Ration formulations containing reduced-fat dried distillers grains with solubles and their effect on lactation performance, rumen fermentation, and intestinal flow of microbial nitrogen in Holstein cows

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ABSTRACT

Sixteen multiparous lactating Holstein cows were used in 2 experiments to evaluate the effects of reduced-fat dried distillers grains with solubles (RFDG) on milk production, rumen fermentation, intestinal microbial N flow, and total-tract nutrient digestibility. In experiment 1, RFDG was fed at 0, 10, 20, or 30% of diet dry matter (DM) to 12 noncannulated Holstein cows (mean $\pm$ standard deviation: 89 $\pm$ 11 d in milk and 674 $\pm$ 68.2 kg of body weight) to determine effects on milk production. In experiment 2, the same diets were fed to 4 ruminally and duodenally cannulated Holstein cows (mean $\pm$ standard deviation: 112 $\pm$ 41 d in milk; 590 $\pm$ 61.14 kg of body weight) to evaluate the effects on rumen fermentation, intestinal flow of microbial N, and total-tract nutrient digestibility. In both experiments, cows were randomly assigned to 4 $\times$ 4 Latin squares over 21-d periods. Treatments (DM basis) were (1) control (0% RFDG), (2) 10% RFDG, (3) 20% RFDG, and (4) 30% RFDG. Feed intake and milk yield were recorded daily. In both experiments, milk samples were collected on d 19 to 21 of each period for analysis of milk components. In experiment 2, ruminal pH was measured; samples of rumen fluid, duodenal digesta, and feces were collected on d 18 to 21. Microbial N was estimated by using purines and DNA as microbial markers. Milk yield was not affected by treatment and averaged 34.0 $\pm$ 1.29 kg/d and 31.4 $\pm$ 2.81 kg/d in experiments 1 and 2, respectively. Percentage of milk protein tended to increase in experiment 1; estimates were 3.08, 3.18, 3.15, and 3.19 $\pm$ 0.06% when RFDG increased from 0 to 30% in the diets. However, milk protein concentration was not affected in experiment 2 and averaged 3.02 $\pm$ 0.07%. Percentage of milk fat

was not affected and averaged 3.66 $\pm$ 0.05% and 3.25 $\pm$ 0.14% in experiments 1 and 2, respectively. Total ruminal volatile fatty acids and ammonia concentrations were not affected by treatment and averaged 135.18 $\pm$ 6.45 mM and 18.66 $\pm$ 2.32 mg/dL, respectively. Intestinal microbial N flow was not affected by treatment; however, purines yielded higher estimates of flow compared with DNA markers. When averaged across treatments, intestinal flow of microbial N was 303 and 218 $\pm$ 18 g of N/d, using purines and DNA as the markers. Dry matter, organic matter, neutral detergent fiber, and nonfiber carbohydrate digestibility tended to increase with increasing inclusion of RFDG. Results from these experiments indicate that dairy rations can be formulated to include up to 30% RFDG while maintaining lactation performance, volatile fatty acids concentration, and intestinal supply of microbial N.

Key words: distillers grains with solubles, lactation, intestinal microbial N, total-tract nutrient digestibility

INTRODUCTION

Over the last decade, the supply of corn milling by-products such as dried distillers grains with solubles (DDGS) has increased (Klopfenstein et al., 2008; Schingoethe et al., 2009). Over that time, extensive research has been conducted to evaluate the effect of this byproduct on lactational performance of dairy cows. A wide array of publications has reported that DDGS can be effectively fed to dairy cows while maintaining milk yield and composition. For example, Ranathunga et al. (2010) reported that, compared with a control diet, the inclusion of 21% DDGS in the ration had no negative effect on milk production or composition. Furthermore, Janicek et al. (2008) reported a linear increase in milk production when DDGS were included from 0 to 30% in dairy diets. Anderson et al. (2006) demonstrated that dairy rations may contain up to 20% DDGS, replacing an equal amount of both ground corn and soybean

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meal, and maintain or enhance milk production. Further research is needed to study effects not only on lactation performance, but also on rumen parameters, nutrient utilization, and intestinal microbial N supply. Recently, the corn milling industry has found new uses for corn oil, which is partially removed from the solubles stream by physical and chemical techniques (e.g., centrifugation or solvent extraction; Majoni et al., 2011); the resulting byproduct is commonly referred to as reduced-fat DDGS (**RFDG**; Mjoun et al., 2010a). With centrifugation technology, the RFDG product contains approximately 5.5% fat. Although the loss of fat may result in a product with less energy, it also contains a lower concentration of PUFA and thus may also lower the potential risk for the development of milk fat depression (Bauman and Griinari, 2003).

Several experiments have evaluated the effect of DDGS on microbial crude protein (**MCP**) synthesis. For example, Kelzer et al. (2009) reported that MCP synthesis was similar for cows fed a control diet with no DDGS, a diet containing 15% DDGS, or a diet containing 15% high-protein DDGS. Chibisa et al. (2012) demonstrated that wheat DDGS may substitute for canola meal as the major protein source in dairy cow diets without negatively affecting MCP production. Furthermore, Janicek et al. (2008) reported that MCP synthesis of cows consuming 30% DDGS was similar compared with those consuming a diet containing no DDGS. Most of these studies have measured MCP by using purine derivatives as a microbial marker and measuring the concentration of this marker in the urine. Although the use of purine derivatives represents a noninvasive method to predict microbial N flow (Harmon and Richards, 1997), one limitation of using this method is thought to be the limited accuracy of the equations for prediction purposes (Firkins et al., 2006). In the present study, microbial N flow was measured in 2 ways: by the use of either purine bases or DNA as microbial markers. The objectives of these experiments were to evaluate the effects of increasing levels of RFDG on lactation performance, rumen fermentation, intestinal flow of microbial N, and total-tract nutrient digestibility; and second to compare the estimates of microbial N using purine bases or DNA as microbial markers. We hypothesize that RFDG can be included in dairy rations with no negative effects on lactation performance, rumen fermentation, intestinal flow of microbial N, or total-tract nutrient digestibility.

MATERIALS AND METHODS

This study was composed of 2 separate experiments. Experiment 1 was conducted to evaluate the effects of RFDG on lactation performance and was conducted at

the Agriculture Research and Development Center of University of Nebraska-Lincoln (Mead). Experiment 2 was conducted to evaluate the effects of RFDG on rumen parameters, intestinal supply of microbial N, and total-tract nutrient digestibility and was conducted in the Dairy Metabolism Facility located in the Animal Science Complex of University of Nebraska-Lincoln (Lincoln). All experimental procedures were approved and cows were cared for according to the guidelines stipulated by the University of Nebraska Institutional Animal Care and Use Committee.

Experiment 1: Experimental Design, Animal Care, and Sampling

Twelve lactating multiparous Holstein cows (mean and SD, 89 ± 11 DIM and 674 ± 68.2 kg of BW) were blocked by milk yield and DIM and randomly assigned to treatment sequences in replicated balanced 4×4 Latin squares according to the method of Kononoff and Hanford (2006) with 21-d periods. The first 14 d of each period were considered an adaptation period and the remaining 7 d were used for data collection. Cows were housed in individual tiestalls equipped with rubber mats and were offered 1 of 4 treatments that differed by the level of RFDG (Poet Nutrition, Sioux Falls, SD) inclusion: (1) control (**CON**), 0% RFDG; (2) 10% RFDG; (3) 20% RFDG; and (4) 30% RFDG. The reported chemical composition of treatments is based upon analyzed chemical composition of individual feed ingredients. Treatments were formulated with the CPM-Dairy model (version 3.0.8.1; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY). A diet (**CON**) not containing RFDG was formulated and designed to be representative of a diet fed in the Great Plains of the United States. Then, a portion of forage, corn, cottonseed, and soy-based feeds were reduced to reach the greatest inclusion of RFDG, 30% of diet DM. Practically, formulations that substitute several ingredients are common when attempts are made to include byproducts. Consequently, formulations were similar in CP but the inclusion of RFDG resulted in a lower concentration of energy, as estimated by the CPM-Dairy model.

Cows were milked using a double-5 herringbone parlor (BouMatic LLC, Madison, WI) at 0730 and 1930 h, milk production was recorded, and milk samples were collected during the a.m. and p.m. milkings of d 19, 20, and 21. Milk samples were stored at room temperature in a vial containing a preserving pellet (2-bromo-2-nitropropane-1,3 diol,) provided by the DHIA (Manhattan, KS). Samples were then analyzed for fat, lactose, and true protein by near-infrared spec-

troscopy (Bentley 2000 Infrared Milk Analyzer; Bentley Instruments, Chaska, MN) by Heart of America DHIA (Manhattan, KS). Chemical methodology based on a modified Berthelot reaction was used to determine MUN concentration (ChemSpec 150 Analyzer; Bentley Instruments). During the last 7 d of each period, daily DMI and milk yield were averaged.

