Diurnal and dietary impacts on estimating microbial protein flow from urinary purine derivative excretion in beef cattle[1]

Grant I. Crawford, Jim C. MacDonald, Andrea K. Watson,[2] Galen E. Erickson,® and Terry J. Klopfenstein

Department of Animal Science, University of Nebraska, Lincoln 68583

ABSTRACT: Two experiments were conducted to determine the effects of diet composition and time of urine spot sampling on estimates of urinary purine derivative (PD) excretion. In Exp. 1, 116 individually fed crossbred heifers (407 ± 32 kg) were arranged in a randomized block design (82 d). Treatments were arranged in a 2 × 3 factorial design, with two urine spot sample collection times (0700 and 1700 hours; AM and PM) and three diets: 85% steam-flaked corn (SFC); 85% SFC + 1.5% urea (UREA); or 25% SFC, 30% wet corn gluten feed, and 30% corn bran (BYPROD). In Exp. 2, six ruminally and duodenally fistulated steers (474 ± 37 kg) were arranged in a replicated 3 × 3 Latin square design, with dietary treatments identical to Exp. 1 (63 d). Treatment diets were selected to result in varied amounts of microbial crude protein (MCP) in order to evaluate the accuracy of using estimates of urinary PD excretion to predict MCP. Urine spot samples were collected at 0700, 1200, 1700, and 2200 hours. No urine collection time × diet interactions occurred ($P > 0.20$) for any variable in either experiment. In Exp. 1, dry matter intake (DMI) was greatest with BYRPOD (10.40 kg/d) and lowest with SFC (7.90 kg/d; $P < 0.05$). Feed efficiencies were greatest for UREA (0.182) and least for SFC (0.141; $P < 0.05$). Urinary PD:creatinine (PD:C) ratio was greatest for BYPROD (1.25) and least for SFC (0.94; $P < 0.05$). Urine spot sampling time had a significant ($P < 0.05$) impact on PD:C, 1.03 for AM and 1.22 for PM samples. In Exp. 2, DMI was greater ($P < 0.05$) with BYPROD than with SFC and tended ($P = 0.07$) to be greater with BYPROD than with UREA. Ruminal pH was greatest for BYPROD (5.94; $P < 0.05$). Flow of MCP was 636, 829, and 1,056 g/d for SFC, UREA, and BYPROD, with BYPROD being greater ($P < 0.05$) than SFC and tending ($P = 0.06$) to be greater than UREA. Urinary PD:C was greater ($P < 0.05$) for BYPROD than SFC and tended ($P = 0.09$) to be greater for UREA than SFC. Urinary PD:C increased linearly ($P < 0.05$) with sampling time. Diets formulated to affect DMI and MCP flow resulted in differences in urinary PD excretion, and these results related well with MCP flow estimated from duodenal purines. Collecting spot samples of urine later in the day resulted in greater estimates of urinary PD excretion; purine and PD flows appear to increase with time after one morning feeding per day. This method is well suited to evaluating relative differences between treatments but should not be extrapolated to assume absolute values.

Key words: cattle, diurnal variation, microbial protein, purine derivatives, spot samples

INTRODUCTION

Ruminal microbial synthesis provides a large proportion of total N flow at the duodenum of cattle (Clark et al., 1992). Therefore, the measurement of microbial crude protein (MCP) flow from the rumen is an important factor in evaluating the nutritional status of ruminants and, furthermore, to optimize production. The most common method for estimating MCP flow from the rumen has been through the measurement of duodenal purines (Stern et al., 1994). The utility of this method, however, is limited in that it requires the use of duodenally fistulated animals in a metabolism setting with limited numbers of animals, and this situation may not adequately represent a typical production setting (Firkins et al., 2006).

An alternative method to duodenal purine collection is the use of urinary purine derivatives (PD) to estimate MCP flow (Elliot and Topps, 1963; Verbic et al., 1990; Chen et al., 1992; Jardstedt et al., 2017). Purine derivatives are the degradation products of purines and consist of allantoin, uric acid, xanthine, and hypoxanthine, although xanthine and hypoxanthine are generally not detected in cattle urine (Martin-Orue et al., 2000; Gonzalez-Ronquillo et al., 2004). In addition, urinary creatinine can be used as a marker of urine output. Several factors can affect urinary creatinine output, but a reasonable assumption is that it is excreted at a rate of approximately 28 mg/kg of animal body weight (BW; Lofgreen and Garrett, 1954; McCarthy et al., 1983; Whittet et al., 2019). The use of urinary PD and creatinine in combination allows for the use of spot samples of urine to estimate MCP flow in cattle, which will allow for experiments to be conducted in a more typical production setting (McDonald et al. 2004; Patterson et al., 2006; MacDonald et al., 2007; Swartz et al., 2016; Brunsvig et al., 2017).

Because duodenal purines have been traditionally used to determine MCP flow, comparing new methods of measuring MCP to estimates from duodenal purines would lend credibility. The objectives of these experiments were to investigate diurnal and dietary effects on estimated urinary PD excretion as measured in spot samples of urine and determine the relationship between duodenal purine flow and estimated urinary PD excretion in cattle. Our hypothesis was that urinary PD:creatinine (PD:C) would improve with the UREA treatment compared with the SFC treatment due to the inclusion of urea to alleviate a rumen degradable protein (RDP) deficiency. We also hypothesized further improvements with the BYPROD treatment due to replacing rapidly digested starch with slower-digesting fibrous corn byproducts.

