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Effects of dietary crude protein level on odour from pig manure

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*The objective of this study was to determine the effects of dietary crude protein (CP) level on odour emission, odour intensity, hedonic tone, and ammonia emission from pig manure and on manure composition (pH, total nitrogen, ammonium, volatile fatty acids, indolic, phenolic and sulphur-containing compounds). An experiment was conducted with growing pigs (*n *= 18) in a randomised complete-block design with three treatments in six blocks. Treatment groups were 12%, 15% and 18% CP diets. Barley was exchanged for soya-bean meal. Crystalline amino acids (AA) were included in the 12% CP diet up to the level of pigs' requirement; the same amount of AA was added to the 15% and 18% CP diets. Pigs with an initial body weight (BW) of 36.5 ± 3.4 kg (mean ± s.d.) were individually penned in partly slatted floor pens and offered a daily feed allowance of 2.8 × maintenance requirement for net energy (NE: 293 kJ/kg* $BW^{0.75}$*). Feed was mixed with water, 1/2.5 (w/w). Faeces and urine of each pig were accumulated together in a separate manure pit under the slatted floor. After an adaptation period of 2 weeks, the manure pits were cleaned and manure was collected. In the 5th week of the collection period, air samples for odour and ammonia analyses, and manure samples were collected directly from each manure pit. Air samples were analysed for odour concentration and for hedonic value and intensity above odour detection threshold. Manure samples were analysed for volatile fatty acids, and indolic, phenolic and sulphurous compounds, ammonium and total nitrogen concentrations. Reducing dietary CP from 18% to 12% lowered odour emission (*$P < 0.05$*) and ammonia emission (*$P = 0.01$*) from pig manure by 80% and 53%, respectively. Reduced dietary CP decreased total nitrogen, methyl sulphide, carbon disulphide, ethanethiol, phenol, 4-ethyl phenol, indole and 3-methyl indole concentrations in the manure (*$P < 0.05$*). Volatile fatty acids and cresols concentrations in the manure of pigs fed different dietary CP levels were similar. A reduction of dietary CP and at the same time providing essential AA is an option to reduce odour emission as well as ammonia emission from pig manure.*

Keywords: diet, odour, pigs, protein

Introduction

Odour emission from pig production facilities causes serious nuisance in the surrounding areas, and should therefore be reduced. Odour is mainly generated by the microbial conversion of feed in the intestinal tract of pigs and by microbial conversion of pigs' excreta under anaerobic conditions in manure storages. A great number of odorous compounds have been identified from animal production facilities. O'Neill and Phillips (1992) summarised 168 compounds in livestock wastes or in the surrounding air. Recently, Schiffman *et al.* (2001) identified a total of 331 different compounds in the air and lagoon water from pig production facilities. They can be classified into four main groups: (1) sulphurous compounds, (2) indolic and phenolic compounds, (3) volatile fatty acids (VFA), (4) ammonia and amines. Many odorous compounds are intermediate or end products of protein degradation (Mackie *et al.*, 1998; Le *et al.*, 2005b). Therefore, protein is an important dietary compound that could be altered to reduce odour emission.

Diets generally contain more protein than the pigs' requirement. The main reason is that the amino acids (AA) composition of dietary protein from feeds does not match the animal's requirement and thus the dietary levels are formulated to supply the minimum level of the most essential and limiting AA. This gives a surplus of other AA in the diet. Usually a large part of dietary protein and its metabolites is excreted via urine and faeces (Jongbloed and Lenis, 1992; Van der Peet-Schwering *et al.*, 1999). Reducing protein or nitrogen (N) concentration in excreta

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decreases the availability of substrates that microbes can metabolise to odorous compounds. It is clear from the literature that ammonia from animal production facilities can be decreased considerably by reducing the amount of protein in the diet and at the same time supplementing most essential AA to balance AA pattern and to maintain animal performance (Hobbs *et al*., 1996; Canh *et al*., 1998b; Zervas and Zijlstra, 2002; Portejoie *et al*., 2004). However, in the case of odour strength and offensiveness the situation is not so straightforward.

Up to now, scientists mainly focused on certain specific odorous compounds in the manure or in the odorous air, e.g. Hobbs *et al.* (1996) and Sutton *et al.* (1998). Little attention has been given to the effect of dietary crude protein (CP) levels on odour emission measured by olfactometry. Odour measured by olfactometry is evaluated through its strength (odour concentration and odour intensity) and offensiveness (odour hedonic value). In this study we measured odour concentration according to the European standard (CEN standard 13 725, 2003). Odour intensity and odour hedonic tone was measured according to The Netherlands standard (NVN 2818, 2005). The main objective of this study was to determine the effects of dietary CP levels on odour strength, odour offensiveness, and ammonia emission from manure of growing pigs and on manure characteristics.

Material and methods

Animals, experimental design and diets

A randomised complete-block arrangement with three treatments in six blocks was used to study effects of dietary CP level on odour concentration, odour emission, odour intensity, odour hedonic tone, and ammonia emission from growing pig manure and on manure characteristics (pH, and concentrations of total N, ammonium, VFA, and indolic, phenolic and sulphurous compounds). There were three dietary CP levels: 12, 15, and 18%. Each treatment was replicated six times, one replicate in each of six blocks, of which a block consisted of samples collected on the same day and from animals with similar initial body weight (BW).

In total 18 growing barrows, Great Yorkshire × (Great Yorkshire × Dutch Landrace) with an initial BW of 36.5 ± 3.4 kg (mean ± s.d.) were randomly allocated to one of the three diets within each of the six blocks. Pigs were penned individually in galvanised steel pens (2.1 × 0.96 m) with a slatted floor at the rear (0.97 × 0.96 m). There was a separate manure pit under the slatted floor of each pen. The volume of the manure pit was 1.35 × 0.91 × 0.36 m (length × width × depth). Pigs were penned in a mechanically ventilated and temperature-controlled room. Temperature and relative humidity were recorded every 5 min. The average temperature and relative humidity of the room during the experimental period were 21.0°C ± 0.84 and 50.0% ± 5.32 (mean ± s.d.), respectively.

