Manure Odor Potential and *Escherichia coli* Concentrations in Manure Slurries of Feedlot Steers Fed 40% Corn Wet Distillers Grains

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This study evaluated feeding 0 and 40% wet distillers grains with solubles (WDGS) diets to cattle and the effects on feedlot manure collected from soil-based pens and incubated for 28 d. Steers (n = 603; 261 ± 32 kg) were fed in eight pens (15 × 150 m) of 75 to 77 steers per pen. Two consecutive experiments were conducted with WDGS—one in which the corn source fed with WDGS was high-moisture and one in which WDGS was fed with dry-rolled corn. We compared odorants (volatile fatty acids [VFAs], aromatic compounds, NH_3, H_2S) and persistence of *Escherichia coli* in feedlot manure slurries stored from 0 to 28 d. From both experiments, manure collected from cattle fed 40% WDGS had lower ($P < 0.05$) total VFAs, including acetate, propionate, and butyrate, all of which continued to be lower to 28 d. However, these slurries had greater concentrations ($P < 0.05$) of branched-chained VFAs (isobutyrate and isovalerate), especially after 14 d of incubations. Similarly, *p*-cresol and skatole concentrations tended to be greater in slurries originating from 40% WDGS diets and increased with incubation time. Indole was initially greater in the slurries from 40% WDGS diets; however, it was metabolized by microbes during incubation. Manure slurries from the 40% WDGS diets had greater quantities of H_2S, NH_3, and P ($P < 0.05$). Levels of *E. coli* in 0 and 40% WDGS manure slurries were similar when high-moisture corn was used in the diets. However, when dry-rolled corn was used, *E. coli* persisted longer in 40% WDGS manure slurries in comparison to 0% WDGS. Results here support earlier studies that suggest feeding WDGS increases odor emissions, N loss, *E. coli* survival, and surface water contamination due to greater potential P runoff.

A cost-effective feed resource for beef cattle is corn (*Zea mays*) wet distillers grains with solubles (WDGS), a byproduct from ethanol production (Stock et al., 2000; Klopfenstein et al., 2008). This process removes starch from corn and concentrates the crude protein, oil, fiber, and minerals approximately 3-fold (Spiehs et al., 2002). When these concentrated nutrients are fed as WDGS to feedlot cattle (typically 20–40% of the diet on a dry matter [DM] basis) in place of corn, it often results in a diet with crude protein, oil, and minerals such as phosphorous (P) and sulfur (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 (Martin, 1982). This was found to be true when cattle were finished on WDGS diets and raised on a concrete floor (Varel et al., 2008) or when cattle were fed individually wheat-based distillers grains (Hao et al., 2009). Recent studies also suggest that there is a positive association between feeding distillers grains and fecal shedding of *E. coli* O157:H7 (Jacob et al., 2008; Wells et al., 2009). The objectives of this study were to evaluate, in a production-scale facility with soil-based pen floors, the effect of WDGS fed at 0 or 40% WDGS (on a DM basis) in two common corn-based diets on the production of malodorant compounds (volatile fatty acids [VFAs], aromatic compounds, NH_3, and H_2S) and the persistence of *E. coli* in fresh manure and manure slurries stored from 0 to 28 d. The corn sources fed with WDGS were high-moisture and dry-rolled corn.

Materials and Methods

Animals and Diets

Detailed information about the animals and diets used in this study were previously published (Wells et al., 2009). Briefly, the study was initiated on 29 Oct. 2007, with 603 steers (261 ± 32 kg body weight) in eight pens (15 by 150 m) of 75 to 77 steers per pen (four pens per treatment). The steers in four control pens (0% WDGS) were fed standard diets through the growing and finishing phases of production. The steers in the four WDGS pens were fed similar diets, but WDGS replaced 47% of the corn

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Abbreviations: CFU, colony-forming unit; DM, dry matter; VFA, volatile fatty acid; WDGS, wet distillers grains with solubles.

grain component (DM basis) in each production phase diet (see below). Pen manure samples were collected four times during the finishing phase of the study. The animals received the finishing diets (0% WDGS or 40% WDGS, DM basis; Table 1) from mid-January 2008 (Day 77) until slaughter (Day 231 or 253). The corn source was high-moisture corn from October 2007 through May 2008 (Day 189). The supply of high-moisture corn was depleted by May 2008; therefore, high-moisture corn was replaced with dry-rolled corn (Day 189–253), and diets were reformulated for DM content differences in corn source. The dry-rolled corn diets were fed for a full 28-d period before the next sampling. Therefore, during Period 1 (high-moisture corn), all pens were sampled twice (15 Apr. and 5 May 2008), and during Period 2 (dry-rolled corn), all pens were again sampled twice (2 and 16 June 2008).

Manure Collection and Analyses

Manure samples were collected from three sections in each pen. Behind the feed trough of each pen was a concrete apron that extended into the pen 3.5 m and across the entire pen. The soil area adjacent to the apron (1.2 by 15 m) was used as the experimental area and was divided into three sections. One composite manure sample (1 kg) was obtained from each of the three sections per pen. The manure layer averaged 12 to 20 cm. Therefore, during each sampling period (15 Apr., 5 May, 2 June, and 16 June 2008), a total of 24 1-kg samples were obtained. Subsamples were used to determine DM, total N, S, and P. A previous study (Spiehs and Varel, 2009) indicated there was no difference in fecal DM output across treatments of 0, 20, 40, or 60% WDGS. From each 1-kg sample, 600 g was weighed and mixed with 300 mL distilled water by blending at high speed with a Waring blender (Waring Inc., New Hartford, CT). This slurry (900 g) was poured into a wide-mouth (10 cm) jar (17 cm tall, 13.5 cm in diameter, 1.6 L volume), with a total of 24 jars for each sampling period. Plastic lids provided with the jars were used to cover approximately 90% of the jar opening to minimize moisture loss over the 28-d experimental incubation period at room temperature (22°C). The contents of the jars were stirred before sampling and were sampled at 0, 2, 4, 7, 14, 21, and 28 d. Little stratification of the slurries occurred due to an active fermentation. Manure slurry pH was obtained 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 mol L^{-1} 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). An autoanalyzer (Model 2700; Yellow Springs Instrument, Yellow Springs, OH) was used to analyze L-lactate and total alcohol, and a gas chromatograph (Hewlett-Packard 6890; 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; Varel et al., 2008). Ammonia was determined using a modification of the Sigma Urea N kit (procedure No. 640; Sigma-Aldrich Chemicals, St. Louis, MO) (Varel and Wells, 2007). Standards and samples were diluted 10-fold, and

