Effects of Acidifying Pig Diets on Emissions of Ammonia, Methane, and Sulfur from Slurry during Storage


Abstract

Ammonia (NH₃) volatilization from intensive livestock production is a threat to natural ecosystems. This study investigated pig diet manipulation by 1% (w/w) benzoic acid (BA) amendment and lowering of dietary electrolyte balance through substituting 1.4% (w/w) CaCO₃ with 2.0% (w/w) CaCl₂. Urine and feces were collected separately from 24 pigs fed one of four diets (Control, +BA, +CaCl₂, +BA+CaCl₂) in metabolic cages and mixed as slurry. During 103 d of storage, all acidifying diets consistently reduced pH in the slurry by 0.4 to 0.6 units. There was a strong relationship between slurry pH and NH₃ emissions, which were considerably reduced by the three acidifying diets. The +BA diet decreased NH₃ emission by 28%, the +CaCl₂ diet by 37%, and the combined +BA and +CaCl₂ diet by 40%. Acidifying diets had no effect on S cycling or emission of volatile S compounds under the prevailing conditions of restricted S feeding. Methane (CH₄) emissions were increased by 73% in diets with CaCl₂. An initial delay in CH₄ emissions was investigated in a separate experiment with manipulation of pH (5.4, 6.7, or 8.8) and inoculation with adapted pig slurry (0, 4, 11, or 19%), which showed that methanogenic potential, rather than inhibitory effects of the chemical environment, caused the delay. In conclusion, NH₃ emissions from slurry could be reduced by addition of BA to pig diets or by controlling the dietary electrolyte balance, but there was no additive effect of combining the two strategies. However, CH₄ emissions from slurry may increase with acidifying diets.

IN MANY PARTS of the world, NH₃ volatilization is of great concern because nitrogen (N) deposition to land can cause eutrophication of natural ecosystems (Fangmeier et al., 1994). The problem is aggravated by a large regional concentration of livestock (Galloway et al., 2004). Acidification of liquid manure (slurry) has proven effective in mitigating NH₃ emissions (e.g., Kai et al., 2008; Petersen et al., 2012, 2013), but acidification is typically achieved by addition of sulfuric acid at a significant cost. Also, acidification of slurry in the slurry pit does not affect losses occurring from wet surfaces, such as slatted floors. It is therefore relevant to consider alternative strategies for acidification, such as diet manipulation.

Slurry acidification through diet manipulation can be attained by different means, one of which is the addition of organic acids (Eriksen et al., 2010; Philippe et al., 2011). Benzoic acid (BA) is approved in the European Union as an ingredient in diets for fattening pigs at a level between 5 and 10 g kg⁻¹ (European Commission, 2007), and BA is effective in decreasing urinary pH by voiding hippuric acid, which is produced in the liver from metabolism of BA (Kristensen et al., 2009; Nørgaard et al., 2010a, 2010b). The use of acids in diets for pigs elicits an antimicrobial effect and increases gain (Kluge et al., 2006). Our previous study showed that when pigs were fed diets supplemented with BA, it reduced NH₃ volatilization by 60 to 70% (Eriksen et al., 2010). However, this study also found an interaction between acidification and dietary sulfur (S), with consequences for the emission of S-containing compounds that are well known to cause malodor (Hansen et al., 2012).

Another potential acidification strategy is to control the dietary cation–anion difference. In many practical situations, the cation–anion balance of the diet is called the dietary electrolyte balance (dEB) and is calculated as dEB = meq (Na + K) – Cl per 100 g diet DM, which is a simplified equation representing the major elements that contribute to keeping the acid–base homeostasis as close as possible to normal (Mongin, 1981). When dEB is reduced (e.g., by increasing dietary Cl⁻), blood pH and bicarbonate concentration go down, indicating metabolic...

Abbreviations: BA, benzoic acid; dEB, dietary electrolyte balance; DMS, dimethyl sulfide; MT, methanethiol; TAN, total ammoniacal nitrogen; VSC, volatile S compounds.
acidity (Patience et al., 1987), because protons are secreted into urine by the kidneys to adjust the acid–base status to normal levels. It has been demonstrated that dietary supplementation of Cl decreases dEB and consequently acidifies urine or slurry, reducing NH₃ emissions (Canh et al., 1998).

