RESEARCH ARTICLE

Relationships among slurry characteristics and gaseous emissions at different types of commercial Spanish pig farms

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Abstract

This study aimed to analyse several factors of variation of slurry composition and to establish prediction equations for potential methane (CH_4) and ammonia (NH_3) emissions. Seventy-nine feed and slurry samples were collected at two seasons (summer and winter) from commercial pig farms sited at two Spanish regions (Centre and Mediterranean). Nursery, growing-fattening, gestating and lactating facilities were sampled. Feed and slurry composition were determined, and potential CH_4 and NH_3 emissions measured at laboratory. Feed nutrient contents were used as covariates in the analysis. Near infrared reflectance spectroscopy (NIRS) was evaluated as a predicting tool for slurry composition and potential gaseous emissions. A wide variability was found both in feed and slurry composition. Mediterranean farms had a higher pH ($p<0.001$) and ash ($p=0.02$) concentration than those located at the Centre of Spain. Also, type of farm affected ether extract content of the slurry ($p=0.02$), with highest values obtained for the youngest animal facilities. Results suggested a buffer effect of dietary fibre on slurry pH and a direct relationship ($p<0.05$) with fibre constituents of manure. Dietary protein content did not affect slurry nitrogen content but decreased ($p=0.003$) total and volatile solids concentration. Prediction models of potential NH_3 emissions ($R^2=0.89$) and CH_4 yield ($R^2=0.61$) were obtained from slurry composition. Predictions from NIRS showed a high accuracy for most slurry constituents ($R^2>0.90$) and similar accuracy of prediction of potential NH_3 and CH_4 emissions ($R^2=0.84$ and 0.68, respectively) to models using slurry characteristics, which can be of interest to estimate emissions from commercial farms and establish mitigation strategies or optimize biogas production.

Additional key words: ammonia; methane; NIRS; animal nutrition; prediction model.

Abbreviations used: ADF (acid detergent fibre); ADL (acid detergent lignin); B_0 (ultimate methane yield); CP (crude protein); DM (dry matter); EE (ether extract); GHG (greenhouse gases); NDF (neutral detergent fibre); NDICP (proportion of N insoluble in NDF); NIRS (near infrared reflectance spectroscopy); RSD (residual standard deviation); SEC (root mean square error of calibration); SECV (root mean square error of cross validation); TS (total solids); VFA (volatile fatty acids); VS (volatile solids).

Introduction

In the past, animal manure was regarded as a scarce and valuable source of plant nutrients to maintain soil fertility. However, at present there is an increasing concern about the impact of high levels of manure fertilization in different parts of the European Union, including some Spanish regions, where pig production is highly concentrated. According to the FAO Corporate Statistical Database (Faostat, 2014), world's pig population has risen to almost 1 billion heads in 2012, and Spain is the sixth world pig producer. In this context, agriculture still plays an essential role in recycling manure nutrients, but also new uses of manure have been developed in recent years (*e.g.* biogas production).

Intensive livestock production constitutes an important source of emissions of ammonia (NH_3) and greenhouse gases (GHG) such as methane (CH_4) and nitrous oxide (N_2O) and, particularly in high producing areas, slurry management has been associated to nitrate contamination of ground and surface waters (Tamminga, 2003). In the European Union (EU-27), it is estimated that livestock contributed in 2012 to about 70% of NH_3 emissions to the atmosphere and pig production contributes to about 15% (EEA, 2014a). The management of livestock manure contributed to about 19% of total CH_4 emission, whereas slurry management in pig production emitted about 5.4% of total EU CH_4 emissions (EEA, 2014b).

It is widely recognized that there is a link between pig slurry composition and gas emission (Møller *et al.*, 2004a; Dinuccio *et al.*, 2008). Therefore, understanding the factors of variation of slurry composition under commercial conditions is essential to predict and control these emissions. However, it has also been reported that pig slurry composition in commercial farms is very heterogeneous and depends on multiple and interacting factors including the animal itself (breed and physiological status), feed composition and consumption, the housing system, manure management practices or environmental conditions (Sánchez & González, 2005; Conn *et al.*, 2007; Moral *et al.*, 2008; Martínez-Suller *et al.*, 2010; Álvarez-Rodríguez *et al.*, 2013).

Experimentally, it has been widely evidenced that nutritional strategies are effective to originate changes in the digestive performance of pigs and therefore influence the composition of excreta and thus the emissions of pollutant gases. The reduction of protein content of feeds affects directly nitrogen excretion and reduces NH_3 emissions (Canh *et al.*, 1998; Hayes *et al.*, 2004). The inclusion of fermentable fibre in feeds have been reported to cause a shift in excreta from urinary to faecal nitrogen (Galassi *et al.*, 2010; Halas *et al.*, 2010) and reduce the pH of excreta (Kerr *et al.,* 2006), thus reducing the emission of NH_3. On the contrary, increasing fermentable fibre content of pig feeds also enhances the emission of CH_4 from enteric origin (Jørgensen, 2007) and the CH_4 emission potential from slurry (Jarret *et al.*, 2012).

A better characterization of the chemical components of pig slurry might improve the prediction of the associated gas emissions either from the animal house, the slurry storage or the soil after slurry application. Conventionally, slurry chemical composition is generally determined by using conventional wet chemical analysis performed at the laboratory which are expensive, time and labour costly, generate chemical wastes, and cannot be applied on-line. At farm level, however, rapid and low cost methods to predict slurry composition are necessary for an efficient use of slurry and as a consequence prediction methods have been developed during the last decade. These may be based on physicochemical models (Chen *et al.*, 2009; Yagüe *et al.*, 2012) or the electrical properties (Bietresato & Sartori, 2013). Also, spectroscopic methods, as near infrared reflectance spectroscopy (NIRS) have found increasing use in the laboratory for low cost and rapid analysis, and offer a great potential for on-farm testing (Saeys *et al.*, 2005). They have recently been applied for pig slurry analyses, and useful and accurate NIRS calibrations have been obtained for dry matter, ammonia N, total N and C (Malley *et al.*, 2002; Saeys *et al.*, 2005; Ye *et al.*, 2005; Sørensen *et al.*, 2007). Predicting methane potential emission using NIRS has also been recently a focus of interest to optimize anaerobic co-digestion processes (Doublet *et al.*, 2013; Triolo *et al.*, 2014). These calibrations need to be checked and updated periodically because of changes induced by variations in the slurry composition, but calibration maintenance, instrument validation, etc. could all be done on-line (Reeves, 2007).

As mentioned before, it is widely accepted that gaseous emissions in commercial farms are affected by a variety of dietary, animal, management and environmental factors. However, quantifying the relevance of factors affecting slurry composition and emissions at commercial level are currently topics of highest interest. Also, there is currently few published information on predicting potential NH_3 and CH_4 emissions from pig slurry at commercial farms using physic-chemical models or NIRS.

The objective of this work was to evaluate the relationships among slurry composition, gaseous emissions and several production factors (feed composition, season and location) in different types of commercial pig farms (nursery, growing-finishing, gestating and lactating sows). These relationships will be analysed throughout a multivariate analysis using a dataset of slurry samples covering a wide range of production conditions in commercial pig farms. Another objective was to establish predicting equations of potential gaseous emissions using physic-chemical models and NIRS.

Material and methods

Description of farm selection and sample collection

A survey protocol of commercial pig farms was established trying to cover the maximum variation in

commercial feeds, which could originate variability in the characteristics of slurries and in the potential NH_3 and CH_4 emissions. Representative feed and slurry samples were collected from 79 commercial pig farms (14 from either gestating, lactating and nursery piglets and 37 from growing-finishing animals) located at two regions in Spain (Centre and Mediterranean). Each farm was sampled once, either in winter or in summer. Farms were selected following three main criteria:

i) reflect the usual conditions of housing and manure management of intensive farming systems in Spain. In this way, dry feeding was generally provided in collective feeders (nursery and fattening pigs) and individual feeders (farrowing and gestation sows), most of farms used pellet feed, access to feeding was restricted only for gestating sows, housing systems for fattening pigs and gestating sows were naturally ventilated, whereas both natural and mechanical ventilation was used for farrowing sows and nursery houses and proportion of slat was close to 100% in fattening, lactating and nursery farms, with lower and more variable values (from 25 to 100%) in gestating facilities).

ii) the slurry accumulated in the pits corresponds to the diets sampled when the survey was done and

iii) get a high variation among feed suppliers. Therefore, this survey protocol did not aim to be representative of the Spanish livestock sector, but to reflect potential variation in feeds, and as a consequence, on slurries and gaseous emissions.

The farms surveyed covered approximately a population of 11,000 sows, 35,000 nursery piglets and 60,000 growing-finishing pigs. The average storage time of slurry under the pits was about one month for lactating and gestating sows, as well as for nursery piglets and 51 days for growing-finishing pigs. The distribution of samples by zone, season and type of farm is shown in Table 1.

