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Evaluation of urinary creatinine excretion to estimate in vivo body composition of Belgian Blue double-muscled bulls[1]

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Abstract — Urinary creatinine excretion (UCE) and chemical body composition of 18 Belgian Blue double-muscled bulls were determined. Partly due to the large fasted live weight (fLW) range (308–710 kg), kg water and protein in the empty body were quite accurately predicted (adjR^2 = 0.94 and 0.97) by UCE. The precision of the prediction of kg empty body fat was less good (adjR^2 = 0.77). However, the empty body composition of the bulls varied only slightly and linearly with increasing live weight so that the fLW was the best parameter to estimate empty body water and protein with an adjR^2 value of 0.99 for both. The empty body fat was estimated from fLW with an adjR^2 = 0.75. The equations predicting relative body composition from UCE had much smaller adjR^2 values, varying from 0.53 for % empty body water to 0.30 for % empty body protein. However, the coefficients of variation were much smaller. The prediction of the relative composition based on UCE was always somewhat better than based on fLW, but the improvement was not enough to justify the work involved in determining UCE. Conclusively, due to the small compositional changes, UCE did not significantly improve the estimation of the body composition from fLW. From the comparison of the results with other studies, it can be concluded that the double-muscled bulls have an extremely high urinary creatinine concentration, which cannot entirely be explained by their increased protein content.

urinary creatinine excretion / double-muscling / body composition estimation / bulls

Résumé —Évaluation de l'excrétion urinaire de créatinine pour estimer in vivo la composition corporelle de taurillons Blanc-Bleu-Belge culards. L'excrétion urinaire de créatinine (UCE) et la composition corporelle de 18 taurillons Blanc-Bleu-Belge culards, de poids au jeûne variant entre 308 à 710 kg ont été déterminées. Les quantités d'eau et de protéines (en kg) dans le poids vif vide

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ont été estimés assez précisément à partir de l'UCE (R^2 ajusté ou adjR^2 = 0,94 et 0,97), en raison de la grande variation de poids. L'estimation de la quantité de gras dans le poids vif vide a été moins précise (adjR^2 = 0,77). La composition corporelle des culards Blanc-Bleu-Belge a changé lentement mais linéairement avec le poids vif entre 300 à 700 kg. En conséquence, le meilleur paramètre pour estimer la quantité d'eau et de protéines (en kg) chez le culard est le poids vif au jeûne (valeur de adjR^2 = 0,99 pour les deux). La précision pour estimer la quantité de gras (kg) à partir de ce même paramètre a été moins bonne (adjR^2 = 0,75) qu'avec l'UCE. Les équations reliant la composition corporelle avec l'UCE ont eu des adjR^2 réduits, entre 0,53 pour la teneur en eau et 0,30 pour la teneur en protéines. Mais les coefficients de variation ont été plus faibles, indiquant que la composition a été estimée plus précisément que la quantité d'eau et de protéines. La prédiction de la composition basée sur l'UCE a toujours été meilleure que celle basée sur le poids au jeûne, mais l'amélioration n'a pas été suffisante pour justifier le travail pour déterminer l'UCE. Lorsque la composition corporelle ne change que légèrement, l'UCE n'améliore pas significativement l'estimation de la composition corporelle du culard Blanc-Bleu-Belge à partir du poids au jeûne. En comparant nos résultats à ceux de la littérature, on peut conclure que la concentration en créatinine urinaire chez les culards Blanc-Bleu-Belge est très élevée. Ces excrétions plus élevées ne s'expliquent pas totalement par le taux de protéines corporelles élevé de ces animaux.

excrétion urinaire de créatinine / culard / estimation de la composition corporelle / taurillons

1. INTRODUCTION

Data about body or carcass composition of live animals are very important in nutritional experiments. Several techniques that were originally developed for human medicine have been adapted for predicting in vivo composition of animals [5]. These techniques (e.g. CT-scan, [17] and NMR, [1]), demand very expensive equipment and cannot be applied to larger ruminants (cows, bulls, etc.), due to the limited size of most apparatus. Therefore, a less sophisticated technique such as urinary creatinine excretion (UCE) is a possible and realisable method for in vivo estimation of body composition in ruminants.

