Effects of dirty housing and a *Salmonella* Typhimurium DT104 challenge on pig growth performance, diet utilization efficiency, and gas emissions from stored manure


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**ABSTRACT:** The objectives of this study were to elucidate the effects of a dirty environment and a *Salmonella* challenge plus associated environmental contamination on pig growth performance, diet utilization efficiency, and gas emissions (CO₂, NH₃, CH₄, N₂O, and H₂S) from stored manure. Twenty-four weaned barrows, aged 31 d at initiation of the trial, were randomly allotted to 3 different treatments in a completely randomized design. Treatments were: pigs housed in cages with manure removed and cages washed daily (Clean); pigs housed in cages sprayed daily with manure slurry mixtures (Dirty); or pigs challenged with *Salmonella* Typhimurium DT104 and housed in cages that were not washed, but manure was removed daily (*Salmonella* challenge). Rectal temperature, body weight, daily feed intake, manure output, manure composition, and gas emissions from stored manure were measured throughout the 24-d animal phase. The Dirty and *Salmonella* challenge treatments were statistically compared to the Clean treatment to evaluate individual effects. Dirty housing tended to decrease ADG from d 1 to 24 (*P* = 0.06) but there were no other effects on pig performance compared with the Clean treatment. In contrast, a *Salmonella* challenge was associated with a marked reduction in each of the measured indicators of pig performance. *Salmonella* challenge increased the carbon to nitrogen ratio, ether extract, and lignin concentrations in excreted manure (*P* = 0.02, 0.01, 0.003, respectively), and increased manure and head space temperatures in manure tanks (*P* < 0.0001). Gas emissions from stored manure of pigs on the Dirty or *Salmonella* treatments were increased for each of the measured gases as compared to the Clean treatment (*P* < 0.01) when expressed per unit of BW gain. When gas emissions from manure of pigs housed in the Dirty treatment were expressed per unit of manure volatile solids (VS), they were increased for NH₃, CH₄, and H₂S (*P* < 0.02). *Salmonella* challenge was associated with increased emissions of CO₂, and N₂O and decreased emissions of H₂S per kilogram manure VS compared to the Clean treatment (*P* = 0.06, 0.03, 0.04, respectively). Collectively, these results indicated that a *Salmonella* challenge and associated housing contamination caused depressed growth rate and increased manure gas emissions, while exposure to a Dirty environment slightly reduced growth performance and clearly increased manure gas emissions per unit of BW gain as compared to Clean control.

**Key words:** ammonia, CO₂, manure, methane, *Salmonella*, stress


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INTRODUCTION

Salmonellosis is considered one of the most serious food-borne zoonoses afflicting an estimated 1.3 million people annually, causing over 500 deaths and $2.4 billion in economic losses in the U.S. (Mead et al., 1999; Scallan et al., 2011). Salmonella is commonly found in the farm environment, and it is reported that 25% to 48% of the U.S. swine herd may be colonized with Salmonella species (Davies et al., 1997; Funk et al., 2001).

Sanitary conditions of the growing environment exert considerable influence on animal production. Le Floc’h et al. (2006) reported that deterioration of sanitary conditions could limit growth performance by inducing a moderate immune response in the absence of clinical signs of illness. Thus, a clean environment should improve growth productivity by reducing the effects of stress and pathogenic challenges (Bassaganya-Riera et al., 2001; Le Floc’h et al., 2009). Although differences in pig growth performance for clean versus dirty environments or pathogen-challenged environments have been evaluated, the effects of these 3 different states on manure gas emissions have not been considered. Animal manure releases carbon dioxide (CO₂), ammonia (NH₃), methane (CH₄), nitrous oxide (N₂O), and hydrogen sulfide (H₂S) during storage and handling which represents approximately 1.3% of total U.S. greenhouse gas emissions (USEPA, 2014). Thus, it is important to quantify the effects of dirty housing and pathogen-challenged environments on swine productivity and the environmental impact of swine operations.

We hypothesized that a Salmonella challenge and associated housing contamination or a dirty environment would reduce pig growth performance and decrease diet utilization efficiency leading to increased nutrients available for conversion to greenhouse gases in stored manure as compared to a very clean environment. The objectives of this work were to quantify the effects of housing sanitation and a Salmonella challenge with associated pathogen contamination of the housing environment on pig growth performance, diet utilization efficiency, and gas emissions from stored manure.

MATERIALS AND METHODS

This study was conducted in accordance with the Federation of Animal Science Societies’ Guide for the Care and Use of Agricultural Animals in Research and Teaching, and approved by the Virginia Tech Institutional Animal Care and Use and Bio-safety Committees.

Animals and Experimental Design

Twenty-four crossbred, weaned barrows (age, 21 d) were randomly assigned to 1 of 3 treatments based on initial BW (7.5 ± 1.1 kg) in a completely randomized design. The treatments were: 1) a clean environment with pigs housed in cages which were washed daily with unheated tap water using a high-pressure washer (Clean); 2) a dirty environment with pigs housed in cages that were sprayed with manure slurry mixtures every day (Dirty); and 3) a Salmonella challenge which encompassed the effects of an oral inoculation with Salmonella Typhimurium DT104 and the resulting contamination of cages with pathogens shed in the feces that was not removed by daily washing (Salmonella). The objective of not washing the Salmonella challenge cages was to more closely replicate on-farm conditions where the facilities cannot be cleaned during the course of an outbreak. Recycling of the pathogen through fecal contamination may prolong the challenge as indicated by rectal temperatures (Balaji et al., 2000; Gebru et al., 2010), thus increasing the loss in production and potential increase in manure emissions.

All animals were from the Virginia Tech swine center and were housed individually in metabolic cages (1.20 m × 1.20 m) in an ABSL-2 facility. Upon receipt of the pigs, rectal swabs were collected individually to screen for the presence of Salmonella. Rectal swabs were incubated at 37°C for 24 h in Gram-negative Hajna broth (BD Bioscience, Franklin Lakes, NJ), then plated onto Brilliant Green Agar (BGA; BD Bioscience) to screen for Salmonella colonies. All pigs were confirmed to be negative for Salmonella presence in feces. After 10 d of adjustment (31 d of age), pigs (n = 8) allotted to the challenge group were orally inoculated with 1 × 10⁹ CFU of Salmonella enterica serotype serovar Typhimurium strain DT104 (ATCC; BAA-185, Manassas, VA) with resistance to 20 ppm novobiocin and 25 ppm Nalidixic acid (S. Typhimurium NalRNovR) in 5 mL of tryptic soy broth (TSB) as previously described (Price et al., 2010). Salmonella challenged pigs were housed in a separate room from nonchallenged pigs within the ABSL-2 facility. Entry into and movement throughout the facility was strictly controlled and managed to prevent contamination of the noninoculated rooms with Salmonella. One pig in the Salmonella group died 2 d after being challenged (33 d of age) due to complications associated with the challenge. Challenged pigs were housed for 24 d postchallenge (55 d of age), and during this time daily quantification of numbers of Salmonella shed in feces (CFU/g) were performed as previously described (Price et al., 2010). Excreted manure was collected daily from each cage. Subsequent to manure collection each day the Clean cages were washed and the Dirty cages were sprayed with manure slurry. The challenged pigs were euthanized using a lethal, intravenous dose of...
sodium pentobarbital (Beuthanasia-D; Schering-Plough, Kenilworth, NJ) at the conclusion of the animal portion of the study. The carcasses were disposed of as regulated medical waste in accordance with Virginia Tech regulations. Animal age was 32 d, 35 d, 43 d, and 55 d on d 1, 4, 12, and 24 postchallenge, respectively.