Cows were individually fed once daily at 0930 h to allow for approximately 5% refusals using a Calan Data Ranger (American Calan Inc., Northwood, NH). Cows were allowed access to feed and fresh water at all times except during milking. Forages were premixed using a 354-12B mixer wagon (Roto-Mix LLC, Dodge City, KS). A control concentrate mix and an RFDG-concentrate mix were prepared. On the farm, the control- or RFDG-concentrate mix was added to the Calan Data Ranger after addition of the premixed forages to achieve 0 or 30% RFDG in the TMR. The intermediate diets were prepared by combining the control- and the RFDG-concentrate mix to achieve 10 or 20% RFDG in the TMR. Feedorts were collected and weighed daily to estimate intake for individual cows. Samples of corn silage, alfalfa haylage, alfalfa hay, brome hay, cottonseed, each concentrate mix, RFDG, and each TMR were collected on d 20 and 21 of each experimental period and stored at -20°C until analysis. Dry matter concentrations of corn silage and alfalfa haylage were determined weekly by heating samples at 100°C for 24 h. Using the resulting DM data, diets were then adjusted to ensure proper inclusion of ingredients in each treatment. Body condition score (1 to 5 scale) and BW were measured on d 20 and 21 of each period. Body condition score was measured by a single, trained individual, and the scoring method used was similar to that of Wildman et al. (1982), but reported to the quarter point.

Experiment 2: Animal Surgeries, Experimental Design, Animal Care, and Sampling

The second experiment was conducted separately after completion of experiment 1. Before initiation of this experiment, 4 multiparous Holstein cows averaging 172 d of gestation were fitted with T-shaped duodenal cannulas. Once cows had completely recovered from duodenal surgeries (approximately 60 d postsurgery), they were fitted with ruminal cannulas. Within the first 2 wk of the following lactation, these cows were transferred to the Dairy Metabolism Facility in the Animal Science Complex of University of Nebraska-Lincoln. At the start of the experiment cows averaged (mean $\pm$ SD) 112 ± 41 DIM and 590 ± 61.14 kg of BW.

Duodenal Cannulations. Duodenal cannulations were performed according to the procedures described by Robinson et al. (1985). Briefly, the right paralumbar

fossa was anesthetized with a paravertebral nerve block using a modification of the Farquharson technique (Turner and McIlwraith, 1989). The right dorsal and ventral branches of the thoracic 13, lumbar 1, and lumbar 2 were blocked with 20 mL of 2% lidocaine hydrochloride (Agri Laboratories Ltd., St Joseph, MO). In addition, recumbent anesthesia and sedation was induced (Anderson and Rings, 2009).

Upon becoming recumbent, the animal was positioned in left lateral recumbency with legs restrained forward and back. A 15-cm skin incision was made in the cranial paralumbar fossa parallel to the last rib with a #20 scalpel. The external abdominal oblique, internal abdominal oblique, and transversus muscles and peritoneum were incised by sharp dissection. Upon entry to the abdomen, the pylorus and proximal 20 cm of the cranial part of the duodenum were exteriorized and separated from the remainder of the incision by moist towels. Site of cannula insertion was centered approximately 10 cm distal to the pylorus. The omentum was elevated from the duodenum for a distance corresponding to the length of the cannula barrel. Care was taken to preserve major branches of duodenal vessels, thus minimizing effects of cannulation on blood flow to the area. A 5- to 6-cm enterotomy was made along the right side of the duodenum in the area previously freed of omentum. The body of the double T-shaped cannula (Bar Diamond Inc., Parma ID) was inserted into the intestinal lumen. The enterotomy was closed adjacent to the barrel of the cannula with 2-0 polydioxanone in a simple interrupted pattern. The cut edges of the enterotomy were inverted and 2 purse-string sutures of the same type of suture material were placed snugly around the cannula barrel. In preparation for cannula exteriorization, a 2-cm-diameter piece of skin was removed from the right body wall in the 10th intercostal space at the level of the frontal plane of the shoulder joint. The cannula barrel was pushed through the body wall at this site and the outer collar was inserted to hold the cannula in place. The peritoneum and transversus were apposed in a simple continuous pattern with #3 chromic gut on a 1/2 circle taper needle. The internal and external abdominal muscles were apposed as a single layer in a simple continuous pattern with #3 gut on a 1/2 circle taper needle. The skin edges were apposed in a Ford continuous interlocking pattern with #3 Braunamid suture (Aesculap Ag & Co. a division of B. Braun, Melsungen, Germany) with an S-curved needle.

Cows were hospitalized and closely monitored until they recovered from the surgical procedure. Body temperature was taken once every day during the 10 d postsurgery. A single dose of 25 mL of Excenell or Exceed (Pfizer Inc., New York City, NY) was ad-

ministered when body temperature exceeded normal range.

Ruminal Cannulations. Ruminal cannulations were conducted according to the procedure described by Noordsy and Ames (2006). Briefly, cows were restrained in a head gate with restraint chute. The left paralumbar fossa was anesthetized by blocking the left dorsal and ventral branches of the thoracic 13, lumbar 1, and lumbar 2 with 20 mL of 2% lidocaine hydrochloride (Agri Laboratories Ltd.).

After anesthesia was applied, a 17-cm linear skin incision was performed to place a 4-inch rumen cannula (Bar Diamond Inc.) in the middle of the paralumbar fossa with a #20 scalpel. The external abdominal oblique, internal abdominal oblique, and transversus muscles were incised parallel to their muscle fibers with sharp or blunt dissections. The peritoneum was entered by blunt dissection and then the peritoneal opening was extended to the size of the skin opening by sharp dissection. The rumen serosa was secured to the underside of the skin with interrupted horizontal mattress sutures. The rumen was resected with a 1-cm margin from the suture line; the cannula was inserted and closed with a plug. Then, cows were returned to their normal diet and closely monitored during the 10 d postsurgery.

Housing of Cannulated Cows. Cows were housed in individual tiestalls having dimensions of 164 cm $\times$ 111 cm that were equipped with rubber mats. At the initiation of the trial, BW of cows was recorded; however, BW was not recorded during the experiment because of difficulties experienced when handling cows through the scale chute. Specifically, the scale chute was too narrow to handle the duodenally cannulated cows and there was a potential risk for cannula disruption by the panels of the chute. Cows were assigned to a single Latin square and randomly assigned to the experimental treatments. Rations were formulated to include the same levels of RFDG fed during experiment 1. Cows were milked at 0700 and 1800 h using individual Delpro 480 DeLaval portable milking units through a pipeline system (DeLaval International AB, Tumba, Sweden). Milk samples were taken on the a.m. and p.m. milkings of d 18, 19, 20, and 21 and processed as described in experiment 1. Cows were individually fed using a Calan Data Ranger; the feeding regimen and feed sample collection was conducted as described previously. Data on DMI and lactation performance were collected daily.

Measurement of Ruminal pH. Ruminal pH was measured according to the protocol described by Penner et al. (2006). Briefly, during d 18 through 21 of each period ruminal pH was continuously measured once every minute using a wireless pH probe (Dascor

Inc., Escondido, CA) placed into the ventral sac of the rumen of each cow. Each probe contained a data logger, 9 V battery, and an electrode cable housed in a watertight capsule. Two 900-g weights were fastened to the bottom of the probe to maintain the electrode in the ventral sac of the rumen. The pH probes were calibrated with pH buffers 4 and 7 and recorded before inserting them into the rumen and after downloading pH data collected into calculation spreadsheets at the end of each period. The initialization of the data logger and the transfer of data were performed using a Windows-compatible software package (Microsoft Corp., Redmond, WA) supplied by Dascor (M1b version 6.1.2h). Ruminal pH measurements were averaged across the 4 collection days so that a period of 24 h beginning at 0930 h was represented. This starting time point was chosen given that TMR were offered at 0930 h after cows returned from a sand-surface exercise pen, where they were temporarily transferred during daily stall cleaning and TMR mixing. From the averaged pH data, the minimum, maximum, and mean ruminal pH were calculated for each cow. Because ruminal pH measurements in this experiment did not reach levels considered to indicate subacute (5.8) or acute ruminal acidosis (5.2; Aschenbach et al., 2011), thresholds of 6.5 and 6.3 were selected as reference levels, and the time and area below these values were computed and are presented.