Borsook and Dubnoff [2] found that 98% of the creatine reserves of the animal are present in the muscles, mainly in the form of phosphocreatine. Between 1.6 [7] and 2.8% [2] of that reserve is daily converted into creatinine, which is entirely [7] excreted in the urine. Dinning et al. [8] concluded that daily excretion of creatinine is not affected by protein intake, but DelGiudice [7] stated that dietary sources of creatine or creatinine (*e.g.* meat) may be important. The differences in excretion between individual animals are larger than the differences between the excretions from day to day [8], while the lean tissue mass of an animal can be considered not to change markedly from day to day in the same animal [13]. As such, several authors [11, 15, 17] have shown that UCE is highly correlated to body weight or to the lean tissue mass of the animal.

To determine UCE the urine is usually collected over several days and a composite sample is analyzed (colorimetric). Except for the collection of the urine the UCE technique is very simple, little time-consuming and requires no high investment.

In this study the adequacy of UCE to estimate body composition of Belgian Blue (BB) double-muscled (dm) bulls was evaluated. Therefore, body composition of 18 bulls, with a large weight range (± 300–700 kg), was analysed after determination of UCE. The resulting equations should predict protein and energy accretion over a large weight range.

2. MATERIALS AND METHODS

2.1. Experimental design

After a two month adaptation period at the Department, 46 bulls used for this experiment were chosen out of 160 bulls. They were purchased at the market at a live weight between 275 and 325 kg. The total experimental period was divided in three phases (ca. 360–460 kg, 460–570 kg, 570–680 kg) to investigate the effect of phase-feeding on animal performance [3]. The animals were divided over four feeding regimens (group 1, 2, 3 and 4), differing in energy and protein content [3]. All diets were fed ad libitum. The negative control group (group 1; $n = 10$) constantly received a low protein level combined with a moderate energy level. With each phase, the protein level of group 2 ($n = 12$) decreased while a constant moderate energy level was fed. The energy level of group 3 increased ($n = 12$) in combination with a constant high protein level. Group 4 ($n = 12$) received rations with increasing energy levels and decreasing protein levels. More details about the diets are given by De Campeneere et al. [3].

At the beginning of each phase (360, 460 and 570 kg) and at the end of the experiment (680 kg), all animals were confined in metabolic cages to determine UCE. In the course of the experiment 18 bulls were slaughtered 5 days after the end of the collection period to determine chemical body composition. These 18 animals were selected from the groups 2, 3 and 4, based on their live weight (LW), in order to have a homogeneous spread over a large LW range (± 300–700 kg). After the first period in the cages, two animals from groups 2, 3 and 4 were slaughtered, with LW ranging from 309 to 405 kg. After the second as well as after the third collection period, one animal out of the groups 2, 3 and 4 was slaughtered. LW varied from 426 to 486 kg and from 543 to 593 kg, respectively. Finally, at the end of the trial, again two animals out of the groups 2, 3 and 4 were slaughtered, with LW ranging from 628 to 723 kg.

2.2. Urinary creatinine excretion

To determine UCE, the bulls were confined in metabolic cages during a seven-day period. The first three days were to adapt the bulls to the cages, the remaining four days total urine and faeces were collected separately. Urine was collected and weighed once daily and a representative sample was taken. Before collecting, diluted sulphuric acid was added to the urine container to reduce pH below 3 and to prevent bacterial destruction of creatinine. Urine samples were stored in plastic bottles and frozen at –20 °C until analysis for creatinine. Creatinine was determined using a colorimetric procedure (Boehringer Mannheim, 1993). UCE was calculated by multiplying the urinary concentration by total urine volume. Four days after the end of the collection period, a blood sample was taken from the jugular vein to determine blood creatinine concentration using the same colorimetric procedure.

2.3. Slaughtering procedure and chemical analyses

The bulls were slaughtered in the experimental slaughterhouse of Ghent University, 5 km from the Department. Bulls were not fasted before slaughter, but fasted LW (fLW) was determined the day before, after a fasting period of 16 hours.

