EFFECT OF DIETARY PROTEIN INTAKE ON CREATININE EXCRETION AND THE CREATININE-NITROGEN RATIO IN BOVINE URINE

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AN easily measured urine component which would reflect nitrogen status of an animal would be valuable. Creatinine was suggested as an index material by Folin (1905a). A close relationship of creatine with protein metabolism was postulated by Chanutin (1926) and confirmed by Beard (1943) and Friedemann et al. (1948). However, Folin (1905b) and Hunter (1922) indicated creatinine is independent of dietary protein levels.

Creatinine excretion apparently is closely correlated with muscle mass when no changes occur in dietary creatine or creatinine (Hobson, 1939); Powell et al., 1961).

Butcher and Harris (1956) illustrated the dependence of urinary nitrogen in contrast to the independence of urinary creatinine upon level of protein intake. As a result, total daily excretion of urinary nitrogen and the urinary creatinine-nitrogen ratio were inversely associated with dietary protein when energy intake was relatively constant.

The objective of this experiment was to study the effects of varying nitrogen intake and the relationship of diurnal variation, total body weight, and total urine volume to urinary creatinine, urinary nitrogen, and their ratio on a relatively constant energy ration. The relationships of total urinary nitrogen, nitrogen intake, and urinary urea nitrogen were also investigated.

Materials and Methods

Animals used were divided into three groups of two lactating Guernsey cows each selected to minimize age, stage of lactation and milk production differences. Housing was in a tie-stall barn. Three treatment groups received either a low (treatment 1—8.9%), or high (treatment 3—14.2%) crude protein grain ration. The amount of grain fed was in accordance with National Research Council (1966) recommendation based on energy requirements. Alfalfa hay (18.4% crude protein and 20.7% crude fiber) and corn silage (3.5% crude protein and 8.5% crude fiber) were individually fed at the daily rate of 6.8 and 13.6 kg, respectively and refusals were weighed back at each feeding to determine intake. Animals were allowed 2 weeks adjustment followed by three, 3-week trial periods. Samples were taken at the end of each 3-week period.

Samples obtained for analyses were: grain, hay and silage for nitrogen, moisture, crude fiber, ether extract and ash; and urine for creatinine, urea nitrogen and total urinary nitrogen. Body weights were recorded at each sampling time. Urine samples were taken every 4 hr. over a 24-hr. period at the end of each 3-week period using the manual stimulation technique of Ashworth and Brody (1933). Samples of natural micturition other than those occurring at the 4-hr. intervals were also collected. The volume of urine and time sampled were recorded. Analyses were made on an individual basis.

Silage energy was calculated by the formula: (75.97 - 0.85 crude fiber) × dry matter, hay energy by the formula: (56.13 - 0.65 crude fiber) × dry matter and grain energy by calculating TDN according to Moore, Irvin and Shaw (1953) in which 1 lb TDN equals 1 megacalorie NE. Urinary urea nitrogen was determined by the diacetyl monoxime method of Friedmann (1953) following a 1:50 dilution of the urine sample. Creatinine was determined by using the Jaffe reaction as described by Hawk, Oser and Summerson (1954). Urine and blood samples were frozen after sampling until analyzed.

The data were evaluated statistically according to Snedecor and Cochran (1967) by analysis of variance, Duncan’s (1955) New Multiple Range Test (DNMRT), and linear regression correlations.
TABLE 1. TREATMENT EFFECTS ON CREATININE, NITROGEN AND CREATININE: NITROGEN*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% Protein</th>
<th>Crea-</th>
<th>Nitrogen</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. mg/ml</td>
<td>mg/ml</td>
<td>ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (low)</td>
<td>8.9</td>
<td>0.518</td>
<td>7.68</td>
<td>1636</td>
</tr>
<tr>
<td>2 (medium)</td>
<td>11.3</td>
<td>0.411</td>
<td>6.85</td>
<td>1391</td>
</tr>
<tr>
<td>3 (high)</td>
<td>14.2</td>
<td>0.555</td>
<td>10.14</td>
<td>1672</td>
</tr>
</tbody>
</table>

*Means within a given column having a common letter in their superscripts are statistically different at P<.05.

