Analysis Of Factors Affecting Ammonia And Methane Emissions From Pig Slurries: Slurry Composition And Dietary Factors

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Abstract

Reducing crude protein is a recommended technique to reduce nitrogen excretion and ammonia emissions. Greenhouse gas emissions from slurry are also affected by nutrient composition. However, there are interactions among nutrients in feeds still not clarified. The objective of this study was to evaluate the impacts of different diets on nitrogen (N) and energy balances. A total of 13 diets were evaluated in digestibility trials using 78 animals. Diets were formulated to fulfil commercial standards, although differing in ingredient composition. Nutrient intake, excretion and potential NH_3 and CH_4 (Biochemical methane potential) emissions from slurry were measured. Animal weight was also monitored. Correlations between emissions and nutrient balance components were done. An analysis of variance was conducted to assess differences in nutrient balance of low, medium and high emitting animals, expressing animals per kg of live weight increased. For the N balance, a two-fold range in faeces to urine N excretion ratio was found throughout the experiments, even considering the low crude protein variations (from 15 to 16%). This was related to the ammonia emissions from slurry ($r = -0.60$, $p<0.001$). In fact, the amount of crude protein ingested to increase 1 kg of metabolic weight was positively correlated with the associated emissions ($r=0.58$, $p<0.001$). However, this was more related to the consumption by the animal than to the crude protein, which was relatively stable among diets. The difference between animals associated with high or low NH3 emissions per weight gain was therefore related to urine losses due to excess N intake. The energy balance shows that methane potential from slurry was mainly related to the excretion of indigested feed components, mainly the fibrous fraction (particularly the soluble fibre). It was clear that animals emitting high amounts of methane were those with higher dry matter and energy ingestion. The results of this study demonstrate relevant effects on N and energy balances at diets formulated according to commercial standards.

Keywords: Feeding strategies, NH_3, CH_4, Slurry, Nutrition balance.

1. Introduction

Animal nutrition plays an essential role in the sustainability of livestock production sector. Apart from being a major cost for most intensive livestock producers, it consumes a relevant share of agricultural crops. Therefore, the supply of feed ingredients is a major issue to be solved in the next decades (Lassaletta et al., 2018). Among others, focus is given to the use of more efficient feeds and to recycled feed ingredients such as agro-industrial by-products.

Using by-products contributes to a circularised economy and lowers the dependency on crops. However, the use of by-products for animal feeding poses several challenges. Crucial aspects to be considered are their availability, homogeneity and nutritional value, which are not always fulfilled by the different feedstocks. Composition of by-products is essential and fibrous by products have lower nutritive value for monogastrics compared to ruminants. Additionally, their productive and environmental implications must be evaluated in detail.

Modifying pig slurry characteristics through diet management is a valuable tool to mitigate gaseous emissions (Aarnink and Verstegen, 2007). Several studies have reported the effects of changing diets (particularly protein) on slurry composition (Rigolot et al., 2010, Jarret et al., 2011; Jarret et al., 2012). The relationship between slurry composition and ammonia (NH_3) and greenhouse gases (GHG) emissions is also evident from these studies.

It has long been demonstrated that crude protein content is positively related to urinary nitrogen excretion and consequently NH_3 emissions. However, it has been observed that for similar crude protein content the excretory pattern may also be altered because of other nutritional effects (e.g. different amount or type of fibre). Therefore, further efforts are needed to quantify the complex relationships among feed constituents, animal performance and emissions.

To this aim, we collected information from several studies evaluating nutrient balance and emissions from diets with similar nutritional content. The objective of this work was to analyse the effect of different diets including by-products on the nutritional (nitrogen and energy) balance at animal level. Effects on the related excretory patterns and gaseous (NH_3 and CH_4) emissions were also examined.

2. Materials and Methods

Description of diets

A meta-analysis was conducted using data from the assays described by Antezana et al. (2015); Beccaccia et al. (2015a, b), which analysed the effect of different sources of protein, fibre and fat on nutrition traits, slurry composition and gaseous emissions. All diets tested in these assays (13 diets; Table 1) were formulated according to commercial standards (FEDNA, 2006).

Table 71. Nutritional content (on fresh matter basis) of the experimental diets tested. The number of repetitions (animals) for each diet was n=6.

