UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Department of Animal Science

PhD Thesis

Análisis de los factores que influyen en las emisiones de amoniaco y metano de purines porcinos: composición del purín y factores nutricionales

Analysis of factors affecting ammonia and methane emissions from pig slurries: slurry composition and dietary factors

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El aumento del conocimiento depende por
completo de la existencia del desacuerdo.

Karl Popper

El planeta puede vivir sin nosotros.
Pero nosotros no podemos vivir sin planeta.

Anónimo

Resumen

La presente Tesis Doctoral está enfocada a la caracterización de las emisiones potenciales de amoniaco (NH_3) y metano (CH_4) a partir de purines porcinos. En concreto, estudia la relación entre el consumo y la excreción de nutrientes, la composición de los purines y las emisiones potenciales de NH_3 y CH_4 y el valor agrícola potencial de los purines. Además, evalúa las metodologías de medición de las emisiones potenciales de NH_3 y CH_4 *in vitro* y propone estrategias para su optimización.

Los objetivos de la presente Tesis Doctoral son: *i)* Caracterizar la composición, el potencial de emisión de NH_3 y CH_4 y el valor agrícola potencial de purines de granjas comerciales de España, *ii)* Evaluar la influencia de la inclusión en el pienso de una fuente de grasa (jabón cálcico de palma) y una fuente de fibra (pulpa de naranja) sobre la composición del purín y emisiones potenciales de NH_3 y CH_4 en cerdos en crecimiento y *iii)* Optimizar las técnicas de medición de las emisiones potenciales de NH_3 y CH_4 *in vitro* de purines de cerdo.

Los resultados de esta Tesis indican que las características fisicoquímicas de purines de granjas comerciales de España varían ampliamente y el tipo de animal (madres vs. animales en crecimiento) afecta de forma relevante a los contenidos de nitrógeno total, nitrógeno amoniacal, potasio, calcio, cobre, sodio, zinc, ácidos grasos volátiles y el pH de los purines, y en consecuencia afectan también sus emisiones potenciales de NH_3 y CH_4. Por lo tanto, la utilización óptima de purines de granjas comerciales como fertilizante agrícola o como insumo para la producción de biogás requiere su previa caracterización. Por otra parte, es probable que factores de manejo y el desperdicio del pienso por los cerdos en granjas comerciales influyan de forma relevante sobre las características de los purines y como tal sobre sus emisiones de NH_3 y CH_4.

La inclusión de jabón cálcico de palma (como fuente de grasa) y pulpa de naranja (como fuente de fibra) en dietas de cerdo de engorde produjo cambios significativos en la composición de purín. La inclusión de jabón cálcico de palma provocó mayores excreciones de extracto etéreo y proteína bruta, así mismo causó mayores emisiones de CH_4 y menores emisiones de NH_3. La inclusión de pulpa de naranja alteró la composición fecal,

pero no afectó significativamente a las emisiones de NH_3 y CH_4. Por otro lado, se encontró evidencias de que menores consumos de proteína bruta (PB) (-14% de PB) permitiría reducir proporcionalmente mayores cantidades de emisiones de NH_3 (-66%). Esta misma tendencia se encontró entre la ingesta de energía bruta y las emisiones de CH_4, de forma que reducciones de -20% en la ingesta de energía bruta permitiría reducciones de -46% de emisiones de CH_4.

Por otra parte, los resultados de la evaluación de las metodologías de mediciones de las emisiones potenciales de NH_3 y CH_4 *in vitro* de purines de cerdo sugieren la posibilidad de reducir hasta en un 50% los recursos (tiempo y reactivos) dedicados a la medición de las emisiones potenciales de NH_3 y CH_4 *in vitro*. Adicionalmente, se proponen modelos de predicción de las emisiones potenciales de NH_3 y CH_4 basados en las características fisicoquímicas de purines y en las dinámicas de emisión *in vitro*.

En su conjunto, la presente Tesis Doctoral proporciona información que permitirá mejorar la utilización de los purines de cerdos, mitigar las emisiones de NH_3 y CH_4, optimizar las mediciones de las emisiones de NH_3 y CH_4 *in vitro*. Al mismo tiempo se encontraron evidencias que indican la posibilidad de optimizar la utilización de la proteína y la energía en la alimentación de cerdos de engorde con una perspectiva sostenible económica y ambientalmente.

Abstract

This PhD Thesis focuses on characterizing potential emissions of ammonia (NH_3) and methane (CH_4) from pig slurries. In particular, it analyses the relationships between nutrient consumption and excretion, slurry composition and potential NH_3 and CH_4 emissions, as well as the potential fertilizer value of slurries. Besides, the methodologies to determine *in vitro* potential NH_3 and CH_4 emissions are analysed and optimization alternatives are proposed.

The objectives of this PhD Thesis are to: *i)* Characterize the composition, potential NH_3 and CH_4 emissions, and potential fertilizer value of slurries from Spanish commercial farms, ii) Evaluate the influence of incorporating to feedstuffs a fat source (calcium soap of palm fatty acid distillate, CSP) and fibre source (citrus pulp, CP) on slurry composition and potential NH_3 and CH_4 emissions in growing pigs, and iii) Optimize the *in vitro* measurement techniques of potential NH_3 and CH_4 emissions from pig slurries.

The results of this Thesis show that physicochemical characteristics of slurries from Spanish commercial farms vary widely, and the type of animal (e.g. sows against growing animals) affect in a relevant way the contents of total nitrogen, ammonia nitrogen, potassium, calcium, cupper, sodium, zinc, volatile fatty acids, as well as the pH of slurries. Consequently, the type of animal also affected potential NH_3 and CH_4 emissions. Therefore, the optimum utilization of slurries from commercial farms, either as fertilizer or as feedstock for biogas production, requires a previous characterization. On the other hand, it was found that management factors and feedstuff waste probably influenced the slurry characteristics and potential NH_3 and CH_4 emissions.

Including CSP as fat source and CP as fibre source in growing pig diets changed significantly slurry composition. Including CSP increased the excretion of ether extract and crude protein. Regarding gaseous emissions from slurries, including CSP increased potential CH_4 emissions and reduced NH_3 emissions. Including CP affected faecal composition, but potential emissions were not affected. On the other hand, evidences were found that lower crude protein consumptions (-14% CP) could be associated to higher

reductions of NH_3 emissions (-66%). The same tendency was found regarding the effect of crude energy intake on CH_4 emissions: a 20% reduction of crude energy would be associated to 46% reductions of CH_4 emissions.

Additionally, the evaluation of *in vitro* measurement techniques for potential NH_3 and CH_4 emissions from pig slurries suggest that it could be possible to reduce up to 50% the resources (time and material) which are used following the standard procedures, without significant effects on the results. Additionally, prediction models of potential NH_3 and CH_4 emissions are proposed, based on the physicochemical characteristics of slurries and the dynamics of *in vitro* emissions.

In overall, this PhD Thesis provides valuable information to improve the use of pig slurries, to mitigate the associated NH_3 and CH_4 emissions, and to optimize *in vitro* measurement methods for potential NH_3 and CH_4 emissions. Additionally, evidences were found on how to optimize the use of protein and energy in growing pigs from a sustainable perspective both in economic and environmental terms.

Resum

La present Tesi Doctoral està enfocada en la caracterització de les emissions potencials d'amoníac (NH_3) i metà (CH_4) a partir de purins porcins. En concret, estudia la relació entre el consum i l'excreció de nutrients, la composició dels purins i les emissions potencials de NH_3 i CH_4 i el valor agrícola potencial dels purins. A més, avalua les metodologies de mesurament de les emissions potencials de NH_3 i CH_4 in vitro i proposa estratègies per a la seua optimització.

Els objectius de la present Tesi Doctoral són: *i*) Caracteritzar la composició, el potencial d'emissió de NH_3 i CH_4 i el valor agrícola potencial de purins de granges comercials d'Espanya, *ii*) Avaluar la influència de la inclusió en el pinso d'una font de greix (sabó càlcic de palma) i una font de fibra (polpa de taronja) sobre la composició del purí i emissions potencials de NH_3 i CH_4 en porcs en creixement i, *iii*) Optimitzar les tècniques de mesurament de les emissions potencials de NH_3 i CH_4 in vitro de purins de porc.

Els resultats d'aquesta Tesi indiquen que les característiques fisicoquímiques de purins de granges comercials d'Espanya varien àmpliament i el tipus d'animal (mares vs. animals en creixement) afecta de forma rellevant als continguts de nitrogen total, nitrogen amoniacal, potassi, calci, coure, sodi, zinc, àcids grassos volàtils i el pH dels purins, i en conseqüència afecten també les seues emissions potencials de NH_3 i CH_4. Per tant, la utilització òptima de purins de granges comercials com a fertilitzant agrícola o com entrada per a la producció de biogàs requereix una caracterització prèvia. D'altra banda, és probable que factors de maneig i el desaprofitament del pinso pels porcs en granges comercials influïxen de forma rellevant sobre les característiques dels purins i com a tal sobre les seues emissions de NH_3 i CH_4.

La inclusió de sabó càlcic de palma (com a font de greix) i polpa de taronja (com a font de fibra) en dietes de porc d'engreix va produir canvis significatius en la composició del purí. La inclusió de sabó càlcic de palma va provocar majors excrecions d'extracte eteri i proteïna bruta, així mateix va causar majors emissions de CH_4 i menors emissions de NH_3. La inclusió de polpa de taronja va alterar la composició fecal, però no va afectar

significativament les emissions de NH_3 i CH_4. D'altra banda, es van trobar evidències que menors consums de proteïna bruta (PB) (-14% de PB) permetria reduir proporcionalment majors quantitats d'emissions de NH_3 (-66%). Aquesta mateixa tendència es va trobar entre la ingesta d'energia bruta i les emissions de CH_4, de manera que reduccions de -20% en la ingesta d'energia bruta permetria reduccions de -46% d'emissions de CH_4.

Comentario [PFR1]: El menos hace falta?. Si hablas de reducciones se supone que va implícito

D'altra banda, els resultats de l'avaluació de les metodologies de mesuraments de les emissions potencials de NH_3 i CH_4 in vitro de purins de porc suggereixen la possibilitat de reduir fins un 50% els recursos (temps i reactius) dedicats al mesurament de les emissions potencials de NH_3 i CH_4 in vitro. Addicionalment, es proposen models de predicció de les emissions potencials de NH_3 i CH_4 basats en les característiques fisicoquímiques de purins i en les dinàmiques d'emissió *in vitro*.

En el seu conjunt, la present Tesi Doctoral proporciona informació que permetrà millorar la utilització dels purins de porcs, mitigar les emissions de NH_3 i CH_4, optimitzar els mesuraments de les emissions de NH_3 i CH_4 *in vitro*. Al mateix temps es van trobar evidències que indiquen la possibilitat d'optimitzar la utilització de la proteïna i l'energia en l'alimentació de porcs d'engreix amb una perspectiva sostenible econòmica i ambientalment.

Abreviaturas utilizadas en español

AGV, ácidos grasos volátiles;
BMP, potencial bioquímico de producción de metano;
CH_4, metano;
EB, energía bruta;
EE, extracto etéreo;
FAD, fibra ácido detergente;
FND, fibra neutro detergente;
FS, fibra soluble;
GEI, gases de efecto invernadero;
LAD, lignina ácido detergente;
MO, materia orgánica;
MS, materia seca;
N-NH_3, nitrógeno amoniacal;
N, nitrógeno;
NH_3, amoniaco;
NH_4^+, amonio;
PB, proteína bruta;
PB IDN, proteína indigestible en detergente neutro;
PV, peso vivo;
ST, sólidos totales;
SV, sólidos volátiles;

List of abbreviations used in English

ADFom, acid detergent fibre without residual ash;
ADL, acid detergent lignin;
AOAC, Association of Official Analytical Chemists;
BEDN, bacterial and endogenous debris nitrogen;
BMP, biochemical methane potential;
CEL, cellulose;
CH_4, methane;
CP, crude protein;
CSP, calcium soap of palm fatty acid distillate.
CV, coefficient of variation,
DE, digestible energy;
CTTAD, coefficient of total tract apparent digestibility;
DE-UE/DE proportion of digestible energy not loss in urine;
DM, dry matter;
EC, electrical conductivity;
F, frequent;
HEM, hemicellulose;
HNO_3, nitric acid;
H_2SO_4, sulfuric acid;
H_3PO_4, ortophosphoric acid;
LF, less frequent;
NH_3, ammonia;
N-NH_3, ammonia nitrogen;
N_2O, nitrous oxide;
OM, organic matter;
OP, orange pulp;
R^2, coefficient of determination;
rAE, relative absolute error;

rRMSE, relative root mean squared error of prediction;

SF, soluble fibre;

SV, volatile solids;

TAN, total ammonia nitrogen;

TKN, total Kjeldahl N;

UDN, undigested dietary nitrogen;

VFA, volatile fatty acids.

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Con formato: Español (alfab. internacional)

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1
Introducción general

1.1 Perspectivas generales de la producción porcina en un contexto de sostenibilidad

La población mundial es de unos 7300 millones de habitantes, y para los próximos diez años se prevé un incremento con una tasa anual de 1.24% (United Nations, 2015). Este aumento de la población mundial implicará una mayor demanda de alimentos en general, y entre ellos de productos de origen animal.

La carne de cerdo es la principal fuente de carne en el mundo y cubre el 37% de la demanda global. En 2014 se produjeron 113 millones de toneladas de carne de cerdo y España se constituyó en el quinto productor mundial con 3.5 millones de toneladas (FAOSTAT, 2015).

En los últimos 10 años, la producción mundial de carne de cerdo ha crecido a una tasa anual de 1.5% (FAO, 2013) y en Europa se prevé, para los próximos 10 años, un crecimiento del 2%. Sin embargo, a nivel mundial para el año 2025, se espera un incremento del consumo de carne del 15% con respecto al actual (European Commission, 2014; EUROSTAT, 2015), por lo que el crecimiento del sector porcino a nivel global podría ser mayor.

El incremento de la producción mundial de carne de cerdo y crecimiento del sector ha implicado la concentración y la intensificación de la crianza de cerdos (EUROSTAT, 2015), conllevando modificaciones en los sistemas de abastecimiento de insumos, de alojamiento, de alimentación y de manejo de los animales, así como de la gestión de los desechos generados por esta actividad. Al respecto, la producción porcina genera aproximadamente 6000 millones de toneladas de purines al año, y su gestión constituye el principal riesgo ambiental de contaminación y de emisión de gases efecto invernadero (GEI) del sector porcino (FAO, 2009).

Por otra parte, la ganadería porcina consume cantidades importantes de recursos, entre ellos materias primas más o menos nobles para la elaboración de los piensos. En ocasiones, la producción de estas materias primas entra en competencia con el uso de suelos para cultivos destinados a alimentación humana o la fabricación de bioenergía (Westhoek et al., 2011). El suministro de las materias primas y piensos necesarios para hacer frente al

aumento de la producción mundial de cerdo constituye uno de los más importantes desafíos a medio plazo para la ganadería.

Por lo tanto, uno de los principales retos del sector porcino es aumentar la producción reduciendo los riesgos y problemas ambientales que genera, tales como las emisiones de GEI o la deposición de nutrientes y materia orgánica al medio ambiente. Esto requerirá la adopción de medidas que posibiliten incrementar la eficiencia de los sistemas productivos incluyendo la alimentación de los propios animales, además de mejorar las estrategias de manejo, gestión y reutilización de las deyecciones.

En la actualidad se estima que la producción de cerdos contribuye con el 9.5% de las emisiones de GEI de la ganadería mundial (Hristov et al., 2013). Por otra parte el 7.1% de las emisiones de la ganadería se derivan de la gestión del estiércol y de esta el 30% corresponden a la gestión de purines de cerdo (FAOSTAT, 2015). Como tal, los purines constituyen la principal carga ambiental de la producción de porcina.

En la práctica, el impacto ambiental global de la producción de cerdos deriva de dos aspectos importantes relacionados a los purines que son la carga mineral y la carga gaseosa. Por un lado, un desequilibrio entre el volumen de purín aplicado al suelo y la demanda de nutrientes del sistema suelo-planta puede generar un exceso en las aportaciones de nitrógeno (N), fósforo (P) y metales pesados al suelo. Los excesos de N podrían provocar la contaminación por nitratos de las aguas subterráneas, mientras que la escorrentía del P podría contribuir a la eutrofización de las aguas superficiales (Aarnink y Verstegen, 2007; Webb et al., 2014). Por último, los metales pesados podrían acumularse en el suelo y constituirse en problemas ambientales de toxicidad a largo plazo (Krupa, 2003; Nicholson et al., 1999; Villamar et al., 2012).

Por otro lado, la carga gaseosa de los purines está dada por las emisiones de amoniaco (NH_3), metano (CH_4), óxido nitroso (N_2O) y olores. Las emisiones de NH_3 y su posterior deposición afectan a los ecosistemas naturales al causar procesos de acidificación de suelos y eutrofización de las aguas que la sustentan (Krupa, 2003). De igual manera, el CH_4 generado en el almacenamiento y la aplicación a campo de purines no puede dejarse de lado, ya que su potencial de calentamiento global es 23 veces mayor al del dióxido de

carbono (CO_2) por lo que es considerado es el gas de efecto invernadero más importante después del CO_2 (Rodhe et al., 2012) Además, contribuye al smog fotoquímico al ser precursor del ozono troposférico (Fiore et al., 2002). Finalmente, el olor asociado a la crianza de cerdos se constituye en una molestia cuando las granjas se encuentran adyacentes a zonas urbanas (Webb et al., 2014).

A pesar de esto, desde hace siglos, los estiércoles han sido utilizados como abono ya que por su composición deben ser considerados una importante fuente de nutrientes para la agricultura si son gestionados adecuadamente (Lobera et al., 1998; Penha et al., 2015). Adicionalmente, los purines son sustratos potencialmente utilizables para producir biogás. Esta última alternativa puede implicar beneficios ambientales, agrícolas y socio-económicos como son la producción de energía renovable, la inactivación de patógenos, la reducción de los olores y la mejora de la calidad del digestato resultante como abono (Holm-Nielsen et al., 2009). En Europa, la producción de biogás se ha incrementado sustancialmente en los últimos años con un aumento de 3.8 millones de toneladas de equivalente de petróleo en 2003 a 13.5 millones de toneladas de equivalente de petróleo en 2013 (EurObserv'ER, 2005, 2014). Por lo tanto en los últimos años se vienen estudiando la co-digestión de purines con diferentes sustratos orgánicos como estrategia para optimizar el proceso de obtención de biogás, constituyéndose en una alternativa sostenible para la gestión de las deyecciones ganaderas (Braun et al., 2008; Ferrer et al., 2014; Flotats et al., 2001).

1.2 Los purines y las emisiones de NH_3 y CH_4

Los purines son una mezcla líquida compuesta por las heces y orina excretadas por los cerdos, así como otros restos orgánicos (pienso, pelos, descamaciones de piel, entre otros) y agua de limpieza y desperdiciada por los animales (Babot et al., 2011). La composición de los purines de granjas comerciales es muy variable y depende de múltiples factores asociados a cada sistema productivo (Moral et al., 2005; Parera i Pous et al., 2010; Teira, 2007). La composición de las heces y la orina puede variar en función de la composición de la dieta, la edad del animal y su metabolismo, principalmente. Adicionalmente, la composición del purín está también condicionada por el sistema de alojamiento (que puede

variar según la orientación productiva), el sistema de alimentación, las prácticas de gestión de estiércol, las condiciones ambientales e incluso la raza y la categoría animal (Lorimor et al., 2000; Conn et al., 2007; Martínez-Suller et al., 2010; Morazán et al., 2015; Sánchez y González, 2005).

En general, los purines contienen entre 1.8% y 6.1% de materia seca (MS), de la cual un 60 a 70% es materia orgánica (MO) y entre 2% y 5 % es N (Abubaker et al., 2015; Moral et al., 2005; Olusegun, 2014; Parera i Pous et al., 2010; Suresh et al., 2009). Además de esto, los purines se caracterizan por producir compuestos fácilmente volatilizables que conllevan a la emisión de gases como el NH_3, CO_2, CH_4 y N_2O (Lorimor et al., 2000). Por ello, como se ha comentado anteriormente, existe una relación directa entre la composición de los purines y la emisión final de gases (Beccaccia et al., 2015c; Fangueiro et al., 2012; Møller et al., 2004).

1.2.1 Emisiones de NH_3

En los purines, el N se encuentra en dos formas, N orgánico (proteína y aminoácidos no digeridos ligados a los componentes de la pared celular, proteína de los piensos desperdiciados por los cerdos y proteína endógena del sistema digestivo) y N inorgánico (urea y nitrógeno amoniacal principalmente). Las emisiones de NH_3 dependen principalmente de la concentración de N mineral disponible en los purines y del pH (Snoek et al., 2014).

El N excretado por los cerdos es el resultado de la diferencia entre el N consumido y el N retenido. El N es excretado de forma orgánica por las heces y de forma inorgánica (principalmente urea) por la orina. Los cerdos de crecimiento y acabado excretan entre un 63% y un 70% del N consumido, y entre un 18% y un 35% del N consumido termina siendo emitido a la atmósfera, principalmente en forma de NH_3 (Van der Peet-Schwering et al.,1999; IPCC, 2003; Aarnink, 1997). Estas pérdidas suponen ineficiencias en el proceso productivo y hacen evidente la necesidad de mejorar la eficiencia de utilización del N en el sistema de alimentación y de gestión de purines.

En la bibliografía se encuentran valores de emisiones diarias de NH_3 que van desde 20 a 222 mg de NH_3 por litro de purín, y la mayoría de estudios indican relaciones directas entre

las emisiones y el contenido de N amoniacal total (TAN) de los purines (Beccaccia et al., 2015; Canh et al., 1998; Hernández et al., 2011; Portejoie et al., 2004).

El proceso de emisión de NH_3 se origina principalmente en la descomposición de la urea excretada en la orina (Figura 1.1), y sólo una pequeña parte proviene de la descomposición de la proteína de las heces (Chowdhury et al., 2014). La descomposición de la urea es un proceso rápido, que se puede completar en cuestión de pocas horas. La velocidad a la que la urea se convierte en NH_3 dependerá de la actividad de la enzima ureasa, producida por bacterias presentes en las heces y en las fosas del purín (Aarnink y Verstegen, 2007). Una vez actúa la ureasa, el NH_3 es liberado a la solución acuosa de purín y se mantiene en equilibrio ácido-base con la forma ionizada (ion amonio, NH_4^+).

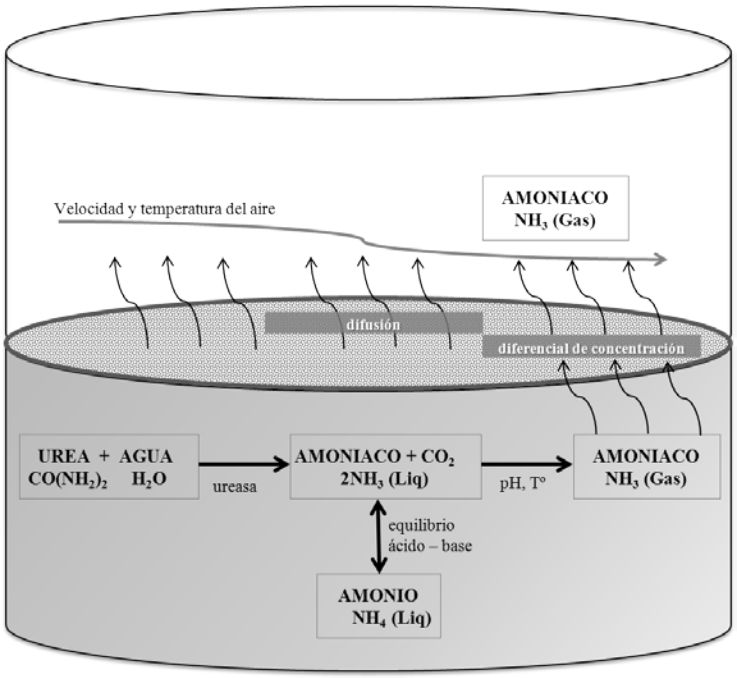

Figura 1.1. Proceso de generación y emisión de NH_3 en purines de cerdo (Fuente: Elaboración propia, adaptado de varios autores)

Las emisiones potenciales de NH_3 están determinadas por el equilibrio ácido-base entre NH_4^+ y NH_3, y consecuentemente están condicionadas por el pH. Así, una disminución en el pH desplaza el equilibrio hacia NH_4^+, reduciéndose la tasa de volatilización de NH_3 (Chowdhury et al., 2014). Adicionalmente, otros factores como la temperatura, la velocidad del aire y la humedad del purín influyen en la tasa de emisión. De forma orientativa, cada disminución de 0.1 puntos de pH puede disminuir la emisión de amoniaco entre un 5 y un 20% en función de la temperatura (Philippe et al., 2011). Adicionalmente, el diferencial de concentraciones de NH_3 del purín y del aire circundante podrían influir en las emisiones (Snoek et al., 2014).

Del mismo modo, la superficie de purín expuesta a emisión y los parámetros físicos de esa superficie también influye en las emisiones de NH_3. Mayores superficies permiten mayores potenciales de volatilización, y por tanto una mayor emisión. Sin embargo, las costras que se producen en la superficie de los purines pueden reducir la difusión del NH_3 a la atmósfera, lo cual las convierte en posibles técnicas de mitigación en granja (Webb et al., 2014).

1.2.2 Emisiones de CH_4: factores que afectan y usos

El CH_4 emitido de los purines de cerdo es el resultado la fermentación anaeróbica de los mismos. Este proceso se puede dar de manera espontánea durante el almacenamiento del purín en la propia granja (emitiéndose CH_4 y CO_2 a la atmósfera), pero también de manera controlada en biodigestores. En este último caso se puede valorizar el CH_4 producido como fuente de energía renovable, y el digestato como un fertilizante más estabilizado.

La formación de CH_4 a partir de los purines (Figura 1.2), se inicia con la hidrólisis de la materia orgánica (lípidos, proteínas y carbohidratos) por acción de enzimas hidrolíticas producidas por microorganismos hidrolíticos-acetogénicos. De esta hidrólisis inicial resultan compuestos solubles como aminoácidos, alcoholes, azúcares y ácidos grasos que son atacados por los microorganismos acidogénicos degradándolos a productos intermedios como son el acetato, ácidos grasos volátiles de cadena corta (propiónico y butírico), etanol, lactato, CO_2 y H_2. A continuación los microorganismos acetogénicos oxidan los ácidos

grasos de cadena corta a acetato, H_2 y CO_2. Finalmente por acción de las bacterias metanogénicas y acetoclásticas se genera el CH_4 y CO_2 (Mao et al., 2015; Weiland, 2010).

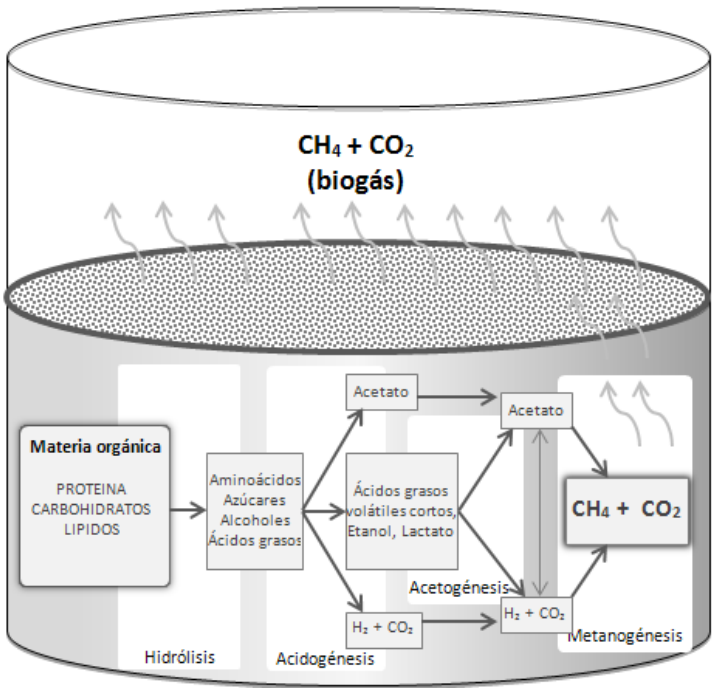

Figura 1.2. Proceso de conversión de la materia orgánica en CH_4 (Fuente: Elaboración propia, adaptado de varios autores)

De acuerdo con IPCC (2006), la emisión de metano en la producción ganadera depende del potencial de emisión específico de cada sustrato (conocido como BMP a partir de sus siglas en inglés, *biochemical methane potential* (Labatut et al., 2011; Strömberg et al., 2014)) y del factor de conversión de metano (*methane conversion factor*, MCF), que a su vez está condicionado por la temperatura ambiente y del sistema de gestión del estiércol. El BMP de purines de cerdo se encuentra, generalmente, entre 390 y 480 mL de CH_4 por g de sólido volátil (SV) (Jarret et al., 2012, 2011; Strömberg et al., 2014). Las estrategias de reducción

de estas emisiones pasan por reducir la cantidad de MO o SV excretados, reducir el BMP o actuar sobre el MCF a través de cambios en la gestión (p.ej. utilizar sistemas de estiércol sólido).

En cambio, la digestión anaerobia del purín persigue maximizar la generación de CH_4 a partir de la fermentación de la materia orgánica, de forma que se obtiene biogás con la siguiente composición aproximada: CH_4 (40% a 70%), CO_2 (30% a 60%) y otros gases (1% de N_2, CO, O_2, NH_3, H_2 y H_2S). Además de producirse estos gases, el sustrato resultante o digestato es una mezcla compuesta por residuos minerales (50% a 70%) y orgánicos (30% a 50%). Los productos finales de este proceso y sus proporciones variarán en función de las características de los purines utilizados (Cepero et al., 2012).

En el proceso de digestión anaerobia del purín intervienen diversos factores que influyen en su rendimiento final y, por tanto, en la emisión de CH_4. Estos factores pueden ser clasificados en factores intrínsecos del purín y factores operacionales del proceso de fermentación.

Los factores intrínsecos más relevantes son la composición fisicoquímica de los purines y en especial la disponibilidad de MO y el pH. En este caso el equilibrio de nutrientes contenidos en los purines permitirá que se desarrollen más o menos las distintas poblaciones microbianas que intervienen en el proceso y el pH condiciona el crecimiento microbiano, siendo ambos determinantes en la producción de CH_4. El crecimiento y acción de las bacterias hidrolíticas se da en pH entre 7.2 y 7.4, las bacterias acetogénicas necesitarán pH entre 7 y 7.2 y las metanogénicas 6.5 y 7.5 (Chen et al., 2008). Otro factor intrínseco que puede inferir en el proceso metanogénico es el contenido de nitrógeno amoniacal ($N\text{-}NH_3$), ya que concentraciones mayores a 200 mg L^{-1} de $N\text{-}NH_3$ en el purín inhiben el crecimiento microbial y con ello la metanogénesis (Angelidaki y Ahring, 1994; Hansen et al., 1998; Mata-Alvarez et al., 2000).

Los principales factores operacionales que influyen en la producción de CH_4 son la temperatura, el tiempo de retención en los digestores anaerobios y la agitación del sustrato. El incremento de la temperatura implica una mayor velocidad del crecimiento de las poblaciones bacterianas y como consecuencia se acelera la producción de CH_4 y CO_2

(biogás). Temperaturas entre 35ºC y 37ºC son reportadas como ideales para el proceso (Parkin y Owen, 1987; van Lier et al., 1993). Del mismo modo otro factor importante del proceso es la mezcla y agitación del purín en fermentación. Esta acción mejora la uniformidad de la densidad de la población bacteriana y el contacto con la materia orgánica en fermentación. En el caso de purines colectados y almacenados en fosas o balsas (en granja), la temperatura, el tiempo de almacenamiento y los niveles de agitación son variables y como tal afectarán el proceso.

1.3 El uso agrícola de purines

Desde hace siglos la aplicación de estiércoles en los campos de cultivo ha constituido la forma principal en la que el ser humano ha sido capaz de restituir la fertilidad de los suelos. En las últimas décadas, como consecuencia de la intensificación de la producción porcina, se han generado grandes cantidades de purines, en ocasiones aplicadas a campos de cultivo en dosis excesivas. Los purines, por su naturaleza y composición fisicoquímica, pueden ser considerados fertilizantes, dado que contienen la mayoría de los nutrientes requeridos por los cultivos (Villar et al., 2004). Sin embargo, no siempre existen terrenos agrícolas adyacentes disponibles para recibir los grandes volúmenes generados por las granjas comerciales convirtiéndose su deposición final en un problema ambiental. Por lo tanto los purines deben ser gestionados eficientemente para evitar impactos negativos al medio ambiente. Una buena gestión implica que sean almacenados, transportados y aplicados a suelos agrícolas disponibles o destinados a la generación de biogás o a plantas de tratamiento, minimizando al máximo la pérdida de nutrientes y las emisiones de GEI.

Diversos ensayos de fertilización de suelos de uso agrícola utilizando purines de cerdo indican buenos resultados productivos, sin alterar negativamente las condiciones del suelo, siempre que su utilización se ajuste a las necesidades de los cultivos y a la capacidad de aporte de macro y micro nutrientes de los purines (Bosch-Serra et al., 2014; Cavanagh et al., 2011; Gomez-Garrido et al., 2014). Al respecto, la Unión Europea ha reglamentado el uso de los purines en agricultura con el objetivo de reducir la contaminación por nitratos de las aguas subterráneas, y ha establecido como límite máximo la aplicación de 170 kg N ha^{-1} (Directiva de 91/676 / CEE). La misma disposición recomienda que se sigan códigos de

buenas prácticas agrarias, de forma que en función de cada cultivo deben seguirse las recomendaciones agronómicas considerando las extracciones de los cultivos y las distintas aportaciones de nutrientes.

Una aplicación excesiva de purines a los suelos puede resultar en la saturación de nutrientes (por ejemplo, N, P, potasio (K)), así como en la acumulación de metales pesados (por ejemplo, cobre (Cu) y zinc (Zn)), sales o incluso residuos de antibióticos, pudiendo llegar a contaminar las aguas subterráneas (Burton y Turner, 2003; Moral et al., 2008; Villamar et al., 2012). Por otro lado, las aplicaciones continuas de purines ricos en sales podrían aumentar la salinidad del suelo e inhibir el crecimiento de las plantas (Alburquerque et al., 2012). En cualquier caso, para usar correctamente los purines como fertilizantes en los campos y evitar problemas ambientales derivados se requiere una monitorización periódica de su contenido de nutrientes (Gomez-Garrido et al., 2014).

Por lo tanto, resulta evidente la necesidad de tener información precisa de la composición de los purines y de sus principales características fisicoquímicas, antes de su utilización.

1.4 Estrategias de alimentación de cerdos para reducir las emisiones gaseosas

En los últimos años se vienen desarrollado alternativas tecnológicas tendientes a mitigar y reducir las emisiones de GEI y NH_3 procedentes de la producción porcina. Estas alternativas implican mejoras en el diseño de las instalaciones productivas como por ejemplo los sistemas de ventilación y captura de las emisiones, los tipos de alojamientos y materiales utilizados, o el diseño y manejo de las fosas de almacenamiento de purines (Cabaraux et al., 2009; Groot Koerkamp et al., 1998). También existen alternativas ligadas al manejo, la gestión y tratamiento de purines, como por ejemplo la acidificación, la separación solido-líquido, o las cubiertas de las balsas de purines, entre otras (Chadwick et al., 2011; Dai y Blanes-Vidal, 2013; Fangueiro et al., 2015; Hjorth et al., 2010; Petersen et al., 2013).

Del mismo modo, se vienen desarrollando alternativas de mitigación de las emisiones con un enfoque nutricional. Estas buscan modificar la composición de las heces y orina (constituyentes principales de los purines) y, como consecuencia, alterar el potencial de

emisión de GEI de los purines (Hristov et al., 2013; Kebreab et al., 2006; Philippe and Nicks, 2015).

En cuanto a las alternativas nutricionales estudiadas para la reducción de las emisiones de NH_3, existe un gran número de trabajos que indican una relación positiva entre el contenido de proteína bruta (PB) de los piensos y las emisiones de NH_3. En este sentido, una reducción del contenido de PB en el pienso de cerdos reduciría las emisiones de NH_3 (Beccaccia et al., 2015b; Canh et al., 1998; Hernández et al., 2011; Portejoie et al., 2004). Al respecto, Aarnink et al. (1993) estimó que la reducción de 10 g kg^{-1} de PB produciría una reducción de 9% en el contenido de N-NH_3 del purín. Por otra parte, Canh et al. (1998) sugieren que la reducción en hasta 4 puntos porcentuales en el contenido de PB del pienso (de un 16.5% a un 12.5%) no afecta a la tasa de crecimiento ni la eficiencia alimentaria en cerdos, siempre y cuando se tengan satisfechos sus requerimientos en aminoácidos esenciales. Por lo tanto, la reducción de la proteína del pienso, contemplando el uso de materias primas proteicas con mayor digestibilidad y la adición de aminoácidos sintéticos podrían mejorar la eficiencia de la utilización del N, reduciéndose su excreción y con ello disminuyendo sus emisiones de NH_3 (Moughan et al., 2003). Del mismo modo Bakker et al. (1996) sugiere que la fermentación de fibra en el intestino grueso de los cerdos podría inducir a que se incorpore urea de la sangre en el intestino grueso. Esa incorporación serviría para promover la fermentación bacteriana, y el N se incorporaría como proteína bacteriana, reduciéndose la excreción de N en la orina e incrementándose la excreción de N en heces.

Adicionalmente, es probable que otros factores como el contenido de extracto etéreo (EE), fibra detergente neutro (FDN), fibra detergente ácido (FDA) en los piensos puedan afectar la composición y el pH de los purines y como consecuencia las emisiones de NH_3 y CH_4 (Canh et al., 1998; Dinuccio et al., 2008; Jarret et al., 2012; Mroz et al., 2000). Al respecto Aarnink y Verstegen (2007) sugieren que el incremento en la proporción de carbohidratos fermentables en los piensos de cerdos provocan la reducción del pH de las excretas y como consecuencia la disminución de la emisión de NH_3, y contrariamente se incrementan las emisiones de CH_4.

