Ruminal fermentation, kinetics, and total-tract digestibility of lactating dairy cows fed distillers dried grains with solubles in low- and high-forage diets

Sanjeeewa D. Ranathunga,^{1*} Kenneth F. Kalscheur,^{1,2†‡} and Kevin J. Herrick^{1§}

¹Dairy and Food Science Department, South Dakota State University, Brookings 57007

²US Dairy Forage Research Center, USDA-Agricultural Research Service, Madison, WI 53706

ABSTRACT

The objective of this study was to investigate the effects of concentrations of forages and corn distillers dried grains with solubles (DDGS) on ruminal fermentation, ruminal kinetics, and total-tract digestibility of nutrients in lactating dairy cows. Four lactating Holstein cows with ruminal cannulas were assigned to a 4 × 4 Latin square in a 2 × 2 factorial arrangement of treatments. Diets were formulated to contain low forage [LF; 17% forage neutral detergent fiber (NDF)] or high forage (HF; 24.5% forage NDF) and DDGS at 0 or 18% (0DG or 18DG) of diet dry matter (DM). Intake of DM was not affected by the diets. Daily mean ruminal pH was affected by forage NDF × DDGS interactions, as the lowest ruminal pH was observed among cows fed LF18DG (6.02). Apparent total-tract digestibility for DM, organic matter, crude protein, NDF, acid detergent fiber, and starch was not affected by diets. Cows fed LF diets had a greater total volatile fatty acid concentration compared with cows fed HF (122 vs. 116 mM). Molar proportions of acetate were greater for HF compared with that of LF diets (62.6 vs. 57.5 mmol/100 mmol) and greater for 0DG diet compared with that of 18DG diets (61.3 vs. 58.7 mmol/100 mmol) diets. The molar proportion of propionate was affected by forage × DDGS interaction as the greatest propionate molar proportion was observed with cows fed LF18DG diet (27.7 mmol/100 mmol). Also, molar proportion of butyrate was affected by forage × DDGS interaction, as the greatest butyrate molar proportion was observed in cows fed HF18DG diet (13.5 mmol/100 mmol). Average fractional dilution rate for all diets was

11.9%/h and was not affected by diets. Fractional passage rate of the solid phase was greater for HF than for LF (4.40 vs. 3.76%/h). The ruminal retention time of solid phase was greater for LF compared with HF diets (27.3 vs. 23.3 h). Fractional passage rate of DDGS was affected by forage × DG interaction, as the highest fractional passage rate of DDGS was observed with cows fed HF18DG diet (7.72%/h). Our results demonstrated that concentrations of forage, DDGS, and their interaction influence ruminal degradation and kinetics of diets fed. Diets formulated at 17% forage NDF at 17% (DM basis) can decrease milk fat concentration compared with diets formulated at 25% forage NDF. Additionally, feeding DDGS at 18% DM basis to lactating dairy cows did not affect milk fat concentration or yield.

Key words: distillers dried grains with solubles, forage, rumen kinetics, metabolism

INTRODUCTION

Distillers dried grains with solubles (DDGS), a co-product of the ethanol industry, has been fed as a protein source (Owen and Larson, 1991; Powers et al., 1995; Kleinschmit et al., 2006) in diets of lactating dairy cows. It has also been evaluated as a non-forage fiber source in the diets of lactating dairy cows (Ranathunga et al., 2010, 2018). Several authors have investigated the optimal use of DDGS in lactating dairy cow diets. Schingoethe et al. (2009) recommended that DDGS could be incorporated at 20% DM basis in lactating dairy cow diets. More recent research from Testroet et al. (2018) showed no differences in performance when feeding a reduced-fat DDGS at 19.5% of diet dry matter. However, there are instances where feeding high-fat DDGS from 20 to 30% on DM basis to lactating dairy cows caused decreased milk fat concentration (Abdelqader et al., 2009; Hippen et al., 2010). Although multifactorial, dietary factors such as inadequate effective fiber and a greater concentration of unsaturated fatty acids could lead to milk fat depression (MFD; Lock, 2010).

A few studies have previously investigated ruminal fermentation characteristics and total-tract digestibil-

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*Current address: Kemin Europa, Animal Nutrition and Health-EMENA, Toekomstlaan 42, Herentals 2200, Belgium.

†Corresponding author: kenneth.kalscheur@ars.usda.gov

‡Current address: US Dairy Forage Research Center, USDA-ARS, 1925 Linden Drive, Madison, WI 53706.

§Current address: POET Nutrition, 4506 N. Lewis Ave., Sioux Falls, SD 57110.

ity of lactating dairy cows fed DDGS (Kelzer et al., 2009; Zhang et al., 2010; Benchaar et al., 2013). However, no published data on ruminal degradation and passage rates of DDGS exists. Therefore, we designed an experiment to investigate the metabolism of DDGS when fed at 18% of the diet (on a DM basis) with low and high forage concentrations. We hypothesized that degradation of DDGS in the rumen would be different and would not cause decreased milk fat concentration when 18% DDGS (on a DM basis) was included in diets formulated with high forage concentration compared with diets with low forage concentration. The primary objective of this study was to investigate the effects of including DDGS with low- or high-forage diets on ruminal fermentation, ruminal kinetics, and total-tract digestibility in lactating dairy cows.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

The study was conducted at the Dairy Research and Training Facility at South Dakota State University, Brookings. All procedures involving animals were approved by the South Dakota Institutional Animal Care and Use Committee. Four multiparous cannulated Holstein cows (initial DIM: 179 ± 56 ; initial BW: 722 ± 36 kg) were assigned randomly to a 4×4 Latin square design balanced for carryover effects in a 2×2 factorial arrangement of treatments with 4-wk periods. The first 2 wk were used for diet adaptation and the last 2 wk for sampling and data collection. Diets were formulated to contain low forage [**LF**; 17% forage neutral detergent fiber (NDF)] or high forage [**HF**; 24.5% forage NDF] and DDGS at 0 or 18% (**0DG** or **18DG**) of diet DM (Table 1). The DDGS (Glacial Lakes Energy LLC, Wattertown, SD) was purchased as a single shipment, to ensure similar composition throughout the experiment. The inclusion of DDGS in 18DG diets resulted in the partial removal of ground corn and total removal of expeller soybean meal and extruded soybeans compared with 0DG diets. A source of rumen inert fat (Energy Booster 100, Milk Specialties Global, Eden Prairie, MN) was added to all diets to increase dietary ether extract (**EE**) concentration. The reference values available in the NRC (2001) were used as the estimates of RDP and RUP, and intestinal values for the feed ingredients. All diets were formulated (NRC, 2001) to be similar in CP (18%), RDP (10.1%), and RUP (7.9%) concentrations on a DM basis.

Forages were premixed in a vertical mixer and blended with concentrates in a Calan Data Ranger (American Calan, Northwood, NH) to mix diets as TMR. Cows

were housed in a free-stall barn and fed once daily (0800 h) using Calan Broadbent individual animal feeders (1 cow assigned per feeder). Cows were trained for 2 weeks to use the feeding system before initiation of the experiment. Amounts of feed offered were adjusted to allow for ad libitum intake and 5 to 10% refusal. Dry matter intake was measured individually using data on daily feed offered and refused.

Markers for Ruminal Flow and Apparent Total-tract Digestibility

Ytterbium-Labeled Corn Silage. Solid passage rates were determined when the experimental diets were fed using Yb. Ytterbium-labeled corn silage was prepared according to the procedure of Varga and Prigge (1982), with some modifications. Initially, 10 L of YbCl_3 solution was prepared, to contain 40 g of Yb/L by adding 460 g of 99% Yb_2O_3 [ytterbium(III) oxide, Sigma-Aldrich, St. Louis, MO] and 640 mL of concentrated HCl (Sigma-Aldrich) into approximately 400 mL of distilled water and then further diluting to 10 L using distilled water. The 10 L of YbCl_3 solution was then diluted to 200 L using tap water, to contain 2g of Yb/L. Approximately 10 kg of corn silage on wet matter basis (50 g of forage sample per 1 L of YbCl_3 solution) was soaked in 200 L of diluted YbCl_3 solution for 48 h, with stirring every 6 h. The corn silage mixture was then strained through 2 layers of cheesecloth, washed 6 times with tap water over a 6-h period to remove any unbound marker, and allowed to air dry for 24 h. Labeled corn silage samples, with average Yb concentration of 3.3 mg/g of DM, were divided into equal doses of approximately 750 g of corn silage on wet matter basis (17.4% DM) with 0.43 g of bound Yb, and stored at -20°C .

