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EMPTY BODY CHEMICAL COMPOSITION OF NELLORE STEERS AT DIFFERENT FEEDING LEVELS ESTIMATED BY INDIRECT METHODS

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Abstract

Thirty-six Nellore steers at 20 months of age on average and initial body weight of 360 kg, fed *ad libitum* for 78 days and two levels of feed restriction for 58 days and posterior *ad libitum* feeding for 78 days were used to estimate body composition using the methods of indirect deuterium oxide or the 9th-10th-11th ribs cut. The body water content was calculated with equations established for Nellore steers. The chemical body composition was different for the ether extract content. The animals subjected to feed restriction presented higher body fat content when estimated by deuterium than by the rib cut. The deuterium oxide or 9th-10th-11th ribs cut methods used for body composition determination were effective to estimate body contents for animals fed *ad libitum*, however, not for animals on feed restriction. Comparatively, the indirect method of deuterium oxide was better than the 9th-10th-11th ribs cut method to estimate body composition in Nellore steers submitted to feed restriction; however, both methods were similar in animals fed *ad libitum*.

Key words

beef cattle, fat thickness, feedlot, feed restriction, rib cut.

INTRODUCTION

Water, fat, protein, and ash are body components of greatest interest for beef cattle nutrition. These components are distributed in a varied way in body tissues according to different factors and are important in dynamic models to estimate body composition (SILVA et al., 2017).

The use of different methods to know the body composition of animals, mainly in the fattening phase (FONSECA et al., 2017; CASTILHOS et al., 2018), suggests a more appropriate choice for each intended objective (SCHOLZ et al., 2015), using direct or indirect methods.

The most traditional indirect method is the use of 9-10-11th ribs, recommended by Hankins and Howes (1946). Many studies have investigated this method and some errors in the estimates were found, although the method has been validated in several circumstances (SILVA et al., 2017; NEVES et al., 2018).

The use of an isotopic marker to track molecules is another indirect method. Deuterium as a substitute for hydrogen in water molecules and has been used in animal studies for different purposes (CHAPMAN et al., 2017; SCHAFF et al., 2017). However, its use is still incipient to determine body constituents for nutritional assessments (GOMES et al., 2012).

For nutrition studies, which do not require knowledge of cut yield or proportion of edible tissues (BARBOSA et al., 2016; CUNNINGHAM et al., 2018), measuring water amount in the empty body may be sufficient to establish the other fractions (CASTILHOS et al., 2018).

Knowing the concentration of main components in the empty body through body water concentration is possible due to relationships between water and other body constituents (NEVES et al., 2018).

Fat is the most variable component in the body. Its distribution is not uniform in the body, varying the deposition location according to the nutritional plan to which the animals were subjected (HANNON and MURPHY, 2019), among other factors, such as weight gain rate, background management, diet type (COX-O'NEILL et al., 2017). Therefore, it becomes easier to express other components in fat -free body or in defatted mass. Relationships between protein and water and between ash and water are constant in dry matter of fat-free empty body (TEIXEIRA et al., 2017).

Based on the premise quantification of water allows knowing other body components effectively, we investigated water concentration by the 9-10-11th rib section and the deuterium oxide dilution methods to use in predetermined equations for Nellore cattle.

This study investigated the suitability of two indirect methodologies to estimate body composition of Nellore steers submitted to feed restriction.

MATERIAL AND METHODS

All procedures involving animals described in this work comply with the Institutional Animal Care and Use Committee Guidelines of FZEA/USP, under Nº 6706080515.

2.1 Animals and installations

Thirty-six Nellore steers at 20 months of average age were used to evaluate the body composition after three levels of dry matter intake (DMI) in a completely randomized design with 3 treatments and 12 replications. Animals were allocated in individual pens and submitted to 28 days of adaptation period for management and diet.

2.2 Diet and treatments

The diet was composed of 20% of sorghum silage and 80% of concentrate (Table 1). After the adaptation period of 28 days, two groups of animals were submitted to feed restriction of 75g DM/kg BW$^{0.75}$ (T75) and 60g DM/kg BW$^{0.75}$ (T60) for 58 days. Afterward, all animals received the diet *ad libitum* for 78 days, totaling 136 feedlot days. Another group of animals was fed *ad libitum* for 78 days - TAL treatment.