Eriksen et al. (2010) found that BA amendment to the diet of pigs caused a reduction, albeit transient, in emissions of CH₄, which has a global warming potential 25 times higher than that of CO₂ (Forster et al., 2007). Hence, dietary manipulation could influence greenhouse gas emissions from manure management. Because CH₄ emissions from fresh slurry often show a lag phase (Møller et al., 2004; Moset et al., 2012), the effects of diet manipulation may be more important for CH₄ emissions during long-term storage. On the other hand, fresh slurry is typically mixed with older material in slurry pits, which could enhance decomposition processes, including CH₄ emissions (Zeeman et al., 1988; Sommer et al., 2007) and possibly the effects of dietary manipulation.

The objective of the present study was to quantify the effects of acidifying pig diets on slurry pH and on NH₃ and other emissions during storage. A diet manipulation experiment was performed with BA amendment and lowering of dEB as strategies to reduce NH₃ emissions. Benzoic acid and CaCl₂ have been investigated previously as acidifying agents but, to our knowledge, not in combination. In view of the different mechanisms involved, it was hypothesized that the effects would be additive. We further monitored the turnover of S-containing compounds and organic acids and concurrent emissions of S-containing gases and CH₄ to characterize treatment effects on metabolic pathways in the slurry. A separate experiment examined the effects of slurry pH and of mixing with aged slurry for short-term CH₄ emissions.

### Materials and Methods

#### Animals and Diets

Twenty-four female crossbred pigs were randomly assigned to four different experimental diets (Table 1). The basic diet (major ingredients: barley, wheat, soybean meal, and fat) was optimized according to Danish recommendations for amino acids, Ca, and P (Jørgensen and Tybirk, 2008). To obtain the four experimental diets, the basic diet was divided into four batches and mixed with BA (Vevo Vitall, DSM Special Products), CaCO₃, or CaCl₂ as follows: diet Control (no BA, 14 g kg⁻¹ CaCO₃), diet +BA (10 g kg⁻¹ BA, 14 g kg⁻¹ CaCO₃), diet +CaCl₂ (no BA, 20 g kg⁻¹ CaCl₂), and diet +BA+CaCl₂ (10 g kg⁻¹ BA and 20 g kg⁻¹ CaCl₂). The amendments of CaCO₃ and CaCl₂ supplied equal amounts of calcium.

Once the pigs reached a weight of 36 kg, they were fed the experimental diets and were housed individually in pens with concrete floors and straw as rooting material. Diets were fed ad libitum, and there was free access to water at all times. From 60 to 66 kg, the pigs were housed in stainless steel balance cages for quantitative collection of urine and feces. After 5 d of adaptation, all pigs were fitted with urine bladder catheters allowing separate collection of feces and urine into a closed container for 7 d. The pigs were fed twice daily at 0800 and 1430 h, and they had free access to demineralized water. To supply the animals with similar amounts of nutrients, allowing estimation of nutrient balance, the offered daily rations were 1800, 1818, 1810, and 1828 g feed d⁻¹ for the treatments Control, +BA, +CaCl₂ and +BA+CaCl₂, respectively. The pigs were weighed at the beginning and at the end of the 12-d period. Urine was weighed and collected once daily. Feed residuals and feces were collected twice daily. Urine and feces was separately pooled from each individual pig and stored at 3°C until the end of the collection period, where

### Table 1. Feedstuff and chemical composition of diets supplemented with 0 or 10 g kg⁻¹ benzoic acid (BA) and two calcium sources (CaCO₃ or CaCl₂).

