ammonia; boars; low crude protein; pig manure; volatile fatty acids

Introduction

Nitrogen emission from pig production takes two forms. Firstly, organic N contained in manure is a major source of potential nitrate pollution (Beckwith *et al.*, 1998). Consequentially, manure N disposal is subject to limits introduced by Council Directive 91/676/EEC (European Council, 1991). However, pig manure can be a useful fertiliser if used correctly. Secondly, the volatilisation of N as ammonia from pig manure is of concern in relation to its environmental impact (Jongbloed and Lenis, 1992). Consequently, targets have been set for European countries to reduce ammonia emissions (United Nations Economic Commission on Europe, 1999). Loss of gaseous N represents an economic loss in terms of the 'fertiliser value' of the manure (Molloy and Tunney, 1983) and exposure to ammonia in pig houses has been reported to affect the behaviour, health and performance of pigs (Drummond *et al.*, 1980), and the health of humans living near or working in pig units (Schiffman, 1998).

Previous studies have indicated that reducing the crude protein (CP) concentration of the diet offers the potential to reduce N excretion (Gatel and Grosjean, 1992; Kerr, 1995) and ammonia emissions (Canh *et al.*, 1998a). Low CP diets have been associated with increased carcass fat (Cromwell, 1996) and previous reports indicated that decreasing the concentration of dietary CP diminished the carcass feed conversion ratio in boars and gilts (Leek *et al*., 2005).

Volatile fatty acids (VFA) are produced by the fermentation of carbohydrate (Topping and Clifton, 2001) and protein (Smith and MacFarlane, 1997) by intestinal microflora. Reducing dietary CP is reported to decrease the concentration (Shriver *et al.*, 2003) and affect the profile of VFA (Sutton *et al.*, 1996) in excreta. However, an increase in VFA production has also been reported in response to decreasing the dietary crude protein concentration (Otto *et al.*, 2003). It has been reported that volatile fatty acids are quantitatively the greatest contributors to manure odour and may provide an indication of manure odour offensiveness (Zhu and Jacobson, 1999).

The objective of the current study was to examine the effect of feeding wheat-soyabean diets containing different dietary CP concentration supplemented with synthetic amino acids on nutrient digestibility, N balance, faecal VFA concentration and *in vitro* ammonia emission during the finishing period.

Materials 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 of Animals Act 1976) Regulations, 1994.

Diets

Dietary composition and analysis is presented in Table 1. Diets A, B, C and D were formulated to contain 130, 160, 190 and 220 g/kg CP, respectively, using

Table 1. Diet ingredient inclusion and proximate analysis

	Diet			
	A	B	C	D
Formulated crude protein concentration (g/kg)	130	160	190	220
Ingredients (g/kg)				
Wheat	886.7	810.0	722.5	637.5
Soyabean meal	60.0	136.7	224.2	309.2
Soya oil	13.3	13.3	13.3	13.3
Limestone (fine)	15.3	20.0	25.3	30.0
Dicalcium phosphate	5.0	5.0	5.0	5.0
Salt	2.5	2.5	2.5	2.5
L-Lysine HCl	8.4	5.9	3.1	–
DL-Methionine	1.9	1.2	0.4	–
L-Threonine	3.4	2.3	1.0	–
L-Tryptophan	1.0	0.6	0.2	–
Trace mineral and vitamin premix[1]	2.5	2.5	2.5	2.5
Analysed composition (g/kg)				
Dry matter	876.2	868.6	877.3	868.5
Crude protein (N × 6.25)	131.9	151.7	183.0	205.6
Ash	37.3	44.6	49.2	54.7
Fibre	41.7	32.7	35.1	33.4
Crude oil	30.75	23.5	22.2	24.3
Neutral detergent fibre	131.6	110.4	118.8	118.3
Acid detergent fibre	40.2	42.5	41.0	46.3
Lysine	11.1	11.2	11.4	11.6
Methionine + cysteine (relative to lysine)	0.57	0.56	0.56	0.60
Threonine (relative to lysine)	0.66	0 .65	0.65	0.65
Tryptophan (relative to lysine)	0.18	0.17	0.18	0.18
Gross energy (MJ)	15.76	15.60	15.70	15.74
Calculated composition (g/kg)				
Calcium	6.9	8.0	7.9	8.0
Phosphorous (total)	5.2	5.7	5.5	5.5
Sodium	1.7	2.3	2.1	2.2
Chloride	4.7	4.5	3.9	3.4
Potassium	4.9	6.3	7.9	9.5
Salt	4.9	5.3	5.4	5.3
Dietary electrolyte balance[2] (mEq/kg)	125.5	180.6	237.3	291.5
Starch	509.1	472.9	427.1	382.6
Sugar	28.2	33.2	38.7	44.1
Non-starch polysaccharides[3]	97.2	110.0	122.0	123.8

