# Factors affecting urinary creatinine in heifers and cows[1]

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**ABSTRACT:** A series of total urine collections were conducted to evaluate the effects of age, diet, gestation, and body condition score (**BCS**) on urinary creatinine (**UC**) and purine derivative (**PD**) excretion in heifers and cows. For each collection, urine was collected over a 5-d period and composited by animal within day. Daily samples were analyzed for UC and PD concentration and averaged over the 5-d period. All animals were fed in individual stanchions at 2.0% of body weight (**BW**). To evaluate the relationship between age and UC excretion, 21 animals ranging from 5 to 80 months of age were fed a forage-based diet supplemented with dried distillers grains (DDG). Creatinine excretion (mg/kg BW) was not correlated with age ($P = 0.37$). To determine if diet alters UC, 11 heifers were sampled for two urine collection periods. In period 1, heifers were fed a forage-based diet supplemented with DDG. In period 2, heifers were fed a finishing diet (90% concentrate, 10% forage). Creatinine excretion (mg/kg BW) and PD:creatinine (**PD:C**) was greater ($P = 0.01$) for heifers when fed the forage-based diet than when fed the concentrate-based diet. Eleven cows fed a forage-based diet supplemented with DDG were sampled to determine the effect of gestation on urinary metabolites. Gestation did not affect UC ($P = 0.42$) or PD:C ($P = 0.30$). To evaluate the relationship between 12th rib fat thickness and metabolite excretion, 40 heifers were fed a common finishing diet. There was no relationship between UC (mg/kg BW; $P = 0.28$) or PD:UC ($P = 0.47$) and 12th rib fat thickness. To evaluate the relationship between BCS and UC, 11 cows were fed a forage diet supplemented with DDG. There was no relationship between BCS and UC (mg/kg BW; $P = 0.99$) or PD:C ($P = 0.84$). To evaluate daily and diurnal variation in UC, nine heifers were fed a forage diet supplemented with DDG. Seven of the heifers were fed a finishing diet (90% concentrate, 10% forage) in a second period. Urine was collected every 2 h from 0600 to 1800 hours. When expressed as mg/kg BW, UC excretion was not different across animals fed the forage-based ($P = 0.40$) or concentrate-based diet ($P = 0.18$). Stepwise regression indicated that at least 3 d of collection were required to estimate UC. Time within day and day within period effects were observed ($P < 0.01$) for UC from 2-h interval samples. The UC varies with type of diet and diurnal variation is present. Variation among animals is relatively small.

**Key words:** beef cattle, creatinine excretion, purine derivatives, urine metabolites, urine output



Transl. Anim. Sci. 2019.3:532–540
doi: 10.1093/tas/txz004

[1]This project is based on research that was partially supported by the Nebraska Agricultural Experiment Station with funding from the Hatch Act (Accession Number 1007896) through the USDA National Institute of Food and Agriculture.

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Received October 17, 2018.
Accepted January 24, 2019.

## INTRODUCTION

Measuring urine output in cattle is important to allow research on N balance and metabolites in the urine such as creatinine and purine derivatives (**PD**). Creatinine, a urine metabolite, has been shown to be an effective marker for estimating urine excretion in beef cattle (Chen et al., 1992; Valadares et al., 1999; McDonald, 2003; Jardstedt et al., 2017). A product of muscle metabolism, urinary creatinine (**UC**) excretion is directly related to muscle mass (Lofgreen and Garrett, 1954; McCarthy et al., 1983; Gopinath and Kitts, 1984; Hayden et al., 1992) and is excreted at a constant rate relative to BW (Brody, 1945). Swartz et al. (2016) and Brunsvig et al. (2017) used UC output to estimate N excretion in grazing heifers and cows. Other researchers have used PD:creatinine (**PD:C**) ratio to estimate microbial crude protein (**MCP**) yield over a grazing season in heifers (MacDonald et al., 2007) and cows (Patterson et al., 2006). Dórea et al. (2017), demonstrated a strong correlation between PD:C and both dry matter intake (**DMI**; $R^2$ = 0.84) and digestible DMI ($R^2$ = 0.85). Galyean and Tedeschi (2014) further showed that MCP yield was highly correlated to DMI ($r^2$ = 0.88) and total digestible nutrients (**TDN**) intake ($r^2$ = 0.89), which suggests a strong relationship between PD and MCP yield.

It is important to understand the factors that influence variability in UC excretion in beef cattle to determine if it could be used as a reliable component in a system to predict urine output. The objectives of these trials were to determine the effects of age, diet, gestation, body condition score (**BCS**), and 12th rib fat thickness on UC excretion and PD:C concentration and total daily excretion.

## MATERIALS AND METHODS

All cattle were managed in accordance with protocols approved by the University of Nebraska Institutional Animal Care and Use Committee.

### *Animals and Diets*

Crossbred heifers and cows, (MARC III composite breed [¼ Angus, ¼ Hereford, ¼ Pinzgauer, and ¼ Red Poll]) were used in a series of total urine collections to determine the effects of age, diet, gestation, BCS, and 12th rib fat thickness on UC and PD:C. Animals were fed a diet (Table 1) of either grass hay (13% crude protein [CP]) supplemented with 30% dried distillers grains (DDG; 30% CP) or a common finishing diet ([16% CP; 77% dry matter [DM]) based on a blend of dry rolled corn and wet corn gluten feed. All animals were fed at 2.0% of body weight (**BW**) once daily at 0800 hours and allowed ad libitum access to water. All animals were fed using individual feed bunks within metabolism stanchions.

