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Indirect calorimetry to estimate energy requirements for growing and finishing Nellore bulls

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Abstract
Determination of nutritional requirements is the basis for diet formulation. The objectives of this study were to determine the net energy requirements for maintenance (NE_m) and weight gain (NE_g) in Nellore bulls during the growing and finishing phases, and to estimate efficiency of metabolizable energy (ME) utilization for maintenance and gain (k_m, k_g). Five Nellore bulls were housed in individual pens at the Universidade Federal de Minas Gerais (Belo Horizonte, Brazil) and evaluated over four experimental periods at 210, 315, 378 and 454 kg shrunk body weight (SBW), approximately. During each period, heat production (HP) was quantified by open circuit indirect calorimetry for three feeding levels: *ad libitum*, restricted and fasting. The NE_m requirement was determined by linear regression between the Log of HP and the ME intake (MEI) for the *ad libitum* and restricted levels. This requirement was also determined by quantifying fasting heat production (FHP). The NE_g requirement was calculated by the difference between MEI and HP during *ad libitum* feeding. The k_m and k_g were calculated by the relationship between net energy (NE) and ME requirements for maintenance and weight gain (ME_m, ME_p), respectively. The NE_m requirements per kg of metabolic empty body weight ($EBW^{0.75}$) fluctuated between 348 and 517 kJ d^{-1}, showing a decreasing trend with age, and were higher than the values reported in the literature. The NE_g requirements ranged between 48.3 and 164 kJ kg^{-1} $EBW^{0.75}$ d^{-1}, and varied according to age and weight gain. The k_m values varied between 58.6 and 69.7%, while k_g varied between 23.4 and 40.2%. We concluded that NE_m and NE_g requirements were influenced by age and possibly by the level of stress, nervousness and activity of animals into the respirometry chamber. Further studies should quantify HP with records of positional changes (time spent standing *vs*. lying down). Additionally, HP quantification should be repeatedly performed in the same experimental period to obtain a representative value of NE_g requirements.

Keywords: calorimetry, efficiency of energy utilization, energy requirements, Zebu cattle

1. Introduction

Energy is the most limiting factor for animal productivity. In 1963, Lofgreen and Garrett introduced a net energy (NE) system for the growing and finishing phases of beef cattle, which separates NE requirements for maintenance (NE_m) from those for weight gain (NE_g) (Lofgreen and Garrett

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1968). This system is the basis of the NRC (2000) model. Determination of nutritional requirements is the basis of diet formulation and is aimed at increasing the expression of genetic potential and improving feed efficiency. Accurate nutritional requirements would promote productive, economic and environmental viability of beef cattle farming.

Between 65–70% of the total energy needed for meat production is used to meet maintenance requirements (Ferrell and Jenkins 1985). The literature suggest that *Bos indicus* have lower energy requirements for maintenance than *Bos taurus* breeds (NRC 2000; Sainz *et al.* 2013). Regarding the requirements for weight gain, Marcondes *et al.* (2010a) indicated that smaller breeds at maturity (i.e., with faster adipose tissue accretion, such as Zebu cattle) have, at the same absolute weight and gain rate, higher NE_g requirements than *B. taurus*.

Brazil has the largest cattle herd in the world with 212.3 million head. Nellore breed and its crosses entail about 50% of this population, which contribute 80% of meat production in the country (IBGE 2015). Given the importance of this breed, determining energy requirements will improve productive efficiency of the herd and the economic return to the farmer. Energy requirements can be determined by calorimetry or comparative slaughter. The latter technique is destructive, laborious and can lead to sampling errors. Furthermore, it requires more animals and time for gathering the data. Calorimetry allows for multiple measurements on the same animal, thus reducing the random error. Calorimetry is questioned for underestimating heat production (HP) *vs*. comparative slaughter, and because it works with animals confined into respirometry chambers (Miller and Koes 1988; Patience 2012). Since comparative slaughter is widely used in Brazil to determine requirements in Nellore cattle, it is necessary to evaluate calorimetry as an alternative technique.

The objectives of this study were to determine NE requirements for maintenance and weight gain in Nellore bulls by calorimetry during growing and finishing, and to establish efficiency of metabolizable energy (ME) utilization for both functions.

2. Materials and methods

2.1. Animals and experimental facilities

The experiment was conducted in the Veterinary School of Universidade Federal de Minas Gerais (UFMG), in Belo Horizonte (Brazil), between June 30, 2008 and January 12, 2010. Belo Horizonte is located at an altitude of 900 m, with 23°C average temperature, 65% relative humidity, and 1600 mm annual rainfall, characterizing a tropical altitude climate.