<table>
<thead>
<tr>
<th>Feedstuff composition, g 100 g⁻¹</th>
<th>Control</th>
<th>+BA</th>
<th>+CaCl₂</th>
<th>+BA+CaCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>56.2</td>
<td>56.2</td>
<td>56.2</td>
<td>56.2</td>
</tr>
<tr>
<td>Wheat</td>
<td>23.0</td>
<td>23.0</td>
<td>23.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Soybean meal, toasted dehulled</td>
<td>17.7</td>
<td>17.7</td>
<td>17.7</td>
<td>17.7</td>
</tr>
<tr>
<td>Fat</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>L-lysine HCl (78%)</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>DL-methionine (99%)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>L-threonine (99%)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Monocalciumphosphate</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Vitamin and mineral mix</td>
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<td>0.20</td>
<td>0.20</td>
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<tr>
<td>Phytase</td>
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<tr>
<td>CaCO₃</td>
<td>1.40</td>
<td>1.40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0</td>
<td>0</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>BA</td>
<td>0</td>
<td>1.00</td>
<td>0</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical composition, g 100 g⁻¹ DM†</th>
<th>Control</th>
<th>+BA</th>
<th>+CaCl₂</th>
<th>+BA+CaCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>0.00</td>
<td>0.82</td>
<td>0.04</td>
<td>0.89</td>
</tr>
<tr>
<td>pH</td>
<td>6.06</td>
<td>5.54</td>
<td>5.20</td>
<td>4.72</td>
</tr>
<tr>
<td>dEB; meq kg⁻¹ DM‡</td>
<td>185</td>
<td>182</td>
<td>182</td>
<td>182</td>
</tr>
</tbody>
</table>

† Analyzed concentration of benzoic acid and measurement of pH as double analysis. DM, dry matter.
‡ Dietary electrolyte balance. Calculated as (Na⁺/23.0 + K⁺/39.1 – Cl⁻/35.5) × 1000, on the basis of analyzed concentrations in g kg⁻¹.
representative samples from each pig were stored at −20°C until analysis of total N and total ammoniacal N (TAN).

Storage Experiment

Immediately before storage (Day 0), a batch of pig slurry from each individual pig (i.e., six replicates per treatment) was prepared by mixing urine and feces in amounts proportional to the excretion, and portions of 20 kg were transferred to 25-L polyethylene containers and stored at 20°C for 103 d. The containers had lids with a single 10-mm hole to allow the escape of gases produced in the slurry during storage.

After 1, 5, 12, 19, 27, 43, 57, 76, and 103 d of storage and after gentle stirring, 100-mL aliquots of slurry were collected from each of the containers for chemical analyses. Then 600-mL subsamples were taken for measuring gaseous emissions as described below. No special precautions were taken to exclude air during sampling. Subsamples for chemical analyses were processed immediately as described below to minimize subsequent oxidation effects.

Chemical Analyses of Stored Slurry

The redox potential (Eh) was measured using a Pt electrode against a calomel reference electrode, and pH was measured with a glass electrode.

For analysis of dissolved sulfide and sulfate (sulfate on Days 1, 12, 27, 43, and 103), a centrifuge tube was filled with slurry, closed with a diffusion-tight lid, and centrifuged for 10 min at 5000 g at 4°C. From the supernatant, 10 mL was quickly transferred to 50 mL of 20% zinc acetate and stored cold until analysis of precipitated ZnS. For this analysis, a 5-g sample of the zinc acetate–preserved sample was transferred to a 300-mL diffusion flask. A test tube with 15 mL oxygen-free 3% alkaline zinc acetate solution was placed inside the diffusion flask before sealing with a rubber stopper. The flask was evacuated and filled with N2 three times to obtain anoxic conditions. Using a syringe with a hypodermic needle, 15 mL oxygen-free concentrated hydrochloric acid was added to the slurry, and the flask was left overnight at 20°C. The sulfide trap was then removed, and sulfide concentrations were determined using the methylene blue method as described by Cline (1969).

For sulfate analysis, 30 mL of the zinc acetate–preserved sample was centrifuged at 12,000 rpm for 2 h, and activated charcoal was added to the supernatant to remove dissolved organic matter. The sulfate content was determined turbidometrically after acidification with hydrochloric acid as described by Hoque et al. (1987). Total S in slurry was determined by turbidimetry after wet-ashing with magnesium nitrate and perchloric acid as described by Hoque et al. (1987). Total S in slurry was determined by turbidimetry after wet-ashing with magnesium nitrate and perchloric acid as described by Hoque et al. (1987). Total S in slurry was determined by turbidimetry after wet-ashing with magnesium nitrate and perchloric acid as described by Hoque et al. (1987).