Three diets with dietary CP levels of 12, 15 and 18% were formulated. Barley was exchanged for soya-bean meal to increase the CP level. Diets had similar contents of net energy (NE), minerals, and vitamins but differed in CP levels by varying the amount of extracted soya-bean meal (Tables 1 and 2). Diets were supplemented with essential AA, e.g. Lys, Trp, Thr and Met. The method of supplementing AA was as follows: first, we supplemented the 12% CP diet with these mentioned AA up to the level of animal requirement based on apparently ileal AA digestibility (Centraal Veevoederbureau, 2004). Then, we supplemented 15 and 18% CP diets with the same amount of these AA as supplemented in the 12% CP diet. This was done to study solely the effect of the CP levels and prevent confounding effects with AA supplementation. Other constituents, for example, fibrous components, and dietary electrolyte balance (dEB), which could possibly affect odour production, were equalised for the same reason.

Experimental diets were analysed for AA composition, ash, DM, CP, minerals, crude fibre, fat, starch, sugar and gross energy. The AA (except Met, Cys, and Trp) were assayed by ion-exchange column chromatography after hydrolysis for 23 h in HCl (6 mol/l). Cys and Met were determined as cysteic acid and methionine sulfone after oxidation with performic acid before hydrolysis (Schram *et al*., 1954). Trp was determined according to Sato *et al.* (1984).

Starch content was determined enzymatically according to the amyloglucosidase/hexokinase method (NEN 3574). Sugar was assayed according to the non-starch polysaccharides (NSP) procedure (Gelder *et al*., 1992). Crude fibre

Table 1 *Ingredient composition of experimental diets (g/kg as-fed basis)*

	Diet		
	12% CP	15% CP	18% CP
Barley	489.8	386.0	282.3
Tapioca meal (starch 62.5 to 65.7%)	300.0	300.0	300.0
Soya-bean meal extracted (crude fibre < 5%)	113.0	192.8	272.5
Wheat middlings	14.8	38.4	62.0
Cane molasses (sugar < 47.5%)	30.0	30.0	30.0
Potasium carbonate	5.7	2.9	0.0
Soya-bean oil	15.2	19.4	23.6
Calcium carbonate	9.9	9.8	9.7
Monocalcium phosphate.H_2O	7.1	6.3	5.5
Salt	4.9	4.9	4.9
Pre-mix[†]	2.0	2.0	2.0
L-Lysine HCl	4.1	4.1	4.1
DL-Methionine	1.5	1.5	1.5
L-Threonine	1.5	1.5	1.5
L-Tryptophan	0.4	0.4	0.4

[†] The vitamin-mineral pre-mix supplied per kg feed included 7000 IU vitamin A, 1700 IU vitamin D3, 20 IU vitamin E, 1.5 mg vitamin K, 1.5 mg vitamin B1, 4 mg vitamin B2, 11 mg D-pantothenic acid, 18 mg niacin, 18 μg vitamin B12, 0.1 mg folic acid, 1.0 mg vitamin B6, 100 mg choline chloride, 75 mg Fe, 10 mg Cu, 65 mg Zn, 30 mg Mn, 0.15 mg Co, 0.75 mg I, 0.30 mg Se.

Table 2 *Nutrient composition of experimental diets (as-fed basis)*

	Unit	Diet		
		12% CP	15% CP	18% CP
Calculated composition				
Crude protein (CP)	g/kg	120	150	180
Faecal digestible CP	g/kg	94.8	124.1	153.3
Ileal digestible CP	g/kg	89.2	115.8	142.3
Net energy	MJ/kg	9.1	9.1	9.1
NSP[†]	g/kg	173.1	179.9	186.7
Digestible NSP	g/kg	89.6	99.7	109.9
Ileal digestible amino acids				
Lys	g/kg	7.4	9.3	11.2
Met	g/kg	2.9	3.2	3.6
Cys	g/kg	1.5	1.8	2.2
Met + Cys	g/kg	4.4	5.1	5.8
Thr	g/kg	4.3	5.3	6.4
Trp	g/kg	1.4	1.8	2.1
Analysed composition				
Dry matter	g/kg	868.0	860.4	871.5
CP (N × 6.25)	g/kg	122.8	142.4	180.3
Ash	g/kg	63.8	60.3	63.7
Crude fibre	g/kg	32.6	35.6	34.5
Crude fat	g/kg	19.9	23.7	38.8
Gross energy	MJ/kg	15.42	15.45	16.00
Sugar	g/kg	57.1	59.9	64.9
Starch	g/kg	410.7	379.8	348.5
NSP	g/kg	193.6	194.3	175.3
Na	g/kg	2.0	1.9	2.0
K	g/kg	10.6	10.4	10.7
Sulphate	g/kg	1.1	1.1	1.1
Chlorine	g/kg	5.2	4.6	4.6
dEB[‡]	meq/kg	213	220	227
dEBS-a[§]	meq/kg	190	196	204
Amino acids				
Ala	g/kg	5.9	6.6	8.5
Arg	g/kg	7.0	9.0	12.2
Asp	g/kg	10.4	13.5	19.5
Cys	g/kg	1.8	1.9	2.5
Glu	g/kg	22.0	25.1	33.6
Gly	g/kg	5.3	6.2	7.6
His	g/kg	4.9	5.4	7.2
Ile	g/kg	4.6	5.7	7.7
Leu	g/kg	8.2	9.7	13.0
Lys	g/kg	8.3	9.9	12.5
Met	g/kg	2.7	3.0	3.4
Phe	g/kg	5.8	7.0	9.1
Pro	g/kg	7.3	7.9	10.0
Ser	g/kg	6.0	7.3	9.0
Thr	g/kg	5.5	6.4	7.3
Trp	g/kg	1.7	2.0	2.5
Tyr	g/kg	4.0	5.0	6.7
Val	g/kg	5.8	6.8	8.9

[†] Non-starch polysaccharides (NSP) were calculated as organic matter − (CP + crude fat + starch + sugar).
[‡] dEB was determined as mEq = Na + K−Cl.
[§] dEBS-a was determined as mEq = Na + K−Cl−2S. dEBS-a does not take into account S present in AA.