The samples were collected from March 2012 to February 2013, following a standardized protocol. Feed samples (1 kg) were taken from feeders or silos depending on their accessibility. Slurry samples were taken during pit discharge through a floor opening at the end of alleys, generally inside the barn. Sampling was made at regular time intervals by pooling a minimum of five aliquots (2-L) in a 15-L container and then subsampled into four 1-L plastic bottles for the different laboratory determinations, and stored at 4ºC until analyzed. They were thoroughly mixed, subsampled into four 1-L plastic bottles for the different laboratory determinations.

Feed and slurry chemical analysis

Slurry samples were maintained at 4ºC and immediately analyzed at the arrival to laboratory (2-3 h after sampling) for pH (GLP21, Crison, Alella, Barcelona, Spain), electric conductivity (HI 98188-02, Hanna Instruments, Eibar, Spain), total solids (TS) and volatile solids (VS). The rest of analysis were made the day after, with samples maintained refrigerated at 4ºC at the lab. Chemical analyses of samples were conducted in triplicate. The TS contents were determined after drying at 103ºC for 24 h, and the VS after ignition in a muffle furnace (12-PR/300, Hobersal, Caldes de Montbui, Barcelona, Spain) at 550ºC for 4 h. The total N and ammonia N concentrations were determined by steam distillation (APHA, 2005) using an automatic analyser (Pro Nitro A, J.P. Selecta S.A, Barcelona, Spain). Volatile fatty acids concentrations were determined by gas chromatography equipped with a flame ionization detector (HP 68050 series Hewlet Packard, USA) following the method described by Jouany (1982) with the addition of an internal standard (4-metil valeric).

The rest of slurry samples were dried at 60ºC for 48 h, and feed and dried slurry samples were ground to pass through a 1-mm mesh screen (Cyclotec 1093 Sample Mill, Foss Electric A/S, Denmark). Dry matter (DM) and ash contents were carried out according to AOAC (2000) procedures 930.15 and 923.03, respectively. Concentration of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined sequentially by using the filter bag system (Ankom Technology, NY) according to Mertens (2002), AOAC (2000; procedure 973.187) and Van Soest *et al*. (1991), using heat stable amylase

Table 1. Number of feed and slurry samples classified by zone, season and type of animals

Type of animal	Centre zone		Mediterranean zone		Total
	Winter	Summer	Winter	Summer	
Gestating sows	3	4	3	4	14
Lactating sows	3	4	3	4	14
Nursery piglets	3	4	3	4	14
Growing-finishing	11	7	11	8	37

(A3306, Sigma) in the case of feed samples, and expressed without residual ash. Concentration of ether extract (EE) was determined by AOAC methods (920.39). Nitrogen was measured in feeds by combustion (method 986.06; AOAC, 2000) using a Leco equipment (model FP-528, Leco Corporation, St. Joseph, MI, USA). The proportion of N insoluble in NDF (NDICP) in feed samples was determined following the standardized procedures of Licitra *et al.* (1996), by analysing the N content (combustion method) in the NDF residues.

Potential gaseous emissions

In vitro potential NH_3 emissions were determined by duplicate in laboratory following the methodology described by Portejoie *et al.* (2004). The samples (0.6 L each) were placed in 1 L closed chambers maintained at constant temperature (25ºC) and connected to an air pump which extracted air from each chamber at an airflow rate of 1.2 L/min. During 15 consecutive days, the air was forced to pass through 2 absorption flasks (impingers) in serial containing 100 mL of 0.1 N H_2SO_4. The acid solution was changed every day during the experiment and analyzed for NH_3 content following 4500 NH3-D procedure (APHA, 2005) using a detection electrode (Orion High Performance NH_3 Electrode, model 9512HPBNWP, Thermo Scientific, USA). The cumulative emission for each sample was calculated by adding the ammonium retained daily in the flasks during the experimental test.

Additionally, ultimate CH_4 yield (B_0) of each slurry sample was determined through biodegradability assays in 125 mL bottles during 100 days by using the methodology described by Vedrenne *et al.* (2007). These assays consisted in incubating different slurry substrates at mesophilic temperatures (35°C) in the presence of inoculum. Inoculum to substrate ratio was at unity or very close to unity on a VS basis (1:1). Each test on pig slurry was carried out in triplicate. Additionally, three blank bottles containing anaerobic sludge-only were also used in order to determine the anaerobic sludge endogenous CH_4 production which was subtracted from the CH_4 produced by the pig slurry on each biogas sampling day. Bottles were then incubated at 35°C for 100 days. During incubation, biogas volume in each bottle was regularly monitored (intervals from 1 to 10 days depending on biogas production) by pressure measurement of the headspace using a manometer (Delta Ohm, HD 9220, Italy). Methane concentration in the biogas was further analysed using a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame ionization detector.

According to the methodology of measuring in vitro emissions, these must not be considered as real emissions but as intrinsic properties of manure defining the potential to generate NH_3 and CH_4. Since all in vitro emissions are obtained in homogeneous environmental conditions, these potentials may be related to slurry characteristics. The effect of other variables (*e.g.* type of farm, season, and location) are therefore addressed in this study only in an indirect way: how these variables may affect slurry composition and thus their potential to emit NH_3 and CH_4.

NIRS determination

Feed and slurry samples were scanned using a Foss NIRSystem spectrophotometer (model 5000, Silver Spring, MD, USA) operating in reflectance mode and equipped with a sample transport device that allows samples be scanned while moving onto the same plane that equipment position. The spectra were acquired at 2 nm intervals over a wavelength range from 1,100 to 2,500 nm using the ISI NIRS 3 software ver. 3.11 (Infrasoft International, Port Matilda, PA, USA), and 32 co-added scans were averaged and collected by sample. Feed samples (undried and ground 1 mm) were scanned using a standard 1/4 sample cell and the equipment in a vertical position. The samples were mixed thoroughly using a homogenizer RW14 (Ika-Werke, Staufen, Germany) during 1 min; then, 80 mL were transferred to handmade polyethylene bags (60 × 230 mm) to a level of 10 cm from the bottom, and the upper part of the plastic bags was sealed after removing air space (Sørensen *et al.*, 2007). The slurry sample bags were scanned by using a large sample cell (200 mm length by 4.7 mm width, and 23 mm depth) with the equipment placed on its back and the sample cell in a horizontal position. In this case, the particles were settled onto the face of cell and did not move out of the path of the light. Each slurry sample was measured in three independent subsamples by preparing three bags that were kept at 4ºC and equilibrated at room temperature (15-20ºC) before scanning. Each subsample was scanned twice mixing the contents to homogenize the sample between scanners, and averaged to provide one spectra per replicate. The average spectra of the three subsamples were used for chemometric analysis.

Statistical analysis

Slurry samples taken from independent facilities were the experimental unit for all the analyses. Descriptive analysis of the variables was performed through

PROC MEANS of SAS (SAS, 2008). Correlation analysis among dietary components and among slurry characteristics and feed composition was done using PROC CORR of SAS.

The prediction model of slurry composition and emissions included type of farm, season and location and their interactions as classified variables, as well as chemical constituents of feeds as linear covariates. PROC GLM of SAS was used to perform all of the analyses. A stepwise variable selection process was conducted using the PROC REG of SAS. To achieve the assumption of normality of the continuous variables analyzed, the Box & Cox (1964) transformation was used with PROC TRANSREG of SAS. The transformations used for each variable of feed composition and slurry characteristic are shown in Table 2. When significant differences of type of farm were detected, the Tukey test was used for mean comparisons.

Calibration models from NIRS for each constituent were performed using WINISI version 1.5 software, by modified partial least square regression based on cross-validation to avoid over-fitting of the equations. Prior to calibration, principal component analysis was performed to remove outliers with a standardized Mahalanobis distance H>3.0 (Shenk & Westerhaus, 1996) and no samples were marked as outliers. Different math pre-treatments of spectral data over three different segments (1,100 to 1,800, 1,200 to 2,400, and 1,100 to 2,500 nm) of the spectral range were tested, including none and three scatter correction techniques, standard normal variate and detrending, multiple, and inverse multiplicative scatter correction (Barnes *et al.*, 1989) together with either or no first or second order derivatives, giving a total of 75 spectral models for each predicted parameter. The statistics used for selecting the best equations were the coefficient of multiple determinations (R^2_{CV}) and the standard error of cross-validation (SECV). The prediction accuracy for a model was based on the ratio of standard deviation (SD) of the reference data to the SECV, which should be at least three for an industrial application (Williams, 2001). The practical accuracy of NIRS calibrations developed was investigated by comparing the SECV to the standard laboratory errors. The repeatability of the predictions from the NIRS method was estimated from the variability of the values predicted in homogeneous analytical conditions from three subsamples.

Results

The average values of main chemical constituents of feeds and its variation (range and standard deviation) within the different types of farms studied are shown

Table 2. Transformations of variables used in the statistical analysis (% DM, except when indicated)

Feed constituents
- Total solids, ether extract, ash: no transformation.
- Crude protein, neutral detergent fibre, acid detergent lignin, neutral detergent insoluble crude protein: logarithmic transformation.