Cobalt EDTA. The passage rate of rumen fluid was determined using cobalt EDTA, prepared according to Udén et al. (1980) using cobalt acetate [Cobalt(II) acetate, Sigma-Aldrich]. For each cow, 10 g of cobalt EDTA was dissolved in 1 L of tap water.

Erbium-Labeled DDGS. Fractional passage rates of DDGS were determined using erbium-labeled DDGS, prepared according to Ellis and Beever (1984). The DDGS was soaked in a solution containing 80 mg of erbium acetate/g of DDGS [erbium(III) acetate hydrate, Sigma-Aldrich], DM basis, in 7.5 mL of distilled water for 24 h. Then the solution was soaked in 10 mL of 0.1 M acetic acid/g of DDGS (Sigma-Aldrich), DM basis, for 6 h, followed by rinsing with distilled water. Labeled DDGS samples were dried at 55°C in a forced-air oven (style V-23, Despatch Oven Co., Minneapolis, MN) for 48 h and divided into equal doses of 90 g on an as-fed

Table 1. Ingredients and nutrient composition of experimental diets¹

Item	Low forage		High forage	
	0DG	18DG	0DG	18DG
Ingredient (% of DM)				
Corn silage	32.8	32.8	48.0	48.0
Alfalfa hay	8.20	8.20	12.0	12.0
Dried ground corn	27.8	21.5	13.6	7.3
DDGS	0	18.0	0	18.0
Soybean meal (44% CP)	10.1	11.6	9.80	11.7
Expeller soybean meal ²	8.21	0	8.70	0
Extruded soybeans	5.00	0	5.00	0
Soybean hulls	5.00	5.00	0	0
Rumen inert fat ³	0.55	0.55	0.75	0.75
Limestone	1.20	1.31	1.05	1.20
Salt, white	0.55	0.55	0.55	0.55
Dicalcium phosphate	0.15	0.05	0.10	0.05
Dairy-micro premix ⁴	0.35	0.35	0.35	0.35
Vitamin E ⁵	0.05	0.10	0.05	0.10
Nutrient (% of DM unless noted)				
DM (% of diet)	55.6	55.5	47.6	47.7
OM	93.3	93.4	93.0	93.1
CP	17.9	18.2	18.0	18.3
NDF	25.0	28.3	29.0	32.3
Forage NDF	16.8	16.8	24.5	24.5
ADF	12.8	13.7	15.1	15.9
NFC ⁶	46.7	42.1	42.0	37.2
Starch	28.1	24.9	23.2	18.8
Ether extract	3.62	4.80	4.11	5.35
Ash	6.73	6.56	6.96	6.95
Ca	0.85	0.84	0.77	0.73
P	0.34	0.37	0.33	0.38
Mg	0.24	0.25	0.25	0.26
K	1.26	1.17	1.37	1.32
S	0.17	0.19	0.18	0.19
NE _L (Mcal/kg) ⁷	1.61	1.61	1.61	1.62
Physical characteristics				
WHC ⁸	3.66	3.75	3.91	4.14
BD ⁹	0.35	0.34	0.32	0.30
X _{gm} (mm) ¹⁰	1.58	1.52	2.04	1.87
S _{gm} (mm) ¹¹	1.56	1.51	2.16	2.00
peNDF ¹²	13.0	13.7	18.5	18.7

¹Diets provided varying concentrations of forage and distillers dried grains with solubles (DDGS): low forage (LF; 41% of diet DM) or high forage (HF; 60% of diet DM) and DDGS at 0 or 18% of diet DM. 0DG = 0% DDGS; 18DG = 18% DDGS.

²SoyPlus (West Central Soy, Ralston, IA).

³Energy Booster 100 (Milk Specialties Global, Eden Prairie, MN).

⁴Contained 10% Mg; 2.6% Zn; 1.7 mg/kg of Mn; 4,640 mg/kg of Fe; 4,712 mg/kg of Cu; 396 mg/kg of I; 119 mg/kg of Co; 140 mg/kg of Se; 2,640,000 IU/kg of vitamin A; 528,000 IU/kg of vitamin D₃; and 10,560 IU/kg of vitamin E (Land O'Lakes, St. Paul, MN).

⁵Vitamin E = 44,000 IU/kg.

⁶NFC = 100 - (% NDF + % CP + % ether extract + % ash; NRC, 2001).

⁷Calculated according to NRC (2001) using analyzed feed values.

⁸WHC = water-holding capacity (kg of water/kg of feed); WHC of corn silage and alfalfa were 5.04 and 6.40 kg of water/kg of feed, respectively.

⁹BD = bulk density (g/cm³); BD of corn silage and alfalfa were 0.23 and 0.30 g/cm³, respectively.

¹⁰X_{gm} = geometric mean length, as calculated by ASAE (2001); X_{gm} of corn silage and alfalfa fed in the study were 4.21 and 1.82 mm, respectively.

¹¹S_{gm} = standard deviation of the geometric mean, as calculated by ASAE (2001); S_{gm} of corn silage and alfalfa fed in the study were 3.79 and 1.88 mm, respectively.

¹²peNDF = pef × NDF content of the sample; pef = physical effectiveness factor (the proportion of particles retained on 1.18-mm screen); peNDF of corn silage and alfalfa fed in the study were 37.1 and 25.4, respectively.

basis, with 0.5 g of bound Er (average Er concentration of 5.6 mg/g of DM).

Labeled corn silage (750 g on wet matter basis), cobalt EDTA (10 g of cobalt EDTA dissolved in 1 L of tap water), and labeled DDGS (90 g on an as-fed basis) were introduced as a single dose via rumen cannula and mixed thoroughly with ruminal contents just before feeding at 0900 h on d 26 of each period.

Titanium Dioxide. Total-tract digestibility of DM, NDF, ADF, CP, starch, and EE was estimated using TiO_2 . Gelatin capsules containing TiO_2 were placed approximately 30 cm into the dorsal area of the rumen via cannula of each cow at 0900 and 2100, at a rate of 20 g/d, from d 19 to 28 of each period.

Sample Collection

Samples of corn silage, alfalfa, concentrate mixes, and TMR were collected for 3 consecutive days at the beginning of wk 3 and 4 of each period and stored at -20°C until analyzed.

Ruminal contents were collected on d 26 of each period from 3 locations (the dorsal, ventral, and caudal areas in the rumen) at 0, 1, 2, 4, 6, 8, 12, 16, 24, 48, and 72 h after feeding. Contents were mixed by hand and filtered through 4 layers of cheesecloth to separate ruminal solids and rumen fluids. Ruminal solids were used for Yb and Er analysis, whereas rumen fluid samples were used for measurements of ruminal pH, VFA, $\text{NH}_3\text{-N}$, and Co concentrations. The pH of ruminal fluid was measured immediately, using a hand-held digital pH meter (Waterproof pH Tester 30, Oaktron Instruments, Vernon Hills, IL). Ten milliliters of rumen fluid was placed in a plastic vial with 200 μL of 9.38 M sulfuric acid for NH_3 analysis. Another 20 mL was collected into a plastic vial for VFA analysis and mixed with 4 mL of 25% metaphosphoric acid. Approximately 30 mL of rumen fluid was collected for Co analysis.

Total ruminal contents were evacuated manually through ruminal cannula approximately 4 h after feeding on the last day of each period. Total ruminal contents were weighed, and representative samples were collected. All ruminal samples were immediately frozen at -20°C until analysis.

A total of 12 fecal grab samples were collected per cow per period, from d 25 to 28 each period. The fecal sampling schedule represented every 2 h of a 24-h period, to account for diurnal variation. At each sampling point, approximately 500 g of feces was collected from each cow. Fecal samples were mixed well, and a representative fecal sample of 100 g on a wet matter basis from each time point was used to make a composite sample per cow per period. Composited samples were frozen at -20°C .