Ruminal Fluid and Intestinal Digesta Sampling. Ruminal fluid and intestinal digesta were collected on d 18 through 21 every 4 h in each experimental period. Whole rumen contents were collected from 4 different regions of the rumen (caudal ventral sac, cranial ventral sac, and 2 samples from the feed mat in the dorsal rumen) and composited. Rumen contents were then strained through 4 layers of cheesecloth; 40 mL of strained rumen fluid was placed in a 45-mL vial and immediately frozen at -20°C for later analyses for ruminal VFA and ammonia-N. Intestinal digesta contents (200 mL) were collected and placed in 250-mL Nalgene bottles (Thermo Scientific Inc., Waltham, MA). Then, samples were composited by cow by day within period and frozen at -20°C for subsequent analyses of intestinal flow of microbial N. Collection time of ruminal fluid and intestinal digesta samples was advanced 1 h in subsequent collection day, so that every 60-min interval in a 24-h period was represented (6 samples per cow per day and a total of 24 samples per cow per period). Specifically, samples were collected on d 18 at 0700, 1100, 1500, 1900, 2300, and 0300 h; on d 19 at 0800, 1200, 1600, 2000, 0000, and 0400 h; on d 20 at 0900, 1300, 1700, 2100, 0100, and 0500 h; and on d 21 at 1000, 1400, 1800, 2200, 0200, and 0600 h.

Collection of Ruminal Bacteria, Ruminal Protozoa, and Yeast Sample. On d 20 and 21 of each experimental period, 1.5 L of whole ruminal digesta contents was collected from 4 different locations within the rumen at 1000 and 1600 h (d 20) and 1200 and 1800 h (d 21). Samples comprised equal volumes of rumen contents taken from the caudal ventral sac, cranial ventral sac, and 2 samples from the feed mat in the dorsal rumen. Then, mixed ruminal bacteria were isolated according to the procedure described by Hristov et al. (2005). Briefly, whole ruminal contents were composited and squeezed through 2 layers of cheesecloth and the filtrate was retained. Solids remaining on the cheesecloth were added to a volume of cold buffer (McDougall, 1948) equal to the volume of filtrate, and shaken manually in a screw-capped jar to dislodge the ruminal microorganisms loosely associated with feed particles. This suspension was then squeezed through 2 layers of cheesecloth and the 2 filtrates were combined (1:1) and preserved with 5% (vol/vol) formalin. From this sample, bacteria were harvested via differential centrifugation (Hristov and Broderick, 1996) with an initial low-speed centrifugation at $400 \times g$ for 5 min at 4°C and a subsequent high-speed centrifugation at $20,000 \times g$ for 15 min at 4°C. Samples were maintained on ice while being processed. Then, the supernatant was discarded and the isolated bacterial pellets were composited by cow and period and frozen at -20°C for later analyses. Rumen protozoa were isolated using a separation funnel according to the procedure described by Shabi et al. (2000). Briefly, the strained ruminal digesta were mixed with 1 volume of warm 0.9% saline and held in a separation funnel for 1.5 h at 39°C. The precipitate was then removed. The protozoal pellet was mixed with 500 mL of warm saline and kept in a separation funnel for another 1.5 h at 39°C. Protozoa were then collected and frozen at -20°C. A sample of dried yeast cells (*Saccharomyces cerevisiae*) utilized in the ethanol production process was obtained and frozen at -20°C until analysis.

Collection of Fecal Samples. Fecal grab samples (approximately 250 g) were taken every 8 h on d 18 to 21 of each period (3 samples per cow per day and a total of 12 samples per cow per period). Samples were then composited by cow, by day, within period. Then, samples were stored at -20°C for later analysis of Cr₂O₃ and chemical composition.

Analysis of Feed, Rumen Fluid, Duodenal Digesta, and Fecal Samples

Feed Particle Size and Feed Chemical Analysis. The Penn State Particle Separator was used to measure the distribution of particle size of TMR according to

Heinrichs and Kononoff (2002). Collected feed ingredients and TMR samples were dried for 48 h at 60°C in a forced-air oven, ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA) and analyzed for chemical composition by an external laboratory (Cumberland Valley Analytical Services, Hagerstown, MD), which included DM (method no. 930.15; AOAC International, 2000), N (method no. 990.03; Leco FP-528 Nitrogen Combustion Analyzer, Leco Corp. St. Joseph, MI), NDF (Van Soest et al., 1991), starch (Hall, 2009), ether extract using diethyl ether (method no. 2003.05; AOAC International, 2006), ash (method no. 942.05; AOAC International, 2000) and phosphorus by inductively coupled plasma emission spectroscopy (method no. 985.01; AOAC International, 2000). In addition, the concentration of yeast (*S. cerevisiae*) protein was measured from subsamples of collected RFDG. This was conducted by using DNA markers as described by Castillo-Lopez et al. (2010).

Ruminal VFA Analysis. At the end of experiment 2, 10 samples of rumen fluid collected from each cow in each period (over the course of the 4 d and representing a 24-h period) were analyzed for VFA by an external laboratory (Rumen Fermentation Profiling Laboratory, West Virginia University, Morgantown). Specifically, rumen samples analyzed for VFA were those collected at 1000, 1100, 1200, 1400, 1600, 1800, 2100, 0000, 0400, and 0900 h. Briefly, analysis of VFA concentrations in effluents was performed according to the gas chromatographic separation procedure (Supelco Inc., 1975). The gas chromatograph was a Varian model 3300 with a flame-ionization detector (Varian Inc., Palo Alto, CA). The column was a 2-m $\times$ 2-mm glass column packed with 10% SP-1200/1% H₃PO₄ on 80/100 Chromosorb WAW (Supelco Inc., Bellefonte, PA).

Ruminal Ammonia Analysis. A portion of the 10 samples of rumen fluid analyzed for VFA were also analyzed for ammonia-N concentration using a phenol-hypochlorite assay (Yang and Varga, 1989). Briefly, 1.5 mL of rumen fluid was centrifuged at $11,000 \times g$ for 20 min at 4°C and then 40 μ L of supernatant was taken in duplicate and combined with 2.5 and 2.0 mL of phenol color reagent and alkaline hypochlorite reagent, respectively. After incubation for 10 min at 37°C, 300 μ L of sample was taken and placed in a 96-well polystyrene plate (Becton Dickinson and Company, Franklin Lakes, NJ) and absorbance was read at 550 nm. Analysis was repeated whenever the coefficient of variation between duplicates exceeded 4.9%.

Intestinal Flow of Microbial N Using Purines as Microbial Marker. Collected duodenal contents were lyophilized and ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas). Then, ground samples were analyzed for DM (100°C oven for

24 h). Subsamples of isolated ruminal bacterial pellets were ground with a mortar and pestle and analyzed for N (AOAC International, 2006; method no. 990.03; Leco FP-528 Nitrogen Combustion Analyzer, Leco Corp.).

Purines (Zinn and Owens, 1986; Broderick and Merchen, 1992) were used as a microbial marker to measure duodenal flow of total microbial N. The analysis of purines was conducted according to the procedure described by Makkar and Becker (1999). Briefly, approximately 50 mg of lyophilized microbial and duodenal samples were placed in Pyrex screw-cap tubes. Then, samples were combined with 0.5 mL of allopurinol internal standard (3 mM allopurinol) and 2.5 mL of 0.6 M HClO₄ and incubated for 1 h. After samples cooled, they were combined with 7.5 mL of 10 mM NH₄H₂PO₄, and the pH was adjusted to between 6.6 and 6.9 using concentrated KOH (8 M). After centrifugation of samples (3,000 × *g* for 10 min), the supernatant was filtered through a 13-mm disposable syringe filter (0.45-μm pore size) with a GHP membrane (Whatman Inc., Florham Park, NJ) into an HPLC vial. Total purines were measured using a 717 HPLC system (Waters Corp. Inc., Milford, MA). Calculation of microbial N was based on the ratio of purine:N obtained from the isolated rumen bacterial pellet and on the concentration of purines in duodenal samples.

Intestinal Flow of Microbial N Using DNA as Microbial Marker. The use of DNA markers for the estimation of microbial N flow has been reported elsewhere (Belanche et al., 2011b). In this study, the sum of bacterial and protozoal N was considered as microbial N. Separate estimates of bacterial N and protozoal N were measured based on (1) the ratio of DNA marker:N in the isolated bacterial or protozoal pellet and (2) the abundance of the targeted bacterial or protozoal DNA marker measured from duodenal samples. From those values, the amount of N originating from bacteria and protozoa was calculated. To do so, subsamples of isolated bacterial and protozoal pellets were analyzed for DM by heating samples at 100°C for 24 h (method no. 930.15; AOAC International, 2000) and N (method no. 990.03; AOAC International, 2006; Leco FP-528 Nitrogen Combustion Analyzer, Leco Corp.). Then, DNA was extracted from the remaining portion of the pellets and from digesta samples according to the extraction method for PCR-quality DNA from digesta samples described by Yu and Morrison (2004). The concentration of DNA (ng/μL) in each sample was measured by spectrophotometry (NanoDrop ND-1000 Spectrophotometer, NanoDrop Technologies Inc., Wilmington, DE) and stored at -20°C in aliquots of 25 μL for later analysis using real-time PCR. The same approach was conducted for the calculation of yeast-based N flow.

