Effects of feeding wheat or corn-wheat dried distillers grains with solubles in low- or high-crude protein diets on ruminal function, omasal nutrient flows, urea-N recycling, and performance in cows

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ABSTRACT

A study was conducted to determine the effects of including either wheat-based (W-DDGS) or corn-wheat blend (B-DDGS) dried distillers grains with solubles as the major protein source in low- or high-crude protein (CP) diets fed to dairy cows on ruminal function, microbial protein synthesis, omasal nutrient flows, urea-N recycling, and milk production. Eight lactating Holstein cows (768.5 ± 57.7 kg of body weight; 109.5 ± 40.0 d in milk) were used in a replicated 4×4 Latin square design with 28-d periods (18 d of dietary adaptation and 10 d of measurements) and a 2×2 factorial arrangement of dietary treatments. Four cows in one Latin square were ruminally cannulated for the measurement of ruminal fermentation characteristics, microbial protein synthesis, urea-N recycling kinetics, and omasal nutrient flow. The treatment factors were type of distillers co-product (W-DDGS vs. B-DDGS) and dietary CP content [15.2 vs. 17.3%; dry matter (DM) basis]. The B-DDGS was produced from a mixture of 15% wheat and 85% corn grain. All diets were formulated to contain 10% W-DDGS or B-DDGS on a DM basis. No diet effect was observed on DM intake. Yields of milk, fat, protein, and lactose, and plasma urea-N and milk urea-N concentrations were lower in cows fed the low-CP compared with those fed the high-CP diet. Although feeding B-DDGS tended to reduce ruminal ammonia-N ($\text{NH}_3\text{-N}$) concentration compared with feeding W-DDGS (9.3 vs. 10.5 mg/dL), no differences were observed in plasma urea-N and milk urea-N concentrations. Additionally, dietary inclusion of B-DDGS compared with W-DDGS did not affect rumen-degradable protein supply, omasal flows of total N, microbial nonammonia N (NAN), rumen-un-degradable protein, and total NAN, or urea-N recycling kinetics and milk production. However, cows fed the low-CP diet had lower N intake, rumen-degradable protein supply, ruminal $\text{NH}_3\text{-N}$ concentration, and omasal

flows of N, microbial NAN, and total NAN compared with those fed the high-CP diet. Feeding the low-CP compared with the high-CP diet also resulted in lower endogenous urea-N production, urea-N recycled to the gastrointestinal tract, and urea-N excretion in urine. In summary, our results indicate that both W-DDGS and B-DDGS can be included as the major protein sources in dairy cow diets without compromising nutrient supply and production performance. However, feeding the low-CP diet lowered omasal flows of microbial protein and metabolizable protein, which, in turn, resulted in lower milk production compared with feeding the high-CP diet.

Key words: dairy cow, dried distillers grains with solubles, microbial protein, omasal nutrient flow

INTRODUCTION

Historically, wheat has been the major feedstock used for ethanol production in western Canada. However, fluctuations in the price of wheat in recent years have resulted in corn being increasingly used in combination with wheat in different ratios based on least cost and availability (Nuez-Ortín and Yu, 2011; Abdelqader and Oba, 2012). Therefore, both wheat-based (**W-DDGS**) and corn-wheat blend (**B-DDGS**) dried distillers grains with solubles are available for use in dairy cow diets. Wheat DDGS can be used to either partially or completely replace barley forage and grain (Zhang et al., 2010a,b) or canola meal (Chibisa et al., 2012) in lactating cow diets without negatively affecting animal performance. However, compared with W-DDGS, limited information exists on the feeding value of B-DDGS. As the proportion of corn to wheat increases in the feedstock, the CP content and ruminal CP degradability of B-DDGS decrease compared with W-DDGS (Boila and Ingalls, 1994a,b; Nuez Ortín and Yu, 2009). Therefore, compared with W-DDGS, the inclusion of B-DDGS in dairy cow diets could potentially reduce dietary RDP supply, thus causing a deficiency in ruminal $\text{NH}_3\text{-N}$, which could suppress microbial protein synthesis in the rumen. In vitro estimates of microbial protein synthesis were lower for B-DDGS

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compared with W-DDGS (138.8 vs. 160.6 g/kg of DM; Nuez-Ortín and Yu, 2010). Abdelqader and Oba (2012) reported a lower ruminal $\text{NH}_3\text{-N}$ concentration when isonitrogenous diets fed to cows contained B-DDGS (a mixture of 50% corn-based DDGS and 50% W-DDGS) compared with 100% W-DDGS, but dietary effects on RDP supply, ruminal microbial protein production, and postruminal flow of MP were not measured. Dietary CP content can also have major effects on RDP supply, as the amount of dietary CP that is degraded in the rumen increases with dietary CP content (Olmos Colmenero and Broderick, 2006b). When dietary CP content is reduced in diets containing either W-DDGS or B-DDGS as the major protein sources (which could potentially alter RDP supply), it is important to determine how changing dietary CP content and RDP supply interact to alter N utilization and production responses.

Urea-N recycling buffers the rumen from low $\text{NH}_3\text{-N}$ concentrations and, thus, can maintain microbial growth when dietary RDP supply is limited (Huhtanen and Hristov, 2009; Agle et al., 2010). It is known that a greater proportion of endogenous urea-N is recycled to the rumen when dietary CP content is reduced (Lapierre and Lobley, 2001). Under such feeding conditions, the fractional contribution of $\text{NH}_3\text{-N}$ from recycled urea-N to microbial protein synthesis increases (Marini and Van Amburgh, 2003). Therefore, the potential decrease in ruminal $\text{NH}_3\text{-N}$ concentration when feeding B-DDGS compared with W-DDGS could increase the rate of urea-N transfer into the rumen and its subsequent sequestration into microbial protein. When corn-based DDGS (a low-RDP source) was compared with urea (a high-RDP source) as a N supplement in steers fed corn-based diets, both urea-N recycling to the gastrointestinal tract (GIT) and the proportion of microbial NAN arriving at the small intestine that was derived from recycled urea-N tended to be greater with corn-based DDGS (Brake et al., 2010). This suggests that recycled urea-N might be a more important source of N for ruminal microbial growth when high-RUP supplemental protein sources such as B-DDGS are fed (Wickersham et al., 2009b; Brake et al., 2010).

The objective of this study was to determine how interactions between the type of distillers co-product (W-DDGS vs. B-DDGS) and diet CP content (15 vs. 17%) alter ruminal N utilization, urea-N recycling to the GIT, omasal nutrient flow, and production performance in lactating dairy cows. We hypothesized that at similar dietary CP content, differences in the RDP content between W-DDGS and B-DDGS would result in differences in ruminal N utilization, urea-N recycling to the GIT, and omasal nutrient flow.

MATERIALS AND METHODS

Animals and Experimental Design

Eight lactating dairy cows (768.5 ± 57.7 kg of BW; 109.5 ± 40.0 DIM) were used in a replicated 4×4 Latin square design with 28-d periods (20 d of dietary adaptation and 8 d of measurements) and a 2×2 factorial arrangement of dietary treatments. Four cows in 1 Latin square were ruminally cannulated for the measurement of ruminal fermentation characteristics, microbial protein synthesis, urea-N recycling kinetics, and omasal nutrient flows. All cows were housed in individual tie-stalls at the Greenbrae Dairy Research Facility (University of Saskatchewan, Saskatoon, SK, Canada). The University of Saskatchewan Animal Care Committee approved the use of cows for this experiment (UCACS Protocol No. 20040048), and they were cared for in accordance with the Canadian Council on Animal Care (1993) regulations.

Experimental Treatments and Feeding Management

The treatment factors were type of distillers co-product (W-DDGS vs. B-DDGS) and dietary CP content (15 vs. 17%, DM basis). In a previous study (Broderick, 2003), feeding diets containing 15.1, 16.7, and 18.4% CP resulted in a linear increase in DMI, but milk yield increased only when feeding diets containing between 15.1 and 16.7% CP. For the current study, therefore, we decided to use dietary CP contents of 15 and 17% to encompass the range in which positive responses in milk yield have been observed. All diets were formulated to contain 10% (on a DM basis) W-DDGS or B-DDGS. Single batches of W-DDGS and B-DDGS were used for the entire study. The batch of W-DDGS was produced from 100% wheat (Terra Grain Fuels, Belle Plaine, SK, Canada), whereas the batch of B-DDGS was produced from 85% corn and 15% wheat (Husky Energy Inc., Minnedosa, MB, Canada). Cows were fed twice daily at 0900 and 1600 h as TMR for ad libitum intake. The forage:concentrate ratio of the TMR was 50:50, with the forage component of the TMR being a mixture of barley silage (65% on a DM basis) and chopped alfalfa hay (35%; Table 1).