An antibiotic free, corn-soybean meal pelleted diet was formulated to meet or exceed NRC (1998) recommendations for nutrients (Table 1). Before feeding, the diet was screened for the presence of Salmonella and confirmed negative. Pigs had ad libitum access to feed and water throughout the trial. The ambient room temperature was maintained at 28°C throughout the study.

**Sample Collection and Preparation**

Feed consumption was recorded daily, and BW was monitored every 4 d. Rectal temperature was assessed for each pig twice per day at 0600 h and 1800 h for 11 d after Salmonella inoculation (d 31 to 42 of age). Salmonella Typhimurium DT104 shedding in feces was monitored only for the challenged group every day for 19 d after challenge (31 to 50 d of age). Manure (feces, urine, waste feed, and spilled water) from 2 adjacent cages in the same treatment group was collected and combined each day. The weight and volume of collected manure was determined daily, and pH was measured using a pH meter (model AB15; Thermo Fisher Scientific Inc., Waltham, MA). Collected manure was mixed and subsampled. One subsample was stored at -20°C until manure composition analysis, and the other representing 20% of the manure volume was loaded into the manure tank allotted to those 2 cages.

**Manure Storage and Gas Collection and Analysis System**

Manure storage tests were run concurrently with the animal experiments. The manure storage and collection system has been previously described (Ni et al., 2010; Page et al., 2014). Briefly, 12 sealed manure tanks (100 cm height and 43 cm diameter) were placed in a separate room within the ABSL-2 facility with ambient temperature maintained at 28°C. Airflow through each tank was adjusted to 7 L min⁻¹ and maintained throughout the storage period. At the beginning of the experiment, 18 L of building tap water was added to each tank. Gases produced were measured over a 24-h period every other day (6 tanks on 1 d and the other 6 on the next day) throughout the trial.

Gases from the manure storage tanks and background air (from the compressor room) were analyzed for CO₂ (Model 410i; Thermo Fisher Scientific Inc.), NH₃ (Model 17i; Thermo Fisher Scientific Inc.), H₂S (Model 450i; Thermo Fisher Scientific Inc.), CH₄ (Model 55i; Thermo Fisher Scientific Inc.), and N₂O (Model 46i; Thermo Fisher Scientific Inc.) concentrations every minute for 17 min during each 2-h period as previously described (Li et al., 2015). Gas emitted from the manure storage tanks was monitored from the beginning of the experiment (d 1) until 11 d after completion of the animal trial. Manure and head space temperatures in the storage tanks were monitored and recorded every 60 s using a HOBO U12 4-external channel data logger (Onset Computer Corporation, Bourne, MA).

Gas emission rates from storage tanks were calculated as gas concentration multiplied by airflow (7 L min⁻¹). The periodic rate measurements were then reduced to simple daily means by tank. Because gas emissions from each tank derived from 20% of the total daily manure collected from 2 pigs, the mean daily tank emission rates were arithmetically expressed per pig per day. Gas emissions per kilogram of manure

### Table 1. Ingredient and nutrient composition of the diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Content, % of AF¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>60.07</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>28.30</td>
</tr>
<tr>
<td>Wheat middlings</td>
<td>5.00</td>
</tr>
<tr>
<td>Lard</td>
<td>1.57</td>
</tr>
<tr>
<td>Blood meal</td>
<td>1.50</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.18</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.07</td>
</tr>
<tr>
<td>Vitamin/Mineral Premix²</td>
<td>0.15</td>
</tr>
<tr>
<td>Salt</td>
<td>0.35</td>
</tr>
<tr>
<td>Selenium premix (0.06%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Lysine (78.8%)</td>
<td>0.36</td>
</tr>
<tr>
<td>D.L.methionine</td>
<td>0.15</td>
</tr>
<tr>
<td>L-threonine (98.5%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Choline Cl (60%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Maxi-Mil³</td>
<td>0.05</td>
</tr>
<tr>
<td>Ronozyme P-CT⁴</td>
<td>0.03</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
</tbody>
</table>

1 AF = air-dried feed.

² Provided per kilogram of diet AF: 348 mg Zn, 397 mg Fe, 54 mg Cu, 1.3 mg I, 157 mg Mn, 11,015 IU Vitamin A, 1,764 IU Vitamin D, 122 IU Vitamin E, 70 mg niacin, 30 mg pantothenic acid, 0.40 mg biotin, 1.6 g choline, 2.1 mg folic acid, 4.4 mg pyridoxine.

³ Anatox, Lawrenceville, GA.

⁴ Novozymes, Bagsværd, Denmark.
volatile solids (VS) and per kilogram of BW gain were calculated as daily gas emissions per pig divided by daily VS output and BW gain per pig, respectively using the mean observed VS and BW gains for the 2 pigs represented in the tank.

**Feed and Manure Nutrient Composition Analysis**

Feed was sampled once per week throughout the trial, and weekly samples were composited across the study. After completion of the trial, frozen manure samples were thawed at room temperature for 2 d in a BSL-2 laboratory, mixed, and composited proportionally to the original excreted volume into 4 samples representing d 31 to 34, 35 to 38, 39 to 46, and 47 to 55 of age. The composited samples were autoclaved for 120 min at 121°C to inactivate the *Salmonella* pathogens so that the samples could be analyzed in a non-BSL-2 laboratory. Manure DM was measured at Virginia Tech (AOAC, 1995). Feed and autoclaved manure samples were sent to Cumberland Valley analytical services (Hagerstown, MD), and assayed for ADF, lignin, ether extract, ash, calcium, and phosphorus. Total carbon and nitrogen was determined at Virginia Tech using a nitrogen and carbon analyzer (Vario EL cube; Elementar Americas Inc., Mount Laurel, NJ). The efficiency of nutrient utilization and N retention were calculated using the formula:

$$\text{Efficiency(\%)} = \frac{\text{DMI} \times \text{NC} - \text{MW} \times \text{NCm}}{\text{DMI} \times \text{NC}} \times 100$$

where DMI is in kg; NC is the dietary concentration of DM, ADF, lignin, ether extract, phosphorus, and total N (% DM basis); MW is the daily manure output (kg); and NCm is the manure nutrient concentration (% DM basis).