was determined gravimetrically after treatment with sulphuric acid and potassium hydroxide (ISO/DIS 6895). For total fat, samples were hydrolysed with chloric acid, followed by extraction of fat with petroleum ether. The Ca, P, Mg, Na, K, Cu and Zn contents were determined using the inductively coupled plasma atomic emission spectrometry (ICP-AES). The Cl content was determined by potentiometric titration of water-diluted solid samples with the chloride specific ion electrode (Jenway Chloride Meter, model PCLM3). For sulphate, samples were extracted with chloric acid. Sulphate was separated with ion chromatography using a water/sodium hydroxide gradient and an Ionpac AS 11 (Dionex) as column. Detection takes place by suppressed electric conductivity. Identification and quantification occur using an external standard solution. The DM was determined gravimetrically after 4 h at 103°C (ISO 6496). The content of ash was determined gravimetrically after ashing at 550°C (ISO 5984). Nitrogen content was determined by the Kjeldahl method (ISO 5983). The analysed dietary CP levels of 12% CP, 15% CP and 18% CP on an as-fed basis were 12.3, 14.2 and 18.0%, respectively (Table 2).

Pigs were fed 2.8 times the maintenance NE requirement (293 kJ/kg $BW^{0.75}$). Water was restrictedly provided by mixing feed with water in the ratio of 1/2.5 (w/w). Apart from water with feed no additional water was given to the pigs. So it was aimed to have the same amount of feed and water intake by the pigs to excrete almost a similar amount of manure. Pigs were fed two times per day at 0800 h and 1500 h. The amount of feed provided was adjusted each day according to the expected BW gain of 750 g/day. Feed intake was recorded every day. Pigs were weighed at the beginning and at the end of the experimental period just before the morning feeding. Daily weight gain and feed efficiency were obtained from the feed intake and the increase in BW during the experimental period.

After an adaptation period of 2 weeks to allow pigs to acclimatise to the experimental diets, pens and manure pits were cleaned. Subsequently, faeces and urine were accumulated together in the manure pit. In the 5th week of the collection period, air samples for odour and ammonia measurements and manure samples were collected for subsequent analyses.

Air sample collection and measurement of odour concentration, odour hedonic tone and odour intensity

Collection of air samples for odour measurement. Air samples were used to measure odour concentration, odour hedonic tone and odour intensity. Air samples were collected directly from air above the manure in the pit. A schematic view of the air sampling set up is shown in Figure 1. In the 5th week of the collection period at about 1000 h each day, a vessel without a bottom was placed in the middle of the manure pit. The bottom of the vessel touched the bottom of the manure pit. The net surface of the vessel was 595 cm^2; and the diameter was 28 cm. The

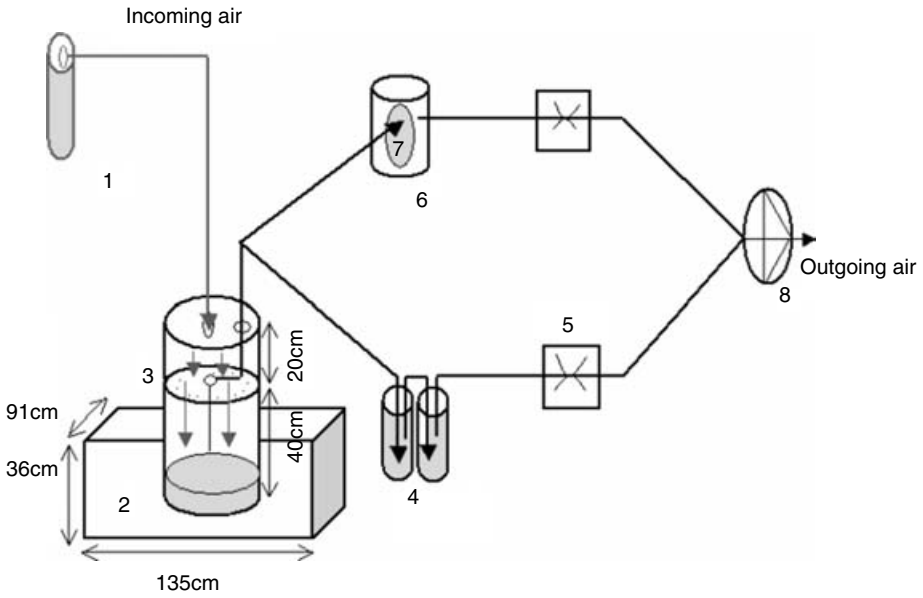

Figure 1 Schematic view of the odour and ammonia sample collection (1 = odour free air pressurised cylinder, 2 = manure pit, 3 = vessel, 4 = impingers, 5 = critical glass capillary, 6 = rigid container, 7 = odour bag, 8 = vacuum pump).

vessel was divided into two compartments by a lid. The net height of the lower compartment was 40 cm and the net height of the upper compartment was 20 cm. On each day, we collected air samples from one block. The experiment had six blocks. Each block consisted of one sample from 12% CP treatment, one sample from 15% CP treatment and one sample from 18% CP treatment.

Air entering the upper compartment of the vessel from a pressurised cylinder was odour-free air. Air entered the lower compartment of the vessel via 24 holes of 1-mm diameter each, located at the edge of the lid. Air was exhausted from the vessel by a hole of 5 mm diameter in the middle of the lid.

The outgoing odour air from the vessel was split into two streams. One stream was used to collect the odour sample. It was connected to an odour-sampling bag placed in a rigid container. This container was connected to a critical glass capillary, which had a flow rate of 0.5 l/min, and then to a vacuum pump. The other stream was used to collect ammonia and was connected to two connected impingers. The outgoing air from the impinger was connected to a critical glass capillary, which had a flow rate of 0.5 l/min, and then to the vacuum pump.

Air samples for odour measurement were collected according to the European standard with the sampling method for delayed olfactometry (CEN standard 13 725, 2003). A 40-l Nalophaan odour sampling bag was placed in a rigid container. The sample bag had been flushed with compressed and odourless air three times before it was placed in a rigid container for collection of the odour sample. The sample bag was used once for each odour sample as recommended by European standard (CEN standard 13 725, 2003). The air was removed from the container by the vacuum pump. The lower pressure in the container caused the bag to fill with a volume of sample air equal to the volume removed from the container (Figure 1). The duration of air sampling for odour was about 80 min.