	Beccaccia et al., 2015a					Beccaccia et al., 2015b			Antezana et al., 2015				
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13
Dry matter	912	902	903	895	899	884	896	891	905	905	909	899	889
Ash	51.8	53	54.3	52.1	51	57.9	52.7	50.7	45	46.4	47.8	49.4	52.1
Crude protein	158	156	154	153	157	153	164	159	146	145	147	146	144
NDICP*	21.4	26.7	21.2	23.8	20.8	9.66	12	17.4	16.1	15.8	21	20.9	26.3
Ether extract	43	53.3	62.9	59.2	72.1	60.1	51.3	47.5	31.2	51.5	83.6	54.6	75.5
Soluble fibre	61.2	75.9	97.3	60.1	71.4	62.3	17.1	25	28.4	44	37.7	95.6	106
NDF*	154	165	158	164	161	148	144	132	167	157	163	169	166
ADF*	45.6	52.3	56.3	61	75.4	73.6	49.4	31.5	50.8	44.7	48	60.3	59.7
Lignin	8	9	10.7	18.9	33.9	4.33	13.6	8.73	11	8.2	9.4	7.9	8.1
GE (MJ/kg)*	16.8	16.9	17	16.9	17.4	16.7	16.9	16.9	16.3	16.8	17.7	16.7	17.2
NE (MJ/kg)*	9.83	9.83	9.83	9.83	9.83	9.91	9.91	9.91	9.2	9.75	10.3	9.41	9.91

*NDICP: Neutral detergent indigestible crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; GE: Gross energy; NE: Net energy.

Experimental procedure

The experimental period consisted in a 14-day adaptation period to diets followed by a period of 7 consecutive days in which faeces and urine were collected individually (21 days in total). After an initial 9-day adaptation period, animals were individually housed in metabolism pens (1.2 × 2 m2) until the end of the study. These pens allowed the measurement of individual feed intake and total and separate collection of faeces and urine. The collection period was divided in two parts to facilitate collections for energy and nutrient balance (Days 1–4) and gaseous emission study (Days 5–7). Pigs were individually weighed at the beginning of the adaptation period, at allocation in metabolism pens (Day 9 of experiment) and at the end of the experiment. Feed consumption was measured individually in the metabolism pens.

During the energy and nutrient balance (4 days), total urine and faeces excreted per animal were collected daily in separate buckets, weighed and stored in a chamber at 4◦C until the end of the collection period. To avoid nitrogen losses due to NH_3 volatilization, urine was collected under sulphuric acid (120 mL of H2SO4 at 10% per bucket and day). Upon final collection the faeces and urine were pooled per pig, mixed, subsampled and stored at −20◦C until laboratory analyses were performed. During the next 3 days, urine and faeces were collected in a similar way, but without any addition of sulphuric acid to urine. At the end of the collection period slurries were reconstituted by mixing urine and faeces from each animal in the same proportion as excreted. A part of these slurries was used in fresh for pH and NH_3 emission measurements and another one was subsampled and frozen (-20 ◦C) for biochemical methane potential (BMP) and slurry characteristics determination.

Feeds and faeces from the nutrient balance period were analyzed for dry matter (DM), ash, neutral detergent fibre, acid detergent fibre and lignin, ether extract, gross energy (GE), nitrogen (N) and neutral detergent insoluble crude protein (NDICP) concentration as described in Beccaccia et al. (2015a). The TAN and TKN were determined by steam distillation (APHA, 2005) using an automatic analyser (2300 Kjeltec, Foss Analytical, Hilleroed, Denmark). To avoid N volatilization, the subsample used for TAN analyses was acidified with HCl immediately after reconstitution. Volatile fatty acids concentration was 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).

Ammonia emission was measured from fresh samples of reconstituted slurry over 11 days using an ammonia trap system. Slurry samples of 0.5 kg from each animal + 50 mL of distilled water to prevent surface crust formation, were placed in a 1 L closed container and maintained at 25◦C in a thermostatic water bath (Selecta, Spain). Containers were connected to an air pump which extracted air at a constant airflow rate of 1.2 L/min.

During 11 consecutive days, the air was forced to pass through two absorption flasks (impingers) in serial containing 100 mL of sulphuric acid 0.1 N. The acid solution was replaced daily during the first 5 days, and every 48 h until the end of the assay (Day 11). The NH_3 trapped in the impingers was analyzed following 4500 NH3-D procedure (APHA, 2005) using a detection electrode (Orion High Performance NH_3 Electrode, model 9512HPBNWP, Thermo Scientific, USA). The cumulative NH_3 emission for each sample was calculated by adding the amount retained daily in the flasks during the experimental test.