Chemical Analysis of Samples

Feed samples were composited by period and dried at 55°C in a forced-air oven (style V-23, Despatch Oven Co.) for 48 h. All samples were ground through a 2-mm screen using a Wiley mill (model 3; Thomas Scientific, Swedesboro, NJ) and then further ground through a 1-mm screen using an ultracentrifuge mill (Brinkmann Instruments, Westbury, NY). All feed samples were analyzed for DM (105°C for 3 h; Shreve et al., 2006). Ash was determined at 450°C for 8 h (AOAC International, 2006). Crude protein was determined using an automated N combustion analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) as described in AOAC International (2006; method 968.06). Concentrations of NDF (Van Soest et al., 1991) and ADF (Robertson and Van Soest, 1981) were determined sequentially, using the Ankom fiber analysis system (Ankom Technology, Macedon, NY). Sodium sulfite and α -amylase (Ankom Technology) were added during NDF extraction. The EE was analyzed according to AOAC procedure (method 920.39; AOAC International, 2006) using an Ankom^{X10} Extractor (Ankom Technology), with diethyl ether as the solvent. Starch concentration was determined using an enzyme-based (thermostable α -amylase and amyloglucosidase) total starch assay (method 996.11, AOAC International, 2002; Total Starch Assay Procedure, Megazyme International Ireland Ltd., Wicklow, Ireland). Composites of corn silage, alfalfa hay, DDGS, and TMR were sent to Dairyland Laboratories Inc. (Arcadia, WI) for mineral analysis (Ca, P, K, Mg, and S). Minerals were quantified according to AOAC procedures (method 953.01; AOAC International, 2006) using an inductively coupled plasma spectrometer (Thermo Jarrell Ash, Franklin, MA). The chemical composition of diets was calculated based on analysis of individual forages (corn silage and alfalfa) and respective concentrate mixes and proportions of each ingredient in the diet.

Rumen fluid samples were thawed and centrifuged at $30,000 \times g$ for 20 min before analysis. Rumen $\text{NH}_3\text{-N}$ concentration was analyzed according to Chaney and Marbach (1962). Ruminal VFA concentration was analyzed using gas chromatography (model 6890, Hewlett Packard, Palo Alto, CA) with a flame-ionization detector. An internal standard, 2-ethylbutyrate, was added to the sample before analysis. The column used for VFA separation was 15 m in length with an inside diameter of 0.25 mm (Nukol, 17926-01C, Supelco Inc., Bellefonte, PA). Split ratio of 100:1 in the injector port was at a temperature of 250°C , with flow rate of 1.3 mL/min of helium. Column and detector temperature were maintained at 140°C and 250°C , respectively.

Ruminal solids samples with rare earth markers (Yb and Er) and rumen samples from ruminal evacuation were lyophilized using an FTS Systems Dura-Dry MP floor-model freeze dryer (FTS Systems Inc., Stone Ridge, NY). Samples were then ground to pass through a 2-mm screen (Wiley mill; Thomas Scientific) and a 1-mm screen (ultracentrifuge mill, Brinkmann Instruments), and stored at room temperature until they were analyzed. Rumen samples from ruminal evacuation were analyzed for DM, NDF, ADF, CP, and starch, as described above.

Ruminal solid samples containing Yb and Er were prepared for analysis by diethylenetriamine pentaacetate (DTPA) extraction method (Karimi et al., 1986), with a few modifications. Briefly, 0.4 g of sample was weighed into a 59-mL plastic vial (Capitol Vial, Thermo Fisher Scientific, Waltham, MA) and stirred continuously for 2 h with 20 mL of 0.1 M diethylenetriaminepentaacetic acid without KCl in a shaking water bath (model 129, Thermo Fisher Scientific). The sample was filtered through a filter paper (Whatman 41, GE Healthcare, Maidstone, UK), and filtrate was analyzed for Yr and Er using an inductively coupled plasma spectrometer (Thermo Jarrell Ash). Rumen fluid samples collected at 0, 1, 2, 4, 6, 8, 12, 16, and 24 h after feeding were thawed and centrifuged ($2,000 \times g$) for 20 min. Then supernatant was filtered through a filter paper (Whatman 41), and filtrate was analyzed for Co using an inductively coupled plasma spectrometer (Thermo Jarrell Ash).

Fecal composites were thawed and lyophilized using a Dura-Dry MP floor-model freeze dryer (FTS Systems Inc.). Then fecal samples were ground to pass through a 2-mm screen (Wiley mill; Thomas Scientific) and a 1-mm screen (ultracentrifuge mill, Brinkmann Instruments). All fecal samples were analyzed for DM, NDF, ADF, CP, and starch, as described above. Fecal titanium dioxide concentration was measured according to the procedure of Myers et al. (2004) with a few modifications by Herrick et al. (2012). These analyses were then used to calculate total-tract digestibility of DM, OM, CP, ADF, and NDF for each cow.

Physical Properties of Feed Samples

Water-holding capacity (**WHC**) of forages and TMR were measured according to the centrifugation method described by Robertson et al. (1980), with a few modifications. Samples used for measuring WHC were dried and ground as previously described. Briefly, 0.5 g of sample was accurately weighed into a pre-weighed 50-mL centrifuge tube and soaked for 24 h in 25 mL of distilled water. The sample was centrifuged at $6000 \times g$ for 20 min, and supernatant was discarded. The

wet sample was weighed, and WHC was calculated as kg water/kg of sample DM. Bulk density (g/mL) was determined by filling a 500-mL container using a filling hopper (Seedburo Equipment Co., Des Plaines, IL) and weighing the amount of feed ingredients or TMR needed to fill the container.

Particle Size Distribution and Physically Effective Fiber

Particle size distribution of individual feed ingredients and TMR was measured using a dry sieving method (Mertens, 1997). Corn silage, alfalfa, and TMR samples composited by period were dried at 55°C in a forced-air oven (style V-23, Despatch Oven Co.) for 48 h before sieving. Other individual feed ingredients were not subjected to drying. Approximately 100 g of each sample was used for sieving. Individual feed ingredients, except corn silage and alfalfa, were sieved through 2.36, 1.70, 1.18, 0.85, 0.60, 0.43, 0.30, 0.212, 0.150, 0.106, 0.075, 0.053, and 0.045-mm screens. Corn silage, alfalfa, and TMR were sieved through 12.7, 9.51, 4.76, 3.35, 2.36, 1.18, 0.60, and 0.075-mm screens of a Rototap shaker (model RX-29, W. S. Tyler, Mentor, OH; ASAE, 2001). The geometric mean (X_{gm}) and the standard deviation of the geometric mean (S_{gm}) were calculated for both methods according to ASAE (2001).

The physical effectiveness factor (**pef**) and physically effective NDF (**peNDF**) were measured using a dry-sieving method (Mertens, 1997). The pef was determined based on the proportion of DM retained on the 1.18-mm sieve ($pef_{1.18}$), and peNDF was calculated as NDF content of the respective individual feed ingredient, TMR or orts, multiplied by pef.

Milk Production and Composition

Cows were milked 3 times per day at 0600, 1400, and 2100 h, and individual milk yields were recorded at each milking. Milk samples were collected from each milking for 2 consecutive days of wk 3 (d 17 and 18) and 4 (d 24 and 25) of each period. All collected milk samples were sent to Heart of America DHIA Laboratory (Manhattan, KS) for milk composition analysis using AOAC International (2006) approved methods. Milk fat, protein, and lactose were analyzed using mid-infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments, Chaska, MN; AOAC International, 2006). Concentration of MUN was analyzed using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments), and SCC were determined using a flow cytometer laser (Somacount 500, Bentley Instruments; AOAC International, 2006). Somatic cell counts were

converted to SCS, where $SCS = \log_{10}(SCC)$. Energy-corrected milk was determined using the following equation (Orth, 1992):

$$[0.327 \times \text{milk yield (kg)}] + [12.95 \times \text{fat yield (kg)}] \\ + [7.2 \times \text{protein yield (kg)}].$$

Mathematical and Statistical Analyses

Ruminal fractional passage rates (K_p) of solid, liquid, and DDGS were calculated according to Grovum and Williams (1973). Natural logarithms of marker concentrations of Yb, Co, and Er at different time points were calculated and plotted against time. It was assumed that the slope of the linear portion of the curve represented K_p of that fraction. Ruminal retention time of solid, liquid, and DDGS was calculated as the reciprocal of the respective K_p :

$$\text{Ruminal retention time} = 1/K_p \text{ of cobalt,} \\ \text{ytterbium, or erbium.}$$

The area below ruminal pH 6.0 was calculated using Microcal Origin, version 6.0 (Microcal Software Inc., Northampton, MA), and h below pH 6.0 was calculated according to the data on area below ruminal pH 6.0.

