

Influence of genotype and diet on steer performance, manure odor, and carriage of pathogenic and other fecal bacteria. III. Odorous compound production^{1,2}

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ABSTRACT: Three beef cattle diets were assessed for their potential to produce odorous compounds from cattle feces excreted during the growing and finishing periods. Eight pens containing 51 steers of varying proportions of Brahman and MARC III genotypes were fed either a chopped bromegrass hay diet or a corn silage diet for a 119-d growing period. After the growing period, all steers were switched to the same high-corn finishing diet (high corn) and fed to a target weight of 560 kg (finishing period). Fecal slurries were prepared from a composite of fresh fecal pats collected in each pen during both periods and incubated anaerobically. In additional flasks, starch, protein, or cellulose was added to the composite fecal subsamples to determine the preferred substrates for fermentation and odorous compound production. The content and composition of the fermentation products varied both initially and dur-

ing the incubation, depending on the diet fed to the steers. The corn silage and high corn feces had the greater initial content of VFA (381.0 and 524.4 $\mu\text{mol/g}$ of DM, respectively) compared with the bromegrass feces (139.3 $\mu\text{mol/g}$ of DM) and accumulated more VFA than the bromegrass feces during the incubation. L-Lactic acid and VFA accumulation in the high corn and corn silage feces was at the expense of starch, based on starch loss and the production of straight-chain VFA. In the bromegrass feces, accumulation of branched-chain VFA and aromatic compounds and the low starch availability indicated that the protein in the feces was the primary source for odorous compound production. Substrate additions confirmed these conclusions. We conclude that starch availability was the primary factor determining accumulation and composition of malodorous fermentation products, and when starch was unavailable, fecal microorganisms utilized protein.

Key words: beef cattle, diet, forage, grain, manure, odor

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INTRODUCTION

Odor emission issues associated with modern cattle production practices pose a challenge to confined feeding operations. Manure accumulation in the feedlot pen and subsequent handling, storage, and application practices all contribute to the anaerobic conditions that lead to odorous compound formation and its subsequent emission. One proposed method to manage

feedlot odor emissions is to modify the diet of cattle (McGinn et al., 2002), an approach that has been shown to minimize excess N and phosphorus excretion (Klopfenstein and Erickson, 2002; Satter et al., 2002; Burkholder et al., 2004). Previous studies of feces from cattle fed a high-corn finishing diet indicate that the availability of microbial substrates within the feces, particularly starch and to a lesser extent protein, are the predominant sources of malodorous fermentation products in stored fecal slurries (Miller and Varel, 2001, 2002). Additional experiments that more closely mimic feedlot pen conditions support these conclusions (Miller and Berry, 2005). Feces from a variety of cattle feedlot diets varying in grain, forage, and by-product composition and processing have yet to be tested for their potential to produce odorous compounds.

The primary objectives of these experiments were to assess the influence of 3 bovine diets (bromegrass or corn silage followed by high corn) on the initial odorous compound composition and the potential production of odorous compounds from fresh feces. Two companion papers address the influences of genotype

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and diet on steer performance, and the carriage of pathogens and fecal indicators (Berry et al., 2006; Ferrell et al., 2006).

MATERIALS AND METHODS

Animals, Feeding, and Sample Collection

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

The breeding, management, feeding, and the experimental diets of the 51 steers utilized for this study are described in detail in the accompanying paper by Ferrell et al. (2006). The 4 genotypes examined were of varying proportions of Brahman and MARC III (*Bos taurus*) genotypes, with 15, 20, 7, and 9 steers of 0, 1/4, 1/2, and 3/4 Brahman, respectively. The MARC III steers are a composite breed composed of 1/4 each of Hereford, Angus, Pinzgauer, and Red Poll. At 28 d after weaning, approximately equal numbers of each breed type were allotted among 8 pens, to become adjusted to using individual Calan-Broadbent electronic headgates (American Calan, Inc., Northwood, NH) over a 28-d period.

During the entire 56-d postweaning period, all steers were fed a 50:50 blend of the 2 experimental grower diets (bromegrass and corn silage). The bromegrass grower diet was 100% chopped bromegrass hay, whereas the corn silage was primarily (87%) corn silage (Ferrell et al., 2006). At the end of the 56-d postweaning period, steers were divided approximately equally between grower diets, with 4 pens assigned to each diet. Steers were individually fed 1 of the 2 grower diets ad libitum for a 119-d growing period (period 1). All steers were then gradually switched over a 2-wk period to the same high-concentrate finishing diet, which was composed primarily of 70% ground corn and 24% corn silage (high corn diet; Ferrell et al., 2006). Steers were fed the high corn diet during the finishing period to a final target weight of 560 kg. Steers were weighed at the beginning and end of the growing period, and at 14-d intervals throughout the growing and finishing periods.

Fecal Slurry Incubations

Fecal slurry incubations to assess diet effects on odorous compound formation were conducted during both the growing and finishing periods. Individual fecal grab samples were of inadequate size for preparation of fecal slurries, so genotype effects on odorous compound production were not determined. Diet effects were determined using pen fecal composites. At least 5 fresh (i.e., noncrusted) intact fecal pats were collected to form a composite fecal sample for each feedlot pen on d 114 of the growing and d 30 of the finishing periods. Thus, time may be a confounding effect when comparing the growing and finishing diets.

Fecal slurries were prepared blending 500 g (as-is) of well-mixed feces from each composite in a Waring blender (Waring Commercial, Torrington, CT) with 500 mL of water. The DM content of the bromegrass, corn silage, and high corn fecal slurries was 9.6, 8.4, and 12.9%, respectively. Roughly 300 mL of the slurry was poured into a 500-mL flask, and the remaining slurry was combined with excess slurry from the other pens on the same diet for substrate addition experiments.

For substrate addition experiments, the slurry composite (1 slurry per diet) was again mixed, and then equally divided (roughly 500 mL each) into 4 blenders, 3 of which received substrate additions. No additions were made to the fourth blender. Casein protein (5 g), starch (10 g), or microcrystalline cellulose (10 g) was added to the 3 remaining blenders, which contained slurry composite. All 4 fecal slurries with or without substrate treatments were blended, and the contents of each blender were equally divided between two 500-mL flasks (2 duplicate flasks per diet \times substrate). The flasks were gassed with N₂, stoppered to limit volatilization losses and ensure anaerobic incubation conditions, and then incubated at room temperature (20 to 23°C). Excess fermentation gas was vented through a needle into a water-filled test tube.

Fecal Slurry Analysis

At periods ranging from daily to weekly, slurry samples were collected from the flasks. Fecal slurry pH was immediately analyzed in the incubation flask using a combination pH electrode (Radiometer Analytical, Westlake, OH). Each flask was swirled to mix the contents, and 3 samples were immediately collected for DM and OM content, substrate content, and fermentation products using an inverted glass pipette (large i.d. to collect fecal particles suspended in the slurry). Details of pH, DM, OM, nonammonia N, starch, and fermentation products analyses have been previously described (Miller and Varel, 2002).

