# Odorant production and persistence of *Escherichia coli* in manure slurries from cattle fed zero, twenty, forty, or sixty percent wet distillers grains with solubles[1,2]

**V. H. Varel,*[3] J. E. Wells,* E. D. Berry,* M. J. Spiehs,* D. N. Miller,† C. L. Ferrell,* S. D. Shackelford,* and M. Koohmaraie*[4]**

*USDA-ARS, US Meat Animal Research Center, Clay Center, NE 68933; and †USDA-ARS, Agroecosystem Management Unit, Lincoln, NE 68583

**ABSTRACT:** Corn ethanol production removes starch and concentrates the remaining nutrients, including CP and minerals. When wet distillers grains with solubles (WDGS) are fed to cattle in place of corn, CP and minerals often exceed dietary needs. This may increase N emission, P run-off, and odor production. These variables are evaluated in this study. Crossbred steers (n = 160; 434 ± 8 kg) were assigned in a completely randomized block design to 9 × 9 m pens with concrete floor (10 animals/pen; 4 pens/treatment). Steers were fed a finishing diet that contained 0, 20, 40, or 60% WDGS on a DM basis, and provided 13.3, 15.5, 20.6, or 24.9% CP, respectively. Two kilograms of manure slurry (14 to 23% DM) were collected from each pen monthly (Aug. 20, Sep. 24, and Oct. 22). Samples were analyzed immediately for odorants, DM, pH, $NH_3$, total alcohol, L-lactate, and concentrations of generic *Escherichia coli*. After incubation of the samples at 22°C for 2, 4, 7, 10, 15, 21, and 28 d, samples were analyzed for methane production in addition to the above characteristics. Before incubation, $NH_3$, $H_2S$, indole, phenol, isovalerate, isobutyrate, and acetate increased ($P < 0.01$) with increasing amounts of WDGS in the diet. Other odorants, including skatole, caproate, valerate, butyrate, and propionate, were greater ($P < 0.01$) in manure slurries from cattle fed 20 or 40% WDGS, compared to 0% WDGS. The L-lactate was greater ($P < 0.01$) in slurries from cattle fed 0% WDGS (447 μmol/g of DM) compared with the other treatment slurries (14 to 15 μmol/g of DM). After incubation, L-lactate contributed to lowered slurry pH (6.3, 7.1, 7.6, and 8.2, respectively, for 0, 20, 40, and 60% WDGS), which inhibited microbial fermentation, *E. coli* persistence, and methane production. Because of the favorable, more neutral pH in the 40 and 60% WDGS slurries, many of the odorant compounds were rapidly converted to methane during a 28-d static incubation. *Escherichia coli* O157:H7 inoculated into subsamples of the manure slurries exhibited behavior similar to that of naturally present generic *E. coli*, surviving in greater numbers longer ($P < 0.05$) in 20 and 40% WDGS slurries than in 0% WDGS. These data indicate feeding WDGS can increase odorants in manure slurries and extend the persistence of *E. coli*.

**Key words:** distillers grains, *Escherichia coli*, feedlot cattle, manure, methane, odor



J. Anim. Sci. 2008. 86:3617–3627
doi:10.2527/jas.2008-1118

## INTRODUCTION

Ethanol production from corn has resulted in a large quantity of wet distillers grains with solubles (**WDGS**), which has proven to be a viable feed resource for beef cattle (Stock et al., 1999; Klopfenstein et al., 2008). This process removes starch from corn and concentrates the CP, oil, fiber, and minerals (Spiehs et al., 2002). When these concentrated nutrients are fed as WDGS to cattle (typically 20 to 40% of the diet, DM basis) in place of corn, it often results in a diet with CP, oil, and minerals such as P and S in excess of dietary needs. These excess nutrients are excreted and can potentially contribute to environmental pollution including elevated N emission, increased P run-off, and greater odor production. Koziel et al. (2006) indicated that *p*-cresol alone appears to cause much of the overall odor impact for swine and beef cattle operations. Cresol originates from the amino acids tyrosine,

[1]Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

[2]Technical assistance of S. Wise, D. Kucera, T. Boman, and J. Long, assistance with statistical analysis of data by D. Light, and secretarial assistance of J. Nierman are appreciated.

[3]Corresponding author: vince.varel@ars.usda.gov

[4]Current address: IEH Laboratories & Consulting Group, Lake Forest Park, WA 98155.

Received April 22, 2008.
Accepted July 22, 2008.

tryptophan (Mackie et al., 1998), and phenylalanine (Mohammed et al., 2003). These 3 AA as well as others are concentrated in WDGS to approximately 3 times that in corn (NRC, 1998; Stein et al., 2006). Therefore, the potential exists for more odor to be generated from manure of cattle fed WDGS than from cattle fed corn. This has not been previously examined. In addition, greater concentrations of methionine, cysteine, and S in WDGS suggest a greater concentration of reduced S ($H_2S$) could be produced in manure from cattle fed WDGS when compared with cattle fed corn. A recent study also suggested that there is a positive association between feeding dried distillers grain and fecal shedding of *E. coli* O157 (Jacob et al., 2008). The objectives of this study were to compare odorants (VFA, aromatic compounds, $NH_3$) and persistence of generic *E. coli* and *E. coli* O157:H7 in manure slurries stored from 0 to 28 d from cattle fed 0, 20, 40, or 60% WDGS on a DM basis.

## MATERIALS AND METHODS

All animal procedures were reviewed and approved by the US Meat Animal Research Center Animal Care and Use Committee before the initiation of the research.

### *Animals and Diets*

One-hundred sixty crossbred steer calves (438 ± 8 kg) were trained to use Calan-Broadbent electronic headgates (American Calan Inc., Northwood, NH), primarily to obtain individual feed intake for another part of this study. Animals were assigned to 1 of 4 dry-rolled corn-based finishing diets with 0, 20, 40, or 60% WDGS on a DM basis (Table 1). The WDGS (31.3% DM; 31.6% CP, 13.7% oil, 0.83% P, and 0.73% S, DM basis) came from a corn ethanol plant in York, NE. There were 16 pens, 10 animals per pen, and 4 pens per treatment. Pens were approximately 9 × 9 m with an entirely concrete floor, and approximately one-third of the pen was under a barn open to the south. The study was conducted between July and November. Pens were cleaned approximately every 3 to 4 wk.

### *Manure Collection and Analyses*

During 3 different periods, approximately 2 kg (as is) of manure slurry was randomly collected across the entire pen, 2 to 3 wk after the pens had been cleaned. The stocking density of the animals (10/pen) kept the manure in a slurry form with a DM in the range of 14 to 23% for each month sampled (Aug. 20, Sep. 24, and Oct. 22). The samples were taken to the laboratory and immediately processed.