Slurry characteristics
- pH: power transformation Y^d, d=9 divided by 10^6.
- Total solids (%), total nitrogen, ammonia nitrogen, ammonia N (%N), ammonia emissions (mg/L/day): logarithmic transformation.
- Volatile solids (%): power transformation Y^d, d=0.21.
- Ash: power transformation Y^d, d=-0.5.
- Neutral detergent fibre, acid detergent fibre, ether extract: no transformation.
- Acid detergent lignin, methane emissions: power transformation Y^d, d=0.5.
- Volatile fatty acids (mg/L): power transformation Y^d, d=0.33.

in Table 3. The results indicate an important variability among the samples analysed. The mean coefficients of variation for crude protein (CP) and NDF were 8.8 and 12.4%, respectively, and rose to 28-36% for NDICP, ADL and EE. The average values of slurry characteristics and its variability for the whole data set studied are presented in Table 4. The coefficients of variation (CV) of chemical components varied from 35-50% (for NDF, EE and total N) to more than 80% in the case of TS, VS, NDICP and ADL. The less variable characteristics were pH (CV=5.6%) and proportion of ammonia N on total N (CV=20%).

Correlation analyses were done: a) among feed constituents and b) between feed composition and some selected slurry characteristics (see results in Table 5). A negative correlation was observed between dietary CP and dietary fibre constituent contents. Dietary fibre components were significant and positively related among them and with ash and NDICP, which instead was little related with dietary CP level. Ether extract concentration in feeds was little related with any of the other components analysed. Otherwise, dietary concentrations of CP and NDICP were negatively associated to TS, VS and NDF, but positively with EE content of the slurry. Fibrous feed constituents were significantly and positively correlated with pH and NDF content in the pig manure and negatively with slurry EE concentration. Dietary EE and ash contents were little related with slurry characteristics, although the later was negatively associated to ammonia N and EE contents in the slurry. Otherwise, electric conductivity was highly correlated ($p<0.001$) with total and ammonia N in the slurry ($r=0.803$ and 0.884, respectively).

Table 3. Variability of feed composition[1] within the different types of farms studied (% DM basis)

	Ash	CP	NDICP	NDF	ADL	EE
Gestation sows, n=14						
Mean	6.25	15.0	2.37	22.6	2.28	4.93
Min	4.43	12.6	1.65	19.4	1.24	2.24
Max	7.99	17.6	5.15	29.3	3.77	7.57
SD	1.04	1.48	0.92	2.72	0.73	1.48
Lactating sows, n=14						
Mean	6.54	17.9	2.26	19.0	1.79	5.85
Min	5.56	15.7	1.45	14.6	1.01	3.49
Max	7.52	21.6	4.14	24.5	2.70	8.01
SD	0.56	1.54	0.65	3.01	0.58	1.25
Nursery piglets, n=14						
Mean	5.90	19.0	1.99	14.0	0.91	5.47
Min	5.01	16.9	1.29	11.3	0.39	2.26
Max	6.97	21.5	3.30	16.2	1.58	7.72
SD	0.49	1.51	0.56	1.48	0.37	1.87
Finishing-growing, n=37						
Mean	5.17	17.1	2.01	16.7	1.31	5.47
Min	3.06	13.7	1.30	11.9	0.41	1.71
Max	6.58	19.9	2.53	20.5	2.51	9.16
SD	0.76	1.52	0.34	1.94	0.51	1.73

[1]CP=crude protein; NDICP=neutral detergent insoluble crude protein; NDF=neutral detergent fibre; ADL=acid detergent lignin; EE=ether extract

Table 4. Variability of slurry among the farms studied (n=79), % DM basis

	pH	Total solids	Ash	Nitrogen	Ammonia-N	NDICP	NDF	ADF	ADL	Ether extract
Gestation sows, n=14										
Mean	7.77	5.87	30.5	13.2	10.1	5.66	39.5	20.7	8.96	6.94
Min	7.54	0.49	21.1	4.12	2.64	1.95	17.3	8.08	4.46	3.58
Max	8.03	15.5	43.3	37.4	34.5	17.2	59.6	36.3	14.1	12.2
SD	0.14	5.27	7.22	9.10	9.01	3.86	15.2	9.28	3.56	2.76
Lactating sows, n=14										
Mean	7.60	3.58	29.2	10.9	7.38	6.17	38.7	19.6	8.88	9.32
Min	6.98	0.86	21.7	6.02	3.11	1.75	24.4	12.3	5.43	4.03
Max	8.16	7.58	43.3	24.5	20.9	18.8	48.9	27.5	16.1	15.5
SD	0.33	2.23	6.82	5.58	5.73	4.66	7.81	4.82	2.66	3.56
Nursery piglets, n=14										
Mean	7.35	3.73	29.7	11.3	7.53	5.98	29.5	12.6	5.12	13.0
Min	6.34	0.57	22.4	5.62	2.52	0.23	3.79	1.07	0.39	6.28
Max	7.92	9.73	42.1	23.0	19.3	16.4	48.0	21.2	10.2	18.8
SD	0.57	2.42	5.91	4.68	4.44	4.93	13.2	6.52	2.73	4.31
Finishing-growing, n=37										
Mean	7.43	5.38	26.7	12.8	8.92	4.24	34.5	16.6	6.78	10.8
Min	6.41	0.75	15.5	4.18	1.32	0.56	5.25	2.24	0.59	2.93
Max	8.05	17.7	44.3	27.9	24.1	14.9	56.6	36.2	18.3	24.9
SD	0.42	4.08	6.87	5.16	4.63	3.22	12.4	7.53	3.57	4.03

NDICP=neutral detergent insoluble crude protein; NDF=neutral detergent fibre; ADF=acid detergent fibre; ADL=acid detergent lignin.

The effects of classified (type of farm, season and location) and of continuous (associated to feed composition) variables on slurry characteristics are shown in Table 6. Mean pH of manure was 7.50. It was not affected either by type of farm or season. An interaction between location and dietary NDF content was observed, as an increase of fibre concentration within type of farm increased linearly pH in farms placed in the

Table 5. Pearson correlation coefficients among some of the dietary and slurry characteristics studied[1, 2]

a) Feed composition (% DM)						
	Ash	**Crude protein**	**NDICP**	**NDF**	**ADF**	**ADL**	**Ether extract**

Feed composition (% DM)	**Ash**	**Crude protein**	**NDICP**	**NDF**	**ADF**	**ADL**	**Ether extract**
Ash	1	–	–	–	–	–	–
Crude protein	**–0.026 (0.818)**	1	–	–	–	–	–
NDICP	**0.260 (0.021)**	**–0.107 (0.347)**	1	–	–	–	–
NDF	**0.409 (<0.001)**	**–0.606 (<0.001)**	0.442 (<0.001)	1	–	–	–
ADF	**0.485 (<0.001)**	**–0.450 (<0.001)**	0.377 (<0.001)	0.864 (<0.001)	1	–	–
ADL	**0.596 (<0.001)**	**–0.443 (<0.001)**	0.489 (<0.001)	0.810 (<0.001)	0.852 (<0.001)	1	–
Ether extract	0.022 (0.848)	0.180 (0.112)	0.129 (0.259)	0.017 (0.879)	–0.129 (0.256)	–0.016 (0.888)	1

b) Slurry characteristics (% DM)						
	pH	**TS (%)**	**VS (%)**	**NDF**	**Total N**	**Ammonia N**	**Ether extract**

Feed composition (% DM)	**pH**	**TS (%)**	**VS (%)**	**NDF**	**Total N**	**Ammonia N**	**Ether extract**
Ash	0.161 (0.156)	0.049 (0.668)	0.018 (0.877)	–0.070 (0.539)	–0.130 (0.252)	**–0.228 (0.042)**	**–0.272 (0.015)**
Crude protein	–0.054 (0.636)	**–0.353 (0.001)**	**–0.357 (0.001)**	**–0.263 (0.019)**	–0.027 (0.815)	–0.059 (0.607)	**0.256 (0.023)**
NDICP	–0.028 (0.807)	**–0.233 (0.038)**	**–0.231 (0.040)**	**–0.207 (–0.067)**	–0.083 (0.464)	–0.129 (0.258)	0.140 (0.219)
NDF	**0.257 (0.021)**	0.142 (0.213)	0.134 (0.240)	**0.238 (0.035)**	0.092 (0.418)	0.116 (0.308)	**–0.471 (p<0.001)**
ADF	**0.343 (0.001)**	0.166 (0.142)	0.147 (0.197)	**0.213 (0.058)**	0.066 (0.560)	0.154 (0.176)	**–0.516 (p<0.001)**
ADL	**0.300 (0.007)**	0.148 (0.193)	0.133 (0.244)	0.132 (0.246)	0.138 (0.223)	0.149 (0.190)	**–0.490 (p<0.001)**
Ether extract	0.064 (0.576)	–0.169 (0.137)	–0.156 (0.171)	–0.124 (0.276)	0.081 (0.473)	0.071 (0.531)	0.065 (0.564)

[1]Values in parentheses indicate *p*-value; bold values indicate significant correlations. [2] NDICP=neutral detergent insoluble crude protein; NDF=neutral detergent fibre; ADF=acid detergent fibre; ADL=acid detergent lignin; TS=total solids; VS=volatile solids;

