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Effect of slow-release urea inclusion in diets containing modified corn distillers grains on total tract digestibility and ruminal fermentation in feedlot cattle

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ABSTRACT: Ruminal degradable intake protein (DIP) deficit may result when cattle are fed diets containing a greater inclusion of processed corn grain and small to moderate inclusion of corn distillers grains (DG). This deficit may arise from greater proportions of rapidly fermentable carbohydrates and RUP in corn grain. Urea-derived N is 100% DIP; however, rates of degradation of carbohydrates and conventional urea (CU) may not match. Therefore, beneficial effects may result from the use of slow-release urea (SRU) sources over CU when added to DIP-deficient diets. An experiment was conducted to evaluate the effect of increasing DIP concentration through inclusion of 1 of 2 SRU sources or CU in DG-containing feedlot diets on ruminal fermentation and total tract digestibility. In addition, an in situ experiment was conducted to characterize N disappearance of urea sources from polyester bags. Four ruminally cannulated steers (initial BW = 588 ± 8 kg) were arranged in a 4 × 4 Latin square design and assigned randomly to 1 of 4 dietary treatments containing 0% (CON) or 0.6% urea in the form of CU (UREA) or SRU as Optigen II (polymer-encapsulated urea; OPTI) or NitroShure (lipid-encapsulated urea; NITRO), and 30% corn earlage, 20% modified corn DG with solubles, 7.8% corn silage, 4.3% dry supplement, and dry-rolled corn (DM basis). Dietary DIP was estimated at 6.6% and 8.3% for CON and urea-containing dietary treatments, respectively. Steers were fed ad libitum once daily. Differences in purine derivatives-to-creatinine (PDC) index between treatments were used as indicators of differences in microbial CP synthesis. Intake of OM, digestibility of OM, NDF, CP, and starch, ruminal pH, total VFA ruminal concentration, and PDC index were not affected by treatment ($P \geq 0.21$). Concentration of ammonia-N noticeably peaked at 4 h after feed delivery for cattle fed UREA (treatment × time, $P = 0.06$) and measured at least 5.5 mg/dL for any treatment and at any hour after feed delivery. During the first 12 h after incubation, N disappearance was greater for CU and NitroShure than Optigen II (urea source × time, $P < 0.01$). Supplementing DIP through inclusion of CU or SRU did not affect feed intake, digestibility, or most of the ruminal fermentation parameters evaluated, which may relate to the lack of need of urea supplementation in the present experiment. More research is warranted to evaluate the use of SRU in DIP-deficient diets.

Key words: degradable intake protein, distillers grains, feedlot cattle, microbial protein, slow-release urea, volatile fatty acids

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INTRODUCTION

Processed corn-based diets containing moderate concentrations (approximately 20%) of corn distillers grains (**DG**) can result in degradable intake protein (**DIP**) deficit due to increased availability of rumen-fermentable carbohydrates and greater proportion of corn RUP. Results from several studies indicated beneficial effects of adding conventional urea (**CU**) to highly fermentable diets with or without DG (Milton et al., 1997; Shain et al., 1998; Ponce, 2010; Wagner et al., 2010; Ceconi et al., 2015) on cattle performance or ruminal fermentation.

Degradation rates for corn starch and DG-derived NDF range from 10% to 40%/h, and 6% to 8%/h, respectively, while that for CU is estimated at 400%/h

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(Sniffen et al., 1992; NRC, 2000). Asynchronous supply of N and energy may result in fermentation occurring largely without microbial growth, thus reducing efficiency of ruminal fermentation and increasing N losses (Dijkstra et al., 1998; Reynolds and Kristensen, 2008). Thus, further benefits may result from the use of slow-release urea (**SRU**) sources over CU when added to DIP-deficient diets. The inclusion of SRU in beef cattle diets has been evaluated, with variable results (Taylor-Edwards et al., 2009; Bourg et al., 2012; López-Soto et al., 2014). Most of the studies involved forage-based diets, and none were designed to compare the effects of CU with those of SRU in concentrate-based, moderate DG-containing diets intended for feedlot cattle.

In vitro N release differs among SRU sources (DiLorenzo and DiCostanzo, 2007a,b). Therefore, we hypothesized that certain SRU would not provide adequate DIP in time to sustain fast carbohydrate fermentation of high-moisture-stored corn. In addition, potentially increased N recycling to the rumen from CU compared with SRU may attenuate differences between urea sources. The objective of this study was to evaluate effects of adding 1 of 2 SRU sources or CU to a concentrate-based diet with 20% DG on ruminal fermentation and feed digestibility.

MATERIALS AND METHODS

Experiments were conducted at the University of Minnesota Rosemount Research and Outreach Center in Rosemount, MN. Animal care and handling procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee.

Experiment 1

Experimental diets and design, cattle handling, and feeding protocol. Dietary treatments consisted of a negative control (**CON**: 30% corn earlage, 20% modified DG with solubles [**MDGS**], 7.8% corn silage, 4.3% dry supplement, and dry-rolled corn [**DRC**]; Table 1), a positive control (**UREA**: CON + 0.6% CU), and 2 SRU-containing diets (**OPTI:** CON + 0.67% Optigen II [polymer-encapsulated urea with 89% urea; Alltech Inc., Nicholasville, KY], and **NITRO**: CON + 0.67% NitroShure [lipid-encapsulated urea with 89% urea; Balchem, New Hampton, NY]). The negative control was included to determine the need for DIP supplementation. Modified DG with solubles used during the entire experiment were obtained from a single ethanol plant. Sources of SRU were chosen based on results from the studies by DiLorenzo and DiCostanzo (2007a,b), in which in vitro ammonia-N (**NH_3–N**) release rates for CU, Optigen II, and NitroShure were determined. Dietary concentration of urea was decided based on results from Ceconi et al. (2015). Dietary CP concentrations measured 13% and 14.7% for CON and the urea-containing diets, respectively (Table 1). Dietary DIP concentrations were estimated using measured CP and published DIP concentrations of each diet ingredient. Published DIP concentrations (CP basis) were 40%, 62%, 75%, 100%, and 49% for DRC, corn earlage, corn silage, urea (NRC, 2000), and MDGS (adapted from Cao et al., 2009), respectively. Dietary TDN concentrations were calculated using the summative energy equation suggested by Weiss et al. (1992). Diets were prepared daily using a portable, electric cement mixer.