All data were analyzed as period means using MIXED procedure in SAS (version 9.3, SAS Institute Inc., Cary, NC) in a 4×4 Latin square design. Weekly means of DMI and milk yield from the final 2 wk of each period were used for statistical analysis. Means for milk composition were determined from individual samples taken at each milking twice weekly during wk 3 and 4. Means for average pH measures, rumen kinetics, and total-tract digestibility were calculated for each period and used for statistical analysis. The statistical model used for feed intake, milk production and composition, average pH measures, rumen kinetics, and total-tract digestibility was as follows:

$$Y_{ijkl} = \mu + \text{forage NDF}_i + \text{DDGS}_j \\ + (\text{forage NDF}_i \times \text{DDGS}_j) + P_k + C_l + \varepsilon_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, forage NDF_i is the effect of forage level i ($i = 1$ to 2), DDGS_j is the effect of DDGS j ($j = 1$ to 2), $(\text{forage NDF}_i \times \text{DDGS}_j)$ is the effect of the interaction of forage level i and DDGS level j , P_k is the effect of period k ($k = 1$ to 4), C_l is the effect of cow l ($l = 1$ to 4), and ε_{ijkl} is the residual error. Cow was used as an experimental unit and was considered as the random variable in the model.

Data on diurnal variation of ruminal pH, $\text{NH}_3\text{-N}$, and VFA concentrations were analyzed using the repeated measures approach, and time was used as the repeated measure. Based on the lowest value according to Akaike's information criterion (Littell et al., 1996), the compound symmetric structure type was selected as the appropriate covariance structure. The statistical model used for repeated measures was as follows:

$$Y_{ijklm} = \mu + \text{forage}_i + \text{DDGS}_j + (\text{forage}_i \times \text{DDGS}_j) \\ + P_k + C_l + \varepsilon_{ijkl} + T_m + (\text{forage}_i \times T_m) \\ + (\text{DDGS}_j \times T_m) + (\text{forage}_i \times \text{DDGS}_j \times T_m) + \omega_{ijklm},$$

where Y_{ijklm} is the dependent variable, μ is the overall mean, forage_i is the effect of forage level i ($i = 1$ to 2), DDGS_j is the effect of DDGS j ($j = 1$ to 2), $(\text{forage}_i \times \text{DDGS}_j)$ is the effect of the interaction of forage level i and DDGS level j , P_k is the effect of period k ($k = 1$ to 4), C_l is the effect of cow l ($l = 1$ to 4), ε_{ijkl} is the whole plot error, T_m is the effect of time m ($m = 1$ to 9), $\text{forage}_i \times T_m$ is the effect of the interaction of forage level i and time m , $\text{DDGS}_j \times T_m$ is the effect of the interaction of DDGS level j and time m , $(\text{forage}_i \times \text{DDGS}_j \times T_m)$ is the interaction between forage i , DDGS j , and time m , and ω_{ijklm} is the subplot error. Cow was used as an experimental unit and was considered as the random variable in the model.

Least squares means were reported for each variable. Interactions of main effects were declared significant at $P \leq 0.05$, and tendencies for main effects were declared at $0.05 < P \leq 0.10$. Mean comparisons using the PDIFF test were performed when there was a significant interaction between the main effects, forage NDF $\times$ DDGS interactions ($P \leq 0.05$).

RESULTS

Dietary Treatments

The ingredient and chemical composition of the diets, concentrate mixes, corn silage, alfalfa hay, DDGS, ground corn, soybean meal, expeller soybean meal, extruded soybeans, and soy hulls are presented in Tables 1 and 2. Data on particle size distribution, pef, peNDF, and physical characteristics of the individual feed ingredients and concentrate mixes are presented in Table 3. The concentrations of OM and CP, as well as NE_L , were similar across diets, averaging 93.2 and 18.1%, and 1.61 Mcal/kg, respectively. Forage NDF concentration for HF and LF diets were 24.5 and 16.8%, respectively. Dietary NDF and ADF concentrations were greater for HF diets than for LF diets by 4.00 and 2.25% units, respectively. Also, dietary NDF and ADF concentrations

were greater for 18DG diets by 3.30 and 0.85% units, respectively, compared with 0DG diets. Starch concentrations of the diets, LF0DG, LF18DG, HF0DG, and HF18DG, were 28.1, 24.9, 23.2, and 18.8%, respectively. Diets were formulated to have similar EE concentration of 4.30% across the diets. However, chemical analysis of diets fed to cows showed that EE concentration of the diets were not similar across the diets, possibly attributable to the differences in the EE value used for diet formulation and value from the actual analysis of DDGS after the study.

Nutrient Intakes

Nutrient intakes of cows fed experimental diets are presented in Table 4. Diet did not affect ($P > 0.10$) intake of DM (23.9 kg/d) or CP (4.32 kg/d). Interactions of main effects were observed for intake of forage NDF ($P = 0.05$) and EE ($P = 0.01$). As expected, total NDF intake was greater for HF diets than for LF diets (7.2 vs. 6.4 kg/d; $P = 0.002$). Cows fed the 18DG diets also had greater NDF intake compared with cows fed the 0DG diets (7.1 vs. 6.5 kg/d; $P = 0.002$). The ADF intake also was greater for HF diets than for LF diets (3.7 vs. 3.2 kg/d; $P = 0.001$). Starch intake was greater for LF diets than for HF diets (6.40 vs. 4.95 kg/d; $P < 0.001$), as well as for 0DG diets compared with 18DG diets (6.25 vs. 5.10 kg/d; $P < 0.001$).

Ruminal pH and Fermentation

Data on ruminal pH and fermentation characteristics are presented in Table 5. There was no forage $\times$ DDGS $\times$ time interaction for ruminal pH ($P = 0.41$; data not shown). The maximum pH was observed with cows fed HF diets (6.87 vs. 6.66 for LF diets; $P = 0.04$). Analysis revealed a tendency to have greater ($P = 0.09$) variance of pH associated with LF diets compared with HF diets. Ruminal pH was below 6 for more than 16 h when cows were fed with LF diets, whereas it was around 6 h when cows were fed with HF diets ($P < 0.01$).

We found no forage $\times$ DDGS $\times$ time interaction for total VFA concentration ($P = 0.45$; data not shown). Cows fed the HF diets had less total VFA (122 vs. 116 mM; $P = 0.03$) than did cows fed LF diets. Molar proportions of acetate were lower for LF compared with HF diets (57.5 vs. 62.6 mmol/100 mmol; $P < 0.001$). Molar proportion of propionate and butyrate were affected by the interaction of the main effects ($P < 0.001$). Molar proportion of isobutyrate was greater for cows fed HF diets than for those fed LF diets (0.96 vs. 0.88 mmol/100 mmol; $P < 0.001$). There was an interaction for the main dietary effects for the valerate molar proportion ($P = 0.02$). Including greater amounts of

Table 2. Concentration of nutrients in concentrate mixes, corn silage, alfalfa hay, distillers dried grains with solubles (DDGS), ground corn, soybean meal, expeller soybean meal, extruded soybeans, and soy hulls used in the diets during the lactation study

Item ¹	Concentrate mix ²									
	LF					HF				
	0DG	18DG	0DG	18DG	Corn silage	Alfalfa hay	DDGS	Ground corn	Soybean meal	Expeller soybean meal ³
DM (%)	88.0	88.0	88.6	88.7	32.4	86.9	89.0	90.0	90.9	90.8
CP	23.6	24.0	30.3	31.0	7.68	18.2	32.5	7.80	51.6	45.2
NDF	13.9	19.6	11.1	19.3	40.8	41.4	35.3	7.76	10.3	20.5
ADF	6.08	7.61	3.87	5.85	20.7	30.1	11.2	2.02	4.54	8.75
Starch	33.2	27.7	26.6	15.7	25.5	2.20	5.53	72.7	1.50	1.31
Ether extract	3.87	5.86	5.39	8.49	3.49	2.33	13.2	3.90	1.85	6.37
Ash	7.54	7.26	9.07	9.02	4.65	9.23	6.73	0.58	7.42	6.88
Ca	1.14	1.11	1.26	1.15	0.23	1.32	0.03	—	—	—
P	0.42	0.48	0.50	0.61	0.23	0.21	0.74	—	—	—
Mg	0.25	0.26	0.28	0.32	0.20	0.33	0.32	—	—	—
K	1.20	1.05	1.42	1.29	1.19	1.96	1.08	—	—	—
S	0.20	0.23	0.24	0.28	0.12	0.19	0.33	—	—	—

¹Nutrients are % of DM unless noted.

