

January 2007

# Evaluating Use of Urinary Purine Derivative to Creatinine Ratio as an Estimate of Microbial Protein Production in Steers

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Crawford, Grant; Luebbe, Matt K.; Erickson, Galen E.; and Klopfenstein, Terry J., "Evaluating Use of Urinary Purine Derivative to Creatinine Ratio as an Estimate of Microbial Protein Production in Steers" (2007). *Nebraska Beef Cattle Reports*. 92.  
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# Evaluating Use of Urinary Purine Derivative to Creatinine Ratio as an Estimate of Microbial Protein Production in Steers

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## Summary

*Six ruminally and duodenally fistulated steers were fed three diets varying in source and concentration of dietary protein to determine impacts on ruminal metabolism, nutrient digestibility, and microbial crude protein (MCP) production as estimated by urinary purine derivative:creatinine (PD:C) ratio. Steers were fed a steam-flaked corn (SFC)-based diet with or without 1.5% urea, or a corn milling byproduct-based diet. Feeding a corn milling by-product-based diet resulted in greater ruminal pH and less time below ruminal pH 5.6, total tract OM digestibility, and ruminal propionate concentration when compared with either SFC-based treatment. The by-product-based treatment produced greater PD:C and MCP production values when compared with the SFC, no urea treatment. Responses in ruminal pH, MCP production, and PD:C indicate the by-product-based diet provided a more favorable rumen environment compared with SFC-based diets, and that urinary PD:C can be used to estimate differences in MCP production.*

## Introduction

Previous research (2007 *Nebraska Beef Report*, pp. 103-105) reported that when 1.5% urea was added to a 85% steam-flaked corn (SFC) diet, ADG, DMI, F:G, and purine derivative:creatinine (PD:C) ratio as an estimate of microbial CP (MCP) production were improved. Further enhancements in DMI and MCP production were observed when a 25% SFC, 60% corn milling by-product diet was fed.

These results were expected based on the composition of the diets, and supported the use of PD:C as an estimate of MCP production. However, because duodenal purines have traditionally been used to determine MCP production, any alternative method must be validated with duodenal purine measurements. Therefore, this metabolism experiment was conducted to confirm the previous findings and explore ruminal fermentation and intake behavior to explain the differences found in the individual feeding experiment.

## Procedure

Six ruminally and duodenally fistulated Holstein steers (1045 ± 82 lb) were used in two 3 x 3 Latin square designs and fed one of 3 diets: 1) a diet containing 85% steam-flaked corn and consisting of 9.6% CP (SFC); 2) the SFC diet with 1.5% supplemental urea resulting in 13.7% CP (UREA); or 3) a corn milling by-product-based diet with 25% SFC, 30% corn bran, and 30% wet corn gluten feed, resulting in 14.1% CP (BYPROD). All diets contained 10% sorghum silage, 320 mg Rumensin<sup>®</sup>/steer daily and 90 mg Tylan<sup>®</sup>/steer daily.

Periods were 21 days in length (16-day diet adaptation and five-day data collection) and all animals were fed for ad-libitum intake. Bunks were read once daily at 0700 hours and feed offerings were adjusted accordingly for feeding at 0730 hours. All feed refusals were removed, quantified, and sampled. Steers were individually fed in free stalls from days 1-16 of each period. In the afternoon of day 16, steers were moved and tethered in individual metabolism stalls and were allowed to acclimate to these stalls overnight. Beginning on day 17, steers were fed in individual feed bunks suspended from load cells con-

nected to a computer equipped with software allowing for continuous data acquisition. 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 DMI, rate of intake, number of meals per day, average meal size, total time spent eating, and average meal length.

Submersible pH electrodes were placed into the rumen of each steer through the ruminal fistula on the morning of day 17 of each period and remained in place through the morning of day 21. Each pH electrode was encased in a weighted, four-wire metal shroud to keep the electrode in a stationary suspended position approximately 5 inches above the ventral floor of the rumen. Electrodes were linked directly to a computer equipped with data acquisition software to record ruminal pH every six seconds and average ruminal pH 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.

Chromic oxide was used as an indigestible marker for determining digestibility and flow. Boluses containing 7.5 g chromic oxide were inserted through the ruminal cannula twice daily (0700 and 1900 hours) from days 8-16. Fecal grab samples were collected 0, 6, and 12 hours post-feeding on days 13-16. On day 20, ruminal fluid samples were collected from each steer immediately before feeding and 3, 6, 9, 12, 18, and 24 hours after feeding for ruminal VFA analyses.