Table 1. Diet composition on dry matter basis

Ingredient	g/100g
Concentrate	80.00
Sorghum silage	20.00
Nutrient	
Total digestible nutrients	76.43
Crude protein	13.62
Metabolizable energy, Mcal/kg	2.76

2.3 Body composition determinations

2.3.1 Water by Deuterium oxide

The body composition was estimated using deuterium oxide after 78 days of *ad libitum* feeding (three days before slaughter) following the methodology described by Leme (1993) through deuterium oxide injection (Sigma D-44501, 99.8% purity, MW 20.03) in the right jugular vein and the amount was calculated based on 0.1g/kg of body weight.

Previously, blood samples were collected in *Vacutainer* 10 ml heparin tubes. Therefore, deuterium oxide was injected into the bloodstream. After administration of deuterium oxide, animals remained grouped without access to food or water. Six hours after administration, a new blood sample was collected in *Vacutainer* 10 ml heparin tubes, refrigerated, transferred to plastic tubes, and stored at –20 °C for posterior analysis.

The deuterium oxide in blood samples was analyzed by mass spectrometry following the methodology described by Coleman et al. (1982) in which water was separated from blood by vacuum distillation, retained in trap at –196 °C, and decomposed by metallic zinc reaction at 500 °C under vacuum system.

The deuterium space (DS) was calculated as follows:

DS = mg D_2O Injected / (mg/ml D_2O Final – mg/ml D_2O Initial).

In order to estimate chemical composition of empty body of Nellore steers from deuterium space (DS), equations described by Leme (1993) were used to calculate the water content in empty body:

Water (%) = 65.9654 + (0.0977 * DS) – (0.0909 * SBW), (R^2 = 0.83 and $S_{y.x}$ = 1.33), where SBW: shrunk body weight, DS: deuterium space.

2.3.2 Water by HH section

After 78 days of feedlot with *ad libitum* feeding, the animals were slaughtered in accordance to Humanitarian Slaughter Guidelines as required by the Brazilian laws.

After slaughter, carcasses were chilled for 24h (0-2 °C). Then, a sample from the 9^{th}-10^{th}-11^{th} ribs was collected from the half right of each carcass (HANKINS and HOWE, 1946). Subsequently, rib cuts were frozen and cut into small pieces with an electrical saw with tape and ground in a large-scale grinder. Next, sub-samples were lyophilized until a constant weight to obtain the water content in the rib cut.

The water content in empty body (EB) for Nellore steers from rib cut composition was estimated by the equation of Lanna (1988):

% Water EB = 24.1936 + (0.6574 * % Water 9-10-11th ribs), (R^2 = 0.91 and $S_{y.x}$ = 0.82).

2.3.3 Ether extract

The ether extract (EE) content in empty body was determined from water content, using an equation described by Leme (1993):

% Ether Extract EB = 93.92968 – (1.27598 * % Water EB), (R^2 = 0.97 and $S_{y.x}$ = 0.62).

2.3.4 Protein and ash

The protein content in the empty body was estimated by the protein and water relationship equal to 0.3009, and ash and water equal to 0.0747, according Leme et al. (1994).

2.3.5 Energy in body weight

To estimate the energy content in empty body, 9.367 kcal/kg was considered for EE and 5.686 kcal/kg for protein, according to NRC (1996).

2.3.6 Empty body weight

During slaughter, the visceral content was emptied, washed, and weighted to determine EBW. Kidney, pelvic, and inguinal fat (internal fat) weights were also registered. From slaughter data, an equation was obtained to estimate EBW from SBW.

2.4 Statistical analysis

The experimental design used was completely randomized with 3 treatments and 12 repetitions. Data were submitted to the variance analysis and treatment means compared by the T Student test. Data were analyzed by the MIXED procedure of SAS software (SAS Institute Inc., Cary, NC, USA) and differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The methods applied considered that determining amounts of water in ribs cut and body water by deuterium was sufficient to estimate other body components, using equations available for Nellore steers (NEVES et al., 2018).

The equation for empty body weight determination was: EBW (kg) = -15.74911 + 0.98517 * SBW (kg), (R^2 = 0.96 and $S_{y.x}$ = 8.64). No differences were observed for final empty body weight between treatments estimated by the equation or observed by the empty gastrointestinal tract (P=0.98; Table 2). Thus, EBW by equation was used to calculate body composition.

Table 2. Descriptive statistics of body weight and carcass traits

Traits	Mean	SD	Minimum	Maximum
Final shrunk body weight, kg	469.2	43.6	385.0	558.0
Empty body weight, kg	446.5	43.8	364.7	539.4
Kidney, pelvic and inguinal fat, kg	12.4	3.2	7.7	19.7
Fat thickness, mm	5.4	2.4	2.0	11.0

The subcutaneous fat thickness in carcass was not different in animals subjected to feed restriction or not (Table 3). There was an effect of treatments on internal fat (P=0.03) and empty body weight (P≤0.01), with greater values for animals on feed restriction when compared to animals fed *ad libitum*.