²Concentrate mixes of diets that provided varying concentrations of forage and distillers dried grains with solubles; low forage (LF; 41% of diet DM) or high forage (HF; 60% of diet DM) and DDGS at 0 or 18% of diet DM (0DG and 18DG, respectively).

³SoyPlus (West Central Soy, Ralston, IA).

Table 3. Particle size distribution, physical effectiveness factor (pef), physically effective NDF (peNDF) based on dry sieving technique, and physical characteristics of concentrate mixes, corn silage, alfalfa hay, dried distillers grains with solubles (DDGS), ground corn, soybean meal, expeller soybean meal, extruded soybeans, and soybean hulls used in the diets during the lactation study

Item	Concentrate mix ¹											
	LF					HF						
	0DG	18DG	0DG	18DG	Corn silage	Alfalfa hay	DDGS	Ground corn	Soybean meal	Expeller soybean meal	Extruded soybeans	Soybean hulls
Sieve (mm)												
12.7	—	—	—	—	5.17	3.21	—	—	—	—	—	—
9.51	—	—	—	—	10.5	2.17	—	—	—	—	—	—
4.76	—	—	—	—	34.2	11.4	—	—	—	—	—	—
2.36	8.25	2.30	14.1	3.18	28.1	21.1	1.17	1.28	3.85	12.4	25.8	0.47
1.18	29.1	27.0	34.5	29.5	13.1	23.6	16.1	12.8	44.6	30.2	36.4	28.2
0.60	35.1	40.7	28.1	39.7	5.63	17.1	46.6	26.1	36.9	27.0	25.2	45.9
Pan	27.5	30.0	23.4	27.6	3.3	21.5	36.1	59.8	14.7	30.5	12.7	25.5
X _{gm} ² (mm)	0.86	0.78	1.00	0.84	4.21	1.82	0.71	0.38	1.05	0.89	1.37	0.80
S _{gm} ³ (mm)	0.73	0.58	0.88	0.59	3.79	1.88	0.42	0.47	0.67	0.84	0.90	0.55
pef ⁴	37.4	29.3	48.6	32.7	91.0	61.4	17.3	14.1	48.4	42.6	62.2	28.6
peNDF ⁵	5.19	5.74	5.40	6.33	37.1	25.4	6.09	1.10	4.99	8.71	5.56	18.0
WHC ⁶	3.25	3.37	3.02	2.75	5.04	6.40	2.97	2.63	4.64	4.43	4.13	7.33
BD ⁷	0.55	0.52	0.61	0.56	0.23	0.30	0.49	0.57	0.64	0.62	0.55	0.30

¹Concentrate mixes of diets that provided varying concentrations of forage and dried distillers grains with solubles; low forage (LF; 41% of diet DM) or high forage (HF; 60% of diet DM) and DDGS at 0 or 18% of diet DM (0DG and 18DG, respectively).

²X_{gm} = geometric mean length, as calculated by ASAE (2001).

³S_{gm} = standard deviation of the geometric mean, as calculated by ASAE (2001).

⁴pef = PHYSICAL effectiveness factor for dry sieving; the proportion of particles retained on 1.18-mm screen.

⁵peNDF = pef × NDF content of the sample.

⁶WHC = water-holding capacity (kg of water/kg of feed).

⁷BD = bulk density (g/cm³).

Table 4. Nutrient intakes of dairy cows fed experimental diets¹

Intake (kg/d)	LF		HF		SEM	P-value ²		
	0DG	18DG	0DG	18DG		F	DG	F × DG ³
DM	25.2	22.9	23.4	23.9	1.35	0.52	0.22	0.06
CP	4.52	4.17	4.20	4.37	0.25	0.66	0.51	0.08
Total NDF	6.26	6.51	6.78	7.71	0.38	0.002	0.01	0.09
NDF (forage)	4.21 ^b	3.84 ^c	5.74 ^a	5.86 ^a	0.26	<0.001	0.26	0.05
NDF (non-forage)	2.05	2.66	1.04	1.85	0.13	<0.001	<0.001	0.31
ADF	3.21	3.15	3.53	3.79	0.18	0.001	0.22	0.08
Starch	7.08	5.72	5.42	4.48	0.37	<0.001	<0.001	0.39
Ether extract	0.91 ^c	1.10 ^b	0.96 ^c	1.28 ^a	0.06	<0.001	<0.001	0.01

^{a-c}Treatment means in same row followed by different superscript letters differ significantly ($P < 0.05$).

¹Diets provided varying concentrations of forage and distillers dried grains with solubles (DDGS); low forage (LF; 41% of diet DM) or high forage (HF; 60% of diet DM) and DDGS at 0 or 18% of diet DM. 0DG = 0% DDGS; 18DG = 18% DDGS.

²F = effect of dietary forage NDF concentration; DG = effect of DDGS concentration; F × DG = interaction of dietary forage NDF concentration and DDGS concentration ($P < 0.05$).

³PDIF test was conducted if $P < 0.05$ for interactions of main effects.

DDGS resulted in lower proportions of ruminal acetate (57.5 vs. 62.6 mmol/100 mmol; $P < 0.001$) but did not affect total VFA concentration ($P = 0.69$). The ratio of acetate to propionate was lower for LF (2.29 vs. 3.16; $P < 0.001$) and 18DG diets (2.53 vs. 2.93; $P < 0.001$) compared with cows fed HF and 0DG diets, respectively. We also discovered a tendency toward lower $\text{NH}_3\text{-N}$ concentration for 0DG diets compared with 18DG diets ($P = 0.10$).

Ruminal Digestion Kinetics

Data on rumen fill and rumen digestion kinetics are presented in Table 6. Ruminal content was affected by forage × DDGS interaction on wet matter basis ($P = 0.03$) and DM basis ($P < 0.001$). Cows fed HF diets had greater ruminal content on wet matter basis (91.6 vs. 81.0 kg; $P < 0.001$) and DM basis (13.4 vs. 12.2 kg; $P < 0.001$) compared with LF diets. Ruminal liquid

Table 5. Ruminal pH, volatile fatty acids (VFA) and ammonia concentrations in dairy cows fed experimental diets¹

Item	LF		HF		SEM	P-value ²		
	0DG	18DG	0DG	18DG		F	DG	F × DG ³
Daily ruminal pH								
Mean	6.17 ^b	6.02 ^c	6.32 ^a	6.36 ^a	0.09	<0.001	0.22	0.03
Minimum	5.68 ^c	5.54 ^d	5.86 ^b	5.95 ^a	0.09	<0.001	0.05	0.001
Maximum	6.81	6.51	6.85	6.89	0.09	0.04	0.17	0.08
Variance	0.17	0.12	0.11	0.11	0.02	0.09	0.11	0.22
pH < 6.0 (h/d)	15.8	16.5	7.50	4.00	3.34	<0.001	0.30	0.13
Area under pH 6.0 (pH × h/d)	2.42	3.35	1.16	0.31	1.00	0.03	0.96	0.28
VFA								
Total VFA (mM)	119	124	117	114	3.59	0.03	0.69	0.14
VFA (mmol/100 mmol)								
Acetate	58.9	56.0	63.8	61.3	0.72	<0.001	<0.001	0.51
Propionate	24.2 ^b	27.7 ^a	19.5 ^d	20.6 ^c	0.88	<0.001	<0.001	<0.001
Butyrate	11.6 ^b	10.9 ^c	12.2 ^b	13.5 ^a	0.58	<0.001	0.14	<0.001
Isobutyrate	0.87	0.88	0.96	0.95	0.04	<0.001	1.00	0.65
Valerate	2.47 ^b	2.83 ^a	1.73 ^c	1.82 ^c	0.23	<0.001	<0.001	0.02
Isovalerate	1.90	1.68	1.86	1.77	0.09	0.47	<0.001	0.07
Acetate:propionate	2.53	2.05	3.32	3.00	0.12	<0.001	<0.001	0.11
$\text{NH}_3\text{-N}$ (mg/dL)	9.98	11.8	11.0	11.1	0.63	0.79	0.10	0.13

^{a-d}Treatment means in same row followed by different superscript letters differ significantly ($P < 0.05$).