For logistical reasons, only three of the six replicates of each treatment were analyzed for volatile S compounds (VSC) and CH4. Volatile S compounds were determined on Days 12, 27, and 43 on a gas chromatograph (Clarus 500, PerkinElmer) with an amperometric S detector. The S-compounds (H2S, methanethiol, dimethylsulfide, dimethyl disulfide, and dimethyl trisulfide) were separated on a capillary column (30 m, 4 μm polydimethylsiloxane film) with ultrapure helium as carrier gas (8.2 mL min−1 at 40°C). For calibration, a permeation chamber (Dynacal) containing dimethyl sulfide (DMS) (release rate: 73 ng min−1 at 50°C) was used. The system has an equimolar response to S compounds according to the supplier. The limit of detection was approximately 10 ppbV, and background samples were run frequently.

The bags were analyzed for CH4 concentration using a Shimadzu GC-14 equipped with a Porapak Q column (50°C) and flame ionization detector (150°C). The carrier was ultrapure helium at 60 mL min−1.

Controls of Short-Term Methane Emissions

A separate incubation experiment was performed to investigate if the initial delay in CH4 emissions observed in all treatments during slurry storage was due to the inhibitory effects of pH on methanogens in fresh slurry (in which case pH adjustment should modify CH4 emissions) or if the delay was rather due to a lack of methanogens adapted to the slurry environment.

Fresh urine and feces were collected from pigs on a diet similar to the control diet in the previous storage experiment. Three pH levels were established by the addition of hydrochloric acid: an unamended control at pH 8.8 and two hydrochloric acid–amended treatments at pH 6.7 and 5.4. These treatments were combined with four levels of inoculum corresponding to 0, 4, 11, and 19% by weight. The inoculum was collected 3 mo into an ongoing storage experiment with slurry from fattening pigs showing CH4 emissions of 5 mg CH4–C kg−1 volatile S h−1 a few weeks before sampling (unpublished data). There was no additional adjustment of pH after this amendment. For each combination of pH1 and inoculum, 25 mL of fresh slurry and inoculum as required was added in triplicate to 120-mL flasks that were then flushed with N2 for 15 min and closed with a septum and screw cap. A hypodermic needle was inserted in the septum to prevent pressure buildup while minimizing exposure to oxygen. The flasks were incubated at 20°C in the dark without agitation. After 0, 2, 4, 8, 10, and 14 d, the needles were removed, the headspace was flushed with N2 for 15 min, and the flasks were closed. For determination of CH4 emission rates, headspace CH4 concentrations were then determined in duplicate and again 2 h later using the procedure described above. Slurry pH was measured in all flasks by the end of the incubation period.
Statistical Analysis

Data on feces, urine, and slurry N content were analyzed using a generalized linear model procedure of SAS (SAS Institute, 1999). Slurry characteristics (pH, sulfide, sulfate, total S, and organic acids) and gas concentrations (NH₃, CH₄, H₂S, and VSC) were analyzed by a repeated measures ANOVA using the REPEATED statement in the PROC MIXED procedure of SAS (Littell et al., 2002) with sampling time, diet, and interactions between those as fixed effects and cage number as random effect. The type of covariance structure was selected based on Akaike’s Information Criterion. The covariance matrix was estimated between those as fixed effects and cage number as random effect. The pH of slurry from pigs on the control diet was initially high (around pH 8.5) (Fig. 1); pH then gradually dropped by 0.4 to 0.6 units compared with the control diet; across the experiment there were no significant differences among these three diets (Fig. 1). The +BA+CaCl₂ diet resulted in an initial decrease of slurry pH of 1 unit, but this effect quickly narrowed to a reduction around 0.5 pH unit. The effect was prolonged, and by the last day of sampling (Day 103) a difference of 0.4 pH units between acidifying diets and the control diet remained.