A 750-g aliquot from the manure slurry of each pen was transferred to a wide mouth (10 cm) jar (17-cm tall, 13.5 cm in diameter, 1.6-L volume), with a total of 16 jars for each sampling period. Plastic lids provided with the jars were used to cover approximately 90% of the jar opening to prevent moisture loss over the 28-d experimental incubation period at room temperature (22°C). Contents of the jars were sampled at 0, 2, 4, 7, 15, 21, and 28 d. Manure slurry pH was obtained by using a combination electrode and PHM 83 pH meter (Radiometer America, Cleveland, OH). A 15-g sample from each jar was dried at 105°C overnight to determine DM. Another 15-g sample was acidified with 15 mL of 0.5 *M* $H_2SO_4$ and stored at −20°C until analyzed for fermentation products (L-lactate, total alcohol, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, phenol, *p*-cresol, indole, and skatole). A YSI Model 2700 autoanalyzer (Yellow Springs Instrument, Yellow Springs, OH) was used to analyze L-lactate and total alcohol, and a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with flame-ionization and mass-selective detectors was used for all other products. Conditions used for analyses of the products have been described previously (Miller and Varel, 2001). Ammonia was determined using a modification of the Sigma urea N kit (procedure No. 640, Sigma-Aldrich Chemicals, St. Louis, MO). Standards and samples were diluted 10-fold, and 5 μL was transferred to a well in a 96-well microtiter plate. This was followed by additions of 50 μL of phenol nitroprusside, 50 μL of alkaline hypochlorite, and 250 μL of 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 (Winooski, VT) Ceres UV900C microplate reader. Hydrogen-sulfide was purged from manure samples using a helium gas stream and trapped in 7 mL of 2% (wt/wt) zinc acetate solution as inert ZnS (APHA, 1965), which was subsequently measured by the formation of methylene blue after reaction of the sulfide with dimethyl-para-phenylenediamine sulfate and ferric ammonium sulfate (Kelly and Wood, 1998). Total N, P, and S were analyzed (Leco combustion, nitric-perchloric acid digestion, and nitric acid digestion, respectively) by a commercial laboratory (Ward Laboratories Inc., Kearney, NE).

Contents of the jars were sampled at 0, 1, 2, 4, 7, 10, 15, 21, and 28 d for the determination of generic *E. coli* concentrations. A 1-g sample of manure slurry from each jar was weighed into a tube containing 9 mL of buffered peptone water (**BPW**; Becton, Dickinson and Company, Sparks, MD). Tube contents were mixed, decimally diluted in additional BPW if needed, and plated onto CHROMagar ECC (DRG International, Mountainside, NJ), by either spread plating or by spiral plating using an Autoplate 4000 spiral plater (Spiral Biotech Inc., Norwood, MA). The CHROMagar ECC (DRG International Inc.) plates were incubated at 37°C for 24 h, and characteristic blue *E. coli* colonies were counted.

A second aliquot (50 g) from the manure slurry of each pen was transferred to a 150-mL serum bottle, gassed with N, and incubated at room temperature (22°C). Methane production was analyzed using a

**Table 1.** Diet formulations

| Item | Wet distillers grains with solubles, % of DM | | | |
|---|---|---|---|---|
| | 0 | 20 | 40 | 60 |
| Ingredient | | | | |
| Alfalfa, hay ground | 10.6 | 10.6 | 10.6 | 10.6 |
| Corn, dry rolled | 82.7 | 68.2 | 48.2 | 28.2 |
| Soybean meal | 5.66 | — | — | — |
| Urea | 0.40 | — | — | — |
| Limestone | 0.56 | 1.1 | 1.1 | 1.11 |
| Vitamin A, D, E[1] | 0.008 | 0.008 | 0.008 | 0.008 |
| Mineral supplement[2] | 0.007 | — | — | — |
| Salt | 0.062 | — | — | — |
| Monensin[3] | 0.030 | 0.030 | 0.030 | 0.030 |
| Thiamin premix,[4] 88 g/kg | 0.023 | 0.023 | 0.023 | 0.023 |
| Composition, analyzed | | | | |
| DM, % | 85.1 | 61.1 | 48.7 | 41.3 |
| CP, % | 13.3 | 15.5 | 20.6 | 24.9 |
| Fat, % | 3.6 | 5.9 | 7.6 | 9.4 |
| Ca, % | 0.41 | 0.64 | 0.70 | 0.68 |
| Cu, mg/kg | 4.75 | 4.50 | 5.50 | 6.25 |
| P, % | 0.35 | 0.45 | 0.57 | 0.66 |
| Mg, % | 0.14 | 0.17 | 0.21 | 0.24 |
| K, % | 0.79 | 0.84 | 0.98 | 1.06 |
| Na, % | 0.06 | 0.08 | 0.12 | 0.15 |
| S, % | 0.16 | 0.28 | 0.42 | 0.54 |
| Composition, calculated[5] | | | | |
| ME, Mcal/kg | 3.11 | 3.06 | 3.05 | 3.02 |
| CP/ME | 43.22 | 47.61 | 61.00 | 74.43 |
| UIP, % CP | 48.07 | 50.46 | 49.80 | 49.00 |
| $NE_m$, Mcal/kg | 2.11 | 2.09 | 2.07 | 2.05 |
| $NE_g$, Mcal/kg | 1.44 | 1.42 | 1.41 | 1.40 |
| ADF, % | 6.14 | 9.75 | 12.50 | 15.23 |
| NDF, % | 12.08 | 19.34 | 23.94 | 28.55 |

[1]The supplement provided 8,800,000 IU of vitamin A; 880,000 IU of vitamin D; and 880 mg of vitamin E per kilogram.
[2]Trace mineral premix contained 13% Ca, 12% Zn, 8% Mn, 10% Fe, 1.5% Cu, 0.2% I, and 0.1% Co.
[3]Rumensin 80 (Elanco Animal Health, Indianapolis, IN).
[4]Provides 200 mg/animal daily.
[5]Used tabular values (NRC, 2000).

8610C gas chromatograph (SRI Instruments, Torrance, CA) as described by Miller and Berry (2005) over a 28-d period.

Additional 600 g aliquots of the manure slurries of each pen were transferred into sterile Whirl-Pak bags (3 bags/pen with 200 g/bag; Nasco, Fort Atkinson, WI) and inoculated with a 5-strain composite of streptomycin-resistant *E. coli* O157:H7. The 5-strain composite inoculum was composed of *E. coli* O157:H7 ATCC 43895 and 4 bovine fecal *E. coli* O157:H7 strains 55AC1, 114AC1, D0F6823, and D0F6853 for which isolation has been described elsewhere (Elder et al., 2000; Berry et al., 2006). For all 5 *E. coli* O157:H7, the streptomycin-resistant mutants were isolated by selective enrichment with increasing antibiotic concentrations using a procedure similar to that described by Eisenstadt et al. (1994). The composite inoculum was prepared by culturing each strain separately in tryptic soy broth (Becton, Dickinson and Company) containing 250 μg/mL of streptomycin, incubated first for 24 h at 37°C, then for 48 h at room temperature. Equivalent volumes of each of the cultures were mixed together and diluted further in BPW, such that addition of 6 mL of inoculum into 600 g of manure slurry yielded initial concentrations of $10^6$ cfu/g of manure. The inocula were mixed thoroughly into the manure by massaging the bags, the bags were loosely closed, and then the bag and its contents were incubated at room temperature. The inoculated manure slurries were sampled at 0, 1, 2, 4, 6, 7, 10, and 14 d. A 1-g sample of manure slurry from each bag was weighed into a tube containing 9 mL of BPW. Following mixing and dilution as needed in BPW, 100 μL volumes were spread-plated onto MacConkey sorbitol agar plates (Difco, Becton, Dickinson and Company) containing 250 μg/mL of streptomycin. Plates were incubated for 20 to 24 h at 37°C, and characteristic nonsorbitol-fermenting *E. coli* O157:H7 colonies were counted. The counts from the triplicate bags from each pen were averaged to derive a mean for the pen at each point in time.