¹Diets provided varying concentrations of forage and distillers dried grains with solubles (DDGS); low forage (LF; 41% of diet DM) or high forage (HF; 60% of diet DM) and DDGS at 0 or 18% of diet DM. 0DG = 0% DDGS; 18DG = 18% DDGS.

²F = effect of dietary forage NDF concentration; DG = effect of DDGS concentration; F × DG = interaction of dietary forage NDF concentration and DDGS concentration ($P < 0.05$).

³PDIF test was conducted if $P < 0.05$ for interactions of main effects.

Table 6. Rumen fill and ruminal digestion kinetics of dairy cows fed experimental diets¹

Item	LF		HF		SEM	P-value ²		
	0DG	18DG	0DG	18DG		F	DG	F × DG ³
Rumen fill ⁴								
Wet content (kg)	78.2 ^c	83.8 ^{bc}	94.1 ^a	87.0 ^{ab}	10.5	<0.001	0.74	0.03
DM (%)	14.4	15.6	14.6	14.8	0.57	0.36	0.06	0.17
Rumen pool (kg)								
Liquid	67.1	70.6	80.2	74.2	8.71	0.04	0.64	0.15
DM	11.2 ^c	13.2 ^{ab}	13.9 ^a	12.9 ^b	1.84	<0.001	0.10	<0.001
OM	10.1 ^c	12.0 ^{ab}	12.5 ^a	11.6 ^b	1.70	<0.001	0.05	<0.001
CP	2.12 ^b	2.59 ^a	2.48 ^a	2.41 ^a	0.35	0.27	0.04	0.01
NDF	6.52 ^b	7.48 ^a	7.56 ^a	7.42 ^a	1.02	0.06	0.10	0.04
ADF	3.78 ^b	4.23 ^a	4.46 ^a	4.30 ^a	0.59	0.01	0.21	0.03
Starch	0.43	0.58	0.74	0.64	0.12	0.05	0.72	0.13
Ether extract	0.55	0.72	0.61	0.62	0.10	0.59	0.04	0.08
Digestion kinetics								
Liquid phase								
Fractional dilution rate (%/h)	10.7	10.6	11.2	11.2	0.53	0.34	1.00	0.95
Retention time ⁵ (h)	9.41	9.47	9.05	8.97	0.43	0.35	0.98	0.88
Solid phase								
Fractional passage rate (%/h)	3.62	3.90	4.25	4.54	0.33	0.03	0.24	0.99
Retention time ⁶ (h)	28.5	26.1	24.3	22.3	1.14	0.01	0.10	0.90
DDGS								
Fractional passage rate (%/h)	6.81 ^{ab}	6.61 ^{ab}	6.41 ^b	7.72 ^a	0.45	0.25	0.13	0.04
Retention time ⁷ (h)	14.8	15.3	15.8	13.2	0.97	0.36	0.18	0.06

^{a-c}Treatment means in same row followed by different superscript letters differ significantly ($P < 0.05$).

¹Diets provided varying concentrations of forage and distillers dried grains with solubles (DDGS); low forage (LF; 41% of diet DM) or high forage (HF; 60% of diet DM) and DDGS at 0 or 18% of diet DM. 0DG = 0% DDGS; 18DG = 18% DDGS.

²F = effect of dietary forage NDF concentration; DG = effect of DDGS concentration; F × DG = interaction of dietary forage NDF concentration and DDGS concentration ($P < 0.05$).

³PDIFF test was conducted if $P < 0.05$ for interactions of main effects.

⁴Manual empty.

⁵Ruminal retention time = 1/rate of passage of cobalt.

⁶Ruminal retention time = 1/rate of passage of ytterbium.

⁷Ruminal retention time = 1/rate of passage of erbium.

pools were greater for HF diets compared with LF diets (77.2 vs. 68.9 kg; $P = 0.04$). Forage × DDGS interactions displayed effects on ruminal pools of OM ($P < 0.001$), CP ($P = 0.01$), NDF ($P = 0.04$), and ADF ($P = 0.03$). Cows fed HF diets had a greater ruminal starch content than did cows fed LF diets (0.69 vs. 0.51 kg/d; $P = 0.03$). Cows fed 18DG had a greater ruminal EE content compared with cows fed LF diets (0.67 vs. 0.58 kg/d; $P = 0.04$).

Average fractional dilution rate and liquid ruminal retention time of diets were 10.9%/h and 9.23 h, respectively, and neither was affected by dietary treatments. Fractional passage rate of solid phase based on corn silage was lower (3.76 vs. 4.40%/h; $P = 0.03$) for cows fed LF diets compared with HF diets. However, the ruminal retention time of solid phase was greater (27.3 vs. 23.3 h; $P = 0.01$) for LF diets than for HF diets, whereas it tended to be greater (26.4 vs. 24.2 h; $P = 0.10$) for 0DG diets than for 18DG diets. An interaction of main effects was observed for the fractional passage rate of the labeled DDGS ($P = 0.04$).

Total-Tract Digestibility

Data on apparent total-tract digestibility and amount of digested nutrients per day are presented in Table 7. Apparent total-tract digestibility of DM, NDF, ADF, CP, and starch was not affected by the diets. Apparent total-tract digestibility of EE was lower for cows fed LF diets compared with HF diets (53.8 vs. 63.5%; $P < 0.001$), whereas it was lower for cows fed 0DG diets than for those fed 18DG diets (55.4 vs. 61.9%; $P = 0.02$).

Forage × DDGS exhibited an interaction for digested EE ($P = 0.003$). Diet did not affect the amount of DM, OM, CP, NDF, or ADF digested by cows. Cows fed LF (6.25 vs. 4.85 kg/d) and 0DG diets (6.12 vs. 4.97 kg/d) digested a greater amount of starch compared with HF and 18DG diets, respectively.

Lactation Performance

Lactation performance of cows during the experiment is presented in Table 8. No significant forage NDF ×

Table 7. Apparent total-tract digestibility of nutrients in dairy cows fed experimental diets¹

Item	LF		HF		SEM	P-value ²		
	0DG	18DG	0DG	18DG		F	DG	F × DG ³
Total-tract digestibility (%)								
DM	72.4	69.5	68.4	67.9	1.64	0.11	0.30	0.46
OM	74.9	71.9	71.7	70.9	1.60	0.21	0.25	0.49
CP	73.0	71.0	71.3	72.0	1.61	0.83	0.70	0.42
NDF	47.7	48.4	45.1	46.8	3.08	0.46	0.67	0.87
ADF	38.9	40.7	38.3	39.3	4.01	0.78	0.69	0.91
Starch	98.0	96.9	97.8	97.9	0.53	0.44	0.37	0.32
Ether extract	51.9	55.7	58.9	68.0	2.08	<0.001	0.02	0.25
Digested (kg/d)								
DM	18.2	16.0	16.0	16.2	0.98	0.19	0.19	0.12
OM	17.5	15.4	15.6	15.7	0.92	0.22	0.16	0.10
CP	3.29 ^a	2.97 ^a	2.97 ^a	3.15 ^a	0.18	0.63	0.53	0.10
NDF	2.97	3.19	3.05	3.60	0.27	0.33	0.15	0.50
ADF	1.23	1.32	1.36	1.49	0.15	0.29	0.42	0.85
Starch	6.94	5.55	5.30	4.39	0.37	0.001	0.002	0.33
Ether extract	0.47 ^c	0.61 ^b	0.57 ^b	0.87 ^a	0.05	<0.001	<0.001	0.003

^{a-c}Treatment means in same row followed by different superscript letters differ significantly ($P < 0.05$).

¹Diets provided varying concentrations of forage and distillers dried grains with solubles (DDGS); low forage (LF; 41% of diet DM) or high forage (HF; 60% of diet DM) and DDGS at 0 or 18% of diet DM. 0DG = 0% DDGS; 18DG = 18% DDGS.

²F = effect of dietary forage NDF concentration; DG = effect of DDGS concentration; F × DG = interaction of dietary forage NDF concentration and DDGS concentration ($P < 0.05$).

³PDIFF test was conducted if $P < 0.05$ for interactions of main effects.

DDGS interactions were observed for any of the reported animal performance measures. Milk production, FCM, MUN, and feed efficiency were not affected by dietary treatments. Cows fed LF diets tended to have lower milk fat content (3.66 vs. 3.02%; $P = 0.08$) and a higher protein content (3.06 vs. 2.99%; $P = 0.01$) than cows fed the HF diets. However, we found no dietary effect on fat or protein yield. Cows fed LF diets tended ($P = 0.07$) to have greater lactose concentration than cows fed LF diets, but there was no effect on lactose

yield. Including DDGS in the diet did not affect milk production or components.