Table 1. Formulated rations and calculated for the finishing phase diets with and without wet distillers grains with solubles fed to steers during study.

	Finishing diets†	
	0% WDGS‡	40% WDGS
	% of DM (DM basis)	
Ingredient		
Corn, grain	82.75	45.15
Corn silage	13.75	13.75
WDGS	0.00	40.00
Soybean meal	2.40	0.00
Urea	0.40	0.00
Limestone	0.57	1.04
Salt	0.062	0.000
Monensin premix§	0.030	0.030
Thiamine premix¶	0.023	0.023
Vitamin supplement#	0.008	0.008
Trace mineral supplement††	0.007	0.000
Nutrient content, formulated		
CP	11.86	17.86
Fat	4.05	6.32
ADF	6.83	19.48
NDF	13.89	27.38
Calcium	0.403	0.451
Phosphorus	0.293	0.339

† Fed Days 78 through 245. The 0 and 40% WDGS diets had respective dry matter levels of 71.07 and 51.98%.

‡ ADF, acid detergent fiber; CP, crude protein; DM, dry matter; NDF, neutral detergent fiber; WDGS, wet distillers grains with solubles.

§ Rumensin 80 (Elanco Animal Health, Indianapolis, IN).

¶ Premix contains 88 g kg^{-1}.

Supplement provides 8,800,000 IU of vitamin A, 880,000 IU of vitamin D, and 880 IU of vitamin E per kilogram.

†† Supplement contains 13% Ca, 12% Zn, 8% Mn, 10% Fe, 1.5% Cu, 0.2% I, and 0.1% Co.

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 microplate reader (Ceres UV900C; Bio-Tek, Winooski, VT). Hydrogen-sulfide was purged from manure samples using a helium gas stream and trapped in 7 mL of 2% (w/w) 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).

Manure slurries were sampled at 0, 1, 2, 4, 7, 10, 14, 21, and 28 d for the determination of *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 (Becton Dickinson and Co., Sparks, MD). Tube contents were mixed, decimally diluted in additional buffered peptone water if needed, and plated onto CHROMagar ECC (DRG International, Mountainside, NJ), 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.

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

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-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 $\log_{10}$ colony-forming unit (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. 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

Data in Table 2 between diets with high-moisture corn and dry-rolled corn are not directly comparable because time of sampling and the environmental conditions confound comparison of the data. Samples collected on 15 Apr. and 5 May 2008 experienced cooler temperatures (3 and 8°C, respectively) than the samples collected on 2 and 16 June 2008 (19 and 24°C, respectively). Thus, any differences observed between the two corn sources may not be a result of the way the corn was processed; rather, it may be influenced by the temperature (see Wells et al., 2009, Fig. 1C for all temperatures) when samples were collected. Therefore, the results are reported separately for each corn source. In general, the odorants acetate, propionate, butyrate, and total VFAs were greater ($P < 0.05$) or the same when high-moisture or dry-rolled corn was fed with no (0%) WDGS in the diet, compared with 40% WDGS (Table 2). The opposite was true for the branched-chain VFAs, isobutyrate and isovalerate; the aromatic odorants, phenol, *p*-cresol, and indole; and H_2S-S and ammonia-N, which were all in greater concentrations ($P < 0.05$) when 40% WDGS was fed. The minerals (N, P, and S) were greater ($P < 0.05$) in the

Table 2. Initial odorants, L-lactate, pH, nitrogen, sulfur, phosphorus, and generic *Escherichia coli* from manure slurries collected over four sampling periods from cattle fed 0 or 40 wet distillers grains with solubles ($n = 24$).

Item	High-moisture corn			Dry-rolled corn		
	Wet distillers grains in diet, % DM† basis					
	0	40	SEM	0	40	SEM
Odorant, mmol kg^{-1} DM						
Acetate	188a‡	158b	5.0	97a	93a	6.0
Propionate	107a	84b	5.7	72a	50a	2.5
isoButyrate	1.4a	2.6b	0.08	2.1a	2.0a	0.12
Butyrate	39a	16b	2.0	61a	28b	4.3
isoValerate	1.8a	2.6b	0.07	2.0a	2.1a	0.11
Valerate	1.3a	1.6b	0.09	4.5a	2.9b	0.30
Caproate	0.36a	0.09b	0.02	0.65a	0.10b	0.05
Total VFAs	339a	266b	9.6	244a	178b	11.6
Phenol	0.07a	0.21b	0.01	0.01a	0.08b	0.01
P-cresol	0.96a	1.28b	0.07	0.47a	0.71b	0.06
Indole	4.68a	5.23a	0.38	1.63a	2.81b	0.32
Skatole	0.49a	0.44a	0.05	0.49a	0.76b	0.08
H_2S-S, mg kg^{-1} DM	0.30a	7.46b	0.54	2.14a	10.19b	0.70
Ammonia-N, g kg^{-1} DM	1.54a	5.19b	0.16	0.85a	2.46b	0.19
pH	6.35a	6.87b	0.05	6.27a	6.53b	0.07
L-lactate, mmol kg^{-1} DM	56.4a	8.8b	5.3	3.4a	5.2b	0.4
Total alcohols, mmol kg^{-1} DM	6.2a	0.51b	0.5	0.84a	0.49a	0.17
Minerals, g kg^{-1} DM						
Total nitrogen	15.5a	17.6b	0.5	16.2a	18.4b	0.5
Phosphorus	4.8a	5.7b	0.15	4.8a	5.9b	0.19
Sulfur	3.4a	4.3b	0.11	3.5a	4.1b	0.15
Generic *E. coli*						
$\log_{10}$ CFU g^{-1} wet manure	6.05a	6.28a	0.10	6.44a	6.94s	0.13