### *Statistical Analyses*

Data for initial pen collections were analyzed as a split-plot with pen as the experimental unit. An initial model was used with all interactions, and after step-

**Table 2.** Odorants, L-lactate, pH, N, S, and P from manure slurries collected over 3 sampling periods from cattle fed 0, 20, 40, or 60% wet distillers grains with solubles (n = 12)

| | Wet distillers grains in diet, % DM basis | | | | |
|---|---|---|---|---|---|
| Item | 0 | 20 | 40 | 60 | SEM |
| Odorant, μmol/g of DM | | | | | |
| Acetate | 252[a] | 421[b] | 621[c] | 685[d] | 12.3 |
| Propionate | 89[a] | 219[b] | 246[b] | 146[c] | 14.0 |
| Isobutyrate | 1.15[a] | 3.12[b] | 6.85[c] | 9.49[d] | 0.2 |
| Butyrate | 89[a] | 527[b] | 298[c] | 125[a] | 21.6 |
| Isovalerate | 1.95[a] | 3.14[b] | 5.39[c] | 9.65[d] | 0.21 |
| Valerate | 2.56[a] | 8.13[b] | 7.96[b] | 5.71[c] | 0.54 |
| Caproate | 0.88[a] | 1.66[b] | 1.42[b] | 1.30[b] | 0.12 |
| Total VFA | 437[a] | 1,184[b] | 1,189[b] | 984[c] | 33.5 |
| Phenol | 0.56[a] | 0.813[b] | 1.435[c] | 2.52[d] | 0.06 |
| Cresol | 10.5[ac] | 9.91[a] | 7.78[b] | 12.0[c] | 0.52 |
| Indole | 55.9[a] | 82.8[b] | 93.7[c] | 118.5[d] | 2.9 |
| Skatole | 1.89[a] | 1.91[a] | 3.07[b] | 4.78[c] | 0.28 |
| $H_2S$-S, μg/g of DM | 1.57[a] | 3.07[a] | 8.85[b] | 16.41[c] | 0.88 |
| Ammonia-N, mg/g of DM | 19[a] | 25[b] | 33[c] | 40[d] | 1.0 |
| pH | 6.28[a] | 7.11[b] | 7.63[c] | 8.19[d] | 0.09 |
| L-Lactate, μmol/g of DM | 447[a] | 14[b] | 14[b] | 15[b] | 17.8 |
| Total alcohols, μmol/g of DM | 10.3[a] | 12.2[ab] | 14.8[b] | 11.0[a] | 1.1 |
| Minerals, mg/g of DM | | | | | |
| Total N | 50.8[a] | 60.9[b] | 66.3[c] | 70.0[c] | 1.7 |
| P | 19.9[a] | 25.8[b] | 33.3[c] | 38.5[d] | 0.5 |
| S | 5.1[a] | 8.7[b] | 11.7[c] | 14.2[d] | 0.3 |

[a–d]Means with different superscripts within each row differ ($P < 0.05$).

down analyses, the final model included the effects of period, pen, dietary treatment, and pen nested within dietary treatment. Dietary treatment was tested with pen nested within dietary treatment as the error term. Data from the incubations of collected pen samples were analyzed as a split-plot in time. Bacterial numbers were transformed to $\log_{10}$ cfu per gram of wet weight before statistical analysis. An initial model was used with all interactions, and after step-down analyses, the final model included the effects of period, pen, dietary treatment, dietary treatment × time, and pen nested within dietary treatment. Dietary treatment was tested with pen nested within dietary treatment as the error term. When dietary treatment × time interaction was observed, differences among means were tested with a protected *t*-test. Means and pooled SEM are presented in the text, tables, and figures. For all statistical analyses, differences were considered significant when the probabilities were less than 0.05. Statistical analyses were conducted using the GLM procedure (SAS Inst. Inc., Cary, NC).

## RESULTS

Many of the initial odorants in manure slurries from cattle fed WDGS increased ($P < 0.01$) with increasing amounts of WDGS in the diet (Table 2). Essentially all odorants analyzed in the manure slurries were greater ($P < 0.01$) from the diets containing WDGS when compared with the diet with 0% WDGS. Exceptions to this were the concentration of butyrate, which was similar between the 0 and 60% WDGS, at 89 and 125 μmol/g of DM, respectively. Also, the *p*-cresol concentration tended to be less ($P < 0.06$) in the manure slurries when 40% WDGS was fed in comparison with 0% WDGS (7.8 and 10.5 μmol/g of DM, respectively). Manure slurry pH, ammonia N, $H_2S$-S, P, and S increased ($P < 0.01$) as the amount of WDGS increased in the diet (Table 2). The concentrations of L-lactate in the manure slurries from cattle fed 20, 40, and 60% WDGS were similar at 14 to 15 μmol/g of DM. However, L-lactate was greater ($P < 0.01$) in the slurries from cattle fed 0% WDGS (447 μmol/g of DM) when compared with the other 3 treatments. Total alcohol concentrations were similar from the 0, 20, and 60% WDGS slurries, but greater ($P < 0.02$) for the 40% slurries.

The accumulation and degradation of VFA and total alcohol fermentation products from in vitro incubation of manure slurries from cattle fed 0, 20, 40, or 60% WDGS are given in Figure 1. Total VFA and alcohol in the slurries from cattle fed 0% WDGS continued to increase for the 28-d incubation periods, as did the 20% WDGS slurries until d 15, and the 40 and 60% WDGS slurries until d 4 and 2, respectively (Figure 1A, 1F). Total VFA and alcohol in these treatments began to decrease once methanogenic microorganisms were established and they converted VFA to methane. Acetate, propionate, and butyrate from the manure slurries of cattle fed 40 and 60% WDGS increased briefly (2 to 7 d) and then declined as methane production in these slurries was initiated (Figure 1B, C, D). Different mi-

