Proceedings, Western Section, American Society of Animal Science

Vol. 62, 2011

WHOLE CORN AND WET DISTILLERS GRAINS SUBSTITUTION IN STEAM-FLAKED CORN DIET ALTERS RUMEN FERMENTATION AND BACTERIAL DYNAMICS

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ABSTRACT: A study evaluated effects of whole shelled corn (WSC) in steam-flaked corn (SFC) finishing diets containing differing amounts of wet distillers grains with solubles (WDGS) on rumen fermentation and shifts in ruminal bacterial populations. A total of 642 heifers (initial BW = 412 ± 18 kg) were blocked by BW and randomly assigned to 36 pens in a 108 d experiment. Treatments were arranged as a 2 × 3 factorial with two amounts of WSC (0 and 20% DM) and three amounts of WDGS (0, 15, and 30% DM) replacing SFC. Ruminal fluid samples, collected from 2 heifers/pen on d 98,were analyzed for VFA, ammonia, pH, and shifts in ruminal bacterial populations using denaturing gradient gel electrophoresis (DGGE) targeting the 16S rDNA gene. Richness and Shannon-Wiener indices evaluated bacterial presence and diversity. Similarity matrix of DGGE banding patterns were constructed using Dice (binary) coefficient. Microbial indices and ruminal samples were evaluated for effects of WSC, WDGS, and interactions using Mixed Procedure of SAS with block as the random variable and pen as experimental unit. Addition of 0 to 15% WDGS to 0% WSC increased total VFA; however, total VFA decreased from 0 to 30% WDGS with 20% WSC (WSC x WDGS interaction, P = 0.02). Ammonia was not affected ($P \geq$ 0.19). Diets with 0% WSC had 3.9% lower pH (P < 0.01), 4.4% less acetate (P = 0.05), 7.4% greater propionate (P = 0.02), and lower acetate:propionate (P< = 0.02). Inclusion of WDGS decreased acetate (quadratic, P < 0.01), increased propionate (quadratic, P = 0.03), and decreased acetate:propionate (quadratic, P = 0.03). Bacterial diversity based on DGGE showed Richness and Shannon-Wiener indices did not differ with inclusion of WSC (P = 0.63), but increased with WDGS (linear, P = 0.01). All samples were 69.02% similar according to Dice. Microbial population shifts and fermentation characteristics imply use of WSC in SFC based diets may negatively alter rumen use of WDGS.

Key words: cattle, wet distillers grains, rumen bacteria

Introduction

Ethanol in recent years has become a popular biofuel. Distillers grains, a coproduct resulting from the ethanol industry, are used extensively in finishing diets of feedlot cattle. Distillers grains can be fed wet (**WDGS**) or dry (**DDGS**) and usually are included to supply energy and (or) protein in the diet. Most feedlot diets use corn grain as the predominant source of energy. Processing methods of the corn varies, but a survey of feedlot nutritionists showed that steam-flaking is the predominant corn processing method in over 11 states (Vasconcelos and Galyean, 2007). Currently there is little data available showing the value of WDGS in steam-flaked corn (**SFC**) diets. When WDGS replaces high moisture corn (**HMC**) or dry rolled corn (**DRC**), ADG and G:F increase (Klopfenstein et al., 2008). Tracey et al. (2010) showed that use of WDGS in SFC-based diets did not negatively alter rumen fermentation or performance characteristics of feedlot steers.

Though steam-flaking is common, processing of corn grain is an added expense to the feedlot. If whole shelled corn (**WSC**) can replace a portion of the SFC in finishing diets containing WDGS without negatively altering animal performance, it might be possible to reduce grain processing costs. This study evaluated the effect of replacing 20% SFC with WSC while adding up to 30% WDGS to the diet on the ruminal bacterial population and rumen fermentation characteristics. A companion-study by McDaniel et al. (2011) evaluated impacts of these treatments on performance and carcass characteristics. We hypothesize that use of WSC in finishing diets containing WDGS would lead to differences in microbial population and rumen fermentation characteristics due to decreased starch availability over SFC-based diets. Our objective was to evaluate the effects of WSC and WDGS on microbial ecology using denaturing gradient gel electrophoresis (**DGGE**) and to compare these results with ruminal fermentation characteristics.