Tal y como se ha comentado con anterioridad, las emisiones de CH_4, se generan a partir de la degradación de la materia orgánica de los purines y, por lo tanto, el potencial de producción de CH_4 de purines podría influirse por la elección de las materias primas y sus proporciones en los piensos. Al respecto Jarret et al. (2012) reporta que el purín resultante del pienso con alto contenido de fibra (4.9% de fibra bruta) produjo mayores emisiones potenciales de CH_4 (126 L animal^{-1} d^{-1}) que los resultantes de la dieta control (2.9% de fibra cruda con emisiones de 76 L animal^{-1} d^{-1}). Contrariamente, las emisiones acumuladas de NH_3 fueron menores en los cerdos alimentados con alto contenido de fibra (3.36 g animal^{-1} d^{-1}) que los alimentados con la dieta control (4.92 g animal^{-1} d^{-1}). Tomando en cuenta estos trabajos, aparentemente existiría un efecto antagónico del contenido de fibra fermentable de los piensos en las emisiones de NH_3 y CH_4 de los purines.

Otros factores dietéticos como la cantidad y la fuente de grasas y aceites también pueden afectar la composición del purín. Sin embargo, aún son escasos los estudios al respecto. Los altos niveles de grasa o aceite en la dieta pueden limitar la disponibilidad de sustratos fermentables a la microflora intestinal (Jørgensen et al., 2011; Patridge y Gill, 2001). Estos elevados niveles de grasa también podrían inhibir la microflora responsable de la emisión de NH_3 (Leek et al., 2004), y la producción de CH_4 (Christensen y Thorberck, 1987).
Por lo tanto, aún se requiere conocer en mayor detalle los efectos de la modificación de las dietas sobre las emisiones potenciales de NH_3 y CH_4, particularmente el efecto del EE, para poder establecer medidas nutricionales de mitigación de las emisiones y mejorar la sostenibilidad de la producción porcina.

1.5 Sistemas de medición de las emisiones de CH_4 y NH_3, en purines de cerdo

Las mediciones de las emisiones de gases de purines de cerdo se han venido realizando en sistemas de cámara estática y de cámara dinámica (Greatorex, 2000). El sistema de cámara estática delimita herméticamente una superficie de emisión por un periodo de tiempo, y las muestras de aire son tomadas del interior de la cámara para la determinación de la concentración de CH_4 y NH_3. En el sistema de cámara dinámica la superficie de emisión es

sometida a un flujo controlado de aire a partir del cual se determina la concentración de CH_4 y NH_3.

La determinación de la concentración de CH_4 y NH_3, se realiza por diferentes metodologías de medición como la cromatografía de gases (Clemens et al., 2006), la espectroscopía infrarroja, laser y fotoacústica (Fukumoto et al., 2003; Haeussermann et al., 2006; Kebreab et al., 2006; Moller et al., 2004) o por sensores electroquímicos (Ni et al., 2000).

En la práctica, para la mitigación de las emisiones de NH_3 y CH_4, resulta esencial poder determinar de manera precisa y eficaz las emisiones de gases procedentes de los alojamientos ganaderos. Sin embargo, como ha sido descrito en los párrafos anteriores, las emisiones de NH_3 y CH_4 de purines dependen de varios factores como son las características fisicoquímicas de los purines y las condiciones ambientales y de almacenamiento. La combinación de estas características resultaría en condiciones particulares para cada establecimiento productivo. Ante esta amplia variación en los factores que afectan a las emisiones, resulta complejo establecer valores absolutos de emisiones y distinguir como afectan los diferentes factores de forma individual para proponer medidas de mitigación.

Por otro lado, en los últimos años se vienen realizando mediciones de las emisiones de NH_3 y CH_4 de purines en condiciones controladas en laboratorio (*in vitro*). Estas técnicas han permitido determinar la emisión potencial de NH_3 y CH_4 de purines de diferentes orígenes en condiciones estandarizadas permitiendo tener una referencia de la capacidad de emisión de un determinado purín. Hasta la actualidad la mayor parte de estudios que reportan el uso de estas metodologías se refieren al efecto de ensayos nutricionales sobre las emisiones (Aarnink y Verstegen, 2007; Hansen et al., 2014; Jarret et al., 2012). Sin embargo estas metodologías, podrían ser usadas para estudiar el efecto de la modificación de los demás factores determinantes de las emisiones, en condiciones controladas y estandarizadas.

La emisión potencial de NH_3 puede ser definida como el NH_3 emitido por unidad de superficie de purín, en condiciones controladas (temperatura, flujo de aire y tiempo). Con frecuencia, esta ha sido determinada utilizando el método de la cámara dinámica con trampas acidas húmedas (Canh et al., 1998) y su aplicación ha estado orientada a establecer

diferencias de emisión de purines resultantes de ensayos de nutrición (Galassi et al., 2010; Jarret et al., 2012; Portejoie et al., 2004). Este método permite estandarizar las variables que intervienen en la emisión de NH_3 (por ejemplo, temperatura, flujo de aire o ratio de emisión por superficie o volumen de purín), y por lo tanto sus resultados son interpretables en términos comparativos, pero no en términos absolutos.

En lo que respecta al potencial de emisión de CH_4 es definido como la producción acumulada de CH_4 por g de MO o SV de sustrato, en condiciones estándar (Angelidaki et al., 2009). La determinación del potencial de emisión de CH_4 de purines se realiza siguiendo la metodología de digestión anaeróbica *in vitro* descrita por Hansen et al. (2004). Dicho potencial se corresponde con el BMP descrito anteriormente, de forma que esta determinación no indica el nivel real de emisión de CH_4, sino que, será posteriormente modificado por el MCF en función de las condiciones ambientales y el sistema de gestión del purín (IPCC, 2006). La aplicación de esta metodología no solo ha estado orientada al estudio de purines resultantes de ensayos nutricionales, sino que, principalmente ha estado orientada a establecer el BMP de purines de diversos orígenes solos y en co-digestión con otros sustratos (Ferrer et al., 2014; Nielfa et al., 2015).

En cualquier caso, la determinación del potencial de emisión de NH_3 y CH_4 *in vitro* en condiciones estandarizadas, permitiría atribuir los cambios en los potenciales de emisión a las variaciones de las características fisicoquímicas del purín o de algún otro factor determinante. Como tal, la aplicación de estas técnicas permitirá referenciar los efectos positivos o negativos de las estrategias de mitigación de emisiones de NH_3 y CH_4.

1.6 Referencias

Aarnink, A.J.A., 1997. Ammonia emission from houses for growing pigs as affected by pen design, indoor climate and behaviour. Ph.D. Thesis, Agricultural University, Wageningen, The Netherlands, 175 p.

Aarnink, A.J.A., Verstegen, M.W.A., 2007. Nutrition, key factor to reduce environmental load from pig production. Livest. Sci. 109, 194-203.

Abubaker, J., Risberg, K., Jönsson, E., Dahlin, A.S., Cederlund, H., Pell, M., 2015. Short-Term Effects of Biogas Digestates and Pig Slurry Application on Soil Microbial Activity 2015.

Alburquerque, J.A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., Bernal, M.P., 2012. Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass and Bioenergy 40, 181-189.

Angelidaki, I., Ahring, B.., 1994. Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. Water Res. 28, 727-731.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, a. J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927.

Babot, D., Hermida, B., Balcells, J., Calvet, S., & Alvarez-Rodríguez, J., 2011. Farm technological innovations on swine manure in Southern Europe. Revista Brasileira de Zootecnia, 40, 316-325.

Bakker, G., Bakker, J, Dekker, R., Jongbloed, R., Everts, H., Van der Meulen, J., Lenis, N. P., 1996. The quantitative relationship between absorption of nitrogen and starch from the hindgut of pigs. J. Anim. Sci, 74 (Suppl 1), 188.

Beccaccia, A., Calvet, S., Cerisuelo, A., Ferrer, P., García-Rebollar, P., De Blas, C., 2015a. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing-finishing pigs. I. Influence of the inclusion of two levels of orange pulp and carob meal in isofibrous diets. Anim. Feed Sci. Technol. 208, 158-169.

Beccaccia, A., Cerisuelo, A., Calvet, S., Ferrer, P., Estellés, F., De Blas, C., García-Rebollar, P., 2015b. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing pigs: II. Effect of protein source in practical diets. Anim. Feed Sci. Technol. 209, 137-144.

Beccaccia, A., Ferrer, P., Ibáñez, M.A., Estellés, F., Rodríguez, C., Moset, V., Blas, C. De, Calvet, S., García-rebollar, P., 2015. Relationships among slurry characteristics and gaseous emissions at different type|s of commercial Spanish pig farms. Spanish J. Agric. 13, 1-15.

Bosch-Serra, À.D., Padró, R., Boixadera-Bosch, R.R., Orobitg, J., Yagüe, M.R., 2014. Tillage and slurry over-fertilization affect oribatid mite communities in a semiarid Mediterranean environment. Appl. Soil Ecol. 84, 124-139.

Braun, R., Weiland, P., Wellinger, a, 2008. Biogas from energy crop digestion. IEA Bioenergy Task.

Burton, C.H., Turner, C., 2003. Manure management. Treatment Strat- egies for Sustainable Agriculture. Lister and Durling, Bedford, UK.

Cabaraux, J.-F., Philippe, F.-X., Laitat, M., Canart, B., Vandenheede, M., Nicks, B., 2009. Gaseous emissions from weaned pigs raised on different floor systems. Agric. Ecosyst. Environ. 130, 86-92.

Canh, T.., Aarnink, a. J.., Schutte, J.., Sutton, a, Langhout, D.., Verstegen, M.W.., 1998. Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing–finishing pigs. Livest. Prod. Sci. 56, 181-191.

Cavanagh, A., Gasser, M.O., Labrecque, M., 2011. Pig slurry as fertilizer on willow plantation. Biomass and Bioenergy 35, 4165-4173.

Cepero, L., Savran, V., Blanco, D., Díaz Piñón, M. R., Suárez, J., Palacios, A., 2012. Producción de biogás y bioabonos a partir de efluentes de biodigestores. Pastos y Forrajes, 35(2), 219-226.

Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B., Misselbrook, T., 2011. Manure management: Implications for greenhouse gas emissions. Anim. Feed Sci. Technol. 166-167, 514-531.

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99, 4044-64.

Chowdhury, A., Rong, L., Feilberg, A., & Adamsen, A.P.S., 2014. Review of ammonia emissions from a pig house slurry pit and outside storage: Effects of emitting surface and slurry depth. Danish Environ. Prot. Agency.

Clemens, J., Trimborn, M., Weiland, P., Amon, B., 2006. Mitigation of greenhouse gas emissions by anaerobic digestion of cattle slurry. Agric. Ecosyst. Environ. 112, 171-177.

Conn, K.L., 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.

Dai, X.R., Blanes-Vidal, V., 2013. Emissions of ammonia, carbon dioxide, and hydrogen sulfide from swine wastewater during and after acidification treatment: effect of pH, mixing and aeration. J. Environ. Manage. 115, 147-54.

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.

European Commission, 2014. Prospects for EU agricultural markets and income 2014-2024 European Union, Direction for Agriculture and Rural Development. http://ec.europa.eu/agriculture/markets-and-prices/medium-term-outlook/2014/fullrep_en.pdf.

EUROSTAT, 2015. Pig farming sector - statistical portrait. Pig farming in the European Union: considerable variations from one Member State to another. Statistics in focus 15/2014 [WWW Document]. Author Pol Marquer, Teresa Rabade, Roberta Forti ISSN2314-9647 Cat. numberKS-SF-14-015-EN-N.

EurObserv'ER, 2014. The state of renewable energies in Europe, edition 2014 In: 14th.

EurObserv'ER 2005. Report, ISSN 2101–9622.Observ'ER, Paris, France.

Fangueiro, D., Hjorth, M., Gioelli, F., 2015. Acidification of animal slurry--a review. J. Environ. Manage. 149, 46-56.

Fangueiro, D., Ribeiro, H.M., Vasconcelos, E., Coutinho, J., Cabral, F., 2012. Influence of animal slurries composition and relative particle size fractions on the C and N mineralization following soil incorporation. Biomass and Bioenergy 47, 50-51.

FAO, 2013. PART 3 Feeding the world. FAO Stat. Yearb. 2013 123-158.

FAO, 2009. El estado mundial de la agricultura y la alimentación.

FAOSTAT, 2015. http://faostat3.fao.org/browse/Q/QA/S.

Ferrer, P., Cambra-López, M., Cerisuelo, A., Peñaranda, D.S., Moset, V., 2014. The use of agricultural substrates to improve methane yield in anaerobic co-digestion with pig slurry: Effect of substrate type and inclusion level. Waste Manag. 34, 196-203.

Fiore, a. M., Jacob, D.J., Field, B.D., Streets, D.G., Fernandes, S.D., Jang, C., 2002. Linking ozone pollution and climate change: The case for controlling methane. Geophys. Res. Lett. 29, 2-5.

Flotats, X., Campos, E., Bonmatí, J.P. a, 2001. Digestión anaerobia de purines de cerdo y co-digestión con residuos de la industria alimentaria. Monogr. Actual. 51-65.

Fukumoto, Y., Osada, T., Hanajima, D., Haga, K., 2003. Patterns and quantities of NH3, N2O and CH4 emissions during swine manure composting without forced aeration—effect of compost pile scale. Bioresour. Technol. 89, 109-114.

Galassi, G., Colombini, S., Malagutti, L., Crovetto, G.M., 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.

Gomez-Garrido, M., Martinez-Martinez, S., Faz Cano, A., Buyukkilic-Yanardag, A., M. Arocena, J., 2014. Soil fertility status and nutrients provided to spring barley (Hordeum distichon L.) by pig slurry. Chil. J. Agric. Res. 74, 73-82.

Greatorex, J.M., 2000. A review of methods for measuring methane, nitous oxide and odour emissions from animal production activities. JTi- Institutet jordbruks-och miljoteknik. Nro. 274.

Groot Koerkamp, P.W.G., Metz, J.H.M., Uenk, G.H., Phillips, V.R., Holden, M.R., Sneath, R.W., Short, J.L., White, R.P.P., Hartung, J., Seedorf, J., Schröder, M., Linkert, K.H., Pedersen, S., Takai, H., Johnsen, J.O., Wathes, C.M., 1998. Concentrations and Emissions of Ammonia in Livestock Buildings in Northern Europe. J. Agric. Eng. Res. 70, 79-95.

Integrated Pollution Prevention and Control (IPPC)., 2003. Reference document on best available techniques for intensive rearing of poultry and pigs. http://eippcb.jrc.ec.europa.eu/reference/BREF/irpp_bref_0703.

IPCC 2006, 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, Eggleston H.S., Buendia L., Miwa K., Ngara T. and Tanabe K. (eds). Published: IGES, Japan.

Haeussermann, A., Hartung, E., Gallmann, E., Jungbluth, T., 2006. Influence of season, ventilation strategy, and slurry removal on methane emissions from pig houses. Agric. Ecosyst. Environ. 112, 115-121.

Hansen, K.H., Angelidaki, I., Ahring, B.K., 1998. ANAEROBIC DIGESTION OF SWINE MANURE: INHIBITION BY AMMONIA. Water Res. 32, 5-12.

Hansen, M.J., Nørgaard, J. V., Adamsen, A.P.S., Poulsen, H.D., 2014. Effect of reduced crude protein on ammonia, methane, and chemical odorants emitted from pig houses. Livest. Sci. 169, 118-124.

Hansen, T.L., Schmidt, J.E., Angelidaki, I., Marca, E., Jansen, J. la C., Mosbaek, H., Christensen, T.H., 2004. Method for determination of methane potentials of solid organic waste. Waste Manag. 24, 393-400.

Henk Westhoek, Trudy Rood, Maurits van de Berg, Jan Janse, Durk Nijdam, Melchert Reudink, E.S., 2011. The protein puzzle: The consumption and production of meat, dairy and fish in the European Union, Netherland. ed. PBL Publishers, The Hague.

Hernández, F., Martínez, S., López, C., Megías, M.D., 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.

Hjorth, M., Christensen, K. V., Christensen, M.L., Sommer, S.G., 2010. Solid—liquid separation of animal slurry in theory and practice. A review. Agron. Sustain. Dev. 30, 153-180.

Holm-Nielsen, J.B., Al Seadi, T., Oleskowicz-Popiel, P., 2009. The future of anaerobic digestion and biogas utilization. Bioresour. Technol. 100, 5478-84.

Hristov, A.N., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz, A., Dell, C., Adesogan, A., Yang, W., Tricarico, J., Kebreab, E., Waghorn, G., Dijkstra, J. & Oosting, S., 2013. Mitigación de las emisiones de gases de efecto invernadero en la producción ganadera – Una revisión de las opciones técnicas para la reducción de las emisiones de gases diferentes al CO2. Producción y Sanidad Animal FAO Documento No. 177. FAO, Roma, Italia, Roma.

IPCC 2006, 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, Eggleston H.S., Buendia L., Miwa K., Ngara T. and Tanabe K. (eds). Published: IGES, Japan.

IPCC, 2003, Integrated Pollution Prevention and Control, July 2003. Reference document on best available techniques for intensive rearing of poultry and pigs.

Jarret, G., Cerisuelo, A., Peu, P., Martinez, J., Dourmad, J.Y., 2012. Impact of pig diets with different fibre contents on the composition of excreta and their gaseous emissions and anaerobic digestion. Agric. Ecosyst. Environ. 160, 51-58.

Jarret, G., Martinez, J., Dourmad, J.-Y., 2011. Effect of biofuel co-products in pig diets on the excretory patterns of N and C and on the subsequent ammonia and methane emissions from pig effluent. Animal 5, 622-631.

Jørgensen, H., Theil, P.K., Erik, K., Knudsen, B., 2011. 27: Enteric Methane Emission from Pigs. Planet Earth 2011 - Glob. Warm. Challenges Oppor. Policy Pract. 605-622.

Kebreab, E., Clark, K., Wagner-Riddle, C., France, J., 2006. Methane and nitrous oxide emissions from Canadian animal agriculture: A review. Can. J. Anim. Sci. 86, 135-157.

Krupa, S. V., 2003. Effects of atmospheric ammonia (NH3) on terrestrial vegetation: A review. Environ. Pollut. 124, 179-221.

Labatut, R.A., Angenent, L.T., Scott, N.R., 2011. Biochemical methane potential and biodegradability of complex organic substrates. Bioresour. Technol. 102, 2255-64.

Lobera, J., Martínez, P., Ferrández, F., Martín, J., 1998. Reutilización agronómica de los purines de cerdo. Comunidad Autónoma de la Región de Murcia Consejería de Agricultura, Agua y Medio Ambiente. Serie técnica y de estudios. 3-162.

Lorimor, J., Powers, W., Sutton, A., 2000. Manure Characteristics. Manure Management System Series. Section 1, MWPS (Midwest Plan Service)-18.Iowa State University Publ., Ames, USA.

Mao, C., Feng, Y., Wang, X., Ren, G., 2015. Review on research achievements of biogas from anaerobic digestion. Renew. Sustain. Energy Rev. 45, 540-555.

Martínez-Suller, L., Provolo, G., Brennan, D., Howlin, T., Carton, O.T., Lalor, S.T.J., Richards, K.G., 2010. A note on the estimation of nutrient value of cattle slurry using easily determined physical and chemical parameters. Irish J. Agric. Food Res. 49, 93-97.

Mata-Alvarez, J., Macé, S., Llabrés, P., 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour. Technol. 74, 3-16.

Møller, H.B., Sommer, S.G., Ahring, B.K., 2004. Methane productivity of manure, straw and solid fractions of manure. Biomass and Bioenergy 26, 485-495.

Moral, R., Perez-Murcia, M.D., Perez-Espinosa, a., Moreno-Caselles, J., Paredes, C., 2005. Estimation of nutrient values of pig slurries in Southeast Spain using easily determined properties. Waste Manag. 25, 719-725.

Moral, R., Perez-Murcia, M.D., 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 Manag. 28, 367-371.

Morazán, H., Alvarez-Rodriguez, J., Seradj, A.R., Balcells, J., Babot, D., 2015. Trade-offs among growth performance, nutrient digestion and carcass traits when feeding low protein and/or high neutral-detergent fiber diets to growing-finishing pigs. Anim. Feed Sci. Technol.

Moughan, P, Fuller, M., D'Mello, J., 2003. Modelling amino acid metabolism and the estimation of amino acid requirements. Amino acids in animal nutrition, (Ed. 2), 187-202.

Mroz, Z., Moeser, a J., Vreman, K., Diepen, J.T. Van, Kempen, T. Van, Canh, T.T., Diepen, J.T.M. Van, Jongbloed, a W., 2000. Effects of dietary carbohydrates and buffering capacity on nutrient digestibility and manure characteristics in finishing pigs The online version of this article , along with updated information and services , is located on the World Wide Web at□: Effects 3096-3106.

Ni, J.-Q., Heber, A.J., Diehl, C.A., Lim, T.T., 2000. SE—Structures and Environment. J. Agric. Eng. Res. 77, 53-66.

Nicholson, F. a., Chambers, B.J., Williams, J.R., Unwin, R.J., 1999. Heavy metal contents of livestock feeds and animal manures in England and Wales. Bioresour. Technol. 70, 23-31.

Nielfa, A., Cano, R., Fdz-Polanco, M., 2015. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. Biotechnol. Reports 5, 14-21.

Olusegun, O.S., 2014. Influence of NPK 15-15-15 Fertilizer and Pig Manure on Nutrient Dynamics and Production of Cowpea, Vigna Unguiculata L. Walp. Am. J. Agric. For. 2, 267.

Parera i Pous, J., Domingo Olivé, F., Mallol Nabot, C., Canut Torrijos, N., 2010. Adaptación del uso de la conductividad eléctrica (CE) para determinar de forma rápida el contenido en nutrientes del purín porcino en Catalunya. Int. Work. Anaerob. Dig. Slaughterh. Waste 67-76.

Parkin, B.G.F., Owen, W.F., 1987. Fundamentals of anaerobic digestion of wastewater sludges 112, 867-920.

Partridge, G., Gill, B., Garnsworthy, P., Wiseman, J., 2001. New approaches with pig weaner diets. Recent developments in pig nutrition 3, 205-237.

Penha, H.G.V., Menezes, J.F.S., Silva, C.A., Lopes, G., de Andrade Carvalho, C., Ramos, S.J., Guilherme, L.R.G., 2015. Nutrient accumulation and availability and crop yields following long-term application of pig slurry in a Brazilian Cerrado soil. Nutr. Cycl. Agroecosystems 101, 259-269.

Petersen, S.O., Dorno, N., Lindholst, S., Feilberg, A., Eriksen, J., 2013. Emissions of CH4, N2O, NH3 and odorants from pig slurry during winter and summer storage. Nutr. Cycl. Agroecosystems 95, 103-113.

Philippe, F.-X., Cabaraux, J.-F., Nicks, B., 2011. Ammonia emissions from pig houses: Influencing factors and mitigation techniques. Agric. Ecosyst. Environ. 141, 245–260.

Philippe, F.-X., Nicks, B., 2015. Review on greenhouse gas emissions from pig houses: Production of carbon dioxide, methane and nitrous oxide by animals and manure. Agric. Ecosyst. Environ. 199, 10-25.

Portejoie, S., Dourmad, J.Y., 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.

Rodhe, L.K.K., Abubaker, J., Ascue, J., Pell, M., Nordberg, Å., 2012. Greenhouse gas emissions from pig slurry during storage and after field application in northern European conditions. Biosyst. Eng. 113, 379-394.

Sánchez, M., González, J.L., 2005. The fertilizer value of pig slurry. I. Values depending on the type of operation. Bioresour. Technol. 96, 1117-1123.

Snoek, D.J.W., Stigter, J.D., Ogink, N.W.M., Groot Koerkamp, P.W.G., 2014. Sensitivity analysis of mechanistic models for estimating ammonia emission from dairy cow urine puddles. Biosyst. Eng. 121, 12-24.

Strömberg, S., Nistor, M., Liu, J., 2014. Towards eliminating systematic errors caused by the experimental conditions in Biochemical Methane Potential (BMP) tests. Waste Manag. 34, 1939-48.

Suresh, a., Choi, H.L., Oh, D.I., Moon, O.K., 2009. Prediction of the nutrients value and biochemical characteristics of swine slurry by measurement of EC - Electrical conductivity. Bioresour. Technol. 100, 4683-4689.

Teira Esmatges, M.R., 2007. Informe para la mejora de la gestión de los purines porcinos en Catalunya. Cons. Assessor per al Desenvol. Sostentenible, stión de los purines porcinos en Cataluña. Volumen 5 de Informes del CADS, 225p.

United Nations, 2015. World Population Prospects, United Nations, Department of Economic and Social Affairs, Population Division (2015). World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241. Cover.

Van der Peet-Schwering, C.M.., Jongbloed, A.., Aarnink, A.J.., 1999. Nitrogen and phosphorus consumption, utilisation and losses in pig production: The Netherlands. Livest. Prod. Sci. 58, 213-224.

Van Lier, J.B., Hulsbeek, J., Stams, A.J.M., Lettinga, G., 1993. Temperature susceptibility of thermophilic methanogenic sludge: Implications for reactor start-up and operation. Bioresour. Technol. 43, 227-235.

Villamar, C.A., Canuta, T., Belmonte, M., Vidal, G., 2012. Characterization of swine wastewater by toxicity identification evaluation methodology (TIE). Water. Air. Soil Pollut. 223, 363-369.

Villar, M.C., Petrikova, V., Díaz-Raviña, M., Carballas, T., 2004. Recycling of organic wastes in burnt soils: Combined application of poultry manure and plant cultivation. Waste Manag. 24, 365-370.

Webb, J., Broomfield, M., Jones, S., Donovan, B., 2014. Ammonia and odour emissions from UK pig farms and nitrogen leaching from outdoor pig production. A review. Sci. Total Environ. 470-471, 865-75.

Weiland, P., 2010. Biogasproduction: current state and perspectives. Appl. Microbiol. Biotechnol. 85, 849.860.

2
Objetivos y estructura de la tesis

El presente trabajo se enmarca en un proyecto mayor financiado por el Ministerio de Ciencia e Innovación que lleva por título "Emisiones de NH_3 y gases efecto invernadero en purín porcino y potencial para producción de biogás o valor fertilizante: variabilidad inducida por estrategias de alimentación (*GASPORC-AGL2011-30023)*.

La presente Tesis está enfocada a la caracterización de las emisiones potenciales de amoniaco (NH_3) y metano (CH_4) a partir de purines porcinos. En concreto, estudia la relación entre el consumo, la excreción de nutrientes, la composición del purín y las emisiones potenciales de NH_3 y CH_4 y el valor agrícola de los purines. Además, evalúa y propone la optimización de las metodologías de medición de las emisiones potenciales de NH_3 y CH_4 *in vitro*.

Los objetivos generales de esta tesis son:

- Caracterizar la composición, el potencial de emisión de CH_4 y NH_3 y el valor agrícola de purines comerciales de España (Capítulo 3).
- Evaluar la influencia de la inclusión en el pienso de una fuente de grasa (jabón cálcico de palma) y una fuente de fibra (pulpa de naranja) sobre la composición del purín y emisiones potenciales de NH_3 y CH_4 en cerdos en crecimiento (Capítulo 4).
- Optimizar las mediciones de las emisiones potenciales de CH_4 y NH_3 *in vitro* de purines de cerdo (Capítulos 5 y 6).

Con el presente trabajo de tesis se pretende contribuir a incrementar la sostenibilidad de la producción porcina, aportando un mayor conocimiento sobre las características de los purines, las relaciones pienso-purín-emisión y sobre la capacidad predictiva de las metodologías de medición de emisiones de NH_3 y CH_4.

En respuesta al primer objetivo, en el tercer capítulo de la tesis *"Composition, potential emissions and agricultural value of pig slurry from Spanish commercial farms"* se estudia la composición/caracterización de los purines de granjas comerciales de España, su potencial de emisiones CH_4 y NH_3 y su aptitud para el uso agrícola.

En relación con el segundo objetivo, en el cuarto capítulo *"Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing pigs: III Influence of varying the dietary level of calcium soap of palm fatty acids distillate with or without orange pulp supplementation"* se estudia los efectos de la inclusión de jabón cálcico de palma (como fuente de grasa) y pulpa de naranja (como fuente de fibra fermentable) en dietas de cerdos de engorde sobre las emisiones potenciales de CH_4 y NH_3 de los purines resultantes.

El tercer objetivo se desarrolla en el quinto y sexto capítulo ("*Ammonia emission quantification from pig slurry using acid wet traps: evaluation and optimization"* y *"Kinetics and prediction of methane production in pig slurry*"). En el capítulo quinto se evalúa la medición de las emisiones potenciales de NH_3 por el método de trampas ácidas y en el capítulo sexto se estudia la cinética de producción de CH_4 de purines in vitro. En ambos casos se plantean posibilidades de optimización de las mediciones y modelos de predicción.

En el séptimo capítulo, con la finalidad de analizar el efecto de factores como el origen y la ingesta de nutrientes sobre la composición de los purines y las emisiones de NH_3 y CH_4, se pone en común los datos obtenidos en los estudios llevados a cabo en el marco de esta tesis y del Proyecto "*GASPORC-AGL2011-30023*". Adicionalmente, las técnicas de determinación de estas emisiones *in vitro* son analizadas en detalle en cuanto a su aplicabilidad y posibilidades de optimización.

3

Composition, potential emissions and agricultural value of pig slurry from Spanish commercial farms

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Abstract. Pig slurry is a valuable fertilizer for crop production but at the same time its management may pose environmental risks. Slurry samples were collected from 77 commercial farms of four animal categories (gestating and lactating sows, nursery piglets and growing pigs) and analyzed for macronutrients, micronutrients, heavy metals and volatile fatty acids. Emissions of ammonia (NH_3) and biochemical methane potential (BMP) were quantified. Slurry electrical conductivity, pH, dry matter content and ash content were also determined. Data analysis included an analysis of correlations among variables, the development of prediction models for gaseous emissions and the analysis of nutritional content of slurries for crop production. Descriptive information is provided in this work and shows a wide range of variability in all studied variables. Animal category affected some physicochemical parameters, probably as a consequence of different slurry management and use of cleaning water. Slurries from gestating sows and growing pigs tended to be more concentrated in nutrients, whereas the slurry from lactating sows and nursery piglets tended to be more diluted. Relevant relationships were found among slurry characteristics expressed in fresh basis and gas emissions. Predictive models using on-farm measurable parameters were obtained for NH_3 (R^2 = 0.51) and CH_4 (R^2 = 0.76), which suggests that BMP may be estimated in commercial farms from easily determined slurry characteristics. Finally, slurry nutrient composition was highly variable. Therefore, complete analyses of slurries should be performed for an effective and environmental friendly land application.

Keywords: ammonia emission, methane emission, fertilizer value, prediction model, slurry characterization.

Abbreviations: BMP, biochemical methane potential; CH_4, methane; DM, dry matter; EC, electrical conductivity; CV, coefficient of variation; NH_3, ammonia; OM, organic matter; SV, volatile solids; TKN, total Kjeldahl nitrogen; TAN, total ammonia nitrogen; VFA, volatile fatty acids.

3.1 Introduction

At present, there is an increasing concern about the environmental impacts associated with intensive and concentrated livestock production. This is especially the case for pig production which currently has a global population of over 1 billion heads, mainly concentrated in intensive producing areas. Spain is the second largest pig producer in Europe and the sixth in the world, with about 26 million animal places (EUROSTAT 2015). Slurry management is influencing emissions of atmospheric pollutants such as ammonia (NH_3), nitrous oxide (N_2O) and methane (CH_4) (Hristov et al. 2013; Viguria et al. 2015) and it is also linked to potentially harmful effects for soil and water (Aarnink and Verstegen, 2007).

Nevertheless, slurry should be considered a valuable resource as fertilizer because it contains significant amounts of most nutrients required by crops (Penha et al., 2015; Villar et al., 2004) plus organic substance which is important for soil fertility. The positive or negative effects of land application of slurry may depend on slurry characteristics (Villamar et al. 2013), dosing (Iguácel et al. 2011) or environmental conditions during application and hereafter (Webb et al. 2014). Moreover, pig slurry is used as a renewable source of energy due to its potential to produce CH_4 under anaerobic conditions (Ferrer et al. 2014; Zhang et al. 2014).

The composition of pig slurry from commercial farms is highly variable due to differences among housing systems, feed composition, climate or farm management (Beccaccia et al. 2015; Moral et al. 2008; Moral et al. 2005b; Sánchez et al. 2005). In recent years, several studies have been published either characterizing physicochemical composition of slurries from commercial pig farms (Parera i Pous et al., 2010; Suresh et al., 2009), or evaluating the potential to emit NH_3 and greenhouse gases (GHG) form slurries obtained under experimental conditions (Antezana et al. 2015; Galassi et al. 2010; Hernández et al. 2013; Jarret et al. 2012 among others). However, there is scarce information on the potential emissions of GHG and NH_3 of slurries from commercial pig farms.

Understanding the relationships between physicochemical properties of slurry can provide a basis for estimating the fertilizer value of slurry, thus facilitating its more efficient use in

agriculture and reducing the potential risks to the environment (Scotford et al. 1998; Díez et al. 2006; Thygesen et al. 2012).

It is therefore necessary to evaluate in detail the physicochemical characteristics of slurries in commercial farms, to analyze their relationships with the emission of CH_4 and NH_3 and to examine the potential fertilizer value of slurries. A previous study (Amanda Beccaccia et al., 2015) analyzed relationships among different feed and slurry characteristics and potential gaseous emissions. The aim of this study was to analyze in depth the variability in mineral composition and gas emission of pig slurry samples from commercial pig farms, to predict potential NH_3 emissions and the biochemical methane potential (BMP) in commercial farms and to explore limitations when using commercial slurries as fertilizers.

3.2 Materials and methods

3.2.1 Description of the selected farms and sample collection

A total of 77 samples of pig slurry from farms located in the Center and East of Spain were analyzed in order to account for potential variations in composition and emissions. A detailed description of the survey protocol and analysis can be found in Beccaccia et al. (2015). Samples were classified according to the animal category: 15 samples from gestating sows, 14 from lactating sows, 14 from nursery piglets and 34 from growing pigs. Table 3.1 summarizes the most relevant characteristics of the surveyed farms.

Sampling was designed to be representative of the slurry extracted from the pits under commercial conditions. A minimum of five aliquots (2 L each) were taken during the pit discharge at equidistant time intervals. Composite samples were then thoroughly mixed in a 15 L container and subsamples were conditioned for the corresponding analyses. All samples were kept in sealed containers at 5ºC until laboratory processing, which was made within 24h.

Table 3.1 Main operation and installation characteristics of the farms surveyed

	Gestating sows	Lactating sows	Nursery piglets	Growing pigs
Number of farms	15	14	14	34
Animal places per farm (average)	770	162	2319	3132
Type of housing	Group	Individual	Group	Group
Feeding type	Dry	Dry (71%) Wet (29%)	Dry	Dry
Feed restriction	Yes	No (79%) Yes (21%)	No	No
Slurry accumulation				
< 1 month	21%	93%	50%	41%
1-3 months	7%	7%	50%	24%
> 3 months	71%	0%	0%	35%
Slat				
Partial	64%	21%	21%	41%
Total	36%	79%	79%	59%
Ventilation type				
Natural	86%	57%	43%	78%
Mechanical	14%	43%	57%	16%

3.2.2 Physicochemical analysis

All pig slurry samples were analyzed to determine dry matter (DM), organic matter (OM) and total Kjeldahl nitrogen (TKN) according to APHA (2005). Also, pH, electric conductivity (EC), total ammonia nitrogen (TAN) (4500 NH_3-B and 4500 NH_3-C procedures) (APHA, 2005), and volatile fatty acids (VFA) (Jouany, 1982) were determined. Volatile fatty acids were analyzed using a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame ionization detector with the addition of an internal standard (4-metil valeric).

Macronutrients, micronutrients and heavy metal concentrations (P, K, Ca, Mg, S, Al, B, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Na, Ni, Pb and Zn) were determined after acid digestion by

inductive-optical coupled plasma spectrophotometry (ICP-OES, ICAP 6500 Duo, Thermo Scientific, Walthamm, MA, USA).

3.2.3 Ammonia emissions

Ammonia emissions assays were performed with an ammonia trap system similar to that described by Ndegwa et al. (2009). Slurry subsamples of 0.6 kg with a volume: surface ratio of 0.0952 m^3/m^2 were placed in duplicate in 1-L closed containers maintained at 25ºC 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^{-1}. 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 each. 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 quantified following 4500 NH3-D procedure (APHA, 2005) using a detection electrode (Orion High Performance NH_3 Electrode, model 9512HPBNWP, Thermo Scientific, USA).

3.2.4 Biochemical methane potential (BMP)

The BMP was determined in a batch assay using 120 mL bottles following the methodology described by Angelidaki et al. (2009). Inoculum from a mesophilic pig slurry anaerobic digester reactor was used. The inoculum was pre-incubated during 15 days at 35ºC in order to deplete the residual biodegradable organic material. Pig slurry and inoculum were mixed to obtain an inoculum to substrate ratio of 1 in OM basis. Each sample was tested out by triplicate. Additionally, three blank bottles containing degasified inoculum were included in the measurements in order to determine the inoculum endogenous CH_4 production. The endogenous CH_4 production from the inoculum was subtracted from the CH_4 produced by the pig slurry on each biogas sampling day. After filling, each bottle was sealed with butyl rubber stoppers and aluminum crimps and the headspace was flushed with pure N_2 for two minutes. Bottles were then incubated at 35°±1ºC for 100 days. 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. Finally, the BMP was expressed per volume of slurry considering the OM content.

3.2.5 Statistical analyses

All slurry components were expressed and analyzed in wet basis. The descriptive statistical analysis of the results of the variables in this study was made by animal category through PROC MEANS of SAS Institute (2008). The effect of the animal type in the studied variables was analyzed using PROC GLM and PROC T TEST of SAS Institute (2008). The Correlation analysis among physicochemical slurry characteristics, as well as NH_3 and BMP, was done using PROC CORR of SAS Institute (2008). A multiple regression analysis was also conducted with a stepwise variable selection process using the PROC REG of SAS Institute (2008) to establish prediction models of potential emissions from easily measurable slurry characteristics (i.e. EC, pH and DM).

From the results of the physicochemical characterization of slurry, potential scenarios of nutrient loads associated to land application were obtained. These scenarios corresponded to limitations of organic manure application according to international regulations, in particular the European Union Directive 91/676/CE which specifies that the livestock manure applied to land each year shall not exceed the amount containing 170 kg N per hectare.