During the slaughtering procedure all non-carcass parts (NCP), including all removable fatty tissues, were separated from the carcass and gathered. Details of the slaughtering procedure were previously described by De Campeneere et al. [6]. Empty body weight (EBW) was calculated as LW minus gut fill. At the end of the slaughtering procedure weight of the right half carcass (CC; including half of the tail) was determined.

CC and the NCP were prepared for analysis according to the procedure described by De Campeneere et al. [6]. Water, protein and fat were analysed in the CC and in the NCP separately. Protein (Kjeldahl) and fat (Soxhlet extraction and gravimetry) were analysed according to the EU methods [9, 10]. From these results, water (EBWa), protein (EBP) and fat (EBF) in the empty body (EB) were calculated. Fat-free body mass (FFBM) was calculated as EBW-EBF.

Due to technical problems, body composition data from one animal were unreliable and therefore excluded from all statistical analysis. Consequently, the compositional data are based on 17 observations.

2.4. Statistical analysis

Means, standard deviations, correlation coefficients and linear regressions were calculated using SPSS 8.0 for Windows [15].

3. RESULTS

In Table I data concerning the weight and the composition of the EB, FFBM, fLW, the blood and urinary creatinine concentration, the UCE ($g \cdot day^{-1}$) and the ratio UCE/fLW are listed. The results show very low fat contents in these dm animals. The maximum fat content in the empty body was just below 10%, while the lowest fat content was 3.5%. In the carcasses the fat content was even lower and varied between 3.1 and 7.4% [4]. The FFBM varied between 260.6 and 618.9 kg and between 90.3 and 96.5% of the EBW.

Daily urinary creatinine excretion averaged 25.2 g and 49.6 mg per kg fLW. This second parameter varied between 44.0 and 53.7 $mg \cdot day^{-1}$.

The correlation coefficients between the different compositional parameters and the creatinine parameters are shown in Table II. To obtain the highest correlation total urine collection is needed, since that parameter was best correlated with all body components (except EBF). However, if total urine collection is impossible, the blood creatinine concentration correlated better with EBWa, EBP, FFBM and fLW than the urinary concentration did.

Table I. Mean, SD and range for the weight and composition of the empty body, the FFBM, the fasted live weight, and different creatinine parameters of the 17 bulls.

	Mean	SD	Range
Empty body weight (kg)	471.6	132.3	276.4–668.8
Empty body			
water (kg)	325.7	85.7	195.4–457.9
protein (kg)	95.2	27.9	54.0–134.7
fat (kg)	31.2	16.4	12.6–64.6
water (%)	69.4	1.9	65.8–72.0
protein (%)	20.1	0.6	18.9–21.2
fat (%)	6.3	1.8	3.5–9.7
FFBM (Fat-free body mass) (kg)	440.5	118.2	260.6–618.9
FFBM (% of empty body)	93.7	1.8	90.3 – 96.5
Fasted LW (kg)	506.0	134.9	308.0–710.0
Blood creatinine concentration ($\mu mol \cdot l^{-1}$)	199.2	43.5	130.0–279.7
Urinary creatinine concentration ($mmol \cdot l^{-1}$)	28.8	9.9	16.6–54.5
UCE (urinary creatinine excretion; $g \cdot d^{-1}$)	25.2	7.4	14.3–37.5
UCE / fasted live weight ($mg \cdot kg^{-1}$)	49.6	2.9	44.0–53.7

Table II. Correlation coefficients between composition (EB = empty body, Wa = Water, P = protein, F = fat, FFBM = fat free body mass) and different creatinine parameters (n = 17).