Data Collection and Sampling

Urea-N recycling kinetics, N balance, apparent total-tract nutrient digestion, ruminal fermentation characteristics, microbial protein synthesis, and omasal nutrient flow were determined using the 4 ruminally cannulated cows in 1 Latin square. On d 21 (0900 h) of each experimental period, the 4 ruminally cannulated

Table 1. Ingredient and chemical composition of diets containing low- or high-CP contents, with either wheat-based dried distillers grains with solubles (W-DDGS) or wheat-corn blend DDGS (B-DDGS) as the major protein source

Item	Low-CP diet		High-CP diet	
	W-DDGS	B-DDGS	W-DDGS	B-DDGS
Ingredient composition (% of diet DM)				
Alfalfa hay	17.8	17.8	17.8	17.8
Barley silage	31.2	31.2	31.2	31.2
Soybean hulls	2.2	2.7	0.45	0.22
Cottonseed hulls	3.6	2.2	0.45	0.22
Barley grain	29.0	29.0	29.0	29.0
W-DDGS	10.0	—	10.0	—
B-DDGS	—	10.0	—	10.0
Canola meal	0.89	1.1	3.3	2.2
Corn gluten meal	0.45	1.1	1.1	2.7
Soybean meal	0.45	0.45	2.2	2.2
Fat canola oil	0.60	0.60	0.60	0.60
Molasses	0.24	0.24	0.24	0.24
Mineral-vitamin mix ¹	1.8	1.8	1.8	1.8
Sodium bicarbonate	0.97	0.97	0.97	0.97
Salt	0.36	0.36	0.36	0.36
Limestone	0.24	0.24	0.24	0.24
Dynamate ²	0.17	0.17	0.17	0.17
Chemical composition				
DM (%)	54.6	54.4	54.9	54.5
OM (% of DM)	94.0	94.1	93.9	94.1
CP (% of DM)	15.0	15.3	17.2	17.4
Fat (% of DM)	3.67	4.65	4.23	4.83
NDF (% of DM)	35.2	34.9	32.6	32.5
ADF (% of DM)	19.2	18.7	17.6	17.0
NE _L ³ (Mcal/kg)	1.68	1.70	1.74	1.75

¹Contained (/kg of premix; DM basis) 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1,000 IU of vitamin E, 16%, Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg of Zn, 1,500 mg of Mn, 535 mg of Cu, 12 mg of Se, and 45 mg of I.

²Dynamate (Eastern Minerals Inc., Henderson, NC) contained 18% K, 11% Mg, and 22% S.

³Estimated using CPM Dairy software (v 3.0.8; Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY) using the chemical analysis of feed ingredients.

cows were fitted with temporary vinyl catheters (0.86-mm i.d. × 1.32-mm o.d.; Scientific Commodities Inc., Lake Havasu City, AZ) in the right and left jugular veins to facilitate continuous isotope infusion and blood sampling. Urea-N transfer to the GIT and whole-body N balance were subsequently determined from d 22 to 26, as described by Lobley et al. (2000). Briefly, urine and fecal samples were collected on d 21 for analysis of ¹⁵N natural abundance. Thereafter, continuous infusion of double-labeled urea (¹⁵N¹⁵N]-urea, 99.8 atom % ¹⁵N; Cambridge Isotope Laboratories Inc., Andover, MA) prepared in 0.15 M sterile saline started at 0800 h on d 22 of each experimental period and ended at 0600 h on d 28. Individual cow N intake from d 13 to 20 was used to determine the daily [¹⁵N¹⁵N]-urea dosage rate, based on a targeted plateau urinary [¹⁵N¹⁵N]-urea enrichment of 0.15 atom percent excess (**APE**). Total fecal and urine collections were conducted from 0800 h on d 22 to 0800 h on d 26 (96 h). Large steel trays placed behind each stall were used to collect feces. On each day,

fecal output for each cow was thoroughly mixed and quantitatively transferred to preweighed plastic tubs for weighing. Subsequently, a 2.5% fecal subsample was collected and stored at −20°C for later chemical analysis. Indwelling Bardex Foley bladder catheters (26 Fr, 75 cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA) used for total urine collection were inserted at 0800 h on d 21 before connection to urine collection tubing at the start of [¹⁵N¹⁵N]-urea infusion (0800 h on d 22). Urine was collected into 20-L carboy polyethylene containers containing 150 mL of concentrated HCl, and this acidification (pH <3) of urine during collection was necessary to prevent microbial growth and volatilization of NH₃-N. Daily urine output was recorded, before collection of a 5% subsample that was pooled by cow for each period and stored at −20°C for later N analysis. A daily 50-mL urine sample was also collected (d 23 to 26) and stored at −20°C for later analysis of urinary enrichments of [¹⁵N¹⁵N]- and [¹⁴N¹⁵N]-urea. Additionally, a 2-mL urine

sample was diluted with 8 mL of distilled water and stored at -20°C until analysis for urea-N.

To measure the flow of nutrients at the omasal canal, YbCl_3 (Siddons et al., 1985; Yang and Beauchemin, 2005) was used as a digesta marker. On d 21 of each experimental period, a 500-mL omasal digesta sample was collected for determination of background ^{15}N (^{15}NB) abundance. A priming dose of YbCl_3 that was equivalent to half the daily dose was then administered into the rumen via the ruminal cannula. Subsequently, a YbCl_3 solution was continuously infused into the rumen using a peristaltic pump (model 205U; Watson-Marlow Pumps Group, Cornwall, UK) for the next 7 d (d 21 to 28) at a constant rate of 1 L/d, providing 3.35 g of Yb per day. The actual amount of marker solution infused each day (~ 1 L) was recorded. During each period, a 50-mL subsample of the marker solution that was infused for each cow was collected and stored at room temperature for Yb analysis. The omasal sampling technique as described by Huhtanen et al. (1997) was used to collect omasal digesta. Briefly, this technique involved locating the omasal canal by hand and then inserting a sampling tube via the ruminal cannula. Sampling tubes were inserted at each sampling time to ensure correct positioning in the omasal canal and to minimize the potential negative effect on digesta passage and normal digestive function if the sampling tubes were left in place between samplings. Once the sampling tube had been inserted in the omasal canal, a 600-mL omasal digesta sample was collected from each cow at 0900, 1500, and 2100 h on d 26; 0300, 1200, and 1800 h on d 27; and 0000 and 0600 h on d 28, such that the collected samples were representative of a 24-h feeding cycle. Omasal samples were stored at -20°C and pooled by cow per period over the 24-h cycle.

To measure ruminal short-chain FA (SCFA) and $\text{NH}_3\text{-N}$ concentrations, and bacterial ^{15}N enrichment, approximately 400 mL of ruminal digesta was also collected at each omasal sampling time from the cranial ventral, caudal ventral, central, and cranial dorsal rumen through the cannula. The ruminal contents were strained through 4 layers of cheesecloth. Two 10-mL subsamples of ruminal fluid were then collected and mixed with chilled 25% (wt/vol) meta-phosphoric acid (H_2PO_4) or 1% H_2SO_4 and stored at -20°C for later determination of SCFA and $\text{NH}_3\text{-N}$ contents, respectively. After straining through cheesecloth, the remaining solid digesta was mixed with 500 mL of 0.15 M saline solution, and homogenized in a blender (NuBlend; Waring Commercial Products, Torrington, CT) for 60 s to dislodge particle-associated bacteria. The blended mixture was squeezed through 4 layers of cheesecloth to obtain a second filtrate. The 2 filtrates were mixed and 375 mL of the mixture were used to isolate mixed ruminal

bacteria by differential centrifugation. Briefly, filtrates were centrifuged at $500 \times g$ for 20 min at 4°C to remove protozoa and residual feed particles. The supernatant was collected and centrifuged at $20,000 \times g$ for 20 min at 4°C to obtain a ruminal bacterial pellet. Bacterial pellets were then pooled by cow per period and stored at -20°C .

To determine ruminal pH, approximately 1,000 mL of ruminal contents were collected from the cranial ventral, caudal ventral, central, and cranial dorsal rumen through the cannula at 0900, 1000, 1100, 1200, 1400, and 1600 h on d 27. The ruminal contents were strained through 4 layers of cheesecloth. Ruminal fluid pH was then measured immediately using a model 265A portable pH meter (Orion Research Inc., Beverly, MA). At the same time points as for ruminal fluid sampling on d 27, blood samples were collected from the contra-lateral jugular vein into 10-mL Vacutainer tubes containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ). The collected samples were centrifuged at $1,500 \times g$ for 15 min at 4°C , and the plasma obtained was stored at -20°C until analyzed for urea-nitrogen (PUN).

Feed intake was recorded daily throughout the experiment. Samples of the TMR and Orts were collected from d 23 to 25 and were stored at -20°C for later analysis. Cows were milked 3 times daily at 0430, 1230, and 1900 h, and milk weights were recorded throughout the experiment. Milk samples were collected daily on 3 consecutive days (d 23, 24, and 25) from all 3 milkings into vials containing 2-bromo-2-nitropropane-1,2-diol as a preservative. Samples were submitted to the Alberta Central Milk Testing Laboratory (Edmonton, Alberta, Canada) for CP, fat, lactose, and MUN analyses.