3.3 Results and discussion

3.3.1 Physicochemical slurry characterization

Summary statistics for the physicochemical characterization of slurry for the different animal categories are presented in Table 3.2. As shown in this table, slurry characteristics varied considerably among and within animal categories. Only for some characteristics the category of animal was found to have a statistically significant effect. Correlations among the main physicochemical characteristics are shown in Table 3.3.

The main results of this study are in agreement with those reported for pig slurries from commercial pig farms in Spain (Moral et al. 2005b; Moral et al. 2005a; Parera i Pous et al., 2010; Sánchez et al. 2005, among others) and other countries (Abubaker et al. 2015; Martínez-Suller et al. 2008; Olusegun 2014; Suresh et al. 2009; Villamar et al. 2012). The EC in pig slurry ranged from 2.65 to 53.46 mS cm^{-1}, and was higher for growing pigs and gestating sows than for lactating sows and nursery piglets ($P < 0.05$). This difference may be attributed to the housing system and management practices such as slurry removal frequency and use of cleaning water. In fact, animal categories showing higher EC are those in which slurries tend to be stored for a longer time in slurry pits (see Table 3.1), thus enhancing drying and mineralization of slurry. On the contrary, lactating sows and weaners are those with shorter slurry accumulation time and tend to use higher volumes of cleaning water. Therefore, slurries from lactating sows and weaners tend to be more diluted, with lower DM contents. Differences in the concentration of protein and minerals in the diet of pigs among animal types (Amanda Beccaccia et al., 2015; Moral et al., 2008) may also be contributing to differences in EC. As shown in Table 3.3 and evidenced by previous studies (Provolo et al. 2007; Yagüe et al. 2012), both DM and EC are significantly correlated with most slurry characteristics. These relationships support the idea of dilution as a main factor affecting nutrient composition of slurries, and thus they have been used to propose prediction models for nutrients (Martínez-Suller et al. 2008). The pH ranged from 6.3 to 8.0 with low variation among animal categories. Slurry pH from gestating sows was on average 7.78 and it was higher than in slurries from other animal categories ($P <0.01$). This value could probably be associated to lower concentrations of VFA (Table 2).

Regarding macronutrients (N, P, K, Ca, Mg and S) their concentration in the slurry tends to be higher for growing pigs and gestating sows, which is statistically significant in the case of N, K and Ca. In this regard, Sánchez et al. (2005) and Moral et al. (2005a) also found higher N concentrations in slurries from growing pigs and gestating sows. It must be considered that nutrient excretion is a consequence of the inefficiency in their use by the animals and therefore it is affected by nutritional factors (Bai et al., 2014; Morazán et al., 2015; Patience et al., 2015), which may differ among animal categories.

A major part of the slurry nitrogen is in inorganic form, mainly as TAN, which represented 65% of the total nitrogen. These values are similar to those obtained by previous studies (Sánchez et al. 2005), which reported values of 57% of TAN. As shown in Figure 1, both N and TAN were positively correlated with EC (R^2 = 0.65 and 0.79, respectively), which supports the conclusions of Martínez-Suller et al. (2008) and Moral et al. (2005b) that these components can be predicted in practice.

In relation to the P content in the slurry, a wide variation was found (ranging from 0.06 to 4.58 kg m^{-3}). The average content was 0.86 kg m^{-3}, but no statistical differences were found among animal categories. In the case of K, the slurry from lactating sows (1.07 kg m^{-3}) had a lower content than for the other categories ($P < 0.05$). Regarding the Ca content, the slurry from gestating sows (on average 1.97 kg m^{-3}) had a higher content than for the other categories ($P < 0.05$). Finally in the case of Mg the average content was 0.56 kg m^{-3}, but no statistical differences were found among animal categories. This is in accordance with previous studies (Moral et al. 2008; Sánchez et al. 2005; Abubaker et al. 2015).

The content of micronutrients and heavy metals in the slurry was not statistically affected by animal category in the case of Al, B, Cd, Co, Cr, Fe, Li, Mn, Mo, Ni and Pb. However, the content of Cu, Zn and Na were affected by the animal category. Particularly, the content of Cu and Zn, which are commonly used as promotors, were highest for nursery piglets (32.8 and 316.8 g m^{-3} respectively, which is about 3 to 6 times higher than other animal category; $P < 0.001$). On the contrary, the Na content was highest ($P < 0.05$) for growing animals (0.53 kg m^{-3}). The content of heavy metals expressed per dry matter basis is within the range reported by Nicholson et al. (1999). These authors reported average concentrations of Cu, Ni, Pb and Cd (351, 10.4, 2.48, 0.30 mg kg^{-1} DM, respectively), which is very similar to this study (297, 8.27, 2.97 and 0.32 mg kg^{-1} DM, respectively). On the contrary, the concentration of Zn and Cr was from 3 to 5 times higher in our study (2540 *vs.* 575 mg kg^{-1} DM for Zn and 10.7 *vs.* 2.82 mg kg^{-1} DM for Cr, respectively).

Table 3.2 Physicochemical characterization of pig slurries by different animal categories (n: 77)

Parameter	All samples (n=77)		Growing pigs (n=34)				Gestating sows (n=15)				Lactating sows (n=14)				Nursery piglets (n=14)			
	Mean	CV	Mean	Min	Max	CV	Mean	Min	Max	CV	Mean	Min	Max	CV	Mean	Min	Max	CV
Physicochemical characteristics																		
EC (mS cm^{-1})	21.8	53	25.7a	6.59	53.5	52	21.6a	10.15	44.6	37	15.5b	6.26	25.6	38	19.0b	2.65	46.6	61
pH	7.52	5.5	7.39a	6.41	8.05	5.7	7.78b	7.54	8.03	1.8	7.66c	7.05	8.16	3.6	7.43ac	6.34	7.92	7.5
DM (kg m^{-3})	49.3	79	57.09	7.5	176.7	72	56.05	4.9	155.4	92	35.6	8.6	75.8	63	36.83	5.7	97.3	67
OM (kg m^{-3})	36.7	83	43.51	4.5	145.1	76	41.1	2.8	120.5	95	25.9	4.9	55.4	66	26.74	3.6	72.4	71
Ash (kg m^{-3})	12.5	71	13.57	3	34.6	62	14.94	2.12	45.6	88	9.72	3.72	20.4	56	10.09	1.88	24.9	60
Macronutrients																		
N (kg m^{-3})	4.79	64	6.01a	1.43	15.1	60	4.36a	1.62	9.09	54	3.37b	1.69	5.97	39	3.71b	0.72	11.7	74
P (kg m^{-3})	0.86	91	0.831	0.104	2.09	63	1.31	0.06	4.58	106	0.704	0.107	1.86	80	0.623	0.111	1.59	68
K (kg m^{-3})	1.66	64	2.05a	0.504	4.62	60	1.53a	0.261	3.81	59	1.07b	0.583	1.58	32	1.42a	0.216	3.85	66
Ca (kg m^{-3})	1.23	96	1.13a	0.227	3.14	72	1.97b	0.115	6.45	108	1.01a	0.198	1.96	61	0.895a	0.149	2.22	67
Mg (kg m^{-3})	0.56	84	0.59	0.081	1.39	62	0.738	0.034	2.66	107	0.477	0.056	1.28	81	0.386	0.082	0.856	63
S (kg m^{-3})	0.3	75	0.34	0.056	0.953	71	0.31	0.043	0.849	88	0.212	0.076	0.47	63	0.313	0.061	0.856	70
Micronutrients and heavy metals																		
Al (g m^{-3})	43.3	85	41.57	3.2	115.9	78	47.9	1.44	152.9	99	53.5	5.59	147.2	83	32.7	2.02	88.1	76
B (g m^{-3})	1.84	69	2.13	0.483	6.48	70	1.55	0.185	3.73	73	1.43	0.408	2.82	56	1.86	0.252	4.33	62
Cd (g m^{-3})	0.013	88	0.011	0.002	0.034	79	0.015	0	0.049	104	0.013	0.001	0.039	96	0.016	0.001	0.043	76
Co (g m^{-3})	0.686	317	0.507	0.014	5.7	208	0.84	0.018	8.9	268	1.37	0.019	16.3	314	0.273	0.023	1.16	129
Cr (g m^{-3})	0.602	112	0.61	0.031	1.95	87	0.88	0.018	4.36	133	0.451	0.037	1.3	90	0.44	0.042	1.68	93
Cu (g m^{-3})	12.7	126	10.4a	0.684	59.75	113	6.12a	0.236	17.6	92	5.3a	0.598	17.2	94	32.8b	3.33	80.6	71
Fe (g m^{-3})	85	93	73.78	5.95	189.6	64	90.52	2.92	302.2	102	107.5	6.47	413.5	113	83.9	5.48	242.9	93
Li (g m^{-3})	0.149	118	0.145	0.017	0.797	126	0.189	0.009	0.963	132	0.145	0.025	0.434	88	0.118	0.015	0.305	82
Mn (g m^{-3})	22.6	89	24.41	3.05	79.5	67	28.59	1.19	123.3	116	15.8	2.12	43.4	83	18.3	2.2	47.9	76
Mo (g m^{-3})	0.313	87	0.355	0.044	1.21	85	0.253	0.008	0.901	98	0.271	0.026	0.988	103	0.318	0.039	0.701	72
Na (kg m^{-3})	0.425	69	0.527a	0.108	1.41	68	0.382a	0.061	0.912	58	0.304b	0.127	0.539	44	0.344c	0.062	0.826	70

Table 3.2 Continued

Parameter	All samples (n=77)		Growing pigs (n=34)				Gestating sows (n=15)				Lactating sows (n=14)				Nursery piglets (n=14)			
	Mean	CV	Mean	Min	Max	CV	Mean	Min	Max	CV	Mean	Min	Max	CV	Mean	Min	Max	CV
Ni (g m^{-3})	0.433	98	0.474	0.047	1.46	77	0.558	0.015	2.69	126	0.303	0.051	0.732	71	0.331	0.031	1.03	90
Pb (g m^{-3})	0.128	112	0.096	0.007	0.335	79	0.115	0.002	0.316	92	0.181	0.009	0.691	105	0.167	0.017	0.893	134
Zn (g m^{-3})	109.9	148	74.33a	3.98	442.8	123	45.92a	1.71	124.9	86	57.9a	5.12	189.4	101	316.8b	35.5	907.1	84
Other characteristics																		
C (Kg m^{-3})	20.2	81	24.5	2.52	77	75	21.06	1.35	56.9	92	13.8	2.45	28.1	66	15.4	2.03	43.1	70
TAN (kg m^{-3})	3.07	65	3.94a	0.673	10.3	59	2.87ab	1.29	4.68	37	1.94c	0.878	3.38	36	2.32b	0.325	7.58	80
Organic N (kg m^{-3})	1.67	88	2.03	0.176	7.87	87	1.62	0.141	5.1	92	1.22	0.213	2.4	58	1.32	0.335	4.44	80
VFA (mol m^{-3})	75.4	106	105.8a	0.867	329.7	88	37.4b	4.21	84.5	88	30.9b	1.589	107.5	102	72.7a	9.77	250	107
Acetic (mol m^{-3})	47.2	112	66.55a	0.618	238.4	94	28.8b	3.65	65.6	80	17.7b	1.45	31.7	64	40.3ab	1.83	165.2	135
Propionic (mol m^{-3})	14.9	101	19.87a	0.114	69.4	82	5.85b	0.231	14	105	6.95c	0.057	36.9	161	18.4a	2.06	40	80
Butyric (mol m^{-3})	6.49	165	10.36a	0.014	47.4	128	0.750b	0.024	3.37	146	2.30b	0.028	14.9	205	5.47ab	0.042	23.8	148
Ratio: A/P	6.42	118	5.4	0.921	45.2	151	8.45	3.59	16.2	57	10.1	0.758	25.7	93	3.28	0.131	11.8	113
Ratio: A/B	53.7	135	28.8a	1.85	118.5	122	121.1b	15.6	325.9	89	75.5b	1.87	293.7	114	32.0a	2.92	142.9	141
Ratio B/P	0.353	96	0.474	0.01	1.28	84	0.153	0.023	0.663	125	0.274	0.02	0.511	75	0.284	0.003	0.645	92
Ratio C/N	4.05	54	4.04	1.24	11.4	55	4.17	0.733	10.5	69	3.81	1.15	6.6	44	4.18	1.81	7.33	43
Ratio N/P	7.82	62	8.65	2.55	26.1	49	7.52	1.41	30.5	97	7.13	2.58	20.2	65	6.79	3.04	12.6	42
Ratio N/K	3.15	37	3.18	1.25	5.67	31	3.23	1.61	6.2	39	3.34	1.75	6.59	41	2.81	1.65	6.53	44
Potential gas emission																		
Emission NH_3 (g m^{-2} d^{-1})	10	4.8	11.6a	3.53	22.7	42	9.37a	5.51	20	41	7.29b	3.32	12.8	35	8.43a	1.47	14.8	52
Emission CH_4 (m^3 m^{-3})	9.73	118	14.4a	0.421	51	98	4.09b	0.224	10.8	103	4.45b	0.223	10.9	101	7.33a	0.822	25.1	107

EC, electrical conductivity; DM, dry matter; OM, organic matter; Ratio: A/P, ratio acetic/propionic; Ratio: A/B, ratio acetic/butyric; Ratio: B/P, ratio butyric/propionic. Means within the same row followed by different letters were significantly different whit ANOVA, test for comparing the animal type (Growing pigs, Gestating sows, Lactating sows and Nursery piglets).

Finally, the content in VFA in the slurry was higher for growing animals than for sows ($p = 0.019$). These results are coherent with those obtained by Conn et al. (2007), who found higher nutrient and VFA contents for finishing animals and attributed these differences to the dilution of slurry. Storage time and temperature have also been reported to reduce the VFA content (Moset et al., 2012; Popovic and Jensen, 2012), while a dependency between animal nutrition and the VFA content of pig slurry was reported Aarnink and Verstegen (2007).

3.3.2 Gaseous emissions

The measured NH_3 emission of slurry was on average 10 g m^{-2} d^{-1}, ranging from 1.47 to 22.7 g m^{-2} d^{-1} (Table 3.2). The emissions were higher for slurries from growing pigs (11.6 g m^{-2} d^{-1}), gestating sows (9.37 g m^{-2} d^{-1}) and nursery piglets (8.43 g m^{-2} d^{-1}), compared to lactating sows (7.29 g m^{-2} d^{-1}) ($p = 0.05$). These results are apparently associated with the amount of TKN ($r = 0.51$) and TAN ($r = 0.65$) in slurries, which in turn is affected by the level of protein used in the diet (Amanda Beccaccia et al., 2015). As mentioned before, the TKN present in slurries is mainly in form of TAN (65% in our slurry), which is the main source of NH_3 emission to the atmosphere. The content of TAN in pig slurries from growing pigs and gestating sows (3.94 and 2.87 kg m^3, respectively) were higher than for lactating sows and nursery piglets (1.94 and 2.32 kg m^3, respectively), which would indicate a greater availability for volatilization of NH_3 in slurry from growing pigs and gestating sows. A positive correlation was also found between NH_3 emissions and EC, but not with slurry pH (Table 3.2 and Figure 3.1). This finding is relevant because pH has been mentioned as an essential factor affecting NH_3 emissions (Snoek et al., 2014), but the results of this study do not reflect a direct effect of pH in a univariate analysis. Considering the variable nature of the samples from commercial farms used in this study, this suggests that the effect of pH may be partially confounded with the effect of TAN. Therefore, the relevance of these factors under commercial management should be further explored.

The BMP showed a wide variation, from 0.22 to 51 m^3 of CH_4 per m^3 of slurry (Table 3.2). The emissions of slurries from growing pigs and nursery piglets had a significantly higher BMP (14.4 and 7.33 $m^3$of CH_4 per m^3 of slurry respectively) than those from gestating and

lactating sows (4.09 and 4.45 $m^3$of CH_4 per m^3 of slurry respectively). As indicated by Gopalan et al. (2013) and Beccaccia et al. (2015), changes in management practices and nutrition may be responsible for these differences between animal categories. The BMP was positively related with EC (r = 0.798; Figure 3.1 and Table 3.3) as well as acetic, propionic, butyric acids (Table 3.3; r = 0.737, 0.561 and 0.581, respectively). As indicated by Adekunle et al. (2015), these VFA are precursors of methanogenic activity and therefore may be potential indicators of BMP. Although accumulation of VFA may inhibit methanogenesis (Weiland 2010), in the conditions reported in this study, it seems that no inhibition was produced. The VFA content showed similar differences among animal categories as the BMP, and was higher for growing animals than for sows (Table 3.2). As indicated by Beccaccia et al. (2015), different animal categories are fed with different contents and types of fibers, which can influence the VFA contents in slurry.

The relationships described above can be used to predict potential emissions from slurry characteristics, as suggested by Yagüe et al. (2012) for other slurry physicochemical characteristics. Prediction equations for emission CH_4 and NH_3 were obtained, from easily measurable characteristics such as EC, pH and DM (Table 3.4). It can be observed that equations for predicting CH_4 emissions had higher coefficients of determination than those for NH_3 emissions (R^2 from 0.64 to 0.76 for CH_4 and from 0.30 to 0.51 for NH_3). However, the prediction equations improved when the models incorporated additional characteristics of the slurry (TKN, OM and TAN). Coefficients of determination including these variables reached 0.9 and 0.8 for CH_4 and NH_3, respectively. Other authors such as (Triolo et al., 2011) proposed prediction equations of BMP based on the content of different fiber fractions. However, the equations presented here can be of highest interest when estimating or comparing potential emissions per volume of slurry at farm level because easily measurable variables have been considered in the prediction models.

Table 3.3. Correlation matrix between the main pig slurry characteristics

Slurry characteristic	pH	EC	DM	Ash	TKN	TAN	OM	VFA
EC	-0.069							
DM	-0.068	0.726						
Ash	0.585	-0.362	-0.557					
TKN	-0.176	0.816	0.867	-0.509				
TAN	-0.134	0.888	0.801	-0.467	0.96			
OM	-0.097	0.729	0.996	-0.595	0.872	0.816		
VFA	-0.523	0.72	0.489	-0.544	0.568	0.656	0.516	
Acetic	-0.409	0.774	0.545	-0.507	0.599	0.698	0.569	0.957
Propionic	-0.444	0.581	0.324	-0.448	0.447	0.509	0.34	0.846
Butyric	-0.677	0.461	0.341	-0.518	0.404	0.459	0.376	0.846
P	0.375	-0.289	-0.085	0.271	-0.216	-0.31	-0.131	-0.504
K	0.243	-0.205	-0.554	0.659	-0.342	-0.237	-0.552	-0.182
Ca	0.452	-0.185	-0.078	0.49	-0.161	-0.251	-0.127	-0.485
C	-0.577	0.416	0.404	-0.75	0.475	0.451	0.426	0.606
ratio C/N	0.43	-0.238	-0.014	0.424	-0.135	-0.177	-0.05	-0.434
Emission NH_3	0.176	0.543	0.312	-0.148	0.513	0.652	0.327	0.362
BMP	-0.316	0.798	0.805	-0.587	0.896	0.903	0.836	0.731

Table 3.3. Continued

Slurry characteristic	Acetic	Propionic	Butyric	P	K	Ca	C	ratio C/N	Emission NH_3
Propionic	0.708								
Butyric	0.741	0.637							
P	-0.438	-0.456	-0.503						
K	-0.187	-0.073	-0.204	-0.025					
Ca	-0.42	-0.499	-0.464	0.6	-0.08				
C	0.541	0.615	0.484	-0.408	-0.37	-0.47			
ratio C/N	-0.375	-0.445	-0.373	0.383	0.156	0.481	-0.54		
Emission NH_3	0.42	0.295	0.162	-0.189	0.124	-0.22	0.137	0.045	
BMP	0.737	0.561	0.581	-0.189	0.124	-0.22	0.137	-0.224	0.612

EC, Electrical conductivity; DM, dry matter; TKN, total Kjeldahl nitrogen; TAN, total ammonia nitrogen; OM, organic matter; VFA, volatile fatty acids; P, phosphorus; K, potassium; Ca, calcium; C, carbon; NH_3, ammonia; E_NH_3, ammonia emission; BMP, biochemical methane potential

Table 3.4. Prediction equations for BMP (L L^{-1}) and NH_3 potential emissions (g m^{-2} d^{-1}) from pig slurry. (n=77)

No.	Equations	R^2	RSD
1	CH_4 emission = -6.29(±0.680) + 0.975(±0.070) EC	0.637	4.6
2	CH_4 emission = 56.18(±13.13) + 0.684(±0.062) EC - 8.17(±1.74) pH	0.658	4.3
3	CH_4 emission = 51.61(±11.17) - 7.51(±1.48) EC + 0.382(±0.076)pH + 1.24(±0.23) DM	0.757	3.5
4	CH_4 emission = 23.79(±7.93) + 0.19(±0.060) EC - 3.54(±1.06) pH - 10.01(±1.40) DM + 1.42(±0.252) TKN + 13.84 (±1.79) OM	0.896	2.4
5	NH_3 emission = 5.17(± 0.919) + 0.217(± 0.037) EC	0.296	30.6
6	NH_3 emission = -12.74(± 7.69) + 0.223(± 0.036) EC + 2.78(± 1.02) pH	0.431	26.9
7	NH_3 emission = -18.48(± 7.12) + 0.172(± 0.056) EC + 2.99(± 0.939) pH – 0.603(± 0.162) DM	0.509	24.6
8	NH_3 emission = -14.67(±4.05) + 2.47(±0.529) pH – 0.623(±0.085) DM + 27.12(±1.81) TAN	0.797	14.4

RSD: residual standard deviation; EC: electric conductivity mS cm-1; DM: dry matter, in percentage per L of slurry; OM: organic matter, in percentage per L of slurry; TKN: total Kjeldahl nitrogen, in percentage per L of slurry; TAN: total ammonia nitrogen, in percentage per L of slurry

3.3.3 Potential fertilizer use

Considering the maximum permissible application rate of livestock manure to land specified in European regulations (170 kg N ha^{-1} $year^{-1}$), Table 3.5 shows nutrient inputs per hectare for land application using the slurries analyzed in this study. The volume of slurry necessary to achieve a supply of 170 kg N ha^{-1} was on average about 52 m^3 ha^{-1}, but was highly variable among farms (from 11 to 236 m^3 ha^{-1}) because of the variation in the concentration of N in the slurries. The corresponding application of OM ranged from 259 to 3520 kg ha^{-1}. As evidenced by previous studies (Fangueiro et al. 2012b; Hernández et al. 2007), the input of OM to soil through pig slurry is relatively low compared with other manure categories due to the relatively small content of organic carbon and the labile nature of the organic compounds. Therefore, according to these authors, the application of pig slurry does not significantly contribute to soil OM but it has been suggested that in the short term it can induce a reactivation of soil microbial activity.

The amount of mineral N incorporated as TAN also varied considerably among animal categories, with different implications. As indicated by Fangueiro et al. (2012a) and evidenced in this study, pig slurry contributes high proportions of TAN, and therefore it may be a valuable fertilizer for supplying N to plants. However, land application of livestock manures is associated to pollution risks related to NH_3 emissions (Krupa, 2003) and N leaching (Mantovi et al., 2006). As mentioned before, due to the high TAN content found in commercial slurries, there is a high potential of NH_3 emission to the atmosphere. For this reason, abatement techniques during land application of pig slurry (e.g. surface application or injection) are of highest relevance (Webb et al., 2005).

The application of P and K varied by a factor 22 and 4, respectively among slurries. The variation in P load was particularly relevant for gestating sows (from 5.6 to 120.6 kg ha^{-1}). Agriculture uses about 80% of global P flows, and an efficient use of this nutrient is essential due to its non-renewable nature and the potential environmental impacts (Schröder et al., 2010). As described by Schoumans et al. (2014) and Dourmad and Jondreville (2007) adopting feeding strategies (use of highly digestible mineral P supplements, use of phytase and phase feeding) may considerably change the absorption and excretion balance of the

animals. Therefore, different implementation of these techniques in commercial farms may be also contributing to the wide variation in the P content of pig slurry.

As indicated before, the concentration of macro nutrients expressed on a wet basis was higher for growing pigs and gestating sows than for nursery piglets and lactating sows. Furthermore, the ratio of major nutrients N:P:K in manure varied between animal categories (growing pigs 1:0.13:0.34; gestating sows 1:0.30:0.34; lactating sows 1:0.20:0.31; and nursery piglets 1:0.16:0.38). Variable ratios of these nutrients in pig slurry have been reported in the literature, particularly those corresponding to N:P ratios. Whereas some authors (Martínez-Suller et al. 2008; Parera i Pous et al. 2010) found relatively high contents of P in pig slurries (N:P ratios 1:0.51 and 1:0.59, respectively), other authors (Abubaker et al. 2015; 31 Sánchez et al. 2005) detected slurries with relatively less P compared to N (N:P ratios 1:0.26, in both cases). In our study, the average N:P ratio was 1:0.18, which means relatively lower P compared to N than the ratios found in the literature for pig slurries. Differences in N:P ratios among studies could be attributed to factors affecting the efficiency in the use of P by the animals (e.g. nutritional factors), but could be also influenced by different slurry storage and sampling strategies among studies. The N:K ratios in the literature vary from 1:0.3 to 1:0.9 (Sánchez et al. 2005; Martínez-Suller et al. 2008), which is consistent with the average ratio found in our study (1:0.3).

These ratios, however, do not necessarily correspond to the crop needs. For example, using the information provided by Van Duivenbooden et al. (1996) for cereals, it can be calculated that the required N:P:K ratio of fertilizer required is 1:0.39:1.34. According to our results, the average slurry would provide insufficient amounts of P and K. Although some particular samples were equilibrated in terms of N and P, most samples (particularly those from growing pigs) would be short of P. In all cases, for cereal production the K content of slurry would be insufficient to cover crop requirements. In this case, the lack of other nutrients in the slurry could be easily complemented with mineral fertilizers to obtain the optimal fertilization ratio. However, in practice fertilization ratios differ widely among crops, cropping systems and soil conditions (Penha et al. 2015) and as discussed here slurry composition is also very variable. Therefore, slurry characterization is required to avoid

oversupply of nutrients and the corresponding environmental consequences, as well as to optimize the efficiency in the use of nutrients.

As regards heavy metals, loading rates were in accordance to those obtained by (Nicholson et al., 1999). Our study also evidences that the variability found in heavy metal concentrations involves variable loading rates of these elements when slurry is applied to soils. Therefore, although evident effects of heavy metals from organic substrates on crop production may not be noticeable (Diacono and Montemurro 2010), it is likely that heavy metal accumulates in areas where pig slurry is continuously applied for years (Nicholson et al., 1999). Regarding the potential effects of heavy metal accumulation, it must be considered that they also depend on their mobility. In fact, long term studies have demonstrated the accumulation of Cu, Zn and Pb in soils (Diacono and Montemurro, 2010), but this did not affect soil productivity. According to these authors, soil properties such as cation exchange capacity, the presence of humic substances or the water and thermic regime are key factors affecting the mobility and thus the effects of heavy metals. The analysis of these properties is however beyond the scope of this study.

Table 3.5. Nutrient inputs per hectare to the soil by an application of pig slurry equivalent to 170 kg N ha^{-1}

Amount of components	All sample (n:77)		Growing pigs (n:34)			Gestating sows (n:15)			Lactating sows (n:14)			Nursery piglets (n:14)		
	Mean	CV	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Volumen (m^3)	51.9	72.2	40.0	11.3	119	50.9	18.7	105	58.1	28.5	101	75.8	14.6	236
Dry matter (Mg)	1.71	51.8	1.64	0.586	4.49	1.89	0.453	4.46	1.69	0.680	2.75	1.72	0.935	2.91
Organic matter (Mg)	1.26	56.1	1.23	0.362	3.47	1.38	0.259	3.52	1.21	0.387	2.13	1.24	0.522	2.23
Ash (Mg)	0.454	44.3	0.408	0.218	1.13	0.517	0.196	0.942	0.473	0.294	0.722	0.483	0.363	0.677
N (kg)	170	0.0	170	170	170	170	170	170	170	170	170	170	170	170
TAN (Kg)	112	22.7	114	54	152	119	75	157	107	69	152	106	72	136
Organic N (kg)	56.9	39.8	55.3	17.4	116	54.1	13.0	103	58.4	17.0	80.7	62.2	32.6	97.2
P (kg)	30.4	66.0	24.7	6.50	66.7	42.1	5.60	121	32.3	8.40	65.9	29.8	13.50	55.8
K (kg)	61.3	36.5	59.9	30.0	136	60.3	27.4	105	58.7	25.8	97.1	68.6	26.0	103
Ca (kg)	43.2	67.2	33.6	7.90	95.4	62.2	10.6	170	47.6	15.9	84.5	41.9	17.5	74.7
Mg (kg)	20.4	67.2	18.8	2.90	67.9	23.9	3.20	69.9	21.8	4.40	45.2	19.0	6.10	36.6
S (kg)	10.9	48.8	9.64	3.10	24.70	10.57	4.00	20.1	10.2	6.00	17.9	15.2	6.00	25.6
Al (kg)	1.62	84.1	1.32	0.300	5.80	1.54	0.100	3.40	2.49	0.400	6.00	1.56	0.200	4.00
B (g)	62.3	86.5	52.9	0.000	200	53.3	0.000	100	64.3	0.000	100	92.9	0.000	100
Cd (g)	0.492	80.4	0.326	0.100	1.00	0.480	0.000	1.10	0.571	0.100	1.40	0.829	0.100	1.80
Co (g)	27.6	345.7	13.9	0.500	147	45.1	1.70	560	59.0	1.50	622	10.7	2.30	39.1

Table 3.5. continued

Amount of components	All sample (n:77)		Growing pigs (n:34)			Gestating sows (n:15)			Lactating sows (n:14)			Nursery piglets (n:14)		
	Mean	CV	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Cr (g)	20.9	96.4	19.1	2.90	106	27.3	1.70	115	19.7	3.00	47.3	19.5	5.10	42.4
Cu (g)	509	132.3	299	79	1518	209	21.8	464	238	47.2	562	1611	148.6	3388
Fe (kg)	3.14	90.0	2.33	0.595	9.57	2.86	0.270	6.32	4.64	0.512	15.2	3.90	0.666	13.7
Li (g)	6.00	126.0	5.20	1.20	38.8	6.13	1.00	23.8	7.98	2.00	30.7	5.84	2.70	22.5
Mn (kg)	0.795	70.4	0.736	0.165	2.34	0.912	0.110	3.24	0.706	0.168	1.43	0.901	0.267	2.71
Mo (g)	11.2	72.2	10.4	2.70	35.7	8.7	0.80	23.7	11.9	2.00	32.4	15.0	4.80	38.4
Na (kg)	15.4	36.5	14.7	5.66	26.9	15.3	6.40	25.4	16.2	4.87	24.8	16.7	5.56	27.7
Ni (g)	15.1	78.7	14.4	4.90	52.2	18.0	1.40	70.8	14.2	4.10	24.9	14.7	6.70	36.7
Pb (g)	5.27	131.6	3.11	0.600	13.6	4.11	0.200	14.3	9.50	0.700	49.0	7.52	2.10	30.4
Zn (kg)	4.58	151.6	2.09	0.267	13.0	1.59	0.158	3.3	2.54	0.409	7.0	15.85	1.43	31.9
C (kg)	688	53.9	687	211	1940	709	125	1776	648	195	1122	711	307	1245

N, nitrogen; TAN, total ammonia nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; S, sulfur; Al, aluminum; B, boron; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; Fe, iron; Li, lithium; Mn, manganese; Mo, molybdenum; Na, sodium; Ni, nickel; Pb, lead; Zn, zinc and C, carbón.

3.4 Conclusions

The composition of pig slurries from commercial farms has a wide range of variation in the main physicochemical characteristics. Some of these characteristics (pH, N, TAN, K, Ca, Cu, Na, Zn and VFA) were significantly affected by the animal category.

Emissions of NH_3 and CH_4 from pig slurries of commercial farms were highly related with the physicochemical composition, and were significantly affected by the animal categories. Potential emissions could be predicted using easily measurable slurry characteristics.

The direct application of pig slurries to cropland require characterization of the slurry, due to the wide variation found in its composition. Supplementation with nutrients or slurry treatment techniques may be necessary depending on slurry composition and crop needs.

3.5 References

Aarnink, A.J.A., Verstegen, M.W.A., 2007. Nutrition, Key Factor to Reduce Environmental Load from Pig Production. Livest. Sci. 109(1-3):194-203.

Abubaker, J. Risberg, K. Jönsson, E. Dahlin, A.S., Cederlund, H., Pell M., 2015. Short-Term Effects of Biogas Digestates and Pig Slurry Application on Soil Microbial Activity. Appl. Environ. Soil Sci. Article ID 658542: 1-15.

Adekunle, K.F., Okolie, J.A., 2015. A Review of Biochemical Process of Anaerobic Digestion. Adv. Biosci. Biotechnol. 6:205-212.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, J., Kalyuzhnyi, S., Jenicek, P., Van Lier, J.B., 2009. Defining the Biomethane Potential (BMP) of Solid Organic Wastes and Energy Crops: A Proposed Protocol for Batch Assays. Water Sci. Technol. 59(5):927–34.

Antezana, W., Calvet, S., Beccaccia, A., Ferrer, P., De Blas, C., García-Rebollar, P., Cerisuelo, A., 2015. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing pigs: III. Influence of varying the dietary level of calcium soap of palm fatty acids distillate with or without orange pulp supplementation. Anim. Feed Sci. Tech. 209:128-136.

APHA. 2005. Standard Methods for the Examination of Water and Wastewater. Centennial. Baltimore, Maryland.

Bai, Z.H., Qin, W., Chen, Q., Oenema, O., Zhang, F.S., 2014. Changes in Pig Production in China and Their Effects on Nitrogen and Phosphorus Use and Losses. Environ. Sci. Technol. 48:12742−12749.

Beccaccia, A., Ferrer, P., Ibáñez, M.A., Estellés, F., Rodríguez, C., Moset, V., De Blas, C., Calvet, S., García-rebollar, P., 2015. Relationships among Slurry Characteristics and Gaseous Emissions at Different Types of Commercial Spanish Pig Farms. Spanish J. Agr. Res. 13(1):1–15.

Conn, K.L., 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(2):440–447.

Diacono, M., Montemurro, F., 2010. Long-Term Effects of Organic Amendments on Soil Fertility . A Review. Agron. Sustain. Dev. 30:401–22.

Díez, J.A., Hernaiz, P., Muñoz, M.J., Torre, A., Vallejo, A., 2006. Impact of Pig Slurry on Soil Properties, Water Salinization, Nitrate Leaching and Crop Yield in a Four-Year Experiment in Central Spain. Soil Use Manage 20(4):444–50.

Dourmad, J-Y. Jondreville, C., 2007. Impact of Nutrition on Nitrogen, Phosphorus, Cu and Zn in Pig Manure, and on Emissions of Ammonia and Odours. Livest. Sci. 112(3):192–98.

EUROSTAT, 2015. Pig Farming Sector - Statistical Portrait .Pig Farming in the European Union: Considerable Variations from One Member State to Another. Statistics in Focus 15/2014. Author: Pol Marquer, Teresa Rabade, Roberta Forti ISSN:2314-9647 Catalogue number:KS-SF-14-015-EN-N.

Fangueiro, D., Lopes, C., Surgy, S., Vasconcelos, E., 2012a. Effect of the Pig Slurry Separation Techniques on the Characteristics and Potential Availability of N to Plants in the Resulting Liquid and Solid Fractions. Biosyst. Eng. 113(2):187–94.

Fangueiro, D., Ribeiro, H., Vasconcelos, E., Coutinho, J., Cabral, F., 2012b. Influence of Animal Slurries Composition and Relative Particle Size Fractions on the C and N Mineralization Following Soil Incorporation. Biomass and Bioenerg. 47:50-51.

Ferrer, P., Cambra-López, M., Cerisuelo, A., Peñaranda, D., Moset, V., 2014. The Use of Agricultural Substrates to Improve Methane Yield in Anaerobic Co-Digestion with Pig Slurry: Effect of Substrate Type and Inclusion Level. Waste Manag. 34:196-203

Galassi, G., Colombini, S., Malagutti, L., Crovetto, G.M., 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. Tech. 161:3-4.

Gopalan, P., Jensen, P.D., Batstone, D.J., 2013. Anaerobic Digestion of Swine Effluent: Impact of Production Stages. Biomass Bioenerg. 48:121-129.

Hernández, D., Fernández, J.M., Plaza, C., Polo, A., 2007. Water-Soluble Organic Matter and Biological Activity of a Degraded Soil Amended with Pig Slurry. Sci. Total Environ. 378:101-103.

Hernández, D., Polo, A., Plaza, C., 2013. Long-Term Effects of Pig Slurry on Barley Yield and N Use Efficiency under Semiarid Mediterranean Conditions. Eur. J. Agron. 44:47-86.

Hristov, A.N., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz, A., Dell, C., Adesogan, A., Yang, W., Tricarico, J., Kebreab, E., Waghorn, G., Dijkstra, J. & Oosting, S. 2013. Mitigation of greenhouse gas emissions in livestock production - A review of technical options for non-CO2 emissions. Edited by Pierre J. Gerber, Benjamin Henderson and Harinder P.S. Makkar. FAO Animal Production and Health Paper No. 177. FAO, Rome, Italy.

Iguácel, F., Yagüe, M.R., Betrán, J., Orús, F., 2011. Ensayos de Fertilización Con Purín Porcino, En Cereales de Invierno de Secano, Dirección General de Desarrollo Rural, Centro de Transferencia Agroalimentaria, Gobierno de Aragón. Informe Técnico 226: 15.

Jarret, G., Cerisuelo, A., Peu, P., Martinez, J., Dourmad, J.Y., 2012. Impact of Pig Diets with Different Fibre Contents on the Composition of Excreta and Their Gaseous Emissions and Anaerobic Digestion. Agri. Ecosys. Environ. 160:51-58.

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

Krupa, S.V., 2003. Effects of Atmospheric Ammonia (NH3) on Terrestrial Vegetation: A Review. Environ. Pollut. 124(2):179–221.