**Table 1.** Composition of experimental diets

| Ingredient | Diet, % dry matter |
|---|---|
| Forage diet | |
| Grass hay | 70.0 |
| Dried distillers grains | 30.0 |
| Finishing diet | |
| Dry-rolled corn | 57.8 |
| Wet corn gluten feed | 30.0 |
| Alfalfa hay | 10.0 |
| Limestone | 1.8 |
| Sodium Chloride | 0.3 |
| Trace mineral premix[a] | 0.05 |
| Vitamin A-D-E premix[b] | 0.02 |
| Rumensin premix[c] | 0.02 |
| Tylan premix[c] | 0.01 |

[a]Premix contained 10% Mg, 6% Zn, 2.5% Mn, 0.5% Cu, 0.3% I, and 0.05% Co.

[b]Premix contained 1,500 IU of vitamin A, 3,000 IU of vitamin D, and 3.7 IU of vitamin E per g.

[c]Finishing diet contained 354 mg/kg monensin and 11 mg/kg tylosin (dry matter basis; Elanco Animal Health, Greenfield, IN).

Heifers and cows were housed in 1.3 × 2.5 m individual metabolism stanchions in a temperature-controlled room (25°C) and tethered for the 5 d collection period. Collection periods consisted of a minimum of 5 d for diet adaptation at ad libitum intake followed by 3 d of adaptation to restricted intake and then 5 d for sample collection at restricted intake (2.0% of BW). Animals were fitted with indwelling Foley urethral catheters (C.R. Bard, Inc., Covington, GA; size 12 to 24) approximately 6 h prior to collection. Urethral catheters were connected to tubing at each animal's hip with Velcro tape. Tubing was suspended by a cable and pulley and led to a 2-liter overnight urine collection bag (C.R. Bard, Inc., Covington, GA) in a cooled thermally-insulated container.

***Trial 1.*** Twenty-one heifers and cows (BW range = 98 to 582 kg) were sampled to test the relationship between age and UC and PD:C. Animals ranged from 5 to 80 mo of age and were fed the grass hay diet supplemented with DDG. Age was approximated to the nearest month at the time of collection.

***Trial 2.*** To determine if diet alters UC and PD:C, heifers were sampled for two urine collection periods. In period 1, 10 heifers (BW = 404 kg, SD = 25) were fed the grass hay diet supplemented with DDG.

In period 2, eight of the heifers (BW = 489 kg, SD = 25) were then fed the common finishing diet containing 87.8% concentrate (dry rolled corn and wet corn gluten feed), 10% forage (alfalfa hay), and 2.2% supplement (fine ground corn carrier with limestone, trace minerals, and Vitamin ADE).

***Trial 3.*** Five gestating (BW = 611 kg, SD = 42) and six non-gestating (BW = 531 kg, SD = 40) cows fed the grass hay diet supplemented with DDG were sampled to determine the effect of gestation on UC and PD:C. Gestation was determined by rectal palpation at the time of collection.

***Trial 4.*** To evaluate the relationship between 12th rib fat thickness and UC and PD:C, heifers ($n$ = 40; BW = 525 kg, SD = 65) were sampled at two stages during the finishing period. Heifers were sampled in blocks of 10 animals and sampling periods were approximately 75 d apart. Heifers were fed the finishing diet containing 87.8% concentrate and 10% forage for both periods. Ultrasound 12th rib fat thickness measurements were made between the 12th and 13th rib (Aloka 500V; Corometrics Medical Systems, Wallingford, CT). Measured 12th rib fat thickness ranged from 0.8 to 2.4 cm.

Eleven cows (BW = 567 kg, SD = 57) were sampled to evaluate the relationship between BCS and UC and PD:C. Cows were fed the grass hay diet supplemented with DDG. Cow BCS was determined at the time of collection. Measured BCS ranged from 3.9 to 6.4.

***Trial 5.*** Nine crossbred heifers (BW = 403 kg, SD = 27) were sampled for two total urine collection periods to determine diurnal (patterns within a 24-h period), daily (comparison between consecutive days), and animal variation in UC and PD:C in growing and finishing heifers. In period 1, nine heifers were fed the forage-based diet supplemented with DDG. In period 2, seven of the heifers (BW = 494 kg, SD = 22) were fed the common finishing diet containing 87.8% concentrate and 10% forage.

### *Sampling*

For all trials, experimental diets were sampled once before each collection period and dried in a 60°C forced-air drying oven for 48 h to determine DM content. Feed refusals were weighed, sampled, and recorded daily before feeding and used to adjust DM offered. Individual feed ingredients were sampled before each collection period and dried at 60°C for 48 h and then ground to pass through a 1-mm screen.

Urine was collected continuously for 5 consecutive days. Urine was drained from each animal's bag at 2-h intervals from 0600 to 1800 hours. Urine was allowed to accumulate from 1800 to 0600 hours when it was then drained. Drainage was measured in a 2 liters graduated cylinder to the nearest 10 ml and recorded. Approximately 45 ml aliquots of urine were collected and stored in 50-ml screw-cap vials. Aliquots were then composited by animal within day by combining 1.0% of each collection interval (overnight sample and 2-h interval samples). Composited daily samples and 2-h interval samples were then initially diluted with 9-part urine diluent (Shingfield and Offer, 1999b) and 1-part urine. All samples were stored at –20°C until subsequent analysis.