Five Nellore bulls with (180±12.4) kg initial body weight (BW) and (10±0.5) mon of age, were individually housed in 3-m^2 covered pens. Animals were adapted to handling, diet, and the respirometry chamber during the first 2 mon. The energy balance tests and determination of requirements started after the adaptation period. The tests included apparent digestibility, urine collection and calorimetry, and were conducted during four different periods, each one lasting 4 mon, approximately. Average shrunk body weight (SBW) in each period was 210, 315, 378, and 454 kg, corresponding to 13.6, 17.8, 21.9, and 26.3 mon of age.

2.2. Feed

The diet consisted of Tifton 85 (*Cynodon* spp.) hay, mineral salt, and a corn-soybean meal supplement. It was formulated to ensure an average daily gain (ADG) of 700 g per animal, following recommendations by Marcondes *et al.* (2010a, b). The roughage/concentrate ratio varied depending on the age of the animals. Diet composition is presented in Table 1.

Animals were fed twice a day at 8:00 and 17:00 throughout the experimental period when they were in the barn, and once a day (at 8:00 h) when they were into the chamber. The amount of feed offered changed, depending on the objectives of the experiment, with *ad libitum* and restricted feeding periods. The daily amount of feed offered was adjusted during *ad libitum* feeding so that rejected feed (orts) represented between 5 and 10% of the offering. Orts were daily weighed and sampled for dry matter (DM) analysis. The dry matter intake (DMI) was calculated as the difference between feed offered and orts. Animals were fed at 1.15 times the NRC (2000) maintenance requirements (0.32 MJ NE_m kg^{-1} metabolic empty body weight, $EBW^{0.75}$) during restricted feeding. The same diet was offered to all animals, changing only the amounts offered.

2.3. Animal handling

Before starting the experiment, animals were treated with ivermectin (1%, w/v, Ourofino, Cravinhos, Brazil) and received an injection of vitamins A, D_3 and E (A-D-E Injetável Emulsificável, Pfizer, São Paulo, Brazil). All animals were weighed at the beginning of the experiment and at 14 d intervals until the end. To determine average SBW, animals were weighed at the same times during 2 consecutive days under a previous 12-h fasting. Additional weighings were conducted during calorimetric measurements.

A performance test was conducted for at least 30 d in each period, prior to the digestibility trials, recording SBW and *ad libitum* DMI. These data were extrapolated to data obtained in the respirometry chamber to estimate NE_g of feed and efficiency of ME utilization for weight gain (k_g).

Table 1 Diet composition and nutrient analysis

Ingredients (as DM%)[1)]	Period							
	1		2		3		4	
Hay (*Cynodon* spp.)	60.0		60.0		70.0		80.0	
Corn	20.0		25.0		18.0		10.0	
Soybean meal	20.0		15.0		12.0		10.0	
Analized nutrient composition (as DM%)[2)]	Hay	Diet	Hay	Diet	Hay	Diet	Hay	Diet
DM	90.1	89.7	89.9	88.9	90.6	89.6	90.6	90.1
CP	7.67	14.5	8.86	15.1	8.09	11.8	11.5	14.5
EE	0.86	1.58	1.92	2.4	1.78	2.17	1.68	2.06
Ash	5.81	5.42	6.31	5.8	6.48	6.47	6.52	6.37
NDF_p	80.4	55.5	70.9	48.4	73.1	56.7	77.2	65.1
ADF	40.6	27.3	33.2	22.2	35.8	27.7	38.4	32.1
CHO_t	85.7	78.5	82.9	76.7	83.7	79.5	80.4	77.1
NFC	5.3	23.0	12.0	28.3	10.6	22.8	3.2	12.0
GE (MJ kg^{-1} DM)	18.9	19.0	18.4	18.4	18.5	18.5	18.8	18.8

[1)] Mineral balance, based on recommendations by Gionbelli *et al.* (2010), determined the amount of the mineral salt offered. Composition per kilogram of mineral salt: 160 g Ca, 60 g P, 110 g Na, 10 g Mg, 50 g S, 82 mg Co, 800 mg Cu, 120 mg I, 3 600 mg Mn, 27 mg Se, 5 200 mg Zn, 4 700 mg Fe, and 600 mg (max) F.

[2)] DM=dry matter; CP=crude protein; EE=ether extract; NDF_p=neutral detergent fiber corrected for protein; ADF=acid detergent fiber; CHO_t=total carbohydrates; NFC=non-fiber carbohydrates; GE=gross energy. The same as below.