Sample Analyses

After the experiment, frozen TMR and fecal samples were thawed overnight at room temperature, pooled per collection period for each cow, and subsequently dried in an oven at 60°C for 48 h (AOAC, 1990; method 930.15). Dried TMR and fecal samples were then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, UK). Samples were analyzed for DM (AOAC, 1990; method 930.15), OM (AOAC, 1990; method 942.05), CP using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05), ether extract (AOAC, 1990; method 920.39), and ADF and NDF (Van Soest et al., 1991). Amylase and sodium sulfite were used for NDF determination. Frozen urine samples were thawed overnight at room temperature and then analyzed for N using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05). The plasma and dilute urine samples were analyzed for urea-N by the diacetyl monoxime method (Marsh

et al., 1957) using a colorimetric urea-N kit (Stanbio Urea Nitrogen Kit, Procedure No. 0580; Stanbio Laboratory, Boerne, TX). Milk samples were analyzed for fat, CP, lactose, and MUN using infrared spectroscopy (MilkoScan 605; Foss Electric A/S, Hillerød, Denmark; AOAC, 1990; method 972.16).

To determine the enrichments of $[^{15}\text{N}^{15}\text{N}]$ - and $[^{14}\text{N}^{15}\text{N}]$ -urea in daily urine samples (50-mL subsample), urinary urea-N was isolated by passing urine containing 1.5 mg of urea-N through a prepacked cation-exchange resin column (AG-50W- $\times$ 8 resin, 100–200 mesh, H^+ form; Bio-Rad Laboratories Inc., Richmond, CA) as described by Archibeque et al. (2001). Following application of urine, 7 mL of N-free water was added to the column before elution of urea-N into test tubes using 20 mL of N-free water. Following air drying (60°C) of the eluate, three 1-mL rinses of N-free water were used to quantitatively transfer urea-N from the test tubes into 17 $\times$ 60-mm borosilicate glass tubes. Subsequently, the urea-N samples were freeze dried and analyzed for $[^{15}\text{N}^{15}\text{N}]$ - and $[^{14}\text{N}^{15}\text{N}]$ -urea enrichment by isotope ratio-mass spectrometry (IRMS; N-15 Analysis Laboratory, University of Illinois, Urbana-Champaign) as described by Lobley et al. (2000). Analytical conditions used during IRMS should lead to the production of ions with mass/charge (m/z) values of 28, 29, and 30 for $[^{14}\text{N}^{14}\text{N}]$ -, $[^{14}\text{N}^{15}\text{N}]$ - and $[^{15}\text{N}^{15}\text{N}]$ -urea, respectively. Standards prepared using $[^{15}\text{N}^{15}\text{N}]$ -urea (99.8 atom % ^{15}N) and $[^{14}\text{N}^{14}\text{N}]$ -urea (natural abundance urea; 0.364 atom % ^{15}N) were also analyzed, and results used to correct for $[^{14}\text{N}^{15}\text{N}]$ -urea produced during non-monomolecular reactions (Lobley et al., 2000). Daily fecal samples were thawed overnight at room temperature before DM analysis (AOAC, 1990; method 930.15). Dried fecal samples were subsequently ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd.) and were analyzed for ^{15}N enrichment by combustion to N_2 gas in an elemental analyzer and continuous-flow IRMS (Lobley et al., 2000).

In preparation for NAN and ^{15}N analyses, the omasal ^{15}NB and bacterial pellet samples were freeze dried. Freeze-dried ^{15}NB samples were then ground through a 1-mm screen (Christy-Norris Ltd.) before being pulverized with a ball mill. Freeze-dried bacterial pellet samples were ground using a mortar and pestle. The composited omasal digesta samples were freeze dried and ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd.). To determine Yb concentration, a 1-g sample was combusted at 550°C for 8 h in a muffle furnace (AOAC, 1990) before nitric acid digestion, as described by Vicente et al. (2004). Subsequently, Yb concentration was measured by atomic absorption spectrophotometry (PerkinElmer 2300; PerkinElmer Corp., Norwalk, CT). The Yb concentration in

omasal digesta was then used to determine omasal DM flow (France and Siddons, 1986). To determine the flow of nutrients to the omasum, the freeze-dried omasal digesta samples were analyzed for OM by combustion in a muffle furnace at 550°C for 8 h, total N (Leco 2000; Leco Instruments Inc., St. Joseph, MI), ADF, and NDF. To quantify $\text{NH}_3\text{-N}$, 10 mL of 0.07 M sodium citrate (pH 2.2) was added to 0.5 g of omasal digesta and the mixture vortexed and subsequently held at 39°C for 30 min in a forced-air oven. The extracts were then centrifuged at $18,000 \times g$ for 15 min at 4°C, and the resultant supernatant was analyzed for $\text{NH}_3\text{-N}$ using the phenol-hypochlorite method as described by Broderick and Kang (1980). Samples of ^{15}NB , bacterial pellets, and omasal digesta were prepared for ^{15}N analysis as described by Brito et al. (2009). Briefly, to volatilize $\text{NH}_3\text{-N}$ before ^{15}N analysis, ^{15}NB , bacterial pellets, and omasal digesta, samples containing approximately 100 μg of N were weighed into 5 $\times$ 9-mm tin capsules (Elemental Microanalysis Ltd., Okehampton, UK). Subsequently, 50 μL of 72 mM K_2CO_3 was added to each capsule before incubation in a forced-air oven at 60°C for 24 h. Enrichment of ^{15}N in NAN of the samples was then measured by combustion to N_2 gas in an elemental analyzer and continuous-flow IRMS. Bacterial pellets were analyzed for OM by combustion in a muffle furnace at 550°C for 8 h.

Frozen ruminal fluid samples were thawed at room temperature and pooled by cow for each period. This was followed by centrifugation at $20,000 \times g$ for 15 min at 4°C to obtain a clear supernatant. For ruminal fluid samples that were preserved with 25% H_2PO_4 , supernatants were filtered through a 0.45- μm membrane and a 0.9-mL portion of the filtered supernatant was mixed with 0.1 mL of 10 mg/mL crotonic acid as an internal standard. Ruminal SCFA were then separated and quantified by gas chromatography (Agilent 6890; Agilent Technologies Canada Inc., Mississauga, ON, Canada) as described by Erwin et al. (1961). For ruminal fluid samples that were preserved with H_2SO_4 , supernatants were analyzed for ruminal $\text{NH}_3\text{-N}$ using a phenol-hypochlorite assay (Broderick and Kang, 1980).

Calculations and Statistical Analyses

Urinary ^{15}N enrichment of $[^{15}\text{N}^{15}\text{N}]$ - and $[^{14}\text{N}^{15}\text{N}]$ -urea and total fecal ^{15}N excretion were used to calculate urea-N kinetics using the model described by Lobley et al. (2000). Based on this model, a fraction of urea-N synthesized in the liver (urea-N entry rate, **UER**) is irreversibly lost in urine (urinary urea-N elimination), whereas the remaining fraction is recycled to the GIT (GIT entry rate, **GER**). Urea-N recycled to the GIT is hydrolyzed by bacterial urease to $\text{NH}_3\text{-N}$, which

has 3 fates: (1) excretion in feces (urea-N in feces); (2) reabsorption into portal blood and reentry into the ornithine cycle (**ROC**), resulting in the formation of 3 urea-N species (i.e., [$^{14}\text{N}^{14}\text{N}$]-, [$^{14}\text{N}^{15}\text{N}$]-, and [$^{15}\text{N}^{15}\text{N}$]-urea); or (3) utilization for anabolic purposes (**UUA**), which is assumed to be primarily ruminal microbial protein synthesis (Lobley et al., 2000).

Omasal true digesta NAN content was calculated as total N – $\text{NH}_3\text{-N}$. Omasal digesta and bacterial pellet ^{15}N enrichment (APE) was calculated as follows: ^{15}N APE = sample ^{15}N percentage – mean background sample ^{15}N percentage. Bacterial NAN flow was then calculated by multiplying omasal NAN flow by the ratio of omasal digesta ^{15}N enrichment to bacterial pellet ^{15}N enrichment (Wickersham et al., 2009a). The omasal flow of bacterial NAN that was derived from recycled urea-N was calculated as bacterial NAN flow $\times$ (bacterial ^{15}N enrichment $\div$ urinary ^{15}N enrichment) (Wickersham et al., 2009a). Apparent ruminal nutrient digestibilities were calculated as nutrient intake – omasal flow of nutrient, with flows expressed as kilograms per day (Reynal and Broderick, 2005). Organic matter truly digested in the rumen was determined as OM intake – (omasal OM flow – microbial OM flow). Omasal flow of nonammonia nonmicrobial N (NANMN), RUP, and RDP were also calculated as described by Reynal and Broderick (2005) as follows: NANMN flow = total NAN flow – microbial NAN flow, RUP flow = total CP flow – microbial CP flow, and RDP supply = total CP intake – RUP flow.