MATERIALS AND METHODS

Experiment 1

The experimental protocol was reviewed and approved by the University of Nebraska Institutional Animal Care and Use Committee. One hundred sixteen crossbred (primarily Hereford and Angus) yearling heifers averaging 407 ± 32 kg initial BW were used in a randomized block design with a 3 × 2 factorial arrangement of treatments. Heifers were housed in an individual feeding facility that is a barn 61 m long with three dividing walls resulting in four identical pens. Each pen was considered a block and has 30 Calan gates (American Calan, Northwood, NH) to accommodate 30 animals. The floor is concrete with a gutter and flush system. The cattle also had access to four identical outdoor pens. Other than the Calan gates, this is similar to many Midwest production facilities and allowed for the measurement of PD excretion in a production setting. Heifers were allotted randomly to pen and, within pen, were stratified by BW and allotted randomly to diet treatment within strata. Each diet treatment was applied to 10 heifers in each pen. Heifers were fed one of three diets (Table 1) formulated to result in differences in MCP production (NRC, 1996), which included: 1) an 85% steam-flaked corn-based diet containing 9.6% CP (SFC); 2) the SFC diet with 1.5% supplemental urea resulting in 13.7% CP (UREA); or 3) a corn-milling byproduct-based diet with 25% SFC, 30% corn

Table 1. Composition of finishing diets fed to heifers (Exp. 1) and steers (Exp. 2; DM basis)

Item	Treatment		
	SFC	UREA	BYPROD
SFC	85.0	85.0	25.0
Sorghum silage	10.0	10.0	10.0
WCGF	0.0	0.0	30.0
Corn bran	0.0	0.0	30.0
Supplement	5.0	5.0	5.0
Fine ground corn	2.68	1.16	2.87
Urea	0.00	1.50	0.00
Limestone	1.42	1.42	1.62
Potassium chloride	0.39	0.41	0.00
Sodium chloride	0.30	0.30	0.30
Tallow	0.13	0.13	0.13
Premix, trace mineral[a]	0.05	0.05	0.05
Premix, vitamin[b]	0.01	0.01	0.01
Premix[c]	0.02	0.02	0.02
Nutrient analysis			
CP, %	9.57	13.73	13.49
NDF, %	13.77	13.62	68.87
Starch, %	59.62	58.59	18.95
Formulation model outputs (NRC, 1996)			
RDP, % of CP	45.5	63.4	74.0
RDP, % of DM	4.35	8.70	9.98
RDP balance, g/d	−284	60	107
Metabolizable Protein balance, g/d	−222	4	194

FGC, finely ground corn.

[a]Premix contained 10% Mg, 6% Zn, 4.5% Fe, 2% Mn, 0.5% Cu, 0.3% I, and 0.05% Co.

[b]Premix contained 1,500 IU vitamin A, 300 IU vitamin D, and 3.7 IU vitamin E/g.

[c]Premix contained Rumensin (320 mg/animal daily) and Tylan (90 mg/animal daily).

bran, and 30% wet corn gluten feed (WCGF), resulting in 13.5% CP (BYPROD). Sorghum silage was included in all diets at 10% of dry matter (DM). Each diet supplied 320 mg Rumensin/heifer daily and 90 mg Tylan/heifer daily. Heifers were fed once daily and implanted with 140 mg trenbolone acetate and 14 mg estradiol (Revalor-H; Merck Animal Health, Madison, NJ) at the beginning of the experiment. The experiment was 84 d in length.

Animals were individually fed once daily (0800 hours) using electronic Calan gates. Heifers were adapted to their respective finishing diet by increasing the amount of feed offered until ad libitum intake was reached. Initially, intake was limited to 1.5% (DM basis) of average initial BW (6.1 kg DM/d). Feed offering was increased by 0.23 kg/d (DM basis) until ad libitum intake was reached (approximately 20 d). Orts were collected weekly and bunks were managed to have crumbs remaining each day at feeding.

Heifers were weighed on three consecutive days at the beginning of the trial. The average initial BW of heifers in pens 1 and 2 was 409 kg and, in pens 3 and 4, was 404 kg. Interim BW measurements were collected on days 26–28 and 54–56, and final BW measurements were collected on days 82–84. At the conclusion of the experiment, heifers were marketed at a commercial abattoir (Tyson Foods, Inc., West Point, NE). Liver scores and hot carcass weight (HCW) were collected on the day of slaughter, while longissimus muscle area, fat depth at the 12th rib, and marbling scores were recorded following a 24-h chill.

Spot samples of urine were collected at the same time heifers were weighed on days 26–28, 54–56, and 84–86. As a result of randomization of individual feeding pens to spot sampling time, all heifers in pens 1 and 2 were spot sampled beginning at 0700 hours, and all heifers from pens 3 and 4 were spot sampled beginning at 1700 hours. Samples were obtained through vulva stimulation with heifers retained in a squeeze chute. Approximately 45 mL of urine was poured into a 50-mL conical vial through two layers of cheesecloth. Samples were immediately placed on ice, transported to the laboratory, and frozen (−20 ºC) for later analyses.

Prior to analysis, urine samples within heifer were composited by period and were diluted with 39 parts urine diluent (distilled water; Shingfield and Offer, 1999) to 1 part urine. Sodium phosphate was added as a buffer. Urine spot samples were analyzed for allantoin, uric acid, xanthine, hypoxanthine, and creatinine by high performance liquid Chromatography (Waters Corp., Milford, MA) following the procedures of Shingfield and Offer (1999). Because urine samples contained negligible amounts of xanthine and hypoxanthine, the sum of allantoin and uric acid is reported as PD. The ratio of PD:C was used to represent relative differences in MCP flow (Shingfield and Offer, 1998). The efficiency of microbial synthesis was calculated as grams of urinary PD per kilogram digestible organic matter intake (DOMI) assuming daily urinary creatinine output of 28 mg/kg (McCarthy et al., 1983; Whittet et al., 2019) and using organic matter (OM) digestibility values from Exp. 2 using the same diets as in the current experiment. The assumption of a fixed value for daily urinary creatinine output is key for this method to be viable.

Experiment 2

Six ruminally and duodenally fistulated Holstein steer calves (initial BW = 474 ± 37 kg)

were assigned randomly to one of three treatments in a replicated 3 × 3 Latin square experimental design. Dietary treatments were formulated to result in differences in MCP flow (NRC, 1996). The three dietary treatments were: 1) SFC; 2) UREA; or BYPROD. All diets contained 10% sorghum silage, 320 mg Rumensin/steer daily and 90 mg Tylan/steer daily (Table 1). Dietary treatments were formulated to be similar to those of Exp. 1. Steers were not implanted in this experiment.