**Statistical Analyses**

Gas and temperature data were assembled and collated by time using SAS (version 9.3; SAS Inst. Inc., Cary, NC) and reduced to daily means. Daily mean data were analyzed using the PROC GLIMMIX procedure of SAS using contrast statements to orthogonally compare Clean versus Dirty, and Clean versus *Salmonella* treatments. As the animals were housed individually, animal was the experimental unit for performance measures (n = 24). However, the manure from 2 pigs was combined for storage gas emissions, and thus storage tank was the experimental unit for gas measurement (n = 12). Growth performance, manure output and composition, diet utilization efficiency, and gas emissions were assessed as continuous variables in time using a repeated measures model,

$$Y_{ijk} = \mu + g_i + d_j + e_{ijk},$$

where $\mu$ was overall mean, $g_i$ was the fixed effect of treatment, $d_j$ was the effect of time after challenge analyzed as a repeated measurement, and $e_{ijk}$ was random error. The spatial covariance structure was used for estimating covariance, and the subject of the repeated measurements was defined as cage or tank nested within treatment. Room and treatment were confounded in our design due to the limited number of rooms in the facility and need to confine the *Salmonella* challenge to the designated population. However, the rooms were all in a common facility, had identical dimensions and configuration, were served by a common air supply, and were environmentally controlled by a common system. Thus, we feel any potential effects of room were very minor. Differences between treatments were declared significant at $P < 0.05$, and a tendency was declared at $P$ values between 0.05 and 0.10. All results were reported as least squares means (LSM).
Linear regression equations for predicting daily CO₂, CH₄, NH₃, N₂O, and H₂S emissions were derived using PROC GLM of SAS with a backward elimination approach using a significance level for retention of terms in the model of 0.1. Manure mass (accumulated daily wet weight), manure composition (percent DM, total N, ADF, lignin, ether extract, ash, calcium, and phosphorus), manure temperature, and manure head space temperature in the tank were chosen to construct the best linear regression equation. Data used for model development were restricted to that from the time the pigs were challenged with *Salmonella* d₁ (31 d of age) to 24 d postchallenge (54 d of age).

**RESULTS**

**Rectal Temperature and *Salmonella* Typhimurium DT104 Shedding**

Rectal temperatures for the 10 d postchallenge are presented in Fig. 1, and fecal shedding of *Salmonella* Typhimurium DT104 is presented in Fig. 2. All pigs were negative for fecal *Salmonella* before inoculation. Rectal temperature increased sharply on d 1 for *Salmonella* challenged pigs relative to the Clean treatment (*P* = 0.01). This coincided with the greatest fecal shedding counts (CFU/g of feces). Rectal temperature gradually decreased to normal on d 5. No significant differences in rectal temperature were observed between Dirty and Clean treatments. All inoculated pigs continued to intermittently shed *Salmonella* for the duration of the experiment likely reflecting the continued presence of the pathogen on cage surfaces.

**Growth Performance, Manure Excretion, and Nutrient Utilization Efficiency**

Data for ADFI, BW, ADG, and G:F are presented in Table 2. Both the Dirty and *Salmonella* treatments had no effects on ADFI from d 1 to 4, d 5 to 12, and d 13 to 24 postchallenge relative to the Clean treatment; however, *Salmonella* challenged pigs housed in unclean condition had a tendency (*P* = 0.08) for decreased ADFI over the entire experimental period (d 1 to 24) relative to the Clean treatment. Body weights did not differ between the Clean and Dirty treatments at any point. The *Salmonella* treatment was associated with reduced BW at d 4, 12, and 24 after challenge compared to the Clean treatment (*P* = 0.0001, 0.05, 0.03, respectively). Depressed ADG were observed for the Dirty treatment after extended exposure (d 13 to 24; *P* = 0.03) which tended to impact ADG for the trial (*P* = 0.06). Compared with the Clean treatment, the *Salmonella* treatment reduced ADG from d 1 to 4 and d 1 to 24 (*P* = 0.002, 0.05) relative to the Clean treatment. Although the Dirty treatment depressed ADG, G:F did not differ

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Clean</th>
<th>Dirty</th>
<th><em>Salmonella</em></th>
<th>SEM</th>
<th>Clean vs. Dirty</th>
<th>Clean vs. Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADFI, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 1 to 4 postchallenge</td>
<td>0.77</td>
<td>0.80</td>
<td>0.63</td>
<td>0.06</td>
<td>0.69</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>d 5 to 12 postchallenge</td>
<td>0.90</td>
<td>0.98</td>
<td>0.95</td>
<td>0.09</td>
<td>0.52</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>d 13 to 24 postchallenge</td>
<td>1.32</td>
<td>1.27</td>
<td>1.20</td>
<td>0.09</td>
<td>0.74</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>d 1 to 24 postchallenge</td>
<td>1.02</td>
<td>1.03</td>
<td>0.88</td>
<td>0.06</td>
<td>0.90</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>BW, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 1 postchallenge</td>
<td>11.73</td>
<td>11.20</td>
<td>11.90</td>
<td>0.41</td>
<td>0.36</td>
<td>0.77</td>
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</tr>
<tr>
<td>d 4 postchallenge</td>
<td>14.10</td>
<td>13.93</td>
<td>12.22</td>
<td>0.28</td>
<td>0.65</td>
<td>0.0001</td>
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</tr>
<tr>
<td>d 12 postchallenge</td>
<td>19.41</td>
<td>19.68</td>
<td>16.99</td>
<td>0.80</td>
<td>0.81</td>
<td>0.05</td>
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<tr>
<td>d 24 postchallenge</td>
<td>29.52</td>
<td>28.29</td>
<td>26.32</td>
<td>0.95</td>
<td>0.36</td>
<td>0.03</td>
<td></td>
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<tr>
<td>ADG, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 1 to 4 postchallenge</td>
<td>0.63</td>
<td>0.57</td>
<td>0.30</td>
<td>0.06</td>
<td>0.46</td>
<td>0.002</td>
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<tr>
<td>d 5 to 12 postchallenge</td>
<td>0.67</td>
<td>0.71</td>
<td>0.60</td>
<td>0.08</td>
<td>0.67</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>d 13 to 24 postchallenge</td>
<td>0.84</td>
<td>0.71</td>
<td>0.78</td>
<td>0.04</td>
<td>0.03</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>d 1 to 24 postchallenge</td>
<td>0.71</td>
<td>0.60</td>
<td>0.59</td>
<td>0.04</td>
<td>0.06</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>G:F, kg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 1 to 4 postchallenge</td>
<td>0.78</td>
<td>0.68</td>
<td>0.37</td>
<td>0.07</td>
<td>0.30</td>
<td>0.001</td>
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</tr>
<tr>
<td>d 5 to 12 postchallenge</td>
<td>0.74</td>
<td>0.74</td>
<td>0.65</td>
<td>0.06</td>
<td>0.98</td>
<td>0.32</td>
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</tr>
<tr>
<td>d 13 to 24 postchallenge</td>
<td>0.65</td>
<td>0.58</td>
<td>0.67</td>
<td>0.04</td>
<td>0.21</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>d 1 to 24 postchallenge</td>
<td>0.70</td>
<td>0.65</td>
<td>0.64</td>
<td>0.04</td>
<td>0.27</td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

1Values are expressed as LSM. *n* = 8.