2.4. Digestibility trials

The apparent digestibility trials were conducted during the four periods tested, before animals went through the respirometry chamber. Animals were fed *ad libitum* and fecal DM (kg d^{-1}) was determined by total collection during 5 consecutive days. Gross energy (GE) of feces was determined on a composite sample collected directly from the anus twice a day (AM and PM) during the 5 d of collection. Percent of digestible energy (DE) was calculated as DE (%)=[(Energy consumed–Energy in feces)/Energy consumed]×100

2.5. Urine collection

Simultaneously with the digestibility trial, urine samples from all animals were obtained during 5 consecutive days by spontaneous micturition or stimulation, approximately 4 h after the morning feeding (Valadares *et al.* 1999). Urine aliquots (100 mL) were collected in jars containing 5 mL 2% HCl and kept frozen at –10°C. Analysis was conducted on a composited sample per animal, obtained at the end of the 5 d.

Urine volume was determined at the end of the digestibility trial, the day before animals entered the respirometry chamber. Urine output was determined by total collection during 24 consecutive hours using a metabolic cage fitted with a plastic tarp funnel, and measured in a graduated cylinder.

2.6. Open-circuit indirect calorimetry

Once digestibility trials and urine collections were completed, animals went through the respirometry chamber for quantification of heat production (HP). The calorimetry equipment has three functional parts: chamber, flow generator, and gas analysis stream (consisting of gas multiplexer, gas analyzer subsampler and gas analyzer). The chamber had side windows and was made of galvanized steel externally covered with polyurethane. Internal dimensions of the chamber were 3.5 m length by 2.5 m width by 2.5 m height, resulting in 21.9 m^3 total volume. The chamber has an air inlet (atmospheric) and outlet. Outgoing air was continuously suctioned at a controlled rate (1 L min^{-1} kg^{-1} SBW) by a mass-flow system (Flowkit-500, Sable Systems International, Las Vegas, NV, USA) that automatically corrected air volume (flow) for temperature, pressure and humidity (RH-300 Analyzer, Sable Systems International). An electronic device equipped with electrovalves (RM8 Intelligent Multiplexer; Sable Systems International) samples both chamber and atmospheric air and sends it to the gas analyzers in an alternative manner. A software automatically records the results (ExpeData-UI2, Sable Systems International). Carbon dioxide (CO_2) and methane (CH_4) concentration were measured with infrared analyzers (CA-10 Carbon Dioxide Analyzer and MA-10 Methane Analyzer, Sable Systems International) with a range of 0.0001 to 10% and 0.001 to 5% for both gases, respectively. Oxygen (O_2) concentration was measured with a paramagnetic analyzer (FC-10A Oxygen Analyzer, Sable Systems International) with a range of 0.0001 to 100%. Gas analyzers (CO_2, CH_4, O_2) were daily calibrated with commercially prepared gases of known concentrations (5% CO_2/95% N_2, 1% CH_4/99% N_2, 21% O_2/79% N_2, respectively; IBG, Sao Paulo, Brazil) before starting gas exchange measurements. Efficiency of the system was checked by injecting pure gases (CO_2, CH_4, and N_2) before starting the study. Injection took 4 h and gas volume was obtained gravimetrically. Measuring stopped when gas concentration inside the chamber equilibrated with the outside air. Calibration factors were calculated by comparing injected with detected gas volume (Rodriguez *et al.* 2007).

The HP was assessed for each period and animal under three feeding levels: *ad libitum* (production level), restricted (maintenance), and fasting level. The HP was quantified in 2 non-consecutive days for each feeding level (obtaining the mean value), except for fasting where HP was quantified only 1 d. The DMI was lowered to 1.15 times the energy requirements for maintenance immediately after quantifying *ad libitum* HP (ALHP). The animals were weighed every week to control the amount of feed needed for maintaining BW. This feed intake lasted for at least 3 wk, following recommendations by the CSIRO (2007), before assessing HP at the maintenance level (MHP). After determining MHP, animals were totally deprived of feed (but not water) for 3 d. Fasting heat production (FHP) was measured on the 3rd d.

Heat production calculation was based on continuous measurement (every 10 min) of O_2 consumption, and CO_2 and CH_4 production over a 22-h period, extrapolating the results to a 24-h period. The classical equation by Brouwer (1965) was used to estimate the HP resulting from respiratory exchange (liters, L), regardless of nitrogen (N) excretion: HP (kJ)=$16.18O_2+5.02CO_2–2.17CH_4$. Elimination of urinary N was justified by the difficulty for collecting urine inside the chamber and because it is generally expected to contribute <1% of the total HP (McLean 1972).

Animals were weighed when entering and exiting the chamber, and the average of both values was used to express HP results. The mean temperature inside the chamber across the experiment was (22.1±0.93)°C, and the mean relative humidity was (63.5±3.72)%.

Once FHP was quantified, animals received an intramuscular dose of B1 vitamin (Fortemil®, Ourofino, Cravinhos, Brazil) to prevent health problems, and the feed offer was gradually increased until the *ad libitum* level was reached. Measurements corresponding to the next period (feed intake, weight gain, and the tests previously described) started approximately 30 d after FHP quantification.