Briefly, potential microbial substrates in the feces, which included nonammonia N, hot-extractable DM (starch and nonstarch), and nonextractable DM, were analyzed in oven-dried, ground samples. Samples for nonammonia N content, analogous to fermentable N (protein and nucleic acids), were made alkaline and dried overnight at 100°C to remove free NH₃ before analysis using a Leco CN-2000 carbon/nitrogen analyzer (Leco, St. Joseph, MI). Hot-extractable DM (starch and nonstarch) and nonextractable DM were measured in a single sample using a gravimetric and enzymatic protocol. Starch was dissolved by autoclaving a portion of the dried sample for 1 h in 0.4 M acetate buffer (pH 4.5), and hydrolyzed during a 4-h digestion with amyloglucosidase. Liberated glucose was measured using a membrane-immobilized enzyme system (YSI Model 27, Yellow Springs Instrument Co., Yellow Springs, OH). Dry matter remaining after the

Table 1. Initial fecal composition by diet

Item	Bromegrass	Corn silage	High corn
DM, % of fecal slurry weight	9.6 ± 0.2 ^a	8.4 ± 0.1 ^b	12.9 ± 0.2 ^c
OM, % of DM weight	87.8 ± 0.1 ^a	88.8 ± 0.1 ^b	92.2 ± 0.3 ^c
pH	7.59 ± 0.03 ^a	6.49 ± 0.08 ^b	6.01 ± 0.08 ^c
L-lactic acid, µmol/g of DM	4.8 ± 2.6 ^{ab}	4.0 ± 0.6 ^b	13.2 ± 2.4 ^c
Total alcohol, µmol/g of DM	2.9 ± 0.9 ^a	2.2 ± 0.8 ^a	9.0 ± 0.5 ^b
Total VFA, µmol/g of DM	139.3 ± 5.5 ^a	381.0 ± 20.6 ^b	524.4 ± 20.3 ^c
Total branched-chain VFA, µmol/g of DM	6.3 ± 0.3 ^a	7.3 ± 0.5 ^a	9.4 ± 1.1 ^a
Total aromatics, µmol/g of DM	1.28 ± 0.10 ^a	1.15 ± 0.26 ^a	1.23 ± 0.12 ^a
Starch, mg/g of DM	1.9 ± 0.3 ^a	91.8 ± 5.1 ^b	180.3 ± 8.1 ^c
CP, ¹ mg/g of DM	94.3 ± 1.1 ^a	122.1 ± 1.4 ^b	150.8 ± 2.5 ^c
Nonstarch hot-extractable of DM, mg/g of DM	67.7 ± 5.8 ^a	138.7 ± 15.1 ^b	203.2 ± 7.8 ^c
Nonextractable DM, mg/g of DM	930.6 ± 5.8 ^a	769.5 ± 14.2 ^b	616.5 ± 12.1 ^c

^{a-c}Within a row, means without a common superscript letter differ, $P < 0.05$.

¹Nonammonia N × 6.25.

low-pH starch hydrolysis incubation (nonextractable DM) was determined by filtering the hydrolysate through F57 filter bags (Ankom Technologies, Macedon, NY) and drying overnight. The nonstarch hot-extractable DM was determined by subtracting the mass of starch measured in the hydrolysate from the mass lost during the low-pH hot extraction.

Fermentation products (L-lactate, ethanol, propanol, isobutanol, butanol, pentanol, hexanol, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, heptanoate, caprylate, phenol, ρ -cresol, 4-ethyl phenol, indole, skatole, benzoate, phenylacetate, and phenylpropionate) were quantified in the liquid phase of the slurries using the YSI analyzer for L-lactate and a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with flame-ionization and mass-selective detectors for all other products. Conditions used for separating, quantifying, and identifying the fermentation products have been previously described (Miller and Varel, 2001).

Statistical Analyses

The GLM procedure of SAS (SAS Inst., Inc., Cary, NC) was used to analyze the initial (d 0) composition of the fecal composites. The model was confined to the no-addition treatments and included the fixed effects of diet (bromegrass and corn silage in the growing period and high corn in the finishing period). A t -test was used to compare the means of each diet, and differences were considered significant at the $P = 0.05$ level.

The MIXED procedure of SAS was used for analysis of the slurry incubation data. The data were analyzed by growth period, with the unit of observation being the slurry flask. For the first diet period (growing), the model was confined to the no-addition treatment and included fixed effects of diet (bromegrass or corn silage), day of incubation, and diet × day of incubation, with day as the repeated measure. During the second diet period (finishing), the model was again constrained to the no-addition treatment and included

day as the repeated measure. The residual was used to test for the significance of day of incubation (growing and finishing periods) and diet × day of incubation (growing period). In the growing period, diet (flask) was used to test for the significance of diet. Differences were considered significant at $P < 0.05$.

The REG procedure of SAS was used to analyze for the effect of substrate treatments (added starch, protein, or cellulose) relative to the no-addition treatment. The data were analyzed by diet and treatment, with day of incubation as the independent variable. Treatment regression coefficients were considered as differing from those of the no-addition treatment if the 95% confidence interval associated with the coefficients did not overlap.

RESULTS AND DISCUSSION

Initial Fecal Composition and Production of Fermentation Products in Fecal Slurries

In the 2 companion studies, diet was found to have a substantial effect on both animal performance and carriage of pathogens and fecal indicator microorganisms (Berry et al., 2006; Ferrell et al., 2006). Diet also profoundly affects manure nutrient composition and can affect greenhouse gas (Harper et al., 1999; Beauchemin and McGinn, 2005) and ammonia emissions (Kellems et al., 1979; Paul et al., 1998) from cattle and cattle production sites. To date, odorous compound production has been investigated primarily in feces from cattle fed finishing rations high in grain (Miller, 2001; Beauchemin and McGinn, 2005; Hao et al., 2005). Feces from transition and grower diets greater in forage or silage content differ in fecal composition from the feces obtained from finishing diets, which likely affects odorous compound production in stored manure. Thus, the primary objective of this study was to examine the variation in initial odorous compound content and subsequent odorous compound production in feces from cattle fed other common diets.

Fresh feces collected from the 3 diets varied considerably in initial composition, microbial substrate content, and concentration of fermentation products (Table 1). The DM and OM content of the fecal slurries differed between the 3 diets, which may have affected the molar concentration of substrates and fermentation products. To minimize the concentration effect, substrate and product concentrations were reported on a fecal DM basis. Differences in fecal pH were largely attributable to VFA and lactic acid content—the more acid, the lower the pH. Of the fermentation products measured (alcohols, VFA, and aromatic compounds), VFA was the principal constituent. Branched-chain VFA and aromatic ring-containing fermentation products, which are attributed to protein fermentation (Barker, 1981; Mackie et al., 1998), were only minor constituents of the fresh feces and did not differ between the different diets.

The content of potential fecal microbial substrates also differed between the diets, although to varying degrees (Table 1). Starch content showed the greatest range (nearly a hundred-fold), whereas CP exhibited a smaller (less than a 2-fold) range of values. All 4 potential microbial substrates were highly correlated to one another ($|r| > 0.999$; $P < 0.025$) with starch, CP, and nonstarch hot-extractable DM positively correlated to one another but negatively correlated with nonextractable DM. Correlations between all potential fecal microbial substrates and both ground corn and soybean meal levels in the diet were also strong ($|r| > 0.907$), but the statistical confidence was considerably less ($P > 0.14$). Nonextracted DM was the largest pool of DM. It was highly organic ($94.2 \pm 0.1\%$ OM) and most likely consisted of cellulose and lignin, which are insoluble in hot, dilute acid. It should be noted that nonammonia N is not a separate DM fraction; rather it would be partitioned between the nonstarch hot-extractable DM and nonextractable DM fractions.