One air sample for odour measurement was collected from each manure pit. During transport and storage, air samples were kept at a temperature above the dew point of the sample to avoid condensation. This was achieved by warming the rigid container of the odour bag to about 4°C above the ambient temperature. The interval between sampling and measuring odour concentration did not exceed 30 h, as recommended by European standard (CEN standard 13 725, 2003).

Measuring odour concentration. Odour concentration was measured by olfactometry according to the European standard (CEN standard 13 725, 2003) as described in detail by Le *et al.* (2005a). Odour concentrations of the examined samples were expressed in European odour units per cubic metre of air (ou_E/m^3). One odour unit is defined as the amount of odour-causing gases which, when diluted in 1 m^3 of air, can just be distinguished from clean air by 50% of the members of an odour panel.

Odour emission was defined as the number of odour units emitted from a manure surface in 1 s and it was obtained by multiplying the ventilation rate with the corresponding odour concentration (equation 1).

$$E_{odour} = (C_{odour} \times V \times 10\,000)/(60 \times 1000 \times 595) \quad (1)$$

where E_{odour} = odour emission per second per m^2 (ou_E/s per m^2), C_{odour} = odour concentration (ou_E/m^3), and V = ventilation rate (l/min), 10 000 = cm^2/m^2, 60 = s/min, 1000 = l/m^3, and 595 = the cm^2 surface area of the manure pit.

Measuring odour hedonic tone and odour intensity. Hedonic tone (H) is used to evaluate the odour offensiveness, which is a measure of the unpleasantness or pleasantness of the perceived odour above the odour detection threshold. Odour intensity (I) refers to the magnitude of the odour sensation and is a measure of the intensiveness of the odour above the odour detection threshold. Odour intensity and hedonic tone were measured at the same time by olfactometry and were determined by the same panel members as for odour concentration. A minimum of four qualified panellists, according to the standard, but generally six were involved in the measurements. The measurements of H and I of each air sample were repeated twice. We used The Netherlands standard (NVN 2818, 2005) to measure odour hedonic tone and odour intensity. The principle of the measurement is to vary the odour concentration and thus to vary hedonic value and intensity. The odour concentration varied randomly in five dilution factors above the detection threshold, with steps of a factor 2, in the range from the dilution at the threshold to 32-fold less diluted air. The five dilution factors above the detection threshold were randomly given to the panellists. The consequence of the former standard procedure is that the five dilutions were different between samples and between treatments, because they had different dilutions at the detection threshold. At each presentation, each panellist was asked to indicate the perceived hedonic value, using a nine-point hedonic scale ranging from -4, extremely unpleasant or offensive through 0, neither pleasant nor unpleasant or neutral odour to $+4$, extremely pleasant. The panellist was also asked to indicate the perceived odour intensity using a seven-point intensity scale ranging from 1, no odour through 2, very faint odour to 7, overwhelming odour. For each odour sample, the hedonic tone and the odour intensity at each odour concentration level above the detection threshold were calculated as the average of the hedonic tone and the odour intensity perceived by all panellists, and plotted against the logarithm of the odour concentration. From the regression lines obtained, the odour concentration at $H = -1$ (mildly unpleasant), $H = -2$ (moderately unpleasant), $I = 1$ (no odour), $I = 2$ (very faint odour), $I = 4$ (distinct odour) were derived. The former procedure is the standard reporting method as given in The Netherlands standard (NVN 2818, 2005). Regression lines of the hedonic tone and the odour intensity were also plotted against the logarithm of the odour concentration for all samples in the same treatment.

Measuring and calculating ammonia emission

Samples for determining ammonia emission were collected at the same time as odour samples. Figure 1 gives a schematic view of the ammonia emission measurement and collection procedure. Ammonia in the outgoing air was removed by passing through two impingers (ammonia trap), each containing about 20 ml 0.5 mol/l HNO_3 solution. The system was run for about 90 min. The ammonia concentration and the volume of the liquid were determined in the first and the second impingers. Ammonia emission per time unit and surface unit was calculated with equation 2.

$$M_{NH3} = (C_{NH3} \times V \times 10\,000)/(T \times 60 \times 595) \qquad (2)$$

where M_{NH3} = mass of ammonia emitted (mg/s per m^2), C_{NH3} = concentration of ammonia in HNO_3 solution (mg/ml HNO_3), V = volume of HNO_3 solution (ml), 10 000 = cm^2/m^2, T = sampling time (min), 60 = s/min, and 595 = the surface area of the manure pit (cm^2).

Collecting and analysing manure samples

Manure samples were analysed to evaluate the effect of the diets on manure characteristics. Analyses included dry matter (DM), ash, total N, ammonium-N, pH, VFA (acetic, propionic, butyric, pentanoic, iso-butyric, iso-pentanoic, hexanoic, and heptanoic acid), indolic (indole and 3-methyl indole) and phenolic compounds (phenol, 4-ethyl phenol, and cresols), and sulphurous compounds (carbon disulphide, methyl sulphide, methyl disulphide, and ethanethiol). Immediately after collecting odour samples, manure in each manure pit was mixed thoroughly before a sample of about 1 kg was collected. Manure samples were stored at -20°C until analysis.

Ammonium was determined spectrophotometrically according to NEN 6472 (Derikx *et al.*, 1994). VFA were measured using a Packard 427 gas chromatograph, equipped with a flame ionisation detector. Manure pH was measured by a pH electrode. For determination of indolic and phenolic compounds and sulphurous compounds, 2.5 g fresh manure was extracted with 15 ml 50% methanol for 2 h. The sample was centrifuged and the supernatant was analysed by high-performance liquid chromatography (HPLC). The HPLC conditions were a water-methanol gradient as elution solution and Alltima C18 (Alltech) as column. Detection was by UV absorption at 200 nm. For identification and quantification, an external standard solution was used.

