



## Creatinine as a metabolic marker to estimate urinary volume in growing goats



Stefanie Alvarenga Santos<sup>a,\*</sup>, Luciana Louzada Prates<sup>b</sup>, Gleidson Giordano Pinto de Carvalho<sup>a</sup>, Antônio Carneiro Santana dos Santos<sup>a</sup>, Sebastião de Campos Valadares Filho<sup>c</sup>, Manuela Silva Libânio Tosto<sup>a</sup>, Lays Débora Silva Mariz<sup>a</sup>, Felipe da Silva Neri<sup>a</sup>, Mariana de Queiroz Sampaio<sup>a</sup>

<sup>a</sup> Universidade Federal da Bahia, 40170-110, Salvador, BA, Brazil

<sup>b</sup> University of Saskatchewan, S7N-5A2, Saskatchewan, SK, Canada

<sup>c</sup> Universidade Federal de Viçosa, 36570-000, Viçosa, MG, Brazil

### ARTICLE INFO

#### Keywords:

Boer  
Carcass  
Spot sample  
Urine

### ABSTRACT

The objectives of this study were to: (1) quantify the relationship between fasting body weight (FBW, kg) and urinary creatinine excretion (UCE, mg/d) in Boer goats; (2) evaluate the urinary volume estimates obtained from creatinine concentrations in the spot samples collected at different time points; (3) compare them with the 24-h observed urine volume. Thirty growing Boer goats ( $18 \pm 2.2$  kg initial BW) were distributed in a complete randomized design. Each collection period fell on 2 consecutive days and collector funnels were used. Spot samples were collected at 0, 4, and 8 h after morning feedings. These procedures were repeated in three runs 25 days apart to obtain different FBWs. All the samples were analyzed to quantify creatinine concentrations. The relationship between UCE and FBW was established by the following equations:  $UCE = 17.39 \times FBW$ ,  $r^2 = 0.96$ ,  $P < 0.01$ ,  $RSD = 40.8$ ;  $UCE = 23.50 \times FBW^{0.9059}$ ,  $R^2 = 0.96$ ,  $P < 0.01$ ,  $RSD = 40.9$ . The null hypothesis was accepted when predicted and observed values were compared ( $P > 0.05$ ). Thus both linear and allometric relationships can be used to predict UCE. The spot samples obtained at 4 h after feeding could be used to estimate urinary volume ( $P < 0.05$ ) instead of at 0 or 8 h. We conclude that UCE can be a metabolic marker to the estimate urinary volume of goats when calculated according to FBW with linear or allometric mathematical relationships.

### 1. Introduction

Experimental trials designed to study nitrogen or energy balance usually require access to 24-h urinary volumes. In general, the urinary urea must be accounted to determine metabolizable protein and energy requirements in ruminants. However, obtaining urine collections is time-consuming and impracticable in grazing animals or dairy goats because they move around pens intensely before daily milking.

An alternative to 24-h urine collections would be a spot sample, using urinary creatinine excretion (UCE) as a marker of urinary volume because its excretion according to fasting body weight (FBW) could be known (Chizzotti et al., 2008). This method is based on the premise that creatinine excretion in the daytime is relatively constant (Chen et al., 1995; Costa e Silva et al., 2014; Liu and McMeniman, 2006). Moreover, UCE is not affected by dietary protein levels or protein degradability (Broderick, 2003; Marini and Van Amburgh, 2003; Brake et al., 2010).

UCE in cattle is well-known, including genetic group differences, and can be estimated from the slope of the regression between FBW and UCE; e.g., 29.0 mg/kg FBW for Holstein cows (Valadares et al., 1999), and 14.4 mg/kg of muscle carcass for Nellore cattle (Costa e Silva et al., 2014). However, this variable has barely been studied recently in goats. Lindberg (1985) reported an UCE of 13.11 mg/kg FBW for male goat kids, and Yañez-Ruiz et al. (2004) reported one of 18.7 mg/kg FBW for adult goats. Moen and Del Giudice (1997) studied factors that affect creatinine ratios in six ruminant species, and proposed that UCE could lower by 30% when animals underwent nutritional restrictions. Yet differences between species have been attributed only to their different metabolic BW because body mass has been used to estimate UCE regardless of species.