Slurry incubations of feces from the 3 diets showed substantial differences in pH change and lactic acid accumulation. The pH declined in all fecal slurries, but to differing degrees depending upon the diet fed (Figure 1A). Feces from corn diets (corn silage or high corn) acidified to a greater extent compared with the bromegrass diet. Corn silage fecal pH differed from the high corn diet feces initially and at the end of the incubation when corn silage fecal pH increased slightly. Lactic acid accumulated and contributed to the large pH declines observed in the corn diets, but it did not accumulate in the bromegrass feces (Figure 1B). The decline in lactic acid at the end of the corn silage incubation corresponded with the observed pH increase.

Odorous fermentation products also accumulated differently in fecal slurries depending on the diet fed the steers (Figure 2). Alcohols and VFA were the primary fermentation products that accumulated during incubation (Figure 2A, 2B). Alcohol and VFA concentrations rapidly accumulated in both the corn silage

and high corn diets compared with the bromegrass diet, which showed only a very small accumulation of alcohol and a more gradual accumulation of VFA. A second phase of VFA accumulation occurred in the corn silage fecal slurries, which was attributed to the conversion of lactic acid to butyrate. Of the 3 major VFA constituents (acetate, propionate, and butyrate comprise 98% of total VFA) in the corn silage fecal slurry, only propionate and butyrate exhibited a substantial rate of increase ($P < 0.01$) during the last 3 wk of incubation, but butyrate accumulation far outpaced propionate accumulation (22.2 and 1.1 $\mu\text{mol/g}$ of DM per d, respectively). Conversion of lactic acid into butyrate has been observed in human and animal bacterial isolates (Counotte et al., 1981; Duncan et al., 2004) and in cattle fecal slurries after a few days of incubation (Miller and Varel, 2001).

Branched-chain VFA and aromatic ring-containing fermentation products are characteristic of protein fermentation (Mackie et al., 1998). The content of these compounds in fecal slurries was relatively unchanged during incubation with the exception of the bromegrass manure (Figure 2C, 2D). Branched-chain VFA accumulation was measured in both bromegrass and corn silage manures (0.35 and 0.02 $\mu\text{mol/g}$ of DM per d) during the incubation period ($P < 0.03$). With regard to aromatic compounds, the bromegrass and the high corn manures accumulated ($P < 0.02$) these compounds at 0.02 and 0.01 $\mu\text{mol/g}$ of DM per d, respectively. Changes in the relative content of branched-chain VFA to total VFA during the manure incubations also indicate that protein fermentation was more important in the bromegrass manure incubations and that carbohydrate fermentation was more important in the corn silage and high corn manure incubations. The initial molar percentages of branched-chain VFA in the bromegrass, corn silage, and high corn manures were 4.5, 1.9, and 1.8% of total VFA, respectively. Over the course of incubation, the percentage in bromegrass fecal slurries increased ($P < 0.001$) at a rate of 0.08% per d, whereas it tended ($P \leq 0.07$) to decrease by 0.02 and 0.01% per d in the corn silage and high corn manures, respectively. Analysis of the branched-chain VFA content in total VFA produced during microbial fermentations of protein yielded 7 to 17% branched-chain VFA (Macfarlane et al., 1992). In the bromegrass fecal slurry, the final content of branched-chain VFA was $7.1 \pm 2.0\%$, indicating extensive use of protein during fermentation. The other 2 diets had a final branched-chain content of less than 1.2% of total VFA.

Odorous compound production from high-concentrate grain diets is largely the result of starch fermentation in the manure (Miller and Varel, 2001, 2002). Although aging the manure or the incorporation of soil tends to enhance protein's role as a fermentation substrate (Miller and Varel, 2001, 2002; Miller and Berry, 2005), the contribution of protein toward total VFA content is minimal compared with that of starch. Four potential microbial substrates were examined in

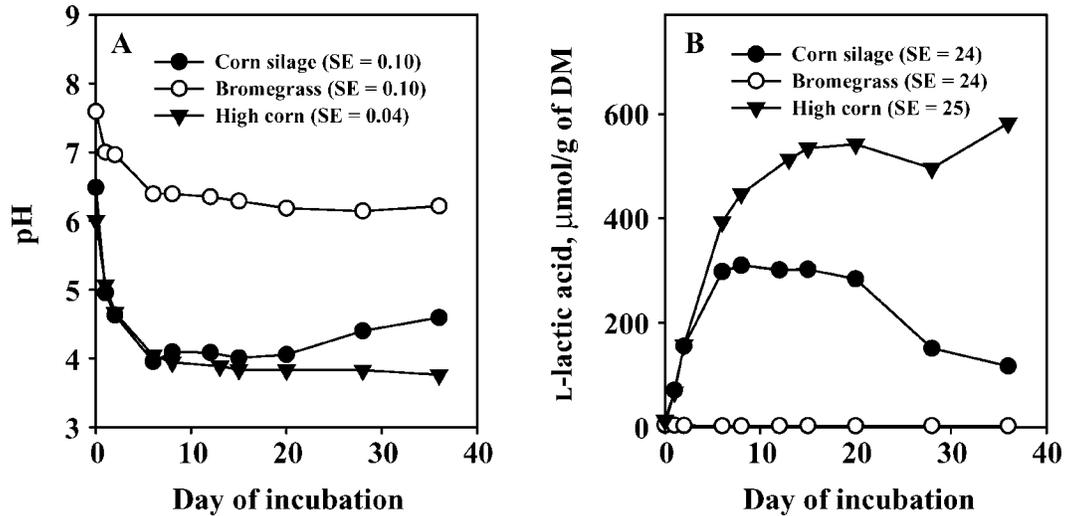


Figure 1. Fecal slurry pH (A) and concentration of L-lactic acid (B) during the fecal slurry incubation. The SE of the LS means ($n = 8$ for bromegrass and corn silage; $n = 16$ for high corn) are presented in the legend key.