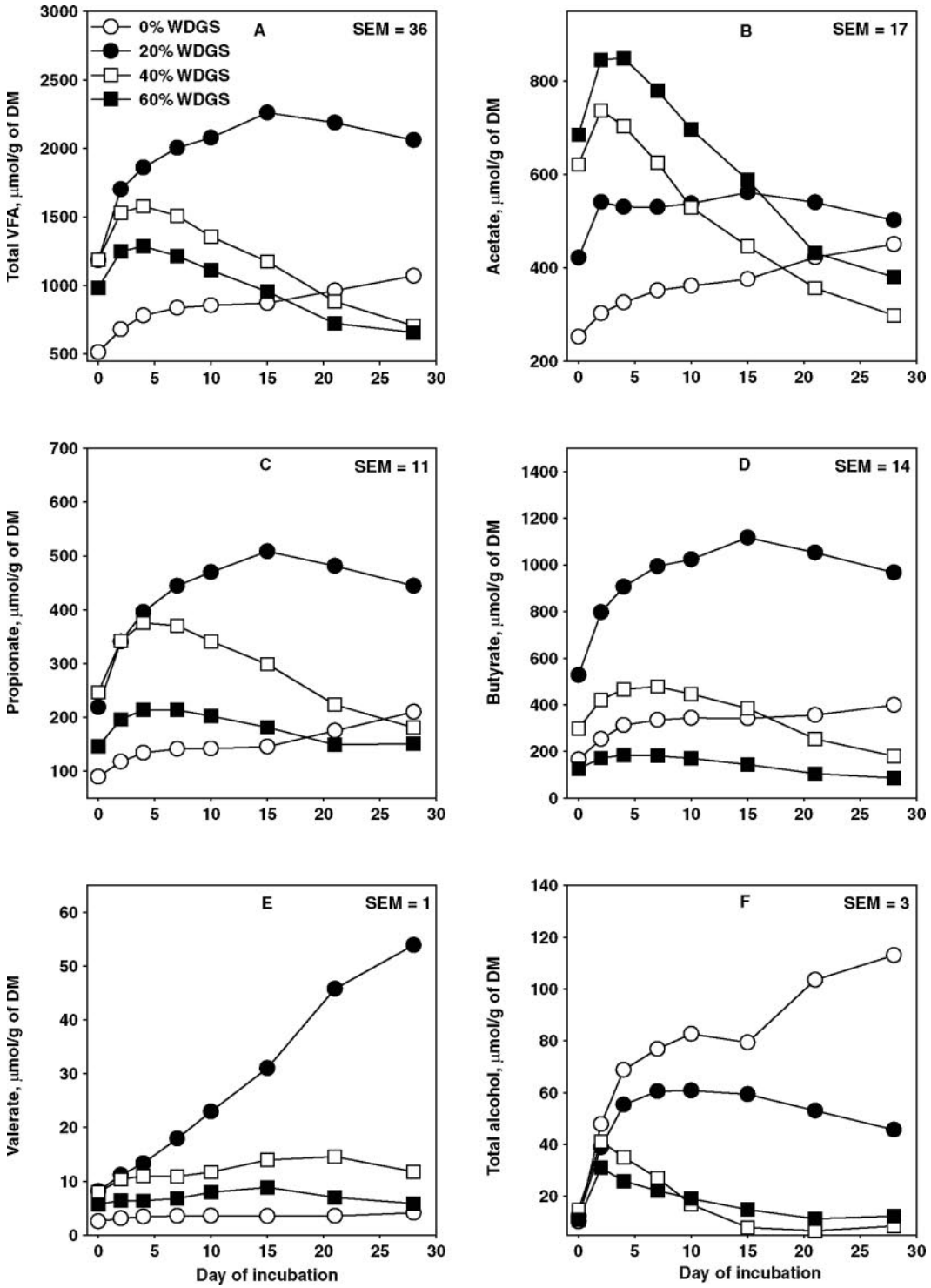


**Figure 1.** Accumulation and degradation of total VFA, individual VFA, and total alcohol fermentation products from in vitro incubation of manure slurries from cattle fed 0, 20, 40, or 60% wet distillers grains with solubles (WDGS). The SEM is the pooled SEM of the least squares means; n = 12.

crobial substrate fermentations occurred in the slurries from the cattle fed 0 and 20% WDGS. Initial VFA concentrations were less in the 0% WDGS slurries than the other treatments (Figure 1A) because of the increased concentration and inhibition of L-lactate (Figure 2A). This caused the pH to decrease to 5.0 by d 7 (Figure 2B). This in turn inhibited methane production (Figure 2D). Slurries from cattle fed 20% WDGS accumulated the greatest concentration of total VFA (Figure 1A), with propionate, butyrate, and valerate (Figure 1C, D, E) primarily accounting for this. Caproate (data not shown) also accumulated to 75 μmol/g of DM after 28 d in the 20% WDGS slurries; however, it was less than 5 μmol/g of DM in the other slurries.

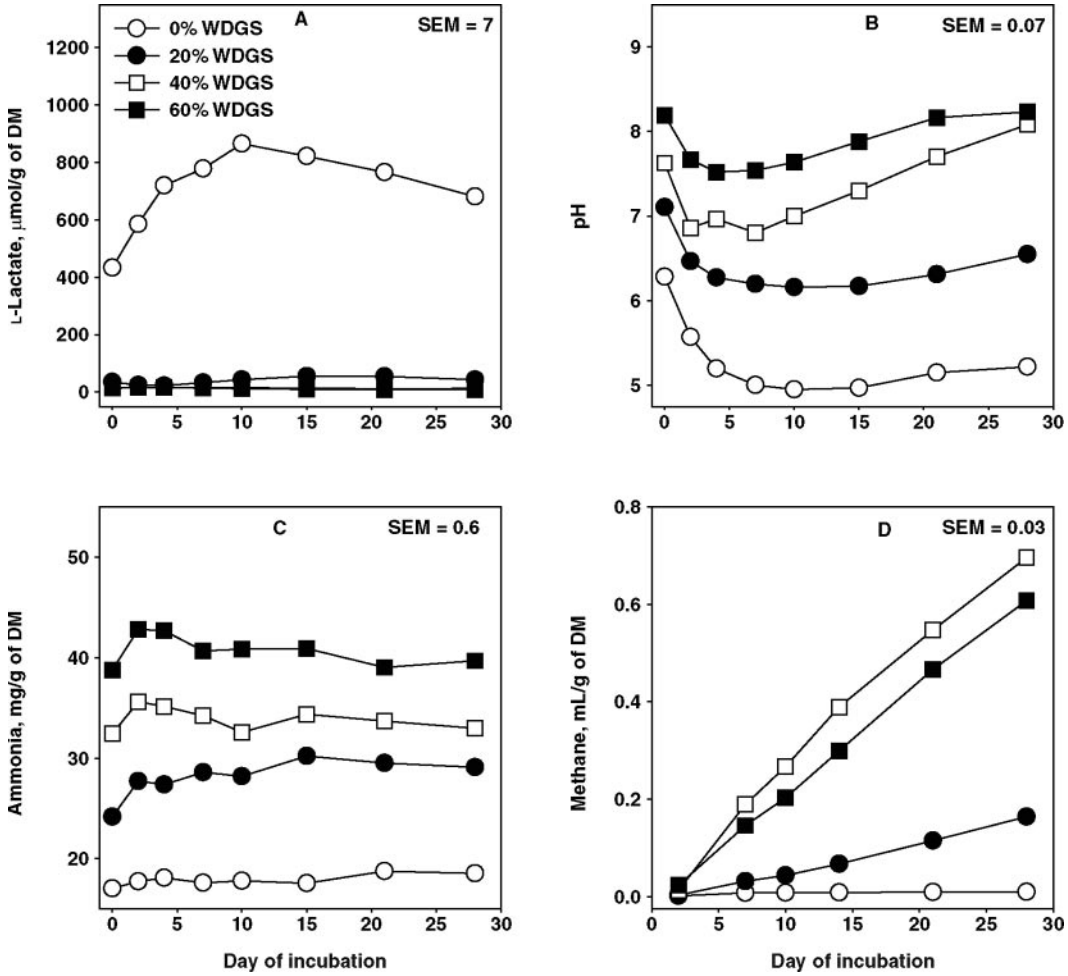


**Figure 2.** L-Lactate, pH, ammonia, and methane values from in vitro incubation of manure slurries from cattle fed 0, 20, 40, or 60% wet distillers grains with solubles (WDGS). The SEM is the pooled SEM of the least squares means; n = 12.