2.7. Laboratory procedures

Samples were analyzed at the Animal Nutrition Laboratory of the Veterinary School, UFMG. Feed samples, orts and feces were partially dried in a forced-air oven at 60°C for 72 or 96 h, ground in a Wiley Laboratory Mill (Arthur H. Thomas Co., Philadelphia, PA) through a 1-mm screen and stored in sealed plastic bottles for subsequent chemical analysis. Feces and orts were analyzed as composited samples for each animal. Samples were composited according to the daily proportion of DM excreted in feces or present in the orts. Feed was analyzed for DM (AOAC 930.15), crude protein (CP) (AOAC 984.13), ether extract (EE) (AOAC 954.02), and ash (AOAC 942.05), according to AOAC (1990). Neutral detergent fiber (NDF) was analyzed using heat stable amylase according with Van Soest *et al.* (1991), and acid detergent fiber (ADF) according to AOAC (1997; AOAC 973.18). The GE was analyzed in an adiabatic bomb calorimeter. Neutral detergent insoluble nitrogen (NDIN) was determined by the Kjeldahl procedure on the NDF residue, with NDF corrected for protein (NDF_p). Total carbohydrates (CHO_t) were obtained by the equation: CHO_t=100–(% CP +% EE+% Ash). Non-fiber carbohydrates (NFC) were obtained by the difference: $CHO_t–NDF_p$. Samples of urine, feces and orts were only analyzed for DM and GE content.

Polyethylene plastic cups of known weight and energy content were used to determine DM and GE in urine. Accordingly, a 10-mL urine aliquot was weighed, dried in a forced-air oven for 96 h, and then burned in the calorimeter, subtracting the interference from the cups.

2.8. Data analysis

Linear regression equations relating *ad libitum* or restricted SBW (*x*) *vs*. BW after 72 h fasting (*y*) were constructed for all periods. The equations were used to predict empty body weight (EBW). Then, EBW raised to 0.75 allowed to express the energy balance results in terms of $EBW^{0.75}$.

The DE intake (DEI) was obtained as the difference between GE intake and energy losses through feces. The ME intake (MEI) was determined by difference between DEI and energy losses through urine and methane, calorimetrically quantified at *ad libitum* feeding. Methane energy content was established at 39.5 kJ L^{-1} (Nkrumah *et al.* 2006). Energy density of feed in terms of DE and ME, expressed in MJ kg^{-1} DM, was obtained by dividing DEI and MEI between DMI *ad libitum*, respectively.

Linear regression models between the logarithm of HP (*y*) and MEI (*x*) (kJ kg^{-1} $EBW^{0.75}$ d^{-1}), LogHP=a+b×MEI, measured in the respirometry chamber at *ad libitum* and maintenance feeding levels, were used to establish the NE_m requirement, which corresponded to the antilog of the intercept and level zero of MEI (Lofgreen and Garrett 1968). This value was compared with the NE_m requirement, directly quantified in the chamber, corresponding to FHP. The MEI required for maintenance (MEI_m) was determined by iteration, from the linear regression equations previously obtained, at the point where HP matched MEI (kJ kg^{-1} $EBW^{0.75}$; Lofgreen and Garrett 1968; Tedeschi *et al.* 2002). The amount of DM required (g kg^{-1} $EBW^{0.75}$) to cover maintenance needs (DMI_m) corresponded to MEI_m (kJ kg^{-1} EBW$^{0.75}$) divided by the ME density of the diet (kJ g^{-1} DM). Then, the NE_m requirement (estimated by linear regression or directly determined in the chamber) was divided by DMI_m to estimate energy density of the feed, in terms of NE_m kg^{-1} DM.

The NE_g, expressed in kJ kg^{-1} EBW$^{0.75}$ d^{-1}, corresponding to the retained energy (RE), was calculated by subtracting ALHP from MEI recorded in the respirometry chamber at *ad libitum* feeding, as described by Nkrumah *et al.* (2006).

The energy density of the diet, in terms of NE_g kg^{-1} DM, was estimated from the differential test described by Lofgreen and Garrett (1968) which established energy retention under two feeding levels. The *ad libitum* DMI was considered as the first feeding level and resulted in the NE_g value determined in the chamber. The DMI corresponding to energy balance (DMI_m) was the second feeding level and corresponded to RE=0. The NE_g kg^{-1} DM was obtained from the relationship between NE_g (kJ kg^{-1} EBW$^{0.75}$) and the difference between the average of *ad libitum* DMI (g kg^{-1} EBW$^{0.75}$) and DMI_m (g kg^{-1} EBW$^{0.75}$). The average *ad libitum* DMI was obtained from the performance tests conducted in each period. The EBW$^{0.75}$ on which *ad libitum* DMI was expressed corresponded to the average EBW recorded during the tests, and was obtained as: [(Initial EBW+Final EBW)/2]$^{0.75}$.