Ruminal and duodenal fistulation surgeries were performed at Oklahoma State University, and steers were transported to the University of Nebraska, Lincoln, after a 1-mo recovery period. Surgical procedures and postsurgical care were reviewed and approved by the Oklahoma State University Institutional Animal Care and Use Committee, and the experimental protocol was reviewed and approved by the University of Nebraska Institutional Animal Care and Use Committee.

Periods were 21 d in length (16-d diet adaptation and 5-d data collection) and all animals were fed for ad libitum intake. Bunks were evaluated once daily at 0700 hours and feed offerings were adjusted accordingly for feeding at 0730 hours. Steers were individually fed in slotted-floor pens (1.5 × 2.4 m) in a temperature-controlled room (25 °C) from day 1 through 16 of each period. In the afternoon of day 16, steers were moved and tethered to individual metabolism stalls in the same room and were allowed to acclimate to stalls overnight. Beginning on day 17, steers were fed in individual feed bunks suspended from load cells (Omega, Stamford, CT) connected to a computer equipped with software allowing for continuous data acquisition (Labtech, Wilmington, MA). Feed weight in each bunk was recorded once every minute and continuously stored for each steer throughout the day. Feed intake measurements (days 17–21 of each period) included dry matter intake (DMI) and rate of intake (Cooper et al., 1999; Erickson et al., 2003).

Prior to feeding on day 17, submersible pH probes (Jenco, San Diego, CA; Sensorex, Garden Grove, CA) were placed into the rumen of each steer through the ruminal fistula and remained in place through the morning of day 21. Each pH probe was encased in a weighted, four-wire metal shroud to maintain the electrode in a stationary suspended position approximately 15 cm above the ventral floor of the rumen. Electrodes were linked directly to a computer equipped with data acquisition software to record ruminal pH every 6 s and averaged every minute throughout the pH data collection phase. On day 21 of each period, the ruminal pH electrodes were removed and steers were returned to their respective free stalls. Ruminal pH measurements included average pH and area of ruminal pH below 5.6 (time below × magnitude below; Cooper et al., 1999; Erickson et al., 2003).

Chromic oxide (Cr_2O_3) was used as an indigestible marker for estimating duodenal flow and fecal output (Merchen, 1988). Boluses containing 7.5 g Cr_2O_3 were inserted through the ruminal fistula twice daily (0700 and 1900 hours) from day 8 through 16 of each period. Urinary creatinine was used as a marker to estimate urine volume, and the ratio of urinary PD allantoin and uric acid to creatinine (PD:C) was used to estimate relative differences in microbial protein production (Shingfield and Offer, 1998).

Sampling. Each experimental diet was sampled daily, composited by period, and dried in a 60- °C oven for 48 h to determine DM concentration. All feed refusals were removed and quantified prior to feeding. During the collection period, orts for individual steers were sampled daily (10% of daily refusal weight), composited by period, and frozen (−20 °C) for later analyses. An additional orts sample (100 g) was dried in a 60- °C oven for 48 h to determine DM concentration.

Spot samples of urine and duodenal flow samples were collected on days 14–16 (0700, 1200, 1700, and 2200 hours). Urine spot samples were collected using a 2-L collection container attached to the steer via a rope fastened around the steer's body, allowing for a free-flowing urine sample. Collection containers were attached to steers for a maximum of 30 min. Urine was filtered through two layers of cheesecloth into 50-mL conical tubes and immediately frozen (−20 °C) for later analyses. Duodenal flow samples (300 mL) were collected at the same time as urine spot samples and were also immediately frozen (−20 °C) for later analyses. Fecal grab samples were collected 0, 6, and 12 h postfeeding on days 13–16 for Cr_2O_3 analyses. For each steer, fecal samples were composited daily on an equal wet-weight basis and frozen (−20 °C) for later analyses. On day 20, ruminal fluid samples (50 mL) were collected from each steer using the suction strainer technique (Raun and Burroughs, 1962) immediately before feeding and 3, 6, 9, 12, 18, and 24 h after feeding. Ruminal fluid samples were immediately frozen (−20 °C) for later analyses.

Laboratory analyses and calculations. Rate of intake was calculated as the slope through the natural log transformation of feed weights recorded each minute during a 24-h collection day. Rate of intake was considered a first-order function and

calculated with the units of %/h (Cooper et al., 1999). Average ruminal pH was calculated by averaging 1,440 measurements recorded during each 24-h collection day. Area of ruminal pH below a value of 5.6 represents the units of magnitude of pH below 5.6 by minute.

Diet, feed ingredient, orts, duodenal, and fecal samples were lyophilized (−50 °C) and ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ) through a 1-mm screen for all laboratory analyses. Laboratory DM of diet, feed ingredient, orts, and fecal samples was determined in a 100- °C oven for 12 h, and OM was determined by ashing samples at 600 °C for 6 h (AOAC, 1996). Crude protein of diet, feed ingredient, orts, duodenal, and fecal samples were determined by the combustion method (AOAC, 1996) using a combustion N analyzer (LECO FP-528, St. Joseph, MI). For chromium analyses, duodenal and fecal samples were ashed, digested with a phosphoric acid–manganese sulfate solution (Williams et al., 1962), and analyzed using an atomic absorption spectrophotometer (SpectrAA-30, Varian, Inc., Palo Alto, CA) with an air-acetylene flame. Ruminal fluid volatile fatty acid (VFA) concentration was determined using a gas chromatograph (HP5890 Series II, Hewlett-Packard Co., Palo Alto, CA) according to Erwin et al. (1961). Starch concentration of diet, feed ingredient, orts, duodenal, and fecal samples was determined using an enzymatic method (Total Starch Assay Kit, Megazyme, Bray, Co. Wicklow, Ireland). Purines were determined in duodenal samples following the procedures of Zinn and Owens (1986) with modifications (Creighton et al., 2000; Mass et al., 2001a). The modifications to the purine assay consisted of a 2-M perchloric acid initial digest (Makkar and Becker, 1999) and the solution used for washing the silver nitrate precipitate was the original precipitation solution (0.2 M ammonium phosphate with 0.002 M silver nitrate; Aharoni and Tagari, 1991). Adenine and guanine were used as standards for purine determination. A SpectraMax 250 spectrophotometer (Molecular Devices Corp., Sunnyvale, CA) was used for purine and starch determination. Neutral detergent fiber (NDF) concentration of ort, diet, feed ingredient, duodenal, and fecal samples was determined according to procedures of Van Soest et al. (1991) using both amylase and sodium sulfite.