Bacterial, protozoal, and yeast DNA markers utilized, real-time PCR reactions, and calculations of marker abundance have been described elsewhere (Yu et al., 2005; Castillo-Lopez et al., 2013). Briefly, for each microbial type, 4 μL of DNA sample was combined with 1 μL of 10 μM forward primer, 1 μL of 10 μM reverse primer, 0.25 μL of 10 μM TaqMan probe, 7.5 μL of TaqMan Master Mix (Applied Biosystems, Foster City, CA), and 1.25 μL of nanopure water. Two samples with no DNA were included and used as nontemplate controls. Each sample was run in duplicate in separate wells of the 384-well, real-time PCR plate.

Duodenal Digesta Flow and Total-Tract Digestibility Measurements. Chromic oxide (Cr₂O₃) was used as an external marker. Briefly, 7.5 g of Cr₂O₃ was weighed (Taylor and Allen, 2005) and placed in gelatin capsules (Torpac Inc., Fairfield, NJ). Then, the capsules were dosed into the rumen via the ruminal cannula twice daily at 0700 and 1900 h from d 10 through 21 of each experimental period. Because of greater than expected variation obtained in the concentration of Cr₂O₃ in duodenal samples, indigestible ADF (**iADF**) was used as the digesta marker (Huhtanen et al., 1994) for the estimation of intestinal DM and microbial N flow. The use of iADF as a single digesta marker was based on reports made by other researchers (Kelzer et al., 2009; Ramirez-Ramirez et al., 2011). The TMR and duodenal samples were weighed (2 g) into 5 × 10 cm Dacron bags with 50-μm pores (No. R510; Ankom Technology, Fairport, NY). The concentration of iADF in samples was determined after a 288-h in situ incubation in the rumen using 2 cows being fed a diet containing 60% forage and 40% concentrate. To estimate fecal output and total-tract nutrient digestibility, however, Cr₂O₃ (Harvatine et al., 2002; Sylvester et al., 2005) was used as the digesta marker and yielded fairly consistent values. Collected fecal samples were composited by cow, by day, within period. Samples were oven-dried (60°C for 48 h) and ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas Co.). Dried fecal samples were analyzed by an external laboratory for chemical composition (Cumberland Valley Analytical Services, Hagerstown, MD). Fecal samples were analyzed for chromium concentration by an external laboratory (Servi-Tech Laboratories, Hastings, NE) by sample digestion in 10 mL of nitric acid and 3 mL of peroxide, with a hydrochloric acid addition and analyzed by inductively coupled plasma emission spectroscopy using a Varian 720-ES spectrophotometer (Varian Inc.). Digesta flow to the duodenum or rectum was calculated based on the amount of the marker fed (iADF) or dosed (Cr₂O₃) and the concentration of the respective marker in duodenal or fecal sample (Harvatine et al., 2002). Apparent total-tract digestion of

DM, OM, NFC, NDF, N, and P were calculated as follows: [(intake of nutrient – fecal output of nutrient)/intake of nutrient] × 100 (May et al., 2010).

Experiment 1: Statistical Analysis

Performance data were analyzed as replicated balanced 4 × 4 Latin squares using the MIXED procedures of SAS (version 9.1, SAS Institute Inc., Cary, NC). Fixed model effects included square, period within square, and treatment, and the random effect was cow within square. Linear and quadratic effects of treatments were tested. The model for this experiment was as follows:

$$Y_{ijkm} = \mu + \tau_m + \beta(\tau)_{im} + \rho(\tau)_{jm} + \alpha_k + \varepsilon_{ijkm},$$

where Y_{ijkm} represents observation $ijkm$; μ represents the overall mean; τ_m represents the fixed effect of square m ; $\beta(\tau)_{im}$ represents the random effect of cow i within square m ; $\rho(\tau)_{jm}$ represents the fixed effect of period j within square m ; and α_k represents the fixed effect of treatment k . The residual term ε_{ijkm} was assumed to be normally, independently, and identically distributed, with variance σ_e^2 .

Experiment 2: Statistical Analysis

Data collected on lactation performance, rumen pH, and duodenal flow were analyzed as a 4 × 4 Latin square using the MIXED procedure of SAS (version 9.1; SAS Institute Inc.). Fixed model effects included treatment and period, with cow as the random effect. The model for this experiment was as follows:

$$Y_{ijk} = \mu + \beta_i + \rho_j + \alpha_k + \varepsilon_{ijk},$$

where Y_{ijk} represents observation ijk ; μ represents the overall mean; β_i represents the random effect of cow i ; ρ_j represents the fixed effect of period j ; and α_k represents the fixed effect of treatment k . The residual term ε_{ijk} was assumed to be normally, independently, and identically distributed, with variance σ_e^2 .

For experiment 2, repeated measurements of rumen ammonia and VFA concentration were analyzed by including a REPEATED model statement, and linear and quadratic effects of treatments were also tested. Treatment means are presented as least squares means and the largest standard error of the mean (SEM) is reported. Statistical significance was declared at $P \leq 0.05$ and tendency was declared if $P > 0.05$ and ≤ 0.15 .

RESULTS

Feed Chemical Composition and TMR Particle Size Distribution

The ingredient composition of experimental treatments fed in experiments 1 and 2 is listed in Table 1. The chemical composition of those treatments is listed in Table 2. Table 3 lists the chemical composition of feed ingredients utilized in the formulation of treatments. Rations contained similar levels of CP and fat. However, the level of NDF increased from 34 to 37% with the highest inclusion of RFDG in the diets. In contrast, the percentage of starch decreased from 26 to 17% with increasing levels of RFDG in treatments. The particle size distribution of dietary treatments fed in both experiments is listed in Table 4. In experiment 1, a linear decrease ($P < 0.01$) of TMR particle size was observed with the inclusion of RFDG. Specifically, the proportion of particles >19.0 mm decreased from 9.38 to 6.75%, the proportion of particles between 19.0 and 8.0 mm decreased from 25.58 to 19.89%, and the proportion of particles between 8.0 and 1.18 mm decreased from 37.86 to 30.20%. However, the proportion of particles <1.18 mm increased from 27.16 to 43.14%. In experiment 2, a similar pattern in TMR particle size distribution was observed. We found a linear decrease ($P < 0.05$) of TMR particle size with the inclusion of RFDG. Specifically, the proportion of particles >19.0 mm decreased from 6.50 to 3.64%, the proportion of particles between 19.0 and 8.0 mm decreased from 26.20 to 18.01%, and the proportion of particles between 8.0 and 1.18 mm decreased from 39.10 to 32.06%. However, the proportion of particles <1.18 mm increased from 27.51 to 46.35% when the inclusion of RFDG increased from 0 to 30%.

DMI, BW, BCS, and Lactation Performance

Data collected in experiment 1 on DMI, BW, BCS, and lactation performance are listed in Table 5. Table 6 lists DMI and lactation performance data collected in experiment 2. In experiment 1, total DMI increased linearly ($P < 0.01$) with the inclusion of RFDG. Total DMI was 25.0, 23.8, 25.9, and 27.9 ± 1.38 kg/d for CON, 10, 20, and 30% RFDG, respectively. In this experiment, an increase in BW ($P < 0.01$) and BCS ($P = 0.05$) was also observed with the inclusion of RFDG in diets. Average BW was 687.2, 687.8, 693.4, and 696.7 ± 18.4 kg, and BCS was 3.06, 3.10, 3.14, and 3.18 ± 0.06 for CON, 10, 20, and 30% RFDG, respectively. In experiment 2, total DMI was not affected ($P = 0.73$) by the inclusion of RFDG, which averaged 21.56 ± 1.65 kg/d across treatments.