Biochemical CH_4 potential from slurry was measured as the cumulative CH_4 production per gram of OM in a batch assay, using 120 mL glass bottles incubated at a mesophilic range (35 ± 1◦C) for 100 days, following the methodology described by Angelidaki et al. (2009). Anaerobic inoculum was pre-incubated during 15 days at 35◦C in order to deplete the residual biodegradable organic material (degasification). A mixture of pooled slurry and inoculum was made to obtain an inoculum to substrate ratio of 1 on OM basis. Slurry samples from each animal were tested by triplicate. Additionally, three blank bottles containing only anaerobic inoculum were also used in order to determine its endogenous CH_4 production. This was subtracted from the CH_4 produced by the slurry on each biogas sampling day. After filling, each bottle was sealed with butyl rubber stoppers and aluminium crimps and the headspace was flushed with pure N_2 for 2 min. During incubation, biogas volume in each bottle was regularly monitored (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 analyzed using a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame ionization detector.

Statistical analysis

A balance of dry matter (DM), nitrogen (N) and gross energy (GE) was calculated at animal level based on live weight increase. As a result, we obtained values for intake, digestion (intake – faeces excretion), retention (intake – urine and faeces excretion), and total nutrient excretion. The amount of NH_3 and CH_4 emitted to increase 1 kg of live weight. The individual data of the pigs used in the three studies were sorted according to the NH_3 and CH_4 emissions. The range of emissions was divided into three categories according to the rank position (higher third, middle third and lower third of NH3 and CH4 emissions emitted per kg LW increase).

An analysis of variance showed that there were no statistical differences among studies for the above mentioned variables. Therefore, all data from the three studies were analysed together. Correlations were obtained between the nutrients ingested, digested, retained, excreted in faeces and urine and the NH_3 and CH_4 emissions using PROC CORR of SAS. For the variables correlated to the emissions, an analysis of variance (PROC GLM of SAS) was used to compare nutrient balance components of ranked emissions (higher, medium and lower) of NH_3 and CH_4.

3. Results and Discussion

A summary of average animal performance parameters and nutrient balance for the different diets is provided in Table 2. It can be observed that animal weight and balance components were different for diets #1 to #5 (Beccaccia et al., 2015a) compared to diets #6 to #13 (Beccaccia et al., 2015b and Antezana et al., 2015). The different animal live weight and average daily gain affected the absolute values of the nutrient balances. On average, nutrient retention was 80%, 55% and 81% for dry matter, nitrogen and gross energy, respectively. The percentage of N retained by the animals ranged from 51% (diet #5) to 64% (diet #11). The percentage of dry matter and energy retained ranged from 77% (diet #13) to 83% (diet #1).

Table 72. Summary of average animal performance, nutrient balance (dry matter, DM; nitrogen, N; gross energy, GE) and emissions for the different diets. The number of replicates was n=6 for each diet.

Diet	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13
LW (kg)	107,1	107,7	105,7	104,7	109,2	55,8	56,9	56,8	62,5	61,5	65,1	61,4	62,8
ΔLW (kg/d)	1,16	1,02	1,00	1,05	1,22	0,91	0,95	1,02	0,92	0,75	0,83	0,67	0,74
DM intake (g/d)	2437	2369	2272	2282	2597	1827	1703	1777	1655	1509	1726	1503	1560
DM excreted as faeces (g/d)	331	355	311	389	479	291	256	281	276	232	313	235	279
DM excreted as urine (g/d)	89,9	103,3	90,1	94,1	114,2	80,4	73,7	66,1	79,4	68,0	60,7	77,8	84,7
N intake (g/d)	67,5	65,5	62,0	62,4	72,6	52,2	49,9	49,2	42,7	38,7	44,7	39,1	40,4
N faeces (g/d)	9,57	11,39	11,44	12,23	15,68	10,38	7,50	11,22	8,79	6,38	8,80	8,82	9,79
N urine (g/d)	18,8	18,3	14,0	18,4	19,6	12,5	12,8	7,7	10,4	8,8	7,2	8,8	8,6
GE intake (MJ/d)	44,9	44,4	42,8	43,1	50,3	34,7	32,1	33,6	29,8	28,0	33,6	27,9	30,2
GE faeces (MJ/d)	6,05	6,58	5,89	7,30	9,19	5,46	4,67	5,22	5,14	4,54	6,70	4,84	6,05
GE urine (MJ/d)	1,01	1,10	1,14	0,93	1,17	0,74	0,77	0,58	0,80	0,67	0,59	0,84	0,95
CH_4 (L/animal/d)	116	126	110	123	139	74	66	83	75	65	106	70	94
NH_3 (g/animal/d)	840	812	597	783	839	488	570	250	412	399	331	373	341