Urine samples were diluted with 1 part urine and 39 parts urine diluent (distilled water) at the time of defrosting with sodium phosphate added as a buffer, and PD and creatinine were analyzed by high performance liquid chromatography (Waters Corp., Milford, MA) according to the procedures of Shingfield and Offer (1999). To determine microbial N flows, we assumed a purine:N ratio of 0.20484 (Crawford et al., 2008). True OM digestibility was corrected for microbial OM reaching the duodenum with the assumption that microbial OM is equal to microbial N/0.0996 (Clark et al., 1992).

Total urinary PD excretion was calculated from urinary allantoin, uric acid, and creatinine output assuming a creatinine output of 28 mg/kg BW (McCarthy et al., 1983; Whittet et al., 2019). These values were regressed upon duodenally absorbed purines assuming an intestinal absorption of 83% for purines reaching the duodenum (Chen and Gomes, 1995).

Statistical Analyses

Experiment 1. Data were analyzed as a randomized block design using the Mixed procedures of SAS (SAS Inst. Inc., Cary, NC). A 3 × 2 factorial treatment structure was used with three dietary treatments (SFC, UREA, and BYPROD) and two urine spot sampling times (0700 and 1700 hours; AM and PM) comprising the factors. Dietary treatment and spot sample collection time were considered fixed effects, and the interaction between the two was initially tested for all variables. Heifer served as the experimental unit for all analyses. Cattle were blocked by the pen they were housed in and block was included in the model as a random effect. There were four pens of cattle with 10 replications of each dietary treatment per pen. Time of sampling was linked to pen, as pens 1 and 2 were sampled in the AM and pens 3 and 4 were sampled in the PM. Least squares means were separated using the PDIFF statement in SAS when protected by a significant ($P < 0.05$) F-test. Tendencies are discussed at a $P < 0.10$.

Experiment 2. Data were analyzed as a Latin square experimental design using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). For total tract nutrient intake and digestibility data, the model included period and dietary treatment as fixed effects and animal as a random effect. Intake pattern, ruminal pH, duodenal purine flow, and urine data were analyzed as repeated measures with day repeated using an autoregressive-1 covariance structure. This covariance structure was the best fit based on least Akaike information

Table 2. Main effects of dietary treatment on live performance and carcass characteristics (Exp. 1)

	Treatment[a]				P-values		
Item	SFC	UREA	BYPROD	SEM	Trt	Time	Trt × time
DMI, kg/d	7.90[c]	8.85[b]	10.40[a]	0.15	<0.01	0.29	0.83
ADG, kg	1.11[b]	1.60[a]	1.68[a]	0.05	<0.01	0.06	0.85
Gain:Feed	0.141[c]	0.182[a]	0.162[b]	0.008	<0.01	0.09	0.50
Carcass weight, kg	327[b]	351[a]	348[a]	4	<0.01	0.57	0.96
Marbling[b]	501[b]	512[ab]	539[a]	10	0.03	0.41	0.93
Longissimus Muscle area, sq cm	90.1	90.6	93.0	2.0	0.54	0.95	0.65
12th rib fat depth, cm	0.97[b]	1.15[a]	1.22[a]	0.05	<0.01	0.28	0.56

[a]SFC = 85% steam-flaked corn, 9.6% CP; UREA = 85% steam-flaked corn + 1.5% urea, 13.7% CP; BYPROD = 25% steam-flaked corn, 30% corn bran, 30% wet corn gluten feed, 13.5% CP.

[b]Marbling score called by USDA grader, where 500 = small[0] and 550 = small[50].

[a,b,c]Values within the same row with uncommon superscripts differ ($P < 0.05$).

criterion. The model included period and dietary treatment as fixed effects with animal as a subject of time included as a random effect. For VFA analysis, a similar repeated measures model was used but with time of day repeated. Least squares means were separated using the PDIFF statement in SAS when protected by a significant ($P < 0.10$) F-test. Regression was used to evaluate the relationship between urinary PD excretion and absorbed purines using PROC REG of SAS.

RESULTS

Experiment 1

No dietary treatment × time of urine collection interactions occurred for any variable ($P > 0.05$), and no live animal performance or carcass characteristic parameter was affected ($P > 0.05$) by urine collection time. Therefore, all live animal performance and carcass characteristic data are presented as the main effect of dietary treatment (Table 2). Dietary treatments were formulated to create treatment differences in MCP flow, which led to expected differences in live performance and carcass characteristics. Heifers consuming the BYPROD treatment had greater ($P < 0.05$) DMI than heifers consuming either the SFC or UREA treatments, and DMI with the UREA treatment was also greater ($P < 0.05$) than that of the SFC treatment, averaging 7.90, 8.85, and 10.40 kg/d for SFC, UREA, and BYPROD, respectively. Average daily gain (ADG) was lower (1.11 kg; $P < 0.05$) with the SFC treatment than with either the UREA (1.60 kg) or BYPROD (1.68 kg) treatments. The UREA and BYPROD treatments did not differ ($P > 0.10$) for ADG. Feed efficiency was poorest ($P < 0.05$) with the SFC treatment, intermediate with the BYPROD treatment, and greatest with the UREA treatment, measuring
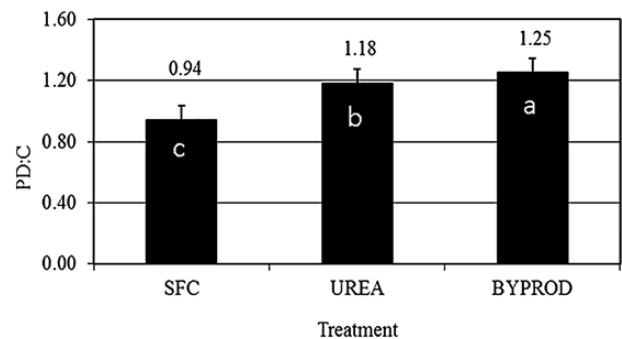

Figure 1. Main effect of dietary treatment on urinary PD:C ratio (Exp. 1). Treatments were SFC (85% steam-flaked corn, 9.6% CP), UREA (85% steam-flaked corn + 1.5% urea), and BYPROD (25% steam-flaked corn, 30% corn bran, and 30% WCGF). Diet $P < 0.01$; diet × urine collection time $P = 0.98$; SEM = 0.03. Unlike superscripts differ ($P < 0.05$).

