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Uso da creatinina urinária como marcador nutricional e de volume urinário em ovinos alimentados com forragem tropical ou temperada

[*Urinary creatinine as a nutritional and urinary volume marker in sheep fed with tropical or temperate forages*]

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ABSTRACT

To test the accuracy of creatinine as a marker for estimating urinary volume and its use as a nutritional index, the possible interference of forage intake and forage quality over creatinine excretion was evaluated. For this, sheep were fed different levels of pearl millet (*Pennisetum americanum* (L) Leeke) or Italian ryegrass (*Lolium multiflorum* Lam). The experiment consisted of a compilation of digestibility trials (n=6) with pearl millet or Italian ryegrass in completely randomized designs with four replications and four forage levels: 1.5, 2.0, 2.5% (kg dry matter (DM)/ 100 kg of live weight (LW)). The trials were repeated at different periods to evaluate how stable the average metabolic excretion of creatinine is. In each trial, total urine collection was performed individually during a period of 24 hours for five consecutive days and subsequently analyzed by colorimetry for creatinine and purine derivatives. The creatinine excretion was not affected (P>0.05) by forage offer or forage type, but there were period effects (P=0.0001). The average creatinine excretion for both forages was 0.21mmol/kg $PV^{0,75}$. Linear regressions between the purine derivatives:creatinine index with total excretion of purine derivatives were detected for pearl millet (P<0.0001, R^2= 0.64) and Italian ryegrass (P=0.02, R^2=0.20). These results demonstrate that creatinine excretion is independent of the type and availability of forage and can be a marker for urinary volume prediction and nutritional measures under grazing systems.

Keywords: digestible organic matter intake, microbial protein synthesis, urine

RESUMO

Para testar a precisão da creatinina como marcador para estimativas de volume urinário e índice nutricional, foram avaliadas a possível influência do consumo e a qualidade da forragem sobre esse marcador. Para isso, ovinos foram alimentados com diferentes níveis de milheto (Pennisetum americanum *(L) Leeke*) *ou azevém* (Lolium multiflorum *Lam). O experimento consistiu de uma compilação de ensaios de digestibilidade (n=6) com milheto ou azevém, em um desenho experimental de blocos completamente ao acaso, com quatro repetições e quatro níveis de forragem: 1,5; 2,0; 2,5% (kg de matéria seca (MS)/ 100kg de peso vivo (PV)). Os ensaios foram repetidos em diferentes períodos, com ambas as forragens, se para avaliar a estabilidade da excreção média de creatinina metabólica. Em cada ensaio, foi coletado o volume total de urina individualmente, durante períodos de 24 horas, por cinco dias consecutivos. Posteriormente, esses ensaios foram analisados por colorimetria para creatinina e derivados de purina. A excreção de creatinina não foi afetada (P>0,05) pelo consumo de forragem ou pelo tipo de forragem, mas foi influenciada pelo período (P=0,0001). A excreção média de creatinina para ambas as forragens foi 0,21mmol/kg* $PV^{0,75}$*. Regressões lineares entre os índices derivados de purina:creatinina com a excreção total de derivados de purina foram detectadas para milheto (P<0,0001; R^2=0,64) e azevém (P=0,02; R^2=0,20). Os resultados demonstraram que a excreção de creatinina é independente do tipo e do consumo de forragem e pode ser usada como marcador preditivo do volume urinário e do* status *nutricional em sistemas de pastejo.*

Palavras-chave: consumo de matéria orgânica digestível, síntese de proteína microbiana, urina

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INTRODUCTION

The quantification of total urine excretion is essential to describe processes such as nitrogen balance and energy intake, as well as estimates of microbial protein synthesis (Kozloski *et al*., 2005). However, under grazing conditions, the quantification of urinary excretion is a very laborious process and most of the time it is not practical. Indirect methods to estimate the total urinary excretion have been suggested, such as the use of urinary creatinine (Faichney *et al*., 1995; Chen *et al*., 2004) as an alternative method of total urine collection.