Results

Data presented in table 1 show significant treatment effects on creatinine concentration (P<.01), nitrogen concentration (P<.005), and their ratio (P<.025), but not on urine volume. Significant differences (P<.05) were found by DNMRT between treatment 2 and treatments 1 and 3 for creatinine, between treatment 3 and treatments 1 and 2 for nitrogen, and between treatment 3 and treatment 1 for the creatinine-nitrogen ratio.

Diurnal variation data show significant differences in creatinine concentration (P<.025), nitrogen concentration (P<.01), urine volume (P<.05) and creatinine-nitrogen ratio (P<.005) as shown in table 2. Significant differences (P<.05) were indicated by DNMRT among means at 12:00 pm (midnight) and 4:00 am in comparison with those at 4:00 pm and 12:00 am on creatinine; among means at 12:00 pm and 4:00 am in comparison with those at 8:00 am and 12:00 am on nitrogen; between the mean at 8:00 am and those at 4:00 pm, 8:00 pm, 12:00 pm, and 4:00 am on urine volume; and between the mean at 4:00 pm and the other five means for the creatinine-nitrogen ratio (table 2). The inverse relationship of urine volume with respect to nitrogen and creatinine concentrations was most evident at 4:00 am when urine volume was near its peak and creatinine and nitrogen concentrations were near their lowest levels. A ratio equivalent to the average creatinine-nitrogen ratio (.0601 as computed on concentration values) occurred shortly after midnight (urine volume of 1650 ml, nitrogen concentration of 6.9 mg/ml, and a creatinine concentration of .415 mg/ml). Figure 1 depicts the relationship of amounts of urinary creatinine, nitrogen, and their ratio based on concentration values in table 2.

The creatinine-nitrogen ratio was significantly different (P<.05) in experimental period 1 (.0512) from those observed in experimental periods 2 (.0622) and 3 (.0666) with an overall period significance of P<.005. No other data showed a significant difference (P<.05) among periods or interaction of diurnal variation with treatments.

Total creatinine, total nitrogen, total urine and creatinine coefficient

\[
\frac{\text{mg creatinine nitrogen}}{\text{kg body weight}}
\]

showed no significant difference (P<.05) with respect to treatment, experimental period, or interaction of treatments with experimental

![Figure 1](image-url)
TABLE 3. DAILY NITROGEN INTAKE VS. REQUIREMENT BY TREATMENTS AND PERIODS

<table>
<thead>
<tr>
<th>Treatment 1</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N-intake Required</td>
<td>N-intake Required</td>
<td>N-intake Required</td>
<td>N-intake Required</td>
</tr>
<tr>
<td>1 (low)</td>
<td>615 g 24.4%</td>
<td>634 g 40.4%</td>
<td>608 g 30.2%</td>
<td>608 g 31.4%</td>
</tr>
<tr>
<td>2 (medium)</td>
<td>753 g 23.9%</td>
<td>729 g 27.2%</td>
<td>708 g 27.6%</td>
<td>708 g 26.1%</td>
</tr>
<tr>
<td>3 (high)</td>
<td>559 g 29.8%</td>
<td>647 g 57.5%</td>
<td>603 g 52.1%</td>
<td>603 g 46.2%</td>
</tr>
</tbody>
</table>

1 Two animals per treatment per period.
2 N-intake reflects differences in levels of milk production, milk composition and body maintenance.

periods. However, nitrogen intake above that required is reflected in the amount of nitrogen excreted by treatment groups. The average daily excretion for all animals was 12,631 ml of urine, 4.01 g of creatinine, and 65.72 g of nitrogen. This resulted in a creatinine-nitrogen ratio of 0.0610 on a total daily basis.

Daily nitrogen intake per treatment and its percentage in excess of daily nitrogen required are presented in Table 3. Daily nitrogen requirements are based on N.R.C. (1966) recommendations for maintenance and milk production. Values for each treatment are averages for the two animals. Treatment 3 shows the largest excess of nitrogen intake above requirement (46.2%) due to the high protein (14.2%) grain ration. Treatment 2 excess above nitrogen requirement (26.1%) is less than treatment 1 (31.4%) because of a decreased voluntary daily intake by animals in treatment 2.

Correlations showed the following significant (P<.005) relationships: nitrogen intake with total urinary nitrogen, .584; nitrogen intake with urinary urea nitrogen, .505; nitrogen requirement with total urinary nitrogen, .634; and urinary urea nitrogen with total urinary nitrogen, .465.