2Pigs were challenged with *Salmonella* Typhimurium DT104.
Table 3. Manure output and composition from pigs raised in Clean or Dirty treatments or challenged with *Salmonella* and housed in initially clean, but unwashed cages.\(^1\)^\(^2\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Clean</th>
<th>Dirty</th>
<th><em>Salmonella</em></th>
<th>SEM</th>
<th>P-value</th>
<th>Clean vs. Dirty</th>
<th>Clean vs. <em>Salmonella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily output, kg DM/pig</td>
<td>0.19</td>
<td>0.22</td>
<td>0.25</td>
<td>0.05</td>
<td>0.55</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Daily output, L/per pig</td>
<td>3.64</td>
<td>3.06</td>
<td>1.68</td>
<td>0.63</td>
<td>0.47</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Output, g DM/g as fed intake</td>
<td>0.11</td>
<td>0.13</td>
<td>0.11</td>
<td>0.02</td>
<td>0.42</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.99</td>
<td>7.13</td>
<td>7.14</td>
<td>0.09</td>
<td>0.24</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>6.53</td>
<td>9.88</td>
<td>8.50</td>
<td>2.07</td>
<td>0.16</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>C:N(^4)</td>
<td>6.24</td>
<td>5.72</td>
<td>8.73</td>
<td>0.65</td>
<td>0.56</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Total C, %</td>
<td>41.68</td>
<td>38.58</td>
<td>41.00</td>
<td>3.35</td>
<td>0.44</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Total N, %</td>
<td>6.01</td>
<td>5.88</td>
<td>5.77</td>
<td>0.17</td>
<td>0.60</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>ADF, %</td>
<td>7.85</td>
<td>9.39</td>
<td>10.16</td>
<td>0.82</td>
<td>0.21</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Lignin, %</td>
<td>1.91</td>
<td>2.43</td>
<td>2.82</td>
<td>0.17</td>
<td>0.05</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Ether extract, %</td>
<td>4.94</td>
<td>5.34</td>
<td>5.95</td>
<td>0.20</td>
<td>0.18</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Ash, %</td>
<td>18.13</td>
<td>21.87</td>
<td>23.56</td>
<td>3.16</td>
<td>0.36</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Calcium, %</td>
<td>1.00</td>
<td>1.39</td>
<td>1.18</td>
<td>0.13</td>
<td>0.06</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>1.24</td>
<td>1.34</td>
<td>1.16</td>
<td>0.08</td>
<td>0.33</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

1. Nutrient concentrations are reported based on the DM. Values are expressed as LSM. \(n = 8\).
2. Manure nutrient concentrations were expressed on a DM basis, whereas DM concentration was reported as percent of manure slurry.
3. Pigs were challenged with *Salmonella* Typhimurium DT104.

between the Clean and Dirty treatments. Compared with the Clean treatment, the *Salmonella* treatment decreased G:F from d 1 to 4 \((P = 0.001)\).

Manure output and composition data are summarized in Table 3. Compared with the Clean treatment, the *Salmonella* treatment decreased the volume of excreted manure pig\(^{-1}\) d\(^{-1}\) \((P = 0.03)\), increased the carbon to nitrogen ratio, and lignin and ether extract concentrations \((P = 0.02, 0.003, 0.01, \text{ respectively})\), and tended to increase ADF concentration in excreted manure \((P = 0.07)\). Pigs exposed to the Dirty treatment had greater lignin and calcium concentrations compared to that of Clean pigs \((P = 0.05, 0.06)\). No treatment effects were observed for manure DM output per pig or per gram of feed intake, manure pH, or any other manure nutrient concentrations.

Nutrient utilization efficiency and N retention are presented in Table 4. No treatment effects were found for DM, ADF, or phosphorus utilization efficiencies, nor for N retention. Decreased lignin disappearance from the gut was observed for the Dirty treatment as compared to the Clean treatment \((P = 0.02)\), and there was a trend for reduced ether extract utilization efficiency \((P = 0.07)\).

**Temperature and Residual Manure Composition from Stored Tanks**

Manure and tank head space temperatures over the period of storage and manure composition in the tanks at the end of the study are presented in Table 5. Both manure and head space temperatures were greater for the *Salmonella* treatment compared to the Clean treatment \((P < 0.01)\). Stored manure from pigs in the Dirty treatment had lower total N and C concentrations versus the Clean treatment \((P = 0.07, 0.06)\). Stored manure from *Salmonella* challenged pigs tended to have greater DM concentrations compared to the Clean treatment \((P = 0.06)\). No treatment effects were observed for stored manure mass and nutrient composition at the end of the study.

**Gases Emitted from Stored Manure**

Gas emissions over time are presented in Fig. 3, and emission data are summarized in Table 6. Gas emissions gradually increased with time and reduced after manure loading was halted. Emissions of NH\(_3\) appeared to increase in association with a secondary fermentation in the storage tanks. The remaining gases had relatively constant emission trends with respect to time. *Salmonella* challenge increased daily CO\(_2\) emissions per pig over the entire experimental period \((P = 0.001, 0.005)\) compared to the Clean treatment. There were no treatment effects for any other gases when expressed per pig per day. When the emissions data were expressed per kilogram of BW gain to reflect the yield per unit of product, both the Dirty and *Salmonella* treatments had significantly increased gas production \((P < 0.001)\) except for H\(_2\)S emissions as compared to the Clean treatment.

Compared with the Clean treatment, the *Salmonella* treatment tended to increase CO\(_2\) emissions per kilogram of manure VS output, increased N\(_2\)O emissions per kilogram of manure VS output, and decreased H\(_2\)S emissions \((P = 0.06, 0.03, 0.04, \text{ respectively})\). The
Dirty treatment markedly increased NH$_3$, CH$_4$, and H$_2$S emissions per kilogram of manure VS output relative to the Clean treatment ($P = 0.01$, 0.0002, 0.02, respectively). No treatment effects were observed for the other gases when expressed in this manner. There were also no significant treatment effects for NH$_3$ and NO$_2$ N emissions per kilogram of total N excreted.