Total branched-chain VFA concentrations in the manure slurries increased ($P < 0.01$) as the concentration of WDGS increased in the diet (Figure 3). Isobutyrate and isovaleric increased similarly for each of the treatments (Figure 3B, C). Isocaproate (data not shown) made up 3 μmol/g of DM or less of the total branched-chain VFA. Unlike the straight-chain VFA, the concentration of branched-chain VFA from the 40 and 60% WDGS slurries did not decline over the 28-d incubation period.

Total aromatic compounds in the manure slurries initially were greater ($P < 0.01$) as the amount of WDGS increased in the diet, with the exception of *p*-cresol (Figure 4). The concentration of these compounds in the 0% WDGS slurries changed little over the 28-d incubation periods, most likely because of the low pH (Figure 2B). This was similarly true for the slurries from the cattle fed 20% WDGS, after the initial increase in concentration between 0 and 2 d. However, with the 40 and 60% WDGS slurries, cresol and indole concentrations declined after the initial increase (Figure 4C, D); presumably they were metabolized to methane (Figure 2D). The concentration of phenol gradually increased in these slurries (Figure 4B), whereas little change occurred with skatole (data not shown).

The initial concentration of ammonia in the slurries (0 d) increased ($P < 0.01$) as the WDGS increased in the diets (Figure 2C). An increase occurred in all slurries between 0 and 2 d, after which only small variations occurred in each treatment over the 28-d incubation periods.

Initial generic *E. coli* concentrations in the manure slurries were similar (Figure 5). With in vitro incubation they decreased rapidly in 0% WDGS slurries to levels near or below the detection limit of 2.0 $\log_{10}$ cfu/g by 7 d. Generic *E. coli* concentrations remained greater for a longer period (to d 15; $P < 0.05$) in 20 and 40% WDGS slurries. The 5-strain composite of *E. coli* O157:H7 inoculated into the manure slurries responded similarly to that of the naturally present generic *E. coli* (Figure 6). *Escherichia coli* O157:H7 concentrations in the 0% WDGS slurries were near or below the detection limit (1.70 $\log_{10}$ cfu/g) by 6 to 7 d. However, again in the 20 and 40% WDGS slurries, *E. coli* O157:H7 concentrations remained greater ($P < 0.05$) than the 0% WDGS slurries through 10 and 14 d, respectively.

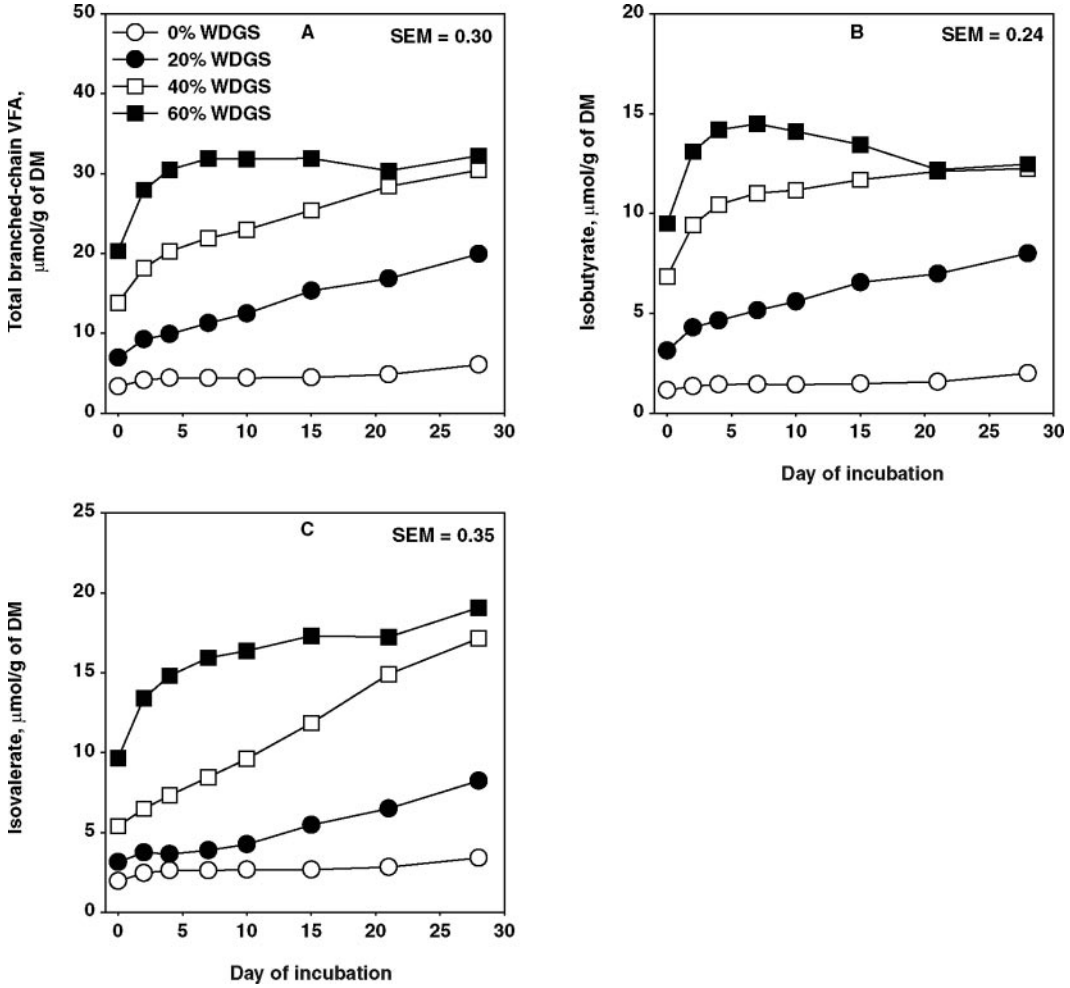


**Figure 3.** Accumulation of total branched-chain VFA, isobutyrate, and isovalerate from in vitro incubation of manure slurries from cattle fed 0, 20, 40, or 60% wet distillers grains with solubles (WDGS). The SEM is the pooled SEM of the least squares means; n = 12.

## DISCUSSION

Data from this study indicate that as the level of WDGS increased in the diet, the concentration of most of the odorants in the slurried manure (feces and urine combined) increased. This is expected when the nutrient composition of the diets is taken into consideration (Hobbs et al., 1996; Sutton et al., 1999). The CP in the diets was 13.3, 15.5, 20.6, and 24.9%, respectively, for the 0, 20, 40, and 60% WDGS expressed on a DM basis. Ethanol production removes starch from corn and, therefore, concentrates the other nutrients including CP, oil, fiber, and minerals. The amino acid concentrations in WDGS are approximately 3 times that in corn (NRC, 1998; Stein et al., 2006). Thus, this excess dietary CP will be excreted by the animal, and the amino acids in the manure slurry will be decarboxylated or deaminated (Mackie et al., 1998). This results in the production of $NH_3$, $H_2S$, VFA, branched-chain VFA from branched-chain amino acids, and aromatic compounds (phenol, cresol, indole, skatole) from aromatic amino acids (tyrosine, tryptophan, phenylalanine). The branched-chain VFA (isobutyrate, isovalerate) and aromatic compounds are well known to have very low odor threshold values and are considered the more offensive and objectionable odorants (Mackie et al., 1998; Miller and Varel, 2002; Koziel et al., 2006). Similarly, diets containing WDGS have a greater S component than a corn diet, plus a greater cysteine and methionine content (S-containing amino acids), which all contribute to sulfide emissions (Mackie et al., 1998). Hydrogen-sulfide is also known to have a very low odor threshold value and is very offensive.