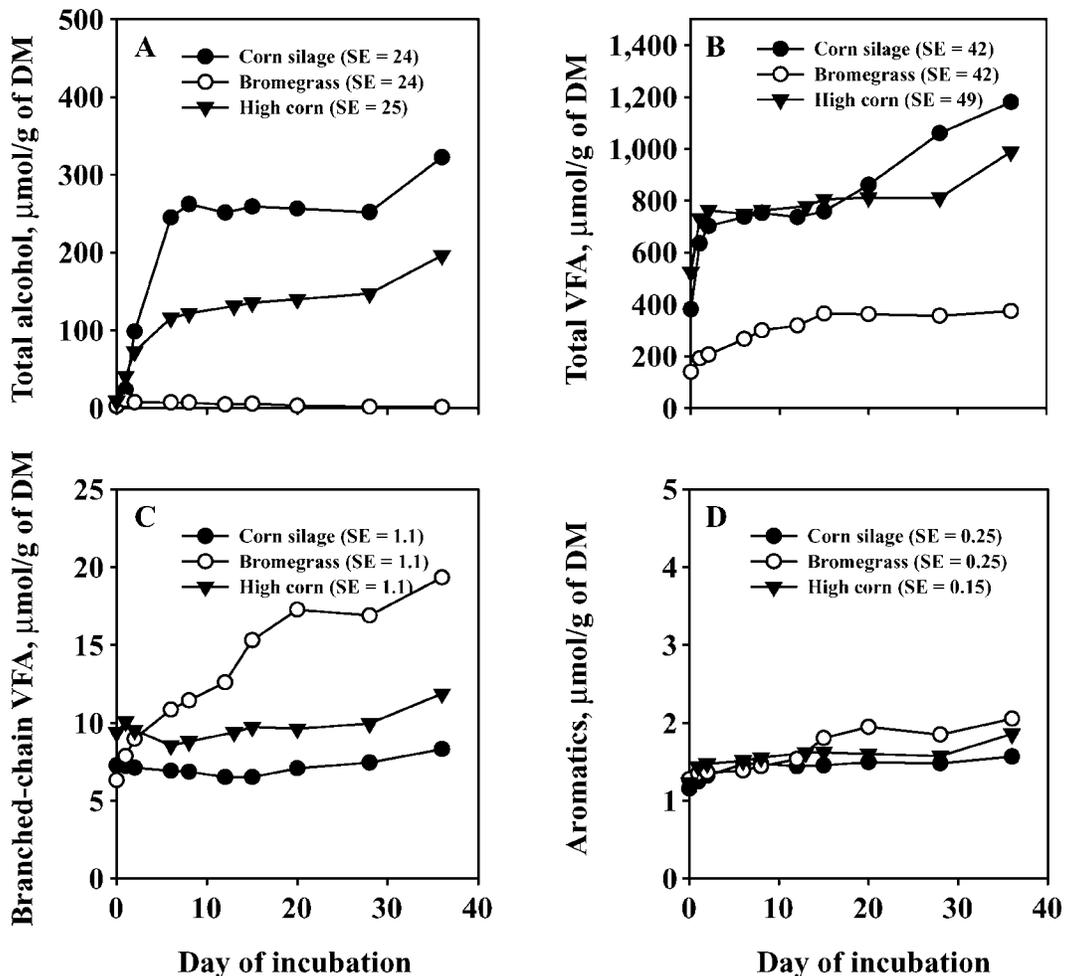


Figure 2. Concentrations of alcohol (A), VFA (B), branched-chain VFA (C), and aromatic compounds (D) in the fecal slurries during the incubation. The SE of the LS means ($n = 8$ for bromegrass and corn silage; $n = 16$ for high corn) are presented in the legend key.

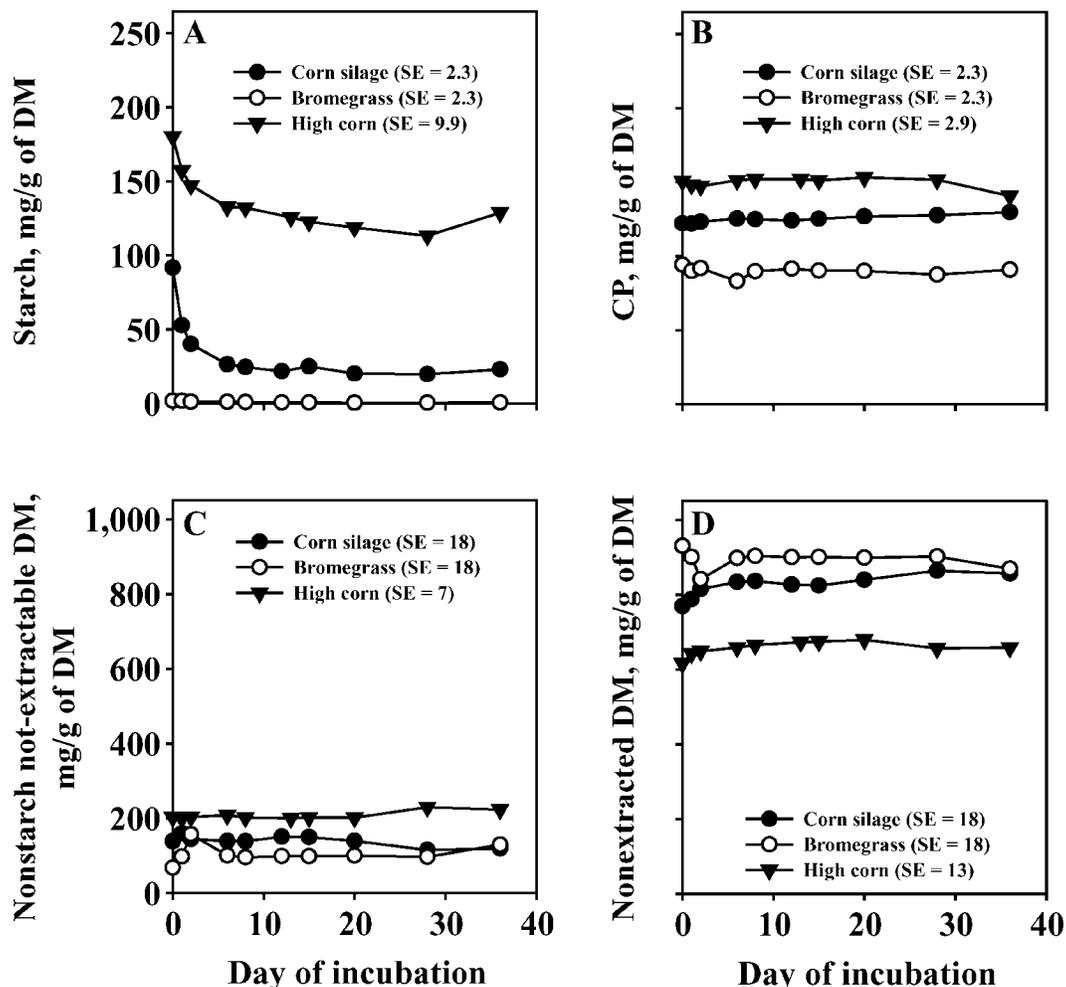


Figure 3. Concentration of potential microbial substrates starch (A), CP (B), nonstarch hot-extractable DM (C), and nonextracted DM (D) in the fecal slurries during the incubation. The SE of the LS means ($n = 8$ for bromegrass and corn silage; $n = 16$ for high corn) are presented in the legend key.

the current study (Figure 3). Starch fermentation (Figure 3A) was clearly the dominant substrate in the corn silage and high corn manures compared with CP (Figure 3B), which was unchanged throughout the course of incubation. Nonextracted DM content actually increased ($P < 0.05$) in the corn silage and high corn manures, reflecting a general enrichment of other DM pools as the starch pool became depleted. One difference between corn silage and high corn was the small rate of decrease ($P = 0.008$) for nonstarch hot-extracted DM in the corn silage fecal slurries and the small rate of increase ($P = 0.001$) for the same DM pool in the high corn fecal slurries. The small rate of decrease (1.4 ± 0.5 mg/g of DM per d) may indicate that hemicellulose, which would be extracted during a low-pH, hot water extraction, is fueling odorous compound production. Regardless, the large starch losses measured in this study far outweigh other potential substrate losses and support earlier findings that starch in the manures from animals fed high-concentrate grain diets fuels odorous compound production (Miller and Varel, 2001, 2002; Miller and Berry, 2005).