[1] The premix (Devenish Nutrition, Belfast, N. Ireland) provided vitamins and minerals (per kg diet) as follows; 4.2 mg retinol, 0.07 mg cholecalciferol, 80 mg DL-alpha tocopherol, 120 mg copper as copper sulphate, 100 μg iron and ferrous sulphate, 100 μg zinc as zinc oxide, 0.3 μg selenium and sodium selenite, 25 μg manganese as manganous oxide and 0.2 μg iodine as calcium iodate on a calcium sulphate/calcium carbonate carrier.

[2] Calculated as ($K^+ + Na^+ - Cl^-$).

[3] Calculated as (organic matter – (crude fibre + crude oil + crude protein + starch + sugar)) (Canh *et al.*, 1998b).

standard feeding values for the ingredients (O'Grady, 1996). Downward adjustment of CP concentration was achieved by increasing the wheat:soyabean meal ratio. Diets were isocaloric and were formulated to an estimated net energy (NE) density of 9.7 MJ/kg. Diets were formulated to have a total lysine concentration of 11.0 g/kg. The dietary concentration of threonine, tryptophan and total sulphur-containing amino acids were maintained at 65, 20 and 60% of lysine, respectively, through the addition of synthetic amino acids. This was similar to the concentration of these amino acids provided in the diet with the highest CP (diet D) and was sufficient to satisfy the ideal protein requirement (van Lunen and Cole, 2001). Diets were milled and mixed on site and fed in meal form. A composite sample was collected during bagging off and retained for analysis.

Animals and housing

Sixteen finishing boars, the progeny of Landrace × Large White sows and meatline sires, were used in this experiment. Four boars were assigned to each dietary treatment. After a 14-day dietary adaptation period, pigs were weighed and transferred to individual metabolism crates. The pigs were allowed a further 5 days to adapt to the metabolism crates. The 7-day collection period was sub-divided into two parts to facilitate studies of apparent digestibility and N balance (days 1 to 5) and ammonia emission (days 6 to 7). Feed and water were offered *ad-libitum*. Collections were replicated at target live weights of 70, 80 and 90 kg, respectively, (C1, C2 and C3, respectively).

Apparent digestibility and N balance study

Urine was collected into a plastic container containing 50 ml of sulphuric acid (25% H_2SO_4), via a funnel below the crate, To avoid N volatilisation, the funnel was washed with weak sulphuric acid (2% H_2SO_4) solution. The urine volume was recorded daily and a 1% sample was collected and frozen for laboratory analysis. A sample of freshly voided faeces was collected daily and frozen. At the end of the collection period the faecal samples were pooled and a sub-sample retained for laboratory analysis. The remaining faeces were oven dried to a constant weight at 100 °C for 24 h.

Ammonia emission study

Four separate total collections of faeces and urine were taken at 12-h intervals over 48 h. 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 ratio in which they were produced to form a manure homogenate. A sample (2 kg) of the manure homogenate from each pig was placed in a sealed container, within a climate-controlled room maintained at 20 °C, fitted with three impingers connected in series. Each impinger contained nitric acid (1 mol/l). Ammonia emission from the manure was measured over 240 h according to the method of Derikx and Aarnink (1993). The contents of the first impinger were collected at 24, 48, 96, 144 and 192 h and replaced with nitric acid; the same was done for the second impinger at 96 h. All impingers were sampled at 240 h. The concentration of ammonia-N (NH_3-N) in the impinger contents was determined by the micro-diffusion technique of Conway (1957).