The efficiency of ME utilization for maintenance (k_m) and k_g were calculated as the relationship between requirements (kJ kg^{-1} EBW$^{0.75}$) of NE_m/ME_m and NE_g/ME_p, respectively, where ME_m and ME_p corresponded to ME for maintenance and production, respectively. The ME_p resulted from the difference between *ad libitum* MEI–MEI_m.

2.9. Statistical analysis

Data were analyzed with the MIXED procedure of SAS (2001) to evaluate the fixed effect of the measurement period and the random effect of the animal. Comparison of means were conducted with the ESTIMATE command. A descriptive statistic analysis, including mean and standard deviation, was also made for all response variables.

3. Results

Regression equations for estimating EBW on the basis of SBW, given *ad libitum* or restricted feeding, and their coefficients of determination (R^2) are shown in Table 2. All animals were included for each equation and a significant linear effect was observed (P<0.05) for all equations. Regression equations showed good fit to the data (R^2>90%).

The average SBW and *ad libitum* DMI during the performance tests are presented in Table 3. The SBW increased with age and was statistically different between experimental periods. The DMI (kg d^{-1}) was lower in Period 1; however, it was lower in Period 4 when expressed as g kg^{-1} EBW$^{0.75}$ d^{-1}, and as a percentage of SBW.

The energy balance during all the periods is presented in Table 4. Each value corresponds to an average of the five experimental units. In correspondence with DMI (kg d^{-1}), intake of GE, DE and ME (MJ d^{-1}), and the HP and energy balance (MJ d^{-1}) were markedly lower in Period 1. When en-

Table 2 Regression equations of empty body weight (EBW) *vs.* shrunk body weight (SBW) under two feeding levels and four experimental periods in Nellore bulls

Feeding level	Period	Equation	R^2	*P*-value
Ad libitum	1	EBW=19.4+0.816SBW	94.4	0.0049
	2	EBW=48.3+0.777SBW	92.8	0.0084
	3	EBW=93.6+0.716SBW	95.7	0.0039
	4	EBW=25.2+0.910SBW	97.6	0.0016
Restricted	1	EBW=29.1+0.769SBW	98.7	0.0005
	2	EBW=44.2+0.804SBW	96.1	0.0028
	3	EBW=16.1+0.911SBW	99.0	0.0004
	4	EBW=21.2+0.924SBW	97.5	0.0017

Table 3 SBW and *ad libitum* dry matter intake (DMI) of Nellore bulls, expressed as kg d^{-1}, g kg^{-1} metabolic empty body weight (EBW$^{0.75}$) d^{-1} and % SBW in four experimental periods

Item	Period							
	1		2		3		4	
	Mean	SD[1)]	Mean	SD	Mean	SD	Mean	SD
SBW (kg)	210 d	18.3	315 c	17.1	378 b	25.3	454 a	25.2
DMI (kg d^{-1})	5.37 c	0.78	8.13 b	0.64	8.65 a	1.07	8.35 ba	0.55
DMI (g kg^{-1} EBW$^{0.75}$ d^{-1})	105 b	11.6	115 a	6.81	104 b	9.88	87.1 c	4.04
DMI (% SBW)	2.55 a	0.27	2.58 a	0.14	2.29 b	0.18	1.84 c	0.09

[1)] SD=standard deviation. The same as below.
Within a row, means without a common letter differ (P<0.0001).

ergy partition was expressed as a percentage of GE intake, significant differences between periods showed variations compared with the partition in absolute terms (MJ d^{-1}).

The NE_m, NE_g and ME_m requirements (kJ kg^{-1} $EBW^{0.75}$ d^{-1}), as well as k_m and k_g (coefficient) are shown in Table 5. The NE_m requirement was greater in Period 1 ($P<0.05$). Except for Period 1, dispersion of the mean value obtained for NE_m was lower when determined by FHP compared with the regression estimate. The NE_g requirement was higher in Period 3, which presented statistical difference with Period 1 ($P<0.05$). The k_m values were different ($P<0.05$) between Periods 2 and 3 when NE_m was determined from FHP.

Energy density of the diets (MJ kg^{-1} DM) is presented in Table 6. Dispersion of mean values varied with the energy expression used. Standard deviations were lower when energy density was expressed in terms of GE versus NE. The NE_m values had lower dispersion than NE_g.