Direct evidence for microbial substrates usage in the bromegrass was more difficult to obtain. Although starch levels were quite low, a small rate of consumption (0.03 mg/g of DM per d) was measured ($P < 0.001$). Initial rates of starch consumption were much larger, because all starch consumption (1.2 mg/g of DM) was complete by d 15. For CP, the accumulation and enrichment of branched-chain VFA and the accumulation of aromatic compounds implies that protein fermentation by bacteria in the manure must be occurring. However, measurable rates of CP loss over the entire incubation period were not observed ($P = 0.66$). The relatively imprecise N assay and the very low production of fermentation products may account for our inability to attribute observed products of protein fermentation to a loss of protein in these samples.

Application of fiber-analysis principles substantially improved our assessment of nonstarch carbohydrates (hemicellulose and cellulose) and lignin as potential manure microbial substrates for odorous compound production. Previously published reports from our laboratory used a modified phenol-sulfuric acid method

(Daniels et al., 1994) to measure total carbohydrate in manure samples, but measurements in replicate samples could be highly variable in samples containing a mixture of soil and manure (Miller and Berry, 2005). Application of fiber-analysis principles has provided insights into feedlot manure composition and manure mineralization in soil (Ward et al., 1978; Van Kessel et al., 2000), but to our knowledge, fiber analysis has not been used to determine which potential microbial substrates are linked to odorous compound production. Future application to other manures and soils should provide a clearer picture of OM transformations and odorous compound production.

Effect of Substrate Additions to Fecal Slurries on Fermentation Product Accumulation

Substrate addition to the bromegrass fecal slurries affected a number of microbial processes depending on the substrate added. Starch addition to bromegrass fecal slurries affected pH, total alcohol accumulation, and total VFA accumulation, particularly after the first week of incubation, compared with the no-addition slurries (Figure 4). Increasing alcohol and VFA concentrations coincided with decreasing added starch concentrations. The increase in total VFA likely accounted for a portion of the observed pH decrease. Starch addition did not alter the low rate of L-lactate or branched-chain VFA accumulation relative to the no-addition slurry. However, a slower rate of accumulation of aromatic compounds was observed ($P < 0.05$) in starch slurries compared with the no-addition slurries (6.6 ± 1.5 and 22.2 ± 5.4 nmol/g of DM per d, respectively). Starch additions also affected the concentrations of other potential microbial substrates by increasing the rate of CP and nonextractable DM accumulation in the starch-amended bromegrass slurries (0.36 ± 0.06 and 2.03 ± 0.34 mg/g of DM per d, respectively) relative to the no-addition bromegrass slurry (-0.03 ± 0.08 and -0.58 ± 0.62 mg/g of DM per d, respectively). Starch additions seemed to enhance the existing fermentations leading to additional accumulation of fermentation products (total alcohols and VFA) and microbial biomass (increased CP and nonextractable DM).

Protein addition to the bromegrass fecal slurries led to greater accumulations of L-lactic acid, total alcohol, total VFA, branched-chain VFA, and total aromatic compounds compared with the no-addition treatment (Figure 4). A rapid decline in CP content mirrored the rise in fermentation product concentrations, confirming that CP was one of the principal substrates for odorous compound formation. Protein addition to the bromegrass fecal slurries did not enhance the rate of microbial biomass accumulation (-0.11 ± 0.26 mg of nonextractable DM/g of DM per d) compared with the no-addition treatments, as was observed in the starch slurries. Rather, existing fermentation reactions were enhanced.

Cellulose addition to the bromegrass fecal slurries enhanced the rate of CP accumulation in a manner similar to starch additions but without production of additional fermentation products. Crude protein content increased in the cellulose treatments but did not change in the no-addition treatments (0.28 ± 0.04 and -0.03 ± 0.08 mg/g of DM per d, respectively). A likely explanation for the CP increase is that microbial biomass increased. The rate of aromatic compound accumulation, however, was lower in the cellulose treatments (-4.3 ± 3.0 and 22.2 ± 5.4 nmol/g of DM per d, respectively) compared with the no-addition bromegrass slurries. Branched-chain VFA formation was also lower ($P = 0.006$) in the cellulose treatment compared with the no-addition slurries (138 ± 61 and 346 ± 40 nmol/g of DM per d, respectively). This result indicates that protein fermentation decreased and implies that an alternative (carbohydrate or lipid) fermentation was enhanced when cellulose was added. No decreases were observed in starch, nonstarch hot-extractable DM, or nonextractable DM (CP content increased) during the incubation [i.e., rates of consumption did not differ ($P > 0.44$) from zero], which led us to conclude that either an unidentified substrate accounts for the very low VFA accumulation observed, or our analysis methods for microbial substrate loss in the starch, nonstarch hot-extractable DM, and nonextractable DM fractions were still not sensitive enough to measure differences in the low activity bromegrass fecal slurries supplemented with cellulose.

Substrate added to the corn silage fecal slurries had a less pronounced effect on microbial fermentations in those fecal slurries compared with the bromegrass fecal slurries (Figure 5). In the corn silage slurries, substrate addition affected the accumulation of total alcohol, branched-chain VFA, and aromatic compounds. Lower peak accumulations of total alcohol were observed in the protein and cellulose additions relative to the no-addition control. A positive rate of branched-chain VFA accumulation was also observed in the all corn silage slurries except in the corn silage slurries spiked with starch ($P = 0.30$). The rate of aromatic compound accumulation in the corn silage slurries was quite low, but measurable in all corn silage slurries except the no-addition control ($P = 0.28$). Measurable rates of nonextractable DM accumulation (0.92 to 2.55 mg/g of DM per d) were observed in all treatments indicating an increase in microbial biomass during the incubation ($P < 0.001$). Positive rates of CP accumulation were calculated for all treatments, but the rate was only statistically significant ($P < 0.006$) in the no-addition and cellulose treatments (0.19 ± 0.05 and 0.20 ± 0.06 mg/g of DM per d). Starch consumption rates were detected ($P < 0.003$) in all treatments (0.55 to 1.15 mg/g of DM per d). Similarly, nonstarch hot-extractable DM consumption rates (0.60 to 1.40 mg/g of DM per d) were indicated ($P < 0.03$) in all treatments except in the corn silage fecal slurries amended with starch, which tended ($P = 0.089$) to consume nonstarch