Increased concentrations of butyrate (>500 μmol/g of DM) were observed in slurries from cattle fed 20% WDGS. Spore-forming clostridia, along with lactic acid-producing bacteria, were found to be the predominant bacterial species in feedlot pad manure in Southern Queensland, Australia (Ouwerkerk and Klieve, 2001). Clostridia are well known to deaminate and decarboxylate amino acids (Mackie et al., 1998) and produce high concentrations of butyrate. Also, their survival techniques such as resistance to desiccation and ability to form endospores are advantageous. Ouwerkerk and Klieve (2001) concluded that the most likely source of the sickly-sweet nuisance odor that emanates from

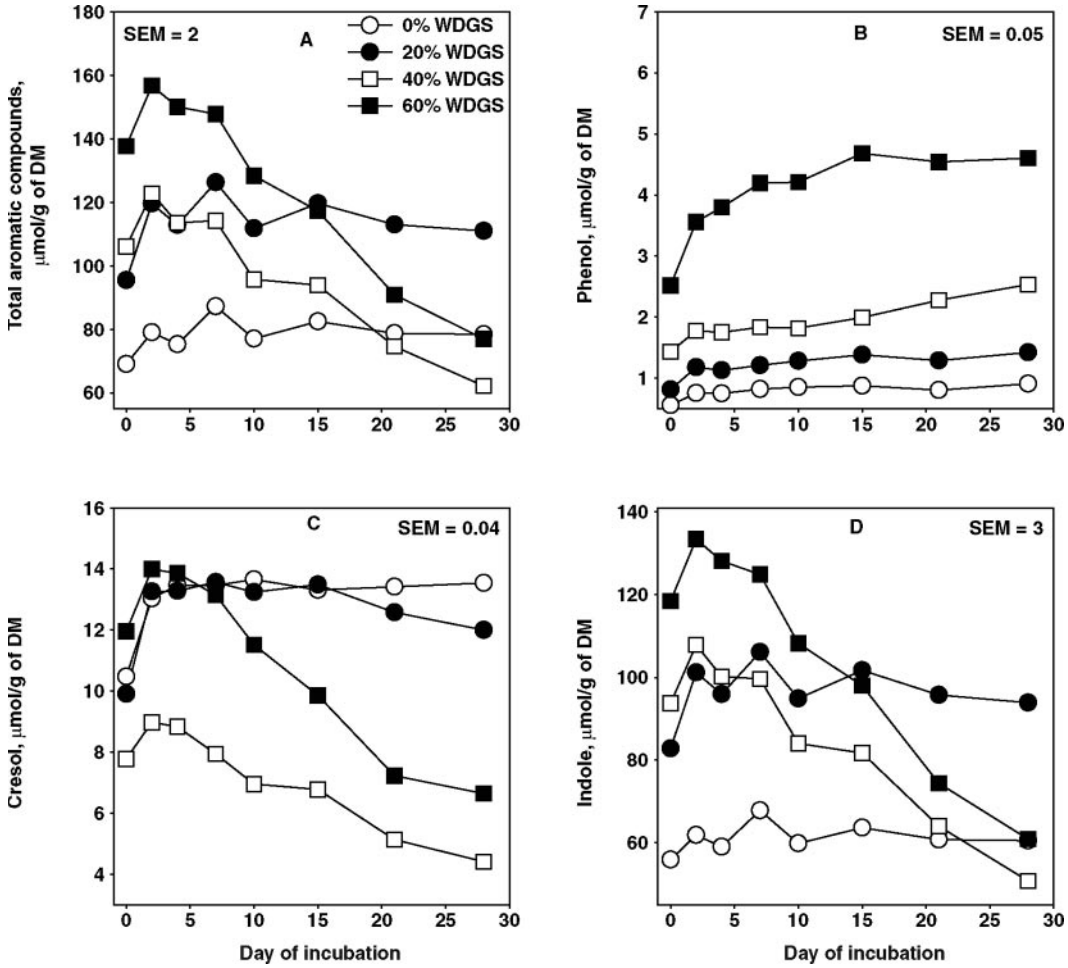


**Figure 4.** Accumulation and degradation of total aromatic compounds, phenol, *p*-cresol, and indole from in vitro incubation of manure slurries from cattle fed 0, 20, 40, or 60% wet distillers grains with solubles (WDGS). The SEM is the pooled SEM of the least squares means; n = 12.

feedlots is anaerobic fermentation by clostridia. The excess dietary CP in the WDGS diets, and the high butyrate found in the WDGS manure slurries, supports this conclusion.

Increased concentrations of the offensive odorant, indole, were also found in the slurries, and it increased with increasing amounts of WDGS in the diet. Indole originates from tryptophan (Attwood et al., 2006). The amount of fiber (ADF and NDF) in the diet increases with WDGS (Spiehs et al., 2002). In swine, dietary fiber increases the excretion of indole in feces (Hawe et al., 1992). Whether this is true with ruminants is unclear. A mixture of rumen bacteria and protozoa produced the same indolic compounds as ruminal bacteria, but with a 2-fold and 5-fold greater concentration of indole and skatole production, indicating that these are positively influenced with the presence of protozoa (Mohammed et al., 2003). Protozoa were not evaluated in our studies; therefore, it is not known whether they were present in greater populations in cattle fed WDGS compared with cattle that were not fed WDGS.

It is unclear why an increase in the concentration of *p*-cresol was not observed in this study as the concentration of WDGS increased in the diet. It originates from dietary metabolism of phenylalanine, tyrosine, and tryptophan and is predominantly excreted in the urine (Martin, 1982; Mackie et al., 1998; Mohammed et al., 2003). It is possible that *p*-cresol is more volatile on the pen surface. This could explain results from some of the studies that suggest *p*-cresol can travel 16 km downwind from a feedlot (Wright et al., 2005; Koziel et al., 2006). Another explanation might be that an active methanogenic fermentation is occurring on the pen surface of cattle fed 40 and 60% WDGS, with some of these aromatic compounds (*p*-cresol) being converted to methane. However, breakdown of aromatic compounds normally does not occur rapidly under anaerobic conditions (Young and Rivera, 1985; Smith and Macfarlane, 1997). There may be enough oxygen or another inorganic electron acceptor in these slurries on the feedlot surface to allow breakdown and metabolism of these ring compounds.