Creatinine is a metabolite formed in the muscle by water removal from creatine phosphate, originating in cellular metabolism (Harper *et al*., 1982). Because creatinine is produced daily and is excreted steadily per kilogram of muscle mass of the animal, it is the most widely used marker for estimating the daily excretion of urine. Furthermore, its use has not been seen as being influenced by nutrient intake and diet composition (Valadares Filho *et al*., 2007).

On the other hand, Liu and McMeniman (2006) showed that variations according to the quality of the diet can be found, and the average daily creatinine excretion by sheep are around 10.7 mg/kg of live weight, with values ranging between 5.0 and 13.6mg/kg of live weight. Other authors (Ørskov and MacLeod, 1982; Hovell *et al*., 1983; Hovell *et al*., 1987) found a relationship between energy intake and creatinine excretion, but the explanation and reason for these observations are still not clear.

Therefore, the aim of this study was to determine whether variations in forage intake and type of forage interfere in sheep urinary creatinine excretion, as well as to examine the use of creatinine as a marker for urinary volume and nutritional index for grazing systems.

MATERIAL AND METHODS

Data from six digestibility trials conducted between the years 2010 and 2011 were compiled. Assays were carried out at the Federal University of Rio Grande do Sul, RS - Brazil. All assays were similar in their experimental design and protocol, varying only in the type/quality of forage and animal group (period). Each digestibility trial was composed of 16 animals randomly allocated to four treatments represented by four levels of herbage allowance (kg dry matter (DM)/100 kg of live weight (LW)): 1.5; 2.0; 2.5 and ad libitum. Each digestibility trial was completely randomized with four treatments and four replicates (animals) per treatment.

The animals used in the trials were crossbred Texel x Ile de France lambs, with average weight of 29.95±5.26kg and around a year old. The animals were fed in the morning (09:00h) and afternoon (18:00h), and the pasture was cut just before animal feeding. We used two forage species, temperate grass: Italian ryegrass (*Lolium* multiflorum Lam) and other tropical grass: pearl millet (*Pennisetum* americanum (L.) Leeke). In experiments with Italian ryegrass the upper half of the plants were cut to simulate the material that would be selected by the grazing animals, while with pearl millet only the leaves were cut due to the large difference in quality between the leaf and stem and the probable selection for green leaves by grazing sheep under non-limiting herbage allowances. For the ad libitum treatment a level of at least 20% of the daily orts was established. The ranges of chemical and morphological composition from the forages used on the digestibility trials can be seen on Table 1.

The experiments were structured as conventional digestibility trials in metabolism cages, with a 10 day adaptation phase and five more days for feces and forage orts collection (Rymer, 2000). Samples of total feces, forage offered and refusals were taken daily. These were dried in an oven with forced air at 55ºC for 72 hours. Subsequently, samples of feces, forage offered and forage orts during the five days were pooled and ground for subsequent laboratory analysis.

Dry matter was determined by drying the material in an oven at 105º C for 12 hours (Easley *et al*., 1965), organic matter by burning in a muffle furnace at 550°C (AOAC methods in. 22,010, and no. 7,010, 1975), and nitrogen (N) by the Kjeldahl method (AOAC methods no.2,036, 1960, no.2049, 1975). The calculation of forage intake was performed by the difference between the forage offered and refusals. Forage digestibility was calculated as the difference between the forage intake and excreted feces, divided by forage intake.

Table 1. Chemical composition and morphological composition of the pearl millet and Italian ryegrass fed to sheep in indoor trials

Parameters	Pearl millet		Italian ryegrass	
	Mean	Range	Mean	Range
Dry Matter (g/kg)	128	119–136	227	170-283
OM[1] (g/kg DM)	890	876–901	915	897-932
CP[2] (g/kg OM)	229	217–241	201	146-256
NDFacp[3] (g/kg OM)	548	500–596	493	418-568
Leaf blades (g/kg DM)			396	175-617
Stem + sheath (g/kg DM)			321	304-338
Seed head (g/kg DM)			283	78-486

[1]OM= organic matter; [2]CP = crude protein; [3]NDFacp = neutral detergent fibre corrected to ash and crude protein.