**Gas Emissions Prediction Model**

Results from regressing gas emissions on manure characteristics are shown in Table 7. Daily CO$_2$ emissions were positively related with accumulated manure weight, manure pH, manure temperature, manure head space temperature, DM content, and ether extract concentration; and negative related with ash concentration. Accumulated manure weight, manure pH, manure temperature, DM content, ether extract concentration, calcium concentration, and phosphorus concentration all had positive relationships with daily CH$_4$ emission. Accumulated manure weight, manure pH, manure temperature, manure head space temperature, DM content, calcium concentration, and phosphorus concentration were positively related with daily NH$_3$ emission, whereas ADF and ash concentrations were negatively related. Hydrogen sulfide emissions were positively related with accumulated manure weight, calcium concentration, and phosphorus concentration, and negatively related with manure temperature, manure head space temperature, ether extract concentration, lignin concentration, total N concentration, and ash concentration. There were positive relationships between N$_2$O emissions and manure temperature, head space temperature, total N concentration, and lignin concentration; and a negative relationship with manure mass, ether extract concentration, and calcium concentration.

**DISCUSSION**

Pigs challenged with *Salmonella* in the unclean environment exhibited an acute febrile response, as indicated by the increased rectal temperature, which is an established indicator of clinical manifestation of salmonellosis in pigs (Balaji et al., 2000; Turner et al., 2002; Gebru et al., 2010). Previous studies reported that a *S. Typhimurium* DT104 challenge resulted in clinical presentation of diarrhea (Scharek-Tedin et al., 2013). Although no attempt to score the severity of diarrhea associated with *Salmonella* challenge was made in the current study, a watery consistency was observed in the challenged group for the first 2–3 d after challenge. *S. Typhimurium* DT104 was also isolated from each inoculated pig in high numbers (4 to 8 log$_{10}$ CFU/g feces) for 5 d after challenge, and all inoculated pigs continued to shed low levels of *Salmonella* for the duration of the experiment likely due to the continued presence of the pathogen in the uncleaned cages. These data confirm that a clinical manifestation of salmonellosis due to challenge with *S. Typhimurium* DT104 was achieved.

Renaudeau (2009) reported reduced BW, ADFI, and ADG when pigs were placed in a Dirty treatment that was attributed to altered feeding behavior. In the current study, the Dirty treatment depressed ADG from d 13 to 24 and d 1 to 24, but no significant effects on BW, ADFI, and G:F or DM and N utilization efficiencies were observed relative to the Clean treatment. Although depressed ADFI was observed in previous studies (Bassaganya-Riera et al., 2001; Le Floc’h et al., 2009), the Dirty treatment did not affect ADFI in the current study. The reason for this difference may be that pigs were reared individually thus reducing social stress and competition for feed. Additionally, although the cages were quite Dirty, the bacterial load did not appear to overwhelm the immune system leading to active infections or pathogens were not present in the environment as evidenced for the lack of elevated body temperatures. However, there was a trend for increased nutritional costs resulting in reduced ADG.

*Salmonella* challenge combined with unclean environment markedly decreased ADG during the first 4 d after inoculation and tended to reduce ADFI. The reduction in ADFI appeared to explain much of the

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**Table 4. Nutrient utilization efficiencies and N retention (%) for growing pigs raised in Clean or Dirty treatments without *Salmonella* challenge or challenged with *Salmonella* and housed in initially clean, but unwashed cages**

<table>
<thead>
<tr>
<th>Item</th>
<th>Clean</th>
<th>Dirty</th>
<th><em>Salmonella</em></th>
<th>SEM</th>
<th>Clean vs. Dirty</th>
<th>Clean vs. <em>Salmonella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>79.86</td>
<td>76.75</td>
<td>77.74</td>
<td>3.98</td>
<td>0.53</td>
<td>0.67</td>
</tr>
<tr>
<td>ADF, %</td>
<td>69.10</td>
<td>57.26</td>
<td>63.84</td>
<td>7.49</td>
<td>0.21</td>
<td>0.57</td>
</tr>
<tr>
<td>Lignin, %</td>
<td>73.95</td>
<td>54.17</td>
<td>63.80</td>
<td>6.83</td>
<td>0.02</td>
<td>0.21</td>
</tr>
<tr>
<td>Ether extract, %</td>
<td>77.89</td>
<td>68.84</td>
<td>72.29</td>
<td>4.53</td>
<td>0.07</td>
<td>0.24</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>70.14</td>
<td>62.28</td>
<td>67.28</td>
<td>5.47</td>
<td>0.29</td>
<td>0.52</td>
</tr>
<tr>
<td>Total N, %</td>
<td>69.22</td>
<td>66.68</td>
<td>65.17</td>
<td>5.38</td>
<td>0.72</td>
<td>0.57</td>
</tr>
</tbody>
</table>

1Values are expressed as LSM, $n = 8$.

2Pigs were challenged with *Salmonella* Typhimurium DT104.
reduced growth rate responses with the decrease in G:F from d 1 to 4 after challenge relative to the Clean treatment due at least in part to a reduction in the dilution of maintenance costs. Salmonella challenge could perturb the endocrine growth axis, immune function, and inflammatory reaction to produce detrimental effects on growth performance (Balaji et al., 2000; Turner et al., 2002; Davis et al., 2010; Gebru et al., 2010; Scharek-Tedin et al., 2013). Balaji et al. (2000) reported Salmonella challenge was responsible for increased plasma cortisol and suppressed plasma IGF-1, which were consistent with observed reductions in feed intake and depressed growth. In the current study, reduced ADFI supplied less energy and nutrients in support of growth which are consistent with decreased ADG and BW. Salmonella challenge could have also depressed growth performance through induction of inflammatory reactions and perturbations of endocrines controlling growth. However, we are unable to determine what proportion of the results of this treatment were due to infection compared with suboptimal housing conditions.

From a performance standpoint, the pigs in the Salmonella challenge within the unclean cages treatment appeared to have recovered from the effects of salmonellosis by d 5 having ADFI and ADG from d 5 to 12 and thereafter that were equivalent to those of the Clean group.

The trend for a reduction in ADFI for the Salmonella challenged pigs housed in unclean environment did not lead to differences in manure DM output, although the Salmonella treatment decreased manure volume output. Given the observation of more watery feces for challenged pigs, the latter observation was surprising. As the manure contained spilled water in addition to animal excreta, it seems likely the reduced manure volume for this treatment resulted from decreased activity and decreased spilled water during consumption rather than a reduction in fecal output. The combination of decreased spilled water and increased water content of the feces (watery consistency) may explain the lack of change in manure DM content.

The trend for an overall reduction in ADFI, the trend for increased ADF, and significant increases in ether extract per unit of manure DM suggest gut function was negatively affected by salmonellosis in the unclean environment. A reduction in lignin efficiency was observed in the Dirty treatment as compared to the Clean treatment likely suggesting that the lignin in ADF was not being liberated from the fiber matrix in the gut lumen resulting in greater lignin excretion.