Diet Effects on Organic Acids

In diets with BA alone or BA+CaCl₂, hippuric acid was elevated (Fig. 2) at the first sampling only (P < 0.001). By Day 7, hippuric acid had disappeared but was almost quantitatively replaced by BA, a product of hippuric acid degradation. Benzoic acid concentrations declined over time (Fig. 2) but were elevated in +BA and +BA+CaCl₂ treatments throughout the study (P < 0.001). The +BA treatment increased acetic (P < 0.001), isobutyric (P < 0.001), butyric (P < 0.001), and valeric acid (P < 0.001) concentrations compared with the other diets and increased succinic acid (P < 0.001) compared with the +CaCl₂ diets. The +CaCl₂ diets caused isovaleric acid concentrations to be lower compared with the +BA (P < 0.001) and the control diet (P < 0.01). There were no effects of the acidifying diets on the concentrations of formic, propionic, isocapronic, and lactic acids.

Diet Effects on Sulfur Compounds

The sulfate content of pig slurry was not affected by type of diet (Fig. 3). From Day 12 and onward, decreasing sulfate contents indicated sulfate reduction activity, initially high but then at declining rates in the last part of the experiment. Also, independent of diet, an accumulation of sulfide was observed (Fig. 3). Total S content was determined at Day 1 and Day 103 (results not shown). There was a significant decline in total S content between these days (P < 0.001), but the total S content and loss of S was independent of diets. The initial total S content decreased by 0.6 units between acidifying diets and the control diet; across the experiment there were no significant differences among these three diets (Fig. 1). The +BA+CaCl₂ diet resulted in an initial decrease of slurry pH of 1 unit, but this effect quickly narrowed to a reduction around 0.5 pH unit. The effect was prolonged, and by the last day of sampling (Day 103) a difference of 0.4 pH units between acidifying diets and the control diet remained.

The redox potential was measured in all samples to evaluate the redox status of each system (results not shown). Following Bohn et al. (1985), pE+pH was calculated, where pE = Eh/59.2. A value of 0 in aqueous systems indicates completely reduced conditions, and a value of 20.78 indicates completely oxidized conditions (Bohn et al., 1985). Except for the first sampling, values for pE+pH were within a narrow range of 3.5 to 4.5, with no significant difference among diets.

Results

Diet Effects on Manure Production, pH, and Redox Potential

The diets significantly affected the production of feces (P < 0.001) and urine (P < 0.05), with 22% less feces and 26% more urine produced by animals fed the CaCl₂-amended diet compared with the control and with 40% less feces in the +BA+CaCl₂ diet (Table 2). The pH of feces was unaffected by diet, whereas the pH of urine was reduced by the +CaCl₂ diet (Table 2). In slurry, the initial pH (Fig. 1) was not significantly reduced by BA, whereas CaCl₂ amendment initially decreased pH by 0.6 units and BA+CaCl₂ amendment by 1 unit.

The pH of slurry from pigs on the control diet was initially high (around pH 8.5) (Fig. 1); pH then gradually dropped by around 1 pH unit before increasing to a final value of around pH 8.2. All acidifying diets consistently (P < 0.001) reduced pH in the slurry by 0.4 to 0.6 units compared with the control diet; across the experiment there were no significant differences among these three diets (Fig. 1). The +BA+CaCl₂ diet resulted in an initial decrease of slurry pH of 1 unit, but this effect quickly narrowed to a reduction around 0.5 pH unit. The effect was prolonged, and by the last day of sampling (Day 103) a difference of 0.4 pH units between acidifying diets and the control diet remained.