Table 1. Ingredient composition (% of DM) of formulated diets containing increasing levels (0 to 30%) of reduced-fat dried distillers grains with solubles (RFDG) fed in experiment 1 and 2

Ingredient	Treatment			
	Control (0%)	10% RFDG	20% RFDG	30% RFDG
RFDG ¹	—	9.7	19.2	29.4
Corn silage	21.4	20.7	20.3	18.7
Alfalfa haylage	9.7	8.9	7.8	6.6
Brome hay	4.9	5.2	5.3	5.5
Alfalfa hay	9.7	8.4	7.1	5.8
Cottonseed	7.8	5.9	4.5	3.0
Ground corn	21.8	17.8	13.8	10.0
Soyhulls	9.6	11.5	13.3	15.5
Soypass ²	5.8	4.3	2.8	1.4
Soybean meal	6.0	4.0	2.0	—
Megalac ³	—	0.6	1.2	1.8
Bloodmeal	1.0	0.6	0.3	—
Limestone ground	1.05	1.05	1.04	1.05
Sodium bicarbonate	0.44	0.44	0.44	0.44
Salt	0.44	0.44	0.44	0.44
Magnesium oxide	0.23	0.23	0.23	0.23
Trace mineral premix ⁴	0.11	0.11	0.11	0.11
Vitamin premix ⁵	0.11	0.11	0.11	0.11

¹Poet Nutrition (Sioux Falls, SD).²LignoTech (Overland Park, KS).³Church & Dwight Co. Inc. (Princeton, NJ).⁴Contained 14% Ca, 0.03% P, 0.42% Mg, 0.20% K, 4.2% S, 0.08% Na, 0.03% Cl, 455 mg/kg Fe, 60,020 mg/kg Zn, 17,374 mg/kg Cu, 43,470 mg/kg Mn, 287 mg/kg Se, 527 mg/kg Co, 869 mg/kg I.⁵Formulated to supply approximately 120,000 IU/d of vitamin A, 24,000 IU/d of vitamin D, and 800 IU/d of vitamin E in total ration.

In experiment 1, milk yield was not affected ($P = 0.78$) by increasing levels of RFDG and averaged 34.1 ± 1.29 kg/d across treatments. The percentage of milk protein tended ($P = 0.07$) to increase with RFDG inclusion with estimates of 3.08, 3.18, 3.15, and $3.19 \pm 0.06\%$ for CON, 10, 20, and 30% RFDG treatments, respectively. The yield of milk protein, however, was similar ($P = 0.23$) across treatments, with an average of 1.05 ± 0.04 kg/d. The percentage ($P = 0.66$) and yield ($P = 0.53$) of milk fat were not affected by treatment and averaged $3.66 \pm 0.09\%$ and 1.24 ± 0.05 kg/d across treatments, respectively. Milk urea N was not affected ($P = 0.85$) by the inclusion of RFDG in diets and averaged 15.98 ± 0.86 mg/dL across treatments. In experiment 2, 1 extra period was added (a total of 5 periods) because 1 cow (consuming the 30% RFDG diet) was removed during the second experimental period because of the presence of excessive levels of foam in the rumen. Statistical differences for lactational performance were not expected; the primary reason for conducting experiment 2 was the study of rumen fermentation, microbial N flow to the duodenum, and major nutrient digestibility. In experiment 2, milk yield was not affected ($P = 0.65$) by increasing levels of RFDG and averaged 31.43 ± 2.81 kg/d across treatments. The percentage ($P = 0.35$) and yield ($P = 0.84$) of milk protein were not affected by RFDG and averaged $3.02 \pm 0.07\%$ and 0.95 ± 0.07

kg/d, respectively. In addition, the percentage ($P = 0.28$) and yield ($P = 0.23$) of milk fat were not affected by RFDG inclusion and averaged $3.25 \pm 0.14\%$ and 1.02 ± 0.11 kg/d across treatments, respectively. Milk urea N tended to increase ($P = 0.14$) according to the inclusion level of RFDG in diets with estimates of 12.64, 13.00, 13.07, and 13.42 ± 0.93 mg/dL, respectively.

Rumen VFA, Ammonia, and pH

Table 7 lists the concentration of VFA in the rumen. We found no significant effect ($P = 0.40$) of treatment on total rumen VFA concentration, with an average of 135.18 ± 6.45 mM across treatments. The molar proportion of acetate decreased ($P < 0.01$) and the molar proportion of propionate increased ($P < 0.01$) with the inclusion of RFDG in diets. We also observed a significant ($P < 0.01$) increase in the molar proportions of isobutyrate and valerate, whereas the molar proportion of butyrate tended to decrease ($P = 0.07$). However, no significant change was observed in the molar proportion of isovalerate. The acetate-to-propionate ratio in the rumen decreased with RFDG inclusion, with values of 2.98, 2.94, 2.52, and 2.39 ± 0.23 for CON, 10, 20, and 30% RFDG, respectively. The concentration of ruminal ammonia (Table 7) was similar ($P = 0.18$), with an average of 18.66 ± 2.32 mg/dL across treatments.

Table 2. Analyzed chemical composition (% of DM unless otherwise noted) of diets containing increasing levels (0 to 30%) of reduced-fat dried distillers grains with solubles (RFDG; Poet Nutrition, Sioux Falls, SD) fed in experiment 1 and 2

Chemical composition ¹ (% of DM)	Treatment			
	Control (0%)	10% RFDG	20% RFDG	30% RFDG
Experiment 1				
DM	71.3	72.0	73.3	75.3
CP	18.3	18.2	18.1	18.0
NDF	35.4	36.5	37.9	38.3
Starch	24.7	22.3	20.0	17.6
Ether extract	3.9	4.0	4.0	4.1
NFC ²	34.0	33.1	32.1	30.7
Ash	8.1	8.2	8.3	8.4
Phosphorous	0.36	0.41	0.46	0.52
ME ³ (Mcal/kg)	2.53	2.51	2.49	2.47
NE _L ³ (Mcal/kg)	1.63	1.62	1.60	1.59
RUP ³ (% of CP)	41.47	43.02	45.91	49.04
Experiment 2				
DM	73.4	74.2	75.9	76.2
CP	18.3	18.2	18.2	18.2
NDF	33.3	34.5	36.2	37.3
Starch	25.8	22.9	20.1	17.2
Ether extract	4.0	4.2	4.3	4.4
NFC ²	35.9	34.8	33.7	31.8
Ash	8.3	8.4	8.4	8.5
Phosphorous	0.35	0.40	0.44	0.51
ME ³ (Mcal/kg)	2.58	2.56	2.53	2.51
RUP ³	41.66	42.26	44.22	49.29

¹Calculated based on analysis of individual feed ingredient and the rate of inclusion to the diet.

²Calculated by difference: 100 – (% NDF + % CP + % fat + % ash).

³According to the CPM Dairy Ration Analyzer v3.0.8.1 (Cornell University, Ithaca, NY; University of Pennsylvania, Kennett

Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY).

Figure 1 illustrates ruminal pH measurements collected and averaged over the course of 4 d. In addition, Table 7 lists minimum, maximum, and mean ruminal pH values as well as time and area below pH 6.5 and 6.3. A decrease ($P = 0.02$) in mean ruminal pH was observed with RFDG inclusion, with estimates of 6.53, 6.49, 6.38, and 6.35 ± 0.12 for CON, 10, 20, and 30% RFDG, respectively. The time ($P < 0.01$) and area ($P = 0.03$) below pH 6.5 increased: estimates of time below pH 6.5 were 546, 834, 941, and $1,040 \pm 293$ min/d and estimates of area below pH 6.5 were 126, 158, 357, and 334 ± 92 pH $\times$ min/d, for CON, 10, 20, and 30% RFDG, respectively. Furthermore, the time below pH 6.3 increased ($P = 0.03$): estimates were 279, 382, 936, and 946 ± 208 min/d, respectively. The area below pH 6.3 tended to increase ($P = 0.13$), with estimates of 45, 47, 180, and 169 ± 64 pH $\times$ min/d, respectively.

Apparent Total-Tract Nutrient Digestibility of TMR

Total-tract digestibility of major nutrients tended to increase with RFDG inclusion (Table 8). Estimates of total-tract DM digestibility ($P = 0.14$) were 65.5, 65.4, 73.0, and $73.4 \pm 4.8\%$ for CON, 10, 20, and 30% RFDG, respectively. In the same order, estimates of

OM digestibility ($P = 0.14$) were 67.7, 67.7, 74.9, and $75.2 \pm 4.4\%$. Estimates of NFC digestibility ($P = 0.07$) were 89.7, 90.1, 92.6, and $92.7 \pm 1.2\%$. Estimates of NDF digestibility ($P = 0.11$) were 44.0, 43.2, 57.0, and $58.0 \pm 7.5\%$. Estimates of N digestibility ($P = 0.06$) were 64.3, 67.7, 74.7, and $76.9 \pm 5.1\%$. In addition, Table 8 lists the output of N and P. The output of N (kg/d) was 0.202, 0.197, 0.152, and 0.146 ± 0.02 ($P = 0.09$); the output of P (g/d) was 46, 54, 53, and 53 ± 7.1 ($P = 0.49$).