### *Laboratory Analyses and Calculations*

Laboratory DM of feed ingredients and feed refusals were determined in a 100°C oven for 24 h. Crude protein was analyzed by the combustion method (FP-528; LECO Corporation, St. Joseph, MI). Diluted urine samples were additionally diluted with 4-parts urine diluent and 1-part diluted urine for analysis. Creatinine and PD (allantoin plus uric acid) concentrations were determined by high-performance liquid chromatography (Waters Corp., Milford, MA) according to the procedure of Shingfield and Offer (1999b).

Daily excretion of creatinine (g/d) and PDs (mmol/d) were calculated for each animal by multiplying the concentration (mmol/liter) by measured 24 h urine volume. Creatinine was also expressed as a coefficient of BW (mg/kg BW) by dividing the daily creatinine excretion by each animal's BW.

### *Statistical Analyses*

Regression analyses using the REG procedure of SAS (SAS Inst. Inc., Cary NC) were conducted to evaluate the relationship between age, BCS, or 12th rib fat thickness and UC and PD:C (trials 1 and 4). The MIXED procedure of SAS was used to determine the effect of diet and gestation on UC and PD:C with animal included as a random effect (trials 2 and 3). For trial 5, data were analyzed using the MIXED procedure of SAS with day as a fixed effect and animal as a random effect. Orthogonal linear, quadratic, and cubic effects within day were tested. Additionally, the STEPWISE and RSQUARE selection methods of the REG procedure of SAS were used to evaluate minimum number of days required for collection. Data were compiled as periods consisting of day 1, 1 to 2, 1 to

3, and 1 to 4 and then regressed against the average of days 1 through 5. In all trials, effects were considered significant when $P \leq 0.05$ and tendencies are discussed when $P \leq 0.10$.

## RESULTS AND DISCUSSION

### *Trial 1*

Regression analysis demonstrated that age was significantly correlated ($r^2 = 0.42$) with UC excretion (g/d). Because BW was correlated ($r^2 = 0.58$) with age, when UC excretion was expressed as a coefficient of BW (mg/kg BW), there was no correlation ($r^2 = 0.04$; $P = 0.37$) between age and UC excretion (Figure 1). Range of UC excretion across all animals was 23.07 to 33.88 mg/kg BW with a mean of 28.64 mg/kg BW. It has been well documented that UC excretion is directly correlated with fat-free mass in ruminant animals (Lofgreen and Garrett, 1954; Van Niekerk et al., 1963; Hayden et al., 1992). Additionally, Gopinath and Kitts (1984) showed an increase in UC excretion (g/d) in growing beef steers with time indicating increased muscle mass with age.

The ratio of molar concentrations of PD and UC decreased ($r^2 = 0.44$) with age. The slope of −0.01 and intercept of 1.31 were both different ($P < 0.01$) from zero (Figure 1). PDs excreted in the urine are primarily exogenous in origin. However, due to tissue ATP and nucleic acid turnover, endogenous PD excretion contributes to total PD excretion. Therefore, when calculating MCP, a correction is made for contribution of endogenous PD to the total supply of PD in the urine based upon metabolic BW ($BW^{0.75}$). However, Shingfield and Offer (1999a) suggest that tissue nucleic acid turnover is associated with an animal's metabolic activity. If metabolic rate decreases as an animal ages, then the decrease observed could be accounted for by endogenous PD excretion.

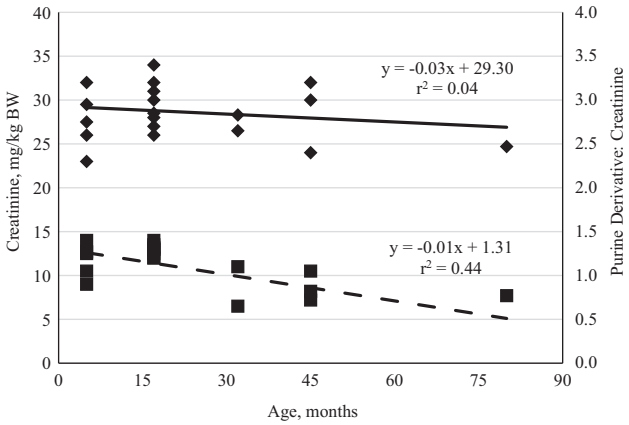


**Figure 1.** Relationships between cattle age (months) and urinary metabolites ($n = 21$; trial 1). Solid line and diamonds (left vertical axis): relationship between age and urinary creatinine excretion (mg/kg body weight). The slope of −0.03 (SE = 0.04) and intercept of 29.3 (SE = 1.16) were both different from zero ($P < 0.01$). Dashed line and squares (right vertical axis): relationship between age and the ratio of molar concentrations of purine derivatives and creatinine. The slope of −0.01 (SE = 0.002) and intercept of 1.31 (SE = 0.06) were both different from zero ($P < 0.01$).