Linear relationships between UCE and FBW have been proposed in cattle (Chizzotti et al., 2008; Valadares et al., 1999), as have allometric functions (Costa e Silva et al., 2014). As UCE is dependent on muscle

\* Corresponding author at: Universidade Federal da Bahia, Escola de Medicina Veterinária e Zootecnia, 40170-110, Salvador, BA, Brazil.

E-mail addresses: [stefanie.alvarenga@ufba.br](mailto:stefanie.alvarenga@ufba.br), [stefanie\\_zootecnia@hotmail.com](mailto:stefanie_zootecnia@hotmail.com) (S.A. Santos).

mass, it could range according to the physiological stage because growing animals of the same species differ in tissue proportion terms in each development stage (Henry and Collingwood, 1998). Thus for a specific metabolic phase like growing, and for a single species like goats, both models could probably be applied because there would be very small differences in metabolic size and, consequently, in the linearity of the relationship between UCE and BW.

Therefore, we hypothesized that: 1) the relationship between UCE and FBW could be fitted to an allometric model; 2) UCE could be used as a marker to quantify 24-h urinary volumes in growing goats; 3) time-point sampling could interfere with this estimate. Thus our study objectives were to quantify the relationship between FBW and UCE in Boer goats, to evaluate the urinary volume estimates obtained from creatinine concentrations in the spot samples collected at different time-points, and to compare them with the observed urine volumes obtained at 24 h.

## 2. Material and methods

### 2.1. Location and ethics procedures

The experiment was carried out at the Experimental Farm of the Federal University of Bahia in São Gonçalo dos Campos city, BA, Brazil. All the procedures followed the ethic and animal welfare guidelines of this same institution and were previously approved by the Ethics Committee for Animal Handling and Care (CEUA; protocol number 28-2014).

### 2.2. Animals, diets and treatments

Thirty crossbreed Boer goats males, with an initial body weight of  $18 \pm 2.2$  kg and  $124 \pm 5.7$  days of age, were kept under the same handling conditions and were allocated as random experimental units. Animals were kept in individual metabolic cages located in a sheep shed, with a slatted floor of approximately  $1.0 \text{ m}^2$  fitted with individual feeders and water containers, and were fed *ad libitum* twice daily. Feed intake was adjusted daily and 15–20% of leftovers were allowed to standardize feed offer to *ad libitum* conditions. Diet was a total mixed ration based on sorghum silage and concentrate at the 50:50 ratio on a dry matter (DM) basis. It was formulated to contain 150 g/kg DM of crude protein (CP) to attend an average daily gain (ADG) of 200 g/day according to NRC (2007). The concentrate ingredients were ground corn, whole maize grain, soybean meal and a mineral mixture, and dietary composition per kg DM was: 497 g neutral detergent fiber, 87.5 g ash, 26.2 g ether extract and 639 g total digestible nutrients.

### 2.3. Experimental protocols and sampling

The total experimental trial lasted 75 days, divided into three periods of 25 days each. The same diet was offered during each period. Urinary samplings were performed on days 24 and 25 during all three periods. This interval was proposed to obtain a variety of FBW during the experiment. Ten animals were randomly submitted to each sampling run. Each sampling run lasted 48 h. Urine was collected using collecting funnels, which were attached to animals and coupled with leading hoses to conduct urine to plastic containers with 100 mL of  $\text{H}_2\text{SO}_4$  20% (v/v) (Chizzotti et al., 2008; Yañez-Ruiz et al., 2004) to preserve N compounds. This solution was added to keep the urine pH below 4.0 (Gonda and Lindberg, 1994), which was monitored during all the collection periods. At the end of each day, the urine pool was weighed, homogenized, filtered through two cheesecloth layers, and an aliquot (50 mL) was sampled and stored at  $-20^\circ\text{C}$  for further creatinine analyses.