Statistical analysis

The effect of dietary CP levels on daily gain, daily feed intake, feed efficiency, odour emission, odour hedonic value, odour intensity, ammonia emission, and manure characteristics were analysed using ANOVA procedure for a randomised complete-block design. Each treatment was replicated six times (six pigs per treatment), one replicate in each of six blocks. A block consisted of samples collected on the same day and with animals with similar initial BW. The individual pig, with the manure pit, was the experimental unit. The analysing model was as following:

$$y_{ij} = \mu + \rho_j + \alpha_i + e_{ij}$$

where y_{ij} = dependent variables; μ = overall mean; ρ_j = effect of block, $j = 1$–6; α = effect of diet, $i = 1, 2, 3$; e_{ij} = experimental error.

Data were presented as either arithmetic or geometric mean. A natural log transformation was applied to odour emission, concentrations of VFA, total N and ammonium,

indolic and phenolic, and sulphurous compounds since they were skewed and not normally distributed.

In each treatment, odour hedonic tone and odour intensity were plotted against the natural logarithm of odour concentration; and odour hedonic tone was plotted against odour intensity. The differences between slopes and between intercepts were tested to decide whether there should be separate regression lines for treatments or a common line for all treatments. The relationship between ammonia emission and odour emission was determined by linear regression. Analyses were computed using GenStat statistical package, seventh version (GenStat VSN International Ltd, 2004).

Results

Effects of dietary CP level on daily gain, daily feed intake and feed efficiency

Results showed no effects of the protein levels on average daily feed intake, daily gain, and feed efficiency ($P > 0.05$), although feed efficiency tended to be improved for the highest CP level (Table 3).

Effects of the dietary CP level on odour strength and offensiveness

Descriptive statistics and analysis of variance of effects of the dietary CP levels on odour strength and offensiveness from pig manure are given in Tables 4 and 5, respectively. Geometric means of odour concentration and odour emission from pig manure were highest for the 18% CP treatment, 31 888 ou_E/m^3 and 4.46 ou_E/s per m^2, respectively and lowest for the 12% CP treatment 7259 ou_E/m^3 and 1.03 ou_E/s per m^2, respectively.

Analyses of variance show that the dietary CP level affected both odour concentration and odour emission from pig manure ($P < 0.05$) (Table 5). The 18% CP treatment had a higher odour concentration and odour emission than the 12% CP treatment ($P < 0.05$). The 15% CP treatment had intermediate results, but did not differ from 12% CP treatment nor from 18% CP treatment ($P > 0.05$).

Table 3 *Effects of dietary crude protein (CP) level on daily gain, feed intake and feed efficiency*

	Diet				
	12% CP	15% CP	18% CP	s.e.[†]	Significance
Initial body weight (BW) (kg)	36.7	36.2	36.5	0.4	
Final BW (kg)	65.7	66.9	68.6	1.2	
Average daily feed intake (kg/day)	1.7	1.7	1.7	0.03	
Average daily gain (g/day)	629	668	697	22.3	
Gain:feed (g/kg)	371	387	409	9.7	[‡]

[†] s.e. = standard errors of the means with 10 d.f. for error.
[‡] Approaching significance ($P = 0.052$).

The odour concentration of the air from manure of pigs fed different dietary CP levels was different at different levels of odour hedonic tone measured above the odour detection threshold ($P < 0.05$). At $H = -1$, odour concentration of manure of pigs fed the 18% CP diet was lower than that of the other two diets ($P < 0.01$), while it was similar for the other two diets. At $H = -2$, there were no differences in odour concentration for pigs fed either the 12% CP or 18% CP diet, while it was higher for pigs fed the 15% CP diet ($P < 0.05$). The odour concentrations were similar for the different treatments with respect to odour intensity at different levels (Table 5).

Relationships between odour concentration and hedonic tone, between odour concentration and intensity, and between intensity and hedonic tone are shown in Figures 2, 3 and 4, respectively. Both intercept and slope of the relationship between hedonic tone and odour concentration were different among treatments ($P < 0.05$). Only the intercepts of the relationship between hedonic tone and intensity and between intensity and odour concentration were different among treatments ($P < 0.05$).

Effects of dietary CP level on manure characteristics and ammonia emission

Tables 6 and 7, respectively, show the descriptive statistics and the analyses of variance of the effects of different dietary CP level on manure characteristics including VFA, indolic and phenolic and sulphurous compounds, total N and ammonium concentrations, pH and ammonia emission. Reduced dietary CP levels decreased total N, methyl sulphide, carbon disulphide, ethanethiol, phenol, indole, 3-methyl indole, and 4-ethyl phenol concentrations ($P < 0.05$), pH ($P < 0.01$) and ammonia emission from pig manure ($P = 0.01$). Reduced dietary CP levels caused a tendency to decrease ammonium concentration in pig manure ($P = 0.07$).

Dietary CP levels did not influence cresols, and total and individual VFA concentrations in pig manure. Although branched-chain VFA concentrations in pig manure: isobutyric, iso-pentanoic acids were reduced by decreasing dietary CP levels, they were not statistically different, $P = 0.08$ and $P = 0.1$, respectively. Methyl disulphide, hexanoic, heptanoic and pentanoic acids were not detected in the manure of the different treatments. The detection limits of methyl disulphide and the three VFA are 0.5 mg/kg and 0.1 g/kg, respectively. The correlation between ammonia and odour emission was 0.1.

Discussion

Diet is the first step in the odour production chain from feed to manure. It is generally accepted that dietary alterations can significantly reduce odour from pig manure (Le *et al.*, 2005b). Dietary protein is one of the most important precursors for odour production thus it is expected that odour produced from pig manure will be

Table 4 *Odour concentration, odour emission and odour concentration at different levels of hedonic tone (H) and at different levels of intensity (I) of air above manure of growing pigs fed different dietary crude protein (CP) levels* (n = *18) (geometric mean (GM) and arithmetic mean (AM) are given)*