† CFU, colony-forming units; DM, dry matter; VFA, volatile fatty acid.

‡ Within corn source, means followed by different lowercase letters within each row differ ($P < 0.05$).

manure slurries when 40% WDGS was included in either of the corn diets than when no (0%) WDGS was in the diet.

Accumulation and degradation of VFAs and aromatic fermentation products from in vitro incubation of manure slurries from cattle fed 0 and 40% WDGS are given in Fig. 1 and 2, respectively, for the high-moisture corn and dry-rolled corn diets. Data in Fig. 1A through 1D indicate that all straight-chain VFAs, with the exception of valerate, were initially lower and continued to be lower ($P < 0.05$) over the 28-d incubation for the diet containing 40% WDGS. The opposite was

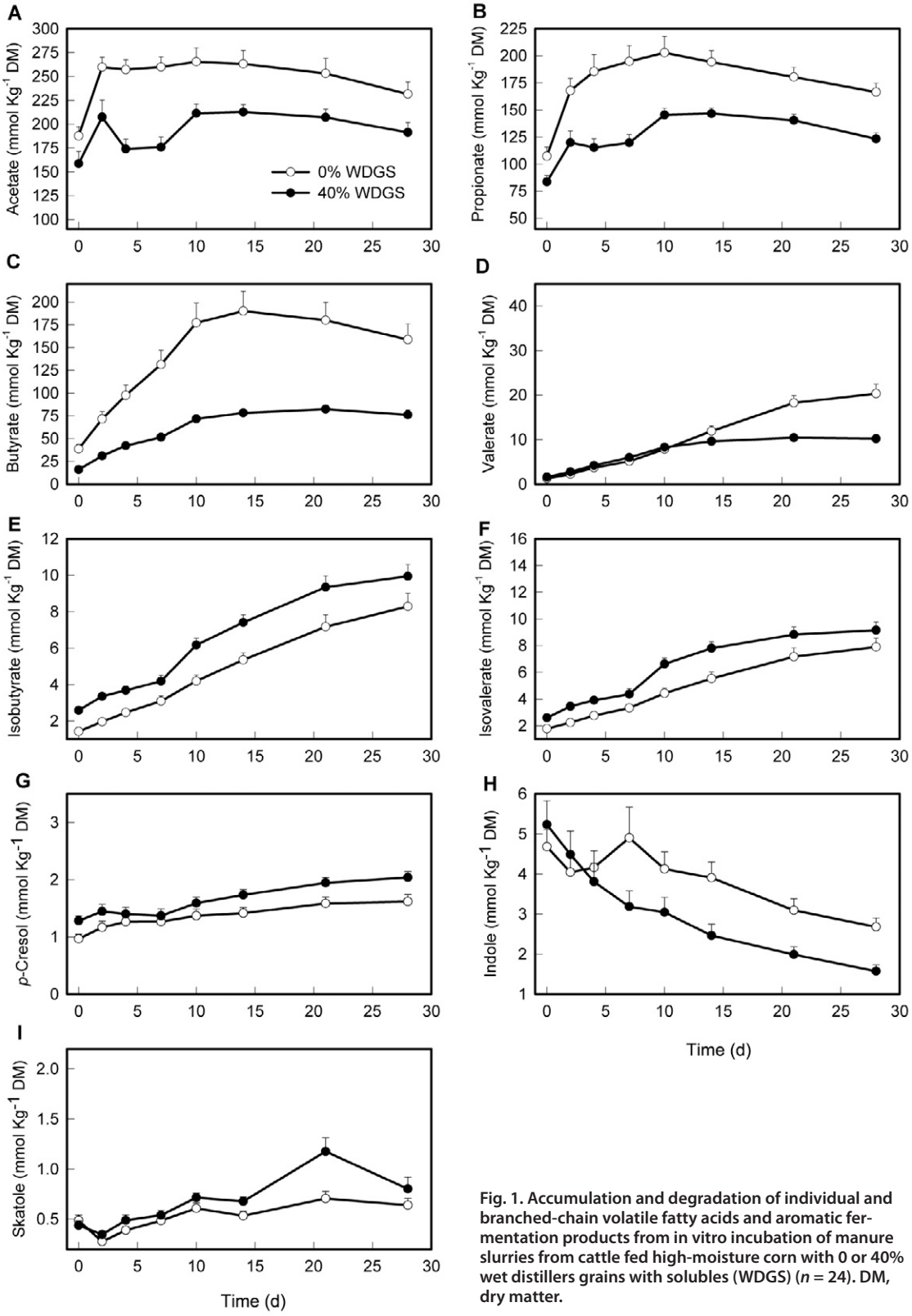

Fig. 1. Accumulation and degradation of individual and branched-chain volatile fatty acids and aromatic fermentation products from in vitro incubation of manure slurries from cattle fed high-moisture corn with 0 or 40% wet distillers grains with solubles (WDGS) ($n = 24$). DM, dry matter.