The observed diet utilization efficiency results were consistent with other observations for healthy pigs, except for ADF and lignin (Le Goff and Noblet, 2001; Ziemer et al., 2012; Kerr et al., 2013). Although greater ADF utilization efficiency and lignin disappearance rate were similar to our previous PRRSV study (Li et al., 2015), we suspect manure handling and autoclaving may have increased their solubility in manure and thus reduced recovery (Edwards, 1973) leading to increased apparent utilization efficiency. Another possible explanation is that lignin and ADF escaped from the containers during fiber determination.

Table 5. Temperature and composition of manure in storage tanks at the end of the trial. Manure loaded into the tanks was generated by pigs raised in Clean or Dirty treatments or challenged with Salmonella and housed in initially clean, but unwashed cages1

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>P-value</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (manure), °C</td>
<td>Clean 17.74</td>
<td>0.08</td>
<td>0.0002 &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dirty 18.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 18.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (head space), °C</td>
<td>Clean 18.84</td>
<td>0.05</td>
<td>0.01 &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dirty 19.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 19.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manure mass, kg</td>
<td>Clean 66.30</td>
<td>12.90</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dirty 52.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 36.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>Clean 2.78</td>
<td>0.61</td>
<td>0.22</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Dirty 6.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 8.94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total C, %</td>
<td>Clean 40.18</td>
<td>0.61</td>
<td>0.07</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Dirty 38.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 40.89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N, %</td>
<td>Clean 5.67</td>
<td>0.20</td>
<td>0.06</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Dirty 5.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 5.53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C:N</td>
<td>Clean 7.12</td>
<td>0.30</td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Dirty 7.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 7.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADF, %</td>
<td>Clean 10.63</td>
<td>1.28</td>
<td>0.55</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Dirty 11.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 11.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignin, %</td>
<td>Clean 2.69</td>
<td>0.35</td>
<td>0.88</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Dirty 2.62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 2.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash, %</td>
<td>Clean 8.74</td>
<td>0.74</td>
<td>0.46</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Dirty 7.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 9.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium, %</td>
<td>Clean 21.84</td>
<td>1.82</td>
<td>0.96</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>Dirty 21.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 20.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>Clean 1.55</td>
<td>0.14</td>
<td>0.15</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Dirty 1.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella 1.59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Nutrient concentrations are reported on a DM basis. Values are expressed as LSM. n = 4.
2 Pigs were challenged with Salmonella Typhimurium DT104.
3 Accumulated manure mass in the tank.
4 Carbon and nitrogen ratio.
due to the particulate matter losses, so their concentrations were underestimated (Krämer et al., 2013).

The shift in gas production rates in the tanks may reflect a change in either rate of fermentation (substrate utilized per unit of time) or the apparent end products of fermentation. For example, a reduction in the yield of methane per unit of substrate may occur when methanogens are inhibited resulting in an increase in CO₂ and H₂ gas production. Assuming the O₂ is captured in water when CO₂ is converted to methane, one would have 1 mole of CO₂ and 4 moles of H₂ for every mole of methane spared, and thus 5 times the gas volume with no change in fermentation rate. Both the Dirty and Salmonella treatments had increased manure and head space temperatures. Given that all the tanks were placed in the same well-ventilated room, it is difficult to envision that the increase in temperature was externally driven, and thus would seem to indicate an increase in fermentation rate leading to increased heat generation. This conclusion is supported by the observed increase in CO₂ production rates per pig for the Salmonella treatment and numerical increases for the Dirty treatment. Manure DM output per pig and the carbon content of the manure were very similar across treatments. Thus, it would seem the substrate loaded into the tanks was not different, and the yield of gas per unit of substrate must be different, which is supported by the trend for increased CO₂ production per unit of VS for the Salmonella treatment. There also is no indication that the production rates were greater due to greater efficiency of nutrient extraction from the manure as the DM and carbon remaining in the tanks at the end of the gas measurements for the Dirty or Salmonella treatments were similar with that of the Clean treatment.

Compared with our previous study (Li et al., 2015), emissions of CO₂, NH₃, and N₂O pig⁻¹ d⁻¹ from manure storage tanks were reduced. Gaseous emissions and emission rates can be affected by the environment conditions, manure output, ventilation rate, surface area, manure handling schemes, fermentation time, and manure composition; however, those factors were largely the same across the studies. In the previous study, 18 L of seed manure was added to each tank which certainly contributed to gas emissions. In the current study, 18 L water was used as a starting point. Ni et al. (2010) reported that reduced manure DM content results in decreased CO₂, NH₃, H₂S concentrations and reduced NH₃ and CO₂ release rates, and thus manure dilution could have contributed to the observed differences in rates. In the present study, average emissions of CO₂, NH₃, CH₄, H₂S, N₂O were 8.9, 0.25, 0.09, 0.02, and 0.01 L pig⁻¹ d⁻¹ (12 to 28 kg BW), respectively. Kaparaju and Rintala

Figure 3. Gas emissions from stored swine manure collected from pigs raised in Clean or Dirty cages or challenged with Salmonella Typhimurium DT104 and housed in initially clean, but unwashed cages. Values are expressed as LSM, and error bars indicate SD.
Housing environment and gas emissions

(2011) reported emissions of CH$_4$ and N$_2$O of 7 L and 30 L pig$^{-1}$ d$^{-1}$, respectively. Sharpe et al. (2001) reported emissions of CH$_4$ of 9 to 60 L pig$^{-1}$ d$^{-1}$. Emissions of CH$_4$, N$_2$O, and CO$_2$ of 3.2, 0.08, 1500 L pig$^{-1}$ d$^{-1}$ were observed from a swine barn (Dong et al., 2007). Aarnink et al. (1996) observed a mean NH$_3$ emission rate of 1.1 L pig$^{-1}$ d$^{-1}$ from an experimental barn with pigs weighing 10.5 to 25.2 kg. Heber et al. (1997) reported mean H$_2$S emission rates of 0.1 L pig$^{-1}$ d$^{-1}$. In the current study, manure fermentation time (35 d) is less than the previous studies which contributes to reduced gas emissions; moreover, the surface area of the tanks may also contribute to the decreased emission rates. However, some of the values in the literature seem impossibly high. For example, 1500 L of CO$_2$ represents 66 moles or 793 g C/d as compared to daily C excretions of approximately 90 g pig$^{-1}$ d$^{-1}$ in the current study.

When gas emissions are expressed per kilogram of BW gain, both growth performance and gas emissions can be evaluated per unit of product. To produce 1 kg live weight, 2 to 3 times more gas was emitted from manure when pigs are reared in the Dirty environment or challenged with *Salmonella* relative to the Clean treatment. The primary reason for increased gas emissions per kilogram of BW gain is that both the Dirty and *Salmonella* treatments depressed ADG ($P = 0.06, 0.05$), and the *Salmonella* challenge increased daily CO$_2$ emissions. Surprisingly, the *Salmonella* challenge did not affect H$_2$S emissions per kilogram of BW gain.