	Diet					
	12% CP		15% CP		18% CP	
	GM	AM	GM	AM	GM	AM
Concentration (ou_E/m^3)	7259	8360	13226	21218	31888	40904
Emission (ou_E/s per m^2)	1.03	1.18	1.85	2.94	4.46	5.76
Concentration at H = −1 (ou_E/m^3)	2.04	2.33	3.52	3.71	1.30	1.47
Concentration at H = −2 (ou_E/m^3)	5.26	5.71	9.68	10.07	4.71	4.98
Concentration at I = 1 (ou_E/m^3)	0.61	0.74	0.90	0.95	0.50	0.51
Concentration at I = 2 (ou_E/m^3)	1.32	1.53	2.01	2.08	1.20	1.23
Concentration at I = 4 (ou_E/m^3)	6.23	6.74	9.87	10.20	7.10	7.47

reduced as dietary CP level decreases (Mackie *et al*., 1998; Sutton *et al*., 1999). In this study, when dietary CP was reduced from 18% to 12% odour concentration and emission decreased by nearly 80%, or from 4.46 to 1.03 ou_E/s per m^2 of the manure pit. Hayes *et al.* (2004) found that odour emission reduced by 31% and 33%, by decreasing dietary CP content from 19% to 16% and from 19% to 13%, respectively. The odour emission rates were 19.6, 13.2, and 12.1 ou_E/s per finishing pig, respectively for the 19%, 16%, and 13% CP diets. Obrock *et al.* (1997), however, found no difference in odour concentration between finishing pigs fed 13% and 9% CP with AA supplemented diets. Possibly, the protein levels in the study of Obrock *et al.* (1997) were so low that they did not cause any difference in odour concentration from pig manure.

Protein is the precursor for the production of different odorous compounds in the gut of animals and in the manure (Le *et al*., 2005b). In our study reduced dietary CP levels decreased the concentrations of indolic and phenolic compounds (phenol, indole, 3-methyl indole and 4-ethyl phenol) and sulphurous compounds (methyl sulphide, carbon disulphide, ethanethiol) in the manure.

When sufficient NSP are available, microbes will use NSP as an energy source and protein or AA as a nitrogen source for their biomass synthesis. This process produces fewer odours than when protein is used as an energy source. If the amount of NSP is relatively low compared with that of protein, the microbes will use protein or AA as an energy source. This situation with insufficient NSP may create more odour than when adequate NSP is available (Gibson and Roberfroid, 1995; Reid and Hillman, 1999). It is important to notice that the three diets had rather low and similar NSP levels. The increased CP levels resulted in higher ratios between fermentable protein and NSP in the gut of animals and in the manure. This can cause more protein or AA to be used by microbes as an energy source resulting in a higher concentration of odorous compounds in the gut of animals and in the manure.

The diets with 15% and 18% CP were supplemented with the same amount of crystalline Lys, Met, Trp and Thr

Table 5 *Effects of the dietary crude protein levels on odour concentration, odour emission and odour concentration at different levels of hedonic tone (H) and at different levels of intensity (I) of air above manure of growing pigs fed different dietary crude protein (CP) levels* (n = *18)*

	Diets				
	12% CP	15% CP	18% CP	s.e.†	Significance
ln‡ (odour concentration)	8.89[a]	9.49[ab]	10.37[b]	0.35	*
ln (odour emission)	0.03[a]	0.62[ab]	1.49[b]	0.35	*
ln (odour concentration at H = −1)	0.71[a]	1.26[a]	0.26[b]	0.16	**
ln (odour concentration at H = −2)	1.66[a]	2.27[b]	1.55[a]	0.16	*
ln (odour concentration at I = 1)	−0.49	−0.10	−0.69	0.21	
ln (odour concentration at I = 2)	0.28	0.70	0.18	0.19	
ln (odour concentration at I = 4)	1.83	2.29	1.96	0.17	

[a,b] Means within rows without a common superscript letter are different at $P < 0.05$.
† s.e. = standard errors of the means with 10 d.f. for error.
‡ Natural logarithm.

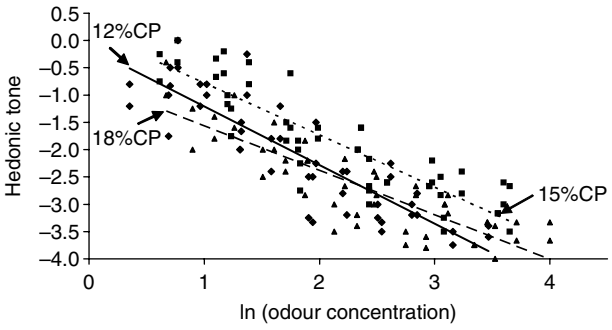

Figure 2 Hedonic tone (H) as a function of odour concentration with regression lines, $H_{12\%CP} = -0.09\ (0.16) - 1.11\ (0.08)$ ln (odour concentration), indicated by ___ ◆; $H_{15\%CP} = 0.18\ (0.18) - 0.95\ (0.07)$ ln (odour concentration), indicated by .....■; $H_{18\%CP} = -0.87\ (0.18) - 0.75\ (0.07)$ ln (odour concentration), indicated by __ __▲, $R^2 = 78.3\%$.

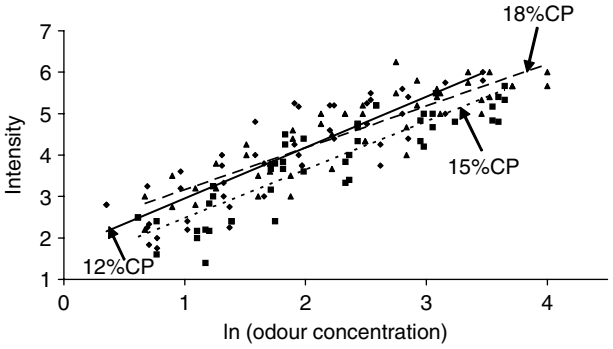

Figure 3 Odour intensity (I) as a function of odour concentration with regression lines, $I_{12\%CP} = 1.95\ (0.13) + 1.13\ (0.05)$ ln (odour concentration), indicated by ___ ◆; $I_{15\%CP} = 1.40\ (0.14) + 1.13\ (0.05)$ ln (odour concentration), indicated by . . . . . ■; $I_{18\%CP} = 1.83\ (0.15) + 1.13$ (0.05) ln (odour concentration), indicated by __ __▲, $R^2 = 77.5\%$.

as the diet with 12% CP. Normally in practice 15% and 18% CP diets are supplemented with less AA. This was done to prevent confounding effects between dietary CP levels and AA supplementation. Le *et al.* (2007) found that especially the level of crystalline sulphur containing AA in the diet affects odour emission from pig manure. Within this study the sulphur containing AA Met was added to the diets. The amount of Met added to the diet was 1.5 g/kg diet that occupied about 36%, 31% and 25% of total sulphur containing AA in 12% CP, 15% CP and 18% CP diets, respectively. When all diets were supplemented with the same amount of crystalline AA, effects of reduced CP content might be a bit less.