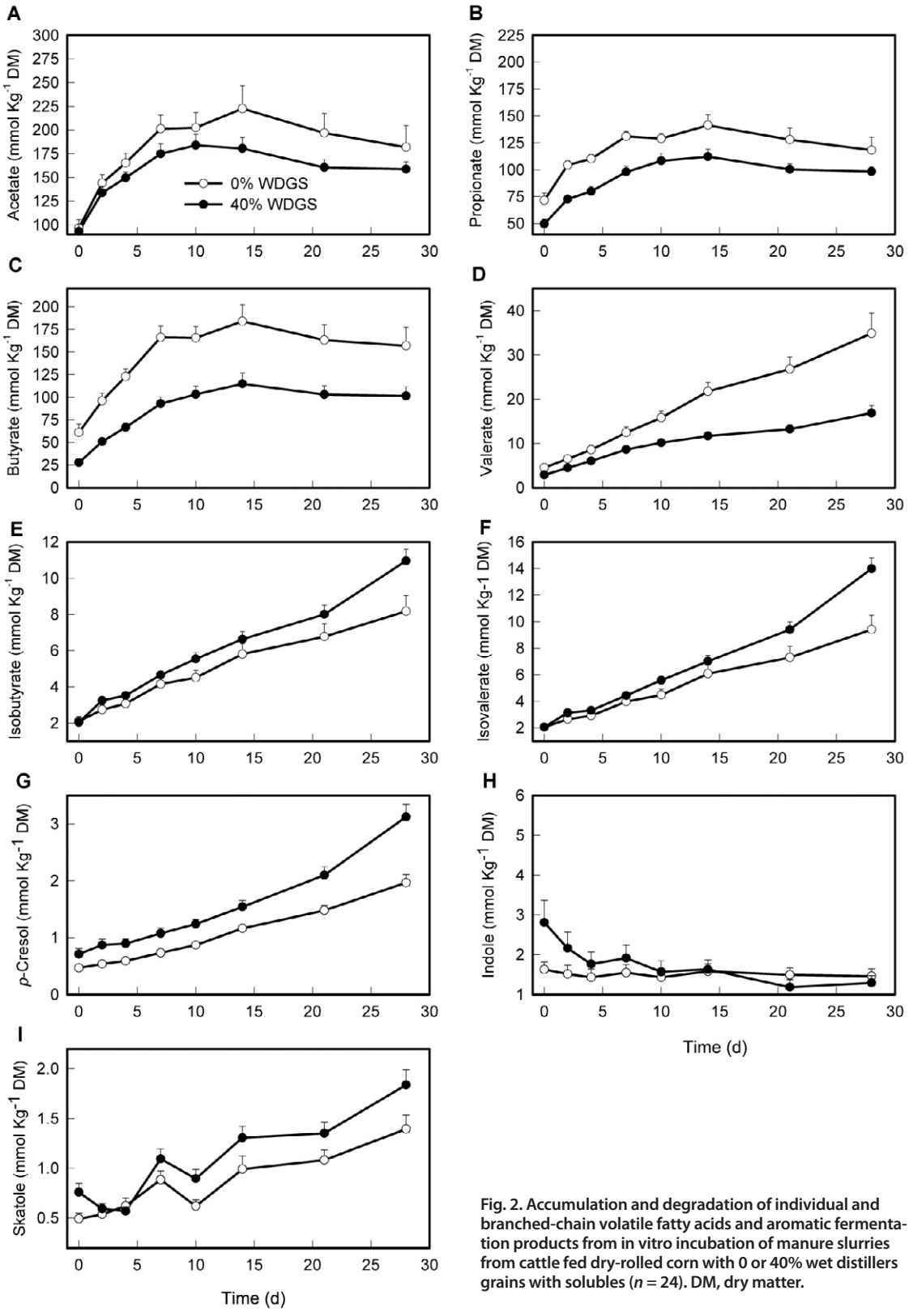

Fig. 2. Accumulation and degradation of individual and branched-chain volatile fatty acids and aromatic fermentation products from in vitro incubation of manure slurries from cattle fed dry-rolled corn with 0 or 40% wet distillers grains with solubles (n = 24). DM, dry matter.

true for the branched-chain VFAs. Isobutyrate and isovalerate concentrations were greater (P < 0.05) over the 28-d incubation for the diet containing 40% WDGS with high-moisture corn (Fig. 1E and 1F). For the dry-rolled corn (Fig. 2E and 2F), isobutyrate and isovalerate concentrations were similar between 0 and 40% WDGS diets up to Day 14; however, at Days 21 and 28, these concentrations were greater (P < 0.05) for the 40% WDGS diet. Aromatic compounds in the manure slurries initially were greater (P < 0.05) with the 40% WDGS diet, irrespective of corn source (Fig. 1G and 1H; Fig. 2G, 2H, and 2I), with the exception of skatole from the high-moisture corn diet (Fig. 1I), which was the same. However, over the 28-d incubation, concentrations of indole declined with the high-moisture and dry-rolled corn diets (Fig. 1H and 2H). The

opposite was true for *p*-cresol and skatole, which increased over the 28-d incubation in slurries from both corn diets (Fig. 1G, 1I, 2G, 2I).