### *Trial 2*

Excretion of UC (g/d) tended to be greater ($P = 0.07$) when heifers were fed the finishing diet compared to when they were fed the forage-based diet (12.92 vs. 12.01 g/d; Table 2). However, when expressed as mg/kg BW, UC excretion was greater ($P = 0.01$) for heifers fed the grass hay diet supplemented with DDG than when fed the finishing diet. When fed the forage-based diet, heifers excreted an average of 29.70 mg/kg BW compared to 26.50 mg/kg BW of UC when fed the finishing diet. The PD:C was greater ($P < 0.01$) for heifers fed the grass hay diet supplemented with DDG than when fed the finishing diet. The PD:C ratio is potentially confounded between age and diet. The range in ages in Figure 1 is nearly 80 mo. The difference in age of the heifers in this trial is less than 3 months so it would have very little effect on the values.

Previous research indicates independence of UC excretion and level of dietary protein (Dinning et al., 1949; Butcher and Harris, 1957; Albin and Clanton, 1966; Ørskov and MacLeod, 1982; Jardstedt et al., 2017) or energy intake (Van Niekerk et al., 1963; Albin and Clanton, 1966). Diets utilized in previous experiments were typically a base diet with added levels of protein or energy intake. Conversely, the two diets fed in the current study were formulated to be quite different. The grass hay diet supplemented with DDG would be expected to give 0.4 kg/d of gain, while the finishing diet would produce 1.5+ kg/d of gain. Furthermore, the forage-based diet supplied fiber as the primary energy

**Table 2.** Effect of diet (trial 2) and gestation (trial 3) on urinary creatinine and purine derivative excretion in heifers (trial 2) and cows (trial 3)

| Diet | Forage | Concentrate | SEM | *P*-value |
|---|---|---|---|---|
| Creatinine, g/d | 12.01 | 12.92 | 0.35 | 0.07 |
| Creatinine, mg/kg BW | 29.70 | 26.50 | 0.86 | 0.01 |
| PD:C molar ratio | 1.27 | 0.95 | 0.03 | <0.01 |
| Gestation | Gestating | Non-gestating | SEM | *P*-value |
| Creatinine, g/d | 17.67 | 14.59 | 0.76 | 0.01 |
| Creatinine, mg/kg BW | 29.20 | 27.67 | 1.35 | 0.42 |
| PD:C molar ratio | 0.76 | 0.85 | 0.06 | 0.30 |

source for the rumen microbes and the resulting end products of fermentation to the heifer. The finishing diet was starch-based, producing a different microbial population and different end products. These two diets represent the extremes, yet the difference in UC excretion (mg/kg BW) was only 11.4%. Using the NRC (1996) model, the heifers were estimated to have 15% body fat when consuming the forage-based diet. After the adaptation and treatment period, heifers were estimated to have 18% body fat. If 30% to 40% of the differences in UC excretion from different diets can be explained by greater percent body fat when the heifers were fed the finishing diet compared to the forage-based diet (NRC, 1996), then the impact of diet on UC excretion is small. The greater PD:C for heifers on the forage diet suggests greater microbial efficiency compared to the finishing diet.

### *Trial 3*

The results of the effect of pregnancy on UC and PD:C in cows are shown in Table 2. Gestating cows had greater ($P = 0.01$) UC excretion (17.67 g/d) than cows that were not gestating (14.59 g/d). However, there was no difference ($P = 0.42$) in creatinine output when expressed as g/kg BW. Creatinine excretion (mg/kg BW) for gestating and non-gestating cows was 29.20 and 27.67, respectively. Based on Anthony et al. (1986), total conceptus weight would range from 20 to 25 kg for the gestating cows. If we account for that weight (subtract it from cow body weight), it would increase the UC by 3% to 4%. However, it is not clear if the conceptus is a source of creatinine. Because of these unknowns and the small number of cows sampled, it might be inappropriate to conclude that gestating and non-gestating cows produce similar amounts of UC.

Similar increases in UC excretion (g/d) were observed by Erb et al. (1977) in dairy cows sampled between 28 and 8 d prepartum and between 8 and 39 d postpartum. However, Erb et al. (1977) also reported differences in the creatinine coefficient in cows sampled 28 and 8 d prepartum (24.96 mg/kg BW) and 8 and 39 d postpartum (21.12 mg/kg BW). Cows sampled in the current study were two groups of animals (gestating and non-gestating), not one group of animals sampled at two stages of production, which would reduce animal variation. Variation among individuals in the current study could account for the inability for a difference to be detected between treatment groups. Additionally, cows sampled in this study ranged from approximately 120 to 200 d pregnant compared to approximately 246 to 277 d pregnant in the study by Erb et al. (1977). Ørskov and MacLeod (1982) reported no differences in UC excretion in relation to BW in cows between 117 and 133 d pregnant and between 220 and 233 d pregnant.

There was no difference in PD:C ($P = 0.30$) between gestating and non-gestating cows (Table 2). The PD:C was numerically lower in gestating cows compared to non-gestating cows (0.76 vs. 0.85) due to UC (g/d) excretion being numerically greater in gestating cows.

### *Trial 4*

Ultrasound 12th rib fat thickness was poorly correlated ($r^2 = 0.14$) with UC excretion (g/d; data not shown). The slope of 1.76 and intercept of 12.89 were both different from zero ($P < 0.05$). However, when expressed as a coefficient of BW (mg/kg BW; Figure 2), no relationship was found ($r^2 = 0.04$; $P = 0.28$) between 12th rib fat thickness and creatinine output. The PD:C was not correlated ($r^2 = 0.02$; $P = 0.47$) with 12th rib fat thickness. There was no relationship between BCS and UC excretion as mg/kg BW ($r^2 = 0.00$; $P = 0.99$; data not shown). Similarly, the PD:C was not correlated with BCS ($r^2 = 0.005$; $P = 0.84$; data not shown).