0.141, 0.182, and 0.162 for SFC, UREA, and BYPROD, respectively.

Carcass characteristics generally followed the live performance results, with heifers consuming the SFC treatment having lower ($P < 0.05$) HCW and 12th rib fat thickness than either the UREA or BYPROD treatments. The BYPROD treatment also resulted in a greater ($P < 0.05$) marbling score than the SFC treatment. No treatment differences ($P > 0.10$) were observed for longissimus muscle area.

Heifers consuming the BYPROD treatment had the greatest ($P < 0.05$) urinary PD:C ratios, with the UREA treatment being intermediate and the SFC treatment being the lowest, measuring 0.94, 1.18, and 1.25 for the SFC, UREA, and BYPROD treatment, respectively (Fig. 1). The efficiency of microbial synthesis was greater ($P < 0.05$) with the UREA and BYPROD treatments than with the SFC treatment and tended ($P = 0.10$) to be greater with the BYPROD treatment than with the UREA treatment (Fig. 2). When urine samples were collected in the PM, measurement of PD:C to estimate MCP flow was greater than when samples were collected
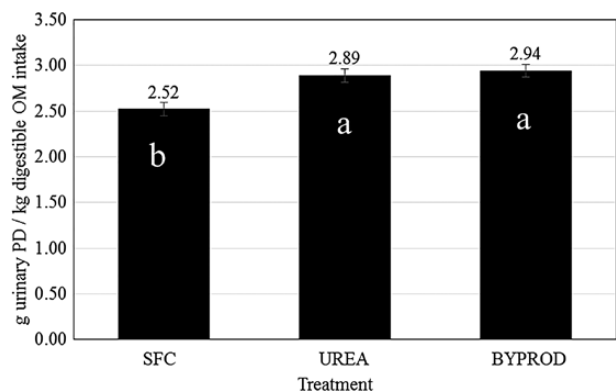
Figure 2. Effect of dietary treatment on estimates of urinary PD relative to digestible OM (Exp. 1). Total urinary PD excretion assuming daily urinary creatinine excretion of 28 mg/kg BW. Treatments were SFC (85% steam-flaked corn, 9.6% CP), UREA (85% steam-flaked corn + 1.5% urea), and BYPROD (25% steam-flaked corn, 30% corn bran, and 30% WCGF). Treatment $P < 0.01$; SEM = 0.07. Unlike superscripts differ ($P < 0.05$).
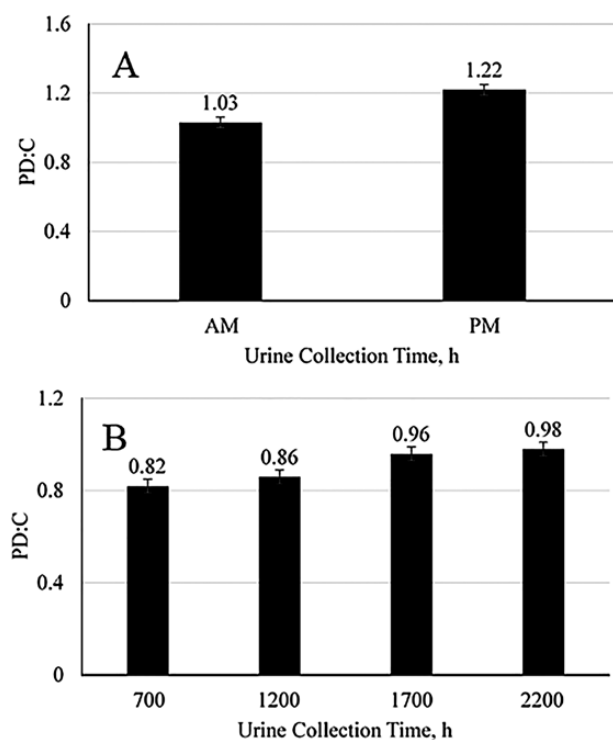

Figure 3. Main effect of urine collection time on urinary PD:C ratio in Exp. 1 (A) and Exp. 2 (B). (A) Urine collection time $P < 0.01$; diet × urine collection time $P = 0.98$; SEM = 0.03. (B) Urine collection time $P < 0.01$; diet × urine collection time $P = 0.21$; SEM = 0.03.

in the AM (Fig. 3), measuring 1.03 and 1.22 for AM- and PM-collected samples, respectively.

Experiment 2

Intake behavior. Feed intake data are presented in Table 3. Dry matter intake was greater ($P < 0.05$) with the BYPROD treatment than with the SFC treatment, measuring 7.96, 8.25, and 9.75 kg/d for SFC, UREA, and BYPROD, respectively. Intake

Table 3. Effect of dietary treatment on feed intake and digestibility (Exp. 2)

Item	SFC	UREA	BYPROD	SEM	*P*-value
	Treatment[a]				
DMI, kg/day	7.96[b]	8.25[ab]	9.75[ab]	0.66	0.09
Rate of intake, %/h[b]	20.4	22.4	24.2	1.4	0.23
OM digestibility, %					
Ruminal	61.2	65.2	62.1	2.8	0.46
Postruminal[c]	62.3[a]	64.9[a]	45.5[b]	4.0	0.01
Total tract	85.3[a]	87.8[a]	79.8[b]	1.2	0.01
NDF digestibility, %					
Ruminal	49.1	54.1	42.3	2.7	0.41
Postruminal[c]	41.3	42.1	42.3	4.9	0.99
Total tract	69.8	72.9	72.6	2.2	0.39
Starch digestibility, %					
Ruminal	83.4[b]	89.8[a]	86.7[ab]	2.4	0.05
Postruminal[c]	94.4	91.9	94.3	1.3	0.33
Total tract	99.0	99.3	99.3	0.2	0.15

[a]SFC = 85% steam-flaked corn, 9.6% CP; UREA = 85% steam-flaked corn + 1.5% urea, 13.7% CP; BYPROD = 25% steam-flaked corn, 30% corn bran, 30% wet corn gluten feed, 13.5% CP.

