Influence of Thymol and a Urease Inhibitor on Coliform Bacteria, Odor, Urea, and Methane from a Swine Production Manure Pit

Vincent H. Varel* and James E. Wells

ABSTRACT

Pathogens, ammonia, odor, and greenhouse gas emissions are serious environmental concerns associated with swine production. This study was conducted in two manure pits (33,000 L each) to determine the influence of 1.5 or 3.0 g thymol L⁻¹ and 80 mg mL⁻¹ urease inhibitor amendments on urea accumulation, coliform bacteria, odor, and methane emission. Each experiment lasted 18 or 19 d, during which time 30 to 36 250-mL samples (six per day) were withdrawn from underneath each pit and analyzed for urea, thymol, volatile fatty acids, coliform bacteria, and Campylobacter. At the end of each experiment, six 50-g samples from each pit were placed in serum bottles, and gas volume and composition were determined periodically for 28 d. Compared with the control pit, volatile fatty acids production was reduced 64 and 100% for the thymol amendments of 1.5 and 3.0 g L⁻¹, respectively. Viable coliform cells were reduced 4.68 and 5.88 log₁₀ colony-forming units kg⁻¹ of slurry for the 1.5 and 3.0 g thymol L⁻¹, respectively, and Escherichia coli were reduced 4.67 and 5.01 log₁₀ colony-forming units kg⁻¹ of slurry, respectively. Campylobacter was not detected in the pits treated with thymol, in contrast to 63% of the samples being positive for the untreated pit. Urea accumulated in the treated pits from Day 3 to 6. Total gas production from serum bottles was reduced 65 and 76% for thymol amendments of 1.5 and 3.0 g L⁻¹, respectively, and methane was reduced 78 and 93%, respectively. These results suggest that thymol markedly reduces pathogens, odor, and greenhouse gas emissions from a swine production facility. The urease inhibitor produced a temporary response in conserving urea.

Ammonia and odor are ranked by the National Research Council of the National Academies in the USA as their highest concerns for emissions from confined animal feeding operations (CAFO) (National Research Council of the National Academies, 2002). Pathogens are also prevalent in wastes produced from CAFOs (Bhaduri et al., 2005; Hutchison et al., 2005). Traditionally, livestock wastes have been spread over land and used as fertilizer for agronomic crops. This practice has come under scrutiny because of potential runoff contamination of ground and surface water and air quality issues related to odor and ammonia emissions. Particulate matter from wastes is known to contain volatile fatty acids (VFA), associated with swine waste can be removed by amending the waste with Fe(III) and a novel dissimilatory Fe(III)-reducing organism. Field applications have not been evaluated. We have amended cattle and swine wastes in the laboratory with plant-derived oils, thymol, carvacrol, and eugenol and conclude that odor and pathogens can be reduced in these wastes (Varel and Miller, 2001; 2004). We have also conducted field studies with a urease inhibitor, N-(n-butyl) thiophosphoric triamide (NBPT), that have demonstrated that urea nitrogen can be retained in beef cattle feedlot waste if it is applied once per week to the feedlot surface (Varel et al., 1999). However, once applications were discontinued, all urea was hydrolyzed, and much of the ammonia nitrogen was emitted into the air. Nonetheless, Parker et al. (2005) concluded that the use of NBPT for reducing ammonia emissions from cattle feedlots looks promising. Here we describe a combination treatment—application of thymol and NBPT—to a swine production manure pit to determine the effect on total coliform bacteria, Escherichia coli, Campylobacter, odor (as represented by volatile fatty acids), urea accumulation, and production of the greenhouse gas methane. The current work is a subsequent study to an in vitro study with cattle waste that suggested that this approach may be successful (Varel et al., 2007).

MATERIALS AND METHODS

Chemicals

Thymol (5-methyl-2-iso-propylphenol) was purchased from Ungerer and Company (Lincoln Park, NJ). N-(n-butyl) thiophosphoric triamide was purchased from Agrotain International (St. Louis, MO). All other chemicals came from Sigma-Aldrich Company (St. Louis, MO).