Table 1. Composition (DM basis) of diets used in Exp. 1 (negative control without urea added [CON], positive control with conventional urea [UREA], and dietary treatments containing 1 of 2 slow-release urea sources: Optigen II[1] [OPTI] or NitroShure[2] [NITRO]) and Exp. 2 (CON)

	Treatment			
Item	CON	UREA	OPTI	NITRO
Dry-rolled corn, %	37.90	37.30	37.23	37.23
Corn earlage,[3] %	30.00	30.00	30.00	30.00
Modified DG w/solubles, %	20.00	20.00	20.00	20.00
Corn silage, %	7.80	7.80	7.80	7.80
Urea, %	–	0.60	–	–
Optigen II, %	–	–	0.67	–
NitroShure, %	–	–	–	0.67
Dry supplement,[4] %	4.30	4.30	4.30	4.30
Analyzed chemical composition,[5] %				
OM	93.6	93.5	93.6	93.6
CP	13.0	14.7	14.7	14.7
NDF	20.8	19.7	20.3	20.8
Starch	45.4	44.7	43.9	44.5
Calculated chemical composition, %				
TDN, %	79.1	79.6	79.2	79.5
NE_g, Mcal/kg	1.28	1.31	1.30	1.31
Basal DIP,[6] %	6.6	6.6	6.6	6.6
Supplemental DIP,[6] %	0.0	1.7	1.7	1.7
Total DIP,[6] %	6.6	8.3	8.3	8.3
eNDF,[6] %	6.6	6.6	6.6	6.6

[1]Contained 89% urea; Alltech Inc., Nicholasville, KY.

[2]Contained 89% urea; Balchem, New Hampton, NY.

[3]Ensiled corn ear: 8.2% CP, 22.4% NDF, and 1.30 Mcal/kg of NE_g (DM basis), and 65.7% DM.

[4]Beef distillers finisher R-800 (Form-A-Feed Inc., Stewart, MN). Contained 800 g/ton of monensin (Rumensin90, Elanco Animal Health, Greenfield, IN), 7% salt, 25% Ca, 3% K, 10 ppm Co, 350 ppm Cu, 9 ppm Se, 1,750 ppm Zn, 148,000 IU/kg of vitamin A, 29,000 IU/kg vitamin D_3, 165 IU/kg vitamin E, and 495 mg/kg of thiamine.

[5]Performed in duplicate on a composite derived from 8 samples.

[6]Based on published degradable intake protein (DIP) and effective NDF (eNDF) concentrations for each ingredient (Lardy et al., 1998; NRC, 2000; Cao et al., 2009).

Four Holstein steers (initial BW = 588 ± 8 kg) fitted with flexible ruminal cannulae were arranged in a 4 × 4 Latin square design and assigned randomly to 1 of the 4 aforementioned dietary treatments. The experiment consisted of four 21-d periods. Steers were weighed before feed delivery on d 1 and 2 of each period. Steers were individually housed in pens from d 1 to 15 and in metabolism stalls from d 16 to 21. During the first 13 d of each period, steers were adapted to their assigned diet. Feed was offered once daily at 0900 h. Bunks were monitored daily to adjust feed offered. From d 14, diets were offered ad libitum and refusals were recorded daily and kept in the bunk unless they represented more than 5% of daily feed delivery. In this case, the refusal was removed, weighed, and sampled. Otherwise, refusals were removed, weighed, and sampled at the end of each period. Diets and feed ingredients were sampled on d 17 and 18 of each period and stored at -20°C.

Fecal, urine, and ruminal fluid sample collection, data collection, and laboratory analyses. To determine apparent total tract digestibility of OM and its components, animals were intraruminally dosed at 0700 and 1900 h daily with 7.5 g of chromic oxide contained in porcine gelatin capsules from d 11 to 21. Fecal grab samples were collected at 0700, 1300, and 1900 h from d 17 to 21 and stored at -20°C. After completion of the experiment, fecal samples were freeze-dried and ground. Ten grams were subsampled from each fecal sample and thoroughly mixed to obtain a sample composited by steer and period. Chromium concentration in feces was determined using an atomic absorption spectrophotometer (AAanlyst 200; PerkinElmer, Walther, MA) based on the procedure suggested by Williams et al. (1962). Daily fecal output was estimated as

$$\frac{\textit{Chromic oxide dose (g/d)} \times \textit{Cr concentration in chromic oxide (g/g)}}{\textit{Cr concentration in feces (g/g)}}.$$

Feed and fecal OM, CP, starch, and NDF contents were determined by methods 942.05, 990.03, 920.40, and 2002.04 (AOAC, 2012), respectively. Digestibility of OM and its components was estimated as

$$\left(\frac{\textit{intake (g)} - \textit{excretion (g)}}{\textit{intake (g)}}\right) \times 100.$$

Urea-derived N that is not utilized by ruminal microbes is partially excreted in the urine. Therefore, to avoid overestimation of CP (N × 6.25) digestibility under potential excess urea-derived N, CP digestibility was also estimated as

$$\left[\frac{\left(\begin{array}{c}\textit{Total CP intake (g)} - \\ \textit{supplemental urea-derived} \\ \textit{CP intake (g)}\end{array}\right) - \textit{CP excretion (g)}}{\begin{array}{c}\textit{Total CP intake (g)} - \\ \textit{supplemental urea-derived} \\ \textit{CP intake (g)}\end{array}}\right] \times 100$$

Daily excretion of OM was calculated based on OM concentration in feces and daily fecal output. Similar calculations were used to estimate excretion of OM components.

Ruminal pH was recorded by sensors programmed to measure and record ruminal pH every 5 min (Omega Engineering Inc., Stamford, CT). Probes were inserted into the rumen of each steer on d 16 and removed at the end of each period. Data were downloaded from individual probes on completion of each period and composited by steer and period on an hourly basis for a 24-h period.