So far, only limited research has been conducted on the impact of dietary CP reduction on odorous compounds in manure, for examples, indolic, phenolic, and sulphurous metabolites. There is hardly any research that correlates the impact of dietary CP reduction on odour strength and offensiveness of the air above the manure or on the excretion of odorous compounds in manure or on the emission of these compounds from the manure (Le *et al.*, 2005b). Previous studies have shown a reduction of phenol, 4-ethyl phenol, indole, 3-methyl indole concentrations

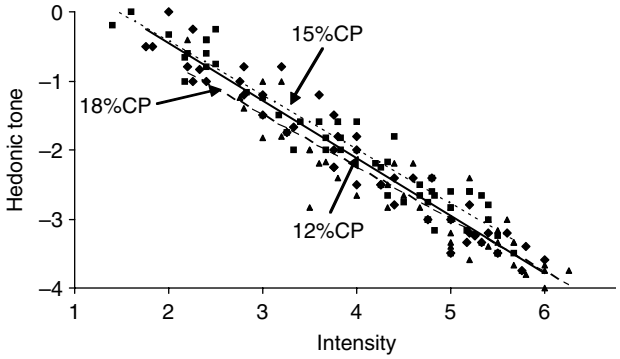

Figure 4 Hedonic tone (H) as a function of odour intensity (I) with regression lines, $H_{12\%CP} = 1.03\ (0.09) - 0.79\ (0.02)$ I, indicated by ___ ◆; $H_{15\%CP} = 1.13\ (0.09) - 0.79\ (0.02)$ I, indicated by . . . . . ■; $H_{18\%CP} = 0.91\ (0.10) - 0.79\ (0.02)$ I, indicated by __ __▲, $R^2 = 91.8\%$.

Table 6 *Descriptive statistics (mean with s.d. in parentheses) of manure characteristics and ammonia emission from manure of pigs fed different dietary crude protein (CP) levels (n = 18)*

	Diet		
	12% CP	15% CP	18% CP
Dry matter (g/kg)	128.2 (24.09)	119.6 (16.95)	123.4 (10.09)
Ash (g/kg)	41.3 (4.41)	37.4 (3.82)	37.8 (2.47)
Total VFA[†] (g/kg)	7.41 (3.73)	6.35 (1.89)	7.58 (2.14)
Acetic acid (g/kg)	4.23 (1.94)	3.98 (0.99)	4.65 (1.12)
Propionic acid (g/kg)	1.72 (0.95)	1.25 (0.5)	1.47 (0.5)
Butyric acid (g/kg)	1.0 (0.82)	0.5 (0.32)	0.7 (0.4)
Iso-butyric acid (g/kg)	0.13 (0.05)	0.20 (0.06)	0.25 (0.11)
Iso-pentanoic acid (g/kg)	0.33 (0.08)	0.45 (0.14)	0.53 (0.18)
Total N (g/kg)	5.78 (0.97)	6.24 (1.09)	7.25 (0.73)
Ammonium (g/kg)	1.91 (0.48)	2.51 (0.5)	2.86 (0.65)
pH	7.10 (0.34)	7.52 (0.17)	7.83 (0.27)
Ammonia emission (mg/s per m^2)	0.008 (0.005)	0.009 (0.001)	0.017 (0.001)
Phenol (mg/kg)	9.10 (2.5)	17.32 (6.97)	32.73 (12.49)
Cresols (mg/kg)	38.18 (8.41)	39.58 (9.72)	41.42 (7.3)
4-ethyl phenol (mg/kg)	1.60 (0.60)	5.84 (1.38)	11.75 (1.58)
Indole (mg/kg)	5.96 (0.92)	9.77 (3.8)	10 (1.7)
3-methyl indole (mg/kg)	5.30 (1.0)	4.80 (0.9)	8.74 (2.21)
Carbon disulphide (mg/kg)	3.33 (0.56)	7.05 (0.71)	9.06 (1.67)
Methyl sulphide (mg/kg)	1.35 (1.42)	8.13 (2.17)	6.48 (2.83)
Ethanethiol (mg/kg)	78.82 (19.1)	81.9 (16.8)	104.6 (14.5)

[†] Total VFA = acetic acid + propinoic acid + butyric acid + iso-butyric acid + iso-pentanoic acid.

in pig manure (Hobbs *et al.*, 1996) as dietary CP levels were reduced from approximately 20 to 13%. However, no measurements on odour strength and offensiveness were conducted in these studies. Sutton *et al.* (1998) reported a reduction of sulphurous compounds (carbon disulphide, dimethyl sulphide, dimethyl disulphide) as dietary CP level

Table 7 *Effects of dietary crude protein (CP) levels on manure characteristics and ammonia emission, only significant effects (P < 0.05) are presented*

	Diet				
	12% CP	15% CP	18% CP	s.e.[†]	Significance
ln[‡] (total N)	1.73^a	1.82^{ab}	1.98^b	0.06	*
ln (ammonium)	7.54	7.81	7.93	0.11	§
ln (methyl sulphide)	0.7^a	2.19^b	1.96^b	0.2	**
ln (carbon disulphide)	1.19^a	1.9^b	2.2^c	0.04	**
ln (ethanethiol)	4.3^a	4.4^a	4.6^b	0.08	*
ln (phenol)	2.18^a	2.8^b	3.43^c	0.14	**
ln (3-methyl indole)	1.65^a	1.55^a	2.14^b	0.08	**
ln (indole)	1.77^a	2.22^b	2.29^b	0.35	*
ln (4-ethyl phenol)	0.20^a	1.64^b	2.46^c	0.19	**
pH	7.1^a	7.52^b	7.83^b	0.4	**
ln (ammonia emission)	-5.03^a	-4.70^a	-4.21^b	0.15	**

[a,b,c] Means within rows without a common superscript letter are different at $P < 0.05$.
[†] Standard errors of the means with 10 d.f. for error.
[‡] Natural logarithm.
[§] Approaching significance ($P < 0.1$).

was reduced from 13% to 8%. The reduction of these odorous compounds is supposed to decrease odour strength and offensiveness from pig manure. This interpretation should, however, be handled with care, because the relationships between the concentration of a single odorous compound or a group of odorous compounds in manure and/or in the odorous air and the odour strength and offensiveness of the odorous air measured by olfactometry are not yet fully clear (Le *et al.*, 2005b). In addition, odour is a complex mixture of many compounds, so the number of compounds analysed in our experiment or in those of others are probably too few to give a general picture of odour strength and offensiveness measured by olfactometry. It could also be that we did not measure the main odorous compounds that determine the odour threshold.