Total urine collection samples, taken every 24 h, were used to obtain a composite sample of each sampling day based on the total volume collected on each sampling day. During the total collection in each

sampling run, urine spot samples of approximately 50 mL were also collected at 0, 4 and 8 h after morning feedings (08:00 h). Urine was collected directly at the end of the hose attached to the funnel after eliciting micturition by manual stimulation. The collected volume of all three spot samples of each day was quantified and the value was recorded. This total volume was added to the final daily volume. All the goats were weighed immediately before each sampling run after 16 h of solids fasting.

### 2.4. Laboratory analyses

Creatinine concentrations were quantified in all the composite urine samples obtained both from the 24-h urine collection and each different collected time point. Samples were analyzed in a semi-automatic biochemistry analyzer (Bio 2000–Bioplus, São Paulo, Brazil) by the colorimetric system for picrate and in an acidifier with an end-point reaction using commercial kits (Doles Reagentes, Goiânia, Brazil).

### 2.5. Calculations

The total urine volume of each animal in each sampling run was considered from a mean volume of 2 collection days. The creatinine concentration (mg/dL) of each animal in each sampling run was obtained from composite samples for both the 24-h urine volume and the spot samples at 0, 4 and 8 h after feeding. True daily UCEs were obtained by multiplying creatinine content and the mean urinary volume, which corresponded to the values of the two consecutive 24-h volumes collected in each sampling run. The expected UCE estimated from the spot samples at each time point were estimated by FBW and the true UCE was obtained after the 24-h collection. The mathematical relationship between them was obtained after the model fitting process described below.

### 2.6. Statistical analyses

The daily UCE was used as the dependent variable, while FBW was considered the independent variable to fit both linear regression models without intercept and an allometric model ( $Y = aX^b$ ). Estimates of the parameters were obtained using PROC REG and PROC NLIN in SAS (Statistical Analysis System, version 9.2). The procedure PROC MIXED was used to investigate the random effects of the sampling runs. The comparison between mathematical adjustments was made using the MES software (Model Evaluation System, version 3.1, Texas A & M University) according to Tedeschi (2006). Predicted and observed UCEs were compared by fitting a simple linear regression model of the observed values (dependent variable) on the predicted values (independent variable) by simultaneous hypothesis testing (Mayer et al., 1994):

$$H_0: \beta_0 = 0 \text{ and } \beta_1 = 1$$

The predicted and observed values were considered similar when the null hypothesis was not rejected. All the statistical procedures were performed using 0.05 as the critical level for Type I error occurrences.

## 3. Results

The daily UCEs according to FBW were obtained by fitting linear regression without intercept and the allometric model according to the following equations:  $\text{UCE} = 17.39 \times \text{FBW}$ ,  $r^2 = 0.96$ ,  $P < 0.01$ ,  $\text{RSD} = 40.8$ ;  $\text{UCE} = 23.50 \times \text{FBW}^{0.9059}$ ,  $R^2 = 0.96$ ,  $P < 0.01$ ,  $\text{RSD} = 40.9$ ; (Fig. 1). The random effect of the sampling run that interacted with BW was not significant ( $P = 0.91$ ), so this correction was not considered. These equations were compared to the values observed to evaluate goodness of fit. The null hypothesis,  $\beta_0 = 0$  and  $\beta_1 = 1$ , was accepted when comparing the predicted and observed values (Table 1) for both equations ( $P > 0.05$ ), which indicates that any of these