[b]Rate of intake calculated from total time spent eating.

[c]Percentage digestion of contents entering duodenum.

[ab]Values within the same row with uncommon superscripts differ ($P < 0.05$).

with BYPROD also tended ($P = 0.07$) to be greater than with UREA, while no differences ($P > 0.10$) in DMI were present between SFC and UREA. Intake per meal and rate of intake were 20.5% and 18.6% greater, respectively, with BYPROD than with SFC; however, the difference was not significant ($P > 0.10$).

Nutrient digestibility. Ruminal OM digestibilities were not different ($P > 0.10$; Table 3). Postruminal and total tract OM digestibilities were not different ($P > 0.10$) between SFC and UREA, and SFC and UREA were each greater ($P < 0.05$) than BYPROD. No treatment differences ($P > 0.10$) were observed for ruminal, postruminal, or total tract NDF digestibility. Ruminal starch digestibility was greater ($P < 0.05$) with UREA than with SFC, and BYPROD was not different ($P > 0.10$) than either treatment. However, there were no differences ($P > 0.10$) among treatments for postruminal and total tract starch digestibility.

Ruminal metabolism. Average ruminal pH was greater ($P < 0.05$) with BYPROD than with SFC or UREA, with no difference ($P > 0.10$) between SFC and UREA (Table 4). Area below pH 5.6, which is a measure of time below pH 5.6, multiplied by the magnitude of pH depression, was lower ($P < 0.01$) with BYPROD than with SFC and tended ($P = 0.09$) to be lower with BYPROD than with UREA.

Table 4. Effect of dietary treatment on ruminal pH, VFA concentration, PD:C ratio, and MCP flow and efficiency (Exp. 2)

Item	Treatment[a]			SEM	*P*-value
	SFC	UREA	BYPROD		
Average pH	5.43[b]	5.58[b]	5.94[a]	0.09	0.01
Area <pH 5.6	374[a]	240[ab]	71[b]	70	0.04
Total VFA, mM	108[a]	103[ab]	82[b]	8	0.06
Acetate, mol/100 mol	42.8[b]	44.2[ab]	50.2[a]	2.4	0.25
Propionate, mol/100 mol	37.0[ab]	39.6[a]	32.3[b]	2.8	0.06
Butyrate, mol/100 mol	11.0	10.0	12.7	1.8	0.79
Acetate: propionate	1.16[ab]	1.12[b]	1.55[a]	0.18	0.08
PD:C	0.75[b]	0.92[ab]	1.06[a]	0.06	0.02
MCP flow, g/d[b]	636[b]	829[ab]	1,056[a]	109	0.02
Microbial efficiency					
g of MCP/ kg OMTD[c]	126	132	148	15	0.48
g of MCP/ kg TDOM[d]	103	119	136	12	0.19

[a]SFC = 85% steam-flaked corn, 9.6% CP; UREA = 85% steam-flaked corn + 1.5% urea, 13.7% CP; BYPROD = 25% steam-flaked corn, 30% corn bran, 30% wet corn gluten feed, 13.5% CP.

[b]MCP flow derived from duodenal purine assay (daily purine flow/purine to N ratio of isolated bacteria [0.205]).

[c]Organic matter truly digested.

[d]Total digestible organic matter intake.

[ab]Values within the same row with uncommon superscripts differ ($P < 0.05$).

Total ruminal VFA concentration was greater ($P < 0.05$) with SFC than with BYPROD and tended ($P = 0.06$) to be greater with UREA than with BYPROD (Table 4). Acetate molar proportion was greater ($P < 0.05$) with BYPROD than with SFC and tended ($P = 0.06$) to be greater with BYPROD than with UREA. Propionate molar proportion was greater ($P < 0.05$) with UREA than with BYPROD, with SFC being intermediate ($P > 0.10$) and not different from the other treatments. This resulted in greater ($P < 0.05$) acetate:propionate ratio with BYPROD than with UREA, with no difference ($P > 0.10$) between SFC and either UREA or BYPROD.

Urinary PD:C was 0.75, 0.92, and 1.06 for SFC, UREA, and BYPROD, respectively, with BYPROD being greater ($P < 0.05$) than SFC and no difference ($P > 0.10$) between UREA and either SFC or BYPROD (Table 4). Flow of MCP was 636, 829, and 1056 g/d for SFC, UREA, and BYPROD, with BYPROD being greater ($P < 0.05$) than SFC and tending ($P = 0.06$) to be greater
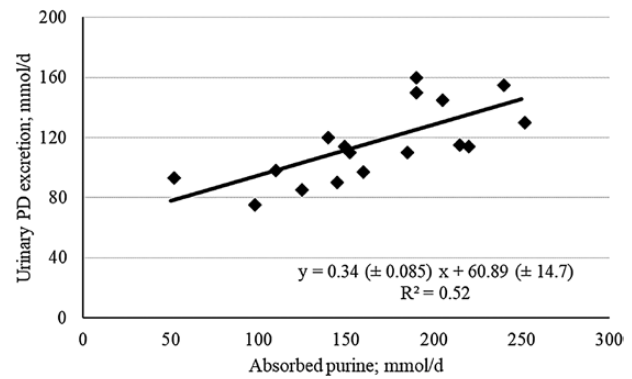

Figure 4. Urinary PD (mmol/d) excretion regressed on absorbed duodenal purines (mmol/d; Exp. 2). Duodenal purine absorption assumed to be 83% of duodenal purine flow (Chen and Gomes, 1995).

than UREA. Microbial efficiency, based on either OM truly digested or total DOMI, did not differ ($P > 0.10$) among treatments; however, numerical increases were observed with UREA greater than SFC and BYPROD greater than UREA (Table 4). Urinary PD:C ratio was greater (linear $P < 0.05$) with samples collected later in the day (Fig. 3). Total urinary PD excretion was regressed on duodenal absorbed purines (both values expressed as mmol/d) and resulted in an equation of $y = 0.3407x + 60.89$ ($R^2 = 0.516$; Fig. 4).