Centre of Spain (from 7.08 to 7.77 in the natural scale between the extreme values of the range studied; *p*<0.001), but not (*p*=0.522) in those located near the Mediterranean (Fig. 1). Total solids and VS concentrations in slurry were 4.85 and 3.61%, as average. They were not affected by either of the classified variables studied, but decreased linearly (by 84% in the natural scale, *p*=0.003) when increasing dietary CP content. Ash slurry concentration on DM basis was affected by location; this variable was negatively transformed, so that values in Table 6 indicate that ash concentration was higher in the Mediterranean than in the Centre located farms (30.0 *vs* 26.1%, respectively, *p*=0.02). Neither total N or total ammonia N content on DM, nor
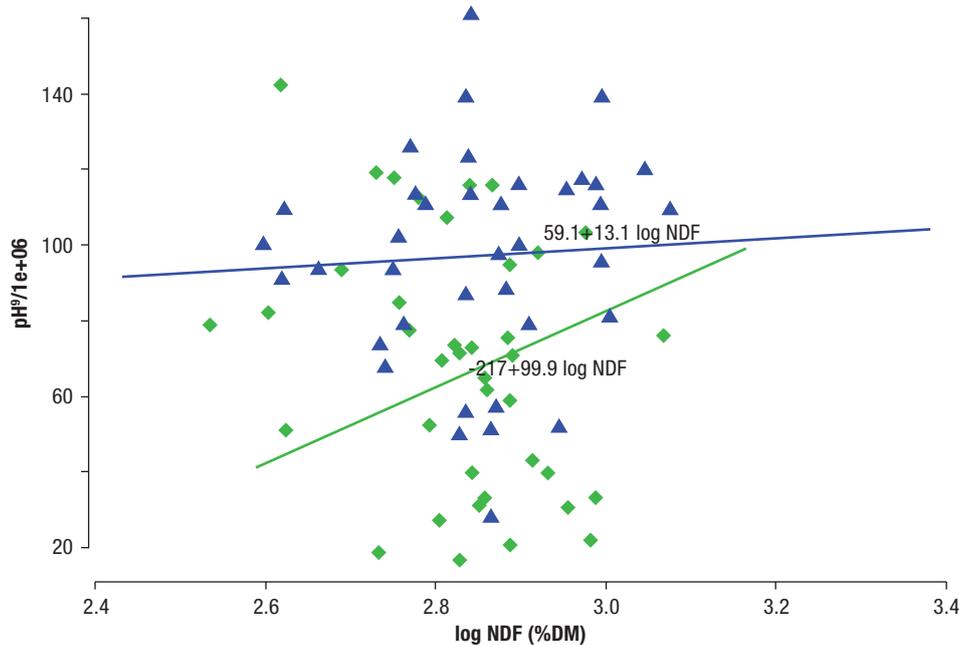
Figure 1. Effect of the interaction between location and dietary NDF (neutral detergent fibre) content on pH of the slurry (Location=Centre ♦; Location=Mediterranean ▲)

Table 6. Effect of the factors studied on slurry characteristics and potential emissions (% DM, except when indicated). Values in table are LS means. Means and regression coefficients of the continuous variables correspond to values transformed as indicated in Table 2

Item[1]	Type of farm[2]				Season[2]		Location[2]		SD	p		
	N	GF	G	L	W	S	C	M		Type	Season	Location
pH[3]	78.1	76.8	98.4	88.0	85.0	85.6	68.4	97.2	28.9	0.406	0.932	**<0.001**
Total solids (%)[4]	1.39	1.39	0.84	1.20	1.22	1.18	1.10	1.13	0.82	0.304	0.837	0.258
Volatile solids (%)[5]	1.27	1.27	1.20	1.22	1.22	1.21	1.20	1.24	0.22	0.302	0.847	0.373
Ash	0.188	0.197	0.180	0.193	0.191	0.188	0.194	0.185	0.021	0.639	0.259	**0.021**
Total N	2.35	2.47	2.39	2.30	2.37	2.38	2.41	2.34	0.22	0.673	0.936	0.554
Ammonia N	1.87	2.05	2.00	1.80	1.92	1.94	1.94	1.92	0.63	0.600	0.890	0.890
Ammonia N (%N)	4.12	4.19	4.21	4.11	4.15	4.16	4.13	4.18	0.22	0.526	0.815	0.388
NDF[6]	29.7	34.3	39.7	38.8	36.9	34.4	37.2	34.1	12.5	0.481	0.416	0.275
ADF[7]	13.3	16.7	20.1	19.4	17.6	17.1	17.8	16.9	7.43	0.372	0.793	0.587
ADL[8]	2.21	2.52	2.89	2.94	2.65	2.63	2.65	2.63	0.64	0.125	0.857	0.857
Ether extract[9]	12.4^a	10.7^{a,b}	7.45^b	9.69^{a,b}	9.32	10.8	9.77	10.3	3.73	**0.016**	0.094	0.538
VFA (mg/L)[10]	18.8^a	16.1^a	7.47^b	12.5^{a,b}	12.9	14.6	15.0	12.5	5.19	**0.002**	0.266	0.068
In vitro ammonia emissions (mg/L/day)[11, 12, 13]	4.62	4.50	4.30	4.38	4.50	4.33	4.29	4.54	0.46	0.754	0.125	**0.027**
Ultimate methane yield (mL/g volatile solids)	18.0^a	16.1^{a,b}	13.1^{b,c}	12.0^c	15.3	14.3	15.4	14.2	4.50	**0.002**	0.311	0.210

[1]NDF=neutral detergent fibre; ADF=acid detergent fibre; ADL=acid detergent lignin; VFA=volatile fatty acids. [2]N=nursery; GF=growing-finishing; G=gestating; L=lactating; W=winter; S=summer; C=centre; M=mediterranean. [3]Interaction between linear effect of dietary NDF content and location: 99.9 ± 29.3 (Centre, *p*=0.001) and 13.4 ± 20.8 (*p*=0.522) at the Mediterranean farms. [4]Linear effect of dietary CP: -3.22 ± 1.06; *p*=0.003. [5]Linear effect of dietary CP: -0.89 ± 0.29; *p*=0.003. [6]Linear effect of dietary NDF: 14.5 ± 7.32; *p*=0.05. [7]Linear effect of dietary NDF: 12.0 ± 4.33; *p*=0.007. [8]Linear effect of dietary NDF: 1.14 ± 0.38; *p*=0.004. [9]Linear effect of dietary ADL (%NDF): -2.25 ± 1.25; *p*=0.08. [10]Linear effect of dietary CP: -25.6 ± 7.67; *p*=0.002. [11]Linear effect of dietary ash: -0.30 ± 0.09; *p*=0.001. [12]Linear effect of dietary CP: -1.51 ± 0.61; *p*=0.015. [13]Linear effect of dietary ADL (%NDF): 0.49 ± 0.19; *p*=0.012.

the percentage of ammonia N on total N in the manure (averaging 12.3, 8.60 and 65.9%, respectively) were affected by any of the variables studied. All the traits related to sequential fibre composition (NDF, ADF and ADL) of slurry DM, were not affected by any of the classified variables, but increased linearly in the natural untransformed scale (by 47.2, 94.4 and 128%, *p*=0.05, 0.007 and 0.004, respectively) with dietary NDF content. Ether extract concentration on manure DM tended to decrease linearly (by 34.7% between extreme values, *p*=0.08) with the degree of lignification of NDF (ADL/NDF × 100) in the diet; it was also affected by type of farm (*p*=0.016) with higher values obtained in young (nursery) with respect to older (gestating and lactating sows) farms, whereas grow-finishing farms gave intermediate values. These results were parallel to those obtained for VFA content and B_0. In addition, VFA decreased linearly with dietary CP content (from 10751 to 466 mg/L, *p*=0.002). Estimates of in vitro ammonia emissions from the slurry decreased in farms located in the Central region (*p*=0.027), but were not affected by the other classified variables studied; they also decreased with dietary ash and CP (by 55.8 and 77.4% in the range sampled in this study, *p*=0.001 and 0.015, respectively) and increased linearly with degree of lignification of NDF (by 137%,

p=0.012), in the range of values studied. No significant effects were detected for any of the interactions among the main factors included in the model on any of the slurry characteristics studied.

A prediction model was developed to estimate the in vitro emissions of ammonia and the potential CH_4 yield from the samples of manure studied, using as independent continuous variables all the chemical traits measured both in feeds and slurry. In this model, only variables associated to manure composition (% DM, except when indicated) were included:

In vitro ammonia emissions (ln mg/L)=$-0.74 (\pm 0.59)$ $+ 0.58 (\pm 0.049)$ ln total N $+ 1.319 (\pm 0.15)$ ln ammonia N (% total N) $+ 2.84 \cdot 10^{-9} (\pm 9.57 \cdot 10^{-10})$ pH[9] (R^2=0.771; RSD=0.269; n=79).