All data on ruminal fermentation parameters, nutrient digestibilities, omasal flow, and excretion, and urea-N kinetics were analyzed as a 4×4 Latin square using PROC MIXED of SAS (SAS Institute, 2004). Production data for the 8 cows were also analyzed using PROC MIXED of SAS for a replicated 4×4 Latin square design. The models included the following independent variables: cow, period, type of distillers co-product (W-DDGS vs. B-DDGS), dietary CP content (low [15.2%] vs. high [17.3%]), and the type of distillers co-product $\times$ dietary CP content interaction. Period and dietary treatment were considered as fixed, whereas cow was considered as random. For dependent variables that had repeated measurements (ruminal pH and PUN concentrations), the repeated measures option within PROC MIXED of SAS (SAS Institute, 2004) was used. Treatment differences were considered significant when $P \leq 0.05$ and tendencies when $0.05 < P \leq 0.10$. Interactions between dietary CP content and type of distillers co-product were not detected for most of the major variables that were measured (see Tables 2 to 7), so only main effects are presented and discussed.

RESULTS

Dietary Characteristics

Dietary ingredient and chemical composition are presented in Table 1. Experimental diets were formulated to contain 15 or 17% CP (DM basis) and chemical analysis showed only marginal maximum deviations (+0.3 and +0.4 percentage units, respectively) from the intended dietary CP contents (Table 1).

Production Parameters

Results on feed intake and milk production that are presented in Table 2 are from all 8 cows that were used in the study. Dietary CP content and type of distillers co-product had no effects ($P \geq 0.29$) on DMI. Cows fed the high-CP diet had greater ($P < 0.01$) N intakes compared with those fed the low-CP diet (Table 2). Cows fed the high-CP diet had greater ($P < 0.01$) milk yield, ECM yield, and feed efficiency compared with those fed the low-CP diet. However, dietary CP content and type of distillers co-product had no effects milk N efficiency ($P > 0.05$). No effects ($P > 0.05$) were observed of dietary CP content and type of distillers co-product on milk fat and lactose contents, but milk protein content was higher ($P = 0.03$) in cows fed the high- compared with those fed the low-CP diet. Feeding the high-CP diet also resulted in greater ($P < 0.01$) milk fat, protein, and lactose yields, and greater MUN and PUN concentrations compared with feeding the low-CP diet.

Ruminal Fermentation Characteristics

Feeding the high-CP diet resulted in a higher ($P = 0.04$) ruminal $\text{NH}_3\text{-N}$ concentration compared with feeding the low-CP diet (Table 3). No effects ($P > 0.05$) were observed of dietary CP content and type of distillers co-product on ruminal total SCFA concentration, molar proportions of propionate, butyrate, and valerate, and acetate:propionate ratio. Ruminal concentration of total branched-chain FA (BCFA; $P < 0.01$) and molar proportions of isobutyrate ($P = 0.03$) and isovalerate ($P = 0.008$) were higher, whereas the molar proportion of acetate ($P = 0.07$) tended to be lower in cows fed the high-CP compared with those fed the low-CP diet. Also, the molar proportion of isovalerate was higher ($P = 0.047$) in cows fed B-DDGS compared with those fed W-DDGS. No effects ($P \geq 0.14$) were observed of dietary CP content and type of distillers co-product on ruminal pH.

Table 2. Dry matter intake and milk yield and composition of cows fed low- or high-CP diets containing either wheat-based dried distillers grains with solubles (W-DDGS) or wheat-corn blend DDGS (B-DDGS) as the major protein source (n = 8)

Variable	Low-CP diet		High-CP diet		SEM	P-value ¹		
	W-DDGS	B-DDGS	W-DDGS	B-DDGS		CP	DDGS	CP × DDGS
DMI (kg/d)	28.3	29.0	29.1	29.2	0.85	0.29	0.40	0.53
N intake (g/d)	684	713	805	814	21.4	<0.01	0.17	0.49
Milk yield (kg/d)	39.5	41.0	44.1	43.7	1.53	<0.01	0.52	0.28
ECM ² (kg/d)	43.8	45.2	48.5	48.0	1.86	<0.01	0.60	0.29
Feed efficiency ³	1.39	1.42	1.52	1.50	0.044	0.01	0.93	0.55
N efficiency ⁴	27.3	27.5	26.7	26.2	1.07	0.30	0.86	0.71
Milk fat (%)	3.65	3.62	3.64	3.72	0.119	0.52	0.78	0.48
Milk fat yield (kg/d)	1.40	1.45	1.56	1.56	0.055	<0.01	0.45	0.37
Milk protein (%)	3.08	3.12	3.19	3.24	0.102	0.03	0.45	0.90
Milk protein yield (kg/d)	1.19	1.25	1.36	1.35	0.049	<0.01	0.57	0.41
Milk lactose (%)	4.61	4.56	4.59	4.62	0.036	0.49	0.68	0.13
Milk lactose yield (kg/d)	1.79	1.84	1.97	1.95	0.089	<0.01	0.79	0.42
MUN (mg/dL)	12.9	12.3	14.8	15.0	0.79	<0.01	0.73	0.56
PUN ⁵ (mg/dL)	16.3	15.4	18.5	19.5	1.12	<0.01	0.95	0.16

¹P-values are for CP content, source of DDGS, and CP × DDGS interaction.

²Energy-corrected milk = [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)] (Orth, 1992).

³Feed efficiency = ECM/DMI.

⁴Nitrogen efficiency = milk N (kg/d)/N intake (kg/d).

⁵Plasma urea-N.

Ruminal Digestion and Nutrient Flow

No effects ($P > 0.05$) were observed of dietary CP content and type of distillers co-product on intakes and omasal flows of DM and OM (Table 4); however, the amount of OM truly digested in the rumen tended to be greater ($P = 0.09$) for cows fed the high-CP compared with those fed the low-CP diet. Intake of NDF ($P = 0.08$) and the amount of NDF apparently digested in the rumen ($P = 0.09$) tended to be lower for cows fed the high-CP compared with those fed the low-CP diet. Intake of ADF ($P = 0.03$) was lower in cows fed the high-CP compared with those fed the low-CP diet.

Also, intake of ADF ($P = 0.06$) tended to be lower in cows fed B-DDGS compared with those fed W-DDGS; however, ruminal digestion and omasal flow of ADF was not affected by dietary CP content and type of distillers co-product.

Intake, Digestibility, and Omasal Flow of Nitrogen Fractions

Cows fed the low-CP diet consumed less N ($P < 0.01$) compared with those fed the high-CP diet (Table 5); however, N apparently digested in the rumen, when

Table 3. Ruminal fermentation characteristics of cows fed low- or high-CP diets containing either wheat-based dried distillers grains with solubles (W-DDGS) or wheat-corn blend DDGS (B-DDGS) as the major protein source (n = 4)

Variable	Low-CP diet		High-CP diet		SEM	P-value ¹		
	W-DDGS	B-DDGS	W-DDGS	B-DDGS		CP	DDGS	CP × DDGS
Ammonia-N (mg/dL)	10.7	11.1	12.7	12.6	0.95	0.04	0.79	0.76
Total SCFA ² (mM)	117.4	117.4	122.8	117.8	4.8	0.26	0.33	0.33
Total BCFA ³ (mM)	2.44	2.54	2.75	2.82	0.18	<0.01	0.32	0.86
SCFA (mol/100 mol)								
Acetate	65.5	65.2	64.3	64.7	0.65	0.07	0.96	0.43
Propionate	19.7	19.8	20.1	19.2	0.46	0.79	0.38	0.35
Butyrate	11.1	11.4	11.7	12.3	0.66	0.13	0.35	0.77
Valerate	1.53	1.47	1.60	1.47	0.057	0.53	0.12	0.53
Isobutyrate	0.86	0.86	0.93	0.93	0.039	0.03	0.88	0.86
Isovalerate	1.22	1.28	1.33	1.46	0.085	0.008	0.047	0.38
Acetate:propionate	3.33	3.30	3.21	3.40	0.101	0.92	0.45	0.33
Mean pH	6.25	6.26	6.16	6.27	0.105	0.26	0.14	0.21

¹P-values are for CP content, source of DDGS, and CP × DDGS interaction.

²SCFA = short-chain FA.

³BCFA = branched-chain FA.