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<table>
<thead>
<tr>
<th>Diet†</th>
<th>Control</th>
<th>+BA</th>
<th>+CaCl₂</th>
<th>+BA+CaCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feces</td>
<td>Production, g d⁻¹</td>
<td>882 ± 43a†</td>
<td>825 ± 49a</td>
<td>699 ± 85b</td>
</tr>
<tr>
<td></td>
<td>Total N, g kg⁻¹ DM‡</td>
<td>33.7 ± 0.8</td>
<td>33.6 ± 0.8</td>
<td>33.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>6.85 ± 0.2</td>
<td>6.56 ± 0.16</td>
<td>7.33 ± 0.18</td>
</tr>
<tr>
<td>Urine</td>
<td>Production, kg d⁻¹</td>
<td>5.4 ± 0.6ab</td>
<td>5.0 ± 0.3b</td>
<td>6.3 ± 0.4a</td>
</tr>
<tr>
<td></td>
<td>Total N, g kg⁻¹</td>
<td>3.5 ± 0.3</td>
<td>3.3 ± 0.1</td>
<td>3.2 ± 0.2</td>
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<tr>
<td></td>
<td>pH</td>
<td>8.24 ± 0.16</td>
<td>7.93 ± 0.3a</td>
<td>6.91 ± 0.2b</td>
</tr>
<tr>
<td>Slurry</td>
<td>Total N, g kg⁻¹</td>
<td>3.6 ± 0.3</td>
<td>3.6 ± 0.1</td>
<td>3.1 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>NH₄-N, g kg⁻¹</td>
<td>2.3 ± 0.2</td>
<td>2.0 ± 0.12</td>
<td>2.2 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>DM content, g kg⁻¹</td>
<td>19.9 ± 2.1b</td>
<td>26.5 ± 1.2ab</td>
<td>19.9 ± 1.3b</td>
</tr>
</tbody>
</table>

† Mean ± SE (n = 6). Values with different letters within rows are significant at P < 0.05.
‡ Dry matter.

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was 200 ± 7 μg S g⁻¹ (mean ± SEM; n = 24), and the final S content was 138 ± 6 μg S g⁻¹ after 103 d of storage.

**Diet Effects on Emissions**

Emissions of three VSC (H₂S, methanethiol [MT], and DMS) were observed, whereas dimethyl disulfide and dimethyl trisulfide could not be detected in this study. The effect of diet were not identified for any of the three S gases (Fig. 4), and only for DMS was there an effect of sampling day (P < 0.001), with significantly highest DMS emission at Day 12.

The N contents of the slurries were not influenced by diet, and on average they contained 3.4 g N kg⁻¹ (SEM = 0.1; n = 24), of which 62% was in the form of NH₄⁺–N (Table 2). As expected, there was a strong relationship between slurry pH and NH₃ emissions. Ammonia release to the airflow established over slurry samples during measurements was considerably reduced (P < 0.05) by all three acidifying diets (Fig. 5). On average, the +BA diet decreased NH₃ emission rates by 28%, the +CaCl₂ diet by 37%, and the combined +BA+CaCl₂ diet by 40%. The concentration measured in air above the control slurry corresponded to an emission rate of 14 g NH₃–N m⁻² d⁻¹.

The pH in pig slurry increased over the first week of storage in all dietary treatments, whereas CH₄ emissions remained low. A gradual decline in pH occurred over several weeks, but temporal dynamics differed among treatments (i.e., pH of the control and +BA treatments reached a minimum after around 60 d, whereas pH in the two treatments with CaCl₂ dropped faster and reached a minimum after 4 wk). A peak in CH₄ emission rates was observed after 8 d, with no apparent lag phase. Emissions were strongly affected by time (P < 0.001), by pH (P < 0.001),
Fig. 3. Concentrations of sulfate and sulfide in stored pig slurry as influenced by dietary acidification using benzoic acid (BA) and CaCl₂. Error bars: SEM (n = 6).

Fig. 4. Volatile sulfur compounds emissions to airflow across the surface of stored pig slurry as influenced by dietary acidification using benzoic acid (BA) and CaCl₂. Error bars: SEM (n = 3).
and by the addition of inoculum ($P < 0.001$), and there were significant interactions between these variables. The intermediate pH always exhibited the highest CH$_4$ emission rates (Fig. 6). Without inoculum, emissions remained low, with minor activity at pH 6.7 only. At 4 and 11% inoculum, the lowest pH (5.4) caused a reduction in CH$_4$ emissions, whereas the intermediate pH (6.7) stimulated CH$_4$ emissions compared with the control. At 19% inoculum there was a stimulation of CH$_4$ emissions at low and, particularly, intermediate pH. Across all three pH levels, the accumulated CH$_4$ emission was, respectively, 15, 27, and 44 times higher in the presence of 4, 11, and 19% inoculum.