Concentrations of VFA and NH_3–N were measured in ruminal fluid samples collected on d 21 at -1, 2, 4, 8, 12, 16, and 24 h after feed delivery. Ruminal fluid samples were collected by a manual suction strainer inserted through the ruminal cannula. Concentrations of VFA were determined using a Hewlett-Packard 6890 gas chromatograph (Agilent Tech., Santa Clara, CA) based on the procedure suggested by Erwin et al. (1961). Concentration of NH_3–N was determined by colorimetry based on the phenol-hypochlorite assay (Broderick and Kang, 1980) and using a spectrophotometer (Gilford Stasar II; Gilford Instrument Laboratories Inc., Oberlin, OH).

Differences in purine derivatives-to-creatinine (**PDC**) index between treatments were used as indicators of differences in microbial CP (**MCP**) synthesis (Chen and Ørskov, 2004). The PDC index was defined as

$$BW^{0.75} \times \frac{PD}{Crt},$$

where purine derivatives (**PD**) and creatinine (**Crt**) were expressed in m*M* and BW in kg (Chen et al., 2004). Purine derivatives from the metabolism of microbial nucleic acids (Chen and Ørskov, 2004) and Crt from muscle metabolism (Lofgreen and Garrett, 1954; Van Niekerk et al., 1963) are excreted in the urine. Therefore, PD (allantoin and uric acid) and Crt were simultaneously determined in urine samples (Shingfield and Offer, 1999) using a high-performance liquid chromatograph (Gilson, Middleton, WI). Urine was sampled at the same time points used for fecal sample collection. Urine samples were collected using a 2-L container attached to the steer via a rope fastened around the steer's body, allowing for a free-flowing urine sample. After collection, the container was removed from the animal and 50 mL of urine were

transferred into a plastic conical tube and immediately stored at -20°C. After completion of the experiment, urine samples were composited by time, steer, and period.

Calculations and data analyses. Microbial efficiency was estimated as

$$\frac{MCP\ flow\ (g/d)}{OM\ digested\ (g/d)} \times 100 .$$

Chen and Gomes (1995) proposed a formula to predict absolute MCP flow from urinary PD. Many of the factors included in this model are highly variable, which may result in estimations inconsistent with MCP flow estimated from duodenal purines (Crawford, 2007). However, urinary PD are reliable markers for estimating relative differences in MCP flow (Südekum et al., 2006). Therefore, treatment effect on microbial efficiency was analyzed using absolute PD-derived MCP flow. However, when treatment effects were significant, differences in microbial efficiency among treatments were reported in relative terms (%). Otherwise, only *P*-value for treatment effect was reported. Absolute MCP flow (g/d) was estimated as

$$\frac{PD_a \times 70 \times 6.25}{0.116 \times 0.830 \times 1000},$$

where $\mathbf{PD_a}$ represented intestinally absorbed PD of microbial origin (mmol/d), 70 was N concentration of purines (mg/mmol), 6.25 was the conversion factor of N to CP, 0.116 was the ratio of purine-N to total N in mixed ruminal microbes, and 0.83 was the intestinal digestibility of microbial purines (Chen and Gomes, 1995). The equation

$$\frac{PD_e - 0.385 \times BW^{0.75}}{0.850}$$

was used to estimate PD_a, where **PDe** was the amount of PD excreted by the animal (mmol/d), 0.385 was the endogenous contribution of PD per kilogram of metabolic BW (kg), and 0.85 represented the proportion of absorbed PD that are recovered in urine; PDe was estimated based on urinary PD concentration and total urine output, assuming that the internal marker Crt was excreted at a rate of 26 mg/kg of BW (Whittet et al., 2004).

Relative differences (%) in microbial efficiency between treatments were estimated as

$$\frac{A_U - A_{CON}}{A_{CON}} \times 100,$$

where $\mathbf{A_{CON}}$ and **AU** were defined as absolute microbial efficiency for CON and urea-containing diets, respectively.

Balances of DIP and MP were calculated for each treatment using Level 1 of the NRC (2000) model. Nitrogen available for synthesis of MCP was represented by DIP supply, which was estimated by the model as a function of DMI and dietary DIP concentration. Rumen-degradable N needs for MCP synthesis were represented by DIP requirements, and DIP requirements were calculated as TDN intake adjusted by microbial efficiency. Microbial efficiency was defined as g of MCP produced per 100 g of TDN. The model assumes that, when present, DIP deficiencies (DIP requirements > DIP supply) are overcome by degradable N supplementation. Therefore, the amount of MCP synthesized in the rumen per day was represented by daily DIP requirements. Additionally, DIP and MP balances were estimated based on some modifications to Level 1 of the NRC (2000) model, which considered inefficiencies in NH_3–N capture by ruminal microbes and contribution of MCP to MP supply under negative DIP balance. Efficiency of NH_3–N capture by ruminal microbes is below 100% due to direct absorption of NH_3–N across the rumen wall and passage of fluid from the rumen (NRC, 1985). Therefore, DIP balance was estimated as

$$DIP\ supply\ (g/d) - DIP\ requirements\ (g/d) \times 1.18,$$

where 1.18 represented the mean RDP to microbial N ratio (NRC, 2001).

When DIP balance results were positive, contribution of MCP to MP supply was calculated as

$$DIP\ requirements\ (g/d) \times 0.64,$$

where 0.64 represented the proportion of true protein in microbial protein (80%) multiplied by its intestinal digestibility (80%; NRC, 2000). When negative, contribution of MCP to MP supply was estimated as

$$DIP\ supply \times 0.85 \times 0.64,$$

where 0.85 represented the ratio between 1.00 and 1.18 (NRC, 2001) or the efficiency in NH_3–N capture by ruminal microbes. Treatment ADG used for MP requirements calculations was estimated using NRC (2000) equations and based on observed DMI and DM digestibility.