Materials and Methods

Animals and Experimental Design. All animal procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. A total of 642 heifers (412 ± 18 kg average BW) were blocked by BW and randomly assigned to one of 36 pens (n = 17 to 18 heifers). Each pen was randomly assigned to one of six treatments. The feeding trial was 108 d in duration. On d 98, two animals from each pen (n = 72) were randomly chosen for sampling of ruminal fluid for analysis of rumen characteristics and microbial dynamics.

Dietary treatments and Sampling Dietary treatments were a 2 × 3 factorial arrangement with two amounts of WSC (0 and 20% DM) and 3 amounts of WDGS (0, 15, and

30% DM) replacing SFC. Animals were fed once per day. Diet ingredients and nutrient composition is in Table 1. All heifers were allowed to adapt to a finishing diet prior to the onset of the 108 d experiment. Ruminal fluid samples were collected once on d 98. Samples were collected via oral lavage with suction strainer (Lodge-Ivey et al., 2009) approximately 4 h post feeding. Samples were placed on ice immediately following collection and were later maintained at -20°C until analysis for ruminal pH, ammonia and VFA concentrations, and extraction of community DNA could be performed.

Laboratory analysis. Ruminal ammonia was analyzed using the phenol–hypochlorite procedure of Broderick and Kang (1980), adapted to a microtiter plate (BioTek Instruments, Winooski, VT). Volatile fatty acid concentration was determined by gas chromatography (Goestch and Galyean, 1983; Varian 3400; Varian Inc., Walnut Creek, CA). Community rumen DNA was extracted from ruminal fluid using the repeated bead beating and column method described by Yu and Morrison (2004b). The quality of the DNA was assessed by electrophoresis on 1.0% agarose gel. Extracted DNA was used as template for subsequent PCR-DGGE.

Each PCR reaction was performed in 25 µL and amplified on DNA Engine PTC-200 (MJ Research, Watertown MA) for each PCR-DGGE analysis. The PCR mixture contained 1.25 U Platinum taq (Invitrogen, Carlsbad CA) 60 mM Tris-SO4 (pH 8.9), 18 mM ammonium sulfate,1.75 mM MgCl2, 250 µM of each deoxynucleoside triphosphate, 0.0674%BSA, 0.5 µM each primer and approximately 100 ng of template DNA. The V3 reigion of the *rrs* gene was amplified using primers 357f (5'-CCTACGGGAGGCAGCAG-3') and 519r (5'-ATTACCGCGGCKGCTGG-3'). The 357f primer has a 40-bp GC clamp attached to the 5' end (CGC CCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGG GGG) to prevent dissociation of the DNA strands (Yu and Morrison, 2004a). To reduce the production of spurious PCR products, touchdown PCR was performed. The PCR cycle consisted of an initial denaturing at 94ºC for 4 minutes, 10 cycles of touchdown PCR wherein the starting annealing temperature of 61ºC was decreased 0.5ºC per cycle for ten cycles to 56ºC. This was followed by 25 cycles with denaturing step at 94ºC for 30 s, annealing at 56ºC 30 s, and a final primer extension at 72º for 30 s. Quality of the PCR products was confirmed visually using a 1.5% agarose gel stained with ethidium bromide.

Using the Bio-Rad D-Code system (BioRad, Hercules, CA), DGGE was performed as described by Simpson et al., 1999. To separate PCR fragments, 30 µL of PCR product was resolved on 7.5 % polyacrylamide gel (37.5:1) containing a 30 to 60% gradient denaturants (100% denaturants consisting of 40% [vol/vol] formamide and 7 M urea). Electrophoresis was performed at 60°C and 82 V for 16 hrs. Additionally, a standard sample was included in each gel to allow for normalization of band migration and gel curvature among different gels (McCracken et al., 2001). After electrophoresis, gels were stained with GelStar (Cambrex, Rockland, ME) according to manufacturer's specifications and the images were captured using Kodak Imaging Systems (New Haven, CT).

Statistical analysis of DGGE banding patterns. Images of DGGE gel patterns were imported as TIFF files into Bionumerics software (version 5.2; Applied Maths, Applied Maths, Inc., Austin, TX). Lanes of each DGGE gel were converted to densiometric curves and an automatic band search was performed on normalized patterns. Gels were normalized using five bands from a 1 kb DNA ladder and a standard sample to ensure the location of bands was consistent across all gels. After visual control of the assigned bands, a band-based analysis was performed using the algorithm for Dice coefficient. To calculate Dice coefficient all bands are divided into classes of common bands and for each band pattern, a particular band class can have two states: present or absent (binary matrix). A 5% band position tolerance was used and is an arithmetic determination of the degree to which banding patterns are alike, (i.e. contain the same bands in similar locations). For cluster analysis similarities were displayed graphically as a dendrogram. The clustering algorithms used to calculate the dendrograms was an unweighted pair group method with average linkages (UPGMA). Clusters (groups) were determined by sequentially comparing patterns and the construction of a related dendrogram reflected relative similarities.