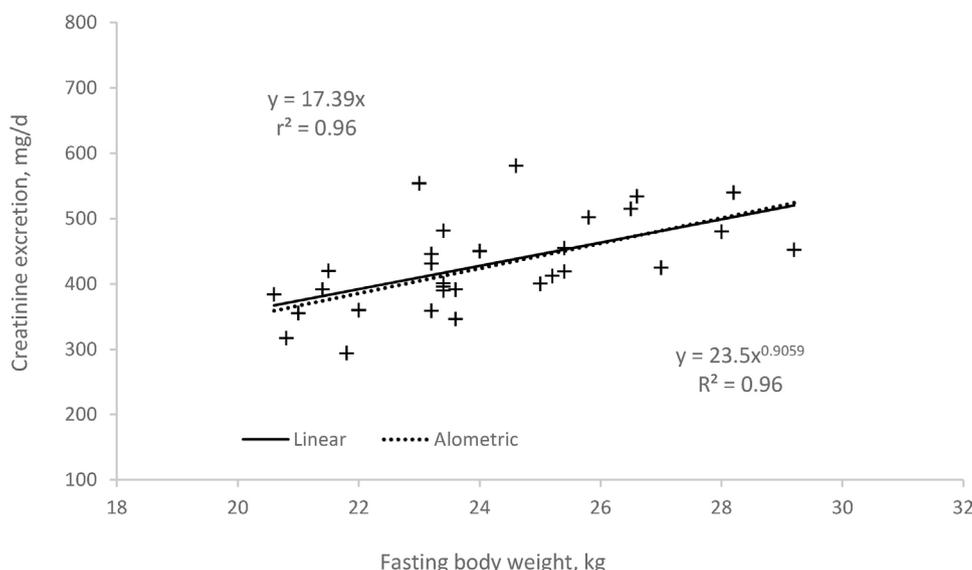


Fig. 1. Observed values of urinary creatinine excretion obtained by 24-h sampling (Y; mg/day) as a function of fasting body weight of growing Boer goats (X; kg) using linear and allometric approaches.

**Table 1**  
Comparative data of observed and predicted urinary creatinine excretions as a function of fasting body weight of growing Boer goats using linear and allometric approaches.

Item	Observed UCE <sup>a</sup>	UCE estimates <sup>b</sup>	
		Linear	Allometric
Average	419.9	419.3	419.8
Standard-deviation	84.1	39.5	37.7
Minimum	131.2	358.2	364.2
Maximum	580.7	507.8	499.5
Regression analysis <sup>c</sup>			
$\beta_0$ estimate	–	38.1	–2.2
$\beta_1$ estimate	–	0.91	1.01
P-value	–	0.97	0.99
Mean square error of prediction <sup>d</sup>	–	6432.1	6418.3
Mean bias	–	0.00	0.00
Systematic bias%	–	0.19	0.00
Random errors%	–	99.8	99.9
Deviation analysis <sup>e</sup>			
Mean deviation	–	–0.55	–0.02
P-Value	–	0.97	0.99

<sup>a</sup> True values of urinary creatinine excretion (mg/day).  
<sup>b</sup> Estimated values obtained for urinary creatinine excretion (mg/day) using Linear approach  $UCE = 17.56 \times FBW$  and allometric approach  $UCE = 16.12 \times FBW^{1.0253}$ .  
<sup>c</sup> Regression analysis proceeded according to Mayer et al. (1994) with predicted (Y) and observed (X) data – Hypothesis tested:  $\beta_0 = 0$  and  $\beta_1 = 1$ , analysis carried out using Model Evaluation System (MES) program.  
<sup>d</sup> MSEP = Estimates of mean square error of prediction, which was decomposed in mean bias, systematic bias and random errors according to Tedeschi (2006).  
<sup>e</sup> Deviation analysis according to Tedeschi (2006).

equations can predict daily UCE in growing goats. The allometric function showed a lower value for the mean squared prediction error (MSPE). However, linear equation without intercept had a lower percentage of systematic bias, and both presented high random error occurrence, which is desirable. The mean deviation of both equations was not significant ( $P > 0.05$ ), which indicates accuracy (Table 1). Consequently, both equations provided precise and accurate estimates for UCE, and any fittings obtained in this study can be recommended.