Data collected after the end of the adaptation period (d 16) were analyzed using the Mixed procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). For pH, VFA and NH_3–N ruminal concentrations, and PDC index a repeated measure structure was utilized. The model included dietary treatment, time, treatment × time interaction, and period as fixed effects and animal as random effect. Degrees of freedom were calculated with the Satterthwaite option. The best covariance structure was selected for each variable following procedure suggested by Casler (2006). Briefly, unstructured matrices of variances, covariances, and correlations were requested. Based on

this information, inadequate structures were ruled out. When more than one structure was potentially suitable, final selection was made based on information criteria (Res Log Likelihood, Akaike's Information Criterion, Schwarz's Bayesian Information Criterion, Hannan and Quinn Information Criterion, and Consistent Akaike's Information Criterion). To decide between models that differed in number of parameters, a χ^2 test involving the *residual log likelihood* criteria was performed. When null hypothesis was not rejected (calculated χ^2 value < critical χ^2 value), data were analyzed based on the reduced model. When transformation of variables was applied to stabilize variances and/or normalize data, statistical analyses were performed on the transformed variable to report *P*-values and on the non-transformed variable to report treatment means and standard errors.

Effects were considered significant when *P*-values were less than or equal to 0.05 and were considered trends when *P*-values were between 0.05 and 0.10. When *P*-value for treatment effect was below 0.10, treatment means were separated using a *t* test (*pdiff* option). When significant, the time effect was evaluated by polynomial contrasts, for which coefficients for unequally spaced sampling times were obtained through the IML procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). Associations between ruminal pH and concentration of total VFA and the former and acetate-to-propionate ratio were analyzed using the Reg procedure of SAS 9.3.

Experiment 2

Cattle and sample handling, experimental design, feeding protocol, and chemical analyses. Two Holstein steers (initial BW = 710 ± 20 kg) fitted with flexible ruminal cannula and individually housed in pens were arranged in a randomized complete block design. Steers were adapted to Exp. 1 CON dietary treatment from d 1 to 7 and were fed ad libitum from d 8 to 10. Steers were fed once daily at 0900 h.

On d 10, N-free polyester bags (5 cm × 10 cm, 50 µm porosity; Ankom Tech., Macedon, NY) containing 0 (blank) or 1 g of CU, Optigen II, or NitroShure with 99.7% DM were introduced in the ventral sac of the rumen of each steer at 0900 h and removed at 1, 2, 4, 6, 8, 12, and 24 h after incubation/feed delivery. Urea samples were analyzed for DM content by oven-drying at 60°C for 48 h. The amount of sample contained within each bag was set considering a relationship of 10 mg of sample/cm^2 of bag (Vanzant et al., 1998). For each incubation time, polyester bags were placed into a weighted mesh bag that was attached to a string. The end of the string was left outside the rumen to facilitate removal. Before incubation in the rumen, bags were placed in a water bath at 39°C for 15 min to simulate insalivation (Nocek and English, 1986; Nocek, 1988). Immediately after retrieval from the rumen, bags were rinsed with cold water until rinsing water was clear. Afterward, bags were dried in a forced-air oven at 60°C for 48 h. Bags corresponding to 0 h after feed delivery were not incubated in the rumen but rinsed and dried after removal from the warm bath. Bags were prepared in duplicate for each combination of blank or urea source, and sampling time.

Disappearance of N from polyester bags was estimated as

$$\frac{N_{IN}\,(g) - N_{OUT}\,(g)}{N_{IN}(g)} \times 100\,,$$

where N_{IN} and N_{OUT} represented the amount of N before and after incubation, respectively. Nitrogen content was determined on dried samples using a 2300 Kjeltec analyzer unit (Foss Tecator AB, Höganäs, Sweden).

Data analyses. Data were analyzed using the Mixed procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC), where animal (block) was considered a random effect. Level of significance was set and treatment means separation was performed as described in Exp. 1.

RESULTS AND DISCUSSION

Unless otherwise specified, treatment × time interactions were nonsignificant ($P > 0.10$). Dietary treatments did not affect ($P \geq 0.53$; Table 2) DM or OM intake; DM intake averaged 2.09% of BW. Similarly, neither digestibilities of NDF, CP, nor starch were affected ($P \geq 0.21$) by urea inclusion, regardless of source. Thus, OM digestibility was similar ($P = 0.67$) among treatments. In agreement with these findings, no beneficial effects on DM or OM digestibility were observed by Garrett et al. (2005), Álvarez Almora et al. (2012), and Holder (2012) when comparing supplementation of CU with NitroShure or with Optigen II, respectively, to diets containing at least 50% forage. However, none of these studies included a negative control to test the effectiveness of different urea sources. Ribeiro et al. (2011) investigated effects of urea and Optigen 1200 supplementation to steers consuming *Brachiaria* hay on ruminal fermentation parameters and feed digestibility. The authors reported similar OM digestibility between cattle fed the negative control, CU-, and SRU-supplemented diets.

Differences in ruminal concentration of NH_3–N among treatments varied across hours after feed delivery (treatment × time, $P = 0.06$; Fig. 1). Concentration of NH_3–N noticeably peaked at 4 h after feed delivery for cattle fed UREA. Ribeiro et al. (2011) reported ruminal concentrations of NH_3–N peaking between 1 and 2 h after

Table 2. Intake and apparent total tract digestibility in cattle fed finishing diets without urea added (negative control [CON]), or added at 0.6% of dietary DM, in the form of conventional urea (positive control [UREA]) or 1 of 2 slow-release urea sources (Optigen II[1] [OPTI] or NitroShure[2] [NITRO])

	Treatment					
Item	CON	UREA	OPTI	NITRO	SEM	*P*-value
n	4	4	4	4	–	–
Intake, kg/d						
DM	14.4	15.4	15.4	14.4	0.7	0.53
OM	13.5	14.3	14.4	13.5	0.7	0.58
Digestibility, %						
OM	67.4	71.0	67.9	70.1	2.6	0.67
NDF	33.4	35.5	32.3	35.4	4.7	0.94
CP	63.1	70.0	66.7	70.8	2.5	0.21
CP[3]	63.1	66.4	62.4	67.3	2.6	0.51
Starch	87.7	88.3	87.3	89.5	2.0	0.56

[1]Contained 89% urea; Alltech Inc., Nicholasville, KY.