Table 3.– Means and standard error (SE) of subcutaneous fat thickness, internal fat, and empty body weight

Traits	*Treatments*			SE	P
	T60	T75	TAL		
Subcutaneous fat thickness, mm	5.87	5.42	4.73	2.37	0.52
Internal fat[1], kg	13.22[a]	13.43[a]	10.43[b]	0.89	0.03
Empty body weight, kg	*458[a]*	468[a]	411[b]	17.9	<0.01

[b] Different letters in the same row indicate significant difference. [1] Kidney, pelvic, and inguinal fat.

In animals with the same fat thickness at slaughter after feed restriction, differences in intramuscular fat deposit or carcass quality are not expected (REZENDE et al., 2012; COX-O'NEILL et al., 2017). However, metabolic and phenotypic changes are observed in animals due to the combination between the amounts of feed intake (CUNNINGHAM et al., 2018) and the time they were subjected to feeding, affecting partition of fat deposition (HANNON and MURPHY, 2019) more than the weight of empty body, which varied as fat internal, but not as subcutaneous fat.

The percentage or relative composition of body components (Table 4; Figure 1) was different between treatments when evaluated by the deuterium method (P<0.01), differing from the estimate by the rib cut method (P=0.11).

Table 4. Means ± standard errors (SE) of percentage composition of empty body weight determined by deuterium oxide (D_2O) or 9-10-11th ribs cut

Traits	*Treatments*		
	T60	T75	TAL
Water			
D_2O	54.6±0.56bB	54.3±0.56bB	58.1±0.58aA
9-10-11th	59.4±0.56aA	58.6±0.56aA	58.1±0.58aA
Ether extract			
D_2O	24.9±0.77aA	25.4±0.77aA	20.0±0.80bA
9-10-11th	18.3±0.77aB	19.4±0.77aB	20.1±0.80aA
Protein			
D_2O	16.4±0.17bB	16.3±0.17bB	17.5±0.17aA
9-10-11th	17.9±0.17aA	17.6±0.17aA	17.5±0.17aA
Ash			
D_2O	4.1±0.04bB	4.1±0.04bB	4.3±0.04bA
9-10-11th	4.4±0.04aA	4.4±0.04aA	4.3±0.04aA
Energy (Mcal/kg)			
D_2O	3.2±0.06aA	3.3±0.06aA	2.9±0.06bA
9-10-11th	2.7±0.06aB	2.8±0.06aB	2.9±0.06aA

a,b Different letters in the same row indicate significant difference (P<0.05). A,B Different letters at the same column indicate significant difference (P<0.05)