According to Canh *et al.* (1998c) fermentable NSP are the most important dietary components determining VFA concentration in the manure. The VFA pool was largely dominated by the short straight−chain VFA such as acetic, propionic and butyric acids which comprised 91% of total VFA in the manure confirming the results of Otto *et al.* (2003) and Le *et al.* (2005a). Branched-chain VFA are only produced from protein metabolism. That could be the reason for the increase of iso-butyric and iso-pentanoic acid concentrations in the manure as dietary CP levels increased from 12% to 18%, although they were not statistically different ($P = 0.08$ and $P = 0.1$, respectively).

The main objective of this study was to determine the effect of dietary CP levels on odour strength and offensiveness, but ammonia emission was considered as well because it is a serious environmental problem. Odour abatements are only of interest if they do not increase other environmental problems as ammonia. Ammonia emission (mg/s per m^2) was decreased by 53% as dietary CP levels were reduced from 18.0% to 12.2% (analysed CP values). This corresponds to about 9.2% ammonia emission reduction for each 1% unit reduction in dietary CP combined with AA supplementation. This figure is very similar to the 10% that was reported by Canh *et al.* (1998b), Kay and Lee (1997), and Sutton *et al.* (1997). Ammonia emission is largely influenced by ammonium concentration, pH and temperature (Aarnink and Elzing, 1998). In this experiment, temperature was controlled and the same for all treatments so the effect of temperature was excluded. Ammonia emission reduction seems to have mainly resulted from lowering manure ammonium concentration and pH. Lowering dietary CP level from 18.0% to 12.2% resulted in a decrease of the ammonium concentration in the manure by 33.2% and a decrease of manure pH by 0.73 unit.

In practice diets with a low CP level generally have a low dEB, as well, because in these diets the concentration of K is generally lower. From research of Canh *et al.* (1998a) it is known that a lower dEB means a lower pH of urine and manure and a lower ammonia emission from the manure. In our study the dEB levels were equalised to solely study the effect of CP. Therefore, with practical diets the effect of a low CP level on ammonia emission could be more pronounced than found in this study. The effect of dEB on odour emission is unknown.

So far, almost all odour studies have focused on concentration and emission of odour and odorous compounds. The odour concentration limits the question of 'how strong and unpleasant an odour is' to a detection threshold and the original odour is characterised in odour units or multiples of the odour concentration at detection threshold. However, this approach has a limitation in comparing different types of odours, because odour concentration does not take into account the other characteristics of odour such as the pleasantness or unpleasantness of odour (Power and Stafford, 2001). Obviously not all odours are similar in their ability to cause annoyance. In our study, not only odour concentration, but also hedonic tone and intensity were measured. The latter two criteria can answer the question how strong and unpleasant an odour is. By using dynamic olfactometry to determine odour concentration and then odour intensity and odour hedonic tone, suitable relationships between them can be determined, allowing different odour types to be compared. The use of odour concentration, odour hedonic tone and odour intensity can give an overall comparison between odours.

A higher odour concentration at $H = -1$ (mildly unpleasant) of odorous air from manure of pigs fed lower CP diets implies that pigs fed the 12% CP or 15% CP diets produced a less offensive odour from the manure, at the same odour concentration, than the 18% CP diet. A higher odour concentration at $H = -2$ (moderately unpleasant) of the 15% CP treatment implies that for this hedonic tone level this odour was less offensive than of the 18% CP and 12% CP treatments. The effect of dietary CP levels on odour concentration at different levels of odour intensity was not significant, in other words air from the different treatments with a similar odour concentration had a similar level of odour intensity.

This study showed a very low correlation between ammonia and odour emission (0.1). It can be explained by the fact that odour is a complex mixture of various compounds such as sulphur-containing compounds, indolic and phenolic compounds, VFA, and ammonia and volatile amines, while ammonia is a single compound. In addition, ammonia is not a very offensive odour (Oldenburg, 1989). This result implies that ammonia emission may contribute minimally to odour emission, and strategies that have been demonstrated to be successful in reducing ammonia emission may not have a similar impact on odour. In literature inconsistent results were found with respect to the correlation between ammonia and odour emissions. Schulte *et al.* (1985) and Miner (1995) found a high correlation between these emissions from pig production facilities. On the other hand Williams (1984), Oldenburg (1989), Liu *et al.* (1993) and Verdoes and Ogink (1997) found only a low correlation for pig production facilities. The inconsistencies in the relationship between ammonia and odour emission likely comes from the fact that ammonia and

odour samples were collected from different farms and at different times. Farms are different in animal types, housing design, and dietary composition, especially the level and type of fermentable carbohydrates may vary a lot among diets. Different times of sample collection imply different environmental factors. These farms and environmental factors play key roles in influencing odour and ammonia emission (Le *et al*., 2005a and b) and consequently the relationship between them. In our study, these sources of variances were prevented, because we collected odour and ammonia samples from the manure of the different treatments in the same animal house, at the same time, and with the same airflow rate.

Implications

This study demonstrates that feeding a diet that more closely meets the protein/AA requirement of the pigs reduced odour concentration, odour emission, odour offensiveness and ammonia emission from pig manure. This can be achieved by reducing the CP content of the diet and supplementing the diet with essential AA. The results of this study were obtained under rather controlled conditions, therefore, they should be validated in a conventional pig facility, measuring not only odour from manure, but from other sources in the animal house, as well.

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