A better fit was obtained when expressing NH_3 emissions on VS basis:

In vitro ammonia emissions (mg/g VS)$^{1/3}$=-3.72 $(\pm 0.92) + 1.85 (\pm 0.12)$ ln total N $+ 0.653 (\pm 0.26)$ ln ammonia N (% total N) $+ 4.83 \cdot 10^{-9} (\pm 1.50 \cdot 10^{-10})$ pH[9] $- 0.046$ EE (R^2=0.888; RSD=0.378; n=79).

In the case of potential CH_4 yield, the fitted model was:

B_0 (mL/g VS)$^{1/2}$=21.1 $(\pm 2.45) + 0.396 (\pm 0.10)$ EE $- 7.07 (\pm 1.21)$ ADL$^{1/2}$ $+ 0.240 (\pm 0.059)$ NDF (R^2=0.610; RSD=3.13; n =79).

According to these results, in vitro ammonia emission was not affected by type of farm, season or location, but increased with total N (*p*<0.001) content, proportion of ammonia N on total N (*p*<0.001) and pH (*p*=0.007) of the slurry from 3.7 to 187; 25.1 to 48.5 and 29.2 to 54.4 mg/g VS and decreased with EE content (*p*<0.001) from 52.8 to 20.1 mg/g VS, for the extreme values of the range studied. Potential methane production was neither affected by any of the classified variables, but increased (*p*<0.001) with EE and NDF manure content (from 149 to 443 and 61.1 to 436 mg/g VS, respectively) and decreased with ADL concentration from 852 to 11.1 mg/g VS in the range of the samples studied.

Figures 2 and 3 represent, respectively, the changes of estimates of ammonia and methane production in the range of values studied, with respect to the slurry characteristics selected in the prediction models. Changes are expressed relatively taking as base=100 the average value of each of the independent variables considered, in the natural untransformed scale.

Calibration and cross validation statistics of prediction of laboratory analyses and ammonia and methane estimations from NIRS analysis are shown in Table 7. The coefficients of determination of calibration for chemical analyses were generally high, above 0.90 for DM, VS, total N and EE, being the lowest (from 0.70 to 0.85) those of ash, pH, and fibrous constituents; the coefficients of determination obtained for cross validation were similar but slightly lower. Coefficients of determination of cross validation for prediction of in vitro ammonia emissions and ultimate methane yield were respectively 0.836 and 0.682.

Discussion

Effects of type of farm, season and location

When considering in the model the effect of the covariates associated to dietary chemical composition, the classified variables studied had a limited influence on the slurry characteristics. Farm location had a significant effect on pH and ash of the manure, with the highest values observed in Mediterranean farms. Although feeding programs, genetic potential and systems of management are similar, there is a major difference in water quality between the two areas sampled. According to the Spanish Ministry of Health (http://sinac.msc.es/SinacV2/), the Mediterranean region has a
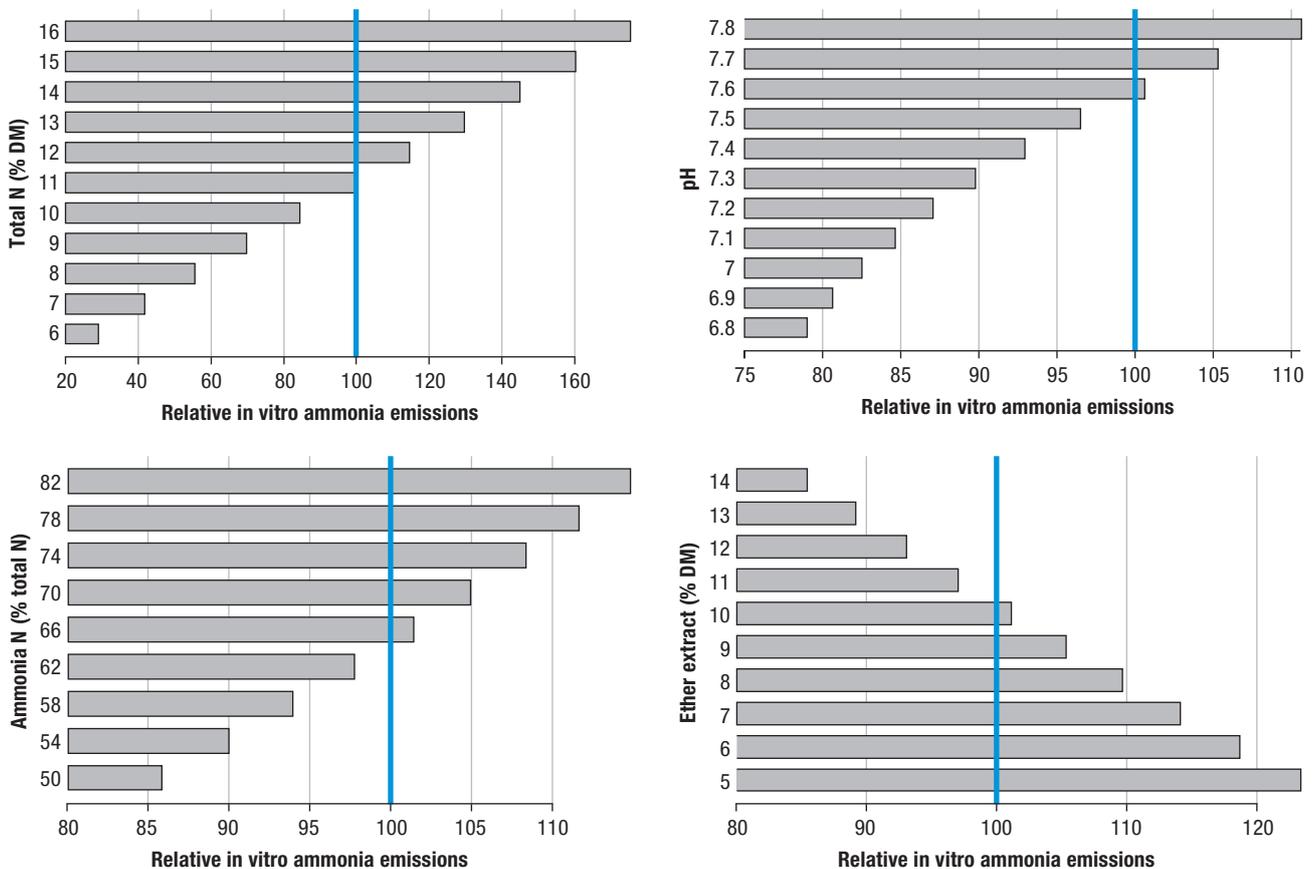
Figure 2. Effect of several slurry characteristics on in vitro ammonia emissions (base 100=average value of each independent variable).
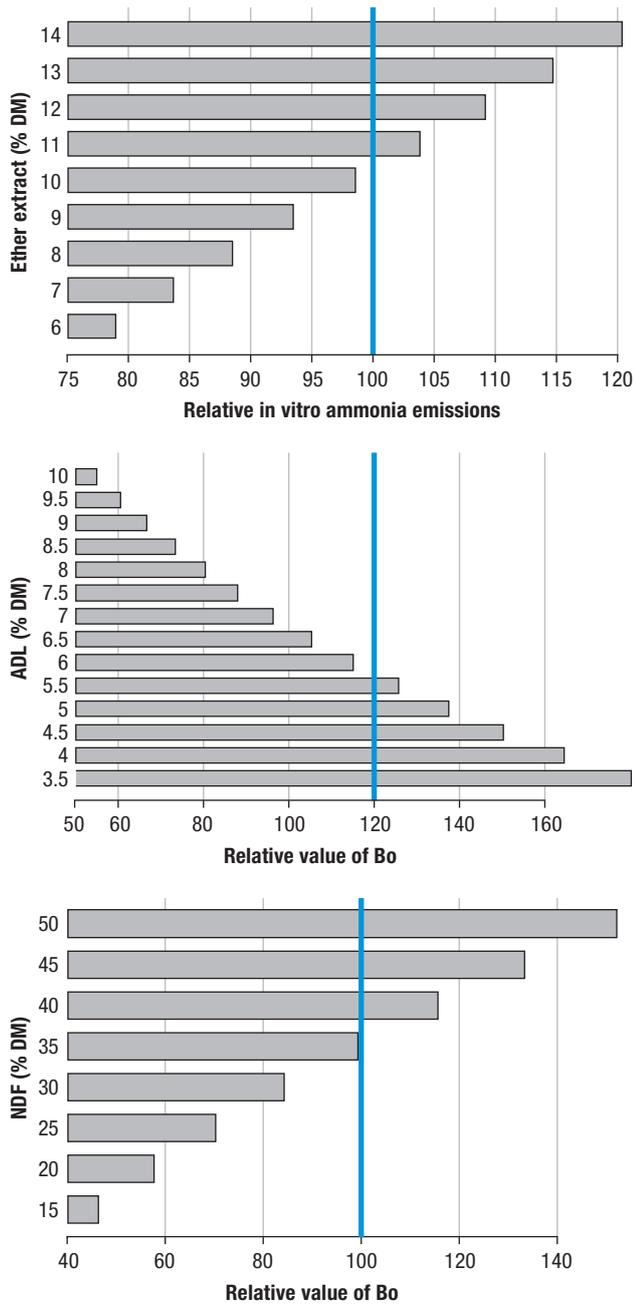
Figure 3. Effect of several slurry characteristics on the ultimate methane yield (B_0). Base 100=average value of each independent variable. ADL=acid detergent lignin; NDF=neutral detergent fibre.

higher mean pH (7.9 *vs* 7.0) and salinity of the drinking water (conductivity 986 *vs* 90 µS/cm) than the Centre region. The current results suggest that these differences may be transferred to the corresponding slurry characteristics (acidity and ash content). The higher mean pH in the slurry from the Mediterranean farms would also explain the higher in vitro potential ammonia emissions (by 31% on average) observed in this area in the present study, as ammonia emissions tend to decrease with acidity (Aarnink & Verstegen, 2007).