The L-lactate concentration was initially higher ($P < 0.05$) for the manure slurries from the 0% WDGS diet with high-moisture corn than the slurries from 40% WDGS (Fig. 3A). However, by Day 14, this difference disappeared, most likely because L-lactate was converted to methane (Fig. 3D). The pH values were higher (Fig. 3B and 4B) for manure slurries from cattle fed 40% WDGS, irrespective of the corn source. This was also true for the ammonia concentrations (Fig. 3C and 4C). Methane production from these slurries (Fig. 3D and 4D) were higher from animals fed 0% WDGS compared with 40% WDGS, irrespective of corn source. Alcohol concentrations from the high-moisture corn diet were initially lower ($P < 0.05$) and continued to be lower over the 28-d incubation for the diet containing 40% WDGS (Fig. 3E). The concentrations from the dry-corn diet (Fig. 4E) were not different. Dry matter was not different between 0 or 40% WDGS with either corn source (Fig. 3F and 4F).

Initial *E. coli* concentrations in the 0 and 40% WDGS manure slurries were not different ($P > 0.05$) when high-moisture corn was the corn source in the diets (Table 2). In addition, the levels of *E. coli* declined similarly, only 1.2 to 1.4 $\log_{10}$ CFU g^{-1} wet manure, in the two WDGS diets during the 28-d incubation (Fig. 5A), and did not differ ($P > 0.05$) on any sampling day. In contrast, when dry-rolled corn was the corn source, the initial *E. coli* concentrations in the slurry were higher ($P < 0.05$) in 40% WDGS diets compared with 0% WDGS (Table 2) and remained higher ($P < 0.05$) during the 28-d incubation (Fig. 5B). Furthermore, *E. coli* levels declined more rapidly in 0% WDGS manure than in 40% WDGS manure, to final levels at 28 d of 2.95 and 4.55 $\log_{10}$ CFU per g wet manure, respectively, when dry-rolled corn was in the diet.

Discussion

Our first study, in which 0, 20, 40, and 60% WDGS diets were fed to cattle finished on concrete floor pens, indicated that essentially all odorants (VFAs, branched-chain VFAs, aromatic compounds, ammonia, and H_2S) increased in the manure slurries with increasing WDGS in the diet (Varel et al., 2008). Our current study, in which 0 or 40% WDGS was included in the diets and the cattle were raised on soil-based pens, indicated that some, but not all, odorants were in greater concentration when 40% WDGS was included in the diet (Table 2). The odorants acetate, propionate, butyrate, and total VFAs were greater in manure slurries when 0% WDGS was in

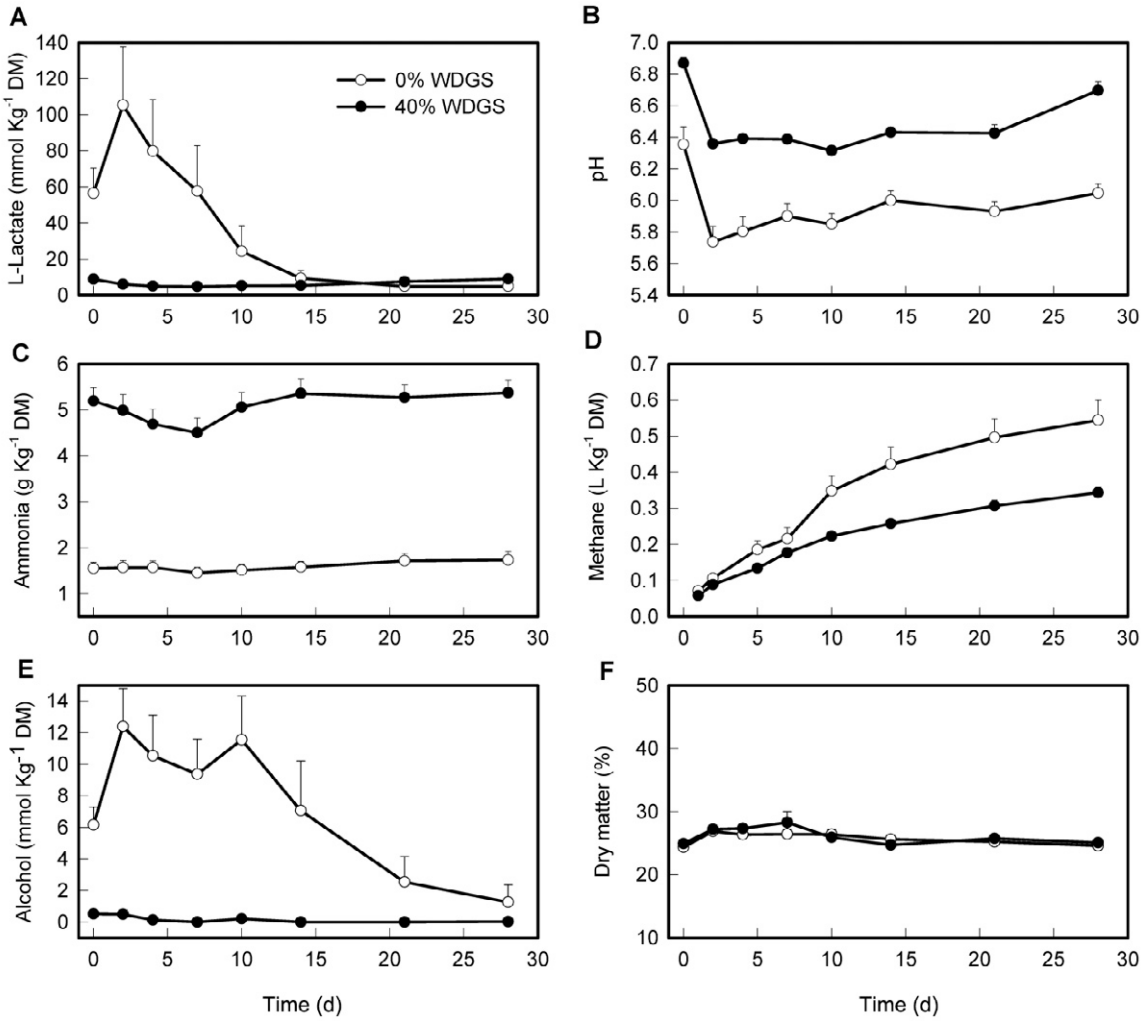

Fig. 3. L-lactate, pH, ammonia, methane, alcohol, and dry matter (DM) values from in vitro incubation of manure slurries from cattle fed high-moisture corn with 0 or 40% wet distillers grains with solubles (*n* = 24).