Diversity indices. Richness (*S)* was determined from the number of bands in each lane using the band matching table generated during the Dice analysis of the DGGE profiles. The Shannon-Wiener index (*H'*) of diversity was used to determine the proportional abundances of bacterial taxa present in the rumen samples of cattle fed increasing levels of WDGS. Shannon-Wiener index was calculated for binary (band presence or absence) using the following equation: $H' = -\Sigma P_i \ln P_i$, where P_i is the importance probability of the bands in a lane, calculated from n_i/N where n_i is the peak height of a band and N is the sum of all peak heights in the densitometric curve.

Statistical analysis. Richness, Shannon-Wiener index, ruminal ammonia, VFA concentrations, and pH were analyzed as a randomized complete block design using the MIXED procedure of SAS version 9.2 (SAS Inst. Inc., Cary, NC). Pen was used as the experimental unit, and block was the random variable. Model included the effect of WSC, WDGS, and WSC × WDGS interactions. Satterthwaite was used as the variance structure. Mean comparisons with *P*-values less than or equal to 0.05 were declared significant, and values less than or equal to 0.10 were considered tendencies. Means were calculated using LSMEANS and separated using PDIFF. Polyorthagonal contrasts were used to evaluate linear and quadratic effects of WDGS concentrations.

Results and Discussion

Distillers grain use has increased over the last decade due to increased ethanol production and the rising price of cereal grains. The use of distillers grains has been evaluated

in different feedlot diet systems including DRC, HMC, and SFC. Steam flaking is a corn processing method designed to increase availability of nutrients; particularly starch. However, the high cost of this method inhibits ability for some to use SFC as the main feed source in their operation. In 2006 the cost of steam-flaking for a 5,000 head feedlot was $9.57/t (Metric ton, DM basis; Macken et al., 2006). Though the NE_g value of SFC is 18% higher than that of whole corn (Zinn et al., 2002) the cost may become inhibitory to today's producer. Because of this, the possibility of including WSC in a SFC diet containing WDGS is an important avenue to explore.

The only interaction between WSC and WDGS was seen in total VFA concentration (WSC x WDGS interaction, $P = 0.02$). When no WSC was in the diet total VFA production increased from 0 to 15% WDGS, but decreased with 30% (99.08, 129.41, and 104.13 ± 5.79 mM for 0, 15, and 30%, respectively). However, when 20% WSC was in the diet, total VFA production decreased linearly as WDGS amounts increased (94.19, 91.84, and 90.95 ± 5.79 m*M* for 0, 15, and 30% WDGS, respectively). A study evaluating the use of DDGS in either DRC or SFC diets showed that SFC diets led to increased total VFA production with an increase in the production of the glucogenic precursor, propionate (May et al., 2009). This increase in propionate, likely contributes to the 18% increase in feeding value of the more highly processed grain source found by Zinn et al. (2002).

Effects of inclusion of WSC and WDGS are summarized in Table 2. Ruminal ammonia concentrations were not affected by WSC ($P = 0.19$) or WDGS inclusion ($P = 0.40$). This was by experimental design as the diets were balanced for RDP. Ruminal pH increased 3.9% from 6.4 to 6.6 with inclusion of WSC ($P = 0.01$). Ruminal pH has been shown to be affected by grain processing level with an increased processing causing a decreased pH (de Vargas et al., 2011). Acetate concentrations were higher ($P = 0.05$) and butyrate concentrations tended ($P = 0.08$) to increase in animals consuming 20% WSC compared to those not receiving WSC. Propionate concentrations decreased 7.4% as the inclusion of WSC increased (40.5 vs 37.5 ± 0.73 for 0 and 20% WSC, respectively; $P = 0.01$). Because of the decrease in acetate and increase in propionate in animals consuming 20% WSC, acetate:propionate was lower in the 0% WSC group ($P = 0.02$). Similar to these results, Sharp et al. (1982) showed acetate decreases, propionate increases, and acetate:propionate decreases as a result of increased corn processing. Similarly, when comparing SFC- and DRC-based diets the inclusion of DDGS had effects on ruminal VFA profiles similar to those in the current study with increased acetate, decreased propionate, and increased acetate:propionate in DRC diets over SFC diets (May et al., 2009). Ruminal pH and VFA profiles are interconnected. Ruminal pH levels may alter fermentation endproducts as individual bacterial species are metabolically active at differing environmental pH. In a continuous flow in vitro fermentation study an induced increased pH led to increased acetate and decreased propionate and butyrate levels (Shriver et al., 1986). This may be due to a difference in active bacterial species due to their optimal pH range.