Compared with the previous studies, much lower CH$_4$ emissions per kilogram of manure VS were observed due to the shorter storage time (35 d). Studies have shown that CH$_4$ emissions per kilogram of manure VS were highly dependent on storage time (Massé et al., 2003; Kaparaju and Rintala, 2011). Kaparaju and Rintala (2011) reported mean CH$_4$ yields of 360 L kg$^{-1}$ manure VS when storage time changed from 180 d to 272 d. In addition, manure temperature and solids content also affect CH$_4$ emissions per kilogram of manure VS (Park et al., 2006; Ni et al., 2010).

### Table 6. Gas emissions from stored manure derived from pigs raised in Clean or Dirty treatments without *Salmonella* challenge or challenged with *Salmonella* and housed in initially clean, but unwashed cages

<table>
<thead>
<tr>
<th>Item</th>
<th>Clean</th>
<th>Dirty</th>
<th><em>Salmonella</em></th>
<th>SEM</th>
<th>Clean vs. Dirty</th>
<th>Clean vs. <em>Salmonella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas emissions per pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO$_2$, L/d</td>
<td>2.87</td>
<td>6.62</td>
<td>17.27</td>
<td>2.03</td>
<td>0.22</td>
<td>0.001</td>
</tr>
<tr>
<td>NH$_3$, L/d</td>
<td>0.18</td>
<td>0.29</td>
<td>0.28</td>
<td>0.07</td>
<td>0.31</td>
<td>0.33</td>
</tr>
<tr>
<td>CH$_4$, L/d</td>
<td>0.04</td>
<td>0.11</td>
<td>0.11</td>
<td>0.04</td>
<td>0.29</td>
<td>0.33</td>
</tr>
<tr>
<td>H$_2$S, L/d</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
<td>0.61</td>
<td>0.78</td>
</tr>
<tr>
<td>N$_2$O, L/d</td>
<td>0.004</td>
<td>0.006</td>
<td>0.012</td>
<td>0.004</td>
<td>0.82</td>
<td>0.22</td>
</tr>
<tr>
<td>Gas emissions, per kg of BW gain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO$_2$, L</td>
<td>6.50</td>
<td>19.48</td>
<td>33.21</td>
<td>4.48</td>
<td>0.002</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>NH$_3$, L</td>
<td>0.21</td>
<td>0.40</td>
<td>0.41</td>
<td>0.03</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>CH$_4$, L</td>
<td>0.05</td>
<td>0.16</td>
<td>0.16</td>
<td>0.01</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>H$_2$S, L</td>
<td>0.019</td>
<td>0.031</td>
<td>0.017</td>
<td>0.002</td>
<td>&lt;.0001</td>
<td>0.30</td>
</tr>
<tr>
<td>N$_2$O, L</td>
<td>0.014</td>
<td>0.017</td>
<td>0.024</td>
<td>0.002</td>
<td>0.01</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Gas emissions, per kg of manure VS$^3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO$_2$, L</td>
<td>87.26</td>
<td>101.37</td>
<td>142.75</td>
<td>23.83</td>
<td>0.62</td>
<td>0.06</td>
</tr>
<tr>
<td>NH$_3$, L</td>
<td>2.84</td>
<td>5.41</td>
<td>2.66</td>
<td>0.64</td>
<td>0.01</td>
<td>0.85</td>
</tr>
<tr>
<td>CH$_4$, L</td>
<td>0.46</td>
<td>2.16</td>
<td>1.06</td>
<td>0.28</td>
<td>0.0002</td>
<td>0.15</td>
</tr>
<tr>
<td>H$_2$S, L</td>
<td>0.25</td>
<td>0.42</td>
<td>0.08</td>
<td>0.05</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>N$_2$O, L</td>
<td>0.17</td>
<td>0.19</td>
<td>0.23</td>
<td>0.03</td>
<td>0.35</td>
<td>0.03</td>
</tr>
<tr>
<td>N emissions, per kg of total N excreted$^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N$_3$H$_3$, g</td>
<td>4.70</td>
<td>6.04</td>
<td>4.94</td>
<td>0.72</td>
<td>0.23</td>
<td>0.82</td>
</tr>
<tr>
<td>N$_3$N$_2$, g</td>
<td>0.78</td>
<td>0.71</td>
<td>0.67</td>
<td>0.10</td>
<td>0.64</td>
<td>0.47</td>
</tr>
</tbody>
</table>

$^1$Values are represented as the LSM. n = 4.

$^2$Pigs were challenged with *Salmonella Typhimurium* DT104.

$^3$VS = volatile solids, which is calculated by subtracting manure ash from manure DM mass.

$^4$N$_3$H$_3$ = NH$_3$ nitrogen; N$_3$N$_2$ = NO$_2$ nitrogen.
fermentation rate which contribute to slightly decreased total C concentration in the terminal manure.

Average daily NH$_3$ and N$_2$O N emissions per kilogram of total N (g N/kg N) excreted were 5.2 g/kg and 0.7 g/kg, which corresponded with previous studies (Külling et al., 2002; Külling et al., 2003; Li et al., 2015). No treatment effects were observed for average daily NH$_3$ and N$_2$O N emissions per kilogram of total N (g N/kg N) excreted, which are consistent with the similar amount of excreted manure total N and released nitric gases among treatments.

The regression coefficients for the gas prediction equations were well defined. Compared with our previous PRRSV study, the regressions in the current study had less variance with respect to time (Fig. 3) resulting in greater than 50% of the variance explained except for the N$_2$O model which explained 23% of the overall variance. Coefficients and magnitudes of the coefficients are slightly different; moreover, more factors were involved in the current regression model. All of the manure mass coefficients were positive except for N$_2$O. Positive coefficients are consistent with a substrate product relationship given that manure mass represents total potential substrate for microbial fermentation. This is further reinforced by the concentration of DM in the manure for CO$_2$, CH$_4$, and NH$_3$, consistent with the observations of decreased manure mass in the storage tanks. The negative coefficients for ash reinforced the substrate product relationship as ash cannot be fermented to yield gas emissions.

Generally, lignin is assumed to be nonfermentable (Hills and Roberts, 1981) particularly at these relatively short storage times, and thus might be expected to have negative coefficients in the regression model. However, it was nonsignificant for CO$_2$, CH$_4$, and NH$_3$, and had a negative effect on H$_2$S and a trend for a positive one on N$_2$O. Thus, knowing lignin input to the fermentation system did not seem to offer a lot of value in predicting gas production and could likely be omitted with minimal impact on prediction quality.