Bromegrass fecal slurry

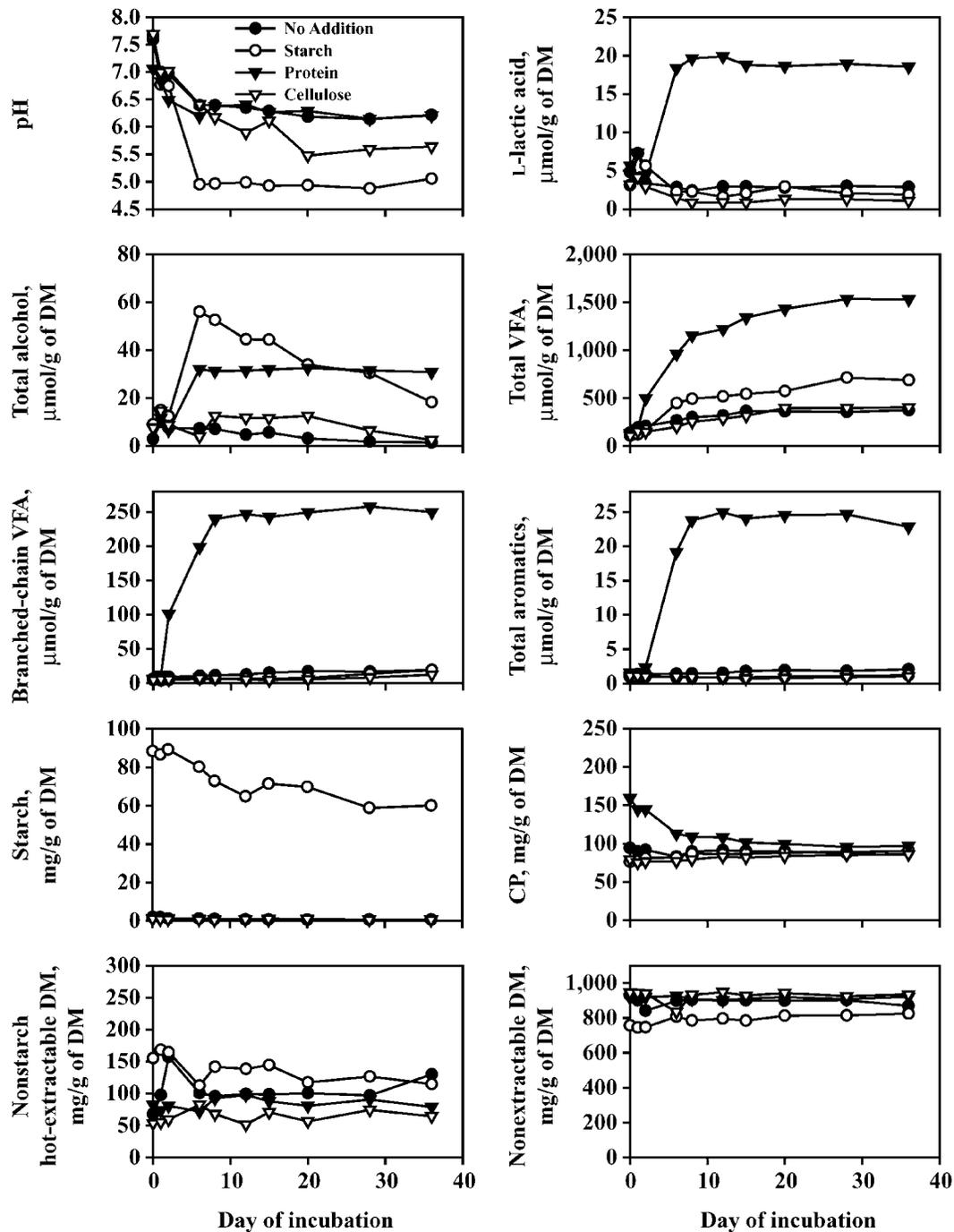


Figure 4. Fecal slurry pH, fermentation product concentration, and potential microbial substrate concentration in bromegrass fecal slurries during incubation with added starch, protein, or cellulose treatments compared with no-addition controls.

hot-extractable DM at a calculated rate of 0.37 mg/g of DM per d. Although the evidence for starch and nonstarch hot-extractable DM as the preferred substrate source was quite strong in all treatments, the accumulation of branched-chain VFA and aromatic compounds, particularly after d 20 in the cellulose treatment, indicates that some endogenous protein fermentation occurred.

In the high corn fecal slurries, substrate additions had very little effect on the accumulation of fermentation products compared with the bromegrass and corn silage slurries (Figure 6). Branched-chain VFA accumulation was detected ($P < 0.001$) in the high corn fecal slurries supplemented with protein or cellulose (58.2 ± 14.4 and 55.2 ± 13.4 nmol/g of DM per d, respectively). Similarly, aromatic compound accumulation

Corn silage fecal slurry

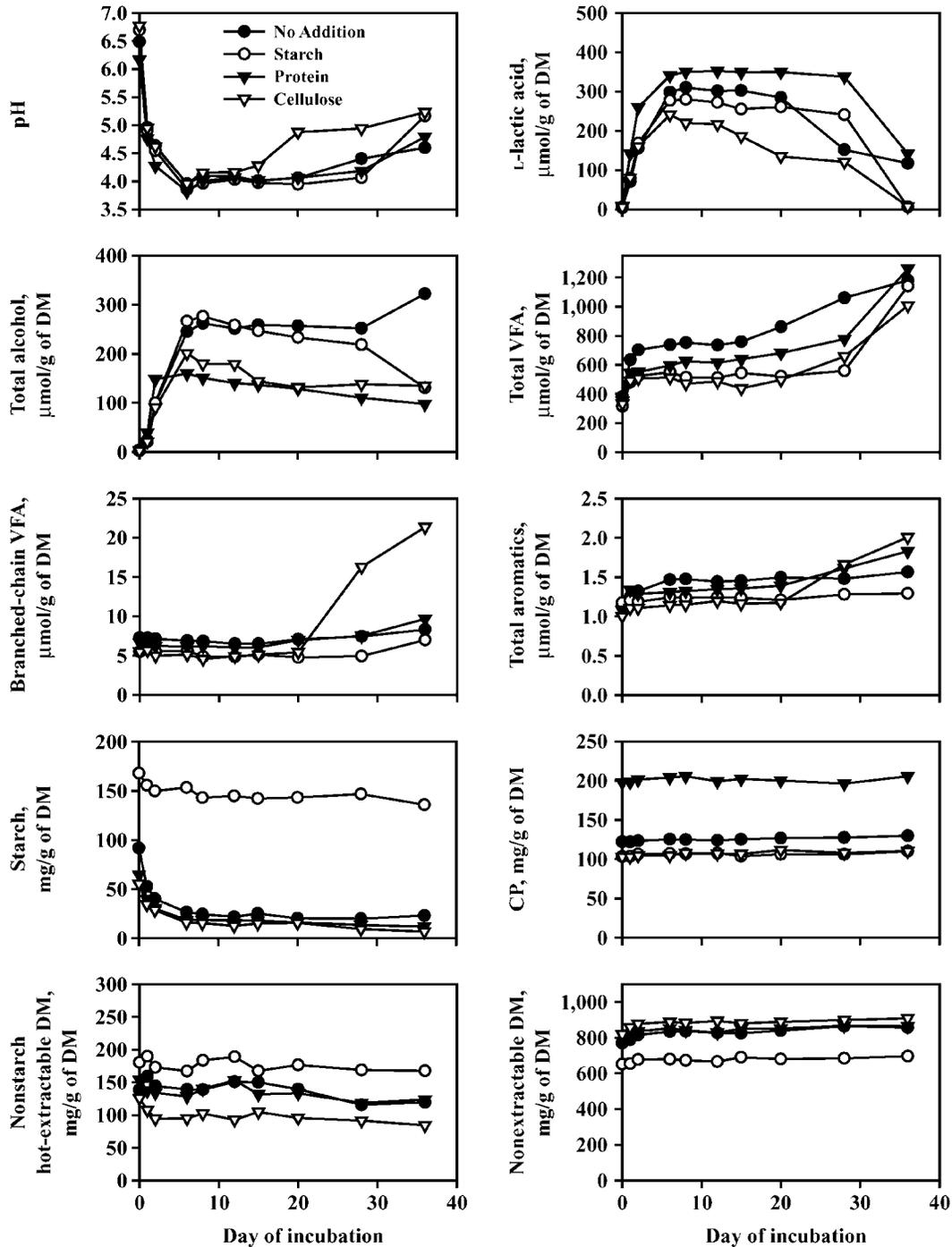


Figure 5. Fecal slurry pH, fermentation product concentration, and potential microbial substrate concentration in corn silage fecal slurries during incubation with added starch, protein, or cellulose treatments compared with no-addition controls.