The previously obtained constant 17.39 mg/kg FBW was used to estimate UCE in each animal. Then the urinary volume from the spot samples was calculated for each sampling time. The observed means of the 24-h urinary volume and the respective estimates from the spot samples (0, 4, and 8 h after feeding) were compared to evaluate creatinine as a marker. The null hypothesis was rejected ( $P < 0.05$ ) when the spot sample was obtained at 0 and 8 h after feeding (Table 2), which

**Table 2**  
Comparative data of observed and predicted urinary volume of growing Boer goats using creatinine as a metabolic marker.

Item	Observed UV <sup>a</sup>	UV estimates <sup>b</sup>		
		0 h	4 h	8 h
Average	0.98	0.87	0.91	0.77
Standard-deviation	0.43	0.37	0.38	0.27
Minimum	0.43	0.40	0.43	0.40
Maximum	2.51	1.98	2.01	1.46
Regression analysis <sup>c</sup>				
$\beta_0$ estimate	–	0.60	0.30	0.10
$\beta_1$ estimate	–	0.40	0.75	1.05
P-value	–	< 0.01	0.20	0.07
Mean square error of prediction <sup>d</sup>	–	0.23	0.13	0.11
Mean bias%	–	14.9	4.5	19.2
Systematic bias%	–	16.3	6.5	0.1
Random errors%	–	68.8	89.0	80.7
Deviation analysis <sup>e</sup>				
Mean deviation	–	–0.11	–0.07	–0.21
P-Value	–	0.03	0.25	0.02

<sup>a</sup> True values of urinary volume (L).  
<sup>b</sup> Estimated values obtained for urinary volume (L) from spot samples collected at 0, 4 and 8 h after feeding, using the constant of 17.56 mg of creatinine/kg FBW.  
<sup>c</sup> Regression analysis proceeded according to Mayer et al. (1994) with predicted (Y) and observed (X) data – Hypothesis tested:  $\beta_0 = 0$  and  $\beta_1 = 1$ , analysis carried out using Model Evaluation System (MES) program.  
<sup>d</sup> MSEP = Estimates of mean square error of prediction, which was decomposed in mean bias, systematic bias and random errors according to Tedeschi (2006).  
<sup>e</sup> Deviation analysis according to Tedeschi (2006).

denotes an estimation error. However, the acceptance of the null hypotheses ( $P > 0.05$ ) for the 4-h time point indicated that the prediction model was not biased at this time point. This sampling time of 4 h after feeding presented low MSPE values and a high percentage of random error. Time points 0 and 8 h contributed well to the model bias (Table 2), which led to a negative mean deviation ( $P < 0.05$ ).

#### 4. Discussion

The average UCE obtained from the 24-h urine sampling in this study agreed with the wide range of 13.11 mg/kg FBW for male goat kids (Lindberg, 1985) to 26.05 mg/kg FBW for lactating goats (Fonseca et al., 2006). As creatinine excretion is related to BW and is proportional to muscle mass, this excretion can consequently alter according to the animal's physiological stages (Valadares et al., 1997; Chizzotti et al., 2008). The linear model is generally adequate among the model

options because creatinine is a byproduct of energy metabolism in muscles, and the relationship between these variables was expected to be linear (Costa e Silva et al., 2014). However, the linear model could be limited when a wide BW interval is studied or when the research theme involves more than one species, which was not the case in this study as it involved only growing goats.

For these cases, the allometric model could cover this amplitude since growth curves are composed of body tissues at different growth rates. This event makes the allometric model a strategic option to evaluate the quantitative differences produced in different physiological phases, e.g., growth and maintenance, together (Fernandes et al., 2007). According to Bonvillani et al. (2010), the allometric coefficient (parameter *b*) can predict late or early characteristics of growing curves. So clearly the similarity between the creatinine excretion estimates obtained with the two evaluated models demonstrates that for a specific BW range, e.g., the growing phase, both the linear and allometric models were adequate to fit the goats data.