DISCUSSION

Nutrient Intake

Low-forage diets often result in greater DMI compared with HF diets (Kalscheur et al., 1997; Oba and Allen, 2000, 2003). However, as we observed in the cur-

Table 8. Lactation performance by dairy cows fed experimental diets¹

Item	LF		HF		SEM	P-value ²		
	0DG	18DG	0DG	18DG		F	DG	F × DG
Production (kg/d)								
Milk	38.9	36.1	37.4	35.7	5.34	0.65	0.28	0.80
ECM ³	33.1	29.5	34.5	35.2	4.84	0.27	0.64	0.49
Milk composition								
Fat (%)	3.01	3.02	3.50	3.81	0.36	0.08	0.62	0.62
Fat (kg/d)	1.17	1.00	1.30	1.40	0.21	0.16	0.84	0.46
Protein (%)	3.07	3.05	2.98	2.99	0.12	0.01	0.75	0.35
Protein (kg/d)	1.19	1.09	1.11	1.06	0.15	0.42	0.26	0.67
Lactose (%)	4.88	4.84	4.77	4.68	0.11	0.07	0.30	0.69
Lactose (kg/d)	1.89	1.77	1.79	1.68	0.28	0.44	0.34	0.94
MUN (mg/dL)	12.4	13.0	12.0	12.7	0.63	0.42	0.22	0.93
Feed efficiency (ECM/DMI)	1.45	1.41	1.58	1.53	0.16	0.32	0.69	0.99

¹Diets provided varying concentrations of forage and distillers dried grains with solubles (DDGS); low forage (LF; 41% of diet DM) or high forage (HF; 60% of diet DM) and DDGS at 0 or 18% of diet DM. 0DG = 0% DDGS; 18DG = 18% DDGS.

²F = effect of dietary forage NDF concentration; DG = effect of DDGS concentration; F × DG = interaction of dietary forage NDF concentration and DDGS concentration ($P < 0.05$).

³ECM = $[0.327 \times \text{milk yield (kg)}] + [12.95 \times \text{fat yield (kg)}] + [7.2 \times \text{protein yield (kg)}]$.

rent study, the proportion of forage in the diets did not affect DMI of lactating dairy cows (Agle et al., 2010; Aguerre et al., 2011; Neveu et al., 2013). Allen (2000) suggested that DMI could be limited by the amount and digestibility of forage and the amount of propionate in the rumen rather than by the specific ratio of forage to concentrate in the diet. The current research contradicts this suggested hypothesis, because cows fed greater amounts of forage had less ruminal propionate and yet we did not observe a DMI response. Interestingly, our data agrees with other researchers who observed no response in DMI when including approximately 20% DDGS in lactating diets (Kleinschmit et al., 2006; Paz et al., 2013; Zanton et al., 2013). In contrast, other studies reported that feeding DDGS at approximately 20% of the diet (DM basis) increased (Kalscheur et al., 2012) or decreased (Ranathunga et al., 2010) DMI of lactating dairy cows.

Rumen pH and Fermentation

Our data support previous studies in which cows were fed HF diets to maintain greater ruminal pH with (Benchaar et al., 2013) or without (Kalscheur et al., 1997; Oba and Allen, 2000, 2003) DDGS in the diet. This indicated that diets with high forage concentration maintain greater ruminal pH with the help of increased chewing and salivation, and greater ruminal buffering capacity, attributed to greater effective fiber concentration. On the contrary, as we observed in our study, low-forage diets with (Ramirez-Ramirez et al., 2012, 2016) or without DDGS (Kalscheur et al., 1997; Oba and Allen, 2000, 2003) could lower the ruminal pH. Interactions between forage and DDGS concentrations observed in our study for the daily mean pH were attributed to the lowest daily mean pH observed with cows fed LF18DG diet. Cumulative effects of ruminal degradation of a higher starch content (25%) and 18% DDGS may have resulted in the lowest ruminal pH for cows fed LF18DG diet. Also, the acidic nature of DDGS (US Grains Council, 2012), attributed to the presence of lactic acid in DDGS (Fron et al., 1996; Kim et al., 2008) or to the addition of sulfuric acid during the dry-grind ethanol production process (US Grains Council, 2012), may have an effect on lowering ruminal pH in the cows fed LF18DG diet.

Effects of forage on total VFA concentration are well documented. Low-forage diets fed with a greater concentration of fermentable carbohydrates yield greater total VFA concentrations, as we observed in our study (Kalscheur et al., 1997; Agle et al., 2010). Contrary to that, Oba and Allen (2003) observed that VFA concentration was not affected by forage level. However, they

noted that LF diets had greater amounts of truly fermented OM compared with HF diets, and they found no relationship between total VFA concentration and true ruminally degraded OM. Total VFA concentration responses to feeding DDGS have been inconsistent. As we observed in our study, Zhang et al. (2010) reported no effect of DDGS on total VFA concentration when DDGS was fed at 20% with low or high forage concentrations. Contrary to our observation, Benchaar et al. (2013) demonstrated a negative effect of DDGS on total VFA production. When they fed increasing levels of DDGS from 0 to 30% of DM, with 60% forage concentration, there was a linear decrease of total VFA concentration from 99.3 to 91.1 mM. In another study, Ramirez-Ramirez et al. (2012) demonstrated the greatest total VFA concentration (130 mM) when DDGS was fed at 30% of DM with 41% of brown midrib corn silage. This could have been attributable to highly digestible fiber from brown midrib corn and DDGS.

Low-forage diets decrease molar proportions of acetate, increase molar proportions of propionate, and lower the ratio of acetate to propionate (Oba and Allen, 2000, 2003; Neveu et al., 2013). Lower acetate and higher propionate molar proportions in LF diets are mostly attributable to starch degradation and its negative effect on fiber digestion. Contrary to our observation, Kalscheur et al. (1997) reported a greater molar proportion of butyrate for LF diets. Other studies have reported no effect of forage concentration on molar proportion of butyrate, whereas its effect on molar proportion on branched VFA is variable (Oba, Allen, 2000, 2003; Neveu et al., 2013).

Previous studies have demonstrated that feeding corn DDGS to lactating dairy cows at $\geq 20\%$ DM can decrease acetate molar proportion and increase propionate molar proportion in the rumen, irrespective of low (Ramirez-Ramirez et al., 2016) or high (Ramirez-Ramirez et al., 2012; Benchaar et al., 2013) forage concentration in the diet. However, in our study, feeding both LF and 18DG diets decreased the acetate molar proportion and increased the propionate molar proportion. Benchaar et al. (2013) suggested that an increase in propionate molar proportion as dietary proportion of DDGS increases is a consequence of a decline in acetate molar proportion rather than an increase in propionate concentration. However, in the current study, cows fed LF18DG demonstrated a greater ruminal propionate concentration (34.5 mM) compared with cows fed other diets, without affecting ruminal acetate concentration (data not shown). Decreased isovalerate molar proportions with feeding DDGS was consistent with other studies (Anderson et al., 2006; Kelzer et al., 2009; Benchaar et al., 2013). The decreased branched

short-chain fatty acids possibly reflected the relatively low content of branched-chain AA in diets high in corn products (Schingoethe et al., 1999). Benchaar et al. (2013) indicated that decreased molar proportion of branched-chain VFA and ruminal NH_3 concentration attributed to feeding DDGS was an indication of reduction of ruminal degradation of protein.

Rumen Fill and Ruminal Digestion Kinetics

Nutrient intake, nutrient pools, and passage rate together may explain how nutrients degraded under different ruminal conditions. The effect of dietary forage concentration on ruminal wet content is variable. A few studies agreed with our data, in which there were more wet content in the rumen when cows were fed HF diets (Voelker et al., 2002; Suarez-Mena et al., 2013); however, other studies reported no effect of forage concentration on ruminal wet content (Yang et al., 2001a; Penner et al., 2009). Greater moisture content and WHC of HF diets, accompanied by increased saliva secretion, may be attributed to greater ruminal wet content and liquid pool compared with LF diets.