[2]Contained 89% urea; Balchem, New Hampton, NY.

[3]Estimated based on non-urea N intake.

feed delivery and being greater for steers receiving urea, intermediate for those supplemented with Optigen 1200, and smallest for the unsupplemented steers during the first 4 h after feed delivery. Similarly, García-González et al. (2007) reported greater ruminal concentration of NH_3–N when adding urea, but not when adding Optigen to 60% corn silage diets fed to cattle. On the contrary, no differences in NH_3–N concentration between urea- and Optigen II-supplemented steers were observed by Álvarez Almora et al. (2012), and the authors attributed this to endogenous urea being recycled back to the rumen and masking potential differences between treatments. Disappearance of N from polyester bags was complete after a 15-min exposure to warm water (0 h incubation) for CU and NitroShure, while it rapidly increased from 27.8% at 0 h to 63.0% at 1 h after incubation and increased almost at a constant rate afterward, up to 93.2% at 24 h after incubation for Optigen II (urea source × time, $P < 0.01$; Fig. 2). Holder (2012) reported that urea disappearance from polyester bags was complete for CU at 1 h after incubation in the rumen while it increased from 0% to about 20% in 1 h and increased thereafter up to 60% at 24 h for Optigen II. The fact that polyester bags were not soaked in warm water before incubation in the study by Holder (2012) may explain a slower Optigen II disappearance compared with that in the present experiment. A complete N disappearance after a 15-min warm-water bath for CU and Nitroshure suggests that derived NH_3–N would be readily available in the rumen.

Results from in vivo and in situ experiments indicate contrasting ruminal concentration of NH_3–N, and N disappearance from polyester bags, between SRU sources;

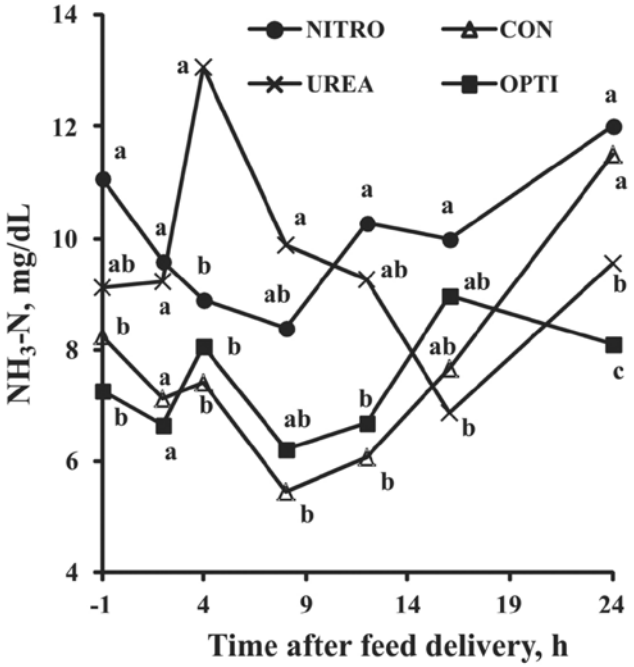

Figure 1. Ammonia-N (NH_3–N) as affected by hours after feed delivery in cattle fed diets without urea added (negative control [CON]), or added at 0.6% of dietary DM, in the form of conventional urea (positive control [UREA]) or 1 of 2 slow-release urea sources (Optigen II [OPTI; 89% urea; Alltech Inc., Nicholasville, KY] or NitroShure [NITRO; 89% urea; Balchem, New Hampton, NY]). [a,b,c]Means with uncommon superscripts differ within hour after feed delivery at $\alpha = 0.08$ (treatment × time, $P = 0.06$); SED at each time point averaged 1.7 mg/dL.

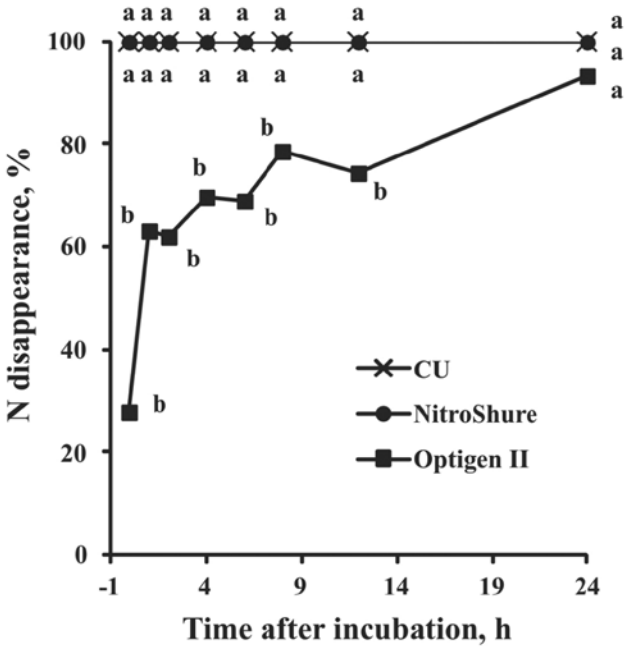

Figure 2. Nitrogen (N) disappearance from polyester bags for conventional urea (CU) and 2 slow-release urea sources (Optigen II [Alltech Inc., Nicholasville, KY] and NitroShure [Balchem, New Hampton, NY]), as affected by hours after incubation in the rumen (Exp. 2). [a,b]Means with uncommon superscripts differ within hour after incubation at $\alpha = 0.05$ (urea source × time, $P < 0.01$); SED at each time point averaged 4.8%.