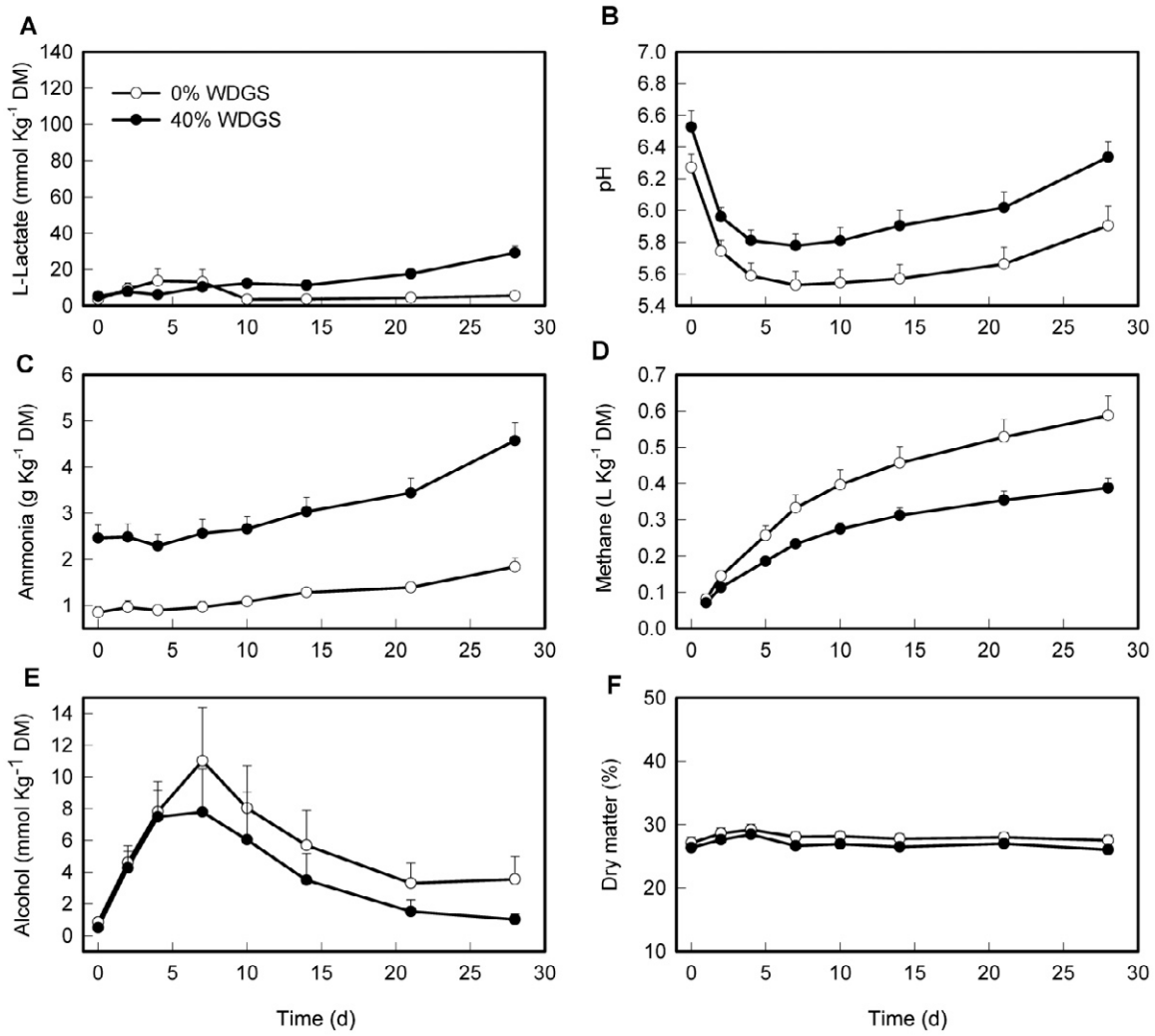

Fig. 4. L-lactate, pH, ammonia, methane, alcohol, and dry matter values from in vitro incubation of manure slurries from cattle fed dry-rolled corn with 0 or 40% wet distillers grains with solubles (WDGS) (*n* = 24).

the diet compared with 40% WDGS. Greater VFA concentrations in the slurries can be explained by a greater concentration of starch in feces when diets contain more corn in the diet (Huntington et al., 2006). On the feedlot surface, starch would rapidly be converted to VFAs. In our first study on concrete floors, when 0% WDGS (82.7% corn) was fed, we likely had significant quantities of starch in the manure, which was rapidly converted to L-lactate, and it limited the formation of VFAs by lowering the pH. Concentrations of L-lactate in the current study, 56.4 and 3.4 mmol kg^{-1} DM, respectively, for 0% WDGS with high-moisture corn and dry-rolled corn (Table 2), are much lower than the 447 mmol kg^{-1} in the 0% WDGS diet in our previous study.