Swine Production Facility and Waste Treatment

A swine barn at the U.S. Meat Animal Research Center, Clay Center, NE, was used in this study. It was equipped with two separate pits (each approximately 45 m long by 2.4 m wide by 0.6 m deep) that have slatted floors on top and six pens (16 sows per pen) over each pit. One pit served as the control pit, and underneath each pit and analyzed for urea, thymol, volatile fatty acids, coliform bacteria, and Campylobacter. At the end of each experiment, six 50-g samples from each pit were placed in serum bottles, and gas volume and composition were determined periodically for 28 d. Compared with the control pit, volatile fatty acids production was reduced 64 and 100% for the thymol amendments of 1.5 and 3.0 g L⁻¹, respectively. Viable coliform cells were reduced 4.68 and 5.88 log₁₀ colony-forming units kg⁻¹ of slurry for the 1.5 and 3.0 g thymol L⁻¹, respectively, and Escherichia coli were reduced 4.67 and 5.01 log₁₀ colony-forming units kg⁻¹ of slurry, respectively. Campylobacter was not detected in the pits treated with thymol, in contrast to 63% of the samples being positive for the untreated pit. Urea accumulated in the treated pits from Day 3 to 6. Total gas production from serum bottles was reduced 65 and 76% for thymol amendments of 1.5 and 3.0 g L⁻¹, respectively, and methane was reduced 78 and 93%, respectively. These results suggest that thymol markedly reduces pathogens, odor, and greenhouse gas emissions from a swine production facility. The urease inhibitor produced a temporary response in conserving urea.

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were boiled 10 min and immediately transferred to a cold-water bath for 5 min. A 300-μL aliquot of each sample was transferred to a well in a 96-well microtiter plate. This was followed by additions of 50 μL phenol nitroprusside, 50 μL alkaline hypochlorite, and 250 μL distilled water. Color was allowed to develop for 20 to 30 min at room temperature. Absorbance at 620 nm was measured using a Bio-Tek Ceres UV900C microplate reader.

Coliform and E. coli were enumerated with 3M Petrifilm Escherichia coli coliform count plates (3M Microbiology Products, St. Paul, MN). Duplicate plates for each of two dilutions were inoculated and incubated at 35°C, and colonies were counted using official methods of the Association of Official Agricultural Chemists as described in the literature provided with the plates. Briefly, total coliform numbers consisted of red and blue colonies associated with gas at 24 h after inoculation. Campylobacter was enriched from 1 g of slurry with 13 mL Bolton Broth with supplement (Oxoid, Hampshire, UK) and Lysed Horse Blood Cells (Lampire Biological Labs, Pipersville, PA). Tubes were gently mixed, capped tightly, and incubated for 4 h at 37°C followed by 44 h at 42°C. A 10-μL aliquot was plated onto Campy-Cephex agar (Stern et al., 1992) and grown using MicroAero Packs in an AnaeroPack System (Mitsubishi Gas Chemical, New York, NY) for 48 h at 42°C. Suspect colonies were verified by agglutination (Campylobacter Test Kit; Oxoid).

Gas volume in serum bottles was determined with a glass syringe as previously described (Miller and Wolin, 1974). Methane was analyzed using a 8610C gas chromatograph (SRI Instruments, Torrance, CA) as described by Miller and Berry (2005).

Statistical Analysis

Data were analyzed with general linear models using SAS (Version 6.12 for Macintosh, SAS Institute Inc., Cary, NC) with pen as experimental unit for the treatments. Main effects that differed over time were subjected to regression analysis, and slopes were analyzed using general linear models. Means and SE reported were determined for the six pen samples for each treatment for each time of collection.