Table 3. Total VFA ruminal concentration, VFA molar proportions, acetate-to-propionate ratio, ruminal pH, and urinary purine derivatives-to-creatinine (PDC) index at different times after feed delivery in cattle fed finishing diets without urea added (negative control [CON]), or added at 0.6% of dietary DM, in the form of conventional urea (positive control [UREA]) or 1 of 2 slow-release urea sources (Optigen II[1] [OPTI] or NitroShure[2] [NITRO])

	Treatment					*P*-value		
Item	CON	UREA	OPTI	NITRO	SEM	Treatment	Time	Treatment × time
n	4	4	4	4	–	–	–	–
pH	5.77	5.83	5.77	5.75	0.08	0.91	< 0.01	0.74
VFA, m*M*								
Total	108.3	105.4	108.7	107.8	12.4	0.99	< 0.01	0.62
Branched-chain[3]	3.39	3.19	2.30	2.89	0.41	0.31	0.43	0.85
Acetate-to-Propionate	1.50$^{a, x}$	1.36$^{ab, x}$	1.08$^{b, y}$	1.42$^{ab, x}$	0.16	0.09	< 0.01	0.80
VFA, mol/100 mol								
Acetate	49.0	47.6	45.7	48.6	1.1	0.18	< 0.01	0.59
Propionate	34.5^{b}	35.8^{b}	42.6^{a}	37.2^{ab}	2.4	0.07	< 0.01	0.99
Butyrate	11.7	11.8	7.5	9.5	2.2	0.40	0.17	0.43
PDC index[4]	161.1	168.5	171.4	175.0	10.6	0.63	< 0.01	0.91

[1]Contained 89% urea; Alltech Inc., Nicholasville, KY.

[2]Contained 89% urea; Balchem, New Hampton, NY.

[3]Summation of isobutyric, isovaleric, and 2-methyl butyric acids.

[4] $PDC\ index = BW^{0.75} \times \frac{PD}{Crt}$; urinary concentrations (m*M*) of purine derivatives (PD) and creatinine (Crt); BW expressed in kg (Chen et al., 2004). Differences in PDC index among treatments indicated differences in microbial CP synthesis (Chen and Ørskov, 2004).

[a,b]Means with uncommon superscripts differ ($P < 0.05$).

[x,y]Means with uncommon superscripts differ ($P < 0.10$).

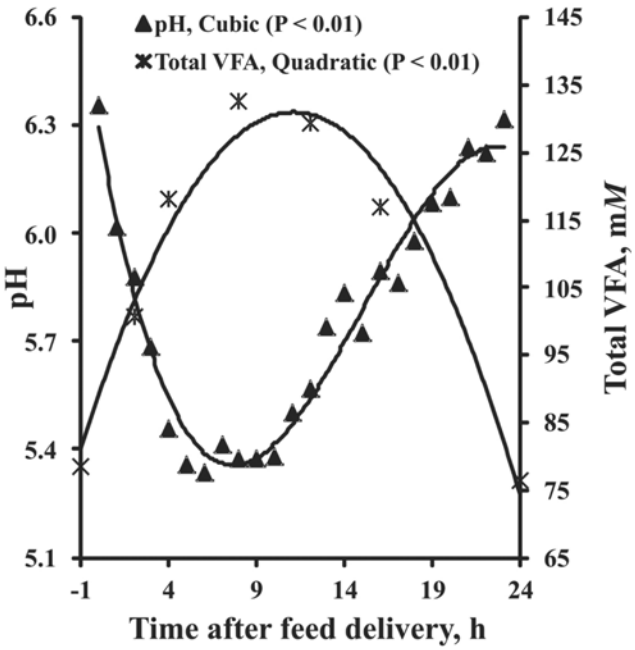

Figure 3. Ruminal pH and total VFA concentration as affected ($P < 0.01$) by hours after feed delivery; SEM at each time point averaged 0.08 for ruminal pH and 10.6 m*M* for total VFA concentration.

N disappearance was similar between NitroShure and CU (Fig. 2), and NH_3–N concentration was similar between cattle fed OPTI and those fed the nonsupplemented diet at most hours after feed delivery (Fig. 1). In in vitro batch culture, DiLorenzo and DiCostanzo (2007a,b) reported that the rate of NH_3–N release was faster for CU compared with NitroShure, while DiLorenzo (unpublished) observed the rate of NH_3–N release being faster for CU, intermediate for NitroShure, and slower for Optigen II.

In agreement with OM digestibility results, total VFA concentration was not affected ($P = 0.99$; Table 3) by treatments, which was consistent with results reported by Garrett et al. (2005) and Álvarez Almora et al. (2012). However, total VFA concentration increased ($P < 0.01$) quadratically with time (Fig. 3). This can be interpreted as microbial activity being stimulated after feed delivery. A concurrent and almost opposite response to time ($P < 0.01$; Table 3) was observed for ruminal pH (Fig. 3), which was not affected ($P = 0.91$) by treatment. Variation in total VFA concentration explained ($P < 0.01$) 84.9% of variation in ruminal pH. This observation is similar to the 86.6% reported by Zinn et al. (2003). Similar to what was observed for total VFA and ruminal pH, the period of increased total VFA concentration mostly related to the period of decreased NH_3–N concentration and vice versa (Fig. 1 and 3). These observations may re-

flect concomitant NH_3–N microbial use during times of increased microbial activity and VFA production.

Branched-chain VFA (**BCVFA**) result solely from the fermentation of branched-chain AA and represent carbon backbone sources for the synthesis of microbial branched-chain AA (Allison and Bryant, 1963; Allison, 1969; Russell, 2002), in particular for cellulolytic bacteria. Based on similar dietary nonurea CP concentration, DMI, and CP digestibility, supply of dietary AA to the rumen might have been similar among treatments. In addition, similar microbial activity, as deduced from similar digestibility of OM and its components, and total VFA concentration among treatments might have resulted in similar microbial utilization of BCVFA. This could explain lack of treatment effect ($P = 0.31$; Table 3) on BCVFA ruminal concentration.

