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Microbial Characteristics, Microbial Nitrogen Flow, and Urinary Purine Derivative Excretion in Steers Fed at Two Levels of Feed Intake

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Summary

Ruminally and duodenally fistulated Holstein steers were fed at 40% and 85% of ad-libitum intake. Microbial purine:N ratio did not differ between intake levels, which makes estimating microbial protein easier in production or experimental settings. Urinary purine derivatives (PD):creatinine (PD:C) ratio and microbial CP flow estimated from the duodenum increased with the higher feeding level. Urinary PD:C and duodenal purine flow were related, suggesting that urinary PD:C ratio can be used to estimate relative differences in microbial CP flow.

Introduction

The urinary purine derivative:creatinine ratio (PD:C) is a useful tool for estimation of microbial CP (MCP) flow in feedlot cattle (*2007 Nebraska Beef Report*, pp. 100-102 and pp. 103-105).

Two major factors limiting the application of the urinary PD:C method to estimate MCP flow are the contribution of endogenous PD to total urinary PD excretion and the purine content of ruminal microbes. Therefore, the objectives of this experiment were to characterize the purine:N ratio of ruminal microbes, estimate endogenous PD contribution to total urinary PD excretion, and evaluate use of spot samples of urine to estimate MCP flow in cattle.

Procedure

Five ruminally and duodenally fistulated Holstein steers (1,254 ± 88 lb) were assigned randomly to one of two treatments. The two treatments were either 40% or 85% ad-libitum intake of a diet consisting of 70% high-moisture corn, 20% corn bran, 5% alfalfa hay, and 5% dry supplement. Steers were fed through automatic feeders programmed to deliver feed every 4 hours in six equal portions throughout the day. Diets contained 33 g/ton monensin and 10 g/ton tylosin. Steers were not implanted for this experiment.

The experiment consisted of four 21-day periods. Each period consisted of a 17-day adaptation phase followed by a 4-day sample collection phase. Chromic oxide was used as an indigestible marker for estimating duodenal flow. Urinary creatinine was used as a marker to estimate urine volume, and the ratio of urinary PD to creatinine was used to estimate relative differences in MCP flow. Spot samples of urine and duodenal flow were collected on days 18 to 20 of each period at 0700, 1200, 1700, and 2200 hours. On day 21, ruminal contents were collected from each steer at 0730 hour.

Ruminal bacteria were isolated from ruminal contents by differential centrifugation to separate bacteria from feed and supernatant. Purines were determined in duodenal and isolated bacterial samples using adenine and guanine as standards. Purines were determined by spectrophotometry, while urinary PD and creatinine were determined by HPLC.

Urinary PD excretion was calculated from urinary PD and creatinine output assuming creatinine output of 28 mg/kg BW. These values were regressed upon duodenally absorbed purines assuming an intestinal absorption of 83% for purines reaching the duodenum.

Data were analyzed using the Proc MIXED procedure of SAS for a crossover design. For ruminal digestibility and microbial characteristics, the model included period, level of intake, and previous period level of intake. Duodenal purine flow and urine data were analyzed as repeated measures. Urine spot sample and duodenal flow sampling time were also analyzed for linear, quadratic, and cubic responses. Treatment differences were considered significant at $P < 0.05$.

Results

Feed offering for the 40% and 85% ad libitum treatments averaged 11.7 and 24.9 lb/day, respectively. No treatment differences ($P > 0.10$) were observed for any microbial characteristic measured (Table 1). Microbial N content averaged 6.12% at the 40% intake level and 6.30% at the 85% intake level. Purine:N ratio averaged 0.195 and 0.208 for the 40% and 85% intake levels, respectively. Among all samples, the purine:N ratio ranged from 0.127 to 0.251, with an overall average of 0.205.

This experiment was designed to produce wide ranges in MCP flows through altering individual animal DMI with the hypothesis that

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Table 1. Ruminal microbial characteristics from steers fed at 40% and 85% of ad libitum DMI[a].

	Level of Intake (%)[a]			
Item	40	85	SEM	*P*-value
OM, % of DM	84.1	85.1	0.7	0.19
Purines, % of DM	1.19	1.34	0.18	0.49
N, % of DM	6.12	6.30	0.44	0.66
Purine:N	0.195	0.208	0.019	0.59

[a]Diets were fed at either 40% or 85% of previously determined ad libitum DMI.

increased DMI would result in an increase in MCP flow and urinary PD excretion. As expected, we observed large ranges in MCP flow estimated from both duodenal purine flow and urinary PD:C (Table 2). Urinary PD:C increased from 0.510 at 40% ad libitum intake to 0.916 at 85% ad libitum intake. Microbial protein flow increased from 305.6 g/day at 40% ad libitum intake to 755.5 g/day at 85% ad libitum intake. Microbial efficiency tended ($P = 0.08$) to improve with the 85% intake level compared with the 40% intake level. An improvement in MCP flow and microbial efficiency at the higher intake level can be attributed at least partially to a greater amount of energy supplied by increased DMI. In addition, a reduction in recycling of nutrients by ruminal bacteria at higher intakes reduces maintenance requirements and provides more nutrients for microbial growth.

Upon regressing urinary PD excretion on absorbed purines (Figure 1), we observed a relationship between the two with an equation of 0.412x + 57.77 where x = absorbed purines in mmol/d and a good fit ($r^2 = 0.60$). This indicates that urinary PD excretion is related to microbial protein flow at the duodenum. Ideally, the slope of this equation would equal 1, which would indicate that all purines absorbed from the small intestine are quantitatively recovered in the urine. However, our value of 0.412 indicates that only 41.2% of absorbed purines are recovered in the urine, and non-renal losses of PD account for 59% of absorbed purines. The intercept of the equation in Figure 1 (57.77 mmol/day) represents endogenous PD contribution to urinary PD excretion. Endogenous PD are PD that do not originate from duodenally absorbed microbial purines, and must be accounted for when making MCP flow estimates from urinary PD:C ratios.