4. Discussion

4.1. Prediction equations of empty body weight from shrunk body weight

The regression equations (Table 2) resulted in average EBW/SBW ratios of 0.899, 0.925, 0.937 and 0.963 for *ad libitum* feeding in Periods 1, 2, 3 and 4, respectively. The EBW/SBW ratio for restricted feeding was 0.894, 0.942, 0.949 and 0.969 in Periods 1, 2, 3 and 4, respectively. According to Owens *et al.* (1995), this ratio can vary from 85 to 95%. The NRC (2000) mentioned a 0.891 ratio, which is

Table 4 Energy balance for Nellore bulls fed *ad libitum* in four experimental periods

Item	Period							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy intake (gross energy, GE, MJ d^{-1})	91.3 c	13.3	132 b	10.5	143 a	17.7	142 a	9.41
Fecal energy (MJ d^{-1})	31.4 c	6.3	42.6 b	5.0	46.6 a	7.2	48.4 a	7.7
Digestible energy (DE, MJ d^{-1})	59.9 c	7.41	89.4 b	5.98	96.4 a	11.8	93.6 ab	3.22
Proportion of GE[1)]	0.656 c	0.026	0.677 a	0.017	0.675 ab	0.025	0.660 bc	0.034
Urinary energy (MJ d^{-1})	3.10 b	0.50	5.56 a	2.30	4.60 ab	1.84	4.44 ab	2.34
Proportion of GE[1)]	0.034 a	0.008	0.042 a	0.016	0.032 a	0.013	0.031 a	0.016
Methane energy (MJ d^{-1})	5.94 b	0.71	6.99 ab	0.67	8.37 a	1.84	8.08 a	1.76
Proportion of GE[1)]	0.065 a	0.006	0.053 b	0.008	0.058 ab	0.009	0.057 ab	0.013
Metabolizable energy (ME, MJ d^{-1})	50.8 b	7.07	76.9 a	5.56	83.4 a	10.7	81.0 a	3.47
Proportion of GE[1, 2)]	0.557 b	0.030	0.581 a	0.020	0.584 a	0.028	0.572 ab	0.033
Proportion of DE[1, 2)]	0.849 a	0.022	0.859 a	0.017	0.865 a	0.027	0.866 a	0.036
Heat production (MJ d^{-1})	48.2 b	5.69	68.7 a	6.74	69.2 a	8.54	70.0 a	5.19
Proportion of GE[2)]	0.529 a	0.020	0.519 ab	0.046	0.484 b	0.022	0.494 b	0.033
Proportion of ME[2)]	0.949 a	0.054	0.893 ab	0.054	0.829 b	0.064	0.864 b	0.058
Energy balance (MJ d^{-1})	2.59 b	2.89	8.20 ab	4.14	14.3 a	5.98	11.1 a	4.77

1) Expressed as coefficient.
2) Metabolicity, *ad libitum* feeding level.
Within a row, means without a common letter differ ($P<0.05$). The same as below.

Table 5 Energy requirements (in kJ kg^{-1} $EBW^{0.75}$ d^{-1}) and efficiency of metabolizable energy for maintenance (k_m) and weight gain (k_g) for four experimental periods in Nellore bulls

Item[1)]	Period							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NE_m[2)]	485 a	62.9	386 b	66.7	385 b	44.7	352 b	111
NE_m[3)]	517 a	107	393 b	14.4	410 b	29.4	348 b	38.9
ME_m	802 a	52.1	659 b	82.5	595 bc	69.7	544 c	110
NE_g	48.3 b	53.6	104 ab	62.1	164 a	76.7	111 ab	56.3
k_m[2, 4)]	0.604 a	0.054	0.586 a	0.066	0.647 a	0.027	0.639 a	0.111
k_m[3, 4)]	0.647 ab	0.137	0.603 b	0.069	0.697 a	0.087	0.650 ab	0.070
k_g[4)]	0.234 a	0.201	0.254 a	0.165	0.395 a	0.114	0.402 a	0.273

1) NE_m=net energy for maintenance, ME_m=metabolizable energy for maintenance, NE_g=net energy for weight gain, k_m=efficiency of metabolizable energy utilization for maintenance, k_g=efficiency of metabolizable energy utilization for weight gain, $EBW^{0.75}$=metabolic empty body weight.
2) Estimated from linear regression between heat production (HP) and metabolizable energy intake (MEI) at *ad libitum* and maintenance feeding levels.
3) Directly established in the respirometry chamber using animals fasted between 48 and 72 h.
4) Expressed as coefficient.