RESULTS

Efforts to calculate and obtain 1.5 g of thymol L⁻¹ of pit manure slurry were within a range of 1.1 to 1.9 g L⁻¹. Sample analyses at Days 3 and 19 gave concentrations of 1.5 and 1.1 g L⁻¹, respectively (Fig. 1A). In the untreated pit, 3.91 mmol kg⁻¹ slurry-d⁻¹ VFA was produced (74.2 mmol kg⁻¹ slurry by Day 19), compared with 1.42 mmol kg⁻¹ slurry-d⁻¹ in the pit treated with thymol and NBPT (27.0 mmol kg⁻¹ slurry by Day 19), providing a reduction of 64% (P < 0.01) (Fig. 1B). Thymol decreased all individual VFA and did not decrease one proportionally more than another. No urea from the urine in the untreated pit was detected (Fig. 1C). In the pit treated with NBPT and thymol, 0.94 g urea kg⁻¹ slurry was present; most of the urea was hydrolyzed by Day 10, and all urea was hydrolyzed by Day 17. The hydrolysis was observed as an increase in ammonia concentration from Day 3 to Day 10 of 2.3 to 3.1 g kg⁻¹ slurry (Fig. 1D).

After 19 d of waste, feed, and water accumulation, the pits were filled to capacity. Therefore, for further analyses we used the sampling from Day 19 to establish in vitro fermentations for another 29 d. Total gas production was reduced over 29 d (from 3.4 to 1.2 L kg⁻¹
slurry for the untreated and treated samples, respectively) or reduced 65% \( (P, 0.01) \) (Fig. 2A). Methane production over 29 d was 0.41 and 0.09 L kg\(^{-1}\) slurry for the untreated and treated samples, respectively, which was a reduction of 78% with thymol treatment \( (P, 0.01) \) (Fig. 2B). Total VFA production over 29 d was reduced from 242 mmol L\(^{-1}\) for the untreated samples to 194 mmol L\(^{-1}\) for the treated samples (Fig. 2C).

Values for pH declined in the untreated samples with the higher VFA concentrations from approximately 6.9 to 6.5 over the 29 d, whereas pH values for the treated samples remained relatively constant at 6.8 (Fig. 2D).

Both pits were drained after 19 d, refilled with water, and amended with a calculated 3.0 g and 0.08 g of thymol and NBPT L\(^{-1}\) of slurry, respectively. In this second experiment, thymol varied between 2.6 and 3.8 g L\(^{-1}\) of slurry. The concentration at Day 4 was 2.6 and at Day 18 was 3.2 g L\(^{-1}\) (Fig. 1E). Rates of VFA production in the treated and untreated pits were 2.65 and \(-0.75\) mmol kg\(^{-1}\) slurry·d\(^{-1}\) (43.9 and \(-12.4\) mmol kg\(^{-1}\) slurry, respec-
tively, by Day 18), indicating no net accumulation in the treated pit (Fig. 1F) because methanogenic organisms used the VFA faster than they were produced. Urea accumulated to 0.86 g kg⁻¹ slurry in the treated pit by Day 6, after which it declined to 0.11 g kg⁻¹ by Day 18 (Fig. 1G). Ammonia increased from 3.8 to 4.5 g kg⁻¹ of slurry between Days 6 and 13 in the treated pit as the accumulated urea was hydrolyzed (Fig. 1H).

The in vitro samples from the second experiment with the higher thymol concentration reduced total gas and methane production from 3.6 to 0.84 and from 0.38 to 0.25 L kg⁻¹ slurry, respectively, or 76 and 93% over the 28 d (P < 0.01) (Fig. 2E and F). Total VFA in the untreated samples increased from 143 to 266 mmol L⁻¹, in comparison to the treated samples, in which VFA decreased from 159 to 127 mmol L⁻¹ (Fig. 2G). The in-vitro samples from the second experiment with the higher thymol concentration reduced total gas and methane production from 3.6 to 0.84 and from 0.38 to 0.25 L kg⁻¹ slurry, respectively, or 76 and 93% over the 28 d (P < 0.01) (Fig. 2E and F). Total VFA in the untreated samples increased from 143 to 266 mmol L⁻¹, in comparison to the treated samples, in which VFA decreased from 159 to 127 mmol L⁻¹ (Fig. 2G).
increasing VFA concentrations in the untreated pit are related to declining pH values (Fig. 2H), whereas in the treated pit decreasing VFA concentrations were related to increasing pH values.