Discussion

As shown in Table 1, urinary creatinine and nitrogen concentrations were lower in treatment 2 than in treatments 1 and 3. However, as is evident in Table 3, when animals were subjected to treatment 2, less nitrogen was consumed as a percent of requirement. This resulted from a decreased daily net feed intake (91.0, 80.0 and 92.4% of silage, hay and grain fed) compared with treatment 1 (98.1, 83.0, and 95.3% of silage, hay and grain fed) which shows greater creatinine and nitrogen concentrations (Table 1). Therefore, considering data in Tables 1 and 3, increased nitrogen intake does significantly increase creatinine (P<.01) and nitrogen concentrations (P<.005) in urine. The effect of nitrogen intake on the creatinine-nitrogen ratio does not follow the same pattern as nitrogen intake expressed as a percent of requirement. The treatment 3 ratio (.0533) is smallest and significantly different (P<.05) from treatment 1 ratio (.0668). Although not significant, treatment 2 ratio is smaller (.0599) than 1 (.0668). This appears to be due to a smaller change in nitrogen (12.1%) than creatinine (26.0%) concentration in treatment 1 compared with treatment 2.

Total urinary creatinine and nitrogen were not significantly (P>.05) affected by treatment, period, or interaction. Total nitrogen more closely reflected nitrogen intake above requirement than did total creatinine (Table 4). Albin and Clanton (1966) reported that creatinine did not fluctuate for individual animals on different protein and energy levels. Increased outputs of urinary creatinine resulted from abrupt, large increases in protein intake while decreased outputs resulted from starvation in a study by Van Niekerk et al. (1963). While total urinary nitrogen did not vary significantly in our study (possibly due to unaccounted nitrogen loss in feces and milk and possible nitrogen gain in body tissues), Albin and Clanton (1966) found that nitrogen intake increased, urinary nitrogen also increased (P<.01). Urinary nitrogen concentration was affected in this way but apparently differences in urine volume or nitrogen intake precluded a similar significant effect on total urinary nitrogen excreted. The total creatinine-nitrogen ratio followed a similar pattern as shown in Table 1 on a concentration basis.

Creatinine and nitrogen concentrations were both significantly (P<.05) lower at 12:00 pm and 4:00 am than at 12:00 am and 4:00 pm for creatinine and 8:00 am and 12:00 am for nitrogen. This may be partially attributed to a higher metabolic activity near midday, and
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also to the possible inverse relationship with urine volume as shown in table 2. The smallest urine volume (1130 ml) occurred at 8:00 am and corresponds to morning milking which preceded the morning feeding. The largest creatinine-nitrogen ratio and highest creatinine concentration occurred during the 4-hr. period ending at 4:00 pm when muscular activity of the animals was likely the greatest. This was expected since most of the creatine in the body (98%) is in skeletal muscle. However, Mitchell and Kruger (1928) indicated that increased muscle catabolism which may be associated with increased creatinine excretion, is not an inevitable consequence of increased muscular activity provided sufficient dietary energy is present for muscular activity. Since administration of creatinine increased the creatine content of muscle, Myers and Fine (1913) concluded that the reaction between creatine and creatinine is reversible. However, subsequent studies by Rose, D嘀 Mitt and Cheatham (1916), Block and Schoenheimer (1939), and Block, Schoenheimer and Rittenberg (1941) have demonstrated that the reaction which changes creatine to creatinine is irreversible. Of this creatine, about 60% is present as phospho-creatine. Moreover, as noted by Borsook and Dubnoff (1947), the conversion of phospho-creatine to creatinine and inorganic phosphate, and the conversion of creatine to creatinine can account for all creatinine excretion in animal species except birds.

The creatinine coefficient, termed "an index of the concentration of creatine in the muscles or body" by Dinning, Gallup and Briggs (1949) did not vary significantly (P<.05) throughout the study (table 4). This would be expected since body weight (average percent change during study 2.05%) and total creatinine were relatively constant for all animals during the study. DeGroot and Aafjes (1960) reported that an increase in body weight will usually cause an increase in creatinine excretion and according to Barakat and Abdalla (1961) a decrease in the creatinine coefficient due to a proportionately greater change in body weight than in the excretion of creatinine.