Table 6 Energy density of diets in four experimental periods (MJ kg^{-1} DM)

Item	Period							
	1		2		3		4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GE	17.0 a	0.05	16.3 c	0.02	16.5 b	0.02	17.0 a	0.02
DE	11.2 a	0.46	11.0 a	0.29	11.1 a	0.42	11.2 a	0.59
ME	9.46 a	0.54	9.46 a	0.33	9.67 a	0.46	9.71 a	0.54
NE_m [1)]	5.73 a	0.42	5.52 a	0.46	6.23 a	0.38	6.23 a	1.17
NE_m [2)]	6.11 ab	1.26	5.73 b	0.84	6.74 a	1.13	6.36 ab	1.00
NE_g	2.26 a	1.97	2.38 a	1.46	3.85 a	1.21	3.89 a	2.51

[1)] Estimated from linear regression between heat production (HP) and metabolizable energy intake (MEI) at *ad libitum* and maintenance feeding levels.
[2)] Directly established in the respirometry chamber using animals fasted between 48 and 72 h.

close to the figure by Marcondes *et al.* (2010a) for Nellore cattle (0.895). The growing trend observed for the EBW/SBW ratio may be due to the reduction in gastrointestinal size and, therefore, its digesta contents as animals age. According to Putrino *et al.* (2006), a reduction in the weight proportion of viscera, liver and especially the digestive tract, is observed when animals reach maturity.

Since the goal of any mathematical model is to predict a biological phenomenon, that prediction is only valid for the data range that used for the equation. For this reason, the intercepts of equations in Table 2 have no biological meaning. They only represent a mathematical adjustment. Regression slope is a mathematical reason expressing the variation of EBW as SBW changes. For restricted feeding, EBW/SBW becomes narrower as age increases, indicating a lower difference in magnitude between EBW and SBW. That trend was not observed when animals were fed *ad libitum*, indicating that this feeding level influences the emptying of the gastrointestinal tract during the fasting period prior to weighing the animals.

4.2. Body weight and dry matter intake

The changes in DMI (Table 3) as a percentage of SBW can be explained by age. Voluntary DMI increases with SBW, but not linearly. Therefore, intake decreases when expressed as a percentage of SBW, which agrees with results by Costa e Silva *et al.* (2013). It is also in agreement with the results of Forbes (1995), who states that growing cattle intake plotted against metabolic weight ($BW^{0.73}$) results in a steady decline with increasing weight. Decreasing DMI (g kg^{-1} $EBW^{0.75}$ d^{-1}) with age could be explained by a reduction in NE_m requirements. Nie *et al.* (2015) reported higher DMI (g kg^{-1} $SBW^{0.75}$ d^{-1}) in ewes during the early compared to the late fattening period, and NE_m requirements also decreased with age, from (260.62±13.21) to (250.61±11.79) kJ kg^{-1} $SBW^{0.75}$ d^{-1}. Although fattening was not evaluated in this study, as fatness increases with BW, and since body fat content can regulate animal intake, a decrease in intake per SBW can be related to an increase in fatness. Lieblelt *et al.* (1965) demostrated the lipostatic control of intake by surgically removing part of the inguinal fat in rats, which resulted in hiperfagia, with later return to the pre-surgery status of fat and body weight.

The highest DMI (g kg^{-1} $EBW^{0.75}$ d^{-1}) occurred in Period 2, while intermediate values were obtained for Periods 1 and 3, and the lowest in Period 4 (Table 3). These values decreased as NDF_p increased (as a percentage of the diet) (Table 1). Voluntary DMI by ruminants may be limited by distention resulting from restricted digesta flow through the gastrointestinal tract. Animal capacity depends on the weight and volume of digesta that causes distention and the flowing rate of digesta at the organ in which distention occurs.

4.3. Energy balance

Apparent GE digestibility decreased (Table 4) with increasing NDF_p content in roughage, regardless of its participation in the diet (Table 1). This behavior was not observed for DE (expressed as a proportion of GE) in relation with NDF_p in the total ration. For Period 4, characterized by greater roughage and NDF_p inclusion (800 and 651 g kg^{-1} DM, respectively), DE was not different (P>0.05) compared to Periods 1 and 3, which had lower roughage/concentrate ratios (60/40 and 70/30, respectively) and lower NDF_p levels (555 and 567 g kg^{-1} DM, respectively). A similar situation occurred for Periods 2 and 3, which differed in roughage/concentrate ratios (60/40 and 70/30, respectively) and NDF_p levels (484 and 567 g kg^{-1} DM, respectively), but had similar DE (P>0.05). From these results we conclude that fiber contents in roughage was the factor that most negatively influenced energy digestibility.

The ME (expressed as a proportion of GE) followed the same dynamics of apparent DE. This supports the NRC (2000) premise indicating that DE and ME are highly correlated, which is why energy losses in urine and gases can be highly predictable from DE. According to Van Soest

(1994), DE losses are about 5–12% in methane and 3–5% in urine. Urinary energy losses in this study ranged from 4.74 to 6.22%, while energy losses in the form of methane ranged from 7.82 to 9.92% of DEI.

The lack of difference (*P*>0.05) between urinary energy losses (as a proportion of GE) supports the assertion by Van Soest (1994), who indicated that they are relatively constant. The difference (*P*<0.05) for methane production (as a proportion of GE) between Periods 1 and 2 reinforces the concept that fibrous diets lead to increased gas production. Period 1 had higher NDF_p in roughage and diet compared with Period 2 (Table 1).