Therefore, UCE can be used as a marker of urinary volume when considering animals in the same maturity stage because this excretion is relatively constant during the day and is not influenced by breeds or dietary factors, e.g., dietary protein level, dietary concentrate level, and offers protein sources (Moen and DeGiudice, 1997; Valadares et al., 1999; Prates et al., 2012).

The results obtained herein still suggest that UCE according to FBW could be estimated using linear or allometric equations. Chizzotti et al. (2008) recommended a linear equation to estimate UCE according to FBW for crossbreed Holstein heifers. Creatinine excretion correlates positively with BW, and concomitantly with muscle metabolism because creatinine is a metabolite that derives from loss of water from creatine-phosphate in a non enzymatic irreversible reaction (Wyss and Kaddurah-Daouk, 2000). It is also assumed that tissue development occurs according to an allometric curve (Smith, 1980). Thus the relationship between UCE and BW is expected to follow an allometric curve once the muscle in animal bodies relates highly with FBW in growing animals (De Campeneere et al., 2000).

Costa e Silva et al. (2012) recommended the following allometric equation  $UCE_{(g/day)} = 0.0345 \times SBW^{0.9491}$  for Nellore cattle, where SBW is shrunk body weight (kg). Valadares Filho et al. (2016) suggested  $UCE_{(mg/day)} = 37.88 \times BW^{0.9316}$  for cattle regardless of genetic group or animal category. These authors conducted studies by a meta-analytical approach, which allowed a larger number of observations than Chizzotti et al. (2008), whose data were obtained from only one study. Nevertheless, more studies on UCE in goats should be carried out.

Despite the possibility of using equations to obtain UCE according to FBW, the better option in spot collection protocols for growing goats must be carefully considered. Urine spot samples are a practicable alternative to 24-h urine samplings for grazing animals or lactating animals (Valadares et al., 1999) since urinary creatinine excretion can be used as a marker of urinary volume. In this study, the estimated volume predicted by spot samples 4 h after feeding was similar to the true 24-h urine collection. However, the spot samples collected at 0 and 8 h after feeding did not show any similarity with the true volume. Pasternack and Kuhlback (1971) observed diurnal variations in UCE in men and excretion rates peaked in the daytime instead of at night, which means that only one spot sampling time might not be enough to estimate 24-h urinary volumes. Chen et al. (1992) found that two, three or four UCE samplings taken at the same time on consecutive days in steers can reduce deviation and increase the correlation coefficient when comparing daily single samples. Samples taken at 15:00 h to 20:00 h tended to give both low deviation and a reasonably high correlation coefficient.

Rennó et al. (2008) also found a difference between the predicted and observed 24-h urine volumes in cattle when using a single sample. It should be noted that this difference does not interfere with UCE estimates, thus spot samples could be used to quantify purine derivatives of excretion. Rennó et al. (2000) observed similar values for estimated

urinary volumes by means of spot collections 4 h after feeding, and obtained a 24-h urine volume. Barbosa et al. (2006) observed that collection period had no effect on urinary creatinine excretion. However, these authors worked with cattle and some variation to periods was observed in goats. George et al. (2011) compared the creatinine concentration in the spot urine samples of various ruminant (cattle, buffalo, sheep and goat) and rabbit species. These authors observed that UCE was affected by neither diet nor the animal's physiological status, but was excreted in proportion to the body weight of all of the studied species. Accordingly, more studies about sampling time should be performed for these species.

## 5. Conclusions

Urinary creatinine excretion can be estimated according to FBW with linear or allometric functions. The average UCE for growing goats was 17.39 mg/kg FBW daily. Urinary volume can be estimated by the urine spot samples obtained at 4 h after feeding. Further research is needed to better investigate the circadian pattern of creatinine concentration in urine and to determine the best urine spot sampling time point in goats.