Table 4. Nutrient flow from and digestion in the rumen of cows fed low- or high-CP diets containing either wheat-based dried distillers grains with solubles (W-DDGS) or wheat-corn blend DDGS (B-DDGS) as the major protein source (n = 4)

Variable	Low-CP diet		High-CP diet		SEM	<i>P</i> -value ¹		
	W-DDGS	B-DDGS	W-DDGS	B-DDGS		CP	DDGS	CP × DDGS
DM								
Intake (kg/d)	27.8	27.5	28.7	28.4	0.87	0.15	0.68	0.96
Omasal flow (kg/d)	21.4	20.4	22.4	22.0	0.96	0.17	0.47	0.72
Apparent digestion (kg/d)	6.43	7.14	6.28	6.42	0.48	0.37	0.38	0.54
Apparent digestion (% of DMI)	23.1	26.1	22.0	22.4	1.76	0.21	0.37	0.48
OM								
Intake (kg/d)	26.0	25.9	26.9	26.8	0.83	0.13	0.78	0.99
Omasal flow (kg/d)	19.2	18.4	20.6	20.0	0.96	0.12	0.42	0.93
Apparent digestion (kg/d)	6.83	7.49	6.29	6.81	0.63	0.32	0.33	0.91
Apparent digestion (% of OM intake)	26.2	29.1	23.4	25.2	2.36	0.19	0.35	0.81
True digestion (kg/d)	13.6	14.4	15.2	14.5	0.60	0.09	0.94	0.14
True digestion (% of OM intake)	52.4	55.8	56.7	54.1	1.93	0.52	0.82	0.14
NDF								
Intake (kg/d)	9.93	9.65	9.40	9.31	0.26	0.08	0.42	0.67
Omasal flow (kg/d)	5.55	4.72	5.32	5.28	0.45	0.68	0.31	0.36
Apparent digestion (kg/d)	4.38	4.94	4.07	4.04	0.42	0.09	0.42	0.36
Apparent digestion (% of NDF intake)	44.1	51.4	43.4	43.1	4.31	0.25	0.35	0.32
ADF								
Intake (kg/d)	5.50	5.16	5.09	4.87	0.15	0.03	0.06	0.61
Omasal flow (kg/d)	2.88	2.45	2.70	2.71	0.26	0.84	0.32	0.29
Apparent digestion (kg/d)	2.62	2.71	2.39	2.16	0.27	0.03	0.63	0.29
Apparent digestion (% of ADF intake)	47.4	52.5	47.2	44.1	4.88	0.21	0.75	0.24

¹*P*-values are for CP content, source of DDGS, and CP × DDGS interaction.

expressed as grams per day or as a proportion of N intake, did not differ ($P > 0.05$) with dietary CP content and type of distillers co-product. The absolute amount of N truly digested in the rumen was higher ($P = 0.03$) in cows fed the high-CP compared with the low-CP diet. The supply of RDP, when expressed as grams per day or as a proportion of DMI, was higher ($P = 0.03$) when cows were fed the high-CP compared with the low-CP diet. Similarly, the amounts of N ($P = 0.02$) and total NAN ($P = 0.03$) flowing at the omasal canal were higher, whereas $\text{NH}_3\text{-N}$ flow tended to be higher ($P = 0.06$) in cows fed the high-CP compared with those fed the low-CP diet. No effects ($P > 0.05$) were observed of dietary CP content and type of distillers co-product on omasal flows of non- NH_3 nonbacterial N and RUP. When cows were fed the high-CP compared with the low-CP diet, omasal flow of bacterial NAN (g/d) tended to be higher ($P = 0.09$). The efficiency of microbial protein synthesis was unaffected ($P > 0.05$) by dietary CP content and type of distillers co-product. The type of distillers co-product did not influence omasal flows of bacterial NAN or total NAN.

Nitrogen Balance and Apparent Total-Tract Nutrient Digestibilities

Cows fed the high-CP diet had greater N intake ($P < 0.01$) and milk N content ($P = 0.04$) compared with those fed the low-CP diet (Table 6). Urinary N and

total N excretion were also higher ($P < 0.01$) in cows fed the high-CP compared with the low-CP diet. Although fecal N excretion did not differ ($P > 0.05$), fecal N excretion expressed as a percentage of N intake was greater ($P < 0.01$) on the low- compared with the high-CP diet. No effects ($P > 0.05$) were observed of dietary CP content and type of distillers co-product on apparent N balance. Apparent total-tract N digestibility was higher ($P < 0.01$), and apparent total-tract DM ($P = 0.10$) and OM ($P = 0.07$) digestibilities tended to be higher when cows were fed the high-CP compared with the low-CP diet. Apparent total-tract N digestibility tended to be higher ($P = 0.06$) in cows fed B-DDGS compared with those fed W-DDGS. Apparent total-tract NDF, ADF, and fat digestibilities were unaffected ($P > 0.05$) by dietary CP content and type of distillers co-product.

Urea-N Recycling Kinetics

Urea-N synthesis (i.e., UER), urea-N loss in urine (urinary urea-N elimination), and urea-N returned to the ornithine cycle (ROC) were higher ($P \leq 0.05$) in cows fed the high-CP compared with those fed the low-CP diet (Table 7). Urea-N that was transferred to the GIT (GER) tended to be higher ($P = 0.06$) in cows fed the high-CP diet compared with those fed the low-CP diet. No effects ($P > 0.05$) were observed of dietary CP content and type of distillers co-product on the

Table 5. Intake, digestibility, and omasal flow of N constituents in cows fed low- or high-CP diets containing either wheat-based dried distillers grains with solubles (W-DDGS) or wheat-corn blend DDGS (B-DDGS) as the major protein source (n = 4)

Variable	Low-CP diet		High-CP diet		SEM	P-value ¹		
	W-DDGS	B-DDGS	W-DDGS	B-DDGS		CP	DDGS	CP × DDGS
N intake (g/d)	677	682	782	791	23.9	<0.01	0.74	0.92
N apparently digested in the rumen								
g/d	−200	−179	−213	−176	24.9	0.85	0.27	0.76
% of N intake	−29.7	−25.9	−27.1	−22.5	3.01	0.34	0.20	0.89
N truly digested in the rumen								
g/d	402	417	551	476	40.5	0.03	0.48	0.29
% of N intake	59.1	61.3	70.4	59.7	4.48	0.31	0.37	0.18
RDP supply								
g/d	2,683	2,800	3,704	3,187	266	0.03	0.47	0.26
% of DMI	9.63	10.2	12.9	11.2	0.83	0.03	0.48	0.19
Flow at omasal canal								
N								
g/d	877	860	995	967	40.9	0.02	0.57	0.88
% of N intake	130	126	127	123	3.01	0.34	0.20	0.89
NH ₃ -N (g/d)	27.7	30.7	42.1	34.3	4.21	0.05	0.58	0.23
NAN								
g/d	849	830	953	933	39.7	0.03	0.60	0.99
% of N intake	126	121	122	118	3.0	0.26	0.23	0.94
NANBN ²								
g/d	248	234	190	281	34.1	0.88	0.29	0.17
% of NAN flow	29.2	28.3	19.9	30.6	4.14	0.43	0.27	0.19
% of N intake	36.8	34.2	24.2	35.9	4.61	0.27	0.35	0.16
% of DMI	0.895	0.845	0.661	0.997	0.1195	0.74	0.26	0.14
RUP								
g/d	1,549	1,462	1,185	1,759	213.2	0.88	0.29	0.17
% of DMI	5.59	5.28	4.13	6.23	0.747	0.74	0.26	0.14
Total bacterial NAN								
g/d	602	596	763	651	58.3	0.09	0.34	0.39
% of NAN	70.8	71.7	80.1	69.4	4.14	0.43	0.27	0.19
Microbial efficiency								
g of microbial N/kg of OMTDR ³	44.2	41.3	48.4	44.9	2.99	0.25	0.34	0.93

¹P-values are for CP content, source of DDGS, and CP × DDGS interaction.²NANBN = non-NH₃ nonbacterial N.³OMTDR = OM truly digested in the rumen.

quantity of urea-N that was used for anabolic purposes (UUA) or lost in feces (urea-N excretion in feces). Dietary CP content and type of distillers co-product had no effects ($P > 0.05$) on fractional urea-N transfers, but the proportion of GER that was lost in feces tended to be higher ($P = 0.07$) in cows fed the low- compared with those fed the high-CP diet. The amount of recycled urea-N that was incorporated into microbial N (g/d) was unaffected by dietary CP content and type of distillers co-product; however, when expressed as a percentage of total microbial NAN, the proportion of recycled urea-N that was incorporated into microbial N was greater ($P = 0.02$) on the low-CP compared with the high-CP diet.