Intestinal Flow of DM, Total N, and Microbial N

Table 9 lists intestinal flow and apparent ruminal digestibility of DM as well as intestinal flow of total N and microbial N. Intestinal flow ($P = 0.41$) and apparent ruminal digestibility ($P = 0.38$) of DM were not affected by treatment and averaged 15.2 ± 1.4 kg/d and $29.8 \pm 3.2\%$, respectively. Intestinal flow of total N was not affected ($P = 0.55$) by treatment and averaged 594.7 ± 68.0 g/d. Likewise, the flow of microbial N was not affected by treatment when estimated using purines ($P = 0.47$) or DNA ($P = 0.65$) markers. However, when averaged across treatments, purines yielded higher ($P < 0.01$) microbial N flow compared with

Table 3. Analyzed chemical composition (mean $\pm$ SD) of feedstuffs used in the formulation of diets containing increasing levels of reduced-fat dried distillers grains with solubles (RFDG; Poet Nutrition, Sioux Falls, SD) fed in experiments 1 and 2

Component	Feed ingredient							
	Corn silage	Alfalfa haylage	Brome hay	Alfalfa hay	Cotton seed	RFDG ¹	Control mix ¹	RFDG-added mix ²
Experiment 1								
DM	32.83 $\pm$ 1.04	32.50 $\pm$ 2.58	88.25 $\pm$ 0.86	87.80 $\pm$ 0.88	89.20 $\pm$ 1.49	90.30 $\pm$ 0.14	88.98 $\pm$ 0.74	90.53 $\pm$ 0.62
CP	8.70 $\pm$ 0.42	22.00 $\pm$ 0.72	10.25 $\pm$ 0.60	20.68 $\pm$ 1.18	21.98 $\pm$ 1.46	31.85 $\pm$ 0.49	21.80 $\pm$ 0.37	20.88 $\pm$ 0.51
NDF	39.30 $\pm$ 1.28	42.85 $\pm$ 2.07	69.85 $\pm$ 4.13	41.88 $\pm$ 1.70	48.60 $\pm$ 2.20	33.90 $\pm$ 3.54	25.03 $\pm$ 1.72	34.77 $\pm$ 0.25
Starch	35.00 $\pm$ 2.38	1.45 $\pm$ 0.86	1.53 $\pm$ 0.83	1.88 $\pm$ 0.67	1.05 $\pm$ 0.39	7.50 $\pm$ 0.57	36.03 $\pm$ 1.18	17.63 $\pm$ 0.61
Ether extract	2.84 $\pm$ 0.17	3.13 $\pm$ 0.20	2.04 $\pm$ 0.31	1.78 $\pm$ 0.37	19.57 $\pm$ 0.43	5.53 $\pm$ 0.53	2.66 $\pm$ 0.25	4.05 $\pm$ 0.24
NFC ³	43.31 $\pm$ 1.21	20.04 $\pm$ 2.55	8.42 $\pm$ 3.72	24.03 $\pm$ 1.72	5.54 $\pm$ 1.29	22.75 $\pm$ 4.44	42.30 $\pm$ 1.43	31.76 $\pm$ 0.55
Ash	5.86 $\pm$ 0.31	11.98 $\pm$ 0.39	9.44 $\pm$ 0.42	11.64 $\pm$ 0.95	4.32 $\pm$ 0.42	5.98 $\pm$ 0.91	8.22 $\pm$ 0.20	8.56 $\pm$ 0.20
Phosphorous YCP ⁴	0.32 $\pm$ 0.03	0.32 $\pm$ 0.01	0.30 $\pm$ 0.01	0.30 $\pm$ 0.03	0.61 $\pm$ 0.04	0.99 $\pm$ 0.05	0.37 $\pm$ 0.02	0.64 $\pm$ 0.03
						1.97 $\pm$ 0.04		
Experiment 2								
DM	35.32 $\pm$ 2.52	50.24 $\pm$ 2.83	87.98 $\pm$ 1.33	86.70 $\pm$ 1.77	88.74 $\pm$ 0.98	90.25 $\pm$ 0.21	88.52 $\pm$ 0.62	91.16 $\pm$ 1.36
CP	8.28 $\pm$ 0.40	22.38 $\pm$ 1.03	10.34 $\pm$ 0.58	19.40 $\pm$ 2.46	21.72 $\pm$ 1.14	32.05 $\pm$ 0.21	21.98 $\pm$ 0.80	21.18 $\pm$ 0.70
NDF	37.22 $\pm$ 2.38	30.44 $\pm$ 1.06	67.66 $\pm$ 2.16	43.86 $\pm$ 4.37	47.56 $\pm$ 4.52	35.80 $\pm$ 0.85	23.90 $\pm$ 1.30	34.02 $\pm$ 1.34
Starch	37.30 $\pm$ 1.81	1.60 $\pm$ 0.26	2.06 $\pm$ 0.45	2.02 $\pm$ 0.19	1.18 $\pm$ 0.50	7.55 $\pm$ 0.49	37.08 $\pm$ 4.03	16.36 $\pm$ 0.87
Ether extract	3.29 $\pm$ 0.25	3.53 $\pm$ 0.41	2.42 $\pm$ 0.34	1.88 $\pm$ 0.27	18.61 $\pm$ 1.57	5.32 $\pm$ 0.23	2.77 $\pm$ 0.38	4.28 $\pm$ 0.51
NFC ³	45.74 $\pm$ 3.09	29.48 $\pm$ 2.01	9.84 $\pm$ 1.50	23.33 $\pm$ 1.90	7.29 $\pm$ 2.91	20.72 $\pm$ 1.53	42.79 $\pm$ 1.19	32.09 $\pm$ 1.55
Ash	5.47 $\pm$ 0.40	14.17 $\pm$ 0.44	9.74 $\pm$ 0.46	11.53 $\pm$ 0.40	4.83 $\pm$ 0.31	6.12 $\pm$ 0.71	8.56 $\pm$ 0.69	8.43 $\pm$ 1.47
Phosphorous YCP ⁴	0.28 $\pm$ 0.02	0.24 $\pm$ 0.01	0.30 $\pm$ 0.01	0.30 $\pm$ 0.03	0.65 $\pm$ 0.02	0.96 $\pm$ 0.01	0.38 $\pm$ 0.02	0.63 $\pm$ 0.02
						1.91 $\pm$ 0.04		

¹Contained 46.8% ground corn, 20.6% soyhulls, 12.5% Soypass (LignoTech, Overland Park, KS), 12.9% soybean meal, 2.1% blood meal, 2.3% limestone-ground, 0.9% sodium bicarbonate, 0.5% salt, magnesium oxide, 0.2% trace mineral premix and 0.2% vitamin premix.

²Contained 48.5% RFDG, 16.5% ground corn, 25.7% soyhulls, 2.3% Soypass (LignoTech), 3.0% Megalac (Church & Dwight Co. Inc., Princeton, NJ), 1.8% limestone-ground, 0.8% sodium bicarbonate, 0.8% salt, 0.4% magnesium oxide, 0.19% trace mineral premix and 0.19% vitamin premix.

³Calculated by difference: 100 – (% NDF + % CP + % fat + % ash).

⁴YCP = yeast crude protein determined by real-time PCR according to Castillo-Lopez et al. (2010).

DNA markers; averages were 303.0 and 218.0 $\pm$ 18.0 g of N/d, for purines and DNA markers, respectively. Intestinal protozoal N flow was not affected ($P = 0.51$) by treatment and averaged 40.7 $\pm$ 13.7 g of N/d. Intestinal yeast N flow, however, increased ($P < 0.01$) with RFDG inclusion; estimates were 0.09, 0.96, 1.20, and 2.50 $\pm$ 0.33 g of N/d for CON, 10, 20, and 30% RFDG, respectively.

DISCUSSION

Lactation Performance and DMI

Potential challenges related to milk fat depression have been suggested when including regular DDGS in dairy rations because of its high content of unsaturated fatty acids (Bauman and Grinari, 2003; Hollmann et al., 2011); therefore, feeding RFDG may reduce these

Table 4. Particle size distribution (% retained, as fed) of formulated diets containing increasing levels (0 to 30%) of reduced-fat dried distillers grains with solubles (RFDG; Poet Nutrition, Sioux Falls, SD) fed in experiments 1 and 2

Particle size ¹	Treatment					P-value ³	
	Control (0%)	10% RFDG	20% RFDG	30% RFDG	SEM ²	Linear	Quadratic
Experiment 1							
>19.0 mm	9.38	7.31	7.90	6.75	0.33	<0.01	0.15
19.0–8.0 mm	25.58	24.05	21.40	19.89	0.44	<0.01	0.97
8.0–1.18 mm	37.86	36.05	33.97	30.20	0.25	<0.01	<0.01
<1.18 mm	27.16	32.57	36.71	43.14	0.54	<0.01	0.36
Experiment 2							
>19.0 mm	6.50	5.21	4.90	3.64	0.54	0.01	0.95
19.0–8.0 mm	26.20	22.33	19.41	18.01	0.72	<0.01	0.11
8.0–1.18 mm	39.10	38.12	35.58	32.06	0.56	<0.01	0.05
<1.18 mm	27.51	34.36	39.84	46.35	0.70	<0.01	0.81

¹Determined using the Penn State Forage Particle Separator as described by Heinrichs and Kononoff (2002).