In contrast to the straight-chain VFA odorants, the branched-chain VFAs isobutyrate and isovalerate; the aromatic odorants *p*-cresol, indole, and skatole; and H_2S-S and ammonia-N were in greater concentrations when 40% WDGS was fed in the current study. Similarly, N, P, and S were all in greater concentrations in the manure slurries when 40% WDGS was included in the diets. These results agree with those of our initial study and with those of Hao et al. (2009) when they fed wheat-based dried distillers grains with solubles to cattle on soil-floor pens. Diets with 40% WDGS normally contain a greater concentration of crude protein when replacing corn grain (11.86 compared with 17.86%, respectively, for 0 and 40% diets) (Table 1). The reason for this is that ethanol is produced from the starch fraction of corn, and in that process the remaining protein, oil, fiber, and minerals are concentrated approximately three times (NRC, 1998; Spiehs et al., 2002; Stein et al., 2006). Thus, with greater protein in the diet, more ammonia is generated by deamination of the amino acids. Similarly, more sulfide is generated from the sulfur-containing amino acids cysteine and methionine (Hobbs et al., 1996). Likewise, with the branched-chain VFAs, isobutyrate and isovalerate concentrations are greater because greater concentrations of branched-chain amino acids, such as leucine, isoleucine, and valine, are degraded (Mackie et al., 1998). Finally, the aromatic amino acids phenylalanine, tyrosine, and tryptophan are degraded to form aromatic compounds, phenol, *p*-cresol, and indole when 40% WDGS is fed (Mohammed et al., 2003; Attwood et al., 2006).

Greater concentrations of branched-chain VFAs, H_2S, and aromatic compounds were observed in this study when 40% WDGS diets were fed. These fermentation products have very low odor threshold values and are considered more offensive and objectionable odorants than straight-chain VFAs (Mackie

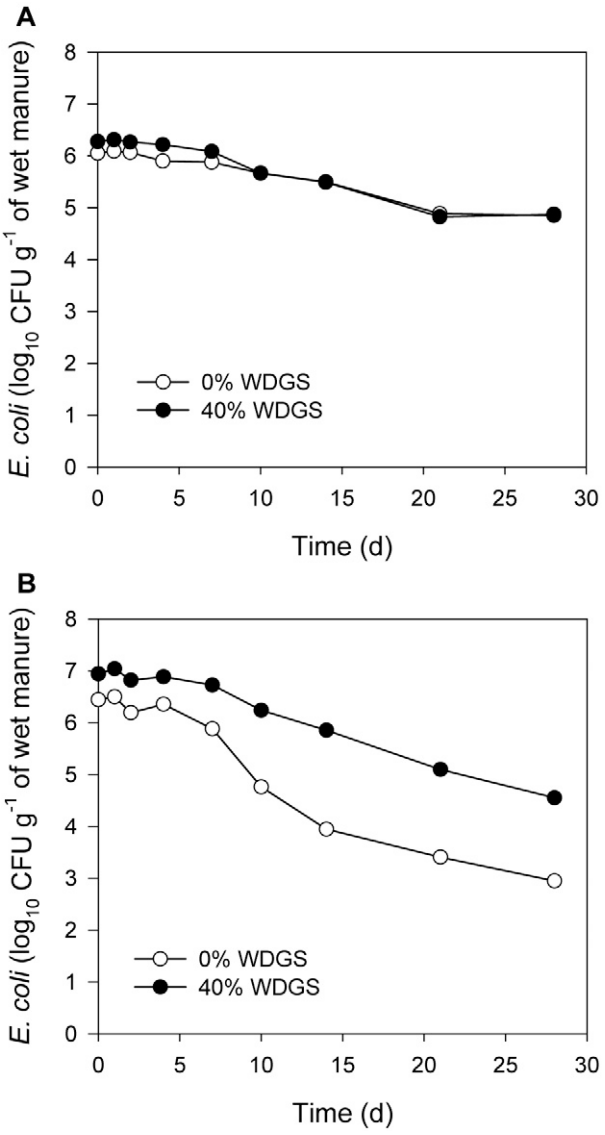

Fig. 5. Persistence of *Escherichia coli* during in vitro incubation of manure slurries from cattle fed 0 or 40% wet distillers grains with solubles (WDGS) with (A) high-moisture corn or (B) dry-rolled corn. CFU, colony-forming units.

et al., 1998; Miller and Varel, 2002; Koziel et al., 2006; Hao et al., 2009). These results strongly support our earlier conclusions and those of Hao et al. (2009) that odor emission is increased when WDGS diets are fed.

Once the manure slurries were incubated for 28 d, the original trends at the beginning of the incubations were maintained until the end of the incubations. In other words, if VFA concentrations were initially greater in the manure slurries from cattle fed 0% WDGS diets, they were greater at the end of 28 d (Fig. 1 and 2). The exceptions to this are the alcohol concentrations (Fig. 3E) and L-lactate (Fig. 3A and 4A). Both of these substrates are readily converted to methane (Fig. 3D and 4D) and are converted more readily than the VFA substrates because of the laws of fermentation thermodynamics (Mackie et al., 1998). The next substrates to be converted to methane are the short-chain VFAs acetate, propionate, and butyrate. This may explain their stationary or gradual decline after Day 14 of incubation (Fig. 1A–1C; Fig. 2A–2C), with a concurrent increase in methane accumulation (Fig. 3D and 4D). Indole disappears during the incubation (Fig. 1H and 2H), but its conversion to methane would be very difficult because the decomposition of aromatic ring-containing substrates during methanogenesis is not as thermodynamically favorable as the utilization of fatty acids (Young and Rivera, 1985). A more likely explanation is its uptake by microorganisms for the production of new protein in microbial biomass.

Manure slurries from cattle fed 40% WDGS had greater pH values and ammonia concentrations when compared with 0% WDGS (Fig. 3B, 3C; Fig. 4B, 4C). This is expected because the 40% WDGS diets contain a greater protein concentration (17.86% compared with 11.86%). Thus, more amino acids are available for deamination (Mackie et al., 1998; Sutton et al., 1999); this raises the ammonia concentration, which in turn increases the buffering capacity of the manure slurries and keeps the pH greater. However, more methane is generated from the 0% WDGS diets than from the 40% WDGS diets because more metabolizable substrates (starch) are available in the manure slurries. These substrates are converted to alcohols, VFAs, and lactic acid, which are subsequently used as substrates by methanogenic organisms (Archibeque et al., 2006; Mackie et al., 1998). Data suggest that methane production was more dependent on substrate availability than differences in pH.