Mantovi, P., Fumagalli, L., Beretta, G.P., Guermandi, M., 2006. Nitrate Leaching through the Unsaturated Zone Following Pig Slurry Applications. J. Hydrology 316:1-4.

Martínez-Suller, L., Azzellino, A., Provolo, G., 2008. Analysis of Livestock Slurries from Farms across Northern Italy: Relationship between Indicators and Nutrient Content. Biosys. Eng. 99(4):540–52.

Moral, R., Moreno-Caselles, J., Perez-Murcia, M.D., Perez-Espinosa, A., Rufete, B., Paredes, C., 2005a. Characterisation of the Organic Matter Pool in Manures. Bioresource Technol. 96(2):153–58.

Moral, R., Perez-Murcia, M.D., Perez-Espinosa, A., Moreno-Caselles, J., Paredes, C., 2005b. Estimation of Nutrient Values of Pig Slurries in Southeast Spain Using Easily Determined Properties. Waste Manag. 25(7):719–25.

Moral, R., Perez-Murcia, M.D., 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 Manag. 28(2):367–71.

Morazán, H., Alvarez-Rodriguez, J., Seradj, A.R., Balcells, J., Babot, D., 2015. Trade-Offs among Growth Performance, Nutrient Digestion and Carcass Traits When Feeding Low Protein and/or High Neutral-Detergent Fiber Diets to Growing-Finishing Pigs. Anim. Feed. Sci. Tech. 207:168-180.

Moset, V., Cambra-López, M., Estellés, F., Torres, A.G., Cerisuelo, A., 2012. Evolution of Chemical Composition and Gas Emissions from Aged Pig Slurry during Outdoor Storage with and without Prior. Solid Separation, Biosys. Eng. 111(1):2–10.

Ndegwa, P.M., Vaddella, V.K., Hristov, N., Joo, H.S., 2009. Measuring concentrations of ammonia in ambient air or exhaust air stream using acid traps. J. Environ. Qual. 38(2):647–653.

Nicholson, F.A., Chambers, B.J., Williams, J.R., Unwin, R.J., 1999. Heavy Metal Contents of Livestock Feeds and Animal Manures in England and Wales. Bioresource Technol. 70(1):23–31.

Olusegun, O.S., 2014. Influence of NPK 15-15-15 Fertilizer and Pig Manure on Nutrient Dynamics and Production of Cowpea, Vigna Unguiculata L. Walp. American. J. Agric. For 2(6):267.

Parera i Pous, J., Olivé, D., Mallol Nabot, C., Torrijos, N.C., 2010. Adaptación Del Uso de La Conductividad Eléctrica (CE) Para Determinar de Forma Rápida El Contenido En Nutrientes Del Purín Porcino En Catalunya. International Workshop on Anaerobic Digestion of Slaughterhouse. Waste, 67–76.

Patience, J.F., Gould, S.A., Koehler, D., Corrigan, B. Elsbernd, A., Holloway, C.L., 2015. Super-Dosed Phytase Improves Rate and Efficiency of Gain in Nursery Pigs. Animal Industry Report: AS 661.

Penha, H.G., Menezes, J.F., Silva, C.A., Lopes, G., Carvalho, C.A., Ramos, S.J., Guilherme, L.R.G., 2015. Nutrient Accumulation and Availability and Crop Yields Following Long-Term Application of Pig Slurry in a Brazilian Cerrado Soil. Nutr. Cycl. Agroecosys. 101(2):259–69.

Popovic, O., Jensen, L.S., 2012. Storage Temperature Affects Distribution of Carbon, VFA, Ammonia, Phosphorus, Copper and Zinc in Raw Pig Slurry and Its Separated Liquid Fraction. Water Res. 46(12):3849–58.

Provolo, G., Martínez-Suller, L., 2007. In Situ Determination of Slurry Nutrient Content by Electrical Conductivity. Bioresource Technol. 98(17):3235–42.

Sánchez, M., González, J.L., 2005. The Fertilizer Value of Pig Slurry. I. Values Depending on the Type of Operation. Bioresource Technol. 96(10):1117–23.

SAS Institute, 2008. SAS/STAT User´s guide, v 9.3, SAS Institute Inc., Cary, NC.

Schoumans, O.F., Chardon, W.J., Bechmann, M.E., Gascuel-Odoux, C., Hofman, G., Kronvang, B., Rubæk, H.G., Ulén, B., Dorioz, J.M., 2014. Mitigation Options to Reduce Phosphorus Losses from the Agricultural Sector and Improve Surface Water Quality: A Review. Sci. Total Environ. 468-469:1255–1266.

Schröder, J.J., Cordell, D., Smit, A.L., Rosemarin, A., 2010. Sustainable Use of Phosphorus. Plant Research International Wageningen UR. Report 357.

Scotford, I.M., Cumby, T.R., White, R.P., Carton, O.T., Lorenz, F., Hatterman, U., Provolo, G., 1998. Estimation of the Nutrient Value of Agricultural Slurries by Measurement of Physical and Chemical Properties. J. Agric. Eng. Res. 71(3):291–305.

Snoek, D.J.W., Johannes, D.S., Ogink, N.W.M., Koerkamp, P.W.G.G., 2014. Sensitivity Analysis of Mechanistic Models for Estimating Ammonia Emission from Dairy Cow Urine Puddles. Biosyst. Eng. 121:12–24.

Suresh, A., Choi, H.L., Oh, D.I., Moon, O.K., 2009. Prediction of the Nutrients Value and Biochemical Characteristics of Swine Slurry by Measurement of EC - Electrical Conductivity. Bioresource Technol. 100:4683–4689.

Thygesen, O., Triolo, J. M., Sommer, S.G., (2012) Indicators of Physical Properties and Plant Nutrient Content of Animal Slurry and Separated Slurry. Biological Engineering Transactions. 5(3): 123-135.

Triolo, J.M., Sommer, S.G., Møller, H.B., Weisbjerg, M.R., Jiang, X.Y., 2011. A New Algorithm to Characterize Biodegradability of Biomass during Anaerobic Digestion: Influence of Lignin Concentration on Methane Production Potential. Bioresource Technol. 102:9395–9402.

Van Duivenbooden, N., De Wit, C.T., Van Keulen, H., 1996. Nitrogen, Phosphorus and Potassium Relations in Five Major Cereals Reviewed in Respect to Fertilizer Recommendations Using Simulation Modelling. Fertilizer Research Wageningen 44:37-49.

Viguria, M., Sanz-Cobeña, A., López, D.M., Arriaga, H., Merino, P., 2015. Ammonia and Greenhouse Gases Emission from Impermeable Covered Storage and Land Application of Cattle Slurry to Bare Soil. Agric Ecosyst Environ 199:261–71.

Villamar, C.A., Canuta, T., Belmonte, M., Vidal, G., 2012. Characterization of Swine Wastewater by Toxicity Identification Evaluation Methodology (TIE). Water, Air, and Soil Pollut. 223(1):363–369.

Villamar, C.A., Rodríguez, D.C., López, D., Peñuela, G., Vidal, G., 2013. Effect of the Generation and Physical-Chemical Characterization of Swine and Dairy Cattle Slurries on Treatment Technologies. Waste Manage. Res. 31(8):820–828.

Villar, M.C., Petrikova, V., Díaz-Raviña, M., Carballas, T., 2004. Recycling of Organic Wastes in Burnt Soils: Combined Application of Poultry Manure and Plant Cultivation. Waste Manage. 24(4):365–70.

Webb, J., Menzi, H., Pain, B.F., Misselbrook, T.H., Dämmgen, U., Hendriks, H., Döhler, H., 2005. Managing Ammonia Emissions from Livestock Production in Europe. Environ. Pollut. 135: 399–406.

Webb, J., Broomfield, M., Jones, S., Donovan, B., 2014. Ammonia and odour emissions from UK pig farms and nitrogen leaching from outdoor pig production. Sci. Total Environ. 470:865–875.

Weiland, P., 2010. Biogas production: current state and perspectives. Appl Microbiol Biotechnol, 85: 849-860.

Yagüe, M.R., Bosch-Serra, A.D., Boixadera, J., 2012. Measurement and Estimation of the Fertiliser Value of Pig Slurry by Physicochemical Models: Usefulness and Constraints. Biosyst. Eng. 111(2):206–216.

Zhang, W., Wei, Q., Wu, S., Qi, D., Li, W., Zuo, Z., Dong, R., 2014. Batch Anaerobic Co-Digestion of Pig Manure with Dewatered Sewage Sludge under Mesophilic Conditions. Appl. Energ. 128: 175–183.

4

Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing pigs: Influence of varying the dietary level of calcium soap of palm fatty acids distillate with or without orange pulp supplementation

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Abstract. The aim of this study was to establish the relationships between faecal fat concentration and gaseous emissions from pig slurry. Five diets were designed to meet essential nutrient requirements: a control and four experimental feeds including two levels (35 or 70 g/kg) of calcium soap fatty acids distillate (CSP) and 0 or 200 g/kg of orange pulp (OP) combined in a 2x2 factorial structure. Thirty growing pigs (six per treatment) were used to measure dry matter (DM) and N balance, coefficients of total tract apparent digestibility (CTTAD) of nutrients, faecal and urine composition and potential emissions of ammonia (NH_3) and methane (CH_4). Increasing dietary CSP level decreased DM, ether extract (EE) and crude protein (CP) CTTAD (by 4.0, 11.1 and 3.5%, respectively, P <0.05), but did not influence those of fibrous constituents. It also led to a decrease (from 475 to 412 g/kg DM, P <0.001) of faecal concentration of neutral detergent fibre (aNDFom) and to an increment (from 138 to 204 g/kg, P <0.001) of EE in faecal DM that was related to greater CH_4 emissions, both per g of organic matter (P=0.021) or on daily basis (P <0.001). Level of CSP did not affect N content in faeces or urine, but increased daily DM (P <0.001), and N (P=0.031) faecal excretion with no effect on urine N excretion. This resulted in lesser (P=0.036) NH_3 potential emission per kg of slurry. Addition of OP decreased CTTAD of EE (by 7.9%, P=0.044), but increased (P <0.05) those of all the fibrous fractions. As a consequence, faecal content of EE increased (from 165 to 177 g/kg DM; P=0.012), whereas that of aNDFom decreased greatly (from 483 to 404 g/kg DM, P <0.001), which in all resulted in a lack of effect of OP on CH_4 potential emission. Inclusion of OP in the diet also led to a significant decrease of CP CTTAD (by 6.85%, P <0.001), and then to an increase of faecal CP concentration (from 174 to 226 g/kg DM, P <0.001), with no significant influence on urine N content. These effects resulted in higher N faecal losses, especially those of the undigested dietary origin, without significant effects on potential NH_3 emission. No significant interactions between CSP and OP supplementation were observed for the gaseous emissions measured.

4.1 Introduction

Intensive pig production is a major contributor to gaseous pollutant emissions. It has been estimated that in the EU it is responsible for 15 and 25% of the total ammonia (NH_3) and methane (CH_4) emissions (EAA; 2014a,b). It is widely recognized that pig slurry characteristics are heterogeneous depending on a number of factors including nutrition. Changes in slurry composition have been associated to gaseous emissions in previous research with modifications of dietary factors, as source and level of fibre (Canh et al., 1998b; Jarret et al., 2012), type of fibre (Triolo et al., 2011; Beccaccia et al., 2015a), level of protein (Canh et al., 1998a; Portejoie et al., 2004; Hernández et al., 2011) and source of protein (Beccaccia et al., 2015b).

Ether extract (EE) is by far the nutrient with the highest potential to generate CH_4 from the slurry through microbial fermentation (Angelidakis and Sanders, 2004). Beccaccia et al. (2015c) reported that EE content in slurry samples from commercial farms increased CH_4 emission potential, but reduced that of NH_3. However, little is known about the relationships among feed composition, faecal fat concentration and gaseous emissions. Fat content in faeces has two origins: indigestible dietary EE, which is mainly related to the source of fat used in the feed as recognized in several research studies (Cera et al., 1989; Wiseman et al., 1990; Kil et al., 2010) and feeding tables (INRA, 2002; CVB, 2004; FEDNA 2010), and endogenous losses that are generally associated to microbial synthesis in the gut. Previous studies (Kreuzer, 1999; Heimendahl et al., 2010) have found an increment of bacterial content in faeces when including a source of fermentable fibre in the diet, although the effects seem to be lesser when diets were compared at the same dietary neutral detergent fibre (NDF) content (Kreuzer et al., 1999; Beccaccia et al., 2015a). Otherwise, fat addition to the diet might hypothetically affect intestinal microbial activity and digestion efficiency of other dietary constituents.

The aim of the current research was to investigate changes in faecal fat concentration induced through supplementation with two industrial food by-products: calcium soap of palm fatty acids distillate and orange pulp, supplying respectively low digestible fat and fermentable fibre, and how these changes affect gaseous emissions from pig slurry.

Keywords: Ammonia emission, Calcium soap of palm fatty acids, Digestion efficiency, Growing pigs, Methane emission, Orange pulp, Slurry.

4.2 Material and methods

4.2.1 Animals, diets and experimental design

Thirty growing male pigs, progeny of Pietrain x (Landrace x Large White) were divided into three series (batches) of 10 animals each and used subsequently in this study. Average and standard deviation of body weight of pigs in batches 1, 2 and 3 at allocation in metabolism pens were 54.0 (±1.46), 61.4 (±1.44) and 72.5 (±3.16) kg, respectively. A control diet (C) was formulated using the most common ingredients used at present in commercial diets for growing-finishing pigs (wheat grain, barley grain, wheat bran and soybean meal). Another four experimental feeds were designed by substituting a mixture of wheat grain and calcium carbonate in the control diet (C) with increasing amounts (35 and 70 g/kg) of calcium soap of palm fatty acids distillate (CSP), alone or with further supplementation of 200 g/kg orange pulp (OP) at each level of fat supplementation. The proportions of the other ingredients were also slightly modified to keep essential nutrient composition of diets above the recommendations of FEDNA (2006) for growing fattening pigs. In particular, levels of essential amino acids per unit of net energy (NE) were maintained as similar as possible among the experimental feeds. The analytical composition of the sample used of orange pulp was described in a companion paper (Beccaccia et al., 2015a); the sample of CSP contained 790 g/kg of EE, 190 g/kg of ash and 90 g/kg of Ca, with an estimated NE concentration of 24.5 MJ/kg (FEDNA, 2010). The ingredient and chemical composition of the experimental diets is presented in Tables 4.1 and 4.2, respectively. As shown, all diets had similar levels of crude protein (CP) and NDF, but the inclusion of CSP was parallel to a slight increase of the dietary concentration of NE, and that of OP to an increment of soluble fibre (SF) content.

Table 4.1. Ingredient composition of the experimental diets (g/kg, as fed basis)

Ingredients	Diets[a]				
	Control	35CSP	70CSP	35CSPOP	70CSPOP
Barley grain	250	250	250	250	250
Wheat grain	468	423	379	301	260
Wheat bran	100	110	120	0	0
Cane molasses	20	20	20	20	20
Soybean meal 45	132	137	141	176	182
Orange pulp	0	0	0	200	200
CSP[a]	0	35	70	35	70
Calcium carbonate	14.5	8.11	1.75	2.36	0
Sodium chloride	1.11	1.5	1.89	1.27	1.23
Monosodium phosphate	6.18	6.25	6.31	7.05	7.18
DL-methionine	0.24	0.48	0.71	0.51	0.83
L-lysine HCL	1.96	2.42	2.88	1.67	2.06
L-threonine	0.43	0.71	0.98	0.45	0.7
L-tryptophan	0	0.09	0.19	0.07	0.17
L-valine	0	0.36	0.72	0.12	0.48
Premix[b]	5	5	5	5	5

[a] CSP = calcium soap of palm fatty acid distillate; OP = orange pulp.

[b] Vitamin and mineral premix supplied per kg complete diet: 5000 IU of vitamin A; 1000 IU of vitamin D3; 3 mg of vitamin B2; 20 mg of vitamin B12; 10 mg of niacin; 4 mg of pantothenic acid; 48 mg of betaine; 30 mg of manganese oxide; 110 mg of zinc oxide; 10 mg of copper sulphate; 0.75 mg of potassium iodide; 0.1 mg sodium selenite; 90 mg of iron carbonate.

Table 4.2. Chemical composition of the experimental diets (g/kg, as fed basis)

	Diets[a]				
	Control	35CSP	70CSP	35CSPOP	70CSPOP
Dry matter	905	905	909	899	889
Ash	45	46.4	47.8	49.4	52.1
Crude protein	146	145	147	146	144
NDICP[b]	16.1	15.8	21	20.9	26.3
Ether extract	31.2	51.5	83.6	54.6	75.5
Soluble fibre[c]	28.4	44	37.7	95.6	106
aNDFom	167	157	163	169	166
ADFom	50.8	44.7	48	60.3	59.7
ADL	11	8.2	9.4	7.9	8.1
Calcium[d]	6.6	7.3	8	8	10.2
Digestible phosphorous[d]	2.5	2.5	2.5	2.5	2.5
Sodium[d]	1.7	1.9	2	2	2
Chlorine[d]	2.1	2.4	2.7	2.1	2.1
Gross energy (MJ/kg)	16.3	16.8	17.7	16.7	17.2
Net energy (MJ/kg)[d]	9.2	9.75	10.3	9.41	9.91
Ileal digestible amino acids[d]					
Lysine	7.1	7.5	7.9	7.2	7.6
Methionine	2.2	2.4	2.6	2.3	2.6
Total sulphur	4.6	4.7	4.9	4.5	4.7
Threonine	4.6	4.8	5.1	4.7	4.9
Tryptophan	1.5	1.6	1.7	1.6	1.7
Isoleucine	4.9	4.9	4.9	5	5
Valine	5.57	6.1	6.4	5.8	6.2

[a] CSP = calcium soap of palm fatty acid distillate; OP =orange pulp. [b] Neutral detergent insoluble crude protein. [c] Calculated as total dietary fibre minus aNDFom corrected for NDICP. [d] Values calculated according to FEDNA (2010).

4.2.2 Experimental procedures, sample preparation, chemical analyses and emissions measurements

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). At the beginning of each trial, pigs were blocked according to live weight, assigned to one of five dietary treatments and placed in conventional pens until Day 9 of the adaptation period. After this period, animals were individually housed in metabolism pens (1.2 × 2 m^2) until the end of the experiment. 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). Feed and water were provided ad

libitum during the adaptation and collecting periods. Feed was provided in dry form (pelleted). 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 per pen until Day 9 of the experimental period and individually until the end of the experiment. Water consumption was measured individually during the collection period. Water was supplied by individual tanks and water spillage was collected and considered to calculate real water consumption.

4.2.3 Nutrient balance trial and sample preparation

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 H_2SO_4 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.

4.2.4 Feed and effluents' chemical analysis

Feeds and faeces from the nutrient balance period were analyzed for dry matter (DM), ash, total dietary fibre (TDF), aNDFom, ADFom and ADL, EE, gross energy (GE), nitrogen (N) and neutral detergent insoluble crude protein (NDICP) concentration. Additionally, faecal N fractionation into undigested and soluble N was determined. Dry matter (930.15), ash (923.03), and TDF (985.29) contents of feeds and faeces were carried out according to AOAC (2000) procedures. Concentration of aNDFom, ADFom and ADL were determined sequentially by using the filter bag system (Ankom Technology Corp., Macedon, NY, USA) according to Mertens (2002), AOAC (2000; procedure 973.187) and Van Soest et al.

(1991), using heat stable amylase (A3306, Sigma–Aldrich, Tres Cantos, Spain), and expressed without residual ash. The contents in soluble fibre were estimated from difference between TDF and aNDFom corrected by CP content in the residue. The contents in hemi- celluloses and cellulose were estimated, respectively, from the differences between aNDFom and ADFom and ADFom–ADL concentrations. Feed and faeces were defatted with petroleum ether prior to fibre analysis. Ether extract content was determined by AOAC methods (920.39). Gross energy concentration was measured in an isoperibol bomb calorimeter (Parr 1356, Parr Instruments Co., Moline, IL, USA). Total N was measured by combustion (method 986.06; AOAC, 2000) using a Leco equipment (model FP-528, Leco Corporation, St. Joseph, MI, USA) and crude protein (CP) estimated as N content × 6.25. The proportion of NDICP in feed and faeces samples was determined following the standardized procedures of Licitra et al. (1996), by analysing the N content (combustion method) in the NDF residues. Nitrogen fractionation of faecal samples was carried out according to the procedure suggested by Kreuzer et al. (1989), by steam distillation (APHA, 2005) using an automatic analyser (2300 Kjeltec, Foss Analytical, Hilleroed, Denmark). In this procedure, total faecal N content is separated after centrifugation between soluble (WSN) and sedimented N, and the bacterial and endogenous debris (BEDN) calculated from the difference between the N sedimented and the undigested N (UDN) estimated from NDICP values. Urine was analyzed for DM, N and GE content. The urine was freeze-dried to obtain its DM content and mixed with benzoic acid before GE analysis to make sure that all sample was burned. Total N was determined by steam distillation (APHA, 2005) using an automatic analyser (2300 Kjeltec, Foss Analytical, Hilleroed, Denmark). Immediately after reconstitution, slurry pH was measured by duplicate with a glass electrode (Crison Basic 20+, Crison, Barcelona, Spain) and samples were taken to analyze DM, ash, total ammonia N (TAN), total Kjeldahl N (TKN) and VFA con- centration. The DM and ash content was analyzed using the same equipment and following the same methodology than that used in faeces analyses. 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).

4.2.5 Gaseous emissions monitoring

Ammonia emission was measured from fresh samples of reconstituted slurry over 11 days using an ammonia trap system similar to that described by Ndegwa et al. (2009). 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 NH3 trapped in the impingers was analyzed following 4500 NH3-D procedure (APHA, 2005) using a detection electrode (Orion High Performance NH3 Electrode, model 9512HPBNWP, Thermo Scientific, USA). The cumulative NH3 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 collected from an anaerobic digester of the Universitat Politècnica de València that treated pig slurry, and 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.

4.2.6 Statistical analysis

Animal was the experimental unit for all the traits studied. The whole data set derived from the five dietary treatments was analyzed in a one factor analysis of variance as a completely randomized design with trial series, type of diet and its interaction as main effects by using PROC GLM of SAS (2008). The effects of diet were analysed as a factorial arrangement by using orthogonal contrasts with level of CSP (35 or 70 g/kg) and inclusion of OP (0 or 200 g/kg) and its interaction as main effects. Contrasts of each of the experimental treatments against the control diet were done by using a Dunnett test. Specific contrasts among means were done when needed. Cumulated CH_4 evolution was analysed by a repeated measures model using PROC MIXED of SAS (2008). Sources of variation included treatment, time, and the treatment × time interaction. The random variable was pig within treatment. Variables were analyzed subjected to 3 covariance structures: compound symmetry, compound symmetry heterogeneous and autoregressive order 1. Using the largest Akaike information criterion and Schwarz Bayesian criterion, the compound symmetry was the structure that fitted the model best.

4.3 Results

4.3.1 Coefficient of total tract apparent digestibility (CTTAD)

Trial series had little influence on any of the traits studied, so that the influence of this factor was excluded from the model. Results in Table 4.3 show that EE digestibility decreased (by 11.1%, P=0.006) when dietary level of CSP increased from 35 to 70 g/kg. This effect was parallel to a decrease of DM, OM and gross energy (GE) digestibility (by respectively 3.89, 3.76 and 3.98%, P <0.001). Level of CSP also decreased digestion

efficiency of CP (by 3.48%, P=0.026), but did not affect those of SF, hemicelluloses (HEM) and cellulose (CEL). The inclusion of 200 g/kg of OP in the experimental feed also led to a decrease of EE digestibility (by 7.9%, P=0.044) and CP (by 6.85%, P <0.001), but did not affect those of DM, OM or GE because of a simultaneous increment of the digestion efficiency of SF, HEM and CEL (by 12.0, 8.11 and 42.3%, respectively, P <0.05). In the case of SF digestibility, a significant interaction was observed (P=0.006), as the improved efficiency observed with OP was greater at the highest level of CSP. In whole, when comparing with C diet, all the treatments increased SF CTTAD values and those of CP and EE only in the case of the diet 35CSP. Otherwise, SF and CEL digestibility improved with respect to diet C with OP addition at any level of CSP. Energy losses in urine expressed as a proportion of digestible energy were not affected by treatments and averaged 0.968.

4.3.2 Composition of effluents

The effect of treatments on the excreta composition is shown in Table 4.4. An increment of CSP level from 35 to 70 g/kg greatly increased (by 48.9%, P <0.001) faecal EE content, but decreased those of aNDFom, ADL, HEM and CEL (by15.1, 15.0, 12.0 and 14.5%, P <0.003). The degree of lignification of NDF in the faecal output was close to 0.108 at both CSP levels. Addition of 200 g/kg of OP to the diet increased (P=0.012) faecal content of EE (by 7.27%) and CP (by 29.6%, P <0.001) and decreased (P <0.001) HEM, CEL and aNDFom contents (by 19.5, 11.9 and 16.3%). A lesser effect was observed on ADL faecal concentration (P=0.057), so that the degree of lignification of NDF tended to increase with OP addition (from 0.104 to 0.113). Treatments did not affect faecal concentration of SF (that was low, averaging 53.6 g/kg DM), neither those of DM and N in urine (mean values of 60.7 g/kg and 121 g/kg DM). Instead, a trend for an interaction (P=0.066) was found on faecal pH, as its increase with OP inclusion (P=0.005) was greater at the highest level of addition of CSP. With respect to the results obtained with the control diet, faecal concentration of EE was higher in all the treatments studied, faecal CP increased in diet CSP35OP and those of aNDFom, HEM and CEL decreased both in diet CSP70 and in the two OP supplemented feeds.

Table 4.3. Effect of diets on the apparent total tract digestibility coefficients and energy balance of the experimental diets

	Diets[a]						Significance[b]		
	Control	35CSP	70CSP	35CSPOP	70CSPOP	SEM[c]	CSP	OP	CSPxOP
Dry matter	0.83	0.856	0.817	0.843	0.816	0.008	0.001	0.394	0.462
Organic matter	0.85	0.872	0.834	0.858	0.831	0.906	<0.001	0.296	0.486
Gross energy	0.822	0.833	0.8	0.827	0.794	0.008	0.001	0.474	0.992
Crude protein[d]	0.79	0.835	0.801	0.773	0.751	0.013	0.026	<0.001	0.427
Ether extract[d]	0.574	0.669	0.603	0.625	0.547	0.023	0.006	0.044	0.795
Soluble fibre[d,e,f,g]	0.68	0.846	0.792	0.905	0.929	0.012	0.25	<0.001	0.006
aNDFom[f,g]	0.518	0.562	0.551	0.646	0.618	0.026	0.477	0.011	0.755
ADFom[f,g]	0.372	0.388	0.388	0.571	0.561	0.031	0.879	<0.001	0.862
Hemicelluloses	0.563	0.613	0.593	0.667	0.62	0.028	0.14	0.039	0.636
Cellulose[f,g]	0.398	0.455	0.437	0.639	0.631	0.03	0.67	<0.001	0.871
(DE-UE)/DE[h]	0.967	0.971	0.977	0.963	0.964	0.006	0.546	0.084	0.729

[a] CSP = calcium soap of palm fatty acid distillate, OP =orange pulp; [b] CSP=effect increasing level of CSP from 35 to 70 g/kg; [c] Standard error of means (n=6); [d]Contrast control vs 30CSP (P <0.05); [e] Contrast control vs 70CSP (P <0.05); [f] Contrast control vs 35CSPOP (P <0.05); [g] Contrast control vs 70CSPOP (P <0.05); [h]Proportion of digestible energy not lost in urine; CSP = calcium soap of palm fatty acid distillate.

Table 4.4. Effects of diets on faeces and urine composition (g/kg DM)

	Diets[a]						Significance[b]		
	Control	35CSP	70CSP	35CSPOP	70CSPOP	SEM[c]	CSP	OP	CSPxOP
Faeces									
Dry matter	378	345	363	375	362	13.6	0.861	0.308	0.266
Organic matter[d,e]	836	844	857	850	844	2.77	0.2	0.244	0.053
Ether extract[d,e,f,g]	85.8	131	199	145	209	4.16	<0.001	0.012	0.659
Crude protein[e]	198	172	176	234	217	6.85	0.362	<0.001	0.156
Soluble fibre	59.2	52	47	63.5	45.6	8.48	0.469	0.731	0.686
aNDFom	521	527	439	423	386	11.5	<0.001	<0.001	0.056
ADFom[f,g]	199	210	176	183	160	5.16	<0.001	<0.001	0.301
ADL	50.1	55	45.5	48.8	42.7	2.18	0.003	0.057	0.445
Hemicelluloses[d,e,g]	322	317	263	240	227	9.08	0.002	<0.001	0.041
Cellulose[d,e,g]	149	155	130	134	117	3.95	<0.001	<0.001	0.348
Urine									
Dry matter	72.9	63.5	47.6	55.2	67.2	7.69	0.793	0.483	0.092
Total Kjeldahl N	138	131	119	110	105	9.34	0.377	0.087	0.696

[a] CSP = calcium soap of palm fatty acid distillate, OP =orange pulp; [b] CSP=effect increasing level of CSP from 35 to 70 g/kg; OP = effect of inclusion of 200g/kg orange pulp. [c]Standard error of means (n=6); [d]Contrast control vs 70CSP (P <0.05); [e]Contrast control vs 35CSPOP (P <0.05); [f]Contrast control vs 35CSP (P<0.05). [g]Contrast control vs 70CSPOP (P <0.05).

Table 4.5. Effects of the diet on daily DM and N balance and on the proportion of faecal N fractions

	Diets[a]						Significance[b]		
	Control	35CSP	70CSP	35CSPOP	70CSPOP	SEM[c]	CSP	OP	CSPxOP
Body weight, kg[d]	62.5	61.5	65	61.4	62.8	0.351	0.001	0.081	0.114
DM balance (g/kg$^{0.75}$)									
Intake	73.9	70	76.2	67.9	69.1	3.67	0.323	0.225	0.499
Faeces	12.3	10	13.8	10.7	12.8	0.644	<0.001	0.75	0.216
Urine	3.48	2.95	2.51	3.67	3.51	0.416	0.478	0.057	0.735
N balance (g/kg$^{0.75}$)									
Intake	1.9	1.79	1.98	1.77	1.79	0.095	0.305	0.278	0.419
Faeces	0.395	0.277	0.389	0.404	0.452	0.034	0.031	0.014	0.368
Urine	0.446	0.383	0.296	0.419	0.454	0.052	0.167	0.384	0.841
Retained	1.06	1.14	1.29	0.946	0.984	0.083	0.267	0.009	0.495
N fractions in faeces (g/g total faecal N)									
UDN[e,f,g]	0.087	0.072	0.062	0.197	0.202	0.025	0.982	<0.001	0.681
BEDN[e,f,h]	0.658	0.586	0.624	0.509	0.483	0.032	0.963	0.005	0.294
WSN[i]	0.255	0.342	0.314	0.293	0.315	0.019	0.926	0.266	0.199

[a]CSP = calcium soap of palm fatty acid distillate, OP =orange pulp; [b]CSP=effect of level of inclusion of CSP, OP = effect of inclusion of 200g/kg of orange pulp; [c]Standard error of means (n=6); [d]Contrast control vs 70CSP (P <0.05); [e]Contrast control vs 35CSPOP (P <0.05); [f]Contrast control vs 70CSPOP (P <0.05); [g]Undigested dietary nitrogen; [h]Bacterial and endogenous debris nitrogen; [i]Water soluble nitrogen.

4.3.3 Dry matter and nitrogen flows

The DM and N daily balances and the separation of faecal N in fractions for each of the experimental diets are presented in Table 4.5. Values were expressed per kg of metabolic weight to correct slight differences among treatments besides to the general increase of pig weight throughout the successive trial series. Treatments did not affect daily DM or N intake that averaged 71.4 and 1.85 g/kg$^{0.75}$, respectively. An increase of dietary CSP level from 35 to 70 g/kg increased in parallel faecal DM excretion (from 10.3 to 13.3 g/kg$^{0.75}$ and day, P <0.001) and N excretion in faeces (from 0.333 to 0.389, P=0.034). However, CSP level did not affect N excreted in urine or the proportions of faecal N fractions. Inclusion of OP in the experimental feeds tended (P=0.057) to increment DM excretion in urine (from 2.73 to 3.59 g/kg$^{0.75}$ and day), but not that of faecal DM. Instead, addition of OP had no significant influence on N excretion in urine, but increased that of N in faeces (from 0.333 to 0.428 g/kg$^{0.75}$ and day, P=0.014). Inclusion of OP in the diet affected the proportion of N fractions determined, as that of undigested N (UDN) increased, whereas that of bacterial and endogenous debris nitrogen (BEDN) decreased. No significant effects of treatments were observed on the proportion of water soluble nitrogen fraction. When comparisons were made against the control diet, results show that BEDN proportion on faecal N decreased in OP diets, in parallel to an increase in UDN fraction.

4.3.4 Slurry characteristics and gaseous emissions

Water intake during the collection period was not affected by treatments, averaging 3.69 ± 1.14 (SD) kg/d. Neither slurry excretion nor the initial slurry characteristics (DM, OM, total ammonia N (TAN), total Kjeldahl N (TKN) and pH did not differ significantly among the different treatments tested (Table 4.6). However, total volatile fatty acids increased (P=0.020) with CSP. This effect was parallel for acetic, propionic and butyric acid, although differences only reached significant levels in the case of acetic acid (P=0.018). Ammonia emission from slurry (g NH_3/kg) was lower in diets formulated with higher levels of CSP (P=0.036) but was not affected by OP inclusion. A trend (P=0.053) was detected for an interaction between treatments on this trait, as the effect of CSP was greater when no OP was added to diets. When slurry characteristics and the derived gaseous emission were

compared with treatment C, a lower pH of diet 70CSP was observed ($P < 0.05$); additionally, NH_3 emission per kg of slurry was lower ($P < 0.05$) in treatments 70CSP, 35CSPOP and 70 CSPOP.

An increment of dietary level of CSP increased potential CH_4 emission BMP from the slurry ($P=0.021$) and the volume of CH_4 emitted per animal and day ($P < 0.001$), but the inclusion of OP did not affect significantly none of these traits. Figure 4.1 shows the evolution of the cumulated CH_4 emission with time in the BMP assay. At day 17 of study, treatments 70CSP and 70CSPOP showed a higher cumulated CH_4 production with respect to the other treatments ($P < 0.05$). From day 24 until the end of the study, treatment 70CSPOP led to higher cumulated CH_4 values than both 35 CSP treatments, with diet 70CSP giving intermediate results. On the other hand, treatment C showed the lowest cumulated CH_4 values, although they were not different from both 35 CSP diets.

Table 4.6. Effect of diets on slurry (faeces + urine) excretion, initial characteristics and derived ammonia (NH_3) emission and biochemical methane potential (BMP).

	Diets[a]						Significance[b]		
	Control	35CSP	70CSP	35CSPOP	70CSPOP	SEM[c]	CSP	OP	CSPxOP
Slurry excretion (kg/d)	1.89	1.78	2.19	2.1	2.04	0.153	0.286	0.602	0.141
Slurry characteristics									
DM (g/kg)	167	146	166	135	144	13.1	0.279	0.203	0.686
OM (g/kg)	134	119	138	108	114	11	0.261	0.13	0.568
Total ammonial N (g/L)	3.61	3.43	2.81	3.04	3.06	0.568	0.605	0.905	0.589
Total Kjeldahl N (TKN, g/kg)	10.2	8.99	8.31	8.79	8.84	0.674	0.622	0.791	0.566
pH[d]	8.52	8.08	7.65	8.13	8.18	0.183	0.354	0.157	0.239
Total volatile fatty acids (mmol/L)	86	69.7	90.6	75.9	96.2	7.82	0.02	0.481	0.971
Acetic acid (mmol/L)	55.5	44.4	57.4	49.7	62.8	4.79	0.018	0.306	0.989
Propionic acid (mmol/L)	14.4	12.4	15.6	12	14.8	1.63	0.088	0.718	0.895
Butyric acid (mmol/L)	7.86	6.63	9.75	7.25	9.72	1.67	0.119	0.865	0.852
Gas emissions									
Ammonia emission assay									
g NH_3/ kg slurry[d, e, f, g]	2.41	2.32	1.59	1.8	1.77	0.188	0.036	0.332	0.053
g N-NH_3/ kg initial TKN[d]	193	218	162	182	166	21.3	0.144	0.504	0.399
mg NH_3/animal and day[h]	412	399	331	373	341	43	0.298	0.879	0.707
Biochemical methane potential									
BMP, mL methane/g OM[g]	305	313	357	346	406	19	0.021	0.063	0.69
L methane/animal and day[d]	77.8	68.7	109	70.6	96.9	5.89	<0.001	0.419	0.276

[a] CSP = calcium soap of palm fatty acid distillate; OP= orange pulp. [b] CSP = effect increasing level of CSP from 35 to 70 g/kg; OP=effect of inclusion of 200 g/kg orange pulp. [c] Standard error of means (n=6). [d] Cumulated (11 days). [e] Contrast Control vs 70CSP (P <0.05). [f] Contrast Control vs 35CSPOP (P <0.05). [g] Contrast Control vs 70CSPOP (P <0.05). [h] 24-h NH_3 emission from the slurry produced by one animal in one day.