From Table 2 it can be observed that animal weight was different among studies and was particularly higher in diets #1 to #5 described in Beccaccia et al. (2015a) compared to diets #6 to #13. Consequently, components of the nutrient balance and emissions expressed per animal were also different among studies. For this reason, in order to evaluate the real influence of feed composition, balance (DM, energy and protein) components and emissions were both expressed per kg live weight (LW) increase. Each experimental data was classified according to the ranked NH_3 and CH_4 emissions, resulting in the distribution per rank and diet shown in Table 3. In general terms, all experiments contributed to all ranks of emissions (low, medium and high).

Table 73. Number of experimental data per diet corresponding to lower, medium and higher NH_3 and CH_4 emissions.

	Beccaccia et al., 2015a					Beccaccia et al., 2015b			Antezana et al., 2015				
Diet	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13
NH_3 Low			1	1		4	2	4	3	2	4	2	3
NH_3 Medium	2	3	3	1	2		1	2	3	2	2	3	2
NH_3 High	4	3	2	4	4	2	3			2		1	1
CH_4 Low	3				2	4	5	3	4	3		2	
CH_4 Medium	2	2	4	4	1	1		3	2	3	1	2	1
CH_4 High	1	4	2	2	3	1	1				5	2	5

An analysis of correlations was conducted among gas emissions, animal performance and diet composition (Table 3). It was observed that CH_4 and NH_3 were positively correlated ($r=0.31$, $p<0.01$). The emission of CH_4 per kg of LW increase was positively correlated with LW ($r=0.36$, $p<0.01$), but inversely correlated with the increment of live weight (-0.37, $p<0.001$). On the contrary, NH_3 emissions per kg of LW increase was not correlated with LW increase, but strongly correlated with live weight ($r=0.52$, $p<0.001$).

Methane production per kg LW increased was positively correlated with diet fat and energy contents, as well as with fermentable fibre. Crude protein was positively correlated with crude and indigestible protein, but was not correlated with dry matter, fat or energy contents of the feed.

Table 74. Coefficients of correlation among gas emissions (expressed per kg live weight increased, ΔLW) animal performance and diet composition. The number of measurements was n=78. Statistical significance is also indicated (ns: not significant; p<0.05 *; p<0.01 **, p<0.001***).

Diet components (g/kg fresh matter)	CH_4 (L/kg LW increase)	NH_3 (g/kg LW increase)
Dry matter	0.12ns	0.20ns
Crude protein	-0.17ns	0.27*
Indigestible CP	0.55***	0.34**
Ether extract	0.43***	-0.10ns
NDF	0.40***	0.08ns
ADF	0.19ns	-0.07ns
Lignin	0.16ns	0.25*
Gross energy	0.41***	0.02ns

Despite the relatively similar nutrient content of diets shown in Table 1, there was a large variation of emissions expressed per kg LW increase for CH_4 (Table 4) and NH_3 (Table 5). For CH_4, emissions in the higher range were on average almost two-fold compared to the lower range. Components of the nutrient balance corresponding to a 1 kg LW increase were strongly correlated with CH_4 emissions associated with that kg LW increase (Table 5). The DM, N and GE intake needed to increase 1 kg of animal LW were higher in animals associated with higher CH_4 emission. Only the ratio of N between faeces and urine was not affected by the emission rank. Remarkably, the retention of DM, N and GE to increase 1kg LW also differed from animals with lower, medium and higher CH_4 emissions.

Table 75. Nutrient balance components expressed per kg live weight (LW) increase, as a function of ranked (low, medium or high) CH_4 emissions expressed as L per kg of live weight increase. The correlation coefficients (r) among balance components and CH_4 emissions are also indicated (p<0.05 *; p<0.01 **, p<0.001***).