rates were detected ($P \leq 0.03$) in the no-addition, starch, and protein treatments (11.0 ± 4.1 , 29.1 ± 12.4 , and 30.3 ± 12.9 nmol/g of DM per d, respectively). No differences in the formation rates of branched-chain VFA or aromatic compounds were detected between treatments. Determining starch, nonstarch hot-extractable DM, and nonextractable DM substrate con-

tents were unfortunately confounded by instrument error. When starch and fiber analyses were conducted on high corn fecal samples receiving casein protein or cellulose, the analytical balance did not function properly, compromising all subsequent mass measurements. Therefore, those results are not reported. Crude protein analyses were conducted using a differ-

ent subsample; thus, a complete data set was collected for CP content. Regression analysis of CP content indicated that a very low rate of protein fermentation occurred during the incubation, which has been observed before in fermentations of high-corn feces (Miller and Varel, 2002). Based on 1) the large accumulation of L-lactic acid, total alcohol, and total VFA, 2) the very minor accumulation of branched-chain VFA or aromatic compounds, and 3) the results of earlier studies of feces from cattle fed a high-corn finishing diet (Miller and Varel, 2002), we believe that starch was the primary substrate for odorous compound formation in the high corn feces.

Substrate additions to the bromegrass, corn silage, and high corn fecal slurries supported our interpretation of the results from the no-addition incubations that starch, protein, and possibly nonstarch hot-extractable DM could fuel fermentations that accumulated malodorous end products. The starch content of feed grains has also been implicated in feedlot odors (Watts and Tucker, 1993), and starch has been identified as a primary substrate for malodorous VFA in cattle feces from finishing diets high in ground corn content (Miller and Varel, 2002; Miller and Berry, 2005). Starch utilization was most evident in corn silage and high corn fecal slurries and in the bromegrass fecal slurries receiving starch additions.

Changes in CP concentration were interpreted in 2 ways; increasing CP concentration indicated an increase in microbial biomass, whereas decreasing CP concentrations indicated that CP might be serving as a substrate for fermentation. The problem is that both of these processes could be happening at the same time. Fortunately, the accumulation of fermentation products specific to protein fermentation (branched-chain VFA and aromatic compounds) could be used as an additional indicator of protein fermentation. Using these 2 indicators (decreasing CP content and increasing protein-specific fermentation products), all manures showed the capacity for protein fermentation. The capacity seemed to be most well developed in the bromegrass fecal slurries, followed by corn silage feces, and then high corn feces.

Results from the nonstarch hot-extractable DM fraction need to be carefully interpreted, because the value is based on the difference of 2 mass measurements corrected for starch content. The method used has many similarities to the ADF analysis originally developed for forage analysis (Van Soest, 1987) but lacks detergent to aid in protein solubilization. Filtration efficiency also differs from the standard method because filter bags are utilized, which would not retain very fine particles that would normally be retained by (and potentially clog) filter crucibles. Furthermore, an increased content of nonstarch hot-extractable DM relative to no-addition treatments was observed in the 3 feces when starch was added. A likely explanation would be that the extra nonstarch hot-extractable DM in the starch treatments is polymeric glucose with in-

completely digested α 1–4 linkages or undigested α 1–6 linkages. Thus, nonstarch hot-extractable DM in these fecal incubations would include hemicellulose, the hot-soluble portion of the CP, and the nondigested starch. Rates of nonstarch hot-extractable DM consumption in corn silage feces (no-addition, protein, and starch treatments) and in the bromegrass starch treatment indicate that potential substrate sources for odorous compound production exist in this fraction. Carbohydrate analysis of the nonstarch hot-extractable DM filtrate from corn silage fecal slurries would provide some insight into the nature of potential odor substrates in the nonstarch hot-extractable DM fraction.

Particulate material remaining after hot-extraction (nonextractable DM) would include predominantly cellulose with a little lignin, ash, and some insoluble protein. Evidence for nonstarch polysaccharide (like cellulose) fermentation associated with odorous compound production has been observed in aged feedlot feces that contained some soil (Miller and Varel, 2002), but large variation between samples was common. In that study, substrate additions similar to the ones used in this study yielded no change in nonstarch polysaccharide. In the fecal incubations reported in this study, nonextractable DM content was either unchanged during the course of the incubation or increased in the fecal slurries. The increase likely reflected an increase in microbial biomass during fermentation. No decreases in nonextractable DM were detected ($P = 0.349$ to 0.964); thus, we conclude that nonextractable DM is not a substrate for odorous compound formation in fresh feces. Adoption of the fiber bag analysis protocol helped to reduce variation in nonextractable DM measurements and enabled a more accurate determination of changes in nonextractable DM substrate content. Cellulose additions confirmed that nonextractable DM had very little influence on odorous compound production in the feces tested in this study and the incubation conditions employed (high fecal content and anaerobic).

How do these differing metabolisms (protein vs. starch) affect perceived odor? Direct measurement of odor parameters (concentration, offensiveness, intensity) using a human panel would be the best standard to compare feces and their chemistries, but unfortunately we did not have direct access to a panel, and serious issues have been raised about the methods used to collect and hold air samples for the long periods needed to transport the gases to an olfactory panel (Koziel et al., 2004). A wide variety of odorous compounds have been detected in the atmospheres of animal production sites (O'Neill and Phillips, 1992; Hartung and Phillips, 1994), and considerable effort has been made to attribute odor to specific airborne chemicals. Volatile fatty acids, aromatic compounds, ammonia, and sulfur compounds have all been implicated, but recent consensus has emerged around volatile organic compounds including VFA and aromatic compounds (Zhu et al., 1999; Zahn et al., 2001). Comparing