Ruminal pH tended (P = 0.07) to decrease linearly with the increase in dietary WDGS; however, as the values ranged from 6.63 to 6.01 it is likely that this is of no biological significance to the animals. Callaway et al. (2010) showed a decrease in ruminal pH with increased DDGs. Ruminal pH in that study ranged from 6.58 to 7.18 which are higher than one might expect in a finishing diet. These pH values, similar to those in our study, may be a result of distillers coproducts having 80% more NDF than corn grain (distillers grains + solubles vs flaked corn grain; NRC, 2000) Al-Suwaiegh et al., (2002) showed no difference in ruminal pH in dairy cattle consuming 30% WDGS vs a control diet. A study evaluating the feeding of corn DDGS at levels of 0 to 1.2% BW showed no change in ruminal pH values (Leupp et al., 2009b). However, an additional study (Leupp et al. 2009a) which evaluated inclusion of 0 to 60% DDGS in a DRC-based diet noted a linear increase in pH from 6.42 to 6.63.

Amounts of WDGS did not affect butyrate concentrations (P =0.37). Acetate concentrations decreased quadratically with increasing inclusion of WDGS (49.5, 45.5, and 47.3 ± 0.91 for 0 15, and 30% WDGS, respectively; $P = 0.01$). Propionate concentrations increased quadratically ($P = 0.03$), with the 30% WDGS diet having the highest concentration at 40.6 compared to 37.0 and 39.3 ± 0.90for 0 and 15% WDGS, respectively. Accordingly, acetate:propionate decreased quadratically, with the highest ratio at 15% WDGS (1.15 vs 1.36 and 1.23 ± 0.05for 0, 15, and 30% WDGS, respectively). Several studies with lactating dairy cows have reported no significant difference in VFAs resulting from the inclusion of distillers grains (Al-Suwaiegh et al., 2002; Anderson et al., 2006). A previous study of ours also showed no difference in VFA production when up to 60% of a SFC-based finishing diet was WDGS (Tracey et al., 2010). The difference in sampling time and duration may have some role in the differences we see between the two studies. In the previous study repeated samples were taken as the animals were being adapted to the WDGS diets, while in the current studies a single sample was taken toward the end of the study period allowing the animals to be fully adapted to the diets and for optimal fermentation to be seen. Leupp et al. (2009a) showed a linear decrease in acetate, linear increase in propionate, a linear decrease in acetate:propionate, and no difference in butyrate concentration with 0 to 60% DDGS.

Microbial diversity indices analyzed included Richness and Shannon-Wiener. Richness was not affected by amount of WSC (P =0.20), but increased linearly with increased WDGS (23.2, 23.3. and 27.1 ± 1.11 for 0, 15, and 30% WDGS respectively; P 0.01). Similarly, Shannon-Wiener increased linearly as WDGS increased (2.40, 2.42, and 2.81 ± 0.11 for 0, 15, and 30% WDGS, respectively; $P = 0.01$), but did not differ with WSC amounts ($P = 0.20$). When dendrograms were constructed using banding pattern based on band presence or absence all samples were 69.02% similar, implying that differences in microbial diversity are occurring due to inclusion of WSC and WDGS. Interestingly, animals consuming no WSC and no WDGS had the least presence/absence similarity amongst themselves. It is understood that changes in diets alters the

ruminal bacterial population. Inclusion of up to 15% condensed distillers coproduct in a DRC-based diet increased fibrolytic and lactilytic bacteria (Fron et al., 1996). While in the current study the bacterial diversity changed with the inclusion of WDGS, in our previous study there was no change in the overall diversity of bacterial population, although there were differences in the number of individuals in each species present (Tracey et al., 2010). A pyrosequencing study showed a change in the proportions of bacterial species present in the rumen and lower gut in animals consuming different levels of DDGS (up to 50% of diet) in a concentrate diet (Callaway et al., 2010).