	EBWa (kg)	EBP (kg)	EBF (kg)	FFBM (kg)	EBWa (%)	EBP (%)	EBF (%)	FFBM (%)
Blood creatinine concentration ($\mu mol \cdot l^{-1}$)	0.888 **	0.874 **	0.820 **	0.888 **	–0.605 *	0.241	0.594 *	–0.594 *
Urinary creatinine concentration ($mmol \cdot l^{-1}$)	0.809 **	0.812 **	0.913 **	0.811 **	–0.740 **	0.203	0.792 **	–0.792 **
Daily creatinine excretion ($g \cdot d^{-1}$)	0.970 **	0.986 **	0.884 **	0.976 **	–0.728 **	0.551 *	0.659 **	–0.659 **
Urinary creatinine / kg fLW ($mg \cdot kg^{-1}$)	0.413	0.481	0.456	0.427	–0.501 *	0.695 **	0.389	–0.389

* P-value < 0.05; ** P-value < 0.01.

When body composition was expressed in percentages, most correlations decreased substantially. For % EBWa, % EBF and % FFBM the highest correlations were found with urinary creatinine concentration. % EBP was best related with mg urinary creatinine per kg fLW.

In Table III the results of the regression analysis are listed. In the upper half of the table relations between UCE or fLW and absolute body composition of the 17 bulls are shown. EBWa and EBP were accurately estimated by UCE. EBP had the highest adjR^2 (0.97) and the lowest RSD value: 4.8 kg (5.0%) vs. 21.4 kg (6.6%) for EBWa. Estimation of the fat content was less successful, with an adjR^2 = 0.77 and a RSD of 25.3%. The FFBM was also accurately predicted by the UCE.

A higher precision was found in estimating the absolute composition of the empty body based on the fLW, with an adjR^2 value of 0.99 for water and protein and a respective RSD (CV) of 9.3 kg (2.9%) and 2.7 kg (2.8%). Again the fat content was less successfully predicted with adjR^2 = 0.75 and RSD (CV) of 8.3 kg (26.6%). The estimation of the EBF was, in contrast with these of EBWa and EBP, slightly better when based on UCE than when based on fLW.

The very high adjR^2 values are partly due to the high variation in EBW. Therefore, body composition was expressed in percentages and the relations between the relative body composition and fLW or UCE were studied (lower half of Tab. III). The precision of prediction did not improve when fLW and UCE were used in one equation to predict absolute or relative body composition.

In comparison with the upper half of the Table, the adjR^2 values were noticeably lower. The correlation between % EBP and UCE or fLW was particularly weak. But, owing to the small compositional variation in this type of bull, the RSD values of the same equations were small, indicating that

Table III. Empty body (EB) composition (water: EBWa, protein: EBP and fat: EBF; kg and % of EB and FFBM (fat-free body mass; kg and % of EB)) estimated from UCE ($g \cdot d^{-1}$) and fLW (fasted live weight, kg) with adjR^2, RSD and CV (coefficient of variation).

Prediction equation		adjR^2	RSD	CV
kg EBWa	= 42.38 + (11.22 × UCE)	0.94	21.4	0.066
kg EBP	= 1.46 + (3.71 × UCE)	0.97	4.8	0.050
kg EBF	= –18.32 + (1.96 × UCE)	0.77	7.9	0.253
kg FFBM	= 47.64 + (15.56 × UCE)	0.95	26.5	0.060
kg EBWa	= 5.80 + (0.632 × fLW)	0.99	9.3	0.029
kg EBP	= –9.07 + (0.206 × fLW)	0.99	2.7	0.028
kg EBF	= –22.68 + (0.106 × fLW)	0.75	8.3	0.266
kg FFBM	= –1.60 + (0.874 × fLW)	0.99	9.4	0.021
% EBWa	= 74.1 – (0.18 × UCE)	0.50	1.33	0.019
% EBP	= 18.9 + (0.05 × UCE)	0.26	0.54	0.027
% EBF	= 2.17 + (0.16 × UCE)	0.40	1.43	0.227
% FFBM	= 97.8 – (0.16 × UCE)	0.40	1.43	0.015
% EBWa	= 74.2 – (0.0096 × fLW)	0.44	1.41	0.020
% EBP	= 19.0 + (0.0022 × fLW)	0.18	0.57	0.028
% EBF	= 1.93 + (0.0086 × fLW)	0.36	1.47	0.233
% FFBM	= 98.1 – (0.0086 × fLW)	0.36	1.47	0.016

it was a good estimation of the composition. From the comparison of the CV and the RSD values it is concluded that the relative equations are always more precise than the absolute equations in predicting body composition, and that UCE gave the best fit to the compositional data, with little difference in precision between UCE and fLW predicting relative composition.