High corn fecal slurry

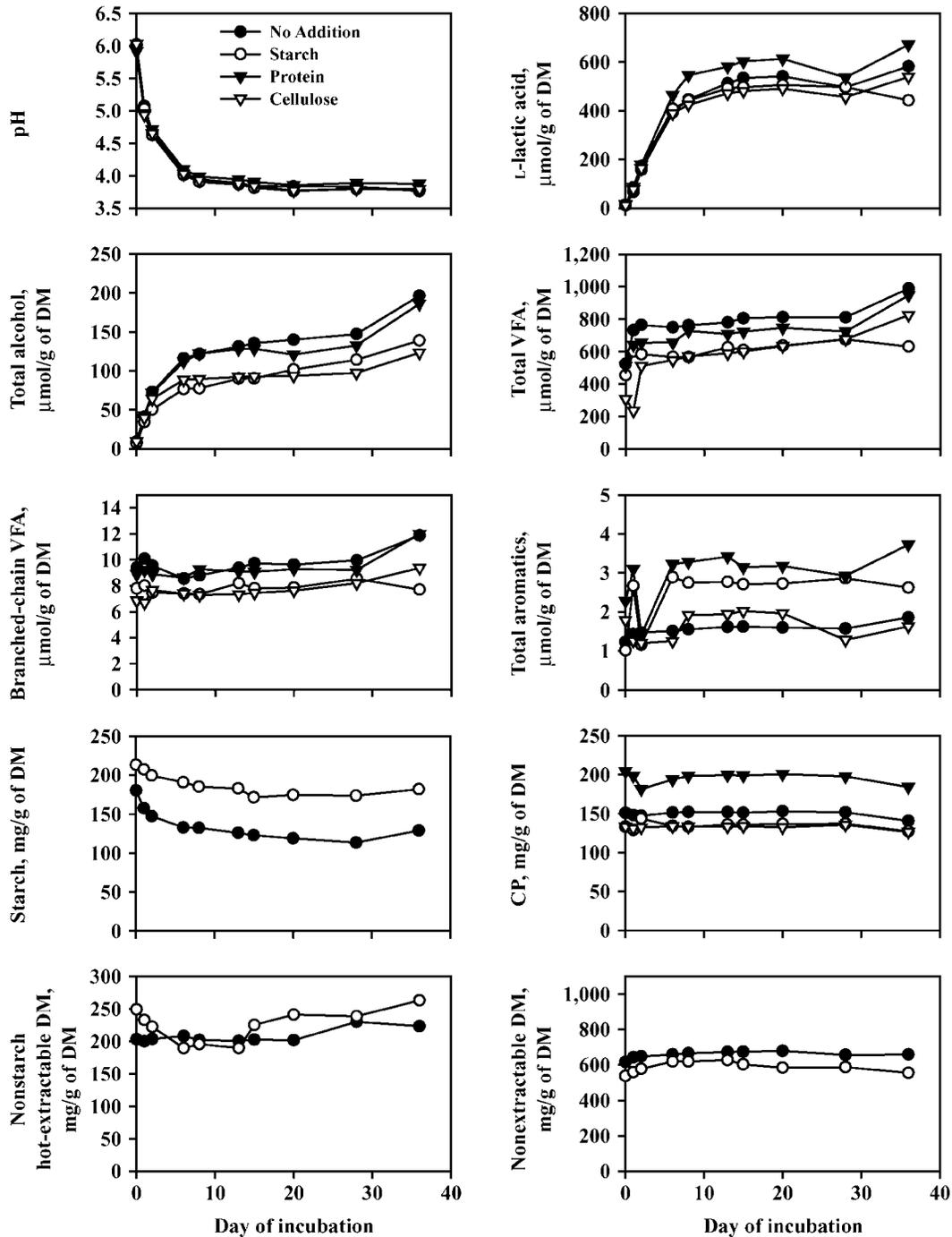


Figure 6. Fecal slurry pH, fermentation product concentration, and potential microbial substrate concentration in high corn fecal slurries during incubation with added starch, protein, or cellulose treatments compared with no-addition controls.

feces on the initial content and accumulation of VFA and aromatic ring compounds would be one option for crudely estimating odor potential. Using this method, bromegrass feces would likely be the least objectionable, because of its low initial VFA content and accumulated VFA content. Another factor that might contribute to the lower odor associated with bromegrass

feces is its high initial pH and greater pH during fecal fermentation, which is partly attributable to the lack of L-lactate accumulation. Greater pH would help to further reduce odorous compound volatilization by reducing the amount of VFA in the nonionic, highly volatile form that occurs at low pH. However, the potential to produce branched-chain VFA and aromatic com-

pounds may enhance odor perception. Those compounds are produced during protein fermentation, have low odor thresholds, and are particularly offensive (Zahn et al., 2001). It seems that this is the conundrum; VFA production is limited by low starch content, which unfortunately encourages protein fermentation and the subsequent production of objectionable branched-chain VFA and aromatic compounds. Determining the point at which any odor mitigation gained by a bromegrass diet (or similar low-starch diet) is eliminated by the production of malodorous protein fermentation products needs to be made using human olfactometry. Further complicating the benefit of a bromegrass diet that limits odorous compound production is the effect of a bromegrass diet on animal performance during the growing period (Ferrell et al., 2006) and the increased emission of methane, a greenhouse gas (Harper et al., 1999).

Although the mechanisms for odorous compound production in fresh manure are becoming clearer, the results of our study have also led to some unanswered questions. In the bromegrass slurries, we expected that pH would also have declined in protein treatments due to the accumulation of acidic fermentation products (Figure 4), but the pH was unchanged relative to the other treatments. One possible explanation is that there was exclusive production of D-lactic acid in the starch and cellulose treatments of the bromegrass feces. Some lactic acid bacteria produce only D-lactic acid (Garvie, 1980; Fukushima et al., 2004), and it would be surprising if only D-lactic acid-producing microorganisms were present in the bromegrass feces. Unfortunately, D-lactic acid is not measured using our method, and thus, this hypothesis will need to be tested in future experiments.

The experimental conditions utilized in this study differ slightly from previously reported fecal or manure slurry experiments both in fecal content and the inclusion of urine in the slurry. Urine has been added to fecal slurries in other studies (at arbitrary concentrations) to better mimic feedlot conditions (Varel and Miller, 2000; Miller and Varel, 2002). We elected to omit urine from our slurries for the following reasons: 1) urine mixing with fresh feces on the feedlot surface would be a rare event; rather urine would more likely affect varying mixtures of aged feces and soil, which cover nearly all of the feedlot surface; and 2) urine composition is dependent on diet and unfortunately urine from bromegrass and corn silage fed cattle was unavailable. In general terms, one could speculate that urine would slightly buffer acidification processes and may provide readily available N for microbial biomass production. The extent that urine may or may not contribute to, or alter, the fecal fermentation process needs to be defined.

Although important insights can be made into odorous compound accumulation from microbial substrates in fresh feces using laboratory slurry incubations, slurry conditions would not encompass the range of

field conditions observed in beef cattle feedlots. Fecal incubations, which were conducted in anaerobic slurries for 5 wk at a constant room temperature, do not reflect the conditions experienced in outdoor feedlot pens common in the United States, where an environment of varying moisture and fecal inputs, substantial temperature swings, and fluctuating aerobic and anaerobic conditions would prevail. Laboratory studies varying only moisture and fecal content have demonstrable effects on multiple odor, nutrient, greenhouse gas, dust, and pathogen issues (Berry and Miller, 2005; Miller and Berry, 2005). Extrapolating the results of these fecal slurry studies to predict emissions from cattle feedlot environments may be misleading. Thus, the insights of these studies should be limited to processes in very fresh feces or to specific areas in feedlot pens at specific times that receive large fecal inputs and are highly saturated (i.e., in areas of the pen immediately after spring thaw where feces and moisture accumulated, or in poorly drained areas near the feed bunk or water source).

IMPLICATIONS

Diet strongly affects feces composition, which in turn affects production of odorous fermentation products. Excreted starch is a primary substrate for odorous compound and lactic acid production, particularly in feces from cattle fed finishing and growing diets high in corn content. In feedlot environments, the combination of high volatile fatty acid and lactic acid would act to reduce pH and enhance volatile fatty acid emission. However, in feces where starch is less available, as was observed in feces from steers fed a bromegrass diet, protein in the feces is increasingly important as a substrate for odorous compound production. As protein is fermented, highly malodorous branched-chain volatile fatty acid and aromatic compounds accumulate at proportionately greater rates than when starch is the primary substrate. Diet manipulation to control cattle feedlot odors is a valid approach, but future application will require human olfactometry to confirm laboratory results.

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