The conditions under which this study was conducted, concrete pen floor and high animal density, provided an ideal environment for microbial activity to occur in the homogeneously mixed manure slurries. These condi-

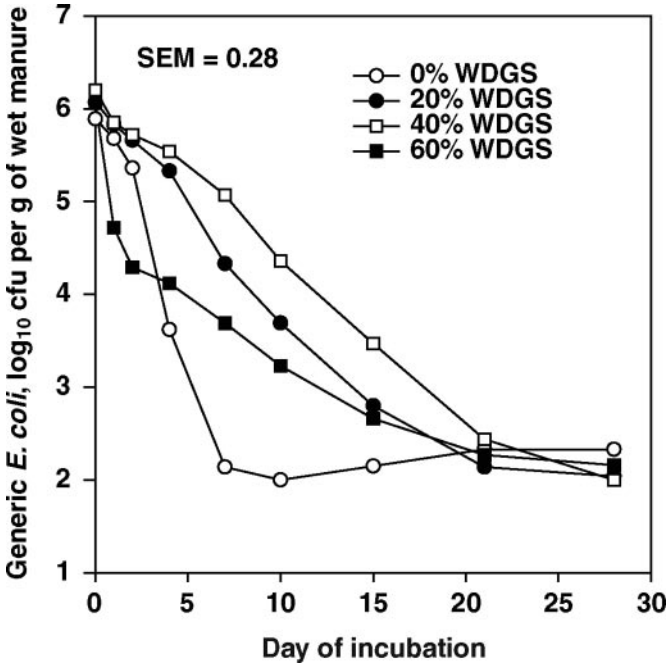


**Figure 5.** Persistence of naturally occurring generic *Escherichia coli* during in vitro incubation of manure slurries from cattle fed 0, 20, 40, or 60% wet distillers grains with solubles (WDGS). The SEM is the pooled SEM of the least squares means; n = 12.

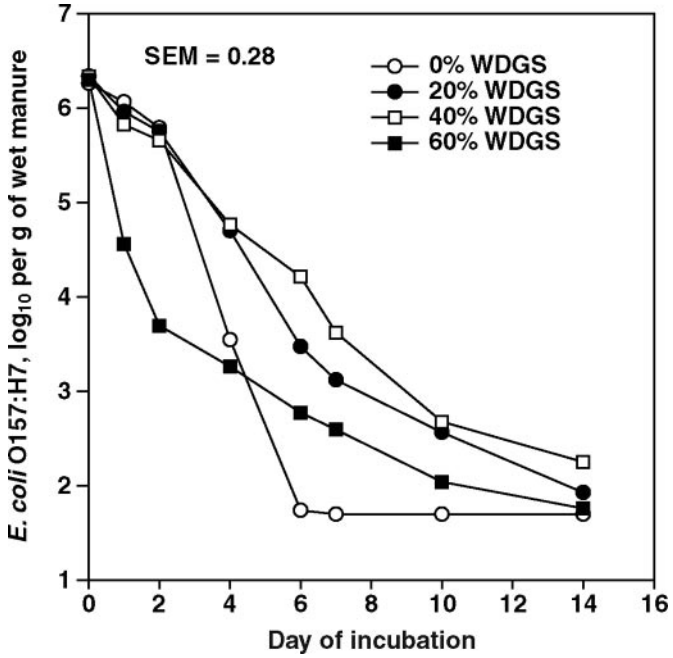


**Figure 6.** Persistence of a 5-strain composite of *Escherichia coli* O157:H7 in manure slurries from cattle fed 0, 20, 40, or 60% wet distillers grains with solubles (WDGS) during an in vitro incubation. The SEM is the pooled SEM of the least squares means; n = 12.

tions are not typically found in a soil-based feedlot pen, which will have alternating wet and dry periods. This will greatly affect the fermentation activity (Miller and Berry, 2005). Rainfall contributed to the low DM in the manure slurries, which ranged from 14 to 23%. This environment allowed the corn starch in manure from cattle fed 0% WDGS to rapidly ferment to lactic acid (Huntington et al., 2006). This consequently reduced manure pH and methane production in comparison with the other diets. Manure slurries from cattle fed the 20, 40, and 60% WDGS had pH values generally above 6.5. This allows manure starch to be converted to VFA as opposed to L-lactate, or if L-lactate is produced, it is likely converted to butyrate by a pathway proposed by Duncan et al. (2004). This could explain the decreased lactate and declining butyrate concentrations in the 20, 40, and 60% WDGS slurries because there is a declining starch concentration in the slurries as the WDGS is increased in the diet. These slurries also had increased ammonia concentrations to serve as a buffering agent against the high VFA concentrations. These conditions are conducive to promoting methanogenic microbial populations that use VFA and possibly some aromatic compounds as substrates to produce methane. The consumption of VFA by methanogenic bacteria has driven the popularity of anaerobic digestion for control of odor and to develop a source of energy from livestock manure (Mackie et al., 1998).

This study confirms results from other studies (Klopfenstein et al., 2008) that indicate WDGS will increase P in the manure when compared with a typical corn finishing diet. The 60% WDGS manure slurries contained roughly 2 times the P concentration compared with the 0% WDGS diet (38.5 and 19.9 g, respectively). This suggests that the land area for applying manure from cattle fed high levels of WDGS must be increased if application is regulated by P as opposed to N.

Recent reports have suggested that feeding distillers grains may increase the prevalence of *E. coli* O157:H7 fecal shedding by cattle (Dewell et al., 2005; Peterson et al., 2007; Jacob et al., 2008). Jacob et al. (2008) hypothesized that 1) feeding dried distillers grains results in decreased starch concentration in the hindgut, which may alter the ecology and favor the growth of *E. coli* O157, or 2) distillers grains may provide a component that stimulates or enhances the growth of the pathogen. We did not quantify naturally occurring *E. coli* O157 in the current work; therefore, our results are not directly comparable with the study of Jacob et al. (2008). In our study, concentrations of both indigenous generic *E. coli* and inoculated *E. coli* O157:H7 were found to survive longer (7 to 8 d) in manure slurries obtained from the pen surface of cattle fed WDGS when compared with 0% WDGS. We believe the reason for this is because slurries from cattle fed WDGS had decreased concentrations of L-lactate (14 to 15 umol/g of DM) and pH values between 6.0 and 8.0. This is in comparison with slurries from cattle fed 0% WDGS (83% corn diet), which initially contained 447 μmol of L-lactate/g of DM and a pH of 6.28, and in which

pH values declined below 6.0. This elevated L-lactate concentration is attributed to greater starch excretion (Huntington et al., 2006) from cattle fed 0% WDGS, compared with 20, 40, or 60% WDGS diets. McWilliam Leitch and Stewart (2002) demonstrated that L-lactate has a significant antimicrobial effect for a wide range of *E. coli* O157 and non-O157 *E. coli* isolates.

Our studies also indicated that *E. coli* persisted somewhat longer in 20 and 40% WDGS slurries than in 60% WDGS slurries. This difference may be due to the high ammonia concentration and high pH in the 60% WDGS slurries. It is well known that a high ammonium ion concentration is toxic for microorganisms. Park and Diez-Gonzalez (2003) indicated a minimal ammonia concentration of 30 mmol/L may be necessary before significant bacterial reduction in manure will occur. The ammonia concentration in our study for the 60% WDGS slurries was over 120 mmol/L, suggesting that it would be toxic for microorganisms.

In the current work, and in those of Jacob et al. (2008) and Peterson et al. (2007), studies were conducted in small experimental pens. Work currently in progress will determine if feeding WDGS to cattle in larger, soil-based feedlot pens (a more typical production system with lower animal density) will provide results consistent with a small pen environment in relation to persistence and prevalence of *E. coli* O157. Odor compounds also may be different from those found in this study when manure is mixed with soil.