Table 2 depicts the significant (P<.05) diurnal variations in creatinine and nitrogen concentrations. Significant diurnal variations in the excretion of urinary nitrogen, and creatinine and in the urinary creatinine-nitrogen ratio were reported by Butcher and Harris (1957). Albin and Clanton (1966) did not observe significant diurnal variations although a diurnal pattern was evident. In their study urinary nitrogen concentration followed a pattern similar to that of creatinine but showed greater variation. Best, Kuhl and Friedemann (1952) found the highest creatinine excretion rate between 2:00 and 10:00 pm; whereas, in this study it was between 12:00 noon and 4:00 pm based on concentration (table 2) or between 4:00 and 8:00 pm based on total amount excreted (figure 1). Additionally, Best et al. (1952) reported that the 6:00 to 10:00 am period more closely reflected the 24-hr. rate; whereas, in this study the 8:00 pm to 12:00 pm period on concentration or total basis was a more accurate reflection of daily excretion.

Nitrogen requirement was found to be more closely correlated (.634) with total urinary nitrogen than was nitrogen intake (.584). This may indicate that excessive nitrogen intake above requirement was accounted for largely by gain in nitrogen in milk or loss of nitrogen in feces. The significant correlation of urinary urea nitrogen with total urinary nitrogen (.465) indicates that urinary urea nitrogen is a significant fraction of total urinary nitrogen. From the above correlations, it would be expected that nitrogen intake would be significantly reflected in urinary urea nitrogen (.505).

Due to diurnal and treatment differences, it is unlikely that a sample of urine collected at any given time of day would yield a creatinine-nitrogen ratio indicative of nitrogen intake. If a total urine collection is made, the creatinine-nitrogen ratio may be a reliable in-

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**TABLE 4. TREATMENT EFFECTS ON CREATININE COEFFICIENT, AND TOTAL URINARY CREATININE, URINARY NITROGEN, URINE VOLUME AND CREATININE-NITROGEN RATIO**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Creatinine coefficient</th>
<th>Creatinine</th>
<th>Nitrogen</th>
<th>N-intake above required</th>
<th>Urine</th>
<th>Creatinine: nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>g</td>
<td>g</td>
<td>%</td>
<td>ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (low)</td>
<td>8.7</td>
<td>4.34</td>
<td>63.00</td>
<td>31.4</td>
<td>11,837</td>
<td>.0688</td>
</tr>
<tr>
<td>2 (medium)</td>
<td>8.3</td>
<td>3.82</td>
<td>62.81</td>
<td>26.1</td>
<td>13,943</td>
<td>.0607</td>
</tr>
<tr>
<td>3 (high)</td>
<td>7.9</td>
<td>3.87</td>
<td>71.34</td>
<td>46.2</td>
<td>12,112</td>
<td>.0542</td>
</tr>
<tr>
<td>S.D.</td>
<td>±2.2</td>
<td>±1.06</td>
<td>±12.43</td>
<td>....</td>
<td>±2,147</td>
<td>....</td>
</tr>
</tbody>
</table>
dicator of nitrogen intake provided nitrogen loss (gain) through other means is considered.

**Summary**

The effects of varying nitrogen intake and the relationship of diurnal variation to creatinine excretion and to the creatinine-nitrogen ratio in bovine urine were studied. Treatment levels were 8.9, 11.3 and 14.2% crude protein in grain mixtures, fed according to N.R.C. energy requirements, with constant amounts of alfalfa hay and corn silage. Diurnal variation was measured for 24 hr. (at 4-hr. intervals) during three experimental periods. Creatinine and nitrogen concentrations and their ratio were significantly affected by both level of nitrogen intake and diurnal variation. Nitrogen intake was reflected (according to amount consumed) in the magnitude of creatinine and nitrogen concentrations. Midday time periods resulted in highest and middle of the night time periods in the lowest concentrations of creatinine and nitrogen. Creatinine-nitrogen ratios based on concentration closely followed treatment levels and diurnal variations. Urine volume varied significantly during the 24-hr. periods and was inversely related to creatinine and nitrogen concentration. Total urinary creatinine and nitrogen, urine, and the creatinine coefficient were not significantly affected by treatment, period, or by their interaction. Correlations indicated significant relationships among nitrogen intakes and dietary requirements, urinary urea nitrogen, and total urinary nitrogen.

**Literature Cited**


Myers, V. C. and M. S. Fine. 1913. The influence of the administration of creatine and creatinine on the creatine content of muscle. J. Biol. Chem. 16:169.