In this study water characteristics were not analysed and therefore further studies in this sense would be necessary to confirm and characterize this effect.

The effect of type of farm on slurry traits was scarce, as the differences in average feed composition among animal categories were accounted by the model co-variates. Even so, a higher EE and VFA concentration and B_0 were observed in the slurry samples taken from the youngest animals (nursery farms). Average feed EE content was similar among the different types of farms (see Table 3), but young pigs show a lower capacity of fat digestion (Soares & López-Bote, 2002) that would lead to a higher fat excretion and slurry concentration. Otherwise, EE is by far the nutrient with the highest capability of microbial fermentation and therefore a nutrient with high potential to generate methane in the slurry (Angelidaki & Sanders, 2004).

Season had no significant influence on any of the slurry components and this could explain the absence of seasonal effect on potential NH_3 and CH_4 emissions. Slurries were collected from indoor slurry pits, where climatic variations are attenuated, thus minimizing the seasonal effect. Other works (Møller *et al.,* 2004b; Pereira *et al.*, 2012) have shown lower methane and ammonia emissions at lower ambient temperatures, probably as a consequence of slower microbial and enzymatic reactions. This study, however, is not comparable since potential emissions are estimated under controlled, laboratory conditions. According to Angelidaki & Sanders (2004) temperature does not influence the ultimate biodegradability of a component, but may reduce the degradation rates. In addition, our results regarding CH_4 emissions are in accordance with Liu *et al.* (2014). In their review, these authors reported that temperature affected CH_4 emissions from lagoons, but was not a significant factor on housing CH_4 emissions from swine. Otherwise, apart from the intrinsic manure characteristics, NH_3 emissions are conditioned by ambient temperature and the convective mass transfer coefficient.

Effects of dietary fibre concentration

A higher fibre supply buffered the decrease of slurry pH in farms located at the Centre of Spain, where mean water and slurry pH were lower than in the Mediterranean area. This result might be explained by the buffering and cation exchange properties of some cell wall constituents (*i.e.* lignin, nitrogen and pectins; Van Soest, 1994), as an increase in dietary NDF concentration also increased slurry content of NDF, and more markedly those of ADL and ADF. These increments reflect the limited digestion efficiency of cell wall constituents in the pig, in inverse relation with its

Table 7. Coefficients of determination and root mean square errors of calibration (R^2_c, SEC) and cross validation (R^2_{CV}, SECV) to predict the chemical composition (%) of the slurry, the in vitro NH3 emissions and the ultimate methane yield (B0)

Item	Scatter correction[a]	Derivative treatments[b]	Wavelenght range, nm	Factors[c]	Calibration		Cross calibration		SD[d]/ SECV
					SEC	R^2_c	SECV	R^2_{CV}	
pH	None	1,10,10,1	1200-2400	8	$11.2 \cdot 10^6$	0.840	$13.2 \cdot 10^6$	0.815	2.33
Total solids	None	1,5,5,1	1100-2400	7	0.178	0.957	0.239	0.926	3.58
Volatile solids	None	2,5,5,1	1100-2400	7	0.055	0.944	0.071	0.915	3.29
Ash	None	1,5,5,1	1100-2400	7	0.010	0.811	0.011	0.753	2.00
Total N	None	2,5,5,1	1200-2400	8	0.077	0.925	0.115	0.842	2.46
Ammonia N (%N)	None	1,10,10,1	1100-2400	8	0.080	0.876	0.079	0.846	2.48
Neutral detergent fibre	None	0,0,1,1	1100-2400	8	0.700	0.831	0.758	0.806	2.25
Acid detergent fibre	None	0,0,1,1	1100-2400	8	0.388	0.765	0.449	0.697	1.76
Lignin acid detergent	None	0,0,1,1	1200-2400	8	0.169	0.709	0.195	0.622	1.60
Ether extract	None	1,10,10,1	1100-2400	8	0.124	0.917	0.131	0.907	3.26
In vitro ammonia emissions (mg/g VS/day)	SNVD	1,10,10,1	1100-2400	8	1.69	0.887	2.04	0.836	2.46
Ultimate methane yield Bo (mL/g VS)$^{1/2}$	MSC	2,10,10,1	1100-1800	8	2.17	0.780	2.64	0.682	1.82

[a]SNVD: standard normal variate and detrending; MSC: multiplicative scatter correction. [b]The first, second, third and fourth numbers denote the order of the derivative function, the spectral segment length over the derivative is calculated and the segment lengths over which the function was smoothed. [c]Factors, number of actual terms used to estimate the parameter. [d]SD=standard deviation

degree of lignification. In contrast with the current results, Canh *et al.* (1997) reported a reduction of pH of slurry in response to the dietary addition of digestible fibre in form of sugar beet pulp, and related it to a higher microbial fermentation in the hindgut. However, a lesser or none effect of dietary NDF on pH was observed in other studies when also supplementing diets with soluble fibre (Halas *et al.*, 2010; Von Heimendahl *et al.*, 2010), or with a mixture of soluble and insoluble fibre (Galassi *et al.*, 2010).

In the same way, there is a general agreement in that inclusion of fermentable fibre, as sugar beet pulp or inuline, leads to a shift in the N excretion from faeces to urine (Aarnink & Verstegen, 2007), which is generally associated to an enhanced metabolic urea retention and excretion as microbial protein in the faeces (Kreuzer *et al.*, 1999). This shift would imply in turn a decrease of ammonia emissions from the slurry with fibre supplementation but also a higher fermentation activity and methane losses, as observed by Montalvo *et al.* (2013). However, Galassi *et al.* (2010) did not observe a significant influence of supplementation of the diet with a mixture of 20% of wheat bran and 4% of sugar beet pulp on ammonia emissions and Triolo *et al.* (2011) reported a high negative correlation (r=-0.952; p<0.001) between lignin content in the manure VS and its biochemical methane potential. Moreover, Bindelle *et al.* (2009) demonstrated that the substitution of sugar beet pulp with a source of insoluble fibre, as oat hulls, decreased the synthesis of bacterial protein in the gut and the ratio faecal N:urinary N to levels similar or lower than those reached with the standard non supplemented diet; these results help to explain the positive relationship found in the current study between degree of lignification of dietary NDF and ammonia emissions from the slurry.

The proportion of soluble/insoluble fibre or the fermentability of the feeds sampled were not measured in the current study, but the average degree of lignification of the NDF was 8.48% (see Table 3), which is similar to those of wheat bran (8.83%) or oat hulls (8.98%), but clearly above to that found in sugar beet pulp (3.9% as average, according to FEDNA, 2010). The relatively lignified type of fibre more frequently used at present in Spanish commercial feeds for pigs would then explain the lack of effect of dietary fibre level on the ratio of ammonia to total N or the ammonia and methane emissions from the slurry.

Effects of dietary N concentration

The lack of influence of dietary protein concentration on ammonia and N content in the slurry differs from most of previous research that generally found a positive relationship between these variables (Canh *et al.*, 1997, 1998; Hayes *et al.*, 2004), although following great changes in dietary protein level (from 4 to 9 percentage units). However, Hernández *et al.* (2011) found little effect of dietary protein level on manure composition and ammonia emissions when working with commercial growing-finishing feeds and a narrower range of CP content (from 14 to 16%). In the same way, neither Portejoie *et al.* (2004) nor Kerr *et al.* (2006)

observed significant differences in ammonia or total N content in the slurry of growing pigs, when comparing diets containing 20 *vs* 16% or 14.5 *vs* 12.0% CP, respectively. This lack of effect might be related to the short range of variation used in commercial studies. In addition, Kerr *et al.* (2006) also suggest that a higher ammonia volatilization might occur in the slurry of pigs fed the greater CP diets before samples were taken. This effect may be particularly relevant for slurries stored for a long time (*e.g.* more than 3 weeks) in manure pits. In the current study, SD of CP content was around 1.5 within type of farms (Table 3), so that most of the diets were in a narrow range of three percentage units with respect to the mean.