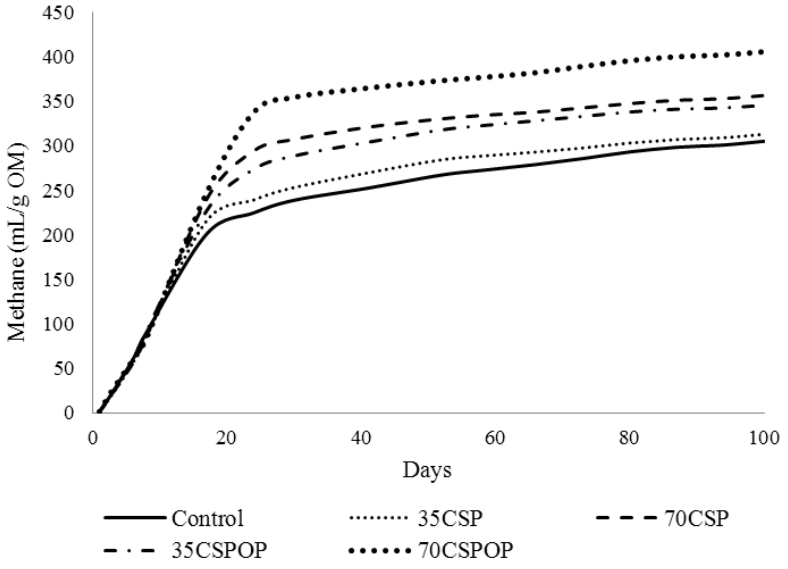

Figure 4.1. Effect of treatments on cumulated methane emission potential from slurry over 100 days (SD=20.1 mL/g OM)

Treatments are: 35CSP = 35 g/kg of calcium soap of palm fatty acid distillate; 70CSP = 70 g/kg of calcium soap of palm fatty acid distillate; 35CSPOP = 35 g/kg of calcium soap of palm fatty acid distillate and 200g/kg of orange pulp; 70CSPOP = 70 g/kg of calcium soap of palm fatty acid distillate and 200 g/kg of orange pulp. At day 17, inclusion of 70 vs 35 g CSP/kg led to increasing methane emission ($P < 0.05$). Beyond day 24, treatment 70CSPOP had higher ($P < 0.05$) values than both 35CSP diets, with 70CSP diet giving intermediate results. Both 70CSP treatments led to higher values ($P < 0.05$) than the control diet from d 17 onwards.

4.4 Discussion

Changes in dietary nutrient composition and digestibility led to significant modifications of faecal composition and then of the substrate for microbial fermentation of slurry. In this way, faecal EE content increased (from 85.8 to 138 and 204 g/kg DM) when CSP addition to the diet increased from 0 (control diet) to 35 and 70 g/kg, as a consequence of the higher EE dietary concentration, but also of a limited digestibility of CSP-fat. This result might be

associated to the high proportion of palmitic acid and to the relatively low ratio of polyunsaturated/saturated fatty acids in palm oil (0.43 and 1.04, respectively; FEDNA, 2010). In addition, dietary contents of free fatty acids and calcium, as occurs in CSP, have been negatively related to fat digestibility in growing pigs (Wiseman and Cole, 1983; Powles et al., 1993). Other studies (Leek et al., 2004 and Cerisuelo et al., 2012) reported no differences on EE digestibility and faecal EE output, respectively, when the level of fat increased in pig diets, indicating that this effect strongly depends on the level and source of fat. Otherwise, inclusion of CSP did not affect CTTAD of fibrous constituents, as also observed Leek et al. (2004) when supplementing a control diet with 45 g/kg of unsaturated or saturated sources of fat.

Inclusion of 200 g/kg of OP also led to a significant (although lesser than for CSP) increment of faecal EE content (from 165 to 177 g/kg DM), which was parallel to a reduction of EE digestibility (from 0.636 to 0.586). This result might be explained by higher fat endogenous losses (Kil et al., 2010). Fermentable fibre is an energy source available for hindgut flora in the pig, so that an increase of low-lignified cell wall constituents in the diet might result in a higher gut microbial growth (Canh et al., 1997; Bindelle et al., 2009; Heimendahl et al., 2010). Inclusion of OP in the diet could therefore imply an increased faecal excretion of microbial fat, which would reduce its apparent faecal digestibility. However, OP inclusion did not affect significantly daily faecal BEDN excretion (0.201 vs 0.212 g/kg$^{0.75}$) in the current study, as the higher faecal N excretion was compensated with a lower proportion of BEDN fraction in the faecal N. The present results confirm those previously obtained using the same methodology by Kreuzer et al. (1999) and Beccaccia et al. (2015a), when inclusion of fermentable fibre was compared in diets containing similar levels of NDF. Otherwise, our results agree with those obtained by Bach Knudsen and Hansen (1991) who observed a decrease of ileal and faecal digestibility of fat when level of soluble fiber in the diet increased; these authors explained this result through a depressed absorption of dietary fat and a lower resorption of bile acids.

Treatments also affected proportion of cell wall constituents in faecal output. In this way, increasing dietary level of CSP from 0 (control diet) to 35 and 70 g/kg, led to a parallel

decrease of faecal aNDFom (from 521 to 475 and 412 g/kg DM) and ADL (from 50.1 to 51.9 and 44.1 g/kg DM) concentrations. Also, inclusion of 200 g/kg of OP resulted in a decrease in faecal aNDFom concentration (from 483 to 404 g/kg DM), although in this case ADL content varied less between dietary OP levels. Treatments had no influence on faecal content of SF because of its high digestibility, as also occurred in previous studies (Graham et al., 1986; Canibe and Bach Knudsen, 1997; Beccaccia et al., 2015a,b).

In whole, the increase in faecal EE content and excretion resulted in greater CH_4 potential emission with CSP dietary level, both per g of OM or on daily basis, despite the parallel decrease in aNDFom content. This result can be explained according to the theoretical estimations of Angelidakis and Sanders (2004) of CH_4 yield of lipids with respect to other slurry components, as cell wall constituents or protein (1.014 vs 0.415 and 0.496 L/g, respectively). In the same way, Beccaccia et al. (2015c) observed higher potential CH_4 emissions in slurry from nursery pigs, characterized by a higher EE content than in other classes of pigs (growing-finishing or adults). Despite causing similar modifications on excreta composition than CSP, the increase in CH_4 potential emissions with dietary OP inclusion observed in the current study did not reach significant levels. This might be related to the lower influence of OP with respect to CSP on faecal EE concentration. In the literature, the inclusion of different fibre sources such as dry distillers grains with solubles, sugar beet pulp or rapeseed meal led to variable effects on faecal or slurry BMP (Jarret et al., 2011a; Jarret et al., 2011b; Jarret et al., 2012; Torres-Pitarch et al., 2014; Beccaccia et al., 2015a) according to differences in fibre, EE or CP content in the slurry. In most of the studies performed, high fibre levels were associated with high levels of fat to obtain isoenergetic feeds. The results of the present experiment suggest that, if this is the case, the effects frequently associated to fibre supplementation might be confounded with those related to a higher dietary and faecal EE content.

Crude protein concentration in faecal DM increased with dietary OP addition in the current study (from 174 to 226 g/kg) in parallel to a decrease of CP digestibility (from 0.818 to 0.762). These results are in agreement with previous research with OP in pigs (Beccaccia et al., 2015a) and might be explained by the synthesis of Maillard compounds because of the

high sugar content of this feedstuff and the high temperature reached during the dehydration process. Addition of OP also increased N excreted daily in faeces, but did not affect significantly N excretion in urine. Consequently, more total N was excreted with OP supplementation (0.864 vs 0.673 g/kg$^{0.75}$, P=0.050), but the increase of the ratio of N excreted in faeces: urine (by 5.15%) did not reach a significant level. Otherwise, increasing CSP dietary inclusion from 35 to 70 g/kg did not affect N concentration in faeces or urine neither daily N urine losses but increased N excretion in faeces, and therefore the ratio faecal: urine N (from 0.83 to 1.14, P=0.014).

Slurry characteristics such as pH, TAN or TKN were not affected by treatments. However, NH_3 emission per kg of slurry decreased with the inclusion of CSP. This effect could be related to an inverse increment in the faecal: urine N ratio, as the urinary N is more easily volatilized than organic N. To the authors' knowledge, processes which may explain the effect of dietary fat content reducing NH_3 emissions have not been described in the literature. Beccaccia et al. (2015c) also reported a negative effect of slurry EE content on NH_3 emission from commercial pig slurries when expressed per g of OM content. However, Leek et al. (2004) did not observe any effect of type or level of dietary oil on N balance or NH_3 emissions from the slurry, but faecal EE concentration was not provided and might not differ as sources of fat used were more digestible than in our study.

4.5 Conclusions

In all, inclusion of two industrial by-products, CSP and OP in isonutritive diets induced significant changes in slurry composition. However, only CSP influenced gaseous emission, increasing BMP and volume of CH_4 per animal and day, and decreasing NH_3 per kg of slurry. These results enhance the interest of modelling characteristics of slurry, as it constitutes the substrate for gaseous emissions. This information might then be used as a tool to manipulate microbial fermentation in order to minimize CH_4 and NH_3 losses.

4.6 References

Angelidaki, I., Sanders, W., 2004. Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Biotecnol. 3, 117-129.

AOAC, 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Washington, DC, USA.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, Centennial ed. APHA, Baltimore, Maryland.

Bach Knudsen, K. E., Hansen, I. 1991. Gastrointestinal implications in pigs of wheat and oat fractions. 1. Digestibility and bulking properties of polysaccharides and other major constituents. Br. J. Nutr. 65, 217–232.

Beccaccia, A.F., Calvet, S., Cerisuelo A., Ferrer, P., García-Rebollar, P., De Blas, C., 2015a. Effects of nutrition on digestion efficiency and gaseous emissions in growing-finishing pigs. I. Influence of the inclusion of two levels of orange pulp and carob meal in isofibrous diets. Anim. Feed Sci. Technol. (submitted)

Beccaccia, A.F., Cerisuelo A., Calvet, S., Ferrer, P., Estellés, F., De Blas, C., García-Rebollar, P., 2015b. Effects of nutrition on digestion efficiency and gaseous emissions in growing-finishing pigs: II. Influence of varying the source of protein in isonutritive diets. Anim. Feed Sci. Technol. (submitted).

Beccaccia, A. F., Ferrer, P., Ibáñez, M.A., Estellés, F., Rodríguez, C., Moset, V., De Blas, C., Calvet, S., García-Rebollar, P., 2015c. Relationships among slurry characteristics and gaseous emissions at different types of commercial Spanish pig farms. Span. J. Agric. Res. (in press, doi: 10.5424/sjar/2015131-6575).

Bindelle, J. Buldgen, A., Delacollette, M., Wavreille, J., Agneessens, R., Destain, J.P., Leterme, P., 2009. Influence of source and concentrations of dietary fiber on in vivo nitrogen excretion pathways in pigs as reflected by in vitro fermentation and nitrogen incorporation by fecal bacteria. J. Anim. Sci. 87, 583-593.

Canh, T.T., Verstegen, M.W.A., Aarnink, A.J.A., Schrama, J.W., 1997. Influence of dietary factors on nitrogen partitioning and composition of urine and faeces of fattening pigs. J. Anim. Sci. 75, 700-706.

Canh, T.T., Aarnink, A.J.A., Schutte, J.B., Sutton, A., Langhout, D.J., Verstegen, M.W.A., 1998a. Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing-finishing pigs. Livest. Prod. Sci. 56, 181-191.

Canh, T.T., Sutton, A.L., Aarnink, A.J.A., Verstegen, M.W.A., Schrama, J.W., Bakker, G.C.M., 1998b. Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs. J. Anim. Sci. 76, 1887-1895.

Canibe, N., Bach Knudsen, K. E., 1997. Digestibility of dried and toasted peas in pigs. 1. Ileal and total tract digestibilities of carbohydrates. Anim. Feed Sci. Technol. 64, 293-310.

Cera, K. R., Mahan, D. C., Reinhart, D. C., 1989. Apparent fat digestibilities and performance responses of postweaning swine fed diets supplemented with coconut oil, corn oil or tallow. J.Anim. Sci. 67, 2040-2047.

Cerisuelo, A., Torres, A., Lainez, M., Moset, V., 2012. Increasing energy and lysine in diets for growing-finishing pigs in hot environmental conditions: Consequences on performance, digestibility, slurry composition and gas emissio. J. Anim. Sci. 90, 1489-1498.

CVB, 2004.Veevoedertabel (Livestock Feed Table). CentraalVeevoeder Bureau, Lelystad, The Netherlands.

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.

FEDNA, 2006. Necesidades nutricionales para ganado porcino: Normas FEDNA. De Blas, C., Gasa, J. Mateos, G. G. (eds). Fundación Española para el Desarrollo de la Nutrición Animal, Madrid, Spain, 55 pp.

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

Graham, H., Hesselman, K., Aman, P., 1986. The influence of wheat bran and sugar beet pulp on the digestibility of dietary components in a cereal-based pig diet. J. Nutr. 116, 242-251.

Heimendahl, E., Breves, G., Abel, Hj., 2010. Fiber-related digestive process in three different breeds of pigs. J. Anim. Sci. 88, 972-981.

Hernández, F., Martínez, S., López, C., Megías, M. D., 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.

INRA, 2002.Valeursnutritives pour les volaillles. In: Sauvant, D., Perez, J. M., Tran, G. (Eds.) Tables de composition et de valeur nutritive des matières premiéres destinées aux animaux d'élevage. INRA Editions, Paris Cedex, France.

Jarret, G., Martinez, J., Dourmad, J.Y., 2011a. Effect of biofuel co-products in pig diets on the excretory patterns of N and C on the subsequent ammonia and methane emissions from pig effluent. Animal. 5, 622-631.

Jarret, G., Cozannet, P., Martinez, J., Dourmad, J. Y., 2011b. Effect of different quality wheat dried distiller's grain solubles (DDGS) in pig diets on composition of excreta and methane production from faeces and slurry. Livest. Sci. 140, 275-282.

Jarret, G., Cerisuelo, A., Peu, P., Martinez, J., Dourmad, J. Y., 2012. Impact of pig diets with different fiber contents on the composition of excreta and their gaseous emissions and anaerobic digestion. Agr. Ecosyst. Environ. 160, 51-58.

Kil, D. Y., Sauber, T. E., Jones, D. B., Stein H. H., 2010. Effect of the form of dietary fat and the concentration of dietary NDF on ileal and total tract endogenous losses and apparent and true digestibility of fat by growing pigs. J. Anim. Sci. 88, 2959–2967.

Kreuzer, M., Roth, F.M., Kirchgessner, M., 1989. Mikrobielle umsetzungen im enddarm von sauen bei intracaecaler infusion hoher Mengen an verschiedenen reinen substraten. 1. Veränderung der Verdaulichkeit von Stickstoff und Aminosäuren. Landwirtsch. Forsch. 42, 72–92.

Kreuzer, M., Wittmann, M., Gerdemann, M.M., 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.

Leek, A. B. G., Beattie, V. E., O'Doherty, J. V., 2004.The effects of dietary oil inclusion and oil source on apparent digestibility, faecal volatile fatty acid concentration and manure ammonia emission. Anim. Sci. 79, 155–164.

Licitra, G., Hernández, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feed. Anim. Feed Sci. Technol. 57, 347–358.

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

Ndegwa, P.M., Hristov, A.N., Joo, H.S., 2009. Measuring concentrations of ammonia in ambient air or exhaust air stream using acid traps. J. Environ. Qual. 38, 647–653.

Portejoie, S., Dourmad, J.Y., 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.

Powles, J., Wiseman, J., Cole, D.J.A., Hardy, B., 1993. Effect of chemical structure of fats upon their apparent digestible energy value when given to growing/finishing pigs. Anim. Prod. 87, 137-146.

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

Torres-Pitarch, V., Moset, V., Ferrer, P., Cambra-López, M., Hernández, P., Coma, J., Pascual, M., Serrano, P., Cerisuelo, A., 2014. The inclusion of rapeseed meal in fattening pig diets, as a partial replacer of soybean meal alters nutrient digestion, faecal composition and biochemical methane potential from faeces. Anim. Feed Sci. Technol. 198, 215-223.

Triolo, J. M., Sommer, S. G., Møller, H. B., Weisbjerg, M. R., Jiang, X. Y., 2011. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: Influence of lignin concentration on methane production potential. Biores.Tech. 2011, 9395-9402.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Wiseman, J., Cole, D., 1983. Interaction between dietary free fatty acids and calcium in growing pigs. In: Proceedings of the 5th World Conference on Animal Production, Tokyo. pp. 423–424.

Wiseman, J., Cole, D.J.A., Hardy, B., 1990. The dietary energy values of soya-bean oil, tallow and their blends for growing/finishing pigs. Anim. Prod. 50, 513-518.

5
Ammonia emission quantification from pig slurry using acid wet traps: evaluation and optimization

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Abstract. Standardized measurement protocols are required to reduce ammonia (NH_3) emissions. In vitro measurement of NH_3 emissions consists in trapping the emission from an emitting source in an acidic solution under controlled conditions. The objective of this study was to assess the *in vitro* NH_3 measurement method from pig slurry with acid wet traps, as regards: (i) the variation between replicates of NH_3 emissions measured in vitro; (ii) the relationships between partial and accumulated emissions; and (iii) the reduction of measurement frequency. For this study, a total of 60 pig slurry samples from different animal types (sows and growing animals) were collected from commercial farms. The coefficient of variation among replicates of accumulated NH_3 emission during 15 days was 6.73%. Emissions tended to decrease with time, and an average reduction of NH_3 emissions about 16% was found in the period 96 - 240 hours respect to the 0 – 96 hours period. However, samples continued emitting considerable amounts of NH_3 after 360 hours. Linear regression models allowed predicting emissions accumulated for 15 days using only the first 8 days ($R^2 > 0.90$). Reducing NH_3 measurement frequency (from 24 to 48 hours) did not significantly affect measured emissions ($P > 0.05$). The results of this study confirm that replication of measurements is required and a coefficient of variation of 10% may be established as quality control requirement. The study also suggests that reducing the duration and frequency of measurements is a practice option to simplify this methodology.

Keywords: ammonia; emissions; impingers; animal slurry, methodology.

Abbreviations: AOAC, Association of Official Analytical Chemists; CH_4, methane; CV, coefficient of variation, (%); DM, dry matter, (%); F, frequent; HNO_3, nitric acid; H_2SO_4, sulfuric acid; H_3PO_4, ortophosphoric acid; LF, less frequent; NH_3, ammonia; N-NH_3, ammonia nitrogen; N_2O, nitrous oxide; OM organic matter, (%); R^2, coefficient of determination; rAE, relative absolute error; rRMSE, relative root mean squared error of prediction; TKN, total Kjeldahl nitrogen, (g L^{-1}); VS, volatile solids (%).

5.1 Introduction

The World Organization for Food and Agriculture (FAO, 2011) recognizes that atmospheric emissions of ammonia (NH_3), nitrous oxide (N_2O) and methane (CH_4) associated with animal production constitute a global problem. Ammonia emissions are directly linked to transboundary air pollution effects such soil acidification, water eutrophication, and changes in the ecosystems (Krupa, 2003). Therefore, researchers are developing options to mitigate emissions and contribute to the sustainability of animal production. To accomplish this aim, accurate, reliable and comparable methods to measure emissions are required.

In recent decades, researchers have used dynamic chambers to measure NH_3 emissions in field conditions from manure storage and slurry application (Hansen et al. 2006; Misselbrook et al. 2005). However, in order to evaluate the effect of animal nutrition strategies on NH_3 emissions from slurries, *in vitro* laboratory measurements of NH_3 emissions are common in the literature (e.g. Galassi et al. 2010; Jarret et al. 2012, among others). During these *in vitro* laboratory assays, slurry samples are kept in chambers under controlled environmental conditions (e.g. temperature and relative humidity). Air is then forced to flow through the headspace of the chamber at a constant airflow (Derikx and Aarnink, 1993; Ni and Heber, 2008). To quantify the NH_3 emitted from samples, exhaust air from the chamber is forced to pass through an acid solution (e.g. using impingers) in which NH_3 is trapped in an acid-base neutralization reaction. The mass of NH_3 captured is later analyzed using conventional analytical methods (e.g. APHA, 2005). Several studies (Derikx and Aarnink, 1993; Canh et al. 1998a) recommend using two acid wet traps in serial. As an indicator of correct operation, these studies suggest that the second trap should accumulate less than 5% of the total NH_3 captured. Replicate chambers have been frequently used as analytical replicates per slurry sample (Derikx and Aarnink, 1993; Pereira et al. 2012). Although these measurements are not representative of field emissions, they are useful to compare the intrinsic emission potential of different samples (Portejoie et al. 2004).

There are several factors influencing the results obtained when using this in vitro NH_3 emission measurement method with acid wet traps. The main ones could be classified as those related to: i) operational parameters: the mass/volume of slurry sample, chamber dimensions, airflow rate, temperature, relative humidity, frequency of replacement of the acid solution and measurement period; and ii) the analytical determination of the NH_3 captured by the acid wet traps. In the case of operational parameters, Ndegwa et al. (2009) studied how this method may be affected by airflow rate, NH_3 concentration, type and size of the acid trap and acid volume and concentration.

Most of these characteristics differ considerably among studies reported in the literature (Table 1): amount of slurry in the chamber from 0.5 to 5.7 kg, the acid nature (nitric, sulfuric or ortophosphoric), acid concentration from 0.02 to 1 M, acid solution volume from 50 to 150 mL, and airflow rate from 1 to 5 L min^{-1}. Regarding the measurement period, reports from three (Pereira et al., 2011) to 23 days (Vaddella et al. 2011) were found. Referring to the frequency of replacement of the acid wet traps, most studies refer to Derikx and Aarnink (1993) who reported a daily frequency of replacement for the first impinger. In the studies carried out by Galassi et al. (2010) and Canh et al. (1998c) the first impinger was replaced daily while the second was replaced after 7 days. However, the most frequent management was replacing the first impinger at 48, 96, 144, 192 and 240 h and the second impinger at 96 h and 240 h (e.g. O'Shea et al. 2009; Hernández et al., 2011; among others). On the contrary, Pereira et al. (2012) replaced the acid solution after 1, 3, 6, 12 h and then every 12 h until 120 h, to measure NH_3 emissions from an emitting layer of faeces and urine of 1 mm thick.

Table 5.1. Methodological characteristics of studies using the in vitro method of acid wet traps for measuring NH_3 emissions from slurry

Slurry Kg	Acid trap			Airflow (L min^{-1})	Measurement period Days	Reference
	Acid	Concentration M	Amount mL			
2	HNO_3	0.5	70	4.2	7[o]	Derikx and Aarnink (1993)
2	HNO_3	1	70	4.2	7 – 16[o]	Canh et al. (1998a) Canh et al. (1998b) Canh et al. (1998c) Canh et al. (1998d)
2	HNO_3	0.5	70	4.2	7[o]	Mroz et al. (2000)
5.7	H_2SO_4	1	50	5	6[o]	Portejoie et al. (2004)
2	HNO_3	1	N. I.	4.2	10[q]	O'Connell et al. (2005)
2	HNO_3	0.5	70	4.2	10[q]	O'Connell et al. (2006)
2	HNO_3	1	N. I.	4.2	10[q]	Lynch et al. (2007)
1	HNO_3	1	70	N. I.	10[q]	O'Shea et al. (2009)
2	HNO_3	1	N. I.	2.5	10[q]	O'Shea et al. (2010)
2	HNO_3	0.5	140	4.2	14[o]	Galassi et al. (2010)
0.7	H_2SO_4	0.05	100	N. I.	10[q]	Hernández et al. (2011)
5	H_2SO_4	1	50	5	16[o]	Jarret et al. (2011)
2	H_3PO_4	0.02	150	4	3[r]	Pereira et al. (2011)
1.1–3.3	H_2SO_4	0.2	150	1	23[s]	Vaddella et al. (2011)
5	H_2SO_4	1	50	5	16[o]	Jarret et al. (2012)
0.5	H_2SO_4	1	100	1.2	15[p]	Beccaccia et al. (2015)

[o] first impinger, replaced daily.
[p] first and second impingers, replaced daily.
[q] first impinger, replaced at 24, 48, 96, 144, 192 and 240 h and the second impinger replaced at 96 and 240 h.
[r] single impinger, replaced at 1, 3, 6, 12 h, and then every 12 h until 72 h.
[s] impinger, replaced at 24, 48, 96, 120, 144, 168, 216, 264, 312, 360, 432, 504 and 552h.

The main drawback related with in vitro NH_3 emission measurement method with acid wet traps is it does not reflect real storage conditions of slurry. Furthermore, there is a lack of standardization in the methodology. There is limited available information on the analytical performance (Ndegwa et al., 2009; Xue et al. 1998), and also on the variation between replicated samples and on the evolution of emissions in time (e.g. Canh et al. 1998a; Portejoie et al., 2004). Particularly, there is no information on the minimum duration of measurements that may provide accurate results in relation with the aim of the study. It has also been reported that minor changes in feed composition influencing nitrogen digestibility

may not be detected as influencing the emissions, probably because of the lack of precision of the method or the influence of other variables (Hernandez et al., 2011). However, this method is widely used and the emission results are considered a reference for understanding emission processes, for establishing emission mitigation strategies and for decision-making (Bittman et al., 2014). Therefore, efforts should be conducted in order to harmonize methodological aspects and to clearly establish the strengths and limitations of this methodology. On the other hand, understanding the effect of measurement period and the frequency of replacement of the acid solution could contribute to optimize the cost and performance of the method.

In this context, this study aims to evaluate the performance of the acid wet traps in vitro method for measuring NH_3 emissions from slurry storage in terms of: (i) the variation between replicates, (ii) the relationships between partial and accumulated emission, and (iii) the effect of reducing the frequency of acid replacement (from 24 h to 48 h) on the emission results.

5.2 Materials and methods

5.2.1 System configuration and slurry characteristics

A total of 60 samples of pig slurry from commercial farms in Spain (finishing - growing, gestation, lactating sows and nursery piglets) were used. Representative slurry samples were collected directly from manure pits, as described by Beccaccia et al. (2015). Samples were transported under refrigeration (4 °C) to the laboratory. Samples composition was variable for dry matter (DM, ranging from 0.5% to 17.7%), volatile solids (VS, ranging from 0.3% to 14.5% in wet basis), total Kjeldahl nitrogen (TKN, ranging from 0.7 g L^{-1} to 15.1 g L^{-1}) and ammonia nitrogen ($N\text{-}NH_3$, ranging from 0.3 g L^{-1} to 9.4 g L^{-1}). More details on slurry sampling procedures and composition of slurries can be found in Beccaccia et al. (2015).

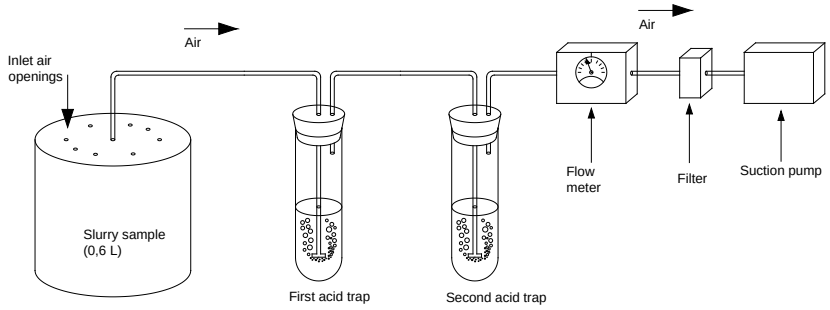

Figure 5.1. Configuration of the measurement system for in vitro measurement of NH_3 emission using acid wet traps

NH_3 emissions were determined using an *in vitro* acid wet trap system similar to that used by Derikx and Aarnink (1993) and Ndegwa et al. (2009) which was installed at the Laboratory of the Institute of Animal Science and Technology (ICTA) of the Universitat Politècnica de València (UPV). One-liter (0.3m diameter and 0.15 m height) closed plastic containers (dynamic chambers), were filled with 0.5 kg of pig slurry (Figure 5.1), remaining a head space of approximately 0.5 L for ventilation. A total of 16 containers were kept in a thermostatic water bath at 25 °C (Precisterm, PJ. Selecta, Spain). Air entered the chambers by eight 3-mm diameter holes located on the edge of the lid. An 8-mm exhaust tube was connected to two acid wet traps (impingers, containing 100 mL of 0.05 M sulfuric acid) in serial and air was drawn from at a constant flow rate of 1.2 L min^{-1} using a suction pump (ZA.32 9,210,003, DVP, Taiwan). Airflow rates were adjusted with regulation valves and it was verified daily using a flow-meter (Rota, Yokogawa, Germany). The acid solution of the impingers was replaced every 24h or 48 h depending on the study as explained below. After this period, NH_3 concentration was determined in these solutions using a selective ion electrode (Model 9512HPBNWP, Thermo Scientific, USA) following method D4500 NH_3 (APHA, 2005). Three studies were conducted to achieve the aims of the work: Study 1: Variation between replicates, Study 2: Relationships between partial and

accumulated emissions, and Study 3: Effect of reducing the frequency of acid replacement (from 24 h to 48 h) on the emission results.

5.2.2 Study I: Variation between replicates

Forty-eight pig slurry samples were used to characterize the variation in NH_3 emissions between replicates of the same sample. In the laboratory, after homogenization, two sub-samples of 0.5 kg were obtained from each slurry sample. For each sub-sample, NH_3 emission was measured during 15 consecutive days using the methodology described in section 2.1. Acid from impingers was replaced each 24 hours. Therefore, two replicate NH_3 emission values were obtained daily for each sample. Data was integrated as follows: daily emission corresponded to the emission at a certain day and the accumulated emission was calculated as the addition of all daily emissions of a certain slurry sample during 15 days. The CV of each pair of replicates (both daily and accumulated for 15 days) was also obtained. The distribution frequency of CV between replicates was calculated (both for daily measurements and for the accumulated 15-day period).

5.2.3 Study II: Relationships between partial and accumulated emission

The NH_3 emissions results generated from the slurry samples used in Study I were used to explore the temporal evolution of emissions. To this aim, partial emissions were calculated and defined as the emission accumulated until a certain day (from day 1 to day 14). Partial emissions were expressed in relative terms to the accumulated emissions and the following linear regression analysis was conducted to explore the emission dynamics (Eq. 5.1):

$$Y\% = \beta_0 + \beta_1 D + \varepsilon \qquad (5.1)$$

Where Y% is the partial emission at a certain day D, expressed in relative terms to the accumulated NH_3 emission, β_0 and β_1 are the model parameters and ε is the model error.

Then, coefficients of correlation were obtained between partial emissions and accumulated emissions in 15-days period. The PROC CORR procedure of SAS (SAS Institute, 2008) was used to this aim.

With the purpose to establish a model to predict the accumulated emissions in 15 days, accumulated and partial emission values were subjected to a regression analysis as a function of time. This analysis was performed using PROC REG of SAS software (SAS Institute, 2008) using Eq. (5.2):

$$Y = \beta_0 + \beta_1 X + \varepsilon \qquad (5.2)$$

Where Y is accumulated NH_3 emission (mg kg^{-1} of slurry in 15 days), X is partial emission (mg kg^{-1} of slurry until a certain day), β_0 and β_1 are the model parameters and ε is the model error.

Predicted and observed emissions were then compared and the following statistical parameters were calculated: coefficient of determination (R^2), relative root mean squared error of prediction (rRMSE), and relative absolute error (rAE).

5.2.4 Study III: Effect of measurement frequency on in vitro NH_3 emission results

To evaluate the effect of the measurement frequency (acid replacement each 24 h vs 48 h) on NH_3 emissions estimation, 12 samples of pig slurry were used. NH_3 emissions were determined during 14 days from two replicates of each sample, following the methodology described in section 2.1. For each replicate, two replacement frequencies of the acid solution were used: every 24 h (Frequent, F, which was considered the reference method) and every 48 h (Less Frequent, LF). The F and LF acid replacements alternated between replicates to avoid potential systematic effects of a replicate.

To evaluate the effect of the sampling frequency (F vs. LF) the results were subjected to an analysis of paired data (paired t-test) using the procedure t-test of SAS (SAS Institute, 2008).

5.3 Results

5.3.1 Study I: Variation between replicates

The emission values (expressed in mg NH_3 per kg of slurry and day) and CV between replicates measured every 24 hours for 15 days are shown in Table 5.2. The accumulated

emissions ranged from 290.9 to 3787 mg kg^{-1} of slurry for the 15-day period. The average CV between daily emissions of replicate samples of slurry along the 15 days of measurement varied between 9.78% (day 1) and 16.60% (day 13). For accumulated emissions in 15 days, the CV was reduced to 6.73%.

Table 5.2. Coefficient of variation (CV) of NH_3 emission between pairs of replicate samples of slurry both daily and in a cumulative period of 15 days (n=48)

Measurement day	NH_3 Emission (mg kg^{-1} of slurry in 24 h)			CV between replicates (%)		
	Mean	Min	Max	Mean	Min	Max
1	188.1	17.3	611.3	9.78	0.14	50.98
2	149.1	18.6	336.3	9.85	0.08	36.02
3	134.3	17.1	277.8	16.57	0.13	89.04
4	131.9	24.5	341.9	13.73	0.00	70.77
5	106.5	23.2	229.9	14.24	0.22	83.04
6	117.8	22.5	232.1	11.05	0.81	45.18
7	127.9	15.2	316.2	15.34	0.89	61.15
8	138.8	20.4	298.6	11.95	0.20	64.52
9	129.8	23.1	292.4	13.65	0.55	59.78
10	136.6	21.3	315.7	10.93	0.36	51.17
11	124.5	22.4	255.3	11.96	0.00	66.92
12	115.6	17.1	241.3	14.03	0.39	103.52
13	106.0	14.8	330.2	16.60	0.43	115.19
14	116.5	11.5	255.2	12.27	0.42	74.51
15	105.9	9.2	283.3	15.05	0.15	107.19
Accumulated emission 15 days	1917.4	290.9	3787.3	6.73	0.28	28.89

Regarding daily measurements, it can be observed that 56% of measurements had a CV lower than 10%, whereas 21% of measurements had a CV above 20%. In the case of accumulated emissions for the 15-day period, 81% of samples had a lower CV than 10% and only 5% of samples had a CV higher than 20%.

5.3.2 Study II: Relationships between partial and accumulated emissions

The evolution of emission in time is shown in Figure 5.2. A highly significant linear relationship ($P < 0.001$, $Y\% = 6.48\ D + 4.47$, $R^2 = 0.97$) was found. All samples continued emitting significant amounts of NH_3 on day 15 (106 mg L^{-1} and day, on average). In

general, most samples emitted constantly in time except in the first two days when emissions were higher.

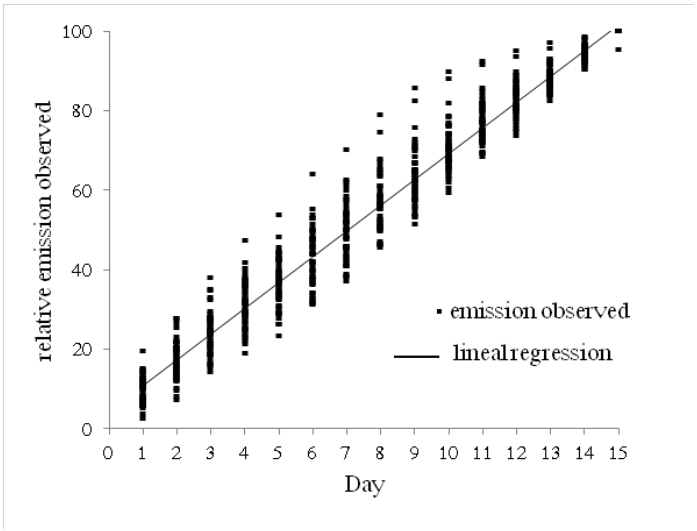

Figure 5.2. Evolution of partial NH_3 emissions (expressed in relative terms to the accumulated emissions in 15 days)

Significant correlations ($P < 0.001$) were obtained between accumulated emissions in 15 days and partial emissions over the previous 14 days (Table 5.3). Correlation coefficients between partial emissions at day 6 and accumulated emissions were above 0.9. Correlation coefficient between accumulated and partial emissions was 0.96 at day 9 and higher than 0.99 at day 11. Predictive models of the accumulated emission (15 days) based on the partial emissions were generated.

Table 5.3. Correlation coefficients between cumulative NH_3 emissions during 15 days and partial cumulative emissions of pig slurry measured in vitro. (n=48)

Partial cumulative (day)	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.95	0.91	0.86	0.84	0.81	0.8	0.78	0.76	0.75	0.75	0.74	0.74	0.73	0.73
2	1	0.97	0.94	0.92	0.9	0.89	0.87	0.86	0.86	0.85	0.85	0.84	0.84	0.84
3		1	0.99	0.98	0.96	0.95	0.93	0.92	0.91	0.89	0.89	0.88	0.87	0.86
4			1	0.99	0.99	0.97	0.96	0.95	0.94	0.93	0.92	0.9	0.9	0.88
5				1	0.99	0.99	0.98	0.97	0.96	0.94	0.93	0.92	0.91	0.89
6					1	0.99	0.99	0.97	0.96	0.94	0.94	0.93	0.92	0.90
7						1	0.99	0.99	0.98	0.97	0.96	0.95	0.94	0.93
8							1	0.99	0.99	0.98	0.97	0.97	0.96	0.95
9								1	0.99	0.99	0.98	0.98	0.97	0.96
10									1	0.99	0.99	0.99	0.99	0.98
11										1	0.99	0.99	0.99	0.99
12											1	0.99	0.99	0.99
13												1	0.99	0.99
14													1	0.99
15														1

As shown in the Table 5.4, the coefficient of determination (R^2) of the model increases with the accumulation of measures, being higher than 0.9 from day 8. The evolution of statistical parameters (R^2, rRMSE and rAE) of the prediction equations increased as more measurement days were considered. When only the first day of measurement is used, a R^2 of 0.53 was obtained. However, R^2 increased to more than 0.90 if emissions until day 8 of measurement were considered, and more than 0.95 at day 10. In Figure 5.3, the predicted and observed NH_3 accumulated emissions for 15 days are plotted for days 1, 3, 5 and 10. As evidenced in Figure 5.3, the prediction of emissions at 15 days using partial emissions improves with the number of measuring days.