An alternative explanation for the low (41.2%) recovery of purines as PD is that the estimate of purine digestibility (83%) from the literature is too high. If PD recovery is regressed on total dudodenal purine flow the

Table 2. Urinary purine derivative:creatinine ratio, microbial protein flow, and microbial efficiency in steers fed at 40% or 85% of ad libitum DMI.

	Level of Intake (%)[a]			
Item	40	85	SEM	*P*-value
PD:C[b]	0.510	0.916	0.027	< 0.01
MCP flow, g/day[c]	305.6	755.5	45.6	< 0.01
Microbial efficiency g of CP/100 g OMTD[cd]	10.2	12.2	0.7	0.08

[a]Diets were fed at either 40% or 85% of previously determined ad libitum DMI.
[b]Urinary purine derivative:creatinine ratio.
[c]Derived from duodenal purine flow assuming purine:N ratio of 0.205.
[d]Organic matter truly digested in the rumen.

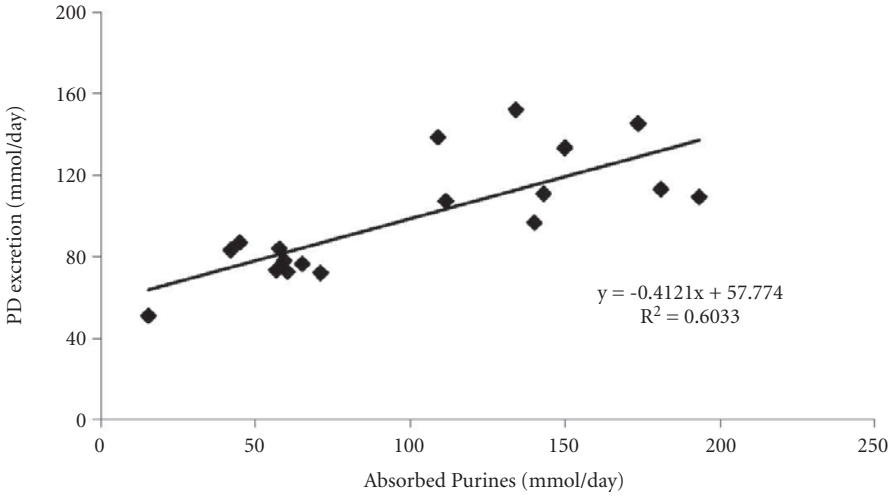

Figure 1. Urinary purine derivative (PD; mmol/day) excretion regressed on absorbed duodenal purines (mmol/day). Duodenal purine absorption assumed to be 83% of duodenal purine flow. SEM: slope ± 0.084; intercept ± 9.5.

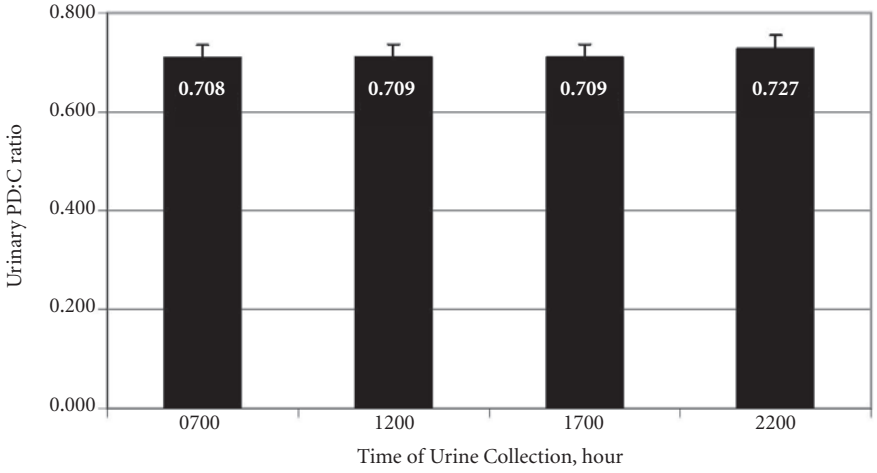

Figure 2. Effect of time of urine spot sample collection on urinary purine derivative:creatinine (PD:C) ratio. Urine collection time linear $P = 0.53$; quadratic $P = 0.65$; cubic $P = 0.83$. Level of intake x urine collection time $P = 0.77$.

recovery of PD declines to 34.2%. The 65.8% of purines not accounted for as PD could be explained by lower digestibility than 83%, secretion in saliva and salvage for use in nucleic acid synthesis in the body. At the present time, we do not know the magnitude of each of these uses (losses).

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. Previous data (*2007 Nebraska Beef Report*, pp. 100-102 and 103-105) suggested that urinary PD:C increased when spot samples of urine were collected in the afternoon rather than the morning. In those studies, animals were fed once daily at either 0730 or 0800 hours. In the current experiment, no diurnal differences in urinary PD:C ratio were observed (Figure 2) when steers were fed meals in six evenly spaced portions throughout the day. These findings suggest that the previously observed diurnal effect is likely a function of feeding time.

In conclusion, a microbial purine:N ratio of 0.205 was observed, and was not affected by level of DMI. Microbial CP flow and urinary PD:C ratio responded similarly to increasing DMI, and the relationship between the two indicates that urinary PD:C ratio from urine spot samples adequately represented relative treatment differences in MCP flow, though lower than expected purine recoveries were observed. Urinary PD:C was not affected by sampling time when steers were fed in six equal and evenly spaced portions throughout the day, indicating that diurnal variation in PD:C was a function of feeding time.

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