UC excretion is highly correlated with empty body protein and fat-free mass (Picón-Reátegui, 1962; Van Niekerk et al., 1963). Therefore, it was expected that ultrasound 12th rib fat thickness measurements and BCS could be used as predictors

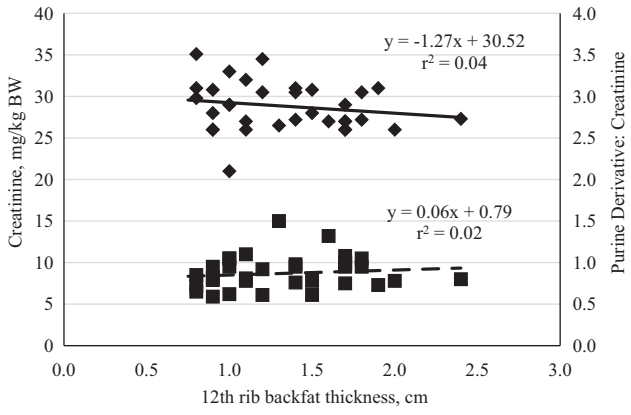


**Figure 2.** Relationships between 12th rib fat thickness (cm) and urinary metabolites ($n = 40$; trial 4). Solid line and diamonds (left vertical axis): relationship between 12th rib fat thickness and urinary creatinine excretion (mg/kg body weight). The slope of −1.27 (SE = 1.15) was not different from zero ($P = 0.28$) while the intercept of 30.52 (SE = 1.61) was different from zero ($P < 0.01$). Dashed line and squares (right vertical axis): relationship between 12th rib fat thickness and the ratio of molar concentrations of purine derivatives and creatinine. The slope of 0.06 (SE = 0.08) was not different from zero ($P = 0.47$) while the intercept of 0.79 (SE = 0.12) was different from zero ($P < 0.01$).

of UC excretion or account for differences among animals due to fluctuations in body fat. However, no relationships were found between ultrasound 12th rib fat measurements or BCS and UC excretion.

### *Trial 5*

Excretion of UC expressed as mg/kg BW was constant across sampling days ($P$ = 0.42), when heifers were fed the forage diet. Range of UC excretion was 28.11 to 30.28 mg/kg BW. The PD:C was not different by day ($P$ = 0.17) across the 5 d period. Excretion of UC expressed as mg/kg BW decreased linearly ($P$ < 0.01) across experimental days when heifers were fed the finishing diet. The PD:C was not different across collection days ($P$ = 0.18) when heifers were fed the finishing diet.

***Variation by day, time, and animal.*** Results for day and diurnal variation are represented in Tables 3 and 4 for UC excretion and PD:C, respectively. Diurnal variation was evaluated using 2-h interval samples from 0800 to 1800 hours for each animal. There was a significant day × time interaction ($P$ < 0.01) for UC excretion (mg/kg BW) when heifers were fed the forage diet (Table 3). Days 2 and 3 had a linear increase in UC excretion ($P$ ≤ 0.04) over time. On days 4 and 5, time of collection resulted in a quadratic ($P$ ≤ 0.01) increase in UC excretion. A significant ($P$ < 0.01) day × time interaction was detected for UC excretion (mg/kg BW) when heifers were fed the finishing diet (Table 3). Time of day resulted in a cubic response ($P$ = 0.01) on days 1 and 2. Days 3, 4, and 5 resulted in quadratic ($P$ ≤ 0.03) increase in UC excretion over time of collection.

There was a significant day × time interaction ($P$ < 0.02) for the PD:C when heifers were fed the forage diet (Table 4). Days 1 and 2 resulted in quadratic ($P$ ≤ 0.03) increases in PD:C over time, while day 4 responded cubically ($P$ = 0.01). There was no effect ($P$ ≥ 0.18) of time of collection on days 3 and 5 ($P$ ≥ 0.18). There was not a significant day × time interaction ($P$ = 0.35) for PD:C when heifers were fed the finishing diet (Table 4). Days 2 and 4 resulted in linear ($P$ = 0.001) increases in PD:C relative to time of collection, while day 1 responded cubically ($P$ = 0.02). There was no effect ($P$ ≥ 0.12) of time of collection on days 3 and 5.

Stepwise regression was used to evaluate the minimum number of days required for an accurate estimate of PD:C from average daily PD:C (Table 4). The objective of stepwise regression was to evaluate a set of variables (days 1 through 5) that were regressed against a dependent variable (average of days 1 through 5). Stepwise regression indicated that 3 or 4 d of collection were required to accurately estimate the PD:C when heifers were fed the forage diet. Adjusted $r^2$ values were 0.69 and 0.80 for 3 and 4 d, respectively. Collecting 4 d resulted in an 11 percentage unit increase in variance explained over 3 d of collection. However, 3 d resulted in a 23 percentage unit increase in variance explained over only 2 d of collection. Stepwise regression indicated that 2 or 3 d of collection were required when heifers were fed the finishing diet. Adjusted $r^2$ values were 0.97 and 0.99 for 2 and 3 d, respectively.