Analysis of samples

Analysis of diets for dry matter (DM) and ash was carried out according to the Association of Official Analytical Chemists (AOAC, 1995). Samples of feed and faeces were analysed for ether extract according to the Soxhlet method, using a Soxtec system (model 1043, Tecator,

Sweden). The gross energy (GE) of feed and faeces were measured using an adiabatic bomb calorimeter (Parr Instruments, IL, USA). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations in feed and faeces were analysed according to the method of van Soest (1976). Dietary crude fibre was analysed by the Weende method (AOAC, 1995) using a Fibertec system (1020 hot extractor; Tecator, Sweden). The dietary concentrations of lysine, threonine, tryptophan and total sulphur amino acids were determined by high performance liquid chromatography (Iwaki *et al*., 1987). The CP (N × 6.25) concentration of feed and the N concentration of the faeces, urine and manure (TKN) were analysed by the macro-Kjeldahl technique using a Buchii distillation apparatus. Total ammoniacal-N ($TAN = NH_3 + NH_4^+$) concentration of the manure was determined by distillation with MgO (Stevenson, 1982). The ammoniacal-N concentration of the urine samples was measured using a diagnostic kit (Randox Laboratories, N. Ireland) by the urease-Berthelot colorimetric method. The pH of the manure sample was measured using a digital hand-held pH meter (pHep5, Hanna Instruments Inc. RI, USA). Thawed faecal samples were analysed for VFA concentration and profile using the method of Leek *et al.* (2004). Analysis of all samples was performed in duplicate.

The digestible energy (DE) density of the diet was calculated from the GE of the feed and faeces. Using DE, the net energy (NE) density of the diet was calculated according to the equation of Noblet (1996): NE = (0.703 × DE) – (0.0041 × CP) + (0.0066 × ether extract) – (0.0041 × crude fibre) + (0.0020 × starch).

Statistical analysis

The data were analysed by repeated measures analysis using PROC MIXED of SAS (Littell *et al.,* 1996). The model included the linear, quadratic and cubic effects of dietary CP concentration and time (collection) and the associated interactions. Individual pigs were the experimental unit. Mean pH values are reported, However, as pH is a log scale, pH was expressed as the hydrogen ion concentration for data analysis.

Results

Apparent digestibility and N balance

One pig was removed from diet D in C3 due to not eating. Average daily gain was not affected by collection period or diet and pigs gained a mean 799 g (s.d. 120.8) live weight per day over the experimental period. Feed intake was higher in C2 than in C1 ($P < 0.01$), but was similar in C3 (2.17 *v.* 2.60 *v.* 2.40 kg/day, s.e. 0.105). Mean pig weight was 71.7, 80.2 and 87.1 kg (s.e. 1.14) during C1, C2 and C3, respectively.

The effect of collection period on apparent digestibility and N balance is presented in Table 2. Intake of NE and N increased between C1 and C2 ($P < 0.01$). The apparent digestibility of N, DM or GE was not affected by collection period. However, due to a numerically lower GE digestibility during C2, dietary NE concentration was also lower ($P < 0.05$). Faecal DM output increased between C1 and C2 ($P < 0.01$). Urine output and faecal DM were not significantly affected by collection period. The efficiency of N retention relative to N consumed or N absorbed was not affected by the collection period. However, N digestion and N retention were significantly higher in C2 than in C1 or C3 ($P < 0.05$). There was no significant relationship between N retention and live weight. Between C1 and C2, there was an increase in the daily excretion of urinary ammoniacal N

Table 2. The effect of balance period on digestibility and nitrogen balance in finishing boars