Spot samples of urine and duodenal content samples were collected on days 14-16 (0700, 1200, 1700, 2200 hours). Urinary creatinine was used as a marker to estimate urine volume,

**Table 1. Effect of dietary treatment on feed intake and ruminal pH characteristics.**

Item	Treatment <sup>a</sup>			SEM	P-value
	SFC	UREA	BYPROD		
DMI, lb/day	17.6 <sup>c</sup>	18.2 <sup>bc</sup>	21.5 <sup>b</sup>	1.5	0.09
Rate of intake, %/h	20.4	22.4	24.2	1.4	0.23
Average ruminal pH	5.43 <sup>c</sup>	5.58 <sup>c</sup>	5.94 <sup>b</sup>	0.09	0.01
Time < pH 5.6, min/day	925 <sup>b</sup>	715 <sup>b</sup>	326 <sup>c</sup>	121	0.01
Area < pH 5.6	374 <sup>b</sup>	240 <sup>bc</sup>	71 <sup>c</sup>	70	0.04

<sup>a</sup>SFC = 85% SFC, 9.6% CP; UREA = 85% SFC + 1.5% urea, 13.7% CP; BYPROD = 25% SFC, 30% corn bran, 30% wet corn gluten feed, 14.1% CP.

<sup>b,c</sup>Values within the same row with uncommon superscripts differ ( $P < 0.05$ ).

**Table 2. Effect of dietary treatment on ruminal VFA concentration, purine derivative:creatinine ratio, and MCP production.**

Item	Treatment <sup>a</sup>			SEM	P-value
	SFC	UREA	BYPROD		
Total VFA, mM	108 <sup>d</sup>	103 <sup>de</sup>	82 <sup>e</sup>	8	0.06
Acetate, mol/100 mol	42.8 <sup>e</sup>	44.2 <sup>de</sup>	50.2 <sup>d</sup>	2.4	0.25
Propionate, mol/100 mol	37.0 <sup>de</sup>	39.6 <sup>d</sup>	32.3 <sup>e</sup>	2.8	0.06
Butyrate, mol/100 mol	11.0	10.0	12.7	1.8	0.79
Acetate:propionate	1.16 <sup>de</sup>	1.12 <sup>c</sup>	1.55 <sup>d</sup>	0.18	0.08
PD:C <sup>b</sup>	0.75 <sup>e</sup>	0.92 <sup>de</sup>	1.06 <sup>d</sup>	0.06	0.02
MCP Production, g/day <sup>c</sup>	235 <sup>e</sup>	309 <sup>de</sup>	395 <sup>d</sup>	41	0.02
Microbial efficiency, g of CP/100 g OM digested	5.89	6.34	7.45	0.90	0.40

<sup>a</sup>SFC = 85% SFC, 9.6% CP; UREA = 85% SFC + 1.5% urea, 13.7% CP; BYPROD = 25% SFC, 30% corn bran, 30% wet corn gluten feed, 14.1% CP.

<sup>b</sup>Purine derivative:creatinine ratio.

<sup>c</sup>Derived from duodenal purine assay.

<sup>d,e</sup>Values within the same row with uncommon superscripts differ ( $P < 0.05$ ).

while urinary PD allantoin and uric acid were used as markers for estimation of MCP production. Purine derivatives and creatinine were analyzed by HPLC. Duodenal purine content was also analyzed for estimation of MCP production.

Data were analyzed as a replicated Latin square experimental design using the Mixed procedure of SAS. For nutrient digestibility the model included period and dietary treatment. Intake, ruminal pH, duodenal purine, VFA, and urine data were analyzed as repeated measures. For intake and ruminal pH analyses, the model consisted of period, dietary treatment, day of collection period, and treatment x day. For VFA, duodenal purine, and urine analyses, the model consisted of period, dietary treatment, time of collection, and dietary treatment x time. All models included steer and steer x treatment x period as random effects. Least squares means were separated using the PDIF statement in SAS when protected by a significant

( $P < 0.10$ ) *F*-test. Time of urine and duodenal content collection were analyzed for linear, quadratic, and cubic responses.

## Results

Intake and pH data are presented in Table 1. Dietary treatments for this experiment were formulated to produce differences in MCP production, allowing for evaluation of the ability of PD:C measurements to estimate MCP production. Therefore, treatment differences for DMI were expected. Dry matter intake was greater ( $P < 0.05$ ) with the BYPROD treatment than with the SFC treatment, measuring 17.6, 18.2, and 21.5 lb/day for SFC, UREA, and BYPROD, respectively. Intake 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. The DMI responses are similar to what was observed with an individual feeding experiment (2007

*Nebraska Beef Report*, pp. 103-105), in which DMI was greatest with BYPROD, intermediate with UREA, and lowest with SFC. Rate of intake was 18.6% greater with BYPROD than with SFC; however, this difference was not significant ( $P = 0.23$ ).