Table 7. Linear regression equations relating gas emissions from stored swine manure to manure loading and composition, pH, and manure temperatures

<table>
<thead>
<tr>
<th>Variable</th>
<th>CO$_2$ (L/d)</th>
<th>CH$_4$ (L/d)</th>
<th>NH$_3$ (L/d)</th>
<th>H$_2$S (ml/d)</th>
<th>N$_2$O (ml/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-85.07 ± 10.45</td>
<td>-0.27 ± 0.04</td>
<td>-0.76 ± 0.11</td>
<td>70.81 ± 11.17</td>
<td>-32.65 ± 13.12</td>
</tr>
<tr>
<td>Manure mass¹, kg</td>
<td>0.12 ± 0.01</td>
<td>0.001 ± 0.0001</td>
<td>0.003 ± 0.0002</td>
<td>0.20 ± 0.01</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>Manure pH</td>
<td>4.63 ± 0.61</td>
<td>0.01 ± 0.004</td>
<td>0.02 ± 0.01</td>
<td>NS³</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.003$</td>
<td>$P &lt; 0.003$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manure temperature, °C</td>
<td>1.16 ± 0.40</td>
<td>0.01 ± 0.002</td>
<td>0.01 ± 0.004</td>
<td>-0.77 ± 0.36</td>
<td>0.87 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.004$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.001$</td>
<td>$P &lt; 0.04$</td>
<td>$P &lt; 0.04$</td>
</tr>
<tr>
<td>Head space temperature, °C</td>
<td>1.54 ± 0.60</td>
<td>NS</td>
<td>0.01 ± 0.006</td>
<td>-2.83 ± 0.56</td>
<td>1.40 ± 0.69</td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.02$</td>
<td></td>
<td>$P &lt; 0.03$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>DM², %</td>
<td>0.23 ± 0.08</td>
<td>0.002 ± 0.0004</td>
<td>0.005 ± 0.003</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.007$</td>
<td>$P &lt; 0.002$</td>
<td>$P &lt; 0.0001$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADF, % of DM</td>
<td>NS</td>
<td>NS</td>
<td>-0.005 ± 0.003</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$P &lt; 0.06$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N, % of DM</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>-2.29 ± 0.89</td>
<td>1.60 ± 0.96</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.1$</td>
</tr>
<tr>
<td>Ether extract, % of DM</td>
<td>1.74 ± 0.33</td>
<td>0.006 ± 0.002</td>
<td>NS</td>
<td>NS</td>
<td>-1.32 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.002$</td>
<td></td>
<td>$P &lt; 0.002$</td>
<td></td>
</tr>
<tr>
<td>Lignin, % of DM</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>-1.52 ± 0.58</td>
<td>2.75 ± 0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.0003$</td>
</tr>
<tr>
<td>Ash, % of DM</td>
<td>-0.20 ± 0.06</td>
<td>NS</td>
<td>-0.002 ± 0.001</td>
<td>-0.15 ± 0.07</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.002$</td>
<td></td>
<td>$P &lt; 0.008$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium, % of DM</td>
<td>NS</td>
<td>0.03 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>9.24 ± 1.23</td>
<td>-4.03 ± 1.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.002$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.002$</td>
</tr>
<tr>
<td>Phosphorus, % of DM</td>
<td>NS</td>
<td>0.03 ± 0.01</td>
<td>0.14 ± 0.02</td>
<td>11.74 ± 1.70</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$P &lt; 0.0003$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>201</td>
<td>227</td>
<td>239</td>
<td>238</td>
<td>219</td>
</tr>
<tr>
<td>$P$-value</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.52</td>
<td>0.64</td>
<td>0.76</td>
<td>0.74</td>
<td>0.20</td>
</tr>
<tr>
<td>RMSE⁴</td>
<td>4.68</td>
<td>0.03</td>
<td>0.05</td>
<td>4.64</td>
<td>5.38</td>
</tr>
</tbody>
</table>

¹Accumulated manure mass added to the storage tank.
²Nutrient composition of the excreted manure.
³NS = nonsignificant, $P > 0.1$.
⁴RMSE=root mean square error.
The same case would seem to be made for ADF as it only had a very marginal effect on NH₃ emissions.

Manure temperature and head space temperature were positive factors for emission rates of CO₂, CH₄, NH₃, and N₂O as would be expected given the temperature dependence of microbial activity and gas exchange between liquid and gaseous phases (Massé et al., 2003). The DM content of manure was positive for all gases, again reflecting increased substrate supply and the effect of greater solids concentrations (Amon et al., 2006; Ni et al., 2010). Ether extract content was positive for CO₂ and CH₄ regressions, and negative for N₂O. The former could represent a substrate effect (Amon et al., 2006), and the latter may represent a negative effect of free fatty acids on microbial fermentation (Long et al., 2012). Ash content was negative for all gases reflecting reduced manure VS supply.

Surprisingly, Ca and P had positive coefficients for CH₄, NH₃, and H₂S. It is unclear why this relationship was observed given that these elements are nonfermentable. It is possible that concentrations of those minerals in the slurry were limiting for microbes producing those gases (Takashima et al., 1990; van Vliet et al., 2006). If that was the case, then this may represent a potential mechanism for reducing the rate of production of these 3 gases. If the availability of these minerals was reduced through the addition of a chelating agent, the rate of gas production may be reduced thereby reducing the carbon and NH₃ footprints of at least the swine growing operation. Manure concentrations of Ca and P could also be controlled to a certain extent through feeding management. Keeping the dietary concentrations of these minerals to levels needed to just meet animal requirements would result in reduced excretion of those minerals (Dourmad and Jondreville, 2007), thus reducing the concentrations in manure.

In summary, the Dirty treatment impaired pig growth rates due to slightly reduced ether extract utilization efficiency and possibly by enhanced stress associated with greater immune activity. Pigs challenged with Salmonella had even greater reductions in performance through decreased ADFI and altered manure C concentration and temperature. Although daily manure volume was significantly decreased by the Salmonella treatment, the manure DM output and DM concentrations were not affected. As suggested by the temperature increase, the fermentability of the manure appeared to be much greater than that of the Dirty treatment. This shift in manure composition was consistent with increased production of CO₂ and N₂O per unit of manure VS associated with the Salmonella challenge. When gas emissions were expressed per unit of BW gain, both the Dirty and Salmonella challenge treatments had increased emissions of CO₂, CH₄, NH₃, and N₂O by as much as threefold compared with the Clean treatment. Housing pigs in a dirty environment resulted in slightly depressed efficiency and clearly increased gas emissions per unit of product produced. Salmonella challenge and the associated pathogen contamination of cages caused an even greater decline in animal performance, and gas production per unit of gain was increased as for the Dirty treatment except for H₂S. Therefore, less than optimal management of swine grower facilities could be expected to more than triple greenhouse gas production per unit of product.

LITERATURE CITED