Variable	Collection period [1]			s.e.	Significance
	C1	C2	C3		
Live weight (kg)	71.7	80.2	87.1		
Dry matter digestibility (kg/kg)	0.897	0.888	0.888	0.0047	
Nitrogen digestibility (g/g)	0.883	0.876	0.877	0.0058	
Gross energy digestibility (MJ/MJ)	0.898	0.889	0.897	0.0047	
Dietary net energy (MJ/kg)	10.18^{b}	10.01^{a}	10.17^{b}	0.049	*
Net energy intake (MJ/day)	22.09^{a}	26.04^{b}	24.32^{ab}	1.030	*
Faecal dry matter (g/kg)	257.0	246.6	249.1	5.60	
Faeces dry matter output (g/day)	194.96^{a}	253.10^{b}	226.36^{ab}	13.83	*
Urine output (l/day)	3.38	3.99	3.77	0.291	
Nitrogen intake (g/day)	58.83^{a}	70.49^{b}	63.13^{ab}	2.846	*
Absorbed nitrogen (g/day)	52.12^{a}	61.57^{b}	55.19^{a}	2.463	*
Urinary nitrogen (g/day)	26.35	28.85	27.67	1.233	
Urinary ammoniacal nitrogen (g/day)	20.82^{a}	23.82^{b}	23.54^{ab}	1.070	*
Faecal nitrogen (g/day)	6.71^{a}	8.92^{b}	7.94^{ab}	0.561	*
Nitrogen excretion (g/day)	33.06^{a}	37.77^{b}	35.61^{ab}	1.594	*
Nitrogen retention (g/day)	25.77^{a}	32.72^{b}	27.52^{a}	1.930	*
Urinary ammoniacal nitrogen/urinary nitrogen excretion (g/g)	0.778	0.803	0.835	0.0143	*
Urinary nitrogen/faecal nitrogen (g/g)	4.01	3.32	3.77	0.235	
Nitrogen retention/nitrogen intake (g/g)	0.447	0.477	0.452	0.0172	
Nitrogen retention/absorbed nitrogen (g/g)	0.508	0.544	0.516	0.0183	

[1] C1, C2 and C3 correspond to periods when liveweight was expected to be 70, 80 and 90 kg, respectively.
[abc] Means, within rows, without a common superscript differ significantly ($P < 0.05$).

($P < 0.05$), faecal N ($P < 0.01$) and total N ($P < 0.05$).

The effect of dietary CP concentration on apparent digestibility and N balance are presented in Table 3. There were cubic effects of dietary CP concentration on the apparent digestibility of DM ($P < 0.05$), N ($P < 0.01$) and GE ($P < 0.05$), but the apparent digestibility of ADF and NDF were not affected by CP concentration. There were cubic effects of dietary CP concentration on the dietary NE density ($P < 0.001$) and NE intake ($P < 0.001$). Individual *t*-test comparisons indicated that the pigs fed diet A consumed a greater quantity of NE than the pigs fed the other diets ($P < 0.001$) and that the pigs fed diet C consumed a greater quantity of NE than the pigs fed either diets B or D ($P < 0.001$). Dietary CP concentration increased the faecal DM concentration (linear, $P < 0.001$; cubic, $P < 0.05$) and urine volume (cubic, $P < 0.01$). Faecal DM output exhibited a cubic response to dietary CP concentration ($P < 0.05$).

N intake decreased (linear, $P < 0.001$) as dietary CP concentration was reduced. Decreasing the dietary CP concentration from 205.6 to 131.9 g/kg reduced the daily amount of N digested (linear, $P < 0.001$), urinary N output (linear, $P < 0.001$; cubic, $P < 0.001$), urinary ammoniacal N output (linear, $P < 0.001$; cubic, $P < 0.001$) and N excretion (linear, $P < 0.001$; cubic, $P < 0.001$). Faecal N output was affected by a cubic response to dietary CP concentration

Table 3. The effect of diet on digestibility and nitrogen balance in finishing boars