**Discussion**

**Ammonia Emissions**

The most important slurry properties controlling NH$_3$ emissions to the atmosphere are TAN and pH. The latter is controlled by the buffering capacity of dissolved carbonates, organic acids, ammoniacal N, and organic particles (Sommer, 2013), all of which are affected by feeding practice. The increase in slurry pH of all dietary treatments during the first week of storage was probably due to mineralization of organically bound

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**Fig. 5.** Ammonia and CH$_4$ emissions to airflow across the surface of stored pig slurry as influenced by dietary acidification using benzoic acid (BA) and CaCl$_2$. Error bars: SEM ($n = 6$).

**Fig. 6.** Effects of pH and addition of aged slurry as inoculum on CH$_4$ emissions from pig slurry during short-term incubation. Error bars: SEM ($n = 3$).
Methane Emissions

A previous report showed a transient inhibition of CH₄ emissions during storage of slurry from pigs on a diet with 2% BA (Eriksen et al., 2010). In the present study there was, relative to the control treatment, no effect of 1% BA (treatment +BA). Toxicity of BA is related to the undissociated form, and, with a pKa of 4.2, the concentrations of undissociated BA in slurry from pigs fed 2% BA would have been ~10 mg kg⁻¹, which is around the threshold where inhibition has been observed with coliform bacteria and a microalga (Knarreborg et al., 2002; Lee and Chen, 2009). Toxic effects of 1% BA would therefore not be expected, leaving only the effects of pH.

With acidifying diets +CaCl₂ and +BA+CaCl₂, there was an increase in CH₄ emissions during the first month of storage. The stimulation of CH₄ emissions in treatments +CaCl₂ and +BA+CaCl₂ was surprising because the difference in pH compared with the +BA treatment was 0.5 units (maximum). Sprott and Patel (1986) found that Ca²⁺ partly alleviated inhibition from NH₃ in pure cultures of several methanogens. This could also have been the case in the storage experiment because initial concentrations of free NH₃ were in a range (50–500 mg kg⁻¹) where methanogens unadapted to NH₃ may be inhibited (Hashimoto, 1986).

All treatments of the storage experiment showed a delay in CH₄ emissions of 1 to 2 wk, indicating that the chemical environment alone did not determine methanogenic activity. The importance of methanogenic potential was investigated in separate incubations of fresh pig slurry with four levels of inoculum and three pH levels (Fig. 6). Here, CH₄ emission rates were positively related to inoculum rate and occurred with no apparent lag phase. The highest CH₄ emission rates always occurred at pH 6.7 (i.e., close to the typical pH optimum of methanogens) (Koster and Koomen, 1988), but in the absence of inoculum CH₄ emissions were insignificant throughout the 14-d incubation period. Methanogens adapted to the slurry environment may tolerate high levels of free NH₃ (van Velsen, 1979; Hashimoto, 1986), and the significant increase in CH₄ emissions when an inoculum of adapted methanogens was introduced implies that under practical storage conditions the emission of CH₄ from fresh excreta would be more sensitive to slurry pH, and thus to dietary manipulation, than observed in the present storage experiment.

Organic Acids

There was initially a rapid accumulation of volatile fatty acids (C₂–C₅) that was probably due to the imbalance between methanogenesis and acidogenesis and acetogenesis. The concentrations of acetic, propionic, butyric, and valeric acid were highest in slurry from pigs fed the BA-amended diet and lowest in slurry from CaCl₂-amended diets. Also, the concentrations of isobutyric, isovaleric, and isoacronic acid, which are products of protein fermentation (Macfarlane and Macfarlane, 2003), were highest in slurry from the BA-amended diets. This may indicate that organic matter degradation was delayed in slurry from the +BA treatment, possibly as a result of inhibition by BA in the digestive system, as indicated above (Knarreborg et al., 2002).