For urine collection each animal was fitted with an abdominal urine collector to avoid contact with the feces. Urine was conveyed to a plastic container with a lid containing 100mL of 20% sulfuric acid. After 24h, the total urine collected was measured, homogenized and 1% of the urine was withdrawn and diluted three times with distilled water to prevent possible crystallization (Chen and Gomes, 1995). The samples were adjusted to pH values below three using 20% sulfuric acid to prevent destruction of the bacterial purine bases and uric acid precipitation. This was frozen at -20 °C until analysis. Urine samples were collected from only 84 animals of the 96 used in the experiments. Those excluded were due to problems with abdominal collectors.

Urinary creatinine was determined by the colorimetric reaction with endpoint in alkaline picrate solution, using commercial kits (Ref: 35, Labtest, Lagoa Santa, MG, Brazil), while the purine derivatives (PD) were determined by colorimetric analysis of allantoin and uric acid as described by Chen and Gomes (1995). Uric acid was determined using a commercial kit (Ref: 73, Labtest, Lagoa Santa, MG, Brazil), xanthine and hypoxanthine are converted to uric acid with xanthine oxidase (Ref: x1875, Sigma-Aldrich Co.).

For estimates of food intake and intestinal flow of microbial purines a purine derivatives:creatinine index (PDC) was calculated. This index was calculated from the urinary concentrations (mmol/L) and creatinine purine derivatives multiplied by metabolic weight as described by Chen *et al*. (2004):

$$PDC\ index = \frac{PD}{Creatinine}\ x\ LW^{0,75}$$

Where creatinine and purine derivatives (PD) are expressed in mmol/L and LW is the liveweight of the animal (kg).

Statistical analysis was performed using SAS version 9.2 statistical package (SAS Institute Inc., Cary, NC, USA). The analysis of the experiment was performed by PROC GLM taking a completely randomized experimental design with four treatments (offers) and four replicates per treatment. The effect of periods (experiments) within the same forage was also tested as a main effect. The comparison of means was performed by Tukey test at a 5% error probability. The regression between the PDC index and intake of digestible organic matter were evaluated by linear regression using the PROC REG procedure of SAS. Cook's distance was calculated, excluding the data of those animals that showed a daily excretion of creatinine per kilogram metabolic weight that was very different from the average of the assay within each level of fodder supply.

RESULTS

Differences for forage intake (Table 2) between the levels of both offers were seen in the experiments with both millet (P<0.0001) and ryegrass (P=0.0079).

In animals fed millet, urinary volume (Uv) and daily excretion of purine derivatives followed the same pattern of response as dry matter intake (DMI), increasing with the increase of forage supply. However, with ryegrass, this behavioral pattern was not statistically different between treatments for Uv (P=0.18) and PD (P=0.21). The average volume of urine was higher for millet than ryegrass (2723 vs 2211 ml; Table 3) but lower for the daily excretion of purine derivatives (13.10 versus 18.71 mmol/day), respectively.

Table 2. Urine volume and urinary excretion of purine derivatives and creatinine by sheep fed different allowances of pearl millet and Italian ryegrass

Parameters	Forage allowance (%LW)				SE[2]	Main effects (Pr>F)	
	1.5	2.0	2.5	AL[1]		Period	Treatment
Pearl millet							
DMI[3] (g/day)	345c	444bc	578b	878a	33.72	0.01	<0.0001
Uv[4] (ml/day)	2042b	2231b	2879ab	3696a	153.01	0.03	0.0001
PD[5] (mmol/d)	8.02b	9.88b	14.07ab	20.11a	1.07	0.002	<0.0001
Creatinine							
mmol/L	1.32	1.26	1.07	0.90	0.07	0.09	0.1529
mmol/day	2.50	2.79	2.97	2.99	0.15	0.02	0.7052
mmol/kg $LW^{0,75}$	0.20	0.21	0.22	0.24	0.01	0.13	0.6615
Italian ryegrass							
DMI[3] (g/day)	458b	556ab	731a	759a	39.57	0.0350	0.0079
Uv[4](ml/day)	1897	2291	1856	2835	184.51	0.0005	0.1852
PD[5] (mmol/d)	14.43	12.85	16.28	30.61	3.34	0.87	0.2153
Creatinine							
mmol/L	1.63	0.94	1.41	0.96	0.15	0.94	0.1355
mmol/day	2.90	2.13	2.17	2.72	0.28	0.0026	0.6833
mmol/kg $LW^{0,75}$	0.23	0.16	0.16	0.21	0.02	0.0001	0.5285