Variable	Diet				s.e.
	A	B	C	D	
Feed intake (kg/day)	2.38	2.37	2.36	2.40	*0.016*
Dry matter digestibility (kg/kg)	0.898	0.886	0.898	0.884	0.0057
Nitrogen digestibility (g/g)	0.870	0.864	0.903	0.877	0.0071
Gross energy digestibility (MJ/MJ)	0.894	0.888	0.907	0.889	0.0056
Dietary net energy concentration (MJ/kg)	10.51	10.07	10.13	9.78	0.063
Net energy intake (MJ/day)	25.07	23.99	24.09	23.20	0.161
Faecal dry matter (g/kg)	220.7	250.3	255.5	277.3	6.25
Faeces dry matter output (g/day)	214.00	235.69	204.17	242.01	11.385
Urine output (l/day)	1.75	3.47	3.73	5.80	0.282
Nitrogen intake[b] (g/day)	50.36	57.43	69.17	79.23	0.603
Absorbed nitrogen[b] (g/day)	43.90	49.59	62.15	69.26	0.636
Urinary nitrogen[be] (g/day)	12.68	26.13	28.38	42.99	1.165
Urinary ammoniacal nitrogen[be] (g/day)	9.39	21.34	22.91	36.87	0.342
Faecal nitrogen[d] (g/day)	6.47	7.84	7.02	9.97	0.486
Nitrogen excretion[be] (g/day)	19.14	33.97	35.40	52.97	1.276
Nitrogen retention (g/day)	31.22	23.46	33.76	26.26	1.297
Urinary ammoniacal nitrogen/urinary nitrogen[c] (g/g)	0.741	0.820	0.806	0.852	0.0173
Urinary nitrogen/faecal nitrogen[bf] (g/g)	2.04	3.45	4.65	4.69	0.301
Nitrogen retention/nitrogen intake[e] (g/g)	0.618	0.402	0.449	0.327	0.0193
Nitrogen retention/absorbed nitrogen[e] (g/g)	0.710	0.467	0.542	0.374	0.0214

[a]Linear effect of crude protein ($P < 0.05$).
[b]Linear effect of crude protein ($P < 0.001$).
[c]Cubic effect of crude protein ($P < 0.05$).
[d]Cubic effect of crude protein ($P < 0.01$).
[e]Cubic effect of crude protein ($P < 0.001$).
[f] Quadratic effect of crude protein ($P < 0.05$).

(cubic, $P < 0.01$). Individual *t*-tests indicated lower ($P < 0.01$) N retention with diets B and D compared to diets A and C. Lower proportions of consumed N and absorbed N were retained with diet D than diet A ($P < 0.01$), but the relationships with dietary CP concentration were cubic ($P < 0.001$). The proportion of urinary N excreted as ammoniacal N responded cubically to dietary CP concentration ($P < 0.05$). Decreasing the dietary CP concentration reduced the urinary N:faecal N ratio (linear, $P < 0.001$; quadratic < 0.01). The excretion of urinary ammoniacal N (*y*, g/day) was related to N intake (*x*, g/day) as:

$$y = -12.64\ (\pm 3.651) + 0.549\ (\pm 0.0555)\ x,\ (R^2\ 0.68)$$

The results of faecal VFA analysis are shown in Table 4. Decreasing dietary CP concentration reduced the concentration of VFA in the faeces ($P < 0.05$). The molar proportions of acetic acid and butyric acid exhibited a quadratic response ($P < 0.01$) to dietary CP concentration. The proportion of acetic acid increased as dietary CP concentration was raised to 183 g/kg and

Table 4. The effect of diet on faecal volatile fatty acid concentration

	Diet[1]				s.e.
	A	B	C	D	
Total volatile fatty acid concentration (mmol/l)[a]	131.83	141.60	149.21	185.03	12.885
Molar proportion of total volatile fatty acid concentration (mmol/100 mmol)					
Acetic acid[b]	47.85	53.93	55.46	51.43	1.619
Propionic acid	25.22	23.48	23.89	22.53	1.087
Isobutyric acid	2.74	3.34	2.49	3.12	0.382
Butyric acid[b]	18.32	15.70	14.46	16.81	0.834
Isovaleric acid	3.96	3.34	2.90	4.82	0.772
Valeric acid	1.91	0.21	0.80	1.29	0.596
Acetic acid:propionic acid	1.93	2.31	2.35	2.28	0.138
Acetic acid:butyric acid[b]	2.63	3.45	3.87	3.13	0.239

[1] See Table 1 for diet definitions.
[a] Linear effect of crude protein ($P < 0.05$).
[b] Quadratic effect of crude protein ($P < 0.01$).

conversely, the proportion of butyric acid decreased as dietary CP concentration was raised to 183 g/kg. Consequently, the ratio between acetic acid and butyric acid was affected by dietary CP concentration (quadratic, $P < 0.01$). Individual *t*-tests indicated that the ratio between acetic acid and propionic acid was higher ($P < 0.05$) in diet C than in diet A.