## Acknowledgment

Funding: This work was supported by the National Council of Technological and Scientific Development of Brazil (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq – Brasil), INCT Animal Science, and Bahia State Research Foundation (Fundação de Amparo à Pesquisa do Estado da Bahia – FAPESB).

## References

- Barbosa, A.M., Valadares, R.F.D., Valadares Filho, S.D.C., Vêras, R.M.L., Leão, M.I., Detmann, E., Souza, M.A.D., 2006. Effect of urinary collection days, concentrate levels and protein sources on creatinine: urea and purine derivatives excretions and microbial protein synthesis in Nellore cattle. *Rev. Bras. Zootec.* 35, 870–877.
- Bonvillani, A., Peña, F., De Gea, G., Gómez, G., Petryna, A., Perea, J., 2010. Carcass characteristics of Criollo Cordobés kid goats under an extensive management system: effects of gender and liveweight at slaughter. *Meat Sci.* 86, 651–659.
- Brake, D.W., Titgemeyer, E.C., Jones, M.L., Anderson, D.E., 2010. Effect of nitrogen supplementation on urea kinetics and microbial use of recycled urea in steers consuming corn-based diets. *J. Anim. Sci.* 88, 2729–2740.
- Broderick, G.A., 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *J. Dairy Sci.* 86, 1370–1381.
- Chen, X.B., Grubic, G., Ørskov, E.R., Osuji, P., 1992. Effect of feeding frequency on diurnal variation in plasma and urinary purine derivatives in steers. *Anim. Prod.* 55, 185–191.
- Chen, X.B., Mejia, A.T., Kyle, D.J., Ørskov, E.R., 1995. Evaluation of the use of the purine derivative: creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: studies in sheep. *J. Agric. Sci.* 125, 137–143.
- Chizzotti, M.L., Valadares Filho, S.C., Valadares, R.F.D., Chizzotti, F.H.M., Tedeschi, L.O., 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. *Livest. Sci.* 113, 218–225.
- Costa e Silva, L.F., Valadares Filho, S.C., Chizzotti, M.L., Rotta, P.P., Prados, L.F., Valadares, R.F.D., Braga, J.M.D.S., 2012. Creatinine excretion and relationship with body weight of Nellore cattle. *Rev. Bras. Zootec.* 41, 807–810.
- Costa e Silva, L.F., Valadares Filho, S.C., Chizzotti, M.L., Rotta, P.P., Zanetti, D., Valadares, R.F.D., Detmann, E., 2014. Models to predict muscle tissue and crude protein in beef cattle. *Livest. Sci.* 160, 186–193.
- De Campeneere, S., Fiems, L., Vanacker, J., Boucqué, C., 2000. Evaluation of urinary creatinine excretion to estimate *in vivo* body composition of Belgian Blue double-muscled bulls. *Annales de zootechnie* 49, 335–342.
- Fernandes, M.H.M.R., Resende, K.T.D., Tedeschi, L.O., Fernandes, J.S., Silva, H.M., Carstens, G.E., Akinaga, L., 2007. Energy and protein requirements for maintenance and growth of Boer crossbred kids. *J. Anim. Sci.* 85, 1014–1023.
- Fonseca, C.E.M.D., Valadares, R.F.D., Valadares Filho, S.D.C., Leão, M.I., Cecon, P.R., Rodrigues, M.T., Araújo, A.M., 2006. Microbial protein synthesis in lactating goats fed diets with increasing levels of dietary protein. *Rev. Bras. Zootec.* 35, 1169–1177.
- George, S.K., Dipu, M.T., Verma, A.K., Singh, P., Mehra, U.R., 2011. Species differences in the concentration of purine derivatives and creatinine in spot urine samples. *J. Indian Vet. Assoc.* 9, 24–26.
- Gonda, H.L., Lindberg, J.E., 1994. Evaluation of dietary nitrogen utilization in dairy cows based on urea concentrations in blood, urine and milk, and on urinary concentration of purine derivatives. *Acta Agr. Scand. A-An.* 44, 236–245.
- Henry, C.J.K., Collingwood, A., 1998. Allometry of fasting urinary nitrogen loss and basal metabolic rate in homeotherms. *Nutr. Res.* 18, 49–60.