Table 5.4. Prediction models of NH_3 emissions accumulated in 15 days (mg kg^{-1} of slurry), based on the partial emission accumulated at different days (from 1 to 11 days)

Variable	Model equation	R^2	rRMSE	rAE
Accumulated emission for 15 days	5.766 x Partial emission in day 1 + 417.7	0.530	0.304	0.240
	4.111 x Partial emission in day 2 + 269.5	0.704	0.241	0.191
	3.326 x Partial emission in day 3 + 176.1	0.771	0.212	0.162
	2.752 x Partial emission in day 4 + 131.4	0.816	0.190	0.148
	2.428 x Partial emission in day 5 + 100.4	0.832	0.182	0.142
	2.129 x Partial emission in day 6 + 81.2	0.839	0.178	0.136
	1.900 x Partial emission in day 7 + 54.8	0.885	0.151	0.116
	1.711 x Partial emission in day 8 + 26.6	0.921	0.125	0.094
	1.545 x Partial emission in day 9 + 17.2	0.944	0.105	0.079
	1.404 x Partial emission in day 10 + 8.0	0.967	0.080	0.060
	1.291 x Partial emission in day 11 + 4.1	0.980	0.066	0.050

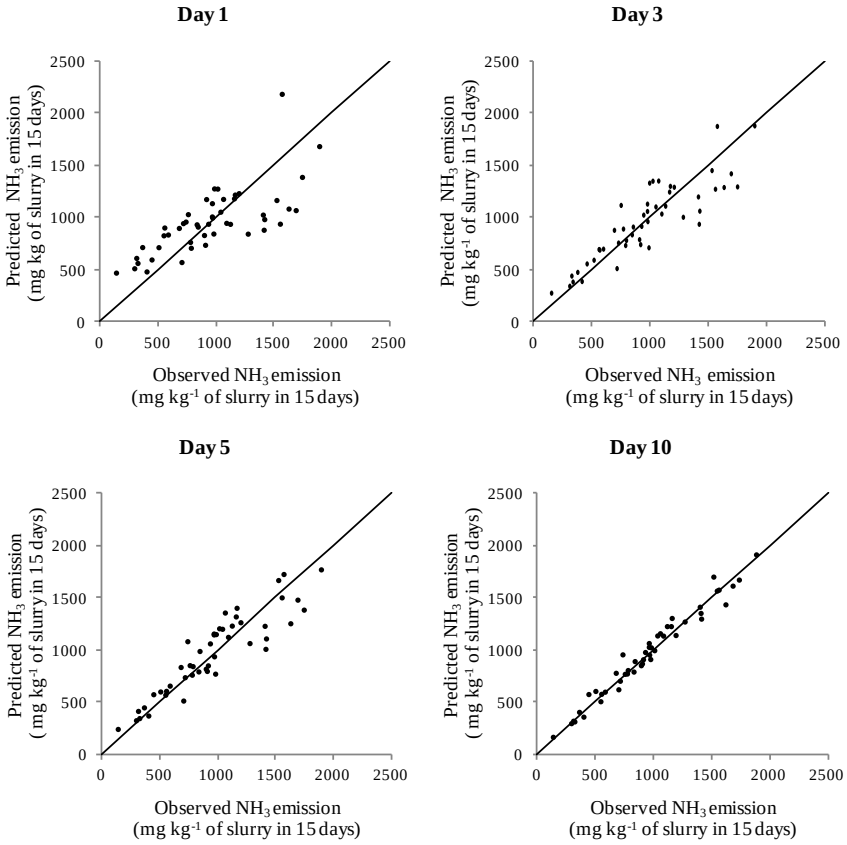

Figure 5.3. Predicted versus observed values of the NH_3 emissions accumulated for 15 days, using partial emission measurement accumulated until days 1, 3, 5 and 10

5.3.3 Study III: Effect of measurement frequency on in vitro NH_3 emission results

For cumulative periods of 48 hours, no statistical difference was found between F (average emission 269.2 mg NH_3 48 h^{-1}) and LF (average emission 277.1 mg NH_3 48 h^{-1}). Differences in NH_3 emissions between treatments were not statistically significant (P = 0.124). Figure 5.4 shows the difference, in relative terms, of a reference approach F (two consecutive measures of 24 hours each) against one single measurement for 48 hours LF. It

can be observed that most of the samples (58%) showed differences between -10% and +10% of the average value. Only less than 12% of samples had deviations of more than 20% (either positive or negative).

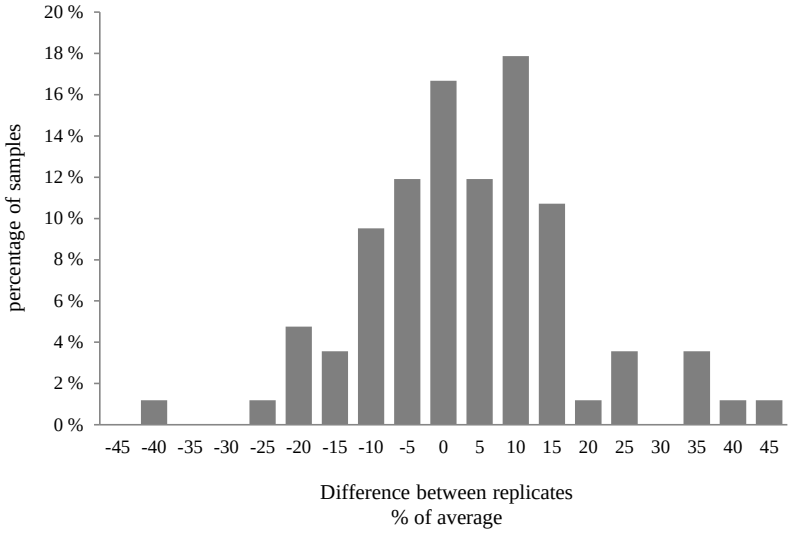

Figure 5.4. Frequency histogram of relative differences between measurements frequent F and less frequent LF, of the NH_3 emission in vitro, for measurement periods of 48 hours

5.4 Discussion

This study provides information about the performance characteristics of the in vitro NH_3 emission measurement method with acid wet traps using pig slurry samples. To the author's knowledge, there are no published studies about the variation between analytical replicates in such measurements, although most published articles use two replicates for measurements. In this study, CV between replicates was quantified to be about 7% for accumulated emissions in 15 days, whereas for daily measurements CV was higher (mostly between 10% and 16%). The lower CV of accumulated emissions for 15 days compared

with daily measurements is a logical consequence of the repetition of measurements, since each accumulated value includes 15 daily repetitions. Therefore, the variation between replicates of a particular sample of slurry may be explained by the intrinsic characteristics and heterogeneity of the substrate, which could arise in analytical errors due to the replication of samples.

In our results, samples showing a high CV (e.g. above 20%) in accumulated emissions corresponded to those showing systematic differences among replicates during the emission period. This suggests that inaccurate replication of samples could add variation factors that are not controlled by the method, such as the chemical composition or intrinsic microbiological characteristics of slurry. Therefore, evaluating the correspondence between replicates is advisable as a quality control of this method.

In order to standardize the in vitro NH_3 emission measurement method with acid wet traps, it seems necessary to define the measurement parameters, conditions of the studies, ways to present the results and margins of acceptable variation between replicates. Several authors recognize the large statistical variation between characteristics associated with biological material (Sokal and Rohl, 2002; Kuehl, 2001; Morris, 1999). Considering the CV found in our study and the biological nature of samples, the authors consider that a CV below 10% between replicates could be an adequate indicator of the analytical precision of measurements. However, further analysis should be conducted to explore the sources of variation and establish particular thresholds according to different measurement objectives.

Regarding the evolution of NH_3 emission, several studies report the emissions accumulated in two periods: 0-96 hours (days 1-4) and 96-240 hours (days 5-10). However, no conclusive results on emission patterns are derived from these studies. On the one hand, Hernández et al. (2011) and Lynch et al. (2007) report almost a constant emission rate among both periods (an average reduction of 2.5% in the 96 – 240 hours accumulation period with respect to the 0 – 96 hours period). On the contrary, the results obtained by O'Connell et al. (2006) show a 25% reduction of emission rate in the 96 – 240 hours period. Considering other experiment durations, Canh et al. (1998c) measured emissions for 7 days and reported an increase in the emission rate until days 2-4, depending on the diet of

the animals, and then a slight decrease until the end of the experiment. However, Portejoie et al. (2004), found relatively constant NH_3 emission rates during 6 days of measurements. In the present study, an average reduction of NH_3 emissions of 16% was found in the period 96-240 hours respect to the 0 – 96 hours period. A further decrease of 10% was measured in the subsequent period 240 – 360 hours. A considerable variation of emission trends among samples was found. The evolution of pH and slurry N-NH_3 content may be crucial factors in this tendency, which should be further explored.

Concerning the measurement period, most studies report variable durations (Table 5.1), depending primarily on the objective of each study. The reported values range from 3 days (Pereira et al., 2011), to 23 days (Vaddella et al., 2011). As mentioned before, in most studies the duration of measurements is 10 days (O'Connell et al. 2005; O'Connell et al., 2006; O´Shea et al., 2010; Hernández et al., 2011; Vaddella et al., 2011). However, our results evidence samples continued emitting relevant amounts of NH_3 on day 15 (on average 105.9 mg/day per kg of slurry). On the other hand this may raise a critical question on the duration of measurements. This *in vitr*o method is normally used for comparisons of slurry samples, but the emission potential of samples beyond the duration of experiment is unknown. So, it would be convenient to explore longer measurement periods to evaluate the evolution of NH_3 emissions from the slurry and establish optimal measurement times.

Considering the high correlations between partial and accumulated emission in 15 days, the possibility of reducing the duration of measurements should be explored and recommended. In this regard, the emission accumulated until day 8 allows calculating the cumulative emissions for 15 days, with a relative error of less than 10% and the coefficient of determination (R^2) above 0.9. It must be considered that CV among replications of accumulated emissions is about 7%, thus increasing measurement time beyond 8 or 10 days seems not to be justified. On the contrary, short measurements (e.g. less than 5 days) could not provide accurate information of the emission potential of the sample.

The evolution of emissions with time must not be extrapolated to real farm storage conditions but is useful to discuss specific methodological aspects such as sampling duration. A number of measurement days are required because of necessary replication, as

discussed above. However, long measurement periods may involve slurry changes derived from the experimental storage such as drying or fermentation and thus, might not be representative of the initial slurry. Container sizes and chamber ventilation rates are essential parameters affecting slurry changes. This study shows that using 8 to 10 days of measurements provides replicable results and no relevant changes in emissions through different days were found. In any case, it seems that the *in vitro* determination of NH_3 emissions is representative of potential emissions in the short term because in the long term the potential emissions are both affected by slurry composition and storage system.

The frequency of replacement of acid samples is also variable among studies. Particularly, some studies propose different replacement frequencies for the first and second impingers (e.g. Galassi et al., 2010; Canh et al. 1998c; O'Shea et al., 2009; Hernández et al., 2011; among others). However, the potential effects of different acid replacement frequencies were not reported in these studies. Although no justification is provided in the literature on how the frequency of acid change is selected, it seems to be chosen according to operative reasons, because normally only aggregate values are reported. These operative reasons may be related mainly to acid saturation. In our study, it was demonstrated that for the range of NH_3 concentrations in the air entering the impingers (from 5 to 85 mg m^{-3}) no systematic effect occurred due to reducing frequency from 24 h to 48 h. However, it is true that conducting daily measurements may provide detailed information on the emission dynamics and its variation as a function of time. In any case, if the stoichiometric capacity of the acid solution is enough to retain the emitted NH_3, acid could be replaced less frequently without affecting the accuracy of results.

Finally, the capture efficiency of acid wet traps used in this study was not limited by the stoichiometric capacity of the acidic solution. According to Ndegwa et al. (2009), there may be other factors involved, such as, the size of air bubbles in the impingers, their immersion time in the acid solution or acid concentration. These factors are directly related to the impinger characteristics and operation conditions (mainly air flow and amount of acid). In this regard, these authors mentioned that airflow rate has a direct effect decreasing capture efficiency. They assumed that the air to water exchange of NH_3 would increase with longer immersion time of bubbles and lower specific surface. In this study only one

model of impinger was used and therefore this effect was not tested. However, the first impinger retained on average 99.97% of the NH_3 captured by the system (first and second impingers). Therefore, it seems that operation conditions in this study were adequate.

5.5 Conclusions

- The variation in ammonia emissions between replicates of the same sample of pig manure indicates the need of at least two replicates. Higher deviations among replicates seem to be related to methodological errors and therefore a CV of 10% could be established as a precision control of this method.
- There is a high correlation between total emissions accumulated for 15 days and partial emissions accumulated for, at least, 8 days. According to the prediction models obtained in this study, 8 to 10 days of measurements might be used.
- Reducing the measurement frequency of ammonia emissions in vitro from 24 hours to 48 hours did not affect the results of the accumulated emission measurement. Therefore, this is a tangible option to reduce labor requirement of this technique.

5.6 References

APHA., 2005. Standard Methods for the Examination of Water and Wastewater. (21st ed.) Baltimore, Md.: Centennial Edition.

Beccaccia, A., Ferrer, P., Ibáñez, M. A., Estellés, F., Rodríguez, C., Moset, V., De Blas, C., Calvet, S., García-Rebollar, P., 2015. Relationships among slurry characteristics and gaseous emissions at different types of commercial Spanish pig farms. Spanish Journal of Agricultural Research, 13(1), 1–15.

Bittman, S., Dedina, M., Howard C.M., Oenema, O., Sutton, M.A., 2014. Options for Ammonia Mitigation: Guidance from the UNECE Task Force on Reactive Nitrogen. Edinburgh, UK. Centre for Ecology and Hydrology.

Canh, T. T., Aarnink, A. J. A., Mroz, Z., Jongbloed, A. W., Schrama, J. W., & Verstegen, M.W.A., 1998a. Influence of electrolyte balance and acidifying calcium salts in the diet of growing-finishing pigs on urinary pH, slurry pH and ammonia volatilization from slurry. Livest. Sci., 56, 1-13.

Canh, T. T., Aarnink, A. J. A., Schutte, J. B., Sutton, A. L., Langhout, D. J., Verstegen, M. W. A., & Schrama, J. W., 1998b. Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing-finishing pigs. Livest. Sci., 56, 181-191.

Canh, T. T., Aarnink, A. J. A., Verstegen, M. W. A., & Schrama, J. W.,1998c. Influence of dietary factors on the pH and ammonia emission of slurry from growing finishing pigs. J. Anim. Sci., 76, 1123-1130.

Canh, T. T., Sutton, A. L., Aarnink, A. J. A., Verstegen, M. W. A., Schrama, J. W., & Bakker, G. C. M., 1998d. Dietary carbohydrates alter the faecal composition and pH and ammonia emission from slurry of growing pigs. J. Anim. Sci. 76, 1887-1895.

Derikx, P. J. L., & Aarnink, A. J. A., 1993. Reduction of ammonia emission from slurry by application of liquid top layers. In: Verstegen, M. W. A., Den Hartog, L. A., Van Kempen, G. J. M., Meltz, J. H. M. European Association for Animal Production Publication; Nitrogen Flow in Pig Production And Environmental Consequences, 69, 344-349.

FAO., 2011. Livestock and environment, in: http://www.fao.org/livestock-environment/en/ Accessed 20.01.16.

Galassi, G., Colombini, S., Malagutti, L., Grovetto, G. M., & 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.

Hansen, M. N., Henriksen, K., & Sommer, S. G., 2006. Observations of production and emission of greenhouse gases and ammonia during storage of solids separated from pig slurry: Effects of covering. Atmos. Environ. 40 (22), 4172-4181.

Hernández, F., Martínez, S., López, C., Megías, M. D., 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(8), 1290–1298.

Jarret, G., Martínez, J., & Dourmad, J., 2011. Effect of biofuel co-products in pig diets on the excretory patterns of N and C and on the subsequent ammonia and methane emissions from pig effluent. Animal, 5(4), 622-631.

Jarret, G., Cerisuelo, A., Peu, P., Martínez, J., & Dourmad, J., 2012. Impact of pig diets with different fibre contents on the composition of excreta and their gaseous emissions and anaerobic digestion. Agric. Ecosyst. Environ. 160, 51-58.

Krupa, S. V., 2003. Effects of atmospheric ammonia (NH3) on terrestrial vegetation: a review. Environ. Pollut. 124, 179-221.

Kuehl, O. Robert, 2001. Design of Experiments. (2nd ed.) Mexico: International Thomson Editores, S.A. de C.V.

Lynch, M. B., Sweeney, T., Callan, J. J., & O'Doherty, J. V., 2007. The effect of dietary barley level on volatile fatty acid concentration and manure ammonia emissions in finishing pigs. Livest. Sci. 109, 236–239.

Morris T.R., 1999. Experimental design and analysis in animal sciences. Wallingford: Ed. CABI.

Mroz, Z., Moeser, A. J., Vreman, K., Van Diepen, J. T., Van Kempen, T., Canh, T. T., & Jongbloed, A. W., 2000. Effects of dietary carbohydrates and buffering capacity on nutrient digestibility and manure characteristics in finishing pigs. J. Anim. Sci. 78, 3096–3106.

Ndegwa, P. M., Vaddella, V. K., Hristov, A. N., & Joo, H. S., 2009. Measuring concentrations of ammonia in ambient air or exhaust air stream using acid traps. J. Environ. Qual. 38, 647-653.

O'Connell, J. M., Sweeney, T., Callan, J. J., & O'Doherty, J. V., 2005. The effect of cereal type and exogenous enzyme supplementation in pig diets on nutrient digestibility, intestinal microflora, volatile fatty acid concentration and manure ammonia emissions from finisher pigs. Anim. Sci. 81, 357-364.

O'Connell, J. M., Callan, J. J., & O'Doherty, J. V., 2006. The effect of dietary crude protein level, cereal type and exogenous enzyme supplementation on nutrient digestibility, nitrogen excretion, faecal volatile fatty acid concentration and ammonia emissions from pigs. Anim. Feed. Sci. Technol., 127, 73–88.

O'Shea, C. J., Lynch, B., Lynch, M. B., Callan, J. J., & O'Doherty, J. V., 2009. Ammonia emissions and dry matter of separated pig manure fractions as affected by crude protein concentration and sugar beet pulp inclusion of finishing pig diets. Agr. Ecosyst. Environ. 131, 154–160.

O'Shea, C. J., Sweeney, T., Lynch, M. B., Gahan, D. A., Callan, J. J., & O'Doherty, J. V., 2010. Effect of β-glucans contained in barley- and oat-based diets and exogenous

enzyme supplementation on gastrointestinal fermentation of finisher pigs and subsequent manure odor and ammonia emissions. J. Anim. Sci., 88, 1411-1420.

Pereira, J., Fangueiro, D., Misselbrook., T., Chadwick, D. R., Coutinho, J., & Trindade, H., 2011. Ammonia and greenhouse gas emissions from slatted and solid floors in dairy cattle houses: A scale model study. Biosyst. Eng. 109(2), 148-157.

Pereira, J., Misselbrook., T., Chadwick, D. R., 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(2), 138-150.

Portejoie, S., Dourmad, J. Y., Martínez, J., & Lebreton, Y., 2004. Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs. Livest. Sci., 91, 45-55.

SAS, 2008. SAS/STAT User's Guide. (Release 9.2). SAS Inst. Inc., Cary NC, USA.

Sokal, R. Robert and Rohlf F. James, 2002. Introduction to biostatistics. 2nd ed. Barcelona: Reverté.

Vaddella, V. K., Ndegwa, P. M., & Joo H. S., 2011. Ammonia loss from simulated post-collection storage of scraped and flushed dairy-cattle manure. Biosyst. Eng. 110, 291 - 296.

Xue, S. K., Chen, S., & Hermanson, R. E., 1998. Measuring ammonia and hydrogen sulfide emitted from manure storages. T. ASAE. 41(4), 1125–1130.

6
Kinetics and prediction of methane production in pig slurry

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Abstract. Pig slurry is a source of atmospheric pollutants such as methane (CH_4). However, it is also a valuable resource for the production of biogas under controlled conditions. Predicting the biochemical methane potential (BMP) is essential to minimize undesired emissions and optimize biogas reactors. In this study, BMP assays of 70 slurry samples from commercial farms and 77 samples of reconstituted slurry from experimental assays were conducted and analyzed for the CH_4 production kinetics. Also, chemical composition (total solids, volatile solids, total Kjeldahl nitrogen, ether extract and fiber fractions) were determined. The CH_4 production kinetics of each sample was adjusted by means of three non-linear models: First order, Gompertz and Mitscherlich. Kinetic parameters of these curves were determined and related to slurry composition. Despite their different origin, both slurries from commercial farms and experimental trials followed a similar tendency. The Mitscherlich model adjusted best to the measured CH_4 production. Ether extract, neutral detergent fiber and volatile fatty acids concentration were found to affect CH_4 kinetics. Also, partial measurements of BMP were found to be representative of the final BMP value. In this regard, measures until day 53 of the BMP assay allowed coefficients of determination (R^2) over 0.9 with BMP. In conclusion, this study provides evidences on the factors affecting the CH_4 emission dynamics and explores prediction models.

Abbreviations: BMP, biochemical methane potential; CH_4, methane; DM, dry matter; EE, ether extract; GHG, greenhouse gasses; OM, organic matter; SV, volatile solids; TKN, total Kjeldahl nitrogen; TAN, total ammonia nitrogen; VFA, volatile fatty acids.

Keywords: methane emission, biochemical methane potential, kinetics of methane production, prediction model, slurry characterization, biogas

6.1 Introduction

Animal slurries are considered a valuable source of nutrients for crops, but at the same time their management is associated to environmental pollution risks. Excessive or inadequate land application may lead to soil and water pollution (Aarnink & Verstegen, 2007), and slurries are considered a relevant source of ammonia (NH_3) and greenhouse gases (GHG), mainly methane (CH_4) and nitrous oxide (N_2O) (Liu et al., 2013). As a consequence of the sustained growth and concentration of the global swine population (FAOSTAT, 2015), in recent years there has been a focus on the sustainable use of nutrients in livestock production systems (Hou et al., 2016) and on the reduction of the associated emissions of atmospheric pollutants (Beccaccia et al., 2015a; Hansen et al., 2014).

Nevertheless, CH_4 can also be produced from slurries under controlled conditions (alone or in combination with other organic wastes) to obtain biogas as a renewable source of energy, as well as more stable fertilizers for crops (Asam et al., 2011; Holm-Nielsen et al., 2009; Molino et al., 2013; Zhang et al., 2014a). The perspectives of scarcity of raw materials, particularly in the agricultural sector, encourages us to explore the most efficient ways for the valorisation of the livestock by-products (Matassa et al., 2015; Schoumans et al., 2015).

The potential production of CH_4 is a fundamental parameter to estimate potential emissions from slurry management and climate change mitigation options (IPCC., 2014), as well as to determine the aptitude for biogas production (Angelidaki et al., 2009). The potential production of CH_4 from a certain substrate is determined by means of the biochemical methane potential (BMP). This assay consists in a laboratory batch digestion of the substrate in the presence of an anaerobic inoculum, at a certain inoculum to substrate ratio (Angelidaki et al., 2009). The mixture is maintained in anaerobic conditions at a certain temperature until biogas production becomes negligible, and during the assay biogas production and CH_4 content of biogas are regularly monitored. As indicated by Strömberg et al. (2015), BMP tests provide information not only on the amount of biogas produced, but also on the decomposition rates of substrates, which is required information for the design and operation of biogas reactors. However, BMP determinations require a long time

(usually up to 3 months) which makes this technique inoperative to assess digestion substrates in real farm conditions.

Recent research has focused on predicting BMP in practice using NIRS (Ward, 2016). However, this author evidences that there are still some obstacles, and particularly stresses that the reference method for BMP is characterized by a low precision. Similar considerations can be adopted for BMP prediction based on the substrate chemical composition (Labatut et al., 2011; Thomsen et al., 2014). Also, as evidenced by Strömberg et al. (2015), these prediction methods do not provide information on the degradation kinetics of the substrates, which is essential for decision-making in slurry management processes (Chadwick et al., 2011; Mähnert and Linke, 2009; Moody et al., 2009; Pham et al., 2013), as well as in the design and operation of real scale biogas reactors (Triolo et al., 2013). Therefore, recent attempts in research aim to develop accurate kinetic models to predict methane emissions from an initial dataset of a BMP assay (Brulé et al., 2014; Kafle and Chen, 2016; Strömberg et al., 2015; Sun et al., 2015a).

To our knowledge, studies reporting the kinetics of CH_4 production and BMP prediction of pig slurries from commercial origins are scarce and those studies reporting kinetics of CH_4 emission include a relatively poor representation of pig slurries. Previous studies have also shown a wide variability in pig slurry composition and emissions, which in turn is affected by a multiple factors (Antezana et al., 2016; Triolo et al., 2013; Zhang et al., 2014b). Therefore, relevant differences in methane kinetics could also be expected among pig slurries. The purpose of this study was to characterize the kinetics of CH_4 production in order to obtain predictive models of the BMP from a wide dataset of pig slurries from commercial farms and nutrition experiments.

6.2 Materials and methods

6.2.1 Slurry Samples

A total of 147 pig slurry samples were evaluated in this study. Slurry samples were obtained from two origins: slurries obtained from slurry pits at commercial farms (n=70)

and reconstituted slurries obtained from experimental feeding assays of growing pigs (n=77).

Slurry samples from Spanish commercial farms were obtained from different animal types: gestating sows (n=30), lactating sows (n=14), nursery piglets (n=13) and growing-finishing pigs (n=13) as explained by Antezana et al. (2016). Briefly, representative slurry samples were obtained by sampling a minimum of five two-liter aliquots at equidistant intervals during the discharge of slurry pits. The composite sample was thoroughly mixed and subsamples were taken for the corresponding analyses.

Reconstituted slurries were obtained from the assays described by Antezana et al. (2015); Beccaccia et al. (2015a, b) , which analyzed the effect of different sources of protein (n=17), fiber (n=30) and fat (n=30), respectively, on nutrition traits, slurry composition and gaseous emissions. All diets tested in these assays were formulated according to commercial standards (FEDNA, 2006). Urine and feces were collected separately in metabolism pens for three days and then slurry was reconstituted according to the original excretion ratio.

6.2.2 Experimental procedures, sample preparation and chemical analyses

Slurries were analyzed for dry matter (DM), ash, fiber fractions (neutral detergent fiber (NDF) and acid detergent fiber (ADF)), ether extract (EE), and total Kjeldahl nitrogen (TKN). Organic matter (OM) was calculated from the difference between dry matter and ash contents. Standard procedures were used (APHA, 2005; AOAC, 2000; Van Soest et al., 1991). All slurry components were expressed and analyzed in dry matter basis. Additionally, pH was measured in fresh slurries (from commercial farms) or immediately after reconstitution (reconstituted slurries) with a glass electrode (Crison Basic 20+, Crison, Barcelona, Spain). More details on the experimental procedures can be found in Beccaccia et al. (2015b) and Beccaccia et al. (2015c).

6.2.3 Biochemical CH_4 potential

For each slurry sample, BMP was determined by triplicate in a batch assay using 120 mL glass bottles following the methodology described by Angelidaki et al. (2009). Inoculum

from a mesophilic pig slurry anaerobic digester reactor was used. The inoculum was pre-incubated during 15 days at 35ºC in order to deplete the residual biodegradable organic material. A mixture of pig slurry and inoculum was made to obtain an inoculum to substrate ratio of 1:1 in OM basis. Additionally, three blank bottles containing only degasified inoculum were also used in order to determine the inoculum endogenous CH_4 production which was subtracted from the CH_4 produced by the pig slurry on each biogas sampling day. After filling, each bottle was sealed with butyl rubber stoppers and aluminum crimps and the headspace was flushed with pure N_2 for two minutes. Bottles were then incubated at 35°±1C for 100 days. 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). CH_4 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.

6.2.4 Statistical analysis and model development

A descriptive analysis of slurry composition and BMP was conducted by type of slurry (from commercial farms vs. reconstituted slurries) through PROC MEANS of SAS Institute (2008).

The statistical analysis explored the correspondence between measured CH_4 production and different models defining the emission kinetics. According to literature, different models can be used to describe the evolution of CH_4 emission by a substrate (Kafle and Chen, 2016; Li et al., 2015; Strömberg et al., 2015; Sun et al., 2015a). For agricultural substrates, it has been reported that the most adequate are the first order kinetic model, the Mitscherlich model and the Gompertz model. Therefore, the suitability of these models was analyzed. Other models also described in the literature (e.g. logistic, exponential or polynomic) were also preliminarily assessed and discarded due to the lack of correspondence with measured data. In the different models, methane yield at a certain day (BMP(t)) was expressed in relative terms to BMP according to Table 6.1

Table 6.1. Models used to describe methane production as a function of time (t, expressed in days)

Model	Equation	Model parameters
First order		k: methane production rate (1/day). e: Euler number. c: shape factor (duration of the lag phase, in days) lag: duration of the lag phase (days)
Mitscherlich		
Gompertz		

The fitting of each model to the real kinetics of CH_4 production was assessed by applying a least squares fit to the equations, using PROC NLIN of SAS Institute (2008). For each model the kinetic parameters were obtained and statistical adjustment was determined using the relative root mean square error of prediction (rRMSE), modeling efficiency (EF) and coefficient of residual mass (CRM) according to the following equations (Triolo et al., 2011).

$$rRMSE = \frac{\sqrt{\frac{\sum_{i=1}^{n}(P_i - O_i)^2}{n}}}{\bar{O}} \qquad (1)$$

$$EF = \frac{\sum_{i=1}^{n}(O_i - \bar{O})^2 - \sum_{i=1}^{n}(P_i - O_i)^2}{\sum_{i=1}^{n}(O_i - \bar{O})^2} \qquad (2)$$

$$CRM = \frac{\sum_{i=1}^{n} O_i - \sum_{i=1}^{n} P_i}{\sum_{i=1}^{n} O_i} \qquad (3)$$

Where O_i is the measured value, $\bar{O}$ is the mean of the measured values, P_i is the predicted value, and n is the number of data points.

Furthermore, the kinetic parameters of the three models for each slurry sample (n=147) were calculated by non-linear regression using the Solver function of Microsoft Excel. To establish potential relationships between the characteristics of the slurry and the kinetic parameters of CH_4 production, a correlation analysis was done using PROC CORR of SAS Institute (2008).

6.2.5 Kinetic prediction of BMP

In order to predict BMP from partial datasets of methane yield production including the initial days of the assay, two non-linear prediction models were explored, using fixed or flexible model parameters. As indicated by Stromberg et al. (2015), preliminary information from the BMP assay can be used as a boundary condition to establish a kinetic model in order to predict the CH_4 production from the remaining part of the assay. First, fixed prediction models were developed using the kinetic parameters of the Gompertz and Mitscherlich models obtained from the whole dataset, as explained before. Thus, the BMP for each slurry sample was calculated according to the general models obtained from all available data. On the contrary, flexible non-linear prediction models were calculated specifically for each sample, using the Solver function of Microsoft Excel.

Fixed prediction models aimed to use the average information of a dataset with multiple samples, thus trying to obtain more robust model coefficients. On the contrary, the flexible models aimed to use the information of each particular curve and therefore it may pose potential benefits in the case of heterogeneous samples such as slurries from commercial farms. In all cases, the BMP calculated and BMP observed were compared and the coefficient of determination (R^2), the rRMSE and the relative average error (rAE) were determined.

6.3 Results and discussion

6.3.1 Pig slurry characteristics

Table 6.2 shows the average properties of slurries and their ranges, separately for slurries from commercial farms and for reconstituted slurries. It can be appreciated that both types of slurries differed in their properties, which is consequence of their different origins.

It was found that the reconstituted slurry was on average 2.8 times more concentrated in total solids than slurries from commercial farms, in which dilution with water occurs as discussed in literature (Antezana et al., 2016; Triolo et al., 2013). Also, more variability was found in composition and BMP of slurries from commercial farms, which can be related to the multiple interrelated and uncontrolled factors under commercial conditions. A deep analysis of the differences between both types of slurries lacks of practical interest and therefore is not objective of this study. However, some interesting remarks can be made here which may affect BMP and CH_4 production kinetics.

Table 6.2. Characteristics of pig slurries and correlation with the BMP.

Characteristics of pig slurry	All samples (n:147)		Commercial slurries (n:70)					Reconstituted slurries (n:77)				
	mean	SD	mean	min	max	SD	Correlation with BMP	mean	min	max	SD	Correlation with BMP
pH	7.50	0.69	7.49	6.34	8.16	0.43	-0.47***	7.51	5.88	8.93	0.86	0.43***
TS (%)	10.0	6.06	5.2	0.5	17.7	3.96	0.12 ns	14.3	5.9	23.0	4.0	-0.18 ns
VS (%)TS	76.5	4.59	72.1	55.7	84.5	6.65	0.32**	80.5	63.8	84.8	2.7	-0.004 ns
TKN (%) TS	9.4	5.24	12.4	3.8	37.7	6.25	-0.05 ns	6.7	3.4	11.1	1.1	0.15 ns
EE (%)TS	9.5	4.03	10.3	2.9	24.9	4.16	0.65***	8.8	4.4	17.8	3.8	0.33**
NDF (%) TS	44.1	16.7	36.0	5.2	59.6	11.7	-0.24*	51.4	19.6	100.1	17.1	-0.01 ns
ADF (%)TS	20.5	8.10	17.6	2.2	36.3	7.33	-0.38**	23.1	8.6	44.5	7.92	-0.04 ns
VFA (mmol g TS^{-1})	0.97	0.85	1.47	1.01	5.01	1.15	0.63***	0.62	0.23	1.03	1.06	0.42***
BMP (mL CH_4 gVS^{-1})	285	101	243	10.3	506	130		330	210	505	60.3	

TS, total solids; VS, volatile solids; TKN, total Kjeldahl nitrogen, EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; VFA, volatile fat acid; BMP biochemical CH_4 potential. *,**,***, significant at $P<0.05$, $P<0.01$, $P<0.001$, respectively; ns, not significant.

When expressed on dry matter basis, it is remarkable that slurries from commercial farms had on average higher contents of TKN (85%) and EE (18%) than reconstituted slurries. Contrarily, they had lower amounts of fiber content (30% and 23% lower contents of NDF and ADF, respectively) than reconstituted slurries from experimental assays. Regarding volatile fatty acids, slurries from commercial farms had on average more than double content (expressed as mmol VFA g TS^{-1}) than slurries from experimental studies. This greater amount of VFA may be due to hydrolysis processes which may have already started in the slurry stored in the pits for several weeks, as indicated by Philippe et al. (2015) and Triolo et al. (2013).

As detailed by Antezana et al. (2016), storage time of slurries at commercial facilities varied between 1 and 4 months. Therefore, it may be expected that a proportion of organic matter has been degraded already and N content has been reduced due to NH_3 emissions. On the contrary, our results show higher nutrient contents on dry matter basis in slurries obtained from uncontrolled (commercial) conditions than from controlled experiments. Therefore, both types of slurries may not only differ due to dilution with spillage or cleaning water, but slurries from commercial farms may also have followed an uncontrolled stabilization process and might also contain a certain amounts of waste food. This contributes to the heterogeneous composition of slurries in commercial conditions described in previous studies (Antezana et al., 2016; Martínez-Suller et al., 2008; Suresh et al., 2009; Triolo et al., 2013; Yagüe et al., 2012).

In all cases, composition of slurries used in this study are in the range of other references on slurry characterization (Aarnink and Verstegen, 2007; Jarret et al., 2012, 2011; Philippe and Nicks, 2015).

6.3.2 Kinetic analysis of CH_4 production

The kinetics of CH_4 production showed on average a similar trend among samples from commercial and experimental origins, but the evolution of CH_4 production was more variable in slurries from commercial farms than in reconstituted slurries (Figure 6.1). On

day 6 the range of CH_4 production varied from 1.41 to 32% and 3.4 to 25% of BMP in commercial and reconstituted slurries, respectively. On day 11 a wider range was found in slurries from commercial farms (4.21 to 71.1% of BMP) compared with the reconstituted slurry (16.3 to 53.2% of BMP).

A short initial lag time was observed at the beginning of the BMP tests, which was apparently lower in slurries from commercial farms. On day 3, 82% of slurry samples from commercial farms and 65% of reconstituted slurry samples produced less than 5% of BMP. However, on day 6, 42% of commercial slurry samples and 39% of reconstituted slurry samples had already produced more than 10% of the BMP. After this initial period, most CH_4 was produced between days 15 and 32, when slurries had already produced on average more than 80% of the BMP. The time required to achieve a 90% of BMP (T_{90}) also ranged widely from 25 days to 74 days in slurries from commercial farms and from 32 to 67 days in reconstituted slurries. In general terms, T_{90} of pig slurry was higher and much more variable in this study than in published references, which report T_{90} values ranging from 17 to 30 days (Kafle and Chen, 2016; Wall et al., 2013; Zhang et al., 2014b).

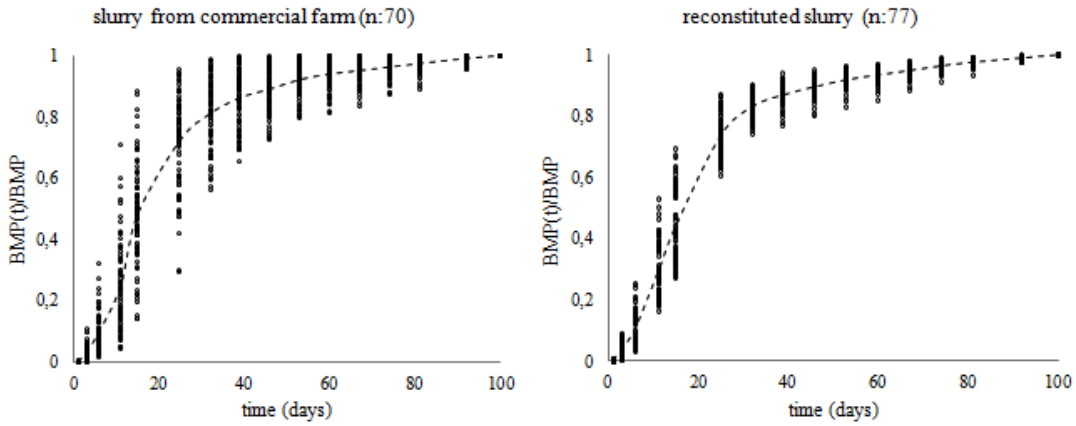

Figure 6.1. Kinetics of the CH_4 production from pig slurries obtained from commercial farms (left) and from reconstituted slurry (right). The evolution is presented in relative terms to the BMP

The model parameters of CH_4 production kinetics are presented in Table 6.3. In accordance with the initial lag phase previously described, the first order model did not fit to the measured CH_4 production rates because this model does not account for a lag phase. In this regard, several studies agree that the models that best describe the dynamics of CH_4 production from slurries or from co-digested slurries are those who consider initial phases of latency. In these studies, kinetic constants and different latency periods are related to the composition of the organic matter of the substrates (Ji et al., 2015; Korazbekova et al., 2015; Li et al., 2015).