Ammonia emission study

The effect of collection period on manure production, composition and ammonia emission is shown in Table 5. The DM concentration of fresh manure increased ($P < 0.01$) between C1 and C3. The TKN concentration of the manure was not significantly affected by collection period. Manure collected during C2 had a lower TAN concentration than manure collected during C1 and C3 ($P < 0.05$). Manure collected during C1 had a higher pH than manure collected during C2 and C3 ($P < 0.05$). Less NH_3–N was emitted from manure collected during C3 than from manure collected during C1 ($P < 0.05$).

Table 5. The effect of collection period on output and composition of manure and on ammonia emission

Variable	Collection period[1]			s.e.	Significance
	C1	C2	C3		
Manure output (g/day)	4888.7	4616.3	4347.5	271.10	
Dry matter (DM, g/kg)	66.6[a]	80.6[b]	92.4[c]	3.69	***
Total Kjeldahl nitrogen (g/kg DM)	118.4	112.5	105.3	5.82	
Total ammoniacal nitrogen (g/kg DM)	64.1[b]	49.9[a]	64.8[b]	4.27	*
pH[2]	8.34[a]	6.79[b]	7.19[b]	–	*
Ammonia nitrogen (g/day collected over 240 h)	0.65[a]	0.58[ab]	0.53[b]	0.036	*
Ammonia nitrogen (mg/g nitrogen intake)	108.16[a]	78.33[b]	78.46[b]	6.093	**

[1] See footnote Table 2.
[2] Measurements were converted to hydrogen ion concentration before statistical analysis, the s.e. on this scale was 50.27 nmol/l (Day 0) and 1.08 nmol/l (Day 10).
[abc] Means, within rows, without a common superscript differ significantly ($P < 0.05$).

The effect of dietary CP concentration on manure composition and ammonia emission are shown in Table 6. The daily volume of manure production was related cubically to dietary CP concentration ($P < 0.001$). The volume of manure produced increased as dietary CP increased from 131.9 to 151.7 g/kg and between 183.0 and 205.6 g/kg. Decreasing dietary CP concentration increased the DM (linear, $P < 0.001$) and reduced the TKN and TAN (linear, $P < 0.001$) in the manure. Manure pH exhibited a quadratic response to dietary CP concentration ($P < 0.001$). Manure pH was highest in the diets containing 183 and 205.6 g/kg CP and decreased as the dietary CP concentration was reduced. Reducing dietary CP concentration decreased NH_3-N emission per pig per day (linear, $P < 0.001$; cubic, $P < 0.01$). Emission of NH_3-N per milligram of N intake decreased (linear, $P < 0.001$; quadratic $P < 0.001$) as the concentration of dietary CP was reduced. The relationship between NH_3-N emission (y, g/day) and the urinary ammoniacal N output (x, g/day) is described by the following equation:

$$y = 0.0229\ (\pm\ 0.005)\ x,\ (R^2\ 0.67).$$

Discussion

The absence of any treatment × period interaction indicates that the pigs responded similarly to all levels of dietary CP concentration during the different collection periods. As the ratio between N retention and absorbed N remained similar during the three collection periods, the high rate of N retention during C2 appears to be associated with higher feed intake and, consequently, N intake during this collection period. Whilst the decrease in feed intake between C2 and C3 was not significant, the result does suggest a curvilinear increase in intake as the pigs grew. This is consistent with the description of feed intake by Siebrits *et al.* (1986). These authors describe feed intake as an allometric function of growth rate and the rate at which protein and fat are deposited in the body. The results of the current study suggest that the peak rate of both net energy intake and N retention occurred between 71.7 kg and 87.1 kg live weight. This pattern concurs with the results of a previous study (Leek, 1999) in which it was estimated that pigs from the same genetic population achieved a maximum rate of N retention between 65 and 80 kg.