Though there were few significant interactions between the amount of WSC and the amount of WDGS, the decrease in favorable fermentation characteristics when WSC is included in SFC-based diets with WDGS implies that ruminal fermentation may be negatively altered, which may ultimately impact performance and carcass characteristics.

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Table 1. Ingredient and chemical composition (DM basis) of experimental diets containing whole shelled corn and wet distillers grains with solubles fed to heifers.

Item	0% Whole Shelled Corn			20% Whole Shelled Corn		
	0% WDGS[1]	15% WDGS	30% WDGS	0% WDGS	15% WDGS	30% WDGS
Ingredient, %						
Steam-flaked corn	78.5	67.6	56.8	58.5	47.7	36.8
Whole shelled corn	-	-	-	20.0	20.0	20.0
WDGS	-	15.0	30.0	-	15.0	30.0
Wheat silage	9.0	9.0	9.0	9.0	9.0	9.0
Soybean meal	7.0	4.0	1.0	7.0	4.0	1.0
Supplement[3]	2.5	2.5	2.5	2.5	2.5	2.5
Tallow	2.0	1.0	-	2.0	1.0	-
Urea	1.0	0.9	0.8	1.0	0.9	0.8
Nutrient, %						
CP	14.5	16.3	18.3	14.2	16.3	18.1
NDF	13.3	16.5	18.9	13.9	16.5	19.6
ADF	7.2	9.7	10.7	7.5	8.8	10.5
EE	4.4	5.1	5.8	4.8	5.2	5.7

[1]WDGS = wet distillers grains with solubles (produced from corn grain, blended with no more than 10% sorghum grain)
[2]Supplement composition (DM): limestone = 75.54%; KCL = 8.01%; salt = 7.71%; MgO = 2.89%; $ZnSO_4$ = 0.86%; selenium premix (0.06% Se) = 0.49%; $MnSO_4$ = 0.42%; $CuSO_4$ = 0.31%; EDDI (4.4%) = 0.04%; vitamin E (500 IU/g, 90% DM) = 0.03%; vitamin A (30,000 IU/g, 90% DM) = 0.03%; $CoSO_4$ = 0.004%; Rumensin (Elanco Animal Health, Indianapolis, IN; 176.4 mg/kg, 90% DM) = 0.72%; Tylan (Elanco Animal Health; 88.2 mg/kg, 90% DM) = 0.48%; mGA 200 (Pfizer Animal Health, New York, NY; 441 mg/kg melengestrolacetate) = 0.39%; and mineral oil = 2.11%
[3]Nutrient composition analyzed by Servi-Tech Laboratories, Amarillo, TX

Table 2. Effect of whole shelled corn (WSC) and wet distillers grains with solubles (WDGS) on ruminal ammonia, pH, VFA production, Richness, and Shannon-Wiener indices in cattle on steam flaked corn diet.

	Treatments							*P* – values			Contrasts[2]	
	WSC			WDGS[1]								
Item	0	20	SE	0	15	30	SE	WSC	WDGS	WSC × WDGS	Lin.	Quad.
Ammonia, m*M*	0.79	1.20	0.32	0.93	0.80	1.39	0.37	0.19	0.40	0.58	0.31	0.37
pH	6.35	6.62	0.07	6.63	6.41	6.43	0.08	0.01	0.09	0.25	0.07	0.19
VFA, mol/100 mol												
Acetate	46.4	48.5	0.74	49.5	45.5	47.3	0.91	0.05	0.01	0.17	0.10	0.01
Propionate	40.5	37.5	0.73	37.0	40.6	39.3	0.90	0.01	0.02	0.14	0.08	0.03
Butyrate	7.92	8.93	0.34	8.04	9.97	8.27	0.48	0.08	0.37	0.63	0.74	0.18
A:P[3]	1.17	1.31	0.04	1.36	1.15	1.23	0.05	0.02	0.03	0.15	0.11	0.03
Richness	23.9	25.3	0.97	23.2	23.3	27.1	1.11	0.20	0.01	0.33	0.01	0.11
Shannon-Wiener	2.47	2.62	0.10	2.41	2.42	2.81	0.11	0.20	0.01	0.33	0.01	0.11

[1]WDGS = wet distillers grains with solubles (corn-based, not exceeding 10% sorghum)
[2]*P*-value for the linear and quadratic effect of increasing WDGS inclusion level
[3]A:P = acetate:propionate

Proceedings

Western Section
American Society of Animal Science

Volume 62

Miles City Montana

June 21–23, 2011