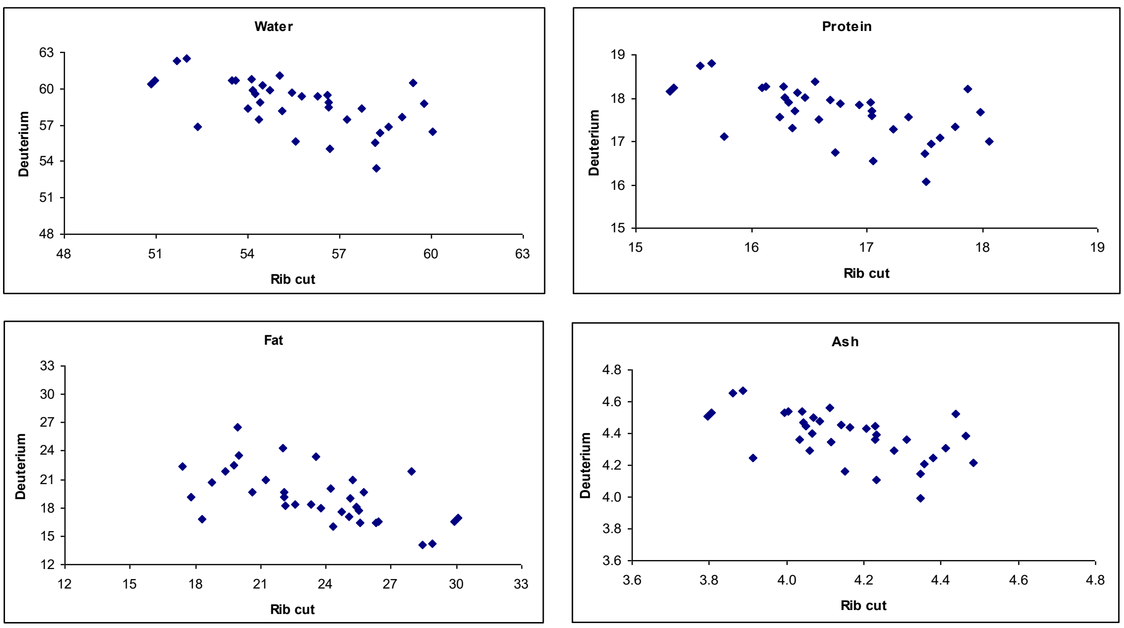

Figure 1. Relationship of empty body components (%) obtained by the deuterium or rib cut methods.

Body composition obtained by section of 9-10-11th ribs cut or deuterium space was the same for animals fed *ad libitum*; however, not for the other two groups with feed restriction. Thus, indirect methods may not identify body composition in the same way, because metabolism during fasting is influenced by the level of nutrition in the previous period, altering the expected body and weight gain composition (HANNON and MURPHY, 2019).

The differential response to nutritional plane by carcass and non-carcass tissues possibly explains some of the inconsistencies in the literature concerning nutritional modification of body composition at a constant weight (HANNON and MURPHY, 2019). In this sense, the data obtained with the deuterium method remained closer to the expected body composition for animals on feed restriction followed by re-alimentation (T60 and T75), because in this condition, internal fat deposition was greater.

Biological responses of each animal to feed restriction vary according to the level of sub-nutrition and posterior re-alimentation as well as the stage of animal development (CUNNINGHAM et al., 2018). In these cases, indirect methods that use carcass composition or subcutaneous fat may not identify these changes in fat deposition, explaining the divergence in literature data (NEVES et al., 2018; CASTILHOS et al., 2018).

Energy concentration and body composition of animals at the end of *ad libitum* feeding period was determined both by the deuterium method and by the section of 9th-10th-11th ribs cut (Figure 2).

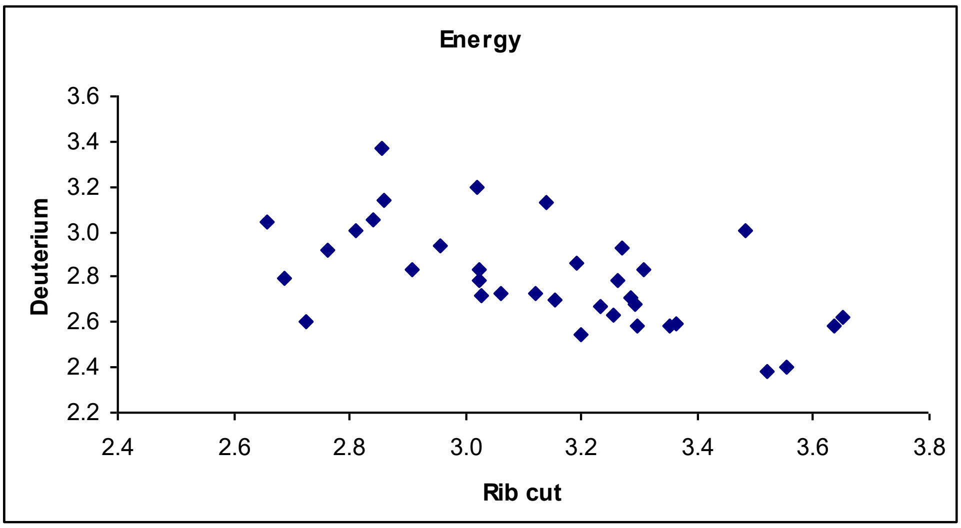

Figure 2. Energy concentration (Mcal) in empty body by the deuterium or rib cut methods.

The weight of chemical components of empty body weight was estimated in a different way by deuterium or rib cut, with higher values for all traits when estimated by rib cut (Figure 3).