- Lindberg, J.E., 1985. Urinary allantoin excretion and digestible organic matter intake in dairy goats. *Swedish J. Agric. Res.* 15, 31–37.
- Liu, Z.J., McMeniman, N.P., 2006. Effect of nutrition level and diets on creatinine excretion by sheep. *Small Rumin. Res.* 63, 265–273.
- Marini, J.C., Van Amburgh, M.E., 2003. Nitrogen metabolism and recycling in Holstein heifers. *J. Anim. Sci.* 81, 545–552.
- Mayer, D.G., Stuart, M.A., Swain, A.J., 1994. Regression of real-world data on model output: an appropriate overall test of validity. *Agric. Syst.* 45, 93–104.
- Moen, R., Delgiudice, G.D., 1997. Simulating nitrogen metabolism and urinary urea nitrogen: creatinine ratios in ruminants. *J. Wildl. Manag.* 1, 881–894.
- NRC, 2007. *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids and New World Camelids*. National Academy Press, Washington, DC (384 pp).
- Pasternack, A., Kuhlback, B., 1971. Diurnal variations of serum and urine creatine and creatinine. *Scand. J. Clin. Lab. Invest.* 27, 1–7.
- Prates, L.L., Valadares, R.F.D., Valadares Filho, S.C., Detmann, E., Santos, S.A., Braga, J.M.S., Barbosa, K.S., 2012. Endogenous fraction and urinary recovery of purine derivatives in Nelore and Holstein heifers with abomasal purine infusion. *Livest. Sci.* 150, 179–186.
- Rennó, L.N., Valadares, R.F.D., Valadares Filho, S.D.C., Leão, M.I., Silva, J.F.C.D., Cecon, P.R., Linhares, R.S., 2000. Plasma urea concentration and urea and creatinine excretions in steers. *Rev. Bras. Zootec.* 29, 1235–1243.
- Rennó, L.N., Valadares Filho, S.D.C., Valadares, R.F.D., Paulino, M.F., Rennó, F.P., Silva, P.A., 2008. Urea levels in diet for steers of four genetic groups: microbial protein production by the urinary purine derivatives, using two collection methodologies. *Rev. Bras. Zootec.* 37, 546–555.
- Smith, R.J., 1980. Rethinking allometry. *J. Theoret. Biol.* 87, 97–111.
- Tedeschi, L.O., 2006. Assessment of the adequacy of mathematical models. *Agric. Sys.* 89, 225–247.
- Valadares Filho, S.C., Silva, Costa E., Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L., Prados, L.F., 2016. BR-CORTE 3.0. *Nutrient Requirements of Zebu and Crossbreed Cattle BR-Corte*, 3rd. ed. UFV, Viçosa (327p.).
- Valadares, R.F.D., Gonçalves, L.C., Rodriguez, N.M., Valadares Filho, S.D.C., Sampaio, I.B.M., 1997. Protein levels in cattle diets. 4. Ruminal ammonia N concentration plasma urea N, and urea and creatinine excretions. *Rev. Bras. Zootec.* 26, 1270–1278.
- Valadares, R.F.D., Broderick, G.A., Valadares Filho, S.C., 1999. Effect of replacing alfalfa with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *J. Dairy Sci.* 8, 2686–2696.
- Wyss, M., Kaddurah-Daouk, R., 2000. Creatine and creatinine metabolism. *Physiol. Rev.* 80, 1107–1213.
- Yañez-Ruiz, D.R., Moumen, A., Martin Garcia, A.I., Molina Alcaide, E., 2004. Ruminal fermentation and degradation patterns protozoa population, and urinary purine derivatives excretion in goats and wethers fed diets based on two-stage olive cake: effect of PEG supply. *J. Anim. Sci.* 82, 2023–2032.