4. DISCUSSION

More data on the body and carcass composition of the Belgian Blue dm bulls were given by De Campeneere et al. [6]. The very low fat content and the small increase of that fat content with increasing LW were the most significant conclusions. Due to the very low fat content, the FFBM was proportionately very large.

The daily UCE in this experiment varied between 14.3 and 37.6 g. Regression analysis indicated that with each kg increase in fLW the daily excretion of creatinine increased by 54.1 mg (UCE ($g \cdot day^{-1}$) = –2.106 + 0.054*fLW; $R^2 = 0.97$). Schroeder [14] estimated a corresponding value of 20.7 mg for a live weight interval from 300–560 kg (UCE = 2.45 + 0.021*fLW). The large difference between both studies could be partly due to the higher protein accretion with each kg increase in fLW for the dm bulls.

The creatinine coefficients (mg creatinine per kg fLW; Tab. I) in this study (range: 44,0–53,7) are also extremely high in comparison with results from Lofgreen and Garret [13]: 24.3–37.3; Schroeder [14]: 24.71–29.22 and Dinning et al. [8]: 9.69–11.72. These three experiments were done with respectively Hereford yearling, crossbred (Simmental × Angus × Charolais; 300–560 kg) and Hereford steers (2 years old). The very low figures of Dinning et al. [8] are quite different from these of Lofgreen and Garret [13], although the same breed was used. The very high protein content in the empty body of the BB dm bulls in our experiment can only partly explain the high values. For each kg protein in the empty body 266 ± 0.012 mg creatinine is excreted. From Schroeder [14], for each of four weight groups (300, 390, 480 and 560 kg) comparable values can be calculated being 150, 176, 166 and 158 $mg \cdot kg^{-1}$ protein respectively. Van Niekerk et al. [16] found from 65 sheep of different breeds that on average 195 g creatinine is excreted for each kg protein in the empty body. These figures prove that the high protein content in the dm bulls causes only part of the difference in the creatinine coefficient. This is in accordance with Hanset and Michaux [12], who found higher blood creatine and creatinine concentrations in dm than in non-dm bulls and concluded that the concentration in the dm type is higher than would be expected from the average hypertrophy of the muscles.

Although the prediction of kg EBWa, kg EBP and kg FFBM from UCE was reasonably good, it was even better when estimated from fLW (Tab. III). The large range in body weight of the animals used in this study and the very small variation of the body composition despite that large weight interval are the most important reasons for this conclusion. Van Niekerk et al. [16] studied with ruminants the precision of estimating EBP from a weight parameter (fLW) and both parameters were highly correlated (0.969), but UCE and EBP were still somewhat better correlated (0.972). Schroeder [14] demonstrated that the addition of LW to the equation containing UCE significantly improved the precision of prediction of FFBM (RSD from 22.2 to 6.63 kg) and EBP (RSD from 5.46 to 1.87 kg).

The bottom part of Table III indicates that the relative body composition is somewhat better predicted based on UCE than on fLW, but the differences are that small that they cannot justify the extra work for the determination of UCE. It indicates however that the technique might be valuable for a population with more variation in body composition. From the CV it can be concluded that despite the very high adjR^2 values of the absolute predictions, the prediction of the relative body composition is more precise.

5. CONCLUSION

In general, UCE is quite accurate in predicting absolute and relative EBWa, EBP and FFBM, but it is less successful in estimating EBF. The estimation of the relative body composition is always somewhat more precise than the estimation of the absolute composition, whether it is based on fLW or on UCE. The best relative predictions are found based on UCE, but the improvement in comparison to the fLW is too small to justify the extra work. Therefore it is concluded that due to the very slight changes in the body composition of the BB dm bulls and the large LW interval, fLW was most useful to estimate the body composition. From the comparison of these results with other studies, it can be concluded that the dm bulls have an extremely high creatinine excretion, which cannot entirely be explained by their increased protein content.

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