Coliform bacteria and *E. coli* in the untreated pit for both experiments averaged 7.88 and 7.71 log10 CFU kg⁻¹ of slurry, respectively (Table 1). *Campylobacter* was observed in 63% of the samples (23/36 samples) taken from the untreated pit and in 58% of the samples (7/12 samples) before the pit was treated. However, it was not detected in the pit treated with 1.5 or 3.0 g of thymol L⁻¹ of slurry. The viable coliform and *E. coli* in the pit treated with 1.5 or 3.0 g thymol L⁻¹ of slurry were relatively constant over the 19 and 18 d for each experiment, and the CFUs reported in Table 1 are from the Day 19 sampling (1.5 g thymol L⁻¹) and the Day 18 sampling (3.0 g thymol L⁻¹). These would be the pathogen loads being transferred to a lagoon or the environment once the pit is drained. The 1.5 g of thymol L⁻¹ of slurry reduced the coliforms and *E. coli* 4.68 and 4.67 CFU kg⁻¹ of slurry, respectively, and the 3.0 g of thymol L⁻¹ of slurry reduced the coliforms 5.88 and 6.01 CFU kg⁻¹ of slurry, respectively.

**DISCUSSION**

Results from this study indicate that our previous laboratory data obtained using thymol to reduce odor and pathogens in swine waste slurries (Varel, 2002; Varel and Miller, 2004) can be replicated on a much larger scale. This is significant because of the minimal labor involved in adding the thymol to the manure pits and the effectiveness in this system. The swine waste pits in this facility are static systems; therefore, no mixing occurs as waste is accumulating. It is unclear whether the lack of mixing in the manure pits contributed to the varying concentrations of thymol that were observed, especially in Experiment 2 (Fig. 1E). The concentration measured on Days 1 and 5 was 2.6 g L⁻¹ of slurry; however, by Days 11 and 13, the concentration was approximately 3.75 g L⁻¹ of slurry. One would assume the concentration of thymol would be diluted with the incoming waste and decrease the concentration. However, a similar trend was observed in the first experiment (Fig. 1A). It is possible that thymol attaches to the solid fraction of the waste, and therefore more thymol was removed in the sampling as the solids content increased in the pit. We have previously observed that thymol partitions with the waste solids instead of the liquid fraction after centrifugation (Varel, 2002). In the future, a greater number of samples may be necessary to more precisely quantify the thymol concentration. However, in spite of the variation in thymol concentrations, profound effects were observed by the two concentrations evaluated.

The effects of 1.5 or 3.0 g of thymol L⁻¹ of swine waste slurry were, respectively, a 64 and 100% reduction in the rate of VFA production, a 65 and 76% reduction of total gas production, a 78 and 93% reduction of methane production, a 4.68 and 5.88 CFU kg⁻¹ of slurry reduction of coliform bacteria, and an elimination of *Campylobacter*. We presume NBPT did not influence any of these parameters because it is added at a very low concentration (80 mg L⁻¹ of slurry), and it is not known to exhibit antimicrobial properties. Zahn et al. (1997) and others (Coates et al., 2005; Zhu et al., 1999) have concluded that C2 to C9 volatile organic acids from swine waste demonstrated the greatest potential for decreased air quality (odor) because these compounds exhibit the highest transport coefficients and highest airborne concentrations. Thus, inhibiting the production of these compounds will significantly curtail odor emissions. Similarly, total gas and methane production are inhibited, indicating that greenhouse gases (CH₄ and CO₂) are greatly reduced. For sustainability of agriculture, recycling of nutrients is critical.

The mode of action of plant essential oils has been recently reviewed (Burt, 2004; Holley and Patel, 2005). It is generally agreed that phenolic components are more effective than the alcohols, aldehydes, or ether components and that the mode of action is primarily as a membrane permeabilizer.