Acetate-to-propionate ratio tended ($P < 0.07$) to be lower for cattle fed OPTI compared with any of the other treatments due to similar ($P = 0.18$) acetate but greater ($P < 0.05$) propionate molar proportions (Table 3). Based on similar DMI, and starch and NDF dietary concentrations and digestibilities among treatments, this result was not expected. Similarly, Álvarez Almora et al. (2012) observed reduced acetate-to-propionate ratio in cattle fed Optigen II compared with CU, when these urea sources were included in forage-based diets. However, Álvarez Almora et al. (2012), as well as Ribeiro et al. (2011), also reported decreased ruminal pH in cattle fed SRU compared with those fed CU. Low pH may negatively impact growth of fiber-fermenting bacteria and might be the result of lower ruminal concentration of NH_3–N, as NH_3–N contributes to ruminal buffering capacity. As mentioned previously, ruminal pH was not affected by treatments in the present experiment; consequently, no association between variations in acetate-to-propionate ratio and ruminal pH across treatments can be established. Contrary to the current findings, however, increased acetate-to-propionate ratio in cattle fed Optigen II compared with those fed CU was reported by Holder (2012). It was suggested that a slower, steadier supply of NH_3–N may favor slow-growing fiber-digesting bacteria. Ammonia-N plays a more important role in nutrition of the relatively slow-growing, fiber-digesting bacteria (Hungate, 1966; Bryant and Robinson, 1963). Therefore, and somewhat opposed to what was suggested by Holder (2012), reduced NH_3–N concentration in cattle fed OPTI compared with other urea sources could have reduced growth of fiber-digesting bacteria and acetate-to-propionate ratio. However, in the current experiment, the association between ruminal concentration of NH_3–N and acetate-to-propionate ratio might be debatable because decreased NH_3–N concentration might reduce growth of cellulolytic bacteria if the decrease in ruminally available N results in an unmet demand of NH_3–N. Based on similar feed digestibility and total VFA concentration among treatments, the existence of an unmet demand of NH_3–N might be arguable.

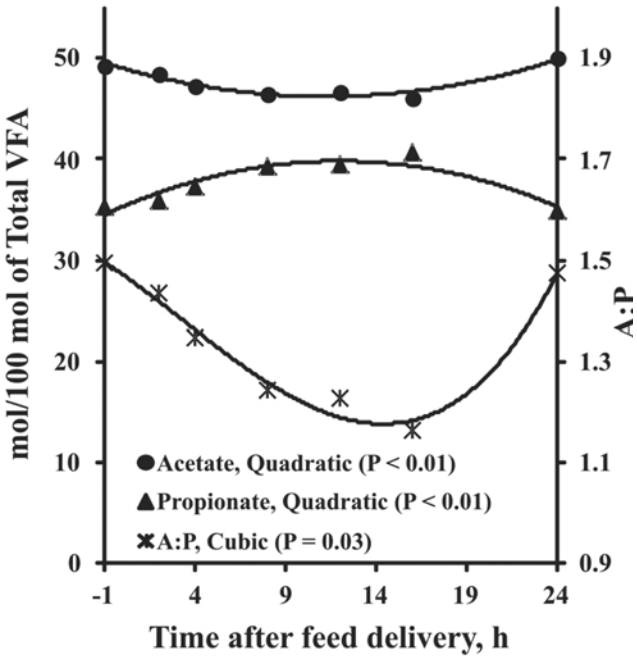

Figure 4. Acetate and propionate molar proportions and acetate-to-propionate ratio (A:P) as affected ($P < 0.01$) by hours after feed delivery; SEM at each time point averaged 0.8 and 2.1 mol/100 mol for acetate and propionate molar proportions, respectively, and 0.1 for A:P.

Ruminal molar proportion of propionate increased ($P < 0.01$; Table 3) while acetate molar proportion decreased ($P < 0.01$) quadratically (Fig. 4) throughout the day. This resulted in a cubic decrease ($P = 0.03$) in the acetate-to-propionate ratio, which could have been associated with a concurrent decrease in ruminal pH (Fig. 1). Ruminal pH can differentially affect ruminal bacteria populations (Russell and Wilson, 1996), which in turn may alter VFA molar proportions, depending on prevailing VFA-production pathways present in each bacteria group. In addition, studies reviewed by Dijkstra et al. (2012) reported that bacteria have the ability to shift VFA-producing pathways in response to changes in pH while fermenting the same substrate. Russell (1998) reported that 25% of the reduction in acetate-to-propionate ratio could be explained by the reduction of pH alone. A similar relationship between acetate-to-propionate ratio and ruminal pH was observed in the present experiment ($P < 0.01$), though the R^2 was smaller (18%).

In agreement with previously described variables, PDC index was not affected ($P = 0.63$; Table 3) by treatment. However, PDC index was smallest ($P < 0.01$) at -2 h (148.6 ± 8.3), intermediate at 4 h (169.3 ± 8.4), and greatest at 10 h after feed delivery (189.1 ± 10.6), resulting in a linear response with time ($P < 0.01$). The effect of time on PDC index was consistent with that on

Table 4. Estimated degradable intake protein (DIP) supply, requirements, and balance (supply − requirements), and MP balance for cattle fed finishing diets without urea added (negative control [CON]), or added at 0.6% of dietary DM, in the form of conventional urea (positive control [UREA]) or 1 of 2 slow-release urea sources (Optigen II[1] [OPTI] or NitroShure[2] [NITRO])

	Treatment			
Item	CON	UREA	OPTI	NITRO
— g/d—				
DIP supply[3]	983	1239	1239	1239
DIP requirements[3]	1023	1016	1015	1015
DIP balance[3]	−40	223	224	224
MP balance[4,5]	549	662	661	661

[1]Contained 89% urea; Alltech Inc., Nicholasville, KY.

[2]Contained 89% urea; Balchem, New Hampton, NY.

[3]Estimated using Level 1 of NRC (2000) model, which assumes that, when present, DIP deficiencies (DIP requirements > DIP supply) are overcome by degradable N supplementation. Therefore, the amount of microbial protein synthesized in the rumen per day was represented by daily DIP requirements.

[4]Estimated using modifications to the Level 1 of the NRC (2000) model: *DIP balance = DIP supply – DIP requirements × 1.18*; when DIP balance resulted positive, contribution of MCP to MP supply was calculated as *DIP requirements × 0.64*; when negative, the contribution was estimated as *DIP supply × 0.85 × 0.64* (NRC, 2001).

[5]Cattle ADG for MP calculations was estimated using NRC (2000) equations and observed DMI and DM digestibility.