DISCUSSION

Dietary treatments in this experiment were formulated to result in differences in MCP flow through modifications in ruminal metabolism. The addition of urea to the SFC diet was expected to provide an RDP source to improve MCP flow, while the BYPROD treatment was expected to improve ruminal pH allowing for a more favorable ruminal environment for MCP synthesis. In Exp. 1, individually fed heifers consuming the BYPROD treatment had greater DMI than heifers consuming either the SFC or UREA treatments. In Exp. 2, differences in DMI were observed among treatments, with an 18.2% improvement for BYPROD compared with UREA and a 22.4% improvement for BYPROD compared with SFC. Similar to the current experiments, increases in DMI have been observed with dietary inclusion of either corn bran or WCGF in beef cattle finishing diets (Scott et al., 1997; Erickson et al., 2002; Adams et al., 2003; Sayer et al., 2005).

In addition to the expected results in DMI, we observed improvements in ADG and gain:feed (G:F) for both UREA and BYPROD treatments compared with SFC. Shain et al. (1998) reported

a 6.6% increase in ADG and a 5.4% increase in G:F at the optimal dietary urea concentration for steers fed 90% concentrate dry-rolled corn (DRC) based diets and supplemented with 0.88%, 1.34%, or 1.96% urea compared with steers fed diets with no urea. In both the current experiments and Shain et al. (1998), metabolizable protein (MP) requirements were met by treatments supplying urea, but the diets without supplemental urea were deficient in RDP. Cooper et al. (2002) reported improvements in steer G:F when urea was included in 90% concentrate, SFC-based diets at 0.4% and 0.8% of DM compared with no urea addition to the same basal diets. No further improvements were observed when urea was supplemented beyond 0.8% of DM. In a separate experiment, Cooper et al. (2002) reported improvements in G:F when urea was supplemented up to 1.5% of the same SFC-based diets, with no further improvements beyond the 1.5% supplementation level.

The increase in DMI is likely related to an improvement in ruminal pH when corn-milling byproducts are included in high-concentrate diets. Inclusion of WCGF in place of corn grain in finishing diets replaces dietary starch with highly digestible fiber, which may result in increased DMI and ADG, as well as decreased incidence and severity of acidosis in feedlot cattle (Krehbiel et al., 1995; Stock et al., 2000). In Exp. 2, we observed a 9.4% improvement in ruminal pH for BYPROD compared with SFC and a 6.5% improvement in ruminal pH for BYPROD compared with UREA. The improvement in ruminal pH with corn byproduct addition has been previously demonstrated in heifers by McDonald et al. (2004), where ruminal pH was 5.44 with a 88% high-moisture corn (HMC) diet and 5.78 with a 68% HMC and 20% corn bran diet. Also, in heifers, Sayer et al. (2005) observed a ruminal pH of 5.75 with a diet containing 75% DRC and 15% corn silage, and 5.98 with a diet containing 20% DRC, 15% corn silage, 45% corn bran, and 15% steep (steep liquor and distillers solubles from wet milling). The 45% corn bran and 15% steep combination in the Sayer et al. (2005) experiment was similar to the BYPROD treatment in the current experiments.

Increasing the concentration of rapidly degradable nonfiber carbohydrates in ruminant diets can lead to several problems with mixed microbial populations (Firkins, 1996). Most importantly, a low ruminal pH resulting from the rapid production of VFA decreases the efficiency of microbial protein synthesis (Russell et al., 1992). In Exp. 2, steers consuming the SFC treatment spent approximately 15.5 h/d with a pH below 5.6, and steers consuming the UREA treatment spent approximately 6.5 h/d with a ruminal pH of less than 5.6. This ruminal pH (5.6) is often recognized as the upper threshold of subacute ruminal acidosis. Krehbiel et al. (1995) reported a 26% decrease in area below pH 6.0 with a steer diet containing 50% DRC and 50% WCGF compared to one containing 100% DRC. Strobel and Russell (1986) observed decreased MCP synthesis with decreased ruminal pH and noted that carbohydrate utilization was also reduced at the lower ruminal pH, though to a lesser extent than MCP synthesis. Those authors concluded that the differences in reductions between MCP synthesis and carbohydrate utilization were due to energy being used for nongrowth functions or energy spilling reactions to maintain H+ balance at lower pH levels.

Much of the research on the use of PD to estimate relative differences in MCP flow has been with the measurement of PD output from total urine collections and most of the research has been done in metabolism settings. The use of spot samples of urine could represent a significant step forward in the estimation of MCP flow in a common production setting. We did observe the relative differences in DMI that we expected, and these differences coincided with Exp. 2 responses in ruminal pH and VFA concentration. Our results agree with other research (Orellana et al., 1998; Cetinkaya et al., 2006; Dórea et al., 2017), where an increase in urinary PD excretion as estimated from PD:C ratio was correlated with an increase in DMI.

In Exp. 1, a reduction in microbial efficiency was observed based on grams of PD excretion per kilogram of DOMI with the SFC treatment compared with the UREA and BYPROD treatments. The BYPROD treatment was numerically greater than the UREA treatment but the difference was not significant. Our objective was not to directly predict purine flow or MCP flow but rather to determine if PD spot samples would estimate relative MCP differences and efficiencies. Firkins et al. (2006) demonstrate the difficulties in predicting purine flow from PD spot samples.