UC excretion was first noted for its constancy when Folin began researching it in 1905. However, since then, researchers have noted significant variability in creatinine output. Best et al. (1952) reported a significant diurnal cycle to creatinine excretion. However, Albin and Clanton (1966) found that N was excreted in a similar diurnal cycle and when expressed as a ratio (N:creatinine), the variability

**Table 3.** Effects of time within day on urinary creatinine excretion (mg/kg body weight) as predicted by 2-h interval samples (trial 5)

| | Time of collection, hours | | | | | | | Orthogonal contrasts | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0800 | 1000 | 1200 | 1400 | 1600 | 1800 | SEM | Linear | Quadratic | Cubic |
| Forage diet | | | | | | | | | | |
| Day 1 | 33.92 | 25.32 | 34.31 | 22.26 | 35.75 | 29.43 | 2.32 | 0.87 | 0.17 | 0.14 |
| Day 2 | 27.38 | 28.34 | 30.69 | 36.48 | 41.42 | 34.94 | 2.81 | 0.01 | 0.30 | 0.05 |
| Day 3 | 21.02 | 30.27 | 34.65 | 23.28 | 39.11 | 31.75 | 3.87 | 0.04 | 0.30 | 0.48 |
| Day 4 | 14.04 | 30.52 | 27.90 | 22.72 | 32.17 | 25.81 | 2.19 | 0.01 | 0.01 | 0.03 |
| Day 5 | 19.03 | 28.41 | 30.21 | 28.40 | 28.29 | 24.18 | 1.95 | 0.16 | 0.01 | 0.20 |
| Finishing diet | | | | | | | | | | |
| Day 1 | 24.03 | 27.75 | 41.39 | 23.47 | 25.97 | 32.97 | 2.59 | 0.33 | 0.24 | 0.01 |
| Day 2 | 23.43 | 26.15 | 42.22 | 24.00 | 27.49 | 33.53 | 2.44 | 0.08 | 0.14 | 0.01 |
| Day 3 | 21.92 | 28.88 | 38.34 | 27.56 | 31.21 | 30.36 | 2.59 | 0.08 | 0.01 | 0.05 |
| Day 4 | 25.22 | 21.50 | 39.13 | 16.22 | 25.87 | 18.96 | 2.49 | 0.04 | 0.03 | 0.35 |
| Day 5 | 13.41 | 21.91 | 26.99 | 33.23 | 23.38 | 27.82 | 1.63 | 0.01 | 0.01 | 0.10 |

**Table 4.** Effects of time within day on the purine derivative to creatinine molar ratio as predicted by 2-h interval samples (trial 5)

| | Time of collection, hours | | | | | | | | Orthogonal contrasts | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0800 | 1000 | 1200 | 1400 | 1600 | 1800 | Mean | SEM | Linear | Quadratic | Cubic |
| Forage diet | | | | | | | | | | | |
| Day 1 | 1.11 | 1.11 | 1.22 | 1.36 | 1.27 | 1.21 | 1.21 | 0.06 | 0.02 | 0.03 | 0.10 |
| Day 2 | 1.10 | 0.99 | 1.20 | 1.10 | 0.91 | 0.83 | 1.02 | 0.06 | 0.01 | 0.01 | 0.70 |
| Day 3 | 1.04 | 1.01 | 1.09 | 0.99 | 0.87 | 0.95 | 0.99 | 0.09 | 0.18 | 0.81 | 0.41 |
| Day 4 | 1.07 | 1.01 | 1.21 | 1.35 | 1.38 | 1.17 | 1.20 | 0.08 | 0.01 | 0.03 | 0.01 |
| Day 5 | 1.23 | 1.31 | 1.33 | 1.22 | 1.21 | 1.15 | 1.24 | 0.09 | 0.20 | 0.26 | 0.44 |
| Finishing diet | | | | | | | | | | | |
| Day 1 | 1.20 | 1.17 | 1.20 | 1.29 | 1.35 | 1.36 | 1.26 | 0.05 | 0.01 | 0.14 | 0.02 |
| Day 2 | 1.16 | 1.16 | 1.18 | 1.23 | 1.27 | 1.29 | 1.22 | 0.06 | 0.01 | 0.43 | 0.33 |
| Day 3 | 1.10 | 1.06 | 1.04 | 1.04 | 1.09 | 1.11 | 1.07 | 0.06 | 0.67 | 0.12 | 0.75 |
| Day 4 | 1.00 | 1.06 | 0.96 | 1.05 | 1.07 | 1.13 | 1.05 | 0.08 | 0.01 | 0.05 | 0.41 |
| Day 5 | 1.13 | 1.11 | 1.11 | 1.11 | 1.11 | 1.12 | 1.12 | 0.07 | 0.91 | 0.41 | 0.80 |

was stabilized. The current data were analyzed to evaluate if there was a common trend in UC (mg/kg BW) excretion and PD:C across times of collection within and across experimental days. If common trends were detected, then the variability in the excretion of each of these components could be accounted for by collecting urine samples at specific times of day. However, there were no common trends in UC (mg/kg BW) excretion or PD:C.

The current results do demonstrate that there was significant variation in UC excretion and PD:C within and across days as predicted by 2-h interval samples. However, Butcher and Harris (1957) also found significant diurnal variation in creatinine and creatinine to N ratios, but when morning and evening samples were composited, they had a similar ratio as total collections. Collecting multiple spot samples within a day may not be feasible in most production settings; however, collecting for multiple days may be practical.