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. The improvement in ruminal pH with the addition of corn milling by-products is likely due to the slower ruminal fermentation rate of by-products compared with that of starch from SFC. Steers consuming the BYPROD treatment spent 325.8 min/day at ruminal pH less than 5.6, which was 65 and 54% lower ( $P < 0.05$ ) than the SFC or UREA treatments, respectively. Area below pH 5.6, which is a measure of time below pH 5.6 multiplied by 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. A ruminal pH of 5.6 is generally considered to be the point at which subacute ruminal acidosis begins to occur, and decreased and erratic DMI are often cited as symptoms of subacute acidosis. We observed 184 and 119% greater time below ruminal pH 5.6 with the SFC and UREA treatments, respectively, than with BYPROD, and this may explain differences observed in DMI among the treatments.

Total and individual VFA molar proportions are presented in Table 2. 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, measuring 108.4, 102.9, and 82.1 mM for SFC, UREA, and BYPROD, respectively. 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 equal ( $P > 0.10$ ) to both treatments. This resulted in a greater ( $P < 0.05$ ) acetate:propionate ratio

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with BYPROD than with UREA, with SFC being equal ( $P>0.10$ ) to both UREA and BYPROD. No differences ( $P>0.10$ ) among treatments were observed for butyrate molar proportion. The depressed total VFA and propionate molar proportion with the BYPROD treatment would be expected due to differences in substrate and the slower-fermenting corn milling by-products replacing starch, which is quickly fermented in the rumen. The greater quantity of acid present in the rumen with the SFC and UREA treatments helps explain the depressed ruminal pH with these treatments compared with the BYPROD treatment.

Production of MCP as estimated by urinary PD:C was greater ( $P<0.05$ ) with BYPROD than with SFC, tended ( $P=0.09$ ) to be greater with UREA than with SFC, and was not different ( $P>0.10$ ) between UREA and BYPROD (Table 2). Urinary PD:C measured 0.75, 0.92, and 1.06 for SFC, UREA, and BYPROD, respectively. The improvement in PD:C between SFC and UREA was 22.7%, while the improvement between SFC and BYPROD was 41.3%. In an individual feeding study (2007 Nebraska Beef Report, pp. 103-105), PD:C was 25.5% greater with UREA than with SFC, and 33% greater with BYPROD than with SFC. In both experiments, the addition of DIP in the form of urea can explain the improvement of UREA over SFC, while the addition of corn milling by-products to provide for a more favorable rumen environ-

ment can explain differences between the BYPROD treatment and the SFC and UREA treatments.

Similar results for MCP production were found with estimates from duodenal purine collections (Table 2). Production of MCP measured 235, 309, and 395 g/day for SFC, UREA, and BYPROD, with BYPROD being greater ( $P<0.05$ ) than SFC and tending ( $P=0.06$ ) to be greater than UREA. Microbial CP flow was 31.4% greater with UREA than with SFC, but the results were not significant ( $P>0.10$ ). Microbial efficiency (Table 2) did not differ ( $P>0.10$ ) among treatments; however numerical improvements were present with measurements of 5.89, 6.34, and 7.45 g of CP/100 g OM ruminally digested for SFC, UREA, and BYPROD, respectively. The numerical improvements in microbial efficiency with BYPROD can be related to improved ruminal pH allowing for increased microbial fermentation.

As was found previously in an individual feeding study (2007 Nebraska Beef Report, pp. 103-105), estimates of MCP from PD:C were greater (linear  $P < 0.05$ ) with samples collected later in the day, measuring 0.82, 0.86, 0.96, and 0.98 when samples were collected at 0700, 1200, 1700, and 2200 hours, respectively, and no treatment x time of urine collection interactions ( $P>0.10$ ) were present. Duodenal purine measurements confirmed these results, with MCP measuring 297, 298, 330, and 328 g/day when duodenal samples were collected at

0700, 1200, 1700, and 2200 hours (linear  $P < 0.05$ ). It is important to note that these steers were fed once daily at 0730 hours, and the PD:C response may be a function of feeding time.

Ruminal OM digestibility measured 61.2, 65.2, and 62.1% for SFC, UREA, and BYPROD, and were not different ( $P>0.10$ ). Post-ruminal and total tract OM digestibilities were not different ( $P>0.10$ ) between SFC and UREA, and both treatments were greater ( $P<0.05$ ) than BYPROD. No treatment differences ( $P>0.10$ ) were observed for ruminal, post-ruminal, 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, post-ruminal starch digestibility was numerically higher with SFC than with UREA, resulting in no differences ( $P>0.10$ ) among treatments for post-ruminal and total tract starch digestibilities.

## Conclusions

The agreement of the PD:C measurements with the duodenal purine data suggest that this method can be effectively used to estimate MCP production in beef cattle.

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