[1]AL= *ad libitum*; [2]SE= standard error; [3]DMI= dry matter intake;[4]Uv= urine volume; [5]PD= purine derivatives
Averages in the same row followed by letters differ by the Tukey test at 5%.

Table 3. Urine volume and urinary excretion of purine derivatives and creatinine by sheep fed different forage types

Parameters	Pearl millet	Italian Ryegrass	Mean	SE[1]	Forage type (Pr>F)
DMI[2] (g/day)	565b	605a	578	26.20	<0.0001
Uv[3] (ml/day)	2723a	2211b	2563	122.04	<0.0001
PD[4] (mmol/d)	13.10b	18.71a	14.87	1.30	0.0011
Creatinine					
mmol/L	1.13b	1.29a	1.18	0.06	0.05
mmol/day	2.81	2.59	2.74	0.14	0.80
mmol/kg $LW^{0,75}$	0.22	0.20	0.21	0.01	0.66

[1]SE= standard error; [2]DMI= dry matter intake; [3]Uv= urine volume; [4]PD= purine derivatives
Averages in the same row followed by letters differ by the Tukey test at 5%.

The daily excretion of creatinine (mmol/kg $LW^{0.75}$) was not affected by the level of forage allowance or type of forage. However, it was not constant over time, varying significantly (P=0.0001) between the digestibility trials (periods) for ryegrass. On average for the two forages the daily creatinine excretion was 0.21mmol/kg $LW^{0.75}$.

The purine derivatives:creatinine index (PDC index) and digestible organic matter intake (DOMI, Fig. 1) were significant for both pearl millet (P<0.0001) and ryegrass (P=0.02) with estimates of DOMI closer to the actual for pearl millet (R^2=0.64) than for ryegrass (R^2=0.20). An inclination angle was also higher for millet (3.14) compared to ryegrass (1.23).