Otherwise, an increase of dietary N concentration led to a linear decrease in TS content of the manure in the current study. Portejoie *et al.* (2004) showed in finishing pigs that lowering dietary protein level (from 20 to 12%) decreased DM concentration in the slurry (from 5.9 to 4.4%), because of a lower water consumption. The same trend (3.24 *vs* 2.51%) was observed by Kerr *et al.* (2006), although in this case the range of variation of protein content in the feed was shorter (from 14.5 to 12.0%) and the differences did not reach significant levels. The observed reduction of DM content in the manure when dietary CP concentration increased would also explain its negative effect on in vitro ammonia emissions per L of slurry, because of the parallel reduction of nutrient content (including ammonia and total N).

Effects of dietary ash concentration

The range of ash content in feeds DM within the different types of farms studied was from 2 to 3.5 percentage units, with SD ranging from 0.5 to 1.0 (Table 3). In commercial diets this variation is mostly related to Ca content (Sánchez & González, 2005), but also to the inclusion of clay in the feeds to improve pelleting characteristics. Some sources of Ca (as its anionic salts, sulphate or chloride) can reduce urine pH and then reduce emissions, but they are of little use in practical pig diets. Otherwise, undigested clay might increase ammonia absorptive properties of the slurry, which could help to explain the reduction of ammonia emissions observed in the current study with increasing ash concentrations in the manure.

Models of prediction of ammonia and methane potential emissions

The accuracy of the prediction of most of the organic constituents of pig slurry from NIRS was gener-

ally high which confirms the findings of other studies (Malley *et al.*, 2002; Saeys *et al.*, 2005; Ye *et al.*, 2005; Sørensen *et al.*, 2007) on the usefulness of this methodology to predict main chemical components of fresh slurry and the biochemical CH_4 potential in a range of organic substrates (Doublet *et al.*, 2013; Triolo *et al.*, 2014). The poor prediction for pH could be related to internal correlations to organic compounds giving no true correlations between pH and spectra absorbance peaks (Huang *et al.*, 2007). The lowest R^2_{cv} values were obtained for fibrous components (as those of Van Soest fibre analysis) where analytical procedures tend to lead to a high analytical error. In addition, the prediction accuracy of chemical traits included as variables in chemical prediction models and those from NIRS help to explain the similar accuracy of the prediction of potential NH_3 and CH_4 emissions from the slurry composition or through NIRS methodology. The ratio of SD to SECV values in Table 7 was 2.46 for ammonia and 1.82 for methane potential emissions; these values are below the level of 3.0 that makes ideally the prediction "good" according to Williams & Sobering (1996), being only useful that of potential NH_3 emissions for screening purposes, and emphasizes the need to enlarge the database used to increase variance of the reference data.

At commercial scale, it must be also considered that relating nutritional factors to gaseous emissions may not be straightforward. Manure composition and therefore gaseous emissions may be also affected by other factors of variation such as slurry management or temperature (Liu *et al.*, 2013; Snoek *et al.*, 2014) in the farm. The type of operation (*e.g.* sows, growing pigs, etc) has also been considered a relevant factor influencing manure composition as a consequence of manure dilution (Conn *et al.*, 2007), but this effect may be confounded with dietary factors, since feed composition differs for animals in different physiological status. However, despite the influence of these variables, the prediction models of emissions in this study showed similar fitting to prediction models of other slurry components reported in the literature. For example, Yagüe *et al.* (2012) reported coefficients of determination ranging between 60 and 90% for physico-chemical models to predict most slurry constituents, whereas Triolo *et al.* (2014) found a coefficient of determination of 84% in predicting biochemical methane production of a wide variety of biomass samples.

In conclusion, the models developed in the current study in a wide range of practical conditions are useful to understand the factors behind changes in slurry characteristics and to predict NH_3 and CH_4 potential emissions. In addition, predictions from NIRS of gaseous emissions showed a similar accuracy to prediction models using slurry composition, and can be therefore

further explored to investigate potential pollution of livestock slurries, as well as for their use as biogas substrates.

Acknowledgments

References

Aarnink AJA, Verstegen MWA, 2007. Nutrition, key factor to reduce environmental load from pig production. Livest Sci 109: 194-203. http://dx.doi.org/10.1016/j.livsci.2007.01.112.

Álvarez-Rodríguez J, Hermida B, Parera J, Morazan H, Balcells J, Babot D, 2013. The influence of drinker device on water use and fertiliser value of slurry from growing-finishing pigs. Anim Prod Sci 53: 328-334. http://dx.doi.org/10.1071/AN12136.

Angelidaki I, Sanders W, 2004. Assessment of the anaerobic biodegradability of macropollutants. Rev Environ Sci Biotechnol 3: 117-129. http://dx.doi.org/10.1007/s11157-004-2502-3.

AOAC, 2000. Official methods of analysis, 15th ed. (Harwitte W, Ed.). Association of Official Analytical Chemists. Washington, USA.

APHA, 2005. Standard methods for the examination of water and wastewater. Centennial Edition, Baltimore, MD, USA.

Barnes RJ, Dhanoa MS, Lister SJ, 1989. Standard normal variate transformation and de-trending of near diffuse reflectance spectra. Appl Spectrosc 43: 772-777. http://dx.doi.org/10.1366/0003702894202201.

Bietresato M, Sartori L, 2013. Technical aspects concerning the detection of animal waste nutrient content via its electrical characteristics. Bioresour Technol 132: 127-136. http://dx.doi.org/10.1016/j.biortech.2012.12.184.

Bindelle J, Buldgen A, Delacollette M, Wavreille J, Agneessens R, Destain JP, Leterme P, 2009. Influence of source and concentrations of dietary fiber on in vivo nitrogen excretion patways in pigs as reflected by in vitro fermentation and nitrogen incorporation by fecal bacteria. J Anim Sci 87: 583-593. http://dx.doi.org/10.2527/jas.2007-0717.

Box GEP, Cox DR, 1964. An analysis of transformations. J R Stat Soc B 26: 211-246.

Canh TT, Verstegen MWA, Aarnink AJA, Schrama JW, 1997. Influence of dietary factors on nitrogen partitioning and composition of urine and faeces of fattening pigs. J Anim Sci 75: 700-706.

Canh TT, Aarnink AJA, Schutte JB, Sutton A, Langhout DJ, Verstegen MWA, 1998. Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing-finishing pigs. Livest Prod Sci 56: 181-191. http://dx.doi.org/10.1016/S0301-6226(98)00156-0.

Chen L, Xing L, Han L, Yang Z, 2009. Evaluation of physicochemical models for rapidly estimating pig manure nutrient content. Biosyst Eng 103: 313-320. http://dx.doi.org/10.1016/j.biosystemseng.2009.04.007.

Conn KL, Topp E, Lazarovits G, 2007. Factors influencing the concentration of volatile fatty acids, ammonia, and other nutrients in stored liquid pig manure. J Environ Qual 36: 440-447. http://dx.doi.org/10.2134/jeq2006.0222.

Dinuccio E, Berg W, Balsari P, 2008. Gaseous emissions from the storage of untreated slurries and the fractions obtained after mechanical separation. Atmos Environ 42: 2448-2459. http://dx.doi.org/10.1016/j.atmosenv.2007.12.022.

Doublet J, Boulanger A, Ponthieux A, Laroche C, Poitrenaud M, Cacho Rivero JA, 2013. Predicting the biochemical methane potential of wide range of organic substrates by near infrared spectroscopy. Bioresour Technol 128: 252-258. http://dx.doi.org/10.1016/j.biortech.2012.10.044.

EEA, 2014a. European Union emission inventory report 1990-2012 under the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP). European Environment Agency, Technical Report 12/2013.

EEA, 2014b. Annual European Union greenhouse gas inventory 1990-2012 and inventory Report 2014. Submission to the UNFCCC Secretariat. European Environment Agency, Technical Report 9/2013.

Faostat, 2014. Production quantities by country, 2012. Food and Agriculture Organization of the United Nations. Available in http://faostat3.fao.org/home/E [accessed April 2014].

FEDNA, 2010. Tablas FEDNA de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos, 3rd ed. (de Blas C, Mateos GG, García-Rebollar P, Eds). Fundación Española para el Desarrollo de la Nutrición Animal, Madrid, Spain, 502 pp.

Galassi G, Colombini L, Malagutti L, Crovetto GM, Rapetti L, 2010. Effects of high fibre and low protein diets on performance, digestibility, nitrogen excretion and ammonia emission in the heavy pig. Anim Feed Sci Technol 161: 140-148. http://dx.doi.org/10.1016/j.anifeedsci.2010.08.009.

Halas D, Hansen CF, Hampson DJ, Kim JC, Mullan BP, Wilson RH, Pluske JR, 2010. Effects of benzoic acid and inulin on ammonia-nitrogen excretion, plasma urea levels, and the pH of faeces and urine of weaner pigs. Livest Sci 134: 243-245. http://dx.doi.org/10.1016/j.livsci.2010.06.153.

Hayes ET, Leek ABG, Curran TP, Dodd VA, Carton OT, Beattie VE, O'Doherty JV, 2004. The influence of diet crude protein level on odour and ammonia emissions from finishing pig houses. Bioresour Technol 91: 309-315. http://dx.doi.org/10.1016/S0960-8524(03)00184-6.