The residual thymol in the waste must be considered. There are several pathways for phenolic compound degradation (Fang et al., 2006). Soil microorganisms are known to degrade some of the monoterpenoid plant essential oils (van der Werf et al., 1999), and some are degraded under anaerobic conditions (Harder and Probian, 1995). Vokou and Liotiri (1999) have concluded that essential oils are used as a carbon and energy source by ubiquitously occurring soil microorganisms and would not accumulate in soil if environmental conditions favor growth of these organisms. These chemicals are also volatile; thus, a fraction of the thymol would be lost once the waste is transferred from a pit to cropland.

One additional potential benefit of using thymol or another plant oil in a livestock waste treatment system is their insecticidal properties (Ibrahim et al., 2001; Isman, 2000). We have routinely observed numerous fly larvae and flies in untreated cattle waste slurries, whereas none appear in samples treated with thymol and other plant essentials oils, such as carvacrol, eugenol, or α-terpinene.

It is unclear from the limited studies with thymol application to manure slurries whether the cost can be jus-

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**Table 1. Effect of thymol and N-(n-butyl) thiophosphoric triamide (NBPT) used as amendments in swine manure pits and their effect on coliforms and *Escherichia coli* numbers and recovery of *Campylobacter* spp. by enrichment and selective plating.**

<table>
<thead>
<tr>
<th>Bacterial population†</th>
<th>Untreated</th>
<th>1.5 g L⁻¹</th>
<th>3.0 g L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliforms (log₁₀ CFU kg⁻¹)‡</td>
<td>7.88 ± 0.10</td>
<td>6.20 ± 0.48</td>
<td>5.00 ± 0.18</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (log₁₀ CFU kg⁻¹)§</td>
<td>7.71 ± 0.13</td>
<td>6.04 ± 0.43</td>
<td>ND§</td>
</tr>
<tr>
<td><em>Campylobacter</em> (% positive)</td>
<td>63</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

† Untreated samples were not significantly different for the two experiments and are combined for this table. The values for 1.5 g L⁻¹ and 3.0 g L⁻¹ amendments are from the Day 19 sampling and the Day 18 sampling, respectively.

‡ Detection limit is 4.7 log₁₀ CFU kg⁻¹ sample.

§ None detected.
Minimally, 1 to 1.5 g thymol kg\(^{-1}\) manure slurry (16% DM) is recommended to obtain beneficial effects (reduced gas odor, and pathogens). Assuming a lagoon (1 million L) contained a slurry with 4% DM, the approximate cost to treat this at 1 g thymol L\(^{-1}\) or kg\(^{-1}\) slurry would be $2500. More work is needed to investigate other plant oils or mixtures of plant oils from byproduct streams, such as the pulp industry, to find a solution that costs less than thymol.

The urease inhibitor NBPT produced a short-term response (6–10 d). Additions of NBPT once per week may be needed to overcome urease activity, similar to what we have observed with cattle waste (Varel et al., 1999). However, McCrory and Hobb (2001) have concluded that urease inhibitors are too expensive and easily broken down or inactivated to bring any economic or practical benefit to livestock producers. Ammonia emissions in Europe have increased by more than 50% during the past 30 yr. Livestock production has been identified as the primary contributor to this increase (McCrory and Hobb, 2001; Pain et al., 1998). Most of the ammonia emissions from livestock waste originate from hydrolysis of urea (Bierman et al., 1999; Van Horn et al., 1996). Further efforts are needed in this area to maximize the return of nitrogen back to agronomic crops, in contrast to allowing this nitrogen to become an air pollutant (Galloway et al., 2003). An estimate for cost of NBPT to treat one metric ton of cattle manure slurry at 80 mg kg\(^{-1}\) is approximately 44 cents (Varel et al., 2007). Therefore, cost is not an issue, although rapid degradation of NBPT remains an issue, as indicated by the current study.

**CONCLUSIONS**

Further regulations on air and water quality may demand some treatment of livestock waste before it is applied to land as fertilizer. Plant oils may serve this role. We have shown that thymol is an effective treatment for stored waste in a swine production facility. The benefits from this treatment are reduced odor, methane, and pathogens.

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**REFERENCES**