When expressed as mg/kg BW, UC output was not different ($P = 0.40$; Figure 3) across experimental animals fed the forage diet. Mean UC excretion was 30.04 mg/kg BW. Additionally, the PD:C was not different ($P = 0.71$) across experimental animals (Figure 3). When expressed as mg/kg BW, UC output was not different ($P = 0.18$) across animals fed the finishing diet (Figure 4). Conversely, PD:C was different ($P < 0.01$) across animals fed the finishing diet (Figure 4).

Research concerning the variation in creatinine excretion across animals is somewhat conflicting. When Folin (1905) first evaluated UC excretion, he noted significant variation between individuals. More recently, Jardstedt et al. (2017) reported no differences in UC excretion due to diet (19.7 mg/kg BW), but relatively high animal to animal variation (12.0

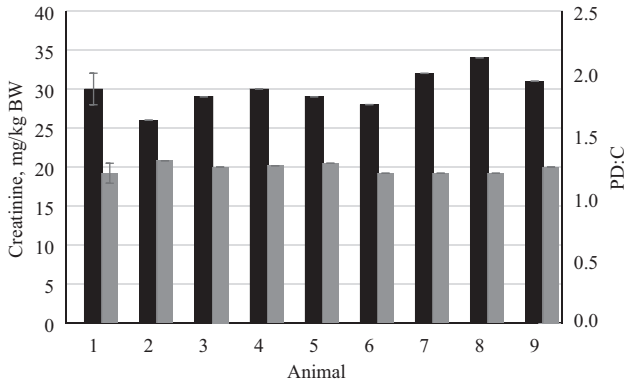


**Figure 3.** Animal variation in urinary creatinine excretion (mg/kg body weight) and the ratio of molar concentrations of PD:C while heifers were fed a hay diet supplemented with dried distillers grains (trial 5). Urinary creatinine excretion (mg/kg body weight) shown in solid black bars following left vertical axis (SEM = 2.02; main effect of animal $P = 0.40$). Ratio of PD:C shown in gray bars following right vertical axis (SEM = 0.08; main effect of animal $P = 0.71$).

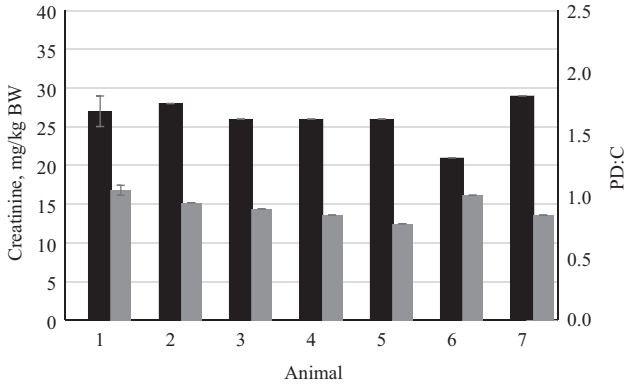


**Figure 4.** Animal variation in urinary creatinine excretion (mg/kg body weight) and the ratio of molar concentrations of PD:C while heifers were fed a finishing diet (trial 5). Urinary creatinine excretion (mg/kg body weight) shown in solid black bars following left vertical axis (SEM = 1.97; main effect of animal $P = 0.18$). Ratio of PD:C shown in gray bars following right vertical axis (SEM = 0.04; main effect of animal $P = 0.002$).

to 32.5 mg/kg BW) using 48 h total urine collection. Albin and Clanton (1966) reported significant animal variation in gestating cows but not in non-gestating cows. Excretion of UC (mg/kg BW) measured in this trial was relatively stable across experimental animals with a mean excretion of 30.05 and 26.21 mg/kg BW for forage and finishing diets, respectively.

Influence of age, diet, and pregnancy on UC excretion is minimal; therefore, creatinine has promise as a marker of urine output and prediction of PD excretion. Variation in excretion of both UC and PD indicates that multiple days of collection are required for an accurate estimate of PD:C. Further efforts in evaluating PD excretion should focus on relationships between spot urine samples and total collections and the ability of spot samples collected on multiple days to estimate average PD excretion. Variation in UC and PD excretion across animals was minimal.

## LITERATURE CITED


Albin, R. C., and D. C. Clanton. 1966. Factors contributing to the variation in urinary creatinine and creatinine-nitrogen ratios in beef cattle. J. Anim. Sci. 25:107–112. doi:10.2527/jas1966.251107x

Anthony, R. V., R. A. Bellows, R. E. Short, R. B. Staigmiller, C. C. Kaltenbach, and T. G. Dunn. 1986. Fetal growth of beef calves. II. Effect of sire on prenatal development of the calf and related placental characteristics. J. Anim. Sci. 62:1375–1387. doi:10.2527/jas1986.6251375x

Best, W. R., W. J. Kulh, and T. E. Friedemann. 1952. Diurnal trend and variation of urinary creatinine excretion. Fed. Proc. 11:188.

Brody, S. 1945. Bioenergetics and growth. New York (NY): Reinhold Publishing Corporation.

Brunsvig, B. R., A. J. Smart, E. A. Bailey, C. L. Wright, E. E. Grings, and D. W. Brake. 2017. Effect of stocking density on performance, diet selection, total-tract digestion, and nitrogen balance among heifers grazing cool-season annual forages. J. Anim. Sci. 95:3513–3522. doi:10.2527/jas.2017.1563

Butcher, J. E., and L. E. Harris. 1957. Creatinine as an index material for evaluating ruminant nutrition. J. Anim. Sci. 16:1020.