This work was part of a larger study that indicated that feeding 40% WDGS was associated with increased *E. coli* 0157:H7 in feces and on hides of cattle (Wells et al., 2009). In our previous work with cattle on concrete-floor pens, generic *E. coli* and *E. coli* 0157:H7 persisted longer in manure slurries of cattle fed 20, 40, and 60% WDGS in comparison to 0% WDGS (Varel et al., 2008). The increased persistence was associated with lower L-lactate concentrations and higher pH in the WDGS diets in comparison to the diet without WDGS, which contained a higher percentage of dry-rolled corn. The results of the current work extend these observations and further indicate that the type of corn (high-moisture vs. dry-rolled) in the diet can affect *E. coli* survival in manure. In comparison to our previous observations, lactate concentrations in the manure slurries were not as high in any diet, regardless of corn type or WDGS, initially or during incubation. The pH differences between 0 and 40% WDGS manures of diets containing high-moisture corn did not affect *E. coli* concentrations, likely because pH values for both these manure slurries remained at pH 5.7 or greater during the 28-d incubations (Wells et al., 2009). In diets with dry-rolled corn, *E. coli* survived longer in 40% WDGS manures, for which pH was 5.8 or greater during incubation, than in 0% WDGS manures, for which pH was 5.6 or below on Days 4, 7, 10, and 14 of incubation. Higher concentrations of VFAs and lower ammonia concentrations presumably contribute to this threshold pH range and lower concentration of *E. coli* in the 0% WDGS slurries. We attribute this lower pH to greater fecal starch and its subsequent fermentation in manure of cattle fed 0% WDGS (Archibeque et. al., 2006; Huntington et al., 2006; Depenbusch et al., 2008). Greater persistence of *E. coli* in manure may be a contributing factor for the observed increases in prevalence of the pathogen *E. coli* 0157:H7 in cattle fed WDGS (Wells et al., 2009). The greater survival of *E. coli* 0157:H7 in the production environment would increase the chances for reinfection or

transmission to additional animals by increasing the opportunities for fecal or oral exposures.

Conclusions

The results of this study conducted in a production-scale setting support earlier studies (Varel et al., 2008; Hao et al., 2009) that suggest that WDGS and dry distillers grains with solubles could increase odor emissions, N loss (NH_3 volatilization), and surface water contamination due to greater P runoff loss. Furthermore, feeding WDGS to cattle may alter the environmental survival of *E. coli* in the manure. Thus, livestock producers should be aware of these potential environmental contaminants, in particular, when they are feeding these byproducts at greater than 20%, as suggested by Hao et al. (2009).

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References

Archibeque, S.L., D.N. Miller, H.C. Freetly, and C.L. Ferrell. 2006. Feeding high-moisture corn instead of dry-rolled corn reduces odorous compound production in manure of finishing beef cattle without decreasing performance. J. Anim. Sci. 84:1767–1777.

APHA. 1965. Standard methods for the examination of water and wastewater. 12th ed. H.P. Orland (ed.) Am. Public Health Assoc., New York.

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.

Depenbusch, B.E., T.G. Nagaraja, J.M. Sargeant, J. S. Drouillard, E.R. Loe, and M.E. Corrigan. 2008. Influence of processed grains on fecal pH, starch concentration, and shedding of *Escherichia coli* 0157 in feedlot cattle. J. Anim. Sci. 86:632–639.

Hao, X., M.B. Benke, D.J. Gibb, A. Stronks, G. Travis, and T.A. McAllister. 2009. Effects of dried distillers' grains with solubles (wheat-based) in feedlot cattle diets on feces and manure composition. J. Environ. Qual. 38:1709–1718.

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 crude protein. 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. p. 31–57. *In* R.S. Burlage, R. Atlas, D. Stahl, G. Geesey, and G. Sayler (ed.) Techniques in microbial ecology. Oxford Univ. Press, New York.

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.

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. National Academy Press, Washington, DC.

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.

Spiehs, M.J., and V.H. Varel. 2009. Nutrient excretion and odorant production in manure from cattle fed corn wet distillers grains with solubles. J. Anim. Sci. 87:2977–2984.

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. 2000. Review of new information on the use of wet and dry milling feed co-products in feedlot diets. J. Anim. Sci. 77:1–12.

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.

Varel, V.H., and J.E. Wells. 2007. Influence of thymol and a urease inhibitor on coliform bacteria, odor, urea, and methane from a swine production manure pit. J. Environ. Qual. 36:773–779.

Varel, V.H., J.E. Wells, E.D. Berry, M.J. Spiehs, D.N. Miller, C.L. Ferrell, S.D. Shackelford, and M. Koohmaraie. 2008. Odorant production and persistence of *Escherichia coli* in manure slurries from cattle fed 0, 20, 40, or 60% wet distillers grains with solubles. J. Anim. Sci. 86:3617–3627.

Wells, J.E., S.D. Shackelford, E.D. Berry, N. Kalchayanand, M.N. Guerini, V.H. Varel, T.M. Arthur, J.M. Bosilevac, H.C. Freetly, T.L. Wheeler, C.L. Ferrell, and M. Koohmaraie. 2009. Prevalence and level of *Escherichia coli* 0157:H7 in feces and on hides of feedlot steers fed diets with or without wet distillers grains with solubles. J. Food Prot. 72:1624–1633.

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