## LITERATURE CITED


APHA. 1965. Standard Methods for the Examination of Water and Wastewater. 12th ed. H. P. Orland, ed. Am. Public Health Assoc., New York, NY.

Attwood, G., D. Li, D. Pacheco, and M. Tavendale. 2006. Production of indolic compounds by rumen bacteria isolated from grazing ruminants. J. Appl. Microbiol. 100:1261–1271.

Berry, E. D., J. E. Wells, S. L. Archibeque, C. L. Ferrell, H. C. Freetly, and D. N. Miller. 2006. Influence of genotype and diet on steer performance, manure odor, and carriage of pathogenic and other fecal bacteria. II. Pathogenic and other fecal bacteria. J. Anim. Sci. 84:2523–2532.

Dewell, G. A., J. R. Ransom, R. D. Dewell, K. McCurdy, I. A. Gardner, A. E. Hill, J. N. Sofos, K. E. Belk, G. C. Smith, and M. D. Salman. 2005. Prevalence of and risk factors for *Escherichia coli* O157 in market-ready beef cattle from 12 U. S. Feedlots Foodborne Pathog. Dis. 2:70–76.

Duncan, S. H., P. Louis, and H. J. Flint. 2004. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl. Environ. Microbiol. 70:5810–5817.

Eisenstadt, E., B. C. Carlton, and B. J. Brown. 1994. Gene Mutation. Pages 297–316 in Methods for General and Molecular Bacteriology. Pro. Gerhardt, R. G. E. Murray, W. A. Wood, and N. R. Krieg, ed. American Society for Microbiology, Washington, DC.

Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. USA 97:2999–3003.

Hawe, S. M., N. Walker, and B. W. Moss. 1992. The effects of dietary fibre, lactose, and antibiotic on the levels of skatole and indole in feces and subcutaneous fat in growing pigs. Anim. Prod. 54:413–419.

Hobbs, P. J., B. F. Pain, R. M. Kay, and P. A. Lee. 1996. Reduction of odorous compounds in fresh pig slurry by dietary control of CP. J. Sci. Food Agric. 71:508–514.

Huntington, G. B., D. L. Harmon, and C. J. Richards. 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J. Anim. Sci. 84(E. Suppl.):E14–E24.

Jacob, M. E., J. T. Fox, J. S. Drouillard, D. G. Renter, and T. G. Nagaraja. 2008. Effects of dried distillers grain on fecal prevalence and growth of *Escherichia coli* O157 in batch culture fermentations from cattle. Appl. Environ. Microbiol. 74:38–43.

Kelly, D. P., and A. P. Wood. 1998. Microbes of the S cycle. Pages 31–57 in Techniques in Microbial Ecology. R. S. Burlage, R. Atlas, D. Stahl, G. Geesey, and G. Sayler, ed. Oxford University Press, New York, NY.

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. BOARD-INVITED REVIEW: Use of distillers byproducts in the beef cattle feeding industry. J. Anim. Sci. 86:1223–1231.

Koziel, J. A., C. Lingshuang, D. W. Wright, and S. J. Hoff. 2006. Solid-phase microextraction as a novel air sampling technology for improved, GC-olfactometry-based assessment of livestock odors. J. Chromatogr. Sci. 44:451–457.

Mackie, R. I., P. G. Stroot, and V. H. Varel. 1998. Biochemical identification and biological origin of key odor components in livestock waste. J. Anim. Sci. 76:1331–1342.

Martin, A. K. 1982. The origin of urinary aromatic compounds excreted by ruminants. 3. The metabolism of phenolic compounds to simple phenols. Br. J. Nutr. 48:497–507.

McWilliam Leitch, E. C., and C. S. Stewart. 2002. *Escherichia coli* O157 and non-O157 isolates are more susceptible to L-lactate than to D-lactate. Appl. Environ. Microbiol. 68:4676–4678.

Miller, D. N., and E. D. Berry. 2005. Cattle feedlot soil moisture and manure content: I. Impacts on greenhouse gases, odor compounds, N losses, and dust. J. Environ. Qual. 34:644–655.

Miller, D. N., and V. H. Varel. 2001. In vitro study of the biochemical origin and production limits of odorous compounds in cattle feedlots. J. Anim. Sci. 79:2949–2956.

Miller, D. N., and V. H. Varel. 2002. An in vitro study of manure composition on the biochemical origins, composition, and accumulation of odorous compounds in cattle feedlots. J. Anim. Sci. 80:2214–2222.

Mohammed, N., R. Onodera, and M. M. Or-Rashid. 2003. Degradation of tryptophan and related indolic compounds by ruminal bacteria, protozoa and their mixture in vitro. Amino Acids 24:73–80.

NRC. 1998. Nutrient Requirements of Swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2000. Nutrient Requirements of Beef Cattle. 8th rev. ed. Natl. Acad. Press, Washington, DC.

Ouwerkerk, D., and A. V. Klieve. 2001. Bacterial diversity within feedlot manure. Anaerobe 7:59–66.

Park, G. W., and F. Diez-Gonzalez. 2003. Utilization of carbonate and ammonia-based treatments to eliminate *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT104 from cattle manure. J. Appl. Microbiol. 94:675–685.

Peterson, R. E., T. J. Klopfenstein, R. A. Moxley, G. E. Erickson, S. Hinkley, G. Bretschneider, E. M. Perberov, D. Rogan, and D. R. Smith. 2007. Effect of a vaccine product containing Type III secreted proteins on the probability of *Escherichia coli* O157:H7 fecal shedding and mucosal colonization in feedlot cattle. J. Food Prot. 70:2568–2577.

Smith, E. A., and G. T. Macfarlane. 1997. Formation of phenolic and indolic compounds by anaerobic bacteria in the human large intestine. Microb. Ecol. 33:180–188.

Spiehs, M. J., M. H. Whitney, and G.C. Shurson. 2002. Nutrient database from distiller's dried grains with solubles produced from new plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639–2645.

Stein, H. H., M. L. Gibson, C. Pedersen, and M. G. Boersma. 2006. Amino acid and energy digestibility in ten samples of distillers

dried grain with solubles fed to growing pigs. J. Anim. Sci. 84:853–860.

Stock, R. A., J. M. Lewis, T. J. Klopfenstein, and C. T. Milton. 1999. Review of new information on the use of wet and dry milling feed co-products in feedlot diets. Proc. Am. Soc. Anim. Sci. http://www.asas.org/jas/symposia/proceedings/0924.pdf Accessed Feb. 19, 2008.

Sutton, A. L., K. B. Kephart, M. W. A. Verstegen, T. T. Canh, and P. J. Hobbs. 1999. Potential for reduction of odorous compounds in swine manure through diet modification. J. Anim. Sci. 77:430–439.

Wright, D. W., D. K. Eaton, L. T. Nielsen, F. W. Kuhrt, J. A. Koziel, J. P. Spirnhirne, and D. B. Parker. 2005. Multidimensional gas chromatography-olfactometry for identification and prioritization of malodors from confined animal feeding operations. J. Agric. Food Chem. 53:8663–8672.

Young, L. Y., and M. Rivera. 1985. Methanogenic degradation of four phenolic compounds. Water Res. 19:1325–1332.