Table 6. The effect of diet on output and composition of manure and on ammonia emission

	Diet[1]				s.e.
	A	B	C	D	
Manure output (g/day)	3093.1	4456.3	4122.1	6801.7	318.61
Dry matter[a] (DM, g/kg)	98.8	79.7	73.8	61.6	4.86
Total Kjeldahl nitrogen[a] (g/kg DM)	77.9	105.6	121.3	144.7	6.87
Total ammoniacal nitrogen[a] (g/kg DM)	30.1	46.9	78.2	84.3	5.56
pH[2]	6.52	8.47	9.19	9.18	–
Ammonia nitrogen[ab] (g/day collected over 240 h)	0.25	0.55	0.57	0.97	0.054
Ammonia nitrogen[ac] (mg/g nitrogen intake)	52.83	89.94	92.45	118.04	7.125

[1]See Table 1 for diet definitions.
[2]Measurements were converted to hydrogen ion concentration before statistical analysis. The s.e. on this scale was 58.03 nmol/l.
[a]Linear effect of crude protein ($P < 0.001$).
[b]Cubic effect of crude protein ($P < 0.01$).
[c]Quadratic effect of crude protein ($P < 0.001$).

The lowest rates of N retention occurred with diets B and D and low apparent digestibilities of N, dry matter and gross energy were recorded with these treatments. This effect was observed during the three collection periods. These results were unexpected and it is not possible to give a reason. However, due to the low rate of N retention in diet B, N excretion was perhaps higher than may have been expected. This introduced a cubic response to dietary CP concentration for several measurements, i.e. N excretion, urinary N, manure volume and ammonia emission, where the values obtained for diet B were similar to those of diet C.

There was an 8.7% reduction in N excretion per 10 g/kg reduction in dietary CP concentration. This is similar to the 8.4% reduction in N excretion per 10 g/kg reduction in dietary CP reported by Kerr (1995) in a review of results from 28 experiments. Due to the positive relationship between urea excretion and ammonia emission (Aarnink, Hoeksma and van Ouwerkerk, 1993), the extent to which dietary CP concentration affected both urinary ammoniacal N output and the daily rate of NH_3-N emission was similar (10.1 *v.* 10.1% per 10 g/kg). The reduction in ammonia emission was greater than the decrease of 8.1% per 10 g/kg observed when similar diets were fed to conventionally housed boars and gilts (Hayes *et al.*, 2004). The results indicate that decreasing the concentration of dietary CP resulted in a larger reduction of ammonia emission than N excretion. Ammonia volatilisation is positively influenced by manure pH and the ammonium concentration of the manure (Canh *et al.*, 1997, 1998b) and the effect of dietary CP concentration on these factors is likely to have affected ammonia emissions. Additionally, Perez-Laspiur *et al.* (2002) reported that the activity of faecal urease, the enzyme responsible for the hydrolysis of urea to ammonium, declines as dietary CP is reduced. Thus, reduced urease activity may contribute to a reduction in NH_3-N emission as dietary CP concentration is reduced.

Manure pH responded quadratically as the concentration of dietary CP decreased and the pH dropped when the concentration of dietary CP decreased below 183.0 g/kg. Manure pH is determined by the level of urea hydrolysis, TAN, the dietary electrolyte balance (dEB) and by the VFA concentration of the excreta (Canh *et al.*, 1998b). Therefore, the combined influence of these factors may explain the quadratic response. A high VFA concentration in the manure from pigs on diet D may explain why the manure pH for diet D was not higher than that of diet C. A high pH had been expected in the former diet, due to the positive influence of a high dEB and a greater concentration of TAN.