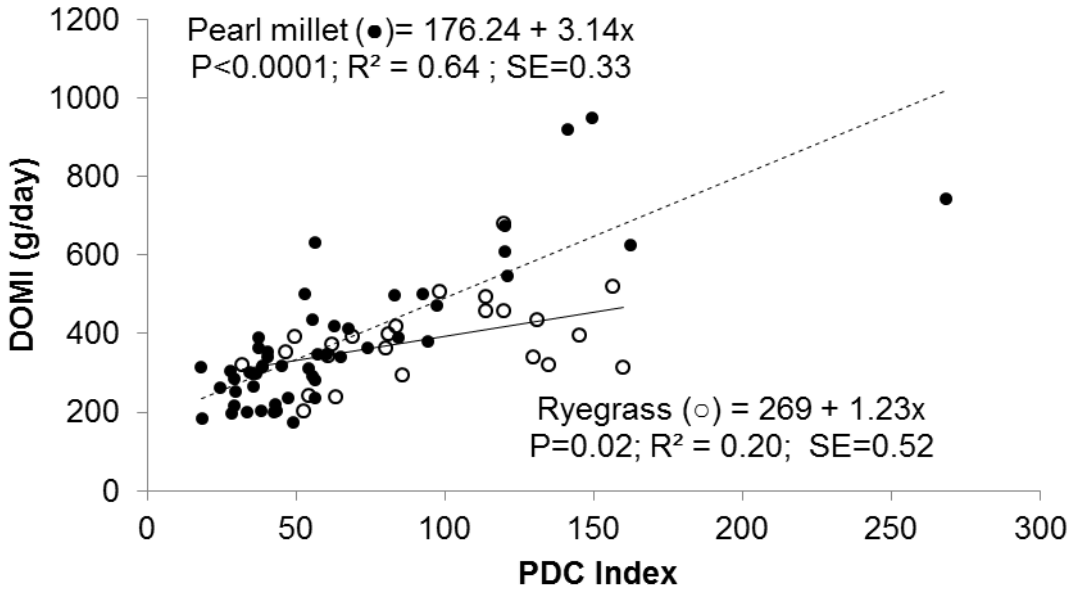

Figure 1. Relationship between the purine derivatives:creatinine index (PDC index) and digestible organic matter intake (DOMI) of sheep in different forage allowances of pearl millet (●) or Italian ryegrass (○).

DISCUSSION

The average excretion of creatinine (0.21mmol/kg/$LW^{0,75}$ or 9.79mg/kg LW) was close to that found by Yu *et al*. (2001) for sheep supplemented with different protein sources (0.20mmol/kg/$LW^{0,75}$), and reported by Liu and McMeniman (2006) for sheep fed different levels of maintenance requirements (9.26mg/kg LW). Reference values for sheep (0.46 to 0.52mmol/kg $LW^{0,75}$) described by Makkar (2004) for daily excretion of creatinine per unit of metabolic weight are well above those found in this study. However, differences between breeds and species are conceivable, mainly due to variations in body weight and proportion of muscle mass (George *et al*., 2006), suggesting the need for caution in generalizing the values.

Experiments with ruminal infusions of nutrients (short-chain fatty acids and protein) have shown a relationship between nutrient intake and excretion of creatinine (Ørskov and MacLeod, 1982; Hovell *et al*., 1983; Hovell *et al*., 1987). Liu and McMeniman (2006) suggest that these differences found between nutrient intake and creatinine excretion may be due to the type of diet provided by different mixtures and concentrations of nutrients infused ruminally rather than strictly by energy consumption. In the present study, however, it is pertinent to remember that two forages with distinct nutritional characteristics were tested, and yet no differences were detected due to forage intake or type of forage. This supports the hypothesis that creatinine excretion rate is constant and dependent solely on muscle mass and therefore proportional to animal weight (Koren, 2000) and it is also expected that the urine volume can be estimated directly from the relationship between the concentration of creatinine in the urine and the average daily excretion.

The PDC index is related to food intake flow and intestinal microbial purine and can thus be used as an indicator of intestinal microbial nitrogen flow (Valadares Filho *et al*., 2007). As such, these estimates must be constructed from the PDC index, by taking into account the animal live weight (Chen *et al*., 2004). The regressions performed, aiming to relate PDC index with DOMI, were significant ($P<0.05$), with coefficients of determination of 0.64 and 0.20 for pearl millet and ryegrass, respectively.

As suggested by Chen *et al*. (2004), from the PDC index, spot samples can be used to estimate microbial protein synthesis in sheep and to establish feeding strategies. However, there are suggestions that this relationship should be used

with caution, since the synthesis of purine derivatives is not constant and can vary considerably according to the type of diet (Makkar, 2004). This is due to differences in the efficiency of microbial protein synthesis due to the greater or lesser capacity of the diet to meet the nutritional requirements of rumen microorganisms. Different slopes for ryegrass and millet (Figure 1) suggest different rates of excretion of purine derivatives (directly related to rumen microbial protein) per unit of consumption of digestible organic matter. Thus, adopting the same PDC index for different forages can lead to under or over estimation of DOMI depending on the efficiency with which it is used in the synthesis of microbial protein.

CONCLUSIONS

Creatinine excretion is not affected by the level of forage allowance, nor the type of forage, suggesting the use of a single value for estimates of urinary volume. The digestible organic matter intake and excretion of purine derivatives present a relationship with the PDC index, and the same can be used as a nutritional indicator. But this index is dependent on the efficiency of microbial synthesis and therefore may vary between different forages.

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