DISCUSSION

Altering dietary CP content had major effects on N utilization, which were largely reflective of the difference in N intake, as has been reported by others (Olmos Col-

menero and Broderick, 2006a; Brito et al., 2007, 2009). The amount of dietary N that was apparently digested in the rumen was negative for all diets, indicating that omasal N flow was greater than N intake. Broderick et al. (2010) reported that zero ruminal N balance (with ruminal N balance calculated as omasal N flow − N intake) occurred when dietary CP and RDP contents were approximately 14.7 and approximately 10.6%, respectively, and that ruminal N balance became positive as dietary CP and RDP contents increased. In the present study, dietary CP and RDP contents were ≥ 15 and $\geq 9.9\%$, respectively, so ruminal N balance would be expected to be largely positive. Others (Reynal et al., 2003; Olmos Colmenero and Broderick, 2006a) have also reported negative apparent N digestion in the rumen in dairy cows. Broderick et al. (2008) attributed the positive ruminal N balance to the N contribution of recycled urea-N to the ruminal NH₃-N pool and its subsequent utilization for microbial protein synthesis. In the present study, urea-N entry to the GIT was

Table 6. Nitrogen balance and apparent total-tract nutrient digestibility in cows fed low- or high-CP diets containing either wheat-based dried distillers grains with solubles (W-DDGS) or wheat-corn blend DDGS (B-DDGS) as the major protein source (n = 4)

Variable	Low-CP diet		High-CP diet		SEM	P-value ¹		
	W-DDGS	B-DDGS	W-DDGS	B-DDGS		CP	DDGS	CP × DDGS
N intake (kg/d)	660	672	766	778	24.4	<0.01	0.64	0.98
Urinary excretion								
Total (kg/d)	37.4	41.5	38.1	42.3	6.46	0.85	0.34	0.99
Total N (g/d)	218	206	270	271	18.9	<0.01	0.66	0.65
Total N (% of N intake)	33.5	30.5	35.3	34.8	2.96	0.22	0.48	0.61
Fecal excretion								
DM (kg/d)	9.47	9.38	9.49	9.53	0.423	0.69	0.88	0.77
N (g/d)	259	246	260	262	14.0	0.31	0.48	0.39
N (% of N intake)	39.3	36.5	34.0	33.6	1.23	<0.01	0.05	0.14
Total N excretion								
g/d	477	452	531	533	25.8	<0.01	0.48	0.42
% of N intake	72.8	67.1	69.3	68.4	3.43	0.70	0.26	0.41
Milk N (g/d)	178	186	207	207	13.5	0.04	0.71	0.73
Milk NE _L ² (Mcal/d)	26.9	27.8	30.0	29.8	1.04	<0.01	0.53	0.34
Apparent N balance (g/d)	19.7	33.6	16.5	36.8	20.0	0.59	0.45	0.70
Productive N ³ (g/d)	183	221	235	246	27.2	0.16	0.36	0.59
Apparent total-tract digestibility (%)								
DM	65.0	65.5	66.2	66.0	0.75	0.10	0.78	0.52
OM	66.7	67.0	67.9	67.7	0.72	0.07	0.86	0.65
N	60.7	63.5	66.0	66.4	1.23	<0.01	0.06	0.14
NDF	45.1	46.1	43.2	44.6	1.36	0.18	0.32	0.85
ADF	39.7	39.4	37.4	38.7	1.57	0.34	0.74	0.59
Ether extract	86.1	84.8	84.3	85.4	1.14	0.64	0.94	0.33

¹P-values are for CP content, source of DDGS, and CP × DDGS interaction.²Milk NE_L (Mcal/d) = kg of milk × (0.0929 × % fat + 0.0563 × % true protein + 0.0395 × % lactose) (NRC, 2001).³Calculated as N secreted in milk plus N apparently retained by the cow.

substantial, ranging from 328 to 383 g/d, so it is not surprising that ruminal N balance was positive.

When expressed in absolute amounts (+704 g/d) or as a proportion of DMI (+2.2 percentage units), RDP supply was greater in cows fed the high-CP diet

compared with those fed the low-CP diet. This was expected because, for a given diet, the amount of CP that is degraded in the rumen (i.e., RDP) increases as dietary CP content increases (Olmos Colmenero and Broderick, 2006b). The lower RDP supply resulted in

Table 7. Urea-N recycling kinetics in cows fed low- or high-CP diets containing either wheat-based dried distillers grains with solubles (W-DDGS) or wheat-corn blend DDGS (B-DDGS) as the major protein source (n = 4)

Variable ¹	Low-CP diet		High-CP diet		SEM	P-value ²		
	W-DDGS	B-DDGS	W-DDGS	B-DDGS		CP	DDGS	CP × DDGS
Urea-N kinetics (g/d)								
Production, UER	428	384	504	486	21.9	<0.01	0.20	0.57
Entry to GIT, GER	347	308	390	375	24.1	0.06	0.31	0.64
Return to ornithine cycle (ROC)	247	214	280	261	16.7	0.05	0.18	0.69
Loss to feces, UFE	14.3	14.0	14.8	13.9	0.89	0.82	0.54	0.77
Loss to urine, UUE	81.5	75.9	114	111	6.54	<0.01	0.52	0.87
Reuse for anabolism, UUA	86.0	79.9	95.3	100	8.79	0.15	0.94	0.56
Fractional urea-N transfers								
UER to urine	0.195	0.197	0.228	0.232	0.0190	0.12	0.88	0.97
UER to GIT	0.805	0.803	0.772	0.768	0.0191	0.12	0.88	0.97
GER to ROC	0.715	0.694	0.717	0.699	0.0121	0.80	0.17	0.90
GER to feces	0.041	0.045	0.038	0.038	0.0024	0.07	0.47	0.36
GER to UUA	0.243	0.260	0.245	0.262	0.0132	0.88	0.25	0.96
Ruminal microbial capture of recycled N								
g of N/d	68.3	69.4	67.0	50.5	6.20	0.14	0.25	0.19
% of total microbial NAN	11.3	11.7	8.80	8.26	1.00	0.02	0.93	0.66

¹UER = urea-N entry rate; GIT = gastrointestinal tract; GER = GIT entry rate; UFE = urea-N excretion in feces; UUE = urinary urea-N elimination; UUA = utilization for anabolic purposes.²P-values are for CP content, source of DDGS, and CP × DDGS interaction.

lower ruminal $\text{NH}_3\text{-N}$ concentrations in cows that were fed the low-CP compared with those fed the high-CP diet. It has been suggested that a ruminal $\text{NH}_3\text{-N}$ concentration between 11.0 and 11.8 mg/dL is required to support maximum microbial protein synthesis (Balcells et al., 1993; Reynal and Broderick, 2005). In the present study, ruminal $\text{NH}_3\text{-N}$ concentration on the 15.2% CP diet was below this threshold by >20%. The ruminal deficiency of $\text{NH}_3\text{-N}$ could have suppressed microbial growth in cows fed the low-CP diet, such that the omasal flow of microbial NAN was 108 g/d lower compared with cows fed the high-CP diet. Olmos Colmenero and Broderick (2006b) also reported a lower microbial NAN flow as dietary CP content was lowered from 19.4 to 13.5%. The choice of W-DDGS or B-DDGS as the major source of dietary protein had no influence on omasal flows of microbial NAN and N fractions. Intakes of N were similar in cows fed W-DDGS and B-DDGS, so it is not surprising that total N flow at the omasal canal was similar because it is primarily dictated by N intake (Reynal and Broderick, 2005; Olmos Colmenero and Broderick, 2006b). Our results show that no differences existed in RDP supply or ruminal $\text{NH}_3\text{-N}$ concentration when W-DDGS or B-DDGS were fed as the major sources of protein. As ruminal $\text{NH}_3\text{-N}$ availability has a major influence on microbial growth, it is not surprising that the omasal flow of microbial NAN was unaffected by source of dietary protein. Across dietary treatments, omasal flow of $\text{NH}_3\text{-N}$ accounted for only 3 to 4% of omasal flow of total N, supporting previous studies (Brito et al., 2007). Omasal flow of $\text{NH}_3\text{-N}$ tended to be greater in cows fed the high-CP compared with the low-CP diet, which was expected, as omasal flow of $\text{NH}_3\text{-N}$ is reflective of ruminal $\text{NH}_3\text{-N}$ concentration (Brito and Broderick, 2007); however, feeding W-DDGS or B-DDGS did not influence the omasal flow of $\text{NH}_3\text{-N}$.

A major objective of the current study was also to determine how interactions between dietary CP content and source of supplemental protein alter urea-N recycling in dairy cows; however, no interactions were detected for the major variables quantifying urea-N recycling kinetics. Our results indicate that endogenous urea production (i.e., UER) was higher in cows fed the high-CP diet compared with those fed the low-CP diet. This is reflective of the differences in N intake, as UER is positively correlated with N intake (Lapierre and Lobley, 2001). For all treatments, the UER:digestible N intake ratios (digestible N intake was calculated using N intake and digestibility data that are presented in Table 5) were 0.90 to 1.07, which fall within the range of 0.43 to 1.23 (mean of 0.88) that was reported previously (Lapierre and Lobley, 2001) for lactating cows at various levels of production and fed a wide variety

of diets. That UER was 0.90 to 1.07 of digestible N intake clearly indicates the magnitude of the transit of N into the urea-N pool and the perpetual reliance of ruminants on urea-N recycling to the GIT to maintain a positive N balance. If no mechanism existed to recapture some of the UER and all of it was lost in urine, then ruminants would be in negative N balance. Indeed, GER:UER ratios (i.e., the proportion of endogenous urea-N that was transferred to the GIT) ranged from 0.772 to 0.805 across dietary treatments and this range is in agreement with previous reports (Marini and Van Amburgh, 2003; Marini et al., 2004). Recycled urea-N buffers the forestomachs from the low ruminal $\text{NH}_3\text{-N}$ concentration that usually prevails when dietary CP content is low, thus providing additional N that can be used to maintain microbial protein synthesis (Lapierre and Lobley, 2001; Reynolds and Kristensen, 2008).