²The highest standard error of treatment means is shown.

³P-values for effects of increasing levels of DDGS in diet.

Table 5. Effects of feeding increasing levels (0 to 30%) of reduced-fat dried distillers grains with solubles (RFDG; Poet Nutrition, Sioux Falls, SD) on DMI, milk production and milk composition (experiment 1)

Item	Treatment				SEM ¹	P-value ²	
	Control (0%)	10% RFDG	20% RFDG	30% RFDG		Linear	Quadratic
DMI (kg/d)	25.0	23.8	25.9	27.9	1.38		
BW	687.2	687.8	693.4	696.7	18.4	<0.01	0.58
BCS ³	3.06	3.10	3.14	3.18	0.06	0.05	1.00
Milk yield (kg/d)	34.4	33.2	34.5	34.2	1.29	0.78	0.46
3.5% FCM (kg/d)	35.1	34.4	35.2	35.3	1.44	0.66	0.56
Fat (%)	3.59	3.74	3.64	3.67	0.09	0.66	0.32
Fat yield (kg/d)	1.24	1.23	1.25	1.26	0.05	0.53	0.80
Protein (%)	3.08	3.18	3.15	3.19	0.06	0.07	0.43
Protein yield (kg/d)	1.06	1.04	1.07	1.09	0.04	0.23	0.44
Lactose (%)	4.80	4.70	4.73	4.73	0.06	0.33	0.20
Lactose yield (kg/d)	1.66	1.58	1.62	1.63	0.07	0.84	0.26
MUN (mg/dL)	16.24	15.54	16.23	15.94	0.86	0.85	0.41

¹The highest standard error of treatment means is shown.²P-values for effects of increasing levels of RFDG in diet.³On a 1 to 5 scale.

challenges. Our results support this concept and agree with data reported previously (Mjoun et al., 2010a,b) in that the inclusion of RFDG in rations of lactating dairy cows had no negative effect on yield or percentage milk fat and tended to increase the percentage of milk protein. Therefore, this study confirms that RFDG is an effective feed ingredient that can be included in rations formulated for dairy cows without affecting production performance.

The increase in DMI observed in experiment 1 may reflect greater palatability of diets containing RFDG compared with CON and may also be a consequence of the reduction in TMR particle size with RFDG inclusion. In conjunction with decreasing particle size, the percentage of forages (consisting of corn silage, alfalfa haylage, brome hay, and alfalfa hay) decreased from 45.7 to 35.8% as RFDG increased from 0 to 30%. This decrease in forage NDF along with a decrease in TMR

particle size allowed more feed to be consumed before reaching rumen fill (Allen and Grant, 2000; Janicek et al., 2008). In addition, based on the CPM Dairy model predictions, the concentration of ME of diets decreased from 2.53 to 2.47 Mcal/kg when RFDG increased from 0 to 30%, possibly because of the reduction in the level of NFC in the rations. Therefore, the increase in DMI may have been a response to compensate for the lower ME ration being consumed and to provide enough nutrients that the cows need for lactation.

VFA and Ruminal pH

The relative concentration of individual ruminal VFA can affect milk composition (Thomas and Martin, 1988). Specifically, increases in supply of acetic acid are associated with increased milk yield and milk fat concentration, and increases in supply of butyric acid

Table 6. Effects of feeding increasing levels (0 to 30%) of reduced-fat dried distillers grains with solubles (RFDG; Poet Nutrition, Sioux Falls, SD) on DMI, milk production and milk composition (experiment 2)

Item	Treatment				SEM ¹	P-value ²	
	Control (0%)	10% RFDG	20% RFDG	30% RFDG		Linear	Quadratic
DMI (kg/d)	21.42	21.78	21.91	21.13	1.65	0.73	0.23
Milk yield (kg/d)	31.41	32.33	30.86	31.13	2.81	0.65	0.76
3.5% FCM (kg/d)	30.28	31.79	29.55	29.21	2.94	0.29	0.38
Fat (%)	3.29	3.37	3.23	3.12	0.14	0.28	0.42
Fat yield (kg/d)	1.03	1.09	0.99	0.97	0.11	0.23	0.33
Protein (%)	2.99	3.01	3.04	3.03	0.07	0.35	0.70
Protein yield (kg/d)	0.94	0.97	0.93	0.94	0.07	0.84	0.80
Lactose (%)	4.76	4.77	4.74	4.69	0.12	<0.01	0.03
Lactose yield (kg/d)	1.49	1.54	1.46	1.46	0.14	0.48	0.64
MUN (mg/dL)	12.64	13.00	13.07	13.42	0.93	0.14	0.98

¹The highest standard error of treatment means is shown.²P-values for effects of increasing levels of RFDG in diet.

Table 7. Effect of feeding increasing levels (0 to 30%) of reduced-fat dried distillers grains with solubles (RFDG; Poet Nutrition, Sioux Falls, SD) on ruminal pH and concentrations of ruminal VFA and ammonia

Item	Treatment				SEM ¹	P-value ²	
	Control (0%)	10% RFDG	20% RFDG	30% RFDG		Linear	Quadratic
Rumen pH							
Minimum	6.08	6.17	6.06	6.03	0.14	0.60	0.53
Maximum	6.95	6.88	6.83	6.77	0.11	<0.01	0.94
Mean	6.53	6.49	6.38	6.35	0.12	0.02	0.88
Time <6.5 (min/d)	546	834	941	1,040	293	<0.01	0.15
Area <6.5 (pH × min/d)	126	158	357	334	92	0.03	0.63
Time <6.3 (min/d)	279	382	936	946	208	0.03	0.72
Area <6.3 (pH × min/d)	45	47	180	169	64	0.13	0.88
Total VFA (mM)	136.22	134.91	138.50	131.10	6.45	0.40	0.30
VFA (mol/100 mol)							
Acetate	63.36	63.41	60.84	59.99	1.48	<0.01	0.24
Propionate	22.12	21.97	25.00	26.11	1.60	<0.01	0.19
Isobutyrate	0.81	0.78	0.70	0.70	0.08	<0.01	0.57
Butyrate	11.24	11.21	10.59	10.73	0.49	0.07	0.74
Isovalerate	0.61	0.61	0.61	0.63	0.07	0.71	0.69
Valerate	1.77	1.92	2.08	2.17	0.12	<0.01	0.65
Acetate:Propionate	2.98	2.94	2.52	2.39	0.23	<0.01	0.55
Ammonia (mg/dL)	19.00	18.80	19.25	17.62	2.32	0.31	0.37

¹The highest standard error of treatment means is shown.²P-values for effects of increasing levels of RFDG in diet.

are also associated with positive effects on milk fat concentration (Firkins et al., 2006). In the present study, however, the data indicated that the decrease in the concentration of ruminal acetate and butyrate did not

negatively affect the percentage and yield of milk fat. On the other hand, the type of carbohydrate digested by ruminants is a decisive factor in determining the ratios of the resultant ruminal VFA. Specifically, in-

Figure 1. Effect of feeding increasing levels (0 to 30%) of reduced-fat dried distillers grains with solubles (RFDG) on variation of ruminal pH. Time after feeding 0 h represents 0930 h, when TMR were offered to cows. Mean pH, $P = 0.02$.

Table 8. Fecal output, nutrient intake and apparent total tract digestibility of nutrients by cows fed increasing levels (0 to 30%) of reduced-fat dried distillers grains with solubles (RFDG; Poet Nutrition, Sioux Falls, SD)

Item	Treatment				SEM ¹	P-value ²	
	Control (0%)	10% RFDG ²	20% RFDG	30% RFDG		Linear	Quadratic
Fecal output							
DM (kg/d)	6.94	7.20	5.65	5.61	0.75	0.15	0.79
N (kg/d)	0.202	0.197	0.152	0.146	0.02	0.09	0.88
P (g/d)	46.00	54.00	53.00	53.00	7.17	0.49	0.53
DM							
Digestibility (%)	65.5	65.4	73.0	73.4	4.8	0.14	0.93
OM							
Intake (kg/d)	19.63	19.92	20.03	19.33	1.50	0.68	0.27
Digestibility (%)	67.7	67.7	74.9	75.2	4.4	0.14	0.96
NFC							
Intake (kg/d)	7.81	7.58	7.41	6.65	0.54	<0.01	0.26
Digestibility (%)	89.7	90.1	92.6	92.7	1.2	0.07	0.87
NDF							
Intake (kg/d)	6.98	7.51	7.81	7.86	0.61	0.01	0.20
Digestibility (%)	44.0	43.2	57.0	58.0	7.5	0.11	0.85
N							
Intake (kg/d)	0.634	0.633	0.634	0.618	0.04	0.58	0.36
Digestibility (%)	64.3	67.7	74.7	76.9	5.1	0.06	0.84
Phosphorous							
Intake (g/d)	73.29	85.92	96.43	108.64	7.68	<0.01	0.90
Digestibility (%)	28.08	35.01	50.18	50.52	11.51	0.08	0.62

¹The highest standard error of treatment means is shown.²P-values for effects of increasing levels of RFDG in diet.

creasing the concentration of starch in diets typically yields more propionate (Evans et al., 1975). In the current study, the inclusion of RFDG decreased the percentage of starch in TMR. In spite of such changes and contrasting our expectations, the molar proportion of ruminal propionate increased with RFDG inclusion, which agrees with reports by Zhang et al. (2010), but contrasts the observations of Anderson et al. (2006) and Ranathunga et al. (2010). This suggests that diets containing RFDG can support or even enhance ruminal propionate production.