Sulfur Turnover

It has been found that acidification of slurry to pH 5.5 inhibits sulfate reduction (Eriksen et al., 2008, 2012; Ottosen et al., 2009). Contrary to these observations, the dietary acidification in this experiment of 0.4 to 0.6 pH units did not appear to influence sulfate reduction (Fig. 3). From an initial level of around 150 μg S g⁻¹ (equivalent to 4.5 mmol L⁻¹ SO₄²⁻), it decreased to around 25 μg S g⁻¹ during the storage period, with a concurrent increase in sulfide for all diets. It thus appears that acidification effects above pH 7 do not greatly affect sulfate reduction in slurry.

For all diets, about 30% of the total S content was lost during the storage period as a result of volatilization of H₂S and organic S compounds. However, the absolute levels were low compared with previous investigations regarding total S content (Eriksen et al., 1995) and S emissions (Eriksen et al., 2008). An initial level of total S of 200 μg S g⁻¹ may therefore indicate that the dietary ingredients, as well as the diets, did not contain excessive levels of S.

Previous studies have demonstrated an interaction between methanogens and S emission probably caused by the ability of methanogens to demethyleate organic S compounds (Higgins et al., 2006; Eriksen et al., 2010). A similar interaction was not evident in our data, although at Day 27 methanogenic activity, as revealed by high CH₄ emission rates in +CaCl₂ and +BA+CaCl₂ treatments, was accompanied by low MT and DMS emission levels. Overall, however, this study did not provide evidence for changes in the odor profile of slurry from acidifying feeding caused by volatile S compounds. It has been demonstrated that elevated sulfate contents in slurry (700–900 μg g⁻¹) due to
abundant S in feed, combined with a pH reduction from 8.5 to 7.5, resulted in a considerable increase in especially MT (Eriksen et al., 2010). This is unfortunate because MT is a very strong odorant with an odor threshold of only 2.2 µg m⁻³, compared with 26 for H₂S (Devos et al., 1990).

**Practical Implications**

Acidifying diets were found to reduce NH₃ emissions by 28 to 40%, and it is thus a means to control emissions from pig production, independently or in combination with other management methods. Methane emissions were stimulated in treatments with CaCl₂, possibly by alleviating inhibition from NH₃. Under practical storage conditions, such an effect of CaCl₂ could be less important if mixing with older slurry material introduces methanogens adapted to high NH₃ levels.

In-house acidification with sulfuric acid in slurry pits (target pH 5.5) may reduce NH₃ emissions from pig production buildings by up to 70% (Kai et al., 2008). About one-third of remaining emissions are estimated to come from feces and urine deposited on surfaces above the slurry pit (Mikkelsen et al., 2011), and acidifying diets could thus further reduce NH₃ emissions from pig production buildings to a total of 80% if combined with in-house acidification. This combination of mitigation options would ensure that CH₄ emissions from the slurry pit are not increased as a result of pH changes or CaCl₂.

A prerequisite for successful use of acidifying diets is that animal performance and health are not impaired. In the present experiment, a tendency for lower feed intake was seen during the collection period when pigs were fed CaCl₂, and this corresponds well with early findings that low dietary electrolyte balance introduces methanogens adapted to high NH₃ levels. This trade-off between NH₃ and greenhouse gas emissions can be avoided by a further lowering of slurry pH in the slurry pit with sulfuric acid.

**Conclusions**

Ammonia emissions from pig slurry could be significantly reduced by the addition of BA to pig diets or by controlling the dietary electrolyte balance through replacement of CaCO₃ by CaCl₂. There was an additive effect of combining the two dietary interventions (BA and CaCl₂ amendment) on initial slurry pH but not on NH₃ emissions. The acidifying diets had no effect on S cycling or emission of VSC under the prevailing conditions of restricted S feeding. In contrast, CaCl₂ amendment, but not BA, significantly increased CH₄ emissions. This trade-off between NH₃ and greenhouse gas emissions can be avoided by a further lowering of slurry pH in the slurry pit with sulfuric acid.

**Acknowledgments**

The authors thank Bodil Steensgaard, Susan Ottesen, Karin Dyberg, and Karin Durup for skilled technical assistance. This study was partly funded by the Ministry of Food, Agriculture and Fisheries.

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