Chen, X. B., G. Grubic, E. R. Ørskov, and P. Osuji. 1992. Effect of feeding frequency on diurnal variation in plasma and urinary purine derivatives in steers. Anim. Prod. 55:185–191. doi:10.1017/S0003356100037442

Dinning, J. S., W. D. Gallup, and H. M. Briggs. 1949. Excretion of creatinine and creatine by beef steers. J. Biol. Chem. 177:157–161.

Dórea, J. R. R., M. A. C. Danés, G. I. Zanton, and L. E. Armentano. 2017. Urinary purine derivatives as a tool to estimate dry matter intake in cattle: a meta-analysis. J. Dairy Sci. 100:8977–8994. doi:10.3168/jds.2017-12908

Erb, R. E., A. H. Surve, R. D. Randel, and H. A. Garverick. 1977. Urinary creatinine as an index of urinary excretion of estrogen in cows prepartum and postpartum. J. Dairy Sci. 60:1057–1063. doi:10.3168/jds.S0022-0302(77)83988-X

Folin, O. 1905. Laws governing the chemical composition of urine. Am. J. Physiol. 13:66–115.

Galyean, M. L., and L. O. Tedeschi. 2014. Predicting microbial protein synthesis in beef cattle: relationship to intakes of total digestible nutrients and crude protein. J. Anim. Sci. 92:5099–5111. doi:10.2527/jas.2014-8098

Gopinath, R., and W. D. Kitts. 1984. Growth, N tau-methylhistidine excretion and muscle protein degradation in growing beef steers. J. Anim. Sci. 59:1262–1269. doi:10.2527/jas1984.5951262x

Hayden, J. M., W. G. Bergen, and R. A. Merkel. 1992. Skeletal muscle protein metabolism and serum growth hormone, insulin, and cortisol concentrations in growing steers implanted with estradiol-17 beta, trenbolone acetate, or estradiol-17 beta plus trenbolone acetate. J. Anim. Sci. 70:2109–2119. doi:10.2527/1992.7072109x

Jardstedt, M., A. Hessle, P. Nørgaard, W. Richardt, and E. Nadeau. 2017. Feed intake and urinary excretion of nitrogen and purine derivatives in pregnant suckler cows fed alternative roughage-based diets. Livestock Sci. 202:82–88. doi:10.1016/j.livsci.2017.05.026

Lofgreen, G. P., and W. N. Garrett. 1954. Creatinine excretion and specific gravity as related to the composition of the 9, 10, 11th rib cut of Hereford steers. J. Anim. Sci. 30:496–500.

MacDonald, J. C., T. J. Klopfenstein, G. E. Erickson, and W. A. Griffin. 2007. Effects of dried distillers grains and equivalent undegradable intake protein or ether extract on performance and forage intake of heifers grazing smooth bromegrass pastures. J. Anim. Sci. 85:2614–2624. doi:10.2527/jas.2006-560

McCarthy, F. D., W. G. Bergen, and D. R. Hawkins. 1983. Muscle protein turnover in cattle of differing genetic backgrounds as measured by urinary N tau-methylhistidine excretion. J. Nutr. 113:2455–2463. doi:10.1093/jn/113.12.2455

McDonald, R. A. 2003. Estimating microbial protein flow in growing and finishing heifers. Ph.D. Diss. Lincoln (NE): Univ. of Nebraska.

National Research Council. 1996. Nutrient requirements of beef cattle. Washington (DC): National Academy Press.

Ørskov, E. R., and N. A. MacLeod. 1982. The determination of the minimal nitrogen excretion in steers and dairy cows and its physiological and practical implications. Br. J. Nutr. 47:625–626. doi:10.1079/BJN19820074

Patterson, H. H., D. C. Adams, T. J. Klopfenstein, and G. P. Lardy. 2006. Application of the 1996 NRC to protein and energy nutrition of range cattle. Prof. Anim. Sci. 22:307–317. doi:10.15232/S1080-7446(15)31113-X

Picón-Reátegui, E. 1962. Creatinine excretion and body composition. Am. J. Clin. Nutr. 10:128–133. doi:10.1093/ajcn/10.2.128

Shingfield, K. J., and N. W. Offer. 1999a. Evaluation of milk allantoin excretion as an index of microbial protein supply in dairy cows. Anim. Sci. 67:371–385. doi:10.1017/S135772980003277X

Shingfield, K. J., and N. W. Offer. 1999b. Simultaneous determination of purine metabolites, creatinine and pseudouridine in ruminant urine by reversed-phase high-performance

liquid chromatography. J. Chromatogr. B. Biomed. Sci. Appl. 723:81–94. doi:10.1016/S0378-4347(98)00549-0

Swartz, J. E., D. W. Brake, E. E. Grings, E. A. Nelson, C. L. Wright, J. A. Walker, E. J. Bloom, and G. A. Perry. 2016. Effects of enzymatically hydrolyzed yeast supplementation and supplementation frequency on nitrogen balance and apparent diet digestibility in periparturient beef cows. J. Anim. Sci. 94(Suppl. 2):362.

Valadares, R. F., G. A. Broderick, S. C. Valadares Filho, and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82:2686–2696. doi:10.3168/jds.S0022-0302(99)75525-6

Van Niekerk, B. D., J. T. Reid, A. Bensadoun, and O. L. Paladines. 1963. Urinary creatine as an index of body composition. J. Nutr. 79:463–473. doi:10.1093/jn/79.4.463