		r	Low CH_4	Medium CH_4	High CH_4	SEM	p-Value
	n		26	26	26		
	CH_4 (L/kg LW increase)		74.6[a]	99.0[b]	141.1[c]	2.90	<0.001
DM balance (g/kg LW increase)	DM intake	0.77***	1792[a]	2113[b]	2436[c]	63.5	<0.001
	DM faeces	0.84***	270[a]	329[b]	417[c]	10.3	<0.001
	DM urine	0.43***	76.6[a]	93.6[b]	105.1[b]	5.25	0.001
	DM retained	0.71***	1445[a]	1691[b]	1914[c]	54.9	<0.001
N balance (g/kg LW increase)	N intake	0.72***	49.2[a]	57.0[b]	65.8[c]	1.86	<0.001
	N faeces	0.75***	8.71[a]	10.81[b]	13.85[c]	0.46	<0.001
	N urine	0.43***	11.7[a]	13.1[a]	16.2[b]	0.99	0.008
	N retained	0.53***	28.8[a]	33.1[b]	35.8[c]	1.25	<0.001
	Ratio N faeces:urine	0.14 ns	0.83	0.91	1.01	0.078	0.25
GE balance (mJ/kg LW increase)	GE intake	0.80***	33.5[a]	39.7[b]	46.4[c]	1.16	<0.001
	GE faeces	0.85***	5.11[a]	6.30[b]	8.30[c]	0.20	<0.001
	GE urine	0.45***	0.75[a]	0.99[b]	1.15[b]	0.065	<0.001
	GE retained	0.72***	27.6[a]	32.4[b]	37.0[c]	1.04	<0.001

There was a variation of more than two-fold for NH_3 emissions expressed per kg LW increase when comparing the higher emission range with the lower range (Table 5). Similar to the ranked CH_4 emissions presented in Table 4, the components of the nutrient (DM, N and GE) balances changed with the NH_3 emissions per kg LW increased. Emissions were higher for animals which needed more nutrients to produce a kg of live weight. On the contrary, lower emissions were critically related with urinary losses, and therefore, negatively correlated with the ratio N urine : N faeces. These findings are coincident with the idea that animals using resources more efficiently reduce their emissions.

Table 76. Nutrient balance components expressed per kg live weight (LW) increase, as a function of ranked (low, medium or high) NH_3 emissions expressed as mg/d per kg of live weight increase. The correlation coefficients (r) among balance components and NH_3 emissions are also indicated (p<0.05 *; p<0.01 **, p<0.001***).

	Variable	r	Low NH_3	Medium NH_3	High NH_3	SEM	p-Value
	n		26	26	26		
	NH_3 (mg/d/kg LW increase)		337[a]	530[b]	860[c]	27.2	<0.001
DM balance (g/kg LW increase)	DM intake	0.52***	1840[a]	2180[b]	2322[b]	72	<0.001
	DM faeces	0.33**	304[a]	348[b]	363[b]	15.0	0.018
	DM urine	0.48***	75.1[a]	93.6[b]	106.7[b]	5.13	<0.001
	DM retained	0.52***	1460[a]	1737[b]	1851[b]	58.3	<0.001
N balance (g/kg LW increase)	N intake	0.55***	49.5[a]	58.4[b]	64.1[c]	1.96	<0.001
	N faeces	0.15 ns	10.3	11.7	11.4	0.61	0.21
	N urine	0.78***	10.1[a]	12.4[b]	18.6[c]	0.78	<0.001
	N retained	0.26*	29.2[a]	34.3[b]	34.2[b]	1.29	0.008
	Ratio N faeces:urine	-0.60***	1.14[a]	0.97[a]	0.62[b]	0.067	<0.001
GE balance (MJ/kg LW increase)	GE intake	0.51***	34.8[a]	40.9[b]	43.8[b]	1.38	<0.001
	GE faeces	0.24*	6.03	6.80	6.88	0.32	0.12
	GE urine	0.47***	0.754[a]	0.961[b]	1.17[c]	0.064	<0.001
	GE retained	0.53***	28.0[a]	33.2[b]	35.7[b]	1.12	<0.001

Remarkably, there was a significant difference in energy and protein balances when comparing low, medium and high CH_4 and NH_3 emissions. Apart from potential measurement uncertainties, results suggest that there might nutritional changes affecting the deposition of dry matter and energy per kg of live weight increase. This would involve relevant productive implications, not only considering animal productivity but also in terms of meat quality.

Finally, it must be stressed that animals within each diet were distributed among the different ranked emissions. This suggests an important individual source of variability (even considering that animals belonged to the same genetics). Feed and microbiome relationships may arise as a relevant way to assess this variability and improve results at practical level, as suggested for poultry (Torok et al., 2011) and cattle (Myer et al., 2015).

4. Conclusions

This study evidences a high effect on NH3 and CH4 emissions for diets formulated with commercial standards, with a low range in crude protein and energy contents. The differences in emissions expressed per kg of live weight increase were related with nutrient consumption patterns and balances. Efforts can be addressed to investigate the interactions between diet and individual effects.

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