High coefficients of determination for Mitscherlich and Gompertz models (R^2> 0.91) were found, and therefore these models described more accurately the kinetics of CH_4 production from pig slurry. Both models consider an inflection point that would represent an initial lag phase in the evolution of CH_4 production. This is also in accordance with results reported by Sun et al. (2015) who evaluated six kinetic models and found lower residual errors in models considering initial lag phases. On the other hand, the methane production rate "k" obtained for the Mitscherlich and Gompertz models was numerically higher for reconstituted slurries than for slurries from commercial farms, but no significant differences were detected. Despite the differences in average composition between both types of slurry, it seems that ageing of slurry related to accumulation in the pits in commercial farms has limited effect on the emission kinetics of pig slurries.

Given that variation in composition and kinetic parameters among slurry samples were detected, we hypothesized that the chemical composition of slurry could affect the kinetic parameters of the models. To explore these relationships, Table 6.4 shows the correlations between experimental data of CH_4 production and the parameters of Mitscherlich and Gompertz models (those with best adjustment to experimental data).

Comentario [VEHE2]: en en la cinetica de primer orden (modelo 1) nos da información acerca de cuanto de tarda en alcanzar el 50% de la B0, a mayor K menos tiempo (de acuerdo con la la tabla 6.3 el purín comercial necesita 35 dias y el reconstituido 37), y esto cuadra con los resultados presentados anteriormente en cuanto a que el purín comercial alcanza antes la Bo
Aunque yo no uso exactamente la misma ecuación de gompertz, en teoría la k en ese modelo da información sobre el punto de pendiente máxima, lo que significa que el purín comercial en su momento de máxima produccion produce mas metano por dia y solido volátil que el reconstituido, y solo puede ser por lo que comentais un poco mas arriba de que tiene mas materia organica fácilmente biodegradable, (justificado por la mayor EE y TKN por gramo de ST, auqnue yo no veo muy asi, como explico arriba) como piensos.
Aunque es un poco raro pq esta envejecido, por lo que yo habría esperado lo contrario, pero al menos los resultados caudran.
El otro modelo no lo he usado nunca y no tengo acceso a ningún articulo donde expliquen el significado "biológico" de la k, pero estaría way sacar algo mas de jugo, auqnue las diferencias no son significativas.
Walter:
Es posible que la K sea mayor en purines reconstituidos por que son heces frescas por un lado y por otro que su concentración por litro es mayor que el de los purines comerciales. y como tal tendrían mayor materia degradable

Table 6.3. Summary of results of kinetic study using three different models of production of CH_4 from pig slurry

Model	parameter kinetics			parameter of model			
Type slurry	k	c	Lag	rRMSE	EF	CRM	R^2
First order							
Commercial farm (n:70)	0.0197			0.345	0.98	0.264	0.513
Reconstituted slurry (n:77)	0.0189			0.352	0.98	0.291	0.438
Mitscherlich							
Commercial farm (n:70)	0.0694	1.982		0.138	1.00	-0.018	0.923
Reconstituted slurry (n:77)	0.0734	2.034		0.074	1.00	-0.014	0.975
Gompertz							
Commercial farm (n:70)	0.0905		2.959	0.134	1.00	-0.032	0.916
Reconstituted slurry (n:77)	0.0945		3.079	0.081	0.97	-0.014	0.970

k, methane production rate (1/day); c, shape factor (duration of the lag phase, in days); lag, duration of the lag phase (days); rRMSE, relative root mean square error; EF, model efficiency; CRM, coefficient of residual mass; R^2, coefficient of determination.

Table 6.4. Correlation coefficients between individual kinetics parameters of CH_4 production and characteristics of the slurry from commercial farm (n: 70) and reconstituted slurry (n: 77)

Model and type slurry	model parameters	correlation coefficients							
		pH	TS	VS	TKN	EE	NDF	ADF	VFA
Mitscherlich									
Slurry from commercial farm									
	k	-0.36**	0.02 ns	0.24*	0.00 ns	0.54***	-0.21*	-0.27*	0.50***
	c	-0.19 ns	0.05 ns	0.08	0.01 ns	0.45***	-0.28*	-0.30*	0.48***
Reconstituted slurry									
	k	-0.14 ns	-0.35**	0.00 ns	0.05 ns	-0.03 ns	-0.53***	-0.12 ns	0.34**
	c	-0.28*	-0.59***	-0.02 ns	0.35*	-0.46***	-0.57***	-0.07 ns	0.44**
Gompertz									
Slurry from commercial farm									
	k	-0.46***	-0.06 ns	0.29*	-0.03 ns	0.63***	-0.21*	-0.30*	0.47***
	Lag	-0.06 ns	0.10 ns	0.02 ns	0.08 ns	0.32**	-0.23*	-0.26*	0.34**
Reconstituted slurry									
	k	0.06 ns	-0.02 ns	0.02 ns	-0.17 ns	0.32*	-0.27*	-0.09 ns	0.10 ns
	Lag	-0.42***	-0.57***	-0.01 ns	0.33*	-0.44**	-0.55***	-0.11 ns	0.36**

TS, total solid (%); VS, volatile solid (%TS); TKN, total Kjeldahl nitrogen (%TS), EE, ether extract (%TS); NDF, neutral detergent fiber (%TS); ADF, acid detergent fiber (%TS); VFA, volatile fatty acids (mmol gST-1); k, methane production rate (day-1); c, shape factor (duration of the lag phase, in days); lag, duration of the lag phase (days).

In slurries from commercial farms, relatively high correlation coefficients between the EE and the methane production rate (k) were found (r = 0.54 and 0.63 for Mitscherlich and Gompertz models, respectively, P<0.001). Similarly, positive correlations were detected between k and VFA (0.50 and 0.47 respectively, P<0.001). Regarding the lag time (c and lag parameters), positive correlations were also obtained with EE content (correlation coefficients from 0.32 to 0.48, P<0.001 and P<0.05, respectively). On the other hand, relatively low and negative correlations were detected between fiber content and the kinetic parameters of the models. These relationships suggest that slurries from commercial farms with higher contents of EE and VFA have higher rates of CH_4 production but concurrently the initial lag times would be lower.

The EE content in slurries is mainly related to the excretion of undigested and endogenous (microbial) fat (Kil et la., 2010). However, under commercial conditions, OM content in the slurry can also arise from waste feed which is mixed with the manure. Fat and other constituents from the feed are expected to involve higher BMP and faster methane production rates, since they have not been subjected to digestion by pigs. Additionally, it must be considered that the amount of OM contained in a commercial feed is around 95% TS (FEDNA 2006) while in slurry this amount is on average much lower (Antezana et al., 2016). Therefore, it is likely that feed waste causes increased BMP of the sludge, because the issuance of raw materials is significantly higher than that of the slurries (Labatut et al., 2011; Li et al., 2013; Luna-del Risco et al., 2011). On the other hand, the VFA contained in the slurry are intermediate products of the degradation of organic matter, and therefore their concentration would be variable depending on the storage conditions and the nutrient content. In the same way, in slurries with higher contents of NDF and ADF, the production rate of CH_4 and the rate of latency would be lower.

In our case, it is possible that higher proportions of NDF in the OM of slurries entail lower concentrations of EE, and consequently the factor k could be lower. This would coincide with the results indicated by Zhang et al. (2014b) in which, “lag” and “c” parameters were higher in the slurries with higher contents of hemicellulose, and consequently obtained lower values for k and BMP. On the other hand greater “lag” and “c” could also suggest

that the inoculum is not fully acclimated to the substrate (NDF, ADF and VFA) (Raposo et al., 2012; Wall et al., 2013).

Contrarily, in the case of the reconstituted slurries the correlation between EE and latency period was negative, whereas non-significant correlations were found between EE and k. Similarly, the content of NDF was negatively correlated with k and latency. In this case, we can assume that the ether extract from reconstituted slurry is mostly indigestible fat; which could be associated to lower CH_4 production.

Regarding VFA, the content in reconstituted slurries was lower than in slurries from commercial farms. As discussed before, this circumstance may be caused by the degradation processes occurring in the pits, which are produced in a lesser extent in fresh reconstituted slurries. Consequently, VFA was positively correlated with k in both types of slurries, but this correlation was lower in reconstituted slurries. Therefore, apparently a high concentration of VFA could be related to longer lag periods in slurries from commercial farms than in reconstituted slurries. In this regard, Weiland, (2010) indicates that an effect of inhibiting the initial methanogenesis could be produced in slurries from commercial farms with high concentrations of VFA. This trend is similar, but to a lesser extent, in reconstituted slurries coinciding with a lower concentration of VFA, probably because these slurries did not undergo a storage period in pits prior to the BMP test.

These results suggest that the kinetics of methane production and BMP are affected by nutritional factors (associated with the ingredients and compositions of diets) and to management conditions (for example slurry storage time or wasted feed). Besides, there may be differences among feed constituents, for example different types and proportions of saturated and unsaturated fats, or the proportions of the different components of NDF and ADF, among others (Philippe et al., 2015; Triolo et al., 2013; Yadvika et al., 2004).

6.4 Prediction of BMP

The results of the comparison of the predicted values versus the observed values with fixed and free modeling are presented in Table 6.6.

Table 6.6 Statistical parameters of the comparison of observed and predicted values of the BMP of pig slurry with different prediction models

Prediction	production accumulated until the day used for the calculation of the BMP							
model	15	25	32	39	46	53	60	74
Fixed								
Gompertz								
R^2	0.486	0.803	0.916	0.953	0.977	0.984	0.988	0.995
rRMSE	1.204	0.592	0.337	0.238	0.162	0.139	0.113	0.076
rAE	0.887	0.402	0.232	0.168	0.118	0.094	0.079	0.051
Mitscherlich								
R^2	0.487	0.804	0.916	0.953	0.977	0.984	0.988	0.995
rRMSE	1.130	0.595	0.346	0.245	0.166	0.137	0.115	0.077
rAE	0.833	0.409	0.238	0.173	0.122	0.097	0.083	0.052
Free								
Gompertz								
R^2	0.145	0.763	0.868	0.891	0.955	0.976	0.983	0.989
rRMSE	1.607	0.592	0.395	0.353	0.223	0.159	0.134	0.106
rAE	0.837	0.369	0.260	0.198	0.148	0.113	0.097	0.075
Mitscherlich								
R^2	0.125	0.655	0.793	0.932	0.951	0.976	0.986	0.993
rRMSE	3.179	0.820	0.562	0.281	0.231	0.161	0.128	0.093
rAE	1.025	0.626	0.386	0.194	0.148	0.110	0.092	0.067

R^2, coefficient of determination; rRMSE, relative root mean squared error of prediction; rAE, relative absolute error.

As expected, the prediction accuracy improves as the BMP progresses. Mitscherlich and Gompertz prediction models performed very similarly for fixed and free models. Using fixed models, BMP early estimates (e.g. from 25 days) would involve relatively high rAE (over 40%), whereas from day 53 it would be possible to obtain rAE lower than 10%. On the contrary, free modelling using Mitscherlich and Gompertz models did not improve the prediction at any stage (e.g. rAE over 11% on day 53). Our results show lower prediction accuracy than similar studies using different substrates (e.g. Kafle and Chen, 2016; Strömberg et al., 2015; Sun et al., 2015b). These differences can be related to the slower methane production rates in this study, where T_{90} is achieved from day 25 to 74, which means that initial days provide poor information on the final behavior of the BMP test. Contrarily, BMP tests reported in the literature show faster methane production rates and therefore a major proportion of CH_4 is produced in earlier stages. It is important to

highlight that the number of slurry samples evaluated in the present study, as well as the diversity of physicochemical characteristics contribute to strengthen the results and their applicability in estimates of the BMP from pig slurries. Our results suggest that it would not be possible to reduce the BMP test time for pig slurries to less than 50 days without increasing the error in 10%.

6.5 Conclusions

- The kinetics of CH_4 production potential from pig slurries fits a sigmoidal function with an initial lag time of about 3 days, followed by a period of intense CH_4 production until day 32, and a final period in which the intensity of CH_4 production is lower.
- The Mitscherlich model describes most accurately the evolution of CH_4 production as a function of time. However, both Gompertz and Mitscherlich models perform similarly to predict BMP from partial measurements.
- Predicting BMP from pig manure is possible using the first 50 days of the BMP tests with less than 10% error.

6.6 References

Aarnink, A.J.A., Verstegen, M.W.A., 2007. Nutrition, key factor to reduce environmental load from pig production. Livest. Sci. 109, 194–203.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, a. J., Kalyuzhnyi, S., Jenicek, P., Van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. Water Sci. Technol. 59, 927–934.

Antezana, W., Calvet, S., Beccaccia, A., Ferrer, P., De Blas, C., García-Rebollar, P., Cerisuelo, A., 2015. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing pigs: III. Influence of varying the dietary level of calcium soap of palm fatty acids distillate with or without orange pulp supplementation. Anim. Feed Sci. Technol.

Antezana, W., De Blas, C., García-Rebollar, P., Rodríguez, C., Beccaccia, a., Ferrer, P., Cerisuelo, a., Moset, V., Estellés, F., Cambra-López, M., Calvet, S., 2016. Composition, potential emissions and agricultural value of pig slurry from Spanish commercial farms. Nutr. Cycl. Agroecosystems.

AOAC, 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Washington, DC, USA.

APHA, 2005. Standard methods for the examination of water and wastewater, Centennial. ed. Baltimore, Maryland.

Asam, Z.-Z., Poulsen, T.G., Nizami, A.-S., Rafique, R., Kiely, G., Murphy, J.D., 2011. How can we improve biomethane production per unit of feedstock in biogas plants? Appl. Energy 88, 2013–2018.

Beccaccia, A., Calvet, S., Cerisuelo, A., Ferrer, P., García-Rebollar, P., De Blas, C., 2015a. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing-finishing pigs. I. Influence of the inclusion of two levels of orange pulp and carob meal in isofibrous diets. Anim. Feed Sci. Technol. 208, 158–169.

Beccaccia, A., Cerisuelo, A., Calvet, S., Ferrer, P., Estellés, F., De Blas, C., García-Rebollar, P., 2015b. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing pigs: II. Effect of protein source in practical diets. Anim. Feed Sci. Technol. 209, 137–144.

Beccaccia, A., Ferrer, P., Ibáñez, M.A., Estellés, F., Rodríguez, C., Moset, V., Blas, C. De, Calvet, S., García-rebollar, P., 2015. Relationships among slurry characteristics and gaseous emissions at different type|s of commercial Spanish pig farms. Spanish J. Agric. 13, 1–15.

Brulé, M., Oechsner, H., Jungbluth, T., 2014. Exponential model describing methane production kinetics in batch anaerobic digestion: a tool for evaluation of biochemical methane potential assays. Bioprocess Biosyst. Eng. 1–12.

Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B., Misselbrook, T., 2011. Manure management: Implications for greenhouse gas emissions. Anim. Feed Sci. Technol. 166-167, 514–531.

FEDNA, 2006. In: De Blas, C., Gasa, J., Mateos, G.G. (Eds.), Necesidades nutricionales para ganado porcino: normas FEDNA. Fundación Española para el Desarrollo de la Nutrición Animal, Madrid, Spain, 55 pp.

Hansen, M.J., Nørgaard, J. V., Adamsen, A.P.S., Poulsen, H.D., 2014. Effect of reduced crude protein on ammonia, methane, and chemical odorants emitted from pig houses. Livest. Sci. 169, 118–124.

Holm-Nielsen, J.B., Al Seadi, T., Oleskowicz-Popiel, P., 2009. The future of anaerobic digestion and biogas utilization. Bioresour. Technol. 100, 5478–84.

Hou, Y., Bai, Z., Lesschen, J.P., Staritsky, I.G., Sikirica, N., Ma, L., Velthof, G.L., Oenema, O., 2016. Feed use and nitrogen excretion of livestock in EU-27. Agric. Ecosyst. Environ. 218, 232–244.

IPCC, 2014: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Summaries, Frequently Asked Questions, and Cross-Chapter Boxes. A Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C.B., V.R. Barros, D.J. Dokken, K.J. Mach, M.D. Mastrandrea, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken,P.R. Mastrandrea, and L.L. White (eds.)]. World Meteorological Organization, Geneva, Switzerland, 190 pp.

Jarret, G., Cerisuelo, A., Peu, P., Martinez, J., Dourmad, J.Y., 2012. Impact of pig diets with different fibre contents on the composition of excreta and their gaseous emissions and anaerobic digestion. Agric. Ecosyst. Environ. 160, 51–58.

Jarret, G., Martinez, J., Dourmad, J.-Y., 2011. Effect of biofuel co-products in pig diets on the excretory patterns of N and C and on the subsequent ammonia and methane emissions from pig effluent. Animal 5, 622–631.

Ji, X., Lin, W., Zhang, W., Yin, F., Zhao, X., Wang, C., Liu, J., 2015. Evaluation of methane production features and kinetics of Bougainvillea spectabilis Willd waste under mesophilic conditions 14, 1910–1917.

Kafle, G.K., Chen, L., 2016. Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. Waste Manag. 48, 492–502.

Korazbekova, K., Bakhov, Z., Mutaliyeva, B., 2015. Influence of Co-substrates for the Methane Production Rate Kinetics in Anaerobic Fermentation of Pig Slurry. Mod. Appl. Sci. 9, 217–225.

Labatut, R.A., Angenent, L.T., Scott, N.R., 2011. Biochemical methane potential and biodegradability of complex organic substrates. Bioresour. Technol. 102, 2255–64.

Li, K., Liu, R., Sun, C., 2015. Comparison of anaerobic digestion characteristics and kinetics of four livestock manures with different substrate concentrations. Bioresour. Technol. 198, 133–140.

Li, Y., Zhang, R., Liu, G., Chen, C., He, Y., Liu, X., 2013. Comparison of methane production potential, biodegradability, and kinetics of different organic substrates. Bioresour. Technol. 149, 565–569.

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.

Luna-delRisco, M., Normak, a., Orupõld, K., 2011. Biochemical methane potential of different organic wastes and energy crops from Estonia. Agron. Res. 9, 331–342.

Mähnert, P., Linke, B., 2009. Kinetic study of biogas production from energy crops and animal waste slurry: Effect of organic loading rate and reactor size. Environ. Technol. 30, 93–99.

Martínez-Suller, L., Azzellino, A., Provolo, G., 2008. Analysis of livestock slurries from farms across Northern Italy: Relationship between indicators and nutrient content. Biosyst. Eng. 99, 540–552.

Matassa, S., Batstone, D.J., Hulsen, T., Schnoor, J., Verstraete, W., 2015. Can direct conversion of used nitrogen to new feed and protein help feed the world? Environ. Sci. Technol. 49, 5247–5254.

Molino, A., Nanna, F., Ding, Y., Bikson, B., Braccio, G., 2013. Biomethane production by anaerobic digestion of organic waste. Fuel 103, 1003–1009.

Moody, L., Burns, R., Wu-Haan, W., Spajić, R., 2009. Use of biochemical methane potential (BMP) assays for predicting and enhancing anaerobic digester performance. Proc. 4th Int. 44th Croat. Symp. Agric. 930–934.

Pham, C.H., Triolo, J.M., Cu, T.T.T., Pedersen, L., Sommer, S.G., 2013. Validation and recommendation of methods to measure biogas production potential of animal manure. Asian-Australasian J. Anim. Sci. 26, 864–873.

Philippe, F.-X., Laitat, M., Wavreille, J., Nicks, B., Cabaraux, J.-F., 2015. Effects of a high-fibre diet on ammonia and greenhouse gas emissions from gestating sows and fattening pigs. Atmos. Environ. 109, 197–204.

Philippe, F.-X., Nicks, B., 2015. Review on greenhouse gas emissions from pig houses: Production of carbon dioxide, methane and nitrous oxide by animals and manure. Agric. Ecosyst. Environ. 199, 10–25.

Raposo, F., De la Rubia, M.A., Fernández-Cegrí, V., Borja, R., 2012. Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures. Renew. Sustain. Energy Rev. 16, 861–877.

SAS, 2008. SAS/STAT User's Guide. (Release 9.2). SAS Inst. Inc., Cary NC, USA.

Schoumans, O.F., Bouraoui, F., Kabbe, C., Oenema, O., van Dijk, K.C., 2015. Phosphorus management in Europe in a changing world. Ambio 44, 180–192.

Strömberg, S., Nistor, M., Liu, J., 2015. Early prediction of Biochemical Methane Potential through statistical and kinetic modelling of initial gas production. Bioresour. Technol. 176, 233–41.

Sun, C., Cao, W., Liu, R., 2015a. Kinetics of Methane Production from Swine Manure and Buffalo Manure. Appl. Biochem. Biotechnol. 177, 985–995.

Sun, C., Cao, W., Liu, R., 2015b. Kinetics of Methane Production from Swine Manure and Buffalo Manure. Appl. Biochem. Biotechnol. 177, 985–995.

Suresh, a., Choi, H.L., Oh, D.I., Moon, O.K., 2009. Prediction of the nutrients value and biochemical characteristics of swine slurry by measurement of EC - Electrical conductivity. Bioresour. Technol. 100, 4683–4689.

The Intergovernmental Panel on Climate Change (IPCC)., 2014. Climate Change 2014: Mitigation of Climate Change, Working Group III Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.

Thomsen, S.T., Spliid, H., Østergård, H., 2014. Statistical prediction of biomethane potentials based on the composition of lignocellulosic biomass. Bioresour. Technol. 154, 80–86.

Triolo, J.M., Sommer, S.G., Møller, H.B., Weisbjerg, M.R., Jiang, X.Y., 2011. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: influence of lignin concentration on methane production potential. Bioresour. Technol. 102, 9395–402.

Triolo, J.M., Ward, A.J., Pedersen, L., Sommer, S.G., 2013. Characteristics of Animal Slurry as a Key Biomass for Biogas Production in Denmark. Biomass Now - Sustain. Growth Use 540.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci. 74, 3583–3597.

Wall, D.M., O'Kiely, P., Murphy, J.D., 2013. The potential for biomethane from grass and slurry to satisfy renewable energy targets. Bioresour. Technol. 149, 425–31.

Ward, A.J., 2016. Near-Infrared Spectroscopy for Determination of the Biochemical Methane Potential: State of the Art. Chem. Eng. Technol. 39, 4, 611–619.

Weiland, P., 2010. Biogasproduction: current state and perspectives. Appl. Microbiol. Biotechnol. 85, 849.860.

Yadvika, Santosh, Sreekrishnan, T.R., Kohli, S., Rana, V., 2004. Enhancement of biogas production from solid substrates using different techniques--a review. Bioresour. Technol. 95, 1–10.

Yagüe, M.R., Bosch-Serra, À.D., Boixadera, J., 2012. Measurement and estimation of the fertiliser value of pig slurry by physicochemical models: Usefulness and constraints. Biosyst. Eng. 111, 206–216.

Zhang, W., Lang, Q., Wu, S., Li, W., Bah, H., Dong, R., 2014a. Anaerobic digestion characteristics of pig manures depending on various growth stages and initial substrate concentrations in a scaled pig farm in Southern China. Bioresour. Technol. 156, 63–9.

Zhang, W., Lang, Q., Wu, S., Li, W., Bah, H., Dong, R., 2014b. Anaerobic digestion characteristics of pig manures depending on various growth stages and initial substrate concentrations in a scaled pig farm in Southern China. Bioresour. Technol. 156, 63–69.

7
Discusión general

7.1 Introducción

En este capítulo se analizan desde una perspectiva global los principales resultados obtenidos en esta Tesis Doctoral. En primer lugar, se discuten las implicaciones relativas a la composición de los purines de diversos orígenes y sus emisiones. En segundo lugar se evalúan de forma general los efectos de la modificación en la composición de la dieta sobre la composición de los purines y las emisiones de amoniaco (NH_3) y metano (CH_4) derivadas de los mismos. Para ello se han analizado de manera conjunta los datos obtenidos en los estudios llevados a cabo en esta tesis y en el Proyecto "*GASPORC-AGL2011-30023"*. Finalmente, se estudia la aplicabilidad y posibilidades de optimización de las técnicas de determinación de las emisiones NH_3 y CH_4 *in vitro*, a partir de la experiencia acumulada en los estudios realizados en esta tesis.

7.2 Los purines: composición y emisiones potenciales

Existen numerosos factores que afectan a la composición de los purines y, en consecuencia, a las emisiones potenciales de gases como el NH_3 y el CH_4. En la presente tesis se estudiaron diversos factores de variación que condicionan dicha composición como son el origen de los purines (granjas comerciales o instalaciones experimentales), la orientación productiva de procedencia (cebo, maternidad, gestación y recría) y la alimentación.

Los resultados obtenidos en los estudios de esta tesis evidencian diferencias entre los purines procedentes de naves de cebo de granjas comerciales y los purines reconstituidos procedentes de ensayos experimentales en el contenido de sólidos totales (ST), sólidos volátiles (SV), nitrógeno total Kjeldahl (NTK), extracto etéreo (EE), fibra neutro detergente (FND), fibra ácido detergente (FAD) y ácidos grasos volátiles (AGV) (Tabla 7.1). En ambos casos la categoría de animales fue la misma, animales de cebo. Además, aunque los piensos ofrecidos a los animales de las granjas comerciales no fueron exactamente los mismos, los piensos experimentales fueron formulados según las recomendaciones FEDNA (2013) para cerdos comerciales. Por lo tanto, se considera que las diferencias en composición se deban, principalmente, a factores como el tiempo de almacenamiento o grado de madurez de los purines y la presencia de otros componentes en el purín como restos de pienso, agua de bebida, agua de limpieza, entre otros. En este sentido, mientras

que los purines reconstituidos fueron una mezcla de heces y orina recién excretadas, los purines procedentes de granjas comerciales estuvieron almacenados, previamente a su recogida, durante períodos variables que oscilaron entre 20 y 120 días. Por otro lado, a nivel comercial, factores como el desperdicio de agua y pienso y el uso de agua de limpieza no fueron controlados. En cambio, en los experimentos con jaulas metabólicas estos factores fueron controlados.

Tabla 7.1. Características de purines de granjas comerciales de cebo y purines reconstituidos en base materia seca

Características de los purines	purines de granjas comerciales (n:31)		purines reconstituidos (n:77)		Significancia
	promedio	SD	promedio	SD	
ST (%)	6.1	4.0	13.1	4.0	***
SV (% ST)	74.1	6.2	80.5	2.7	***
NTK (% ST)	12.6	5.6	6.7	1.1	***
EE (% ST)	11.0	4.2	8.8	3.8	**
FND (% ST)	35.7	11.7	51.4	17.1	***
FAD (% ST)	17.4	7.4	23.1	7.9	**
AGV (mmol g ST^{-1})	1.61	1.2	0.62	0.16	***
Emisión NH_3 (mg L^{-1})	116	26	170	32	**
BMP (ml CH4 g ST^{-1})	309	16.4	330	60.3	ns

ST, sólidos totales; SV. Sólidos volátiles; NTK, nitrógeno total Kjeldhal; EE, extracto etéreo; FND, fibra neutro detergente; FAD, fibra ácido detergente; AGV, ácidos grasos volátiles, BMP, potencial bioquímico de producción de metano.
*, **, ***, significancia a P <0.05, P <0.01, P <0.001, respectivamente; ns: no significativo.

Según se observa en la Tabla 7.1., los purines obtenidos de granjas comerciales de cerdos de engorde tuvieron en promedio un contenido de ST 53% menor al de los purines reconstituidos (en promedio 6% y 13%, respectivamente), por lo tanto los purines de granjas comerciales tuvieron mayores contenidos de agua. Esta agua adicional sería proveniente del agua de limpieza y el agua de bebida, desperdiciada por los animales. Según estos resultados estimamos que se han incorporado aproximadamente 1.5 litros de agua por cada litro de heces y orina excretada por los cerdos en engorde en condiciones comerciales.

En base seca también se encontraron diferencias en la composición entre ambos orígenes (Tabla 7.1). Así, los purines de granjas comerciales de cerdos de engorde tuvieron menor

cantidad de SV que los purines reconstituidos (74% y 80%, respectivamente), pero resultaron con mayores contenidos de NTK (12.6% y 6.7%, respectivamente), EE (11% y 8.8%, respectivamente) y AGV (1.6 y 0.6 mmol g MS^{-1}, respectivamente). Las diferencias en el contenido de SV podría deberse a que durante el almacenamiento de los purines comerciales parte de la materia orgánica (MO) sería degradada por la acción de los microorganismos que se desarrollan en el purín (Martinez et al., 2003), mientras que los purines reconstituidos estuvieron exentos de este efecto. En el caso de los otros dos componentes (NTK y EE), el aporte de MO procedente del pienso desperdiciado podría estar jugando un papel fundamental en este aspecto. Por otro lado, es de destacar que el mayor contenido de NTK y EE en purines de granjas comerciales con respecto a los purines reconstituidos no se dio en la misma proporción (+90% y +25%, respectivamente). Considerando la hipótesis de la degradación de los compuestos orgánicos que se produce durante el almacenamiento de los purines de origen comercial, parece razonable que la degradación anaeróbica de los componentes grasos de los piensos desperdiciados sea más rápida que de los componentes proteicos (Labatut et al., 2011) y como consecuencia se dieron estos resultados. Por otra parte, la mayor concentración de AGV en purines comerciales confirmaría una actividad de las bacterias hidrolíticas presentes en el purín que degradarían los compuestos orgánicos, previa a la metanogénesis, que se estaría dando en estos purines (Popovic y Jensen, 2012). Por el contrario, los purines reconstituidos estuvieron exentos de pienso desperdiciado y no fueron almacenados. Las muestras de heces y orina de los purines reconstituidos fueron conservadas a 4ºC un máximo de 3 días antes de la reconstitución por lo que cabría considerar que los procesos de descomposición de la MO serían despreciables tal como lo considera Sommer et al. (2007).

Así, además de los mencionados efectos del tiempo de almacenamiento y la dilución con agua de desperdicio y limpieza, parece que el pienso desperdiciado por los cerdos en granjas comerciales, sería un factor relevante a tener en cuenta para la reducción de emisiones. Esta relevancia aumenta aún más, si consideramos que en granjas comerciales el purín puede ser acumulado durante un ciclo completo (hasta 4 meses en cerdos de engorde) y como tal acumularía el pienso desperdiciado durante todo ese tiempo, alterando la

composición de los purines y, por tanto, las emisiones potenciales además de las pérdidas económicas que este desperdicio podría representar (Goodband et al., 2008).

Por otro lado, el mayor contenido de FND y FAD en purines reconstituidos podría ser consecuencia de la composición de los piensos empleados en los diferentes tratamientos experimentales que, en muchos casos, se basaron en la modificación de las fuentes, tipos y niveles de fibra, a pesar de haberse formulado los piensos siguiendo estándares comerciales. A esto se añadiría el efecto del almacenamiento de los purines de granjas comerciales durante el cual parte de la MO fermentable probablemente haya sido degradada, contribuyendo a estas diferencias (Martinez et al., 2003).

Las emisiones potenciales de NH_3 y CH_4 de los purines evaluados estuvieron relacionadas con sus características fisicoquímicas. Las emisiones potenciales de NH_3 en purines de granjas comerciales fueron muy inferiores a las registradas en los purines reconstituidos (116 mg de NH_3 L^{-1} y 170 mg de NH_3 L^{-1}, respectivamente, Tabla 7.1). Dado que en ambos casos los ensayos de emisión se realizaron por unidad de volumen de purín, esta diferencia estaría relacionada principalmente con el menor contenido de NTK de los purines de granjas comerciales con respecto a los reconstituidos.

Por otro parte, la emisión potencial de NH_3 expresada sobre el contenido de NTK del purín, fue menor para las muestras procedentes de granjas comerciales que para las de purines reconstituidos (15 y 19 mg de NH_3 por g NTK d^{-1}, respectivamente). Al respecto, es probable que parte del NTK presente en purines de granjas comerciales se haya emitido ya durante el almacenamiento en fosa como NH_3, restando así una fracción de NTK menos volatilizable. En este sentido, Moset et al. (2012) reporta una reducción de 12% en el contenido de NTK del purín después de 9 semanas de almacenamiento. Entretanto, en purines reconstituidos la emisión de NH_3 se dio únicamente durante las determinaciones *in vitro*, en las que todo el N ureico estuvo a disposición para ser degradado a NH_3.

En cuanto a las emisiones potenciales de CH_4, los purines de granjas comerciales tuvieron una emisión potencial menor que los reconstituidos (con medias de 309 y 330 ml g SV^{-1}, respectivamente), aunque esta diferencia no fue estadísticamente significativa. Por lo tanto, inicialmente, la MO de ambos purines sería igualmente degradable en condiciones

anaerobias. En la práctica, un purín envejecido contiene una MO menos degradable, es decir, un menor contenido en PB y EE y un mayor contenido en fibra indigestible y, por lo tanto, debería emitir menos gases entre ellos CH_4. Sin embargo, como ya se ha comentado con anterioridad, en situaciones comerciales la contaminación con el pienso desperdiciado podría incrementar la cantidad de MO y su potencial de emisión original.

7.3 Relación entre el consumo de nutrientes, excreción de nutrientes, características del purín y las emisiones potenciales de NH_3 y CH_4.

Los datos obtenidos en el capítulo 4 de la presente tesis se evaluaron conjuntamente con los datos de otros dos estudios similares realizados en el contexto del proyecto GASPORC-AGL2011-30023 (Beccaccia et al., 2015a, 2015b) con la finalidad de analizar y discutir las relaciones de causa y efecto entre el consumo de nutrientes, la excreción de nutrientes, las características de los purines y las emisiones potenciales de NH_3 y CH_4. De esta forma, se analizaron 78 registros correspondientes a 78 cerdos alimentados con 13 dietas formuladas con diferentes fuentes y niveles de proteína, fibra y grasa. En estos estudios, se reconstruyeron purines a partir de heces y orina colectadas individualmente de cada animal en jaulas metabólicas. Por lo tanto, los posibles efectos del desperdicio de pienso o agua fueron minimizados y se eliminó el efecto correspondiente al tiempo de almacenamiento en fosas.

Utilizando los 78 registros individuales de consumo y excreción de nutrientes (g kg $PV^{-0.75}$ en base seca), las características del purín reconstituido (en base fresca) y emisiones de NH_3 (expresadas en mg kg $PV^{-0.75}d^{-1}$ y como potenciales, g L^{-1}) y emisiones de CH_4 (expresadas en L kg $PV^{-0.75}$ d^{-1} y como potenciales, BMP) se realizó un análisis de correlaciones con el procedimiento PROC CORR del SAS (2008) con el fin de identificar las inter-relaciones existentes entre los diferentes parámetros estudiados. Estos resultados se presentan en la Tabla 7.2.

Tabla 7.2. Correlaciones entre los nutrientes consumidos (g kg $PV^{-0.75}$ d^{-1}), nutrientes excretados (g kg $PV^{-0.75}$ d^{-1}) en base seca, características de los purines resultantes (g L^{-1}) y las emisiones potenciales de CH_4 y NH_3, en cerdos de cebo

variables	(n:78)		coeficientes de correlación				
				emisiones			
	Promedio	SD	Ratio N H/O	CH_4 L kgPV$^{-0.75}$ d^{-1}	NH_3 mg kgPV$^{-0.75}$ d^{-1}	BMP	NH_3 g L^{-1}
nutrientes consumidos							
PB[a]	14.3	2.47	0.13 ns	0.42***	0.28*	-0.23*	-0.21 ns
EB[b]	1.58	0.25	0.26*	0.55***	0.19 ns	-0.13 ns	-0.25*
PB IDN[a]	1.79	0.44	-0.02 ns	0.53***	0.21 ns	0.49**	0.06 ns
EE[a]	5.35	1.47	0.45**	0.65***	-0.09 ns	0.21 ns	-0.42***
FS[a]	8.47	5.22	-0.16 ns	0.25*	0.17 ns	0.46**	-0.01 ns
FND[a]	11.1	5.04	0.49**	0.16 ns	-0.22 ns	-0.21 ns	-0.18 ns
FAD[a]	3.65	2.17	0.58***	0.18 ns	-0.29**	-0.13 ns	-0.33**
LAD[a]	3.02	2.90	-0.37**	0.12 ns	0.29**	0.22*	0.09 ns
nutrientes excretados (heces y orina)							
PB[a]	5.76	1.42	-0.13 ns	0.20 ns	0.62***	-0.27*	0.19 ns
EB[b]	0.27	0.05	0.47***	0.70***	0.01 ns	0.12 ns	-0.19 ns
EE[a]	1.354	0.65	0.47***	0.45***	-0.30**	0.34*	-0.14 ns
FND[a]	5.53	1.27	0.20 ns	0.47***	0.21 ns	-0.13 ns	0.00 ns
FAD[a]	3.64	1.86	0.02 ns	0.18 ns	0.17 ns	-0.22*	-0.17 ns
LAD[a]	0.26	0.28	-0.27*	0.14 ns	0.31*	0.08 ns	0.06 ns
características del purín							
pH	7.537	0.85	-0.03 ns	0.02 ns	-0.04 ns	0.39*	0.22*
NTK[c]	8.46	2.13	0.11 ns	0.02 ns	-0.16 ns	-0.06 ns	0.48***
N NH_3[c]	2.95	1.46	-0.06 ns	0.07 ns	-0.13 ns	0.16 ns	0.27*
EE[c]	13.30	7.96	0.44**	0.28**	-0.47**	0.31*	-0.01 ns
FND[c]	23.56	9.52	0.40***	0.01 ns	-0.35**	-0.08 ns	0.27*
FAD[c]	10.48	3.74	0.35**	0.04 ns	-0.33*	-0.14 ns	0.17 ns
LAD[c]	3.05	1.74	0.00 ns	-0.03 ns	-0.01 ns	-0.19 ns	0.19 ns
excreción del N							
Ratio H/O	0.91	0.40		0.40***	-0.60***	0.15 ns	-0.50***
emisiones potenciales							
CH_4[d]	3.811	0.87			-0.09 ns	0.50***	-0.21 ns
NH_3[e]	20.24	7.90				-0.20 ns	0.59***
BMP	332	59					-0.13 ns
NH_3 g L^{-1}	1.87	0.61					

PB, proteína bruta; EB, energía bruta; PB IDN, proteína bruta indigestible en detergente neutro; EE, extracto etéreo; FS, fibra soluble; FDN fibra neutro detergente; FAD, fibra ácido detergente; LAD, lignina ácido detergente; SD, desviación estándar; ratio N H/O, ratio de excreción de nitrógeno en heces: nitrógeno en orina; BMP, potencial bioquímico de producción de metano.