The slope of the regression of estimated urinary PD excretion (mmol/d) on duodenal purine flow was 0.34 ± 0.09. Crawford et al. (2008) reported a slope of 0.41 ± 0.08. Both values are lower than some reported values [Chen and Gomes, 1995 (0.85); Hristov, et al., 2005 (0.97)] but similar to others [Martin-Orue et al., 2000 (0.67)]. These data, along with those of Crawford et al. (2008), suggest that PD recovery in urine is approximately 40% in cattle on

high-grain feedlot diets. Variation around this value could be due to several factors, including procedures used; exogenous purines were infused in some experiments, while others were conducted with animals that were intragastrically infused. Incomplete recovery of purines could also be due to the secretion of PD via saliva into the rumen, entrance of PD into the gut via nonsalivary routes, and salvage of PD. Potentially, all these factors could interact with diet and intake (Crawford et al., 2008). Alternatively, in nonlactating cattle, Firkins at al. (2006) suggested that urinary allantoin could be predicted from RDP intake and used to calculate duodenal purine flow with no other significant effects across trials. Because purine flow at the duodenum fluctuates throughout the day, the relationship between purine derivatives and purine flow can also fluctuate throughout the day. It is important to note that Fig. 4 was developed from samples taken across time.

The PD:C increased with time on feed. As cattle progress through the finishing period, body fat increases to approximately 28% (Guiroy et al., 2002). It is not clear how this increased fattening affects the excretion of creatinine on a BW basis or endogenous PD on a metabolic BW basis. Whittet et al. (2019) reported that fattening did not affect creatinine excretion on a BW basis. Gonzalez-Ronquillo et al. (2004) reported 70% recovery of duodenal purines as PD in late lactation of dairy cows but only 56% recovery in early lactation. This suggests that the physiological state (level of production) may affect purine recovery as PD in urine. While ratios are not quantitative, the increase in ratios suggests that greater recovery of PD occurred at 85 d in the feeding period than at 28 d.

As was observed with the urinary PD:C ratio, the BYPROD treatment had the greatest MCP flows among the three treatments (Exp. 1), and the addition of 1.5% urea to the SFC treatment supplied an RDP source to improve MCP flow compared with the SFC treatment. When regressing PD excretion on duodenal purine flow, we observed an R^2 value of 0.52. Mass et al. (2001b) reported an R^2 value of 0.74 when allantoin excretion by steers was regressed on duodenal purine flow. Mass et al. (2001b) utilized PD:C ratio and an assumed daily creatinine excretion based on animal BW. The lower R^2 observed in our experiment could be due to the compounding errors associated with using PD and creatinine as markers for urinary PD excretion, along with errors associated with using multiple markers to estimate duodenal purine flow.

The intercept of our equation (Fig. 4) represents endogenous PD contribution and equates to 562 µmol PD/kg $BW^{0.75}$. Endogenous PD result from catabolic pathways of nucleic acid turnover. Crawford et al. (2008) reported endogenous PD contribution of 501 µmol PD/kg $BW^{0.75}$ when the same steers from the present experiment were fed a HMC-based diet at 40% and 85% of ad libitum intake. Giesecke et al. (1993) reported endogenous PD contribution of 560 µmol PD/kg $BW^{0.75}$ when PD excretion was measured in steers using a rumen emptying technique. Other researchers (Fujihara et al., 1987; Chen et al., 1990; Verbic et al., 1990; Bowen et al., 2006) have reported values ranging from 414 to 514 µmol PD/kg $BW^{0.75}$.

One of the major concerns with the use of the PD:C ratio to estimate MCP flow in ruminants is the possible impact of diurnal variation in PD and creatinine excretion. McDonald et al. (2004) reported a linear increase in allantoin:creatinine ratios when spot samples of urine were collected from heifers at 0800, 1100, 1400, and 1700 hours. In the McDonald et al. (2004) experiments, cattle were fed once daily, diurnal variation was present with both urine and duodenal samples, and there were no dietary treatment × spot sampling time interactions. When steers were fed equal amounts of an HMC-based diet every 4 h throughout the day, PD:C ratio did not differ in urine spot samples collected at 0700, 1200, 1700 and 2200 hours and no treatment × time interactions were observed for PD:C (Crawford et al., 2008). These data suggest that purine flow has a diurnal pattern, increasing with time after once per day feeding. Therefore, the PD:C ratio increases with time after once per day feeding. Whittet et al. (2019) reported no day × time of day interaction for PD:C ratio and suggested that 2 or 3 d of spot samples of urine should be collected to minimize variation.

The 1996 NRC model (NRC, 1996) predicts microbial efficiencies of 4.59% (assuming equal to RDP), 9.82%, and 11.5%, respectively, for SFC, UREA, and BYPROD, respectively (grams of MCP per kilogram of total digestible nutrients). The Beef Cattle Nutrient Requirements Model (The National Academies of Sciences, Engineering, and Medicine, 2016) predicts respective microbial efficiencies of 4.54%, 9.27%, and 9.33%. Neither model accounts for N recycling, so microbial efficiencies for the SFC are underestimated. Nitrogen recycling in this situation would be very difficult to model. The difference between the two models is in the prediction of the BYPROD diet. The 1996 model accounts for ruminal pH and, therefore, predicts greater microbial efficiency for the BYPROD diet than the UREA diet. The 2016 model does

not account for ruminal pH. Calculated microbial efficiencies in the current trial (Exp. 1) were 12.3%, 14.1%, and 14.4% for the SFC, UREA, and BYPROD diets, respectively (grams of MCP per kilogram of digestible OM).

In Exp. 2, microbial efficiency measured by duodenal purine flow was numerically greater for the BYPROD diet ($P = 0.19$) compared to the UREA diet. In Exp. 1, microbial efficiency was numerically greater for the BYPROD diet than the UREA diet. These data suggest that ruminal pH does influence microbial efficiency, but the effect may not be as great as predicted by the 1996 NRC model.

SUMMARY

Treatment differences as estimated by urinary PD:C ratios were confirmed by MCP flow measurement from duodenal purine collection and through regression of urinary PD excretion on duodenal purine flow, suggesting that PD:C ratios from spot samples of urine may be used to estimate relative differences in MCP flows among treatments. Diurnal variation in PD:C measurement was present but did not interact with treatment, suggesting that comparisons between treatments can be made from samples collected at similar times of the day. A critical distinction is that these estimates of microbial efficiency are good predictors of relative differences between treatments but should not be taken as absolute values.

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