Consistent with previous studies, we observed slower passage rates for cows fed LF diets compared with HF diets (Colucci et al., 1990; Huhtanen and Jaakkola, 1993) and no dietary effects on fractional dilution rates (Krämer et al., 2013). Ruminal fermentation of starch with highly digestible non-forage NDF from DDGS could rapidly decrease ruminal pH, thus suppressing fiber-digesting microflora. Fibrolytic bacteria are sensitive to ruminal pH, and when ruminal pH decreases below a critical value, rumen fiber digestion decreases rapidly. Fiber digestion is impaired when ruminal pH drops below approximately 6.0 to 6.3 (Erdman, 1988; Mouriño et al., 2001). In addition, a greater unsaturated FA load from DDGS (Schingoethe et al., 2009) may overwhelm the rumen environment and the antibacterial action of polyunsaturated FA (Chalupa et al., 1984), which could also reduce the fiber-digesting bacteria population. This could decrease fiber digestibility and leave undigested fiber fractions in the rumen for a longer time. Also, the lack of “scratch factor” attributed to lower forage NDF intake may disturb rumination and mastication, thus decreasing saliva flow to affect the buffering capacity of the rumen (Dijkstra et al., 2012).

Feeding DDGS with HF concentrations may affect ruminal nutrient degradability differently than DDGS fed with LF concentrations. Greater intake of total NDF, forage NDF, ADF, and EE, and higher solid passage rates in the rumen may indicate lower degradability of particular nutrients in the rumen. However,

in situ degradability data from our companion study revealed that the HF18DG diet had the greatest NDF and ADF effective degradabilities (Ranathunga et al., 2011). Also, greater milk fat concentration from cows fed HF18DG diet indicated that greater unsaturated fatty acid concentration from the DDGS diet did not negatively affect biohydrogenation of the unsaturated fatty acids, and there was lower negative effect on fiber degradability in the rumen. High-forage diets form a thick rumen mat, which scratches the rumen wall to stimulate the rumination process. This process leads to increased mastication and salivation and higher ruminal pH. Increased mastication breaks down longer fiber particles and allows greater degradability and faster passage from the rumen (Dijkstra et al., 2012). A faster passage rate of solid phase means that fiber particles stay in the rumen for a shorter time. However, improved fiber degradability indicated greater microbial activity on fiber digestion. Grant (1997) suggested that the thick rumen mat attributed to HF diets may retain non-forage particles to slow down passage from the rumen, as non-forage particles are characterized by smaller particle size and greater specific gravity than forage particles. The smaller average particle size (0.46–1.18 mm; Kleinschmit et al., 2007) and greater specific gravity (1.24; Bhatti and Firkins, 1995) of DDGS suggest that DDGS would stay longer in the rumen with HF diets. However, fractional passage rates estimated using Er-labeled DDGS indicated that DDGS stayed in the rumen for a similar amount of time across the diets. In situ degradability data from our companion study demonstrated that DDGS had greater NDF and ADF effective degradabilities when DDGS was fed with HF concentrations (Ranathunga et al., 2011). This could be attributed to the presence of more fiber-digesting bacteria, owing to more favorable rumen conditions such as higher ruminal pH.

Apparent Total-Tract Digestibility

Apparent total-tract digestibility data demonstrated that when they were fed 4 different diets, cows depended on different sources of nutrients to obtain their nutritional requirements. For example, starch digested as a portion of DM was greatest for cows fed LF0DG at 38%, whereas it was 35, 33, and 27% for LF18DG, HF0DG, and HF18DG diets, respectively. Lower total-tract digestibilities for fiber were reported for cows fed LF diets, possibly attributable to unfavorable ruminal conditions for fiber-digesting microorganisms, such as lower ruminal pH (Kalscheur et al., 1997; Yang et al., 2001b). However, our data indicated no dietary effect on total-tract digestibility for NDF and ADF. Lower

NDF intake and greater ruminal retention time may have allowed cows fed the LF0DG diet to digest fiber even though lower ruminal pH was unfavorable for fiber-digesting microorganisms compared with cows fed HF diets. However, cows fed HF18DG digested numerically greater amounts of NDF and ADF (NDF and ADF kg digested per d) compared with cows fed the other diets. This could be possibly attributed to higher ruminal pH, which favors fiber-digesting microorganisms. Also, greater digestibility of EE contributed a significant portion of required energy for cows fed HF and 18DG diets compared with cows fed LF and 0DG diets, respectively. The HF18DG diet exhibited the greatest amount of EE digested (kg digested per d), indicating that HF dietary conditions were conducive to digestibility of the unsaturated fatty acid load from DDGS. Because rumen microorganisms perform extensive metabolism of lipids, existing ruminal conditions such as ruminal pH may have affected the variation in the digestibility and amount of digested EE (Harfoot and Hazlewood, 1997). Lower ruminal pH observed in cows fed LF diets may have negatively affected the extent of lipid hydrolysis (Van Soest and Demeyer, 1996a, b; Harfoot and Hazlewood, 1997), thus decreasing the total-tract digestibility of EE. The reason for improved EE digestibility among cows fed 18DG compared with 0DG diets is not known.

Lactation Performance

We observed higher SEM values for milk production and milk component data in the current study than in our parallel production study with 12 lactating cows in mid lactation with an initial DIM of 107 ± 17 (Ranathunga et al., 2018). We conducted the production study during the same time frame as the current study and used the same experimental design and same diets to feed cows. However, none of the statistical interpretations of dietary effect on milk production and milk components in the current study were different compared with the other study.

Low-forage and high-concentrate diets often result in greater milk yield (Oba and Allen, 2000, 2003; Voelker Linton and Allen, 2007). In our study, we observed no dietary effect on milk and energy-corrected milk, indicating that all cows spent similar amounts of energy to produce milk. Inclusion of DDGS in lactating dairy cow diets has variable effects on milk yield. Meta-analyses of previous studies on feeding distillers grains (both wet and dried) to lactating cows and reviews of distillers grains indicated that cows fed diets containing distillers grains produced either similar or improved amounts of milk compared with cows fed control diets with 0%

distillers grains (Schingoethe et al., 2009; Hollmann et al., 2011; Kalscheur et al., 2012; Paz et al., 2013), as we observed in our study. Also, our present data support our previous data on partial replacement of ground corn and soybean feeds with DDGS, demonstrating that diets formulated with DDGS were able to sustain milk production compared with a control diet including 0% DDGS (Ranathunga et al., 2010).

Feeding diets with LF with the higher unsaturated fat from the DDGS is thought to cause MFD. In the current study, milk fat concentration and yield for cows fed LF diets decreased by 17.5 and 19.6%, respectively, compared with cows fed HF diets. This agrees with previously reported associations of MFD with LF diets (Griinari et al., 1998; Peterson et al., 2003; Rico and Harvatine, 2013). Our data demonstrated that adequate physically effective fiber is necessary to maintain higher ruminal pH. Limited fiber degradation in LF diets may limit precursors for fat synthesis, such as acetate and butyrate. Also, incomplete biohydrogenation of polyunsaturated fatty acid in the rumen may inhibit *de novo* fatty acid synthesis (Bauman and Griinari, 2001). Another study demonstrated that elevated ruminal propionate concentration associated with LF diets may have a role in MFD (Maxin et al., 2011). Contrary to LF diets, HF diets maintain normal rumen conditions and ferment the fiber fraction to precursors of acetate and butyrate. Also, increased milk fat concentration, along with numerically high milk fat yield, indicated increased biohydrogenation of unsaturated FA when DDGS was fed with HF diets, and this combination exhibited no negative effect on *de novo* FA synthesis.

CONCLUSIONS

This study demonstrated that forage and DDGS inclusion affected the chemical characteristics of diets and nutrient availability in the rumen. Cows fed LF diets exhibited lower ruminal pH, which we attribute to greater ruminal degradation of rapidly degrading carbohydrates. Under such ruminal conditions, fiber digestion for cows fed LF diets may have negatively affected the fiber fraction so that it was retained in the rumen for a longer time. By contrast, cows fed HF diets may have maintained a higher ruminal pH and increased ruminal fiber digestion. Greater solid passage rates for HF diets indicated that fiber was retained in the rumen for a shorter period. However, total-tract digestibility of the nutrients was not affected by diet, except for EE. These results demonstrated that providing forage NDF at 17% on a DM basis can decrease milk fat concentration, whereas providing forage NDF at 25% DM basis can sustain milk fat concentration. Additionally, feed-

ing DDGS at 18% DM basis to lactating dairy cows did not affect milk fat concentration or yield when included at either forage NDF concentration.

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