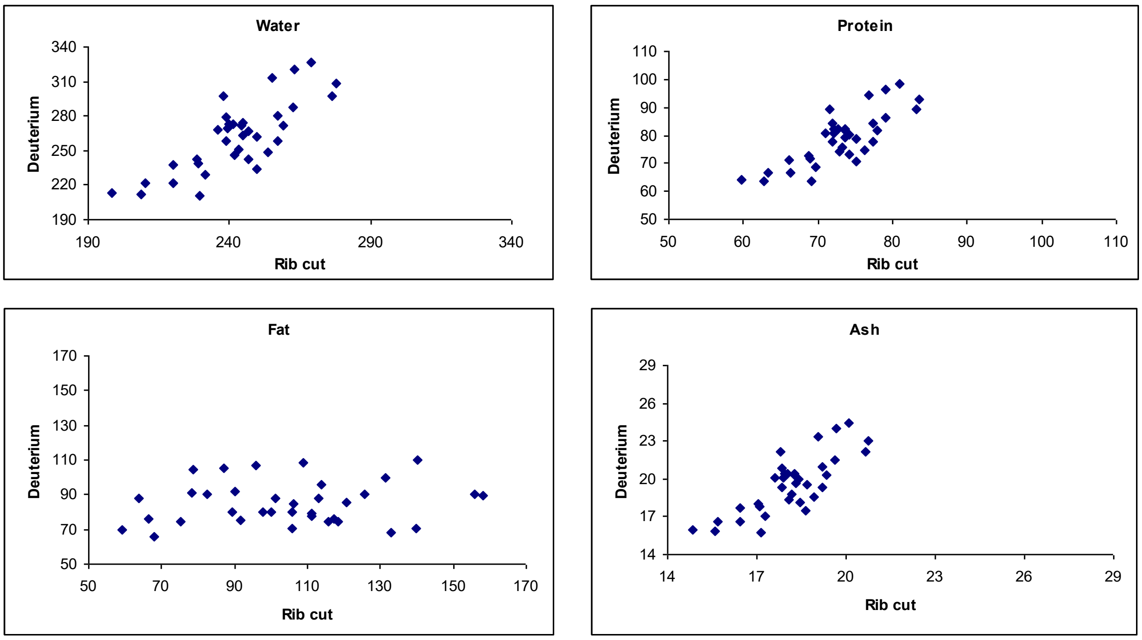

Figure 3. Chemical components (kg) of empty body weight by the deuterium or rib cut methods.

The methods used in this study differ for water, protein, and ash in empty body (P<0.01), although differences were not observed between treatments for these characteristics (P=0.15). The chemical body composition presented interaction (P<0.01) between treatments and methods only for the EE content (Figure 3). The methods showed difference for the EE content in the treatments subjected to feed restriction (P<0.01), however, there was no difference for the *ad libitum* feeding treatment (P=0.76).

The fat free empty body composition is constant, especially in animals that reached chemical maturity (NRC, 1996) and could be an equilibrium indicator of these traits, confirming the applicability of the method used. In our study, we verified the same constancy in the percentage of these elements, regardless of animals on feed restriction or on *ad libitum* feeding, with 80.1% for protein and 19.9% for ash in fat-free dry matter of empty body.

The normal growth pattern is evidenced by the fat and protein net synthesis and the extent that nutrition alters the "normal" pattern needs investigation. The increased efficiency during re-alimentation may have a connection with the change in carcass composition (COX-O'NEILL et al., 2017). In this study, animals subjected to feed restriction presented higher fat content in the body when estimated by deuterium (P<0.01), which was not verified with the estimate from the rib cut method (P=0.34).

The method of body composition prediction by water content in the rib cut was effective to determine body composition using data from the treatment without feed restriction, as different body fractions in percentage presented similar values when estimated by the deuterium or rib cut methods.

Therefore, comparison of estimates from both methods showed difference for data regarding the treatments of feed restriction, where values obtained by deuterium oxide were better adjusted to the conditions of animals subjected to compensatory gain and presented higher internal fat ($P<0.05$) at slaughter, despite the similarity observed for subcutaneous fat thickness in the treatments.

In the experimental conditions of this study, both deuterium oxide and 9th-10th-11th ribs cut methods used to estimate empty body chemical composition produced similar results for animals fed *ad libitum*; nevertheless, not for animals subjected to feed restriction, which showed no continuous growth. In this case, composition estimated by deuterium oxide was more consistent with the nutritional plane and could be used in situations where slaughter is not applicable (CASTILHOS et al., 2018).

Therefore, there is a need for models to know body composition that take into account physiological changes in the growth of animals caused by different nutritional conditions to which they were subjected (TEIXEIRA et al., 2017).

CONCLUSIONS

Comparatively, the indirect method of deuterium oxide was better than 9th-10th-11th ribs cut method to estimate body composition in Nellore steers subjected to feed restriction; however, the methods were similar for animals fed *ad libitum*.

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