Increasing dietary CP concentration is reported to increase water consumption (Pfeiffer *et al.*, 1995). Higher water intake appears to be reflected in this study by the increase in urine volume and manure volume in response to increasing dietary CP concentration. Consequently, manure dry matter decreased as dietary CP concentration increased, which lessened the effect of reducing dietary CP concentration on manure N concentration and may have had a negative influence on NH_3-N emission. However, increases in manure volume will increase the required manure storage capacity and the cost of disposal. Higher water intakes may also be a response to increasing dEB (Patience and Chaplin, 1997), although decreased faecal dry matter is also expected (Dersjant-Li *et al.*, 2001). Indeed, there was a linear increase in faecal dry matter with increasing dietary CP content. VFAs stimulate

colonic fluid and electrolyte transport (Topping and Clifton, 2001) and greater VFA concentrations associated with increasing dietary CP may be responsible for the increase in faecal DM.

Although the diets were formulated to contain a similar level of NE, the results of this study indicate that there were differences in NE intake. The differences arise when, in contrast to using theoretical values, the results of actual dietary analysis and energy digestibility are used in the calculation of NE, which reflect the analysed composition and measured gross energy digestibility of the individual diets. Consequently, pigs fed diet A exhibited the highest NE intake which was mainly due to a higher energy digestibility than had been predicted when the diet was formulated. The result is consistent with the increased backfat depth and lower lean meat proportion reported in the previous study (Leek *et al.*, 2005) when pigs were fed a similar diet containing 131.6 g/kg CP.

The supply of nitrogenous substrates affects the fermentative activity of the intestinal microflora and the production of VFA in the pig (Marounek *et al*., 2002). Whilst acetic acid, propionic acid, butyric acid and valeric acid are the products of both protein and carbohydrate fermentation in the intestine, the branched-chain VFAs, i.e. isobutyric acid and isovaleric acid, are uniquely produced by protein fermentation (Smith and MacFarlane, 1997). Surprisingly, our results did not detect a change in the proportion of the branched-chain VFAs. As they are poorly utilised as energy substrates by the pig, branched-chain VFA may be excreted via the urine. However, urinary excretion of VFA was not quantified in this study. Sutton *et al.* (1996) did not observe a change in the concentration of branched-chain VFA in freshly excreted manure in response to low-CP diets.

Dietary factors other than CP concentration also affect the production and profile of VFA. Shriver *et al.* (2003) demonstrated the contribution made by the fermentation of dietary fibre irrespective of dietary CP concentration. The apparent digestibility of both NDF and ADF was similar between the diets. This suggests that the observed reduction in total faecal VFA concentration was attributable to reduced fermentation of protein rather than fibre, as dietary CP concentration decreased. However, differences in the fermentation of other dietary components not included in the ADF or NDF measurements, i.e. non-starch polysaccharides and resistant starch, may have occurred as the dietary inclusion of wheat and soyabean meal were adjusted to manipulate the CP concentration. Soyabean non-starch polysaccharides are highly fermentable (Just, 1982) and, consequently, lower inclusion of soyabean meal as dietary CP concentration was reduced may have contributed to the decreased concentration of total VFA. Replacing the soyabean meal with wheat increased the starch concentration of the diet. It is likely that more starch enters the hindgut as consumption increases and the subsequent fermentation of this starch by the hindgut microflora may promote the production of butyric acid (Topping and Clifton, 2001). Indeed, Otto *et al.* (2003) reported an increase in the faecal concentration of butyric acid, isobutyric acid, valeric acid and isovaleric acid when soyabean meal was replaced with maize starch in low-CP diets. Thus, changes in the supply of fermentable dietary carbohydrate in addition to the level of dietary CP are likely to affect the VFA profile, resulting in a quadratic response to CP concentration.

The decrease in faecal VFA concentration represents a 28.8% reduction between

diets containing 205.6 and 131.9 g/kg CP. In an earlier study, Hayes *et al.* (2004) reported that odour emission from conventionally housed pigs fed diets containing similar dietary CP concentrations decreased by 24.5%.

Decreasing dietary CP offers a means of reducing both atmospheric-N and organic-N outputs. Use of low-CP diets could assist pig producers in meeting the requirements of environmental legislation. Reducing the dietary CP concentration may also provide some additional benefit in reducing the volume of manure produced.

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