Although cows fed the high-CP diet tended to have greater GER compared with those fed the low-CP diet, most of the recycled urea-N was reabsorbed as $\text{NH}_3\text{-N}$ (i.e., ROC). Consequently, the proportion of GER that was used for anabolic purposes (i.e., UUA) was unaffected by dietary CP content. The anabolic utilization of recycled urea-N is primarily via microbial incorporation of $\text{NH}_3\text{-N}$ arising from urea-N degradation (Lobley et al., 2000). Our observations indicate that the amount of recycled urea-N that was recovered as microbial NAN was low, ranging from 60.0 to 68.9 g/d, or 8.5 to 11.5%, when expressed as a proportion of microbial NAN. In dairy cows consuming 350 to 414 g of N/d, estimates obtained using ^{15}N isotope dilution techniques indicated that 34 to 41 g (or 12 to 25%, when expressed as a proportion of microbial NAN) of duodenal microbial NAN was derived from recycled urea-N (Ouellet et al. 2002; Lapierre et al., 2008; Ouellet et al., 2010), which is higher than our own estimates. Differences in N intakes and, as a consequence, ruminal $\text{NH}_3\text{-N}$ pool sizes could explain the discrepant results between studies in the proportion of recycled urea-N that is used for microbial NAN production. The mean N intake in our study (~ 719 g of N/d) was approximately 337 g/d greater than the mean N intake (~ 382 g/d) in the previous studies above. Apart from recycled urea-N, ruminal $\text{NH}_3\text{-N}$ for microbial NAN synthesis can also be derived from the degradation of dietary N. As dietary N intakes increases, therefore, ruminal bacteria become less dependent on recycled urea-N as a source of $\text{NH}_3\text{-N}$. Feeding W-DDGS or B-DDGS as the major source of protein did not influence urea-N kinetics. Although a tendency existed for ruminal $\text{NH}_3\text{-N}$ concentration to be higher in cows fed W-DDGS compared with those fed B-DDGS, we can surmise that the difference in ruminal $\text{NH}_3\text{-N}$ concentration were not

large enough to elicit the inhibitory effects of $\text{NH}_3\text{-N}$ on urea-N transfer into the rumen.

Our results showed that most of the positive responses in animal performance (i.e., milk yield and composition, and N efficiency) that we observed were due to changes in dietary CP content, rather than changes in the major source of dietary CP. Perusal of the literature indicates that reducing dietary CP content is beneficial in reducing feed costs and N losses to the environment (Olmos Colmenero and Broderick, 2006b; Hristov et al., 2011); however, responses in milk production to reducing dietary CP content have been equivocal. In our study, feeding a diet containing 15.2% CP compared with 17.3% CP lowered milk yield by 3 kg/cow per day. Also, milk protein content and yield decreased by 0.12 percentage units and 140 g/d, respectively. This decrease in production performance that we observed with the low-CP diet is undesirable for economic reasons, and it could primarily be attributed to a deficiency in MP supply at the small intestine, which was 103 g/d lower compared with the high-CP diet. As discussed previously, the lower RDP supply in cows fed the low-CP diet could be responsible for the lower MP supply. Others (Firkins et al., 1986; Allen, 2000) have reported that an insufficient RDP supply can also decrease milk production by reducing fiber digestion, which, in turn, can result in reduced DMI and nutrient supply for milk production. In the present study, DMI was unaffected by dietary RDP content, and apparent ruminal fiber digestion was greater in cows fed the low-CP compared with those fed the high-CP diet; however, total-tract fiber digestion was similar across RDP levels, thus indicating that the lower ruminal fiber digestion with the high-RDP diet was offset by higher postruminal fiber digestion. Apparent total-tract digestibility of DM and N were actually lower in cows fed the low-RDP compared with those fed the high-RDP diet, suggesting that the lower milk yield in cows fed the low-RDP diet could partly be attributed to a reduction in nutrient supply.

Feed efficiency (i.e., milk yield/DMI) was improved when cows were fed the high-CP diet, a response that can be partly attributed to the higher milk yield, as DMI did not change. As expected, cows fed the high-CP diet excreted more N compared with cows fed the low-CP diet, with this response due to higher urinary N excretion (rather than fecal N excretion), as has been reported by others (Olmos Colmenero and Broderick, 2006b; Lee et al., 2011). However, milk N efficiency (i.e., milk N/N intake) did not improve when we fed the low-CP diet because the lower N intake was matched by a lower milk N content, as has been reported previously (Haque et al., 2012). Across dietary treatments, total N

excretion as a proportion of N intake ranged from 67.7 to 71.0%, which falls within the range that is typically reported in the literature (Castillo et al., 2001). Other indicators of N use that we measured were PUN and MUN concentrations. Feeding the low-CP diet resulted in lower PUN and MUN concentrations compared with feeding the high-CP diet, as has been reported in other studies in which dietary CP content has been manipulated (Olmos Colmenero and Broderick, 2006b; Lee et al., 2011). Low concentrations of PUN and MUN suggest efficient use of dietary CP (Olmos Colmenero and Broderick, 2006b).

Feeding W-DDGS or B-DDGS as the major protein source did not influence production responses. Our results indicate that RDP contents in diets containing W-DDGS and B-DDGS were similar at 11.3 and 10.7%, respectively, and were above the recommended range of 9.5 to 10.5% for maximum microbial protein synthesis (NRC, 2001). It is not surprising, therefore, that the source of dietary protein did not influence microbial NAN and MP supply and, as a consequence, milk production was unaffected by source of dietary protein.

CONCLUSIONS

Results from this study indicate that feeding a diet containing 15.2% CP compared with 17.3% CP lowered milk production. This response was partly attributed to an insufficient supply of RDP that suppressed microbial NAN synthesis in the rumen, thus resulting in a lower intestinal MP supply. Our results also indicate that feeding up to 10% of dietary DM as B-DDGS or W-DDGS results in similar levels of omasal MP supply and milk production in dairy cows; thus, W-DDGS and B-DDGS are comparable as sources of protein in dairy cow diets.

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REFERENCES

- Abdelqader, M. M., and M. Oba. 2012. Lactation performance of dairy cows fed increasing concentrations of wheat dried distillers grains with solubles. *J. Dairy Sci.* 95:3894–3904.
- Agle, M., A. N. Hristov, S. Zaman, C. Schneider, P. M. Ndegwa, and V. K. Vaddella. 2010. Effect of dietary concentrate on rumen function, digestibility, and nitrogen losses in dairy cows. *J. Dairy Sci.* 93:4211–4222.

- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624.
- AOAC (Association of Official Analytical Chemists). 1990. *Official Methods of Analysis*. 15th ed. AOAC, Arlington, VA.
- Archibeque, S. L., J. C. Burns, and G. B. Huntington. 2001. Urea flux in beef steers: Effects of forage species and fertilization. *J. Anim. Sci.* 79:1937–1943.
- Balcells, J., J. A. Guada, C. Castrillo, and J. Gasa. 1993. Rumen digestion and urinary excretion of purine derivatives in response to urea supplementation of sodium-treated straw fed to sheep. *Br. J. Nutr.* 69:721–732.
- Boila, R. J., and J. R. Ingalls. 1994a. The ruminal degradation of dry matter, nitrogen and amino acids in wheat-based distillers dried grains in sacco. *Anim. Feed Sci. Technol.* 48:57–72.
- Boila, R. J., and J. R. Ingalls. 1994b. The post-ruminal digestion of dry matter, nitrogen and amino acids in wheat-based distillers dried grains and canola meal. *Anim. Feed Sci. Technol.* 49:173–188.
- Brake, D. W., E. C. Titgemeyer, M. L. Jones, and D. E. Anderson. 2010. Effect of nitrogen supplementation on urea kinetics and microbial use of recycled urea in steers consuming corn-based diets. *J. Anim. Sci.* 88:2729–2740.
- Brito, A. F., and G. A. Broderick. 2007. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. *J. Dairy Sci.* 90:1816–1827.
- Brito, A. F., G. A. Broderick, and S. M. Reynal. 2007. Effects of different protein supplements on omasal nutrient flow and microbial protein synthesis in lactating dairy cows. *J. Dairy Sci.* 90:1828–1841.
- Brito, A. F., G. F. Tremblay, H. Lapierre, A. Bertrand, Y. Castonguay, G. Bélanger, R. Michaund, C. Benchaar, D. R. Ouellet, and R. Berthiaume. 2009. Alfalfa cut at sundown and harvested as baleage increases bacterial protein synthesis in late-lactation dairy cows. *J. Dairy Sci.* 92:1092–1107.
- Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci.* 86:1370–1381.
- Broderick, G. A., P. Huhtanen, S. Ahvenjärvi, S. M. Reynal, and K. J. Shingfield. 2010. Quantifying ruminal N metabolism using the omasal sampling technique in cattle—A meta-analysis. *J. Dairy Sci.* 93:3216–3230.
- Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63:64–75.
- Broderick, G. A., N. D. Luchini, S. M. Reynal, G. A. Varga, and V. A. Ishler. 2008. Effect on production of replacing dietary starch with sucrose in lactating dairy cows. *J. Dairy Sci.* 91:4801–4810.
- Canadian Council on Animal Care. 1993. *Guide to the Care and Use of Experimental Animals*. Vol. 1. Canadian Council on Animal Care (CCAC), Ottawa, Ontario, Canada.
- Castillo, A. R., E. Kebreab, D. E. Beever, and J. France. 2001. A review of efficiency of nitrogen utilization in lactating dairy cows and its relationship with environmental pollution. *J. Anim. Feed Sci.* 9:1–32.
- Chibisa, G. E., D. A. Christensen, and T. Mutsvangwa. 2012. Effect of replacing canola meal as the major protein source with wheat distillers grains with solubles on ruminal function, microbial protein synthesis, omasal flow, and milk production in cows. *J. Dairy Sci.* 95:824–841.
- Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acids analyses of blood and rumen fluid by gas chromatography. *J. Dairy Sci.* 44:1768–1771.
- Firkins, J. L., L. L. Berger, N. R. Merchen, G. C. Fahey Jr., and D. R. Nelson. 1986. Effects of feed intake and protein degradability on ruminal characteristics and site of digestion in steers. *J. Dairy Sci.* 69:2111–2123.
- France, J., and R. C. Siddons. 1986. Determination of digesta flow by continuous marker infusion. *J. Theor. Biol.* 121:105–120.
- Haque, M. N., H. Rulquin, A. Andrade, P. Faverdin, J. L. Peyraud, and S. Lemosquet. 2012. Milk protein synthesis in response to the provision of an “ideal” amino acid profile as 2 levels of metabolizable protein supply in dairy cows. *J. Dairy Sci.* 95:5876–5887.
- Hristov, A. N., M. Hanigan, A. Cole, R. Todd, T. A. McAllister, P. M. Ndegwa, and A. Rotz. 2011. Review: Ammonia emissions from dairy farms and beef feedlots. *Can. J. Anim. Sci.* 91:1–35.
- Huhtanen, P., P. G. Brotz, and L. D. Satter. 1997. Omasal sampling technique for assessing fermentative digestion in the forestomach of dairy cows. *J. Anim. Sci.* 75:1380–1392.
- Huhtanen, P., and A. N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *J. Dairy Sci.* 92:3222–3232.
- Lapierre, H., and G. E. Lobley. 2001. Nitrogen recycling in the ruminant: A review. *J. Dairy Sci.* 84(E. Suppl.):E223–E236.
- Lapierre, H., D. R. Ouellet, R. Berthiaume, R. Martineau, G. Holtrop, and G. E. Lobley. 2008. Distribution of ^{15}N in amino acids during ^{15}N -leucine infusion: Impact on the estimation of endogenous flows in dairy cows. *J. Dairy Sci.* 91:2702–2714.
- Lee, C., A. N. Hristov, K. S. Heyler, T. W. Cassidy, M. Long, B. A. Corl, and S. K. R. Karnati. 2011. Effects of dietary protein concentration and coconut oil supplementation on nitrogen utilization and production in dairy cows. *J. Dairy Sci.* 94:5544–5557.
- Lobley, G. E., D. M. Bremner, and G. Zuur. 2000. Effects of diet quality on urea fates in sheep as assessed by refined, non-invasive ^{15}N urea kinetics. *Br. J. Nutr.* 84:459–468.
- Marini, J. C., J. D. Klein, J. M. Sands, and M. E. Van Amburgh. 2004. Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. *J. Anim. Sci.* 82:1157–1164.
- Marini, J. C., and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. *J. Anim. Sci.* 81:545–552.
- Marsh, W. H., B. Fingerhut, and E. Kirsch. 1957. Determination of urea nitrogen with the diacetyl method and an automatic dialyzing apparatus. *Am. J. Clin. Pathol.* 28:681–688.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Nuez Ortín, W. G., and P. Yu. 2009. Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. *J. Sci. Food Agric.* 89:1754–1761.
- Nuez-Ortín, W. G., and P. Yu. 2010. Estimation of ruminal and intestinal digestion profiles, hourly effective degradation ratio and potential N to energy synchronization of co-products from bioethanol processing. *J. Sci. Food Agric.* 90:2058–2067.
- Nuez-Ortín, W. G., and P. Yu. 2011. Using the NRC chemical summary and biological approaches to predict energy values of new co-product from bio-ethanol production for dairy cows. *Anim. Feed Sci. Technol.* 170:165–170.
- Olmos Colmenero, J. J., and G. A. Broderick. 2006a. Effect of dietary crude protein concentration on ruminal nitrogen metabolism in lactating dairy cows. *J. Dairy Sci.* 89:1694–1703.
- Olmos Colmenero, J. J., and G. A. Broderick. 2006b. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.* 89:1704–1712.
- Orth, R. 1992. Sample day and lactation report. DHIA 200 Fact Sheet A-2. Mid-States Dairy Records Processing Center (DRPC), Ames, IA.
- Ouellet, D. R., R. Berthiaume, G. Holtrop, G. E. Lobley, R. Martineau, and H. Lapierre. 2010. Effect of method of conservation of timothy on endogenous nitrogen flows in lactating dairy cows. *J. Dairy Sci.* 93:4252–4261.
- Ouellet, D. R., M. Demers, G. Zuur, G. E. Lobley, J. R. Seoane, J. V. Nolan, and H. Lapierre. 2002. Effect of dietary fiber on endogenous nitrogen flows in lactating dairy cows. *J. Dairy Sci.* 85:3013–3025.
- Reynal, S. M., and G. A. Broderick. 2005. Effect of dietary level of rumen-degradable protein on production and nitrogen metabolism in lactating dairy cows. *J. Dairy Sci.* 88:4045–4064.
- Reynal, S. M., G. A. Broderick, S. Ahvenjärvi, and P. Huhtanen. 2003. Effect of protein supplements of differing degradability on omasal flow of microbial and undegraded protein. *J. Dairy Sci.* 86:1292–1305.
- Reynolds, C. K., and N. B. Kristensen. 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. *J. Anim. Sci.* 86(E. Suppl.):E293–E305.

- SAS Institute. 2004. SAS/STAT 9.1 User's Guide. SAS Institute Inc., Cary, NC.
- Siddons, R. C., J. Paradine, D. E. Beever, and P. R. Cornell. 1985. Ytterbium acetate as a particulate-phase digesta-flow marker. *Br. J. Nutr.* 54:509–519.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Vicente, F., A. Sarraseca, A. de Vega, and J. A. Guada. 2004. Performance of several Cr and Yb analytical techniques applied to samples of different biological origin (digesta or faeces). *J. Sci. Food Agric.* 84:2035–2040.
- Wickersham, T. A., E. C. Titgemeyer, and R. C. Cochran. 2009a. Methodology for concurrent determination of urea kinetics and the capture of recycled urea nitrogen by ruminal microbes in cattle. *Animal* 3:372–379.
- Wickersham, T. A., E. C. Titgemeyer, R. C. Cochran, and E. E. Wickersham. 2009b. Effect of undegradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. *Br. J. Nutr.* 101:225–232.
- Yang, W. Z., and K. A. Beauchemin. 2005. Effects of physically effective fiber on digestion and milk production by dairy cows fed diets based on corn silage. *J. Dairy Sci.* 88:1090–1098.
- Zhang, S. Z., G. B. Penner, M. Abdelqader, and M. Oba. 2010a. Effects of feeding alfalfa hay on chewing, rumen pH, and milk fat concentration of dairy cows fed wheat dried distillers grains with solubles as a partial substitute for barley silage. *J. Dairy Sci.* 93:3243–3252.
- Zhang, S. Z., G. B. Penner, W. Z. Yang, and M. Oba. 2010b. Effects of partially replacing barley silage or barley grain with dried distillers grains with soluble on rumen fermentation and milk production of lactating dairy cows. *J. Dairy Sci.* 93:3231–3242.