VFA and NH_3–N concentrations and ruminal pH, suggesting increased microbial activity and MCP synthesis after feed delivery. Similarly, Crawford (2007) reported greater PD-to-Crt ratio at 9 compared with -1 h after feed delivery. As a result of similar OM intake and digestibility, the amount of OM digested was similar ($P = 0.82$) among treatments. Similar amount of OM digested and PD-derived MCP flow ($P = 0.63$) resulted in similar ($P = 0.50$) microbial efficiency among treatments.

Level 1 of the NRC (2000) model estimates microbial efficiency by estimating ruminal pH. In turn, ruminal pH is estimated based on dietary effective NDF (**eNDF**) concentration. Since dietary eNDF was similar across treatments (6.6%; Table 1), model-estimated pH and resulting microbial efficiency did not differ among treatments. Similar dietary TDN (Table 1), DMI (Table 2), and model-estimated microbial efficiency resulted in similar DIP requirements across diets (Table 4). Because dietary DIP concentration was purposefully altered by urea dietary inclusion, DIP supply was less for cattle fed CON (983 g/d; Table 4) than any of the urea-supplemented diets (1239 g/d). Consequently, estimated DIP balances were −40, 223, 224, and 224 g/d for cattle fed CON, UREA, OPTI, and NITRO, respectively (Table 4). Estimated DIP balance for cattle fed CON decreased from −40 to -224 g/d (data not shown) when inefficiencies in NH_3–N capture by ruminal microbes were considered (NRC, 2001).

Despite estimated DIP deficit for cattle fed CON, supplementation of any form of urea did not improve feed digestibility or ruminal fermentation parameters. These results would suggest that CON was not deficient in DIP. Satter and Slyter (1974) reported that microbial protein yield, measured as tungstic acid-precipitable N, increased linearly with supplementary urea until NH_3–N started to accumulate and that increasing NH_3–N concentration beyond 5 mg/dL had no effect on microbial protein production. In the current experiment, NH_3–N concentrations for cattle fed CON were above 5 mg/dL (Fig. 1), again supporting lack of DIP deficit.

Other microbial-affecting factors, not considered in the NRC (2000) model, may have offset the projected DIP deficit. Relative DMI (2.09% BW) was less than expected (2.24% to 2.45% BW) based on previous studies (Ceconi et al., 2015), which may have decreased outflow rate (Evans, 1981a,b; Sniffen and Robinson, 1987) and microbial efficiency (Isaacson et al., 1975; Russell and Baldwin, 1979; Meng et al., 1999), thus resulting in DIP requirements being smaller than expected. In addition, reduced rate of passage and a concomitantly increased rumen retention time in the present experiment may have permitted greater dietary CP degradability than expected.

Fermentation rate is positively associated with microbial efficiency (Van Soest, 1994) because greater fermentation rate permits faster microbial growth and reduces microbial maintenance requirements. For example, based on rumen fermentability of carbohydrates, Cooper et al. (2002) estimated that minimum dietary DIP concentration that resulted in greatest animal performance was 10.0%, 8.3%, or 6.3% for HMC-, SFC-, or DRC-based finishing diets, respectively. Even though corn earlage provided HMC to the diet, which is highly fermentable, it also contributed a considerable amount of fiber (22.4% NDF; Table 1), which may have reduced diet fermentation rate and microbial efficiency.

Yield of ATP is greatest for glucose derived from polysaccharides (fiber and starch), intermediate for soluble carbohydrates (sugars), and smallest for protein (Van Duinkerken et al., 2011). Consequently, dietary inclusion of 20% MDGS containing 30.6% CP might have reduced microbial efficiency, thus reducing DIP requirements and need for DIP supplementation.

Finally, a DIP deficit can be partially or totally reversed by a positive intestinal MP balance, as urea recycled to the rumen (NRC, 2000). As emphasized by Valkeners et al. (2004), Cabrita et al. (2006), and Reynolds and Kristensen (2008), short-term effects of asynchrony between carbohydrates and N rumen availability, such as a DIP deficit, may be overcome by N recycled via hepatic

synthesis of urea. In that regard, slower diet fermentation rate may have allowed a positive MP balance (Table 4) to recycle urea to the rumen in time to alleviate a potential DIP deficit. Based on NRC (1985) calculations, recycled urea-CP was estimated at 392 g/d for animals fed the CON diet. Jenkins et al. (2011) evaluated the effect of adding CU to a DRC-based diet containing 10% dried DG on finishing cattle performance. Balances of DIP and MP for the no-urea diet were estimated by the authors at −92 and 268 g/d, respectively. Because MP requirements were met, it can be assumed that N from the excess 268 g of MP would be transformed to urea in the liver and that a high proportion of the urea synthesized would be recycled back to the rumen (Reynolds and Kristensen, 2008) to overcome an unmet demand of 92 g of DIP. Lack of effect of urea supplementation on ADG and feed efficiency may indicate that excess MP absorbed at the intestinal level may have been able to supply recycled N in time when carbohydrates from DRC were fermented in the rumen. Similar results were observed by Stalker et al. (2004) when supplementing urea to forage-based diets containing 25% or 30% DG for which the need of DIP supplementation (negative DIP balance) and excess MP (positive MP balance) were calculated to be similar. However, N recycled from excess MP may not have been able to overcome a DIP deficit generated by HMC- or DRC+HMC-, 0%-urea-containing diets, based on the positive response of animal performance, ruminal fermentation, and feed digestibility to urea supplementation (Cooper et al., 2002; Ceconi et al., 2015). This result can be interpreted in terms of inadequacy of excess MP to meet, in time, the DIP deficit generated by rapidly fermentable diets.

In conclusion, results from these experiments did not identify beneficial effects of Optigen II or NitroShure in place of CU. The unsupplemented control diet may not have elicited the DIP deficit originally predicted. Previous studies have demonstrated improved ruminal fermentation, feed digestibility, and animal performance when supplementing DIP through the inclusion of CU to rapidly fermentable, moderate-DG-containing diets; thus, more research is warranted to evaluate the use of SRU in diets for which a DIP deficit is actually expressed.

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