Hernández F, Martínez S, López C, Megías MD, López M, Madrid J, 2011. Effect of dietary crude protein levels in a commercial range on the nitrogen balance, ammonia emission and pollutant characteristics of slurry in fattening pigs. Animal 5: 1290-1298. http://dx.doi.org/10.1017/S1751731111000115.

Huang G, Han L, Liu X, 2007. Rapid estimation of the composition of animal manure compost by near infrared reflectance spectroscopy. J Near Infrared Spec 15: 387-394. http://dx.doi.org/10.1255/jnirs.745.

Jarret G, Cerisuelo A, Peu P, Martinez J, Dourmad JY, 2012. Impact of pig diets with different fibre contents on the composition of excreta and their gaseous emissions and anaerobic digestion. Agr Ecosys Environ 160: 51-58. http://dx.doi.org/10.1016/j.agee.2011.05.029.

Jørgensen H, 2007. Methane emission by growing pigs and adult sows as influenced by fermentation. Livest Sci 109: 216-219. http://dx.doi.org/10.1016/j.livsci.2007.01.142.

Jouany JP, 1982. Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermentor contents. Sci Alimen 2: 131-144.

Kerr BJ, Ziemer SL, Trabue SL, Crouse JD, Parkin TB, 2006. Manure composition of swine as affected by dietary protein and cellulose concentrations. J Anim Sci 84: 1584-1592.

Kreuzer M, Wittmann M, Gerdemann MM, Hanneken H, Abel H, Machmuller A, 1999. Re-examination of the metabolizable energy contents of various rations containing different types and levels of bacterially fermentable substrates in digestibility experiments with growing pigs. J Anim Physiol Anim Nutr 82: 33-49. http://dx.doi.org/10.1046/j.1439-0396.1999.00218.x.

Licitra G, Hernández TM, Van Soest PJ, 1996. Standardization of procedures for nitrogen fractionation of ruminant feed. Anim Feed Sci Technol 57: 347-358. http://dx.doi.org/10.1016/0377-8401(95)00837-3.

Liu Z, Powers W, Liu H, 2013. Greenhouse gas emissions from swine operations: Evaluation of the Intergovernmental Panel on Climate Change approaches through meta-analysis. J Anim Sci 91: 4017-4032. http://dx.doi.org/10.2527/jas.2012-6147.

Malley DF, Yesmin L, Eilers RG, 2002. Rapid analysis of hog manure and manure-amended soils using near-infrared spectroscopy. Soil Sci Soc Am J 66: 1677-1686. http://dx.doi.org/10.2136/sssaj2002.1677.

Martinez-Suller L, Provolo G, Carton OT, Brennan D, Kirwan L, Richards KG, 2010. The composition of dirty water on dairy farms in Ireland. Irish J Agr Food Res 49: 67-80.

Mertens DR, 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing beakers or crucibles: collaborative study. J AOAC Int 85: 1217-1240.

Møller HB, Sommer SG, Ahring BK, 2004a. Methane productivity of manure, straw and solid fractions of manure. Biomass Bioenerg 36: 485-495. http://dx.doi.org/10.1016/j.biombioe.2003.08.008.

Møller HB, Sommer SG, Ahring BK, 2004b. Biological degradation and greenhouse gas emissions during pre-storage of liquid animal manure. J Environ Qual 33: 27-36. http://dx.doi.org/10.2134/jeq2004.2700.

Montalvo G, Morales J, Pineiro C, Godbout S, Bigeriego M, 2013. Effect of different dietary strategies on gas emissions and growth performance in post-weaned piglets. Span J Agric Res 11: 1016-1027. http://dx.doi.org/10.5424/sjar/2013114-3185.

Moral R, Perez-Murcia MD, Perez-Espinosa A, Moreno-Caselles J, Paredes C, Rufete B, 2008. Salinity, organic content, micronutrients and heavy metals in pig slurries from South-eastern Spain. Waste Manage 28: 367-371. http://dx.doi.org/10.1016/j.wasman.2007.01.009.

Pereira J, Misselbrook TH, Chadwick DR, Coutinho J, Trindade H, 2012. Effects of temperature and dairy cattle excreta characteristics on potential ammonia and greenhouse gas emissions from housing: A laboratory study. Biosyst Eng 112: 138-150. http://dx.doi.org/10.1016/j.biosystemseng.2012.03.011.

Portejoie S, Dourmad JY, Martinez J, Lebreton Y, 2004. Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs. Livest Prod Sci 91: 45-55. http://dx.doi.org/10.1016/j.livprodsci.2004.06.013.

Reeves JB, 2007. The present status of "quick tests" for on-farm analysis with emphasis on manures and soil: What is available and what is lacking? Livest Sci 112: 224-231. http://dx.doi.org/10.1016/j.livsci.2007.09.009.

Saeys W, Mouazen AM, Ramon H, 2005. Potential for onsite and online analysis of pig manure using visible and near infrared reflectance spectroscopy. Biosyst Eng 91: 393-402. http://dx.doi.org/10.1016/j.biosystemseng.2005.05.001.

Sánchez M, González JL, 2005. The fertilizer value of pig slurry. I. Values depending on the type of operation. Bioresour Technol 96: 1117-1123. http://dx.doi.org/10.1016/j.biortech.2004.10.002.

SAS Inst., 2008. SAS/STAT® User's guide, v 9.3, SAS Institute Inc., Cary, NC, USA.

Shenk JS, Westerhaus MO, 1996. Calibration of ISI way. In: Near infrared spectroscopy: the future waves (Davies AMC, Williams P, eds). NIR Publ., Chichester, West Sussex, UK, pp: 198-202.

Snoek DJ, Stigter JD, Ogink NW, Groot Koerkamp PW, 2014. Sensitivity analysis of mechanistic models for estimating ammonia emission from dairy cow urine puddles. Biosyst Eng 121: 12-24. http://dx.doi.org/10.1016/j.biosystemseng.2014.02.003.

Soares M, López-Bote CJ, 2002. Effect of dietary lecithins and fat unsaturation on nutrient utilization in weaned pigs. Anim Feed Sci Technol 95: 167-177. http://dx.doi.org/10.1016/S0377-8401(01)00324-8.

Sørensen LK, Sørensen P, Birkmose TS, 2007. Application of reflectance near infrared spectroscopy for animal slurry analyses. Soil Sci Soc Am J 71: 1398-1405. http://dx.doi.org/10.2136/sssaj2006.330.

Tamminga S, 2003. Pollution due to nutrient losses and its control in European animal production. Livest Prod Sci 84: 101-111. http://dx.doi.org/10.1016/j.livprodsci.2003.09.008.

Triolo JM, Sommer SG, Møller HB, Weisbjerg, Jiang XY, 2011. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: Influence of lignin concentration on methane production potential. Bioresour Technol 102: 9395-9402. http://dx.doi.org/10.1016/j.biortech.2011.07.026.

Triolo JM, Ward AJ, Pedersen L, Løkke MM, Qu H, Sommer SG, 2014. Near infrared reflectance spectroscopy (NIRS) for rapid determination of biochemical methane potential of plant biomass. Appl Energ 116: 52-57. http://dx.doi.org/10.1016/j.apenergy.2013.11.006.

Van Soest PJ, Robertson JB, Lewis BA, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74: 3583-3597. http://dx.doi.org/10.3168/jds.S0022-0302(91)78551-2.

Van Soest PJ, 1994. Nutritional ecology of the ruminant, 2nd edition. Cornell Univ Press, USA, 476 pp.

Vedrenne F, Béline F, Dabert P, Bernet N, 2007. The effect of incubation conditions on the laboratory measurement of methane producing capacity of livestock wastes. Bioresour Technol 99: 146-155. http://dx.doi.org/10.1016/j.biortech.2006.11.043.

Von Heimendahl E, Breves G, Abel H, 2010. Fiber-related digestive processes in three different breeds of pigs. J Anim Sci 88: 972-981. http://dx.doi.org/10.2527/jas.2009-2370.

Williams PC, Sobering D, 1996. How do we do it: a brief summary of the methods we use in developing near infrared calibrations. In: Near infrared spectroscopy: the future waves (Davies AMC, Williams P, eds). NIR Publ., Chichester, West Sussex, UK, pp: 185-188.

Williams PC, 2001. Implementation of near-infrared technology. In: Near-infrared technology in the agricultural and food industries, 2nd ed (Williams PC, Norris K, Eds.). Am Assoc Cereal Chemists Inc., St. Paul, MN, USA, pp: 145-169.

Yagüe MR, Bosch-Serra AD, Boixadera J, 2012. Measurement and estimation of the fertiliser value of pig slurry by physicochemical models: Usefulness and constraints. Biosyst Eng 111: 206-216. http://dx.doi.org/10.1016/j.biosystemseng.2011.11.013.

Ye W, Lorimor JC, Hurburgh C, Zhang H, Hattey J, 2005. Application of near-infrared reflectance spectroscopy for determination of nutrient contents in liquid and solid manures. T ASAE 48: 1911-1918. http://dx.doi.org/10.13031/2013.20000.