The reduction in ruminal pH with RFDG inclusion may be partially explained by the reduction of TMR particle size, which likely decreased the time cows spent chewing and thus producing less saliva needed to buffer pH in the rumen. Overall, measurements indicate that ruminal pH was highest just before feeding and declined for approximately 8 to 9 h thereafter before gradually increasing and return to similar values before the next feeding. During this time, a gradual decrease in acid production and increased VFA absorption (Whitelaw et al., 1970), combined with salivary buffer production during rumination (Maekawa et al., 2002), returned the pH to near its previous value before feeding (Palmonari et al., 2010). The exact cause of the excessive foam formation and low rumen pH (as low as 5.5) observed in the cow consuming the 30% RFDG diet is still unclear. Previous research has indicated that development of stable, persistent ruminal foams, possessing sufficient

strength for bloat to occur, depends on ruminal pH, protein concentration, temperature, and the concentration and source of starch in the diet (Buckingham, 1970; Branine and Galyean, 1990; Cheng et al., 1998). To our knowledge, no reports have been made on the development of acidosis or excessive foam in the rumen when including RFDG in dairy rations. The reduction in TMR particle size may represent a risk for the development of ruminal acidosis (Pereira et al., 1999). In the present study, the greatest reduction in particle size was observed in the 30% RFDG TMR. In this treatment, the proportion of particles <1.18 mm in experiments 1 and 2 averaged 44.8%, a value considerably higher than the established guidelines (Heinrichs and Kononoff, 2002), which suggest that this proportion should not be greater than 20%. It is important to note that, in spite of this reduction in TMR particle size, the average rumen pH values observed in this study did not fall below 6.

Apparent Total-Tract Nutrient Digestibility and Intestinal Flow of Microbial N

In this study, although treatment did not affect apparent ruminal DM digestibility, increases in apparent total-tract digestibility of OM (from 67.7 to 75.2%), NFC (from 89.7 to 92.7%), NDF (from 44.0 to 58.0%), and N (from 64.3 to 76.9%) were observed when the level of RFDG increased from 0 to 30%. An enhance-

Table 9. Intestinal flow and apparent ruminal digestibility of DM, intestinal flow of total N and microbial N in cows fed increasing levels (0 to 30%) of reduced-fat dried distillers grains with solubles (RFDG; Poet Nutrition, Sioux Falls, SD)

Item	Treatment				SEM ¹	P-value ²	
	Control (0%)	10% RFDG	20% RFDG	30% RFDG		Linear	Quadratic
Intestinal flow							
DM (kg/d)	15.1	16.3	14.6	14.7	1.47	0.41	0.42
Ruminal DM digestibility (%)	29.4	25.6	34.5	29.9	3.2	0.38	0.87
Total N (g/d)	592	636	581	570	68.0	0.55	0.50
Purine markers							
Microbial N ³ (g/d)	301	340	279	296	26	0.47	0.62
Microbial N (% of total N)	49.0	53.0	49.0	50.0	3.35	0.94	0.61
DNA markers							
Bacterial N (g/d)	164	190	193	167	22.3	0.89	0.27
Protozoal N (g/d)	43	30	39	51	13.7	0.51	0.28
Microbial N ⁴ (g/d)	202	222	232	220	30.9	0.65	0.60
Microbial N (% of total N)	34.0	35.4	40.8	38.5	4.9	0.46	0.76
Yeast N (g/d)	0.09	0.96	1.20	2.5	0.33	<0.01	0.50
Calculated diet RUP ⁵ (%)	49.1	46.5	50.9	48.1			
Predicted microbial N ⁶ (g/d)	340	360	353	335			

¹The highest standard error of treatment means is shown.²P-values for effects of increasing levels of RFDG in diet.³Microbial N estimated using purines as microbial marker.⁴Microbial N estimated using DNA as microbial markers and it is the sum of bacterial and protozoal N.⁵Calculated based on CP intake and intestinal flow of total N and microbial N (when using purines as the microbial marker), not corrected with endogenous protein, which is assumed to be equal across treatments.⁶Predicted by the CPM Dairy Ration Analyzer v3.0.8.1 (Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY).

ment in total-tract nutrient digestibility has been reported elsewhere (Martínez-Pérez et al., 2013) when supplementing cattle with DDGS. We suggest that the low fat levels of the RFDG improved NDF digestion (Ueda et al., 2003). In addition, the smaller particle size of RFDG increased the surface area, enhancing total-tract nutrient digestibility (Kitessa et al., 1999). The maintained ruminal DM digestibility and the sustained production of rumen ammonia when feeding RFDG may have contributed to maintain the growth of rumen microorganisms, which prefer ammonia as their source of nitrogen (Hristov and Ropp, 2003).

One of the factors that may affect rumen microbial growth is protein degradability of the diet (Stern and Hoover, 1979). Based on the intake of CP and intestinal flow of total N and microbial N (when using purines as the microbial marker), we calculated the RUP of diets fed. It was calculated by subtracting intestinal flow of microbial protein from intestinal flow of total protein; this result represented intestinal flow of feed protein. Then, the percentage of feed CP consumed and reaching the intestine was obtained. Estimates of RUP were 49.1, 46.5, 50.9, and 48.1% for CON, 10, 20, and 30% RFDG, respectively. In this study, the percentage of MP of microbial origin (percentage of intestinal total protein flow that was represented by microbial protein) was not affected by treatment. Specifically, when purines were used as the microbial marker, es-

timates were 49.0, 53.0, 49.0, and 50.0%; when DNA was used as the microbial marker, estimates were 34.0, 35.4, 40.8, and 38.5% for CON, 10, 20, and 30% RFDG diets, respectively. This agrees with reports from other researchers (Kelzer et al., 2009; Chibisa et al., 2012), who reported that cows consuming DDGS synthesized similar amounts of MCP compared with a control diet. When averaged across treatments, duodenal protozoal N flow represented 13.5% of total intestinal microbial N. The contribution of protozoa N observed in this study was similar to that observed by Sylvester et al. (2005), who reported that protozoal N accounts for 11.9% of the duodenal flow of microbial N. Intestinal flow of yeast N ($P < 0.01$) was 0.09, 0.96, 1.20, and 2.5 ± 33 g/d for CON, 10, 20, and 30% RFDG diets, respectively. Considering the concentration of yeast-based N in RFDG, animals consumed 6.5, 13.0, and 19.0 g of yeast N/d when feeding 10, 20, or 30% RFDG, respectively. Therefore, our results indicate that yeast cells contained in corn ethanol byproducts are extensively degraded in the rumen. Estimates of intestinal microbial N flow obtained in this study were lower compared with values predicted by the CPM Dairy model, which predicted 340, 360, 353, and 335 g of microbial N/d for CON, 10, 20, and 30% RFDG diets, respectively. The higher yield of intestinal microbial N when using purines as microbial marker may indicate that nonmicrobial purines are reaching the duodenum

(Belanche et al., 2011b). It is also likely that the DNA markers used are not present in all rumen microbial species, resulting in an underestimation of microbial N. It has been suggested, however, that the use of DNA markers represents a good option when the purpose is the estimation of bacterial and protozoal N separately (Belanche et al., 2011a,b).

CONCLUSIONS

This study was designed (1) to evaluate the effect of RFDG on lactation performance, rumen fermentation, intestinal microbial N supply, and total-tract nutrient digestibility; and (2) to compare the estimates of microbial N using purines or DNA as microbial markers. Results indicate that DNA markers yield lower microbial N compared with purines, possibly because the DNA markers utilized in this study did not account for all rumen microbial species reaching the small intestine. In addition, when RFDG was included in dairy rations, lactation performance was maintained, rumen VFA concentrations and intestinal microbial N supply were not affected, and total-tract nutrient digestibility tended to increase. Overall, this study demonstrates that RFDG is an effective alternative energy and protein feed source for the dairy industry.

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