[a:] g kg $PV^{-0.75}d^{-1}$; [b:] MJ kg $PV^{-0.75}$ d^{-1}; [c:] g L^{-1}, [d:] L kgPV$^{-0.75}$ d^{-1}, [e:] mg kg $PV^{-0.75}$ d^{-1}.; *, **, ***, significancia a P<0.05, P<0.01, P<0.001, respectivamente; ns, no significativo.

Además, con el propósito de cuantificar de manera práctica y delimitar las variables involucradas en la emisión de NH_3 y CH_4 (por kg de $PV^{-0.75}$ d^{-1}), los registros individuales de los 78 cerdos fueron clasificados en función a la amplitud del rango de las emisiones en tres clases: emisiones bajas (el tercio inferior del rango), emisiones medias (el tercio medio del rango) y emisiones altas (el tercio superior del rango). Con los registros clasificados se realizó un análisis de varianza (PROC ANOVA, del SAS, 2008) tomando como factor de variación la clase (emisiones bajas, emisiones medias y emisiones altas) y como variables dependientes el consumo de nutrientes, los nutrientes excretados y las características del purín. Este procedimiento se siguió particularmente para las emisiones potenciales de NH_3 y CH_4. Los resultados son presentados en la Tabla 7.3.

Con la finalidad de aproximar un balance de la utilización del N y la EB teniendo en cuenta la eficiencia de utilización de estos nutrientes para incrementar el PV promedio día, el consumo, la retención y la excreción de N y EB fueron expresados en función al incremento de peso diario (g de N por incremento de PV d^{-1} y MJ por incremento de PV d^{-1}), los resultados son presentados en la figura 7.1 y 7.2, respectivamente.

7.3.1 Emisiones de NH_3

De acuerdo a los resultados de la correlación entre las variables evaluadas presentados en la Tabla 7.2, los cerdos que consumieron mayores cantidades de proteína bruta (PB) y lignina ácido detergente (LAD) producirían mayores emisiones potenciales de NH_3 (todo expresado por kg de $PV^{-0.75}$). Por el contrario, los cerdos que consumieron mayores cantidades de FAD tendrían las menores emisiones de NH_3, coincidiendo con los resultados obtenidos por Garry et al. (2007) y O'Connell et al. (2006). A este efecto inicial se adiciona, con mayor significancia, la relación de la emisión de NH_3 (mg kg $PV^{-0.75}$ d^{-1}) con la excreción de PB (r: 0.62, $P<0.001$). Esto sugiere que no sólo el aporte de proteína en el pienso condiciona las emisiones de NH_3, sino que también intervienen otros factores relacionados con la calidad de la proteína ingerida y su digestibilidad, (Aarnink y Verstegen, 2007; O'Connell et al., 2006), ya que estos condicionarán la parte de la proteína de la dieta que llega al purín. Por otra parte, simultáneamente, podría existir un efecto contradictorio del consumo de FAD. Es probable que mayores niveles de FAD produzcan

un incremento de la tasa de fermentación del alimento en el ciego y aumenten la incorporación de N en las heces (N bacteriano). Por lo tanto, el consumo de FAD podría provocar mayor excreción de N en heces (r: 0.53, P<0.001) y menor excreción de nitrógeno en orina (r: -0.25, P<0.05), coincidiendo con los reportes de Hernández et al. (2011). La correlación negativa (r: -0.60, P<0.001) entre las emisiones de NH_3 y el ratio de excreción de N heces/orina coinciden con los reportes de Canh et al. (1998); Galassi et al. (2010); Jarret et al. (2011); Le et al. (2009), entre otros.

En ese sentido, desde el punto de vista nutricional, la digestibilidad de la PB y la fermentación de la fibra serían factores determinantes para las emisiones de NH_3. El primer factor se asocia con la PB insoluble en fibra neutro detergente (PB FND) y el último factor se asocia con el consumo de FAD tal y como lo menciona Chowdhuryet al. (2014). En el presente estudio, si tenemos en cuenta que los niveles de proteína en los piensos fueron similares (15.3% en promedio y rango entre 14.4% y 16.4%), la cantidad de N excretado en la orina sería el resultado de diferencias en la asimilación de la proteína consumida. La proteína digerida excedente a las necesidades metabólicas y productivas de los cerdos serían excretados en la orina como N inorgánico, en forma de urea principalmente (Canh et al., 1998), constituyéndose en la principal fuente de emisiones de NH_3. Por otro lado, el N orgánico excretado en las heces tendría menor facilidad de emisión de NH_3 dado que los procesos de mineralización necesarios para la formación de NH_3 requerirán mayor tiempo (Philippe et al., 2011). Por lo tanto un segundo factor importante a considerar para la reducción de las emisiones potenciales de NH_3 sería la reducción de excreción del N principalmente urinario y como tal del ratio N heces/orina.

Respecto a la correlación entre las características del purín y las emisiones potenciales de NH_3 (g L^{-1} purín) los resultados indicarían que los purines con mayores contenidos de NTK, nitrógeno amoniacal (N-NH_3), FND y pH tendrían mayores emisiones potenciales de NH_3. Estos resultados confirman la relación directa entre el contenido de N del purín y las emisiones de NH_3, así como el efecto de la acidez del medio (Chowdhury et al., 2014; Philippe et al., 2011).

Tabla 7.3. Emisiones de NH_3 y CH_4 por kg de $PV^{-0.75}$ d^{-1} de purines de cerdos de cebo: Relación con el consumo y la excreción de nutrientes (orina+heces)

Factor de clasificación	emisiones bajas promedio ± SE		emisiones medias promedio ± SE		emisiones altas promedio ± SE		P-value
Emisión de NH_3 (mg kg $PV^{-0.75}$ d^{-1})	(n:20) 11.4 ± 0.84		(n:45) 20.0 ± 0.55		(n:13) 33.6 ± 1.01		
Consumo, PB[a]	13.5 ± 0.54	e	14.2 ± 0.36	ef	15.6 ± 0.67	f	0.047
Consumo, FAD[a]	4.76 ± 0.47	e	3.28 ± 0.31	f	3.2 ± 0.58	f	0.026
Excretado, PB[a]	4.82 ± 0.24	e	5.47 ± 0.16	f	6.65 ± 0.29	g	0.001
N en orina[a]	0.367 ± 0.03	e	0.473 ± 0.02	f	0.682 ± 0.03	g	0.001
Contenido en el purín, EE[c]	18.2 ± 1.62	e	12.9 ± 1.08	f	7.2 ± 2.01	g	0.001
Emisión de CH_4 (L kg$PV^{-0.75}$ d^{-1})	(n:18) 2.70 ± 0.09		(n:42) 3.76 ± 0.06		(n:18) 5.03 ± 0.09		
Consumo, PB[a]	12.4 ± 0.53	e	14.5 ± 0.35	f	15.5 ± 0.53	f	0.001
Consumo, EB[b]	1.37 ± 0.05	e	1.60 ± 0.03	f	1.75 ± 0.05	g	0.001
Consumo, PB IDN[a]	1.41 ± 0.09	e	1.82 ± 0.06	f	2.08 ± 0.09	g	0.001
Consumo, EE[a]	4.38 ± 0.28	e	5.11 ± 0.18	f	6.87 ± 0.28	g	0.001
Excretado, PB[a]	4.49 ± 0.63	e	5.79 ± 0.63	f	5.84 ± 0.63	f	0.001
Excretado, EB[b]	0.21 ± 0.01	e	0.27 ± 0.01	f	0.32 ± 0.01	g	0.001
Excretado, EE[a]	1.08 ± 0.14	e	1.25 ± 0.09	e	1.86 ± 0.14	f	0.001
Excretado, FND[a]	4.41 ± 0.26	e	5.73 ± 0.17	f	6.19 ± 0.26	f	0.001
N en heces[a]	0.313 ± 0.02	e	0.400 ± 0.01	f	0.483 ± 0.02	g	0.001
Contenido en el purín, EE[c]	12.1 ± 1.82	e	12.1 ± 1.19	e	17.4 ± 1.82	f	0.002

PB, proteína bruta; EB, energía bruta; PB IDN, proteína bruta indigestible en detergente neutro; EE, extracto etéreo; FDN fibra neutro detergente; FAD, fibra ácido detergente; N, nitrógeno; SE, error estándar de la media

[a] g kg $PV^{-0.75}d^{-1}$; [b] MJ kg $PV^{-0.75}d^{-1}$; [c] g L^{-1}

Letras diferentes (e, f, g) junto a los promedios de una misma fila indican diferencias estadísticas significativas entre clases

Cuando las emisiones de NH_3 (mg kg $PV^{-0.75}d^{-1}$) se clasificaron en emisiones potenciales bajas, medias y altas (Tabla 7.3) se evidencia que, las emisiones potenciales bajas se correspondieron a cerdos que consumieron en promedio 15% menos PB que los cerdos con altas emisiones (13.5 g y 15.6 g de PB kg $PV^{-0.75}d^{-1}$, respectivamente). Por lo tanto, a nivel práctico, la menor ingestión de PB (-15%) implicarían mitigar la emisión de NH_3 en un -66% aproximadamente (de 33.6 mg a 11.4 mg de NH_3 kg $PV^{-0.75}d^{-1}$, respectivamente). Ahora bien, son necesarios ensayos productivos para evaluar el efecto de estas estrategias en los diferentes parámetros productivos.

Por otra parte, el contenido de EE en los purines tuvo una correlación negativa con las emisiones de NH_3, en este caso es probable que este resultado sea consecuencia de un efecto asociado al consumo y excreción de FND ya que los piensos con mayores contenidos de fibra también tuvieron mayores contenidos de EE y como tal el consumo y la excreción de estos dos nutrientes estarían asociados.

7.3.2 Emisiones de CH_4

Al evaluar las relaciones entre la emisión de CH_4 y el consumo de nutrientes encontramos que los cerdos que consumieron mayores cantidades de PB, EB, PB IDN, EE y FS (g kg $PV^{-0.75}$ d^{-1}, respectivamente) producirían mayores emisiones de CH_4 (L kg $PV^{-0.75}$ d^{-1}). La correlación entre la emisión de CH_4 (L kg $PV^{-0.75}$ d^{-1}) y el consumo de EE y EB fueron significativamente mayores a la de los otros nutrientes ($r > 0.55$, con $P<0.001$, en ambos casos). En este sentido, si tenemos en cuenta que las dietas evaluadas fueron similares en sus contenidos de proteína y energía (entre 144 g y 164g de PB y 16.3MJ y 17.7 MJ de EB, por kg de pienso respectivamente), es posible que la asociación encontrada entre las emisiones de CH_4 y la proteína se deba, en realidad, a su relación con la energía.

A estas relaciones iniciales entre el consumo de nutrientes y las emisiones de CH_4 podemos agregar el efecto de la excreción de nutrientes. Al respecto se encontró que la emisión de CH_4 tuvo una correlación altamente significativa (r: 0.7, $P<0.001$) con la excreción de EB y también con la excreción de EE y FND (r: 0.45 y r: 0.47, $P<0.001$ respectivamente). Por lo tanto, parece que la digestibilidad de la EB y de sus principales fuentes (EE en mayor medida y FDN) son determinantes en las emisiones de CH_4 (L kg $PV^{-0.75}$ d^{-1}). En todo caso

es muy probable que mayores excreciones de energía resulten en mayores emisiones de CH_4 (L kg $PV^{-0.75}$ d^{-1}) (Jarret et al., 2012).

Cuando las emisiones de CH_4 (L kg $PV^{-0.75}d^{-1}$) se clasificaron en emisiones potenciales bajas, medias y altas (Tabla 7.3) se encontró que, las emisiones potenciales bajas se correspondieron a cerdos que en promedio consumieron menos energía (-27%) que los cerdos con altas emisiones (1.37 MJ y 1.75 MJ de EB kg $PV^{-0.75}d^{-1}$, respectivamente). Si tenemos en cuenta la asociación de la EB con la PB en los piensos, menores consumos de EB implicó también menores consumos de PB (-25%) (12.4 g y 15.5 g de PB kg $PV^{-0.75}d^{-1}$, respectivamente). Por lo tanto el menor consumo de EB podría implicar reducir en 46% las emisiones de CH_4 kg $PV^{-0.75}d^{-1}$ respecto a los animales con mayores consumos de EB (de 5.03 L a 2.7 L de CH_4 kg $PV^{-0.75}$ d^{-1}).

Por otra parte los animales con altas emisiones de CH_4 tuvieron mayores excreciones de EB, EE y FND. Por lo tanto es de suponer que, de la relación entre el consumo y la excreción de EB, así como de nutrientes asociados (EE y FND), depende en gran parte las emisiones de CH_4 (Elmholt, 2013). Además de esto, la BMP de purines dependería de la composición y la naturaleza de los SV del purín. Estos son una proporción de la MO no digerida por los cerdos, por lo que su variación estaría supeditada a los ingredientes utilizados en la fabricación del pienso. Para los resultados evaluados parece ser que el contenido de EE y FND de los purines fue determinante.

7.3.3 Balance de nutrientes y emisiones de NH_3 y CH_4

Las Figuras 7.1 y 7.2 muestran respectivamente, de forma esquemática, los balances de la utilización del N y la EB en cerdos de cebo en función al incremento de PV y las emisiones de NH_3 y CH_4, respectivamente.

En el caso del N (Figura 7.1), se observan diferencias en el consumo y la excreción total de N entre los cerdos con emisiones de NH_3 altas medias y bajas. A pesar de estas diferencias, la retención de N fue similar en los tres casos (40.1 g, 39.5 g y 39.4 g de N incremento de kg PV, para emisiones altas medias y bajas, respectivamente). Esta misma tendencia se encontró en la excreción de N en heces (11.6 g, 11.4 y 10.8 g de N incremento de PV,

respectivamente), por lo tanto las diferencias principales estuvieron en la excreción de N en la orina.

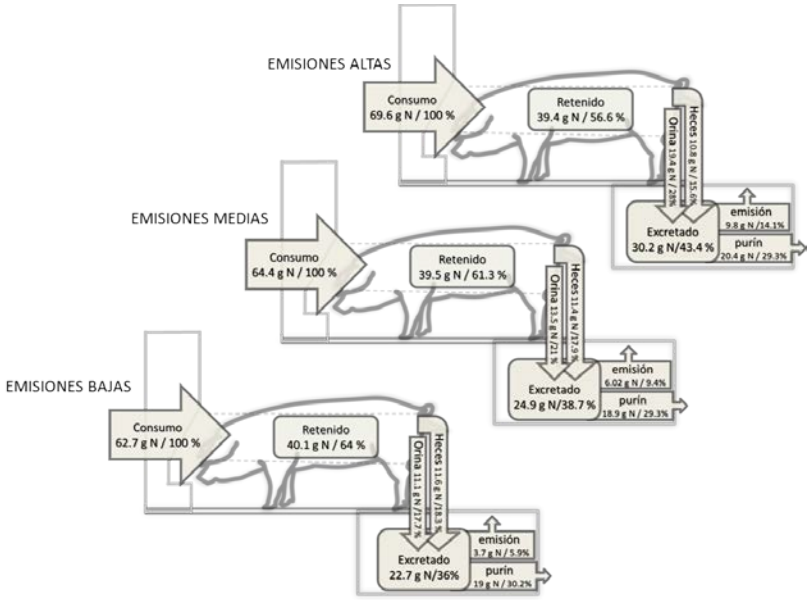

Figura 7.1. Utilización del N en cerdos de cebo (g de N por incremento de kg PV) y emisiones de NH_3 (g kg PV). Fuente: Autor.

En el caso de los cerdos clasificados en altas emisiones, habrían digerido el 84% del N consumido (69.6 g de N ingerido), de esta cantidad habrían utilizado 39.4 g N para sus procesos metabólicos y productivos y el exceso habría sido excretado por la orina (19.4 g de N) y como consecuencia se tuvieron mayores emisiones de NH_3, emitiendo el 32% del N excretado. En el caso de los cerdos clasificados en emisiones medias, consumieron 64.4 g N, habrían digerido el 82% del N consumido, habrían utilizado 39.5 g de N para sus procesos metabólicos y productivos, el exceso habría sido excretado por la orina (13.5 g N) y se habría emitido el 24% del N excretado. Para el caso de los cerdos clasificados en emisiones bajas, consumieron 62.7 g N, habrían digerido el 81% del N consumido, habrían utilizado 40.1g de N para sus procesos metabólicos y productivos, el exceso habría sido excretado por la orina (11.1 g N) y se habrían emitido el 16 % del N total excretado. Se

observa además que las diferencias absolutas de nitrógeno urinario excretado se corresponden con las diferencias en el NH_3 potencialmente emitido. Estas relaciones confirmarían que los factores determinantes en las emisiones serían el consumo y la digestibilidad del N, ambos factores determinarían la disponibilidad final de nitrógeno (heces/orina) en los purines.

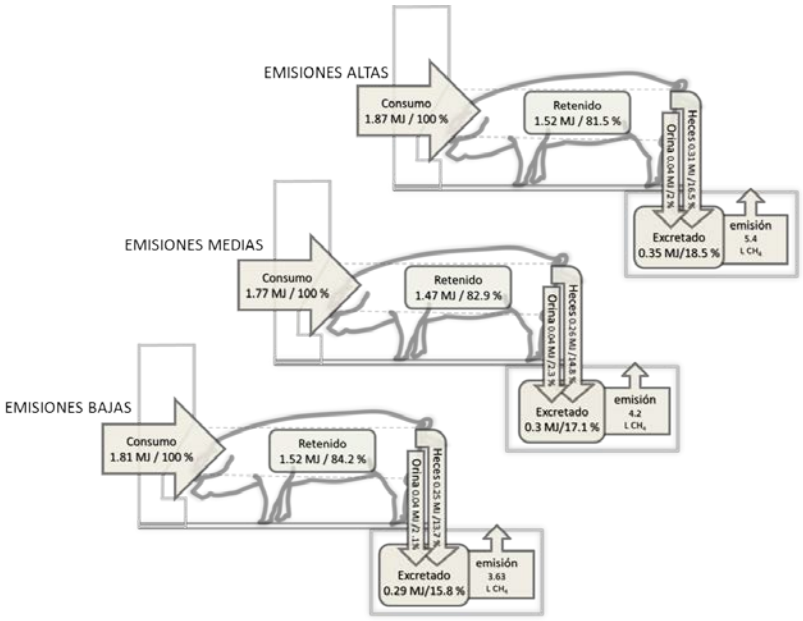

Figura 7.2. Utilización de la EB (MJ por incremento de PV kg) y las emisiones de CH_4 (L MJ^{-1}) en la alimentación de cerdos de cebo. Fuente: Autor

En el caso de la utilización de la EB se encontró que, en promedio, el 83% de la EB consumida fue retenida, probablemente para sus procesos metabólicos y productivos. El 17% de la EB fue excretada (heces y orina) y se emitió 4.2 L de CH_4 por MJ (Figura 7.2). Se observan diferencias en el consumo de EB para los cerdos con emisiones de CH_4 altas, medias y bajas (1.87, 1.77 y 1.81 MJ, respectivamente), sin embargo la retención y la excreción de energía en orina fue similar en las tres categorías. Por lo tanto, es probable que los cerdos evaluados hayan utilizado alrededor de 1.49 MJ en sus necesidades

metabólicas y productivas por kg de incremento de PV. Por lo tanto, la diferencia principal entre las categorías sería en la excreción de energía en heces.

Los cerdos con emisiones altas excretaron mayores cantidades de energía en heces (0.31 MJ) que los cerdos con emisiones medias y bajas (26 MJ y 25 MJ por incremento de PV kg, respectivamente). La energía excretada en heces es principalmente la energía no digerida por el animal, y por lo tanto la cantidad de energía consumida y su digestibilidad serían los factores determinantes en las emisiones de CH_4.

A la luz de los resultados, una mejora de la eficiencia de utilización del N y de la EB, reduciría la excreción de N y EB de los piensos y de las emisiones de NH_3 y CH_4.

7.4 Optimización de la medición del potencial de emisiones de NH_3 y CH_4

La medición de las emisiones potenciales de NH_3 *in vitro* por el método de trampas ácidas húmedas (Ndegwa et al., 2009) implica el registro de la emisión de NH_3 diariamente durante 11 días. La medición del potencial de emisión de CH_4 *in vitro* (metodología descrita por Angelidaki et al. 2009) replica procesos controlados de fermentación anaeróbica de purines en botellas de 120 ml durante 100 días. En ambos casos, los métodos permiten determinar las emisiones potenciales en condiciones estandarizadas, como tal permitirían diferenciar el potencial de emisiones de purines de diferentes orígenes.

Sin embargo, en el caso de la aplicación de la metodología de trampas ácidas húmedas *in vitro*, no se encontraron reportes de las implicaciones de la frecuencia de medición de las emisiones parciales de NH_3 sobre la precisión de los resultados de las emisiones acumuladas. En el caso de la determinación del potencial de emisión de CH_4 uno de los inconvenientes del método podría ser la duración del ensayo (100 días). No obstante se han desarrollado modelos de predicción que pueden ser utilizados para estimar las emisiones a partir de características del purín como el pH, ST, EE, LAD, FND (Beccaccia et al., 2015; Godin et al., 2014), pero aún son escasos aquellos que se basan en calcular las emisiones potenciales tomando en cuenta la dinámica de producción de CH_4 (Kafle y Chen, 2016).

En el caso de las emisiones de NH_3 los resultados encontrados en el capítulo 5 evidencian que el día 15 aún se sigue emitiendo NH_3 y como es probable que los purines sigan emitiendo más allá de 15 días. Sin embargo queda clara la posibilidad de reducir la frecuencia de reemplazo de las trampas ácidas, de una frecuencia diaria a una frecuencia interdiaria, sin afectar significativamente la estimación de la emisión acumulada. Complementariamente también es evidente la posibilidad de reducir el tiempo de medición de 15 a 11 días y calcular la emisión "restante" con el modelo de predicción propuesto en el Capítulo 5 (Emisión potencial de NH_3 = 1.2912 x emisión parcial en el día 11 + 4.1, R^2 = 0.98) con un alto grado de certeza.

Los resultados de la evaluación metodológica del método de trampas ácidas nos indican la factibilidad de reducir en un 50% el tiempo dedicado a la medición de las emisiones de NH_3, esto no solo incluye la mano de obra, si no también incluye el uso de todos los insumos y equipos asociados a esta técnica (solución ácida, solución alcalina, energía eléctrica).

En el caso de la determinación del potencial de producción de CH_4, conforme a los resultados presentados en el capítulo 6 podemos indicar que, las dinámicas de emisión de CH_4 de purines de granjas comerciales y purines reconstituidos fueron similares. En ambos casos, al día 53 de iniciado la determinación in vitro de la BMP, se ha producido más del 95% de la emisión potencial, el 5% de la emisión restante se produciría entre los días 54 a 100. Por lo tanto, para la determinación de la BMP de purines de cerdo se requerirían mediciones hasta el día 50. Las emisiones restantes podrían ser calculadas con los métodos predictivos basados en la cinética de producción de CH_4 desarrollados en la presente tesis. En referencia a la dinámica de la BMP, se correspondió a un modelo asintótico y la determinación de la BMP a diferentes tiempos podría ser estimada por modelización usando los registros de emisión de CH_4 hasta el día 32.

La aplicación de los resultados del modelado de la cinética de producción de CH_4 y la predicción de la BMP permitirían reducir en un 50% el tiempo dedicado a la medición de la BMP. Esta reducción implica a todos los recursos y medios involucrados como son el personal técnico de laboratorio (para el seguimiento, registro de la producción de biogás,

toma de muestras y determinación de la concentración de CH_4), recursos, equipos e insumos asociados a esta técnica.

Por otra parte, las características de los purines estudiados y sus emisiones potenciales de NH_3 y CH_4 han tenido un amplio rango en su origen, y composición por lo tanto los modelos de predicción desarrollados serían robustos.

7.5 Referencias

Aarnink, A.J.A., Verstegen, M.W.A., 2007. Nutrition, key factor to reduce environmental load from pig production. Livest. Sci. 109, 194-203.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, a. J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59, 927.

Antezana, W., Calvet, S., Beccaccia, A., Ferrer, P., De Blas, C., García-Rebollar, P., Cerisuelo, A., 2015. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing pigs: III. Influence of varying the dietary level of calcium soap of palm fatty acids distillate with or without orange pulp supplementation. Anim. Feed Sci. Technol.

Beccaccia, A., Calvet, S., Cerisuelo, A., Ferrer, P., García-Rebollar, P., De Blas, C., 2015a. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing-finishing pigs. I. Influence of the inclusion of two levels of orange pulp and carob meal in isofibrous diets. Anim. Feed Sci. Technol. 208, 158-169.

Beccaccia, A., Cerisuelo, A., Calvet, S., Ferrer, P., Estellés, F., De Blas, C., García-Rebollar, P., 2015b. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing pigs: II. Effect of protein source in practical diets. Anim. Feed Sci. Technol. 209, 137-144.

Beccaccia, A., Ferrer, P., Ibáñez, M.A., Estellés, F., Rodríguez, C., Moset, V., Blas, C. De, Calvet, S., García-rebollar, P., 2015. Relationships among slurry characteristics and gaseous emissions at different type|s of commercial Spanish pig farms. Spanish J. Agric. 13, 1-15.

Canh, T.., Aarnink, a. J.., Schutte, J.., Sutton, a, Langhout, D.., Verstegen, M.W.., 1998. Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing–finishing pigs. Livest. Prod. Sci. 56, 181-191.

Chowdhury, A., Rong, L., Feilberg, A., & Adamsen, A.P.S., 2014. Review of ammonia emissions from a pig house slurry pit and outside storage: Effects of emitting surface and slurry depth. Danish Environ. Prot. Agency.

Elmholt, S., 2013. Report Methane emission from pigs. Aarhus University, Faculty of Agricultural Sciences, Department of Animal Health and Bioscience, Research Centre Foulum Blichers Allé 20, DK-8830 Tjele, Denmark.

Galassi, G., Colombini, S., Malagutti, L., Crovetto, G.M., 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.

Garry, B.P., Fogarty, M., Curran, T.P., O'Doherty, J. V., 2007. Effect of cereal type and exogenous enzyme supplementation in pig diets on odour and ammonia emissions. Livest. Sci. 109, 212-215.

Godin, B., Mayer, F., Agneessens, R., Gerin, P., Dardenne, P., Delfosse, P., Delcarte, J., 2014. Biochemical methane potential prediction of plant biomasses: Comparing chemical composition versus near infrared methods and linear versus non-linear models. Bioresour. Technol. 175C, 382-390.

Hernández, F., Martínez, S., López, C., Megías, M.D., 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.

Jarret, G., Cerisuelo, A., Peu, P., Martinez, J., Dourmad, J.Y., 2012. Impact of pig diets with different fibre contents on the composition of excreta and their gaseous emissions and anaerobic digestion. Agric. Ecosyst. Environ. 160, 51-58.

Jarret, G., Martinez, J., Dourmad, J.-Y., 2011. Effect of biofuel co-products in pig diets on the excretory patterns of N and C and on the subsequent ammonia and methane emissions from pig effluent. Animal 5, 622-631.

Kafle, G.K., Chen, L., 2016. Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. Waste Manag. 48, 492-502.

Labatut, R.A., Angenent, L.T., Scott, N.R., 2011. Biochemical methane potential and biodegradability of complex organic substrates. Bioresour. Technol. 102, 2255-64.

Le, P.D., Aarnink, a. J. a, Jongbloed, a. W., 2009. Odour and ammonia emission from pig manure as affected by dietary crude protein level. Livest. Sci. 121, 267-274.

Martinez, J., Guiziou, F., Peu, P., Gueutier, V., 2003. Influence of Treatment Techniques for Pig Slurry on Methane Emissions during Subsequent Storage. Biosyst. Eng. 85, 347-354.

Moset, V., Cambra-López, M., Estellés, F., Torres, A.G., Cerisuelo, A., 2012. Evolution of chemical composition and gas emissions from aged pig slurry during outdoor storage with and without prior solid separation. Biosyst. Eng. 111, 2-10.

Ndegwa, P.M., Vaddella, V.K., Hristov, a N., Joo, H.S., 2009. Measuring concentrations of ammonia in ambient air or exhaust air stream using acid traps. J. Environ. Qual. 38, 647-653.

O'Connell, J.M., Callan, J.J., O'Doherty, J. V., 2006. The effect of dietary crude protein level, cereal type and exogenous enzyme supplementation on nutrient digestibility, nitrogen excretion, faecal volatile fatty acid concentration and ammonia emissions from pigs. Anim. Feed Sci. Technol. 127, 73-88.

Philippe, F.-X., Cabaraux, J.-F., Nicks, B., 2011. Ammonia emissions from pig houses: Influencing factors and mitigation techniques. Agric. Ecosyst. Environ. 141, 245-260.

Popovic, O., Jensen, L.S., 2012. Storage temperature affects distribution of carbon, VFA, ammonia, phosphorus, copper and zinc in raw pig slurry and its separated liquid fraction. Water Res. 46, 3849-58.

SAS, 2008. SAS/STAT User's Guide. (Release 9.2). SAS Inst. Inc., Cary NC, USA.

Sommer, S.G., Petersen, S.O., S??rensen, P., Poulsen, H.D., M??ller, H.B., 2007. Methane and carbon dioxide emissions and nitrogen turnover during liquid manure storage. Nutr. Cycl. Agroecosystems 78, 27-36.

8
Conclusiones y líneas de investigación futuras

8.1 Conclusiones

La composición y las emisiones potenciales de NH_3 y CH_4 de purines de granjas comerciales fueron variables. La orientación productiva de las granjas comerciales afectó significativamente los contenidos de nitrógeno total, nitrógeno amoniacal, potasio, calcio, cobre, sodio, zinc, ácidos grasos volátiles y el pH de los purines, y en consecuencia afectó también sus emisiones potenciales de NH_3 y CH_4. Por lo tanto la utilización de purines como enmienda agronómica o como insumo para la producción de biogás requiere determinar su composición.

La inclusión de grasa en la dieta de cerdos (jabón cálcico de palma) provocó mayores excreciones de extracto etéreo y proteína bruta, así mismo causó mayores emisiones de CH_4 y menores emisiones de NH_3. La inclusión de una fuente de fibra (pulpa de naranja) alteró la composición fecal, pero no afectó significativamente a las emisiones de NH_3 y CH_4.

La composición de los purines de cerdo y sus emisiones de NH_3 y CH_4 varían con la alteración de factores nutricionales (composición del pienso, ingesta y digestibilidad). El análisis global de los resultados indica que las diferencias de emisión de NH_3 se deben a cambios en la excreción de N en la orina, mientras que los cambios de emisión de CH_4 se deben principalmente a la excreción fecal de EE y FND. No obstante, en condiciones de manejo comercial estos factores se interrelacionaron de forma compleja con otros tales como el desperdicio de pienso o el uso de agua de limpieza.

En cuanto a las metodologías de determinación de las emisiones potenciales de NH_3 y CH_4 utilizadas en la presente Tesis Doctoral se ha determinado la factibilidad de reducir en 50% los recursos y el tiempo dedicados a la medición de las emisiones potenciales *in vitro*. Así mismo se ha constatado la posibilidad de predecir las emisiones potenciales de NH_3 y CH_4 a partir de las características fisicoquímicas de purines y de registros de emisión de las primeras etapas de ensayos *in vitro*.

8.2 Líneas de investigación futuras

Después de haber respondido las preguntas de investigación planteadas en cada uno de los objetivos de la esta Tesis Doctoral han surgido nuevas incógnitas que de ser resueltas permitirán mejorar el entendimiento de los mecanismos de mitigación de los efectos derivados de las emisiones ganaderas y en particular de las generadas por el sector porcino.

En este sentido creemos que es necesario mejorar el conocimiento de las emisiones de GEI en condiciones de granja comercial y determinar cómo los factores asociados a las instalaciones y a los sistemas de manejo influyen sobre las emisiones potenciales de las excretas generadas por los cerdos. De igual modo es necesario conocer la magnitud de las pérdidas de pienso por desperdicio en los diferentes sistemas de manejo y alojamiento y sus efectos sobre la composición de los purines las emisiones de NH_3 y CH_4 además de estimar las pérdidas económicas que estas implican a los sistemas productivos. En base a esta información podría desarrollarse tecnologías que permitan minimizar estas pérdidas y optimizar los sistemas productivos.

El estudio de los factores nutricionales y sus efectos en la composición de los purines y sus emisiones ha aportado resultados en gran medida coincidentes con la literatura. Sin embargo, los resultados obtenidos respecto al efecto de la grasa abren líneas de investigación de gran interés. En primer lugar, es importante profundizar en cómo afectan los distintos tipos de grasa a la digestión, composición de purines y sus emisiones. En segundo lugar, son complejos los mecanismos de interacción entre los distintos componentes del pienso (por ejemplo, la grasa con la fibra), y sus efectos en las emisiones, de forma que deberían revisarse y explorarse estas relaciones en mayor profundidad.

Teniendo como base la información generada en el marco de esta Tesis Doctoral y de los estudios adicionales realizados en el marco del proyecto *GASPORC-AGL2011-30023* es necesario conocer las implicaciones productivas y económicas de la implementación de las estrategias nutricionales evaluadas. Esto permitiría establecer nuevas recomendaciones nutricionales que tomen en cuenta consideraciones productivas, económicas y ambientales viables.

Del mismo modo queda por explorar los límites de las emisiones potenciales de NH_3 *in vitro* de purines de cerdo ya que, es probable que las emisiones continúen más allá de los 15 días evaluados en la presente Tesis Doctoral.

Por otro lado, consideramos que aún quedan por precisar detalles importantes de los procesos de fermentación microbiana en el tracto intestinal posterior de cerdos (intestino grueso y ciego), sobre todo de aquellos que pudieran darse con la inclusión de fuentes de fibra y grasa u otros insumos en el pienso y sus implicaciones en la excreción final de energía y nitrógeno y en la eficiencia del sistema.

Lista de publicaciones

Antezana, W., Calvet, S., Beccaccia, A., Ferrer, P., De Blas, C., García-Rebollar, P., Cerisuelo, A., 2015. Effects of nutrition on digestion efficiency and gaseous emissions from slurry in growing pigs: III. Influence of varying the dietary level of calcium soap of palm fatty acids distillate with or without orange pulp supplementation. Anim. Feed Sci. Technol., 209:128-136.

Antezana, W., De Blas, C., García-Rebollar, P., Rodríguez, C., Beccaccia, A., Ferrer, P., Cerisuelo, A., Moset, V., Estellés, F., Cambra-López, M., Calvet, S., 2016. Composition, potential emissions and agricultural value of pig slurry from Spanish commercial farms. Nutr. Cycl. Agroecosystems, 104:159-173.

Antezana, W., Ferrer, P., Cambra-López, M., Estellés, F., Calvet, S.2015. Ammonia emission quantification from pig slurry using acid wet traps: evaluation and optimization. Aceptado en: Water, Air, & Soil Pollution.

Presentaciones en Congresos Nacionales

Antezana, W., Cerisuelo, A., Ferrer, P., Estellés, F., Moset, V., García-Rebollar, P., De Blas C. y S. Calvet. Efecto de la variación en la cantidad de nitrógeno ligado a la fibra en piensos de cerdos sobre las características del purín resultante y su potencial de producción de NH3, biogás y CH4. Workshop REMEDIA, Abril de 2014, Valencia, España.

Antezana, W., Calvet, S., Ferrer, P., Estellés, F., Cambra-López, M., Beccaccia, A., García-Rebollar, P., De Blas, C. and Cerisuelo, A. Modificación del nivel y tipo de fibra en piensos de cerdos mediante la inclusión de subproductos agroindustriales sobre las características del purín y su potencial de producción de amoniaco, biogás y metano. Workshop REMEDIA, Abril de 2014, Valencia, España.

Estellés, F., **Antezana, W.**, Ferrer, P., Cerisuelo, A., García-Rebollar, P., De Blas, C. and Calvet, S. The effect of slurry composition on ammonia potential emissions from fattening pig slurries: a review of three nutrition assays. Workshop REMEDIA, mayo de 2015, Madrid, España.

Cerisuelo, A., **Antezana, W.**, Estellés, F., Ferrer, P., Beccaccia, A., García-Rebollar, P., De Blas, C. and Calvet, S. The effect of slurry composition on methane potential emissions from fattening pig slurries: a review of three nutrition assays. Workshop REMEDIA, mayo de 2015, Madrid, España.

Presentaciones en Congresos Internacionales

Estelles, F., Sanz-Cobeña, A., Beccaccia, A., **Antezana, W**., Cambra-López, M., Ferrer, P., Cerisuelo, A., Garcia-Rebollar, P., Vallejo, A., De Blas, C. and S. Calvet. Dietary fibre in pigs diets: effects on greenhouse gas emissions from slurry storage to field application. Conference Animal Change, Madrid, Spain, May 2014.

Antezana, W., Ferrer, P., Calvet, S., and Estellés, F. Measuring ammonia emissions from pig slurry using acid wet traps with two different protocols. Proc. Int. Conf. Agricultural Engineering, AgEng2014, Zurich.

Cerisuelo, P., Ferrer, A., Beccaccia, S., Calvet, S., Cambra-López, M., Estellés, F., **Antezana, W.**, García-Rebollar, P. and C. De Blas. Dietary from agroindustrial by-products in pig diets: effects on nutrient balance and ammonia and methane emissions. 65 th Annual Meeting of the European Federation of Animal Science. Copenhagen, Denmark, May, 2014.

Calvet, S., **Antezana, W.**, Ferrer, P., Estellés, F., Cambra, M., Beccaccia, A., Cerisuelo A., García-Rebollar P. and C. De Blas. Potential NH3 and CH4 Emissions from Pig Slurries produced in Commercial and Experimental Sites. 18 th World Congress of Commission International Biosyst. Eng. Beijing, China, September, 2014.

Antezana , W., Estellés, F., Cerisuelo, A., Ferrer, P., Beccaccia, A., García-Rebollar, P., De Blas, C. and S. Calvet. The effect of slurry composition on NH3 potential emissions from fattening pig slurries: a review of three nutrition assays. 16 th International Conference, Rural-Urban Symbiosis RAMIRAN. Hamburg, Germany, September 2015.

Antezana, W., Estellés, F., Cerisuelo, A., Ferrer, P., Beccaccia, A., García-Rebollar, P., De Blas, C. and S. Calvet. The effect of slurry composition on CH_4 emissions from fattening pig slurry: a review of three nutrition assays. 16 th International Conference, Rural-Urban Symbiosis RAMIRAN. Hamburg, Germany, September 2015.

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