Energy losses in urine and gas accounted between 8.80 and 9.90% of GE intake, and between 13.4 and 15.1% of DE (Table 4). The ARC (1980) suggests that ME represents approximately 82% of the DE, while CSIRO (2007) and NRC (2000) suggest 81 and 80%, respectively. The higher values observed in this study, between 84.9 and 86.6%, are in the range reported by the AFRC (1993): between 81 and 86%.

The RE, estimated as the difference between MEI-HP, corresponded to 2.84, 6.20, 9.99 and 7.79% of the GE intake in Periods 1, 2, 3 and 4, respectively (Table 4). These low percentages were due to the high HP with regard to ME, fluctuating between 82.9 and 94.9%. Similar values, between 75 and 91%, were obtained by Lapierre *et al.* (1992). In the present study, HP accounted for the largest energy loss — between 48.4 and 52.9% of the GE intake.

4.4. Energy requirements for maintenance and weight gain

Requirements of NE_m and ME_m The decrease of maintenance requirements with increasing BW (Table 5) can be explained by the lower weight proportion of organs and body protein as age increases. Ferrell (1988) noted that heart, liver, kidney, digestive tract and nervous tissues receive a high proportion of the cardiac output and use a high amount of the total energy expenditures relative to their mass compared with muscle, adipose, skin or other tissues. In relation to body protein, it is much more metabolically active than fat tissue and may account for differences in maintenance requirements per kilogram between development stages.

In the classic comparative slaughter experiment by Lofgreen and Garrett (1968) using *B. taurus* steers and heifers, NE_m requirements were estimated to be 322 kJ kg^{-1} $EBW^{0.75}$, figure accepted by the NRC (2000). In the present study, regardless of the method for estimating NE_m and the evaluated period, results exceeded the reference value suggested by those researchers. This difference increases considering that NRC (2000) and CSIRO (2007) maintenance requirements for *B. indicus* are 10 and 20% lower, respectively, than for *B. taurus* because of lower genetic potential for production of *B. indicus*. However, several literature reports do not support the above concept. Tedeschi *et al.* (2002) indicated that Nellore steers and bulls (*B. indicus)* have the same NE_m requirements as the NRC (2000). Chizzotti *et al.* (2008), through a meta-analysis using 16 comparative slaughter studies, determined that the NE_m requirement of Nellore purebred and Nellore×*B. taurus* crossbreds growing bulls, steers, and heifers was 314 kJ kg^{-1} $EBW^{0.75}$. Freitas *et al.* (2006) found no differences in NE_m requirement among Nellore purebred and Nellore×Angus, Nellore×Brown Swiss, and Nellore×Simmental growing bulls, which was 331 kJ kg^{-1} $EBW^{0.75}$. Ferrell and Jenkins (1998) reported a NE_m requirement of 312 kJ kg^{-1} $EBW^{0.75}$ for *B. indicus* crossbred steers.

In the present study, stress, nervousness and activity of animals into the respirometry chamber could mostly explain the increased NE_m demands. According to Forbes (1925), the maintenance requirement while standing is about 16% higher compared to lying down. According to ARC (1980), heat emission of standing animals can increase up to 70%. For future studies, it is suggested to record the time spent standing and lying down, and correct HP based on this. Although the animals were acclimated to the respirometry chamber before starting the experiment, and they went through it repeatedly during the experimental periods, animal behavior was altered with respect to the former housing conditions. In this regard, ARC (1980) stated that fasting metabolism can be increased to 30% when animals are not accustomed to the experimental procedure. Analysis of serial experiments by Blaxter (1967) suggests that day- to-day variations in heat production may be ±2l8 kJ per 24 h, reflecting differences in animal activity.

The FHP was determined after restricted feeding lasting 35.6, 43.2, 57.6 and 52 d for Periods 1, 2, 3 and 4, respectively, obtaining daily gains of 83.4, –138, 191 and 61.5 g, respectively. Other possible explanation for the greater NE_m demands is the length of the restrictive feeding periods, which was not enough to decrease metabolic rate, although it exceeded the minimum of 21 d suggested by the CSIRO (2007) and weight gain was reduced compared with *ad libitum* feeding. According to Williams and Jenkins (2003), production increases vital functions, pointing to the fact that energy processes (i.e., digestion, circulation, drainage, maintenance of concentration gradients, muscle tone, dynamic replacement tissue) ensured by food for true maintenance (constant BW and body composition) are increased in the productive animal, in line with its higher nutrition level. Ferrell and Jenkins (1985) indicated that the proportion of tissues such as liver, gastrointestinal tract and kidney increase in response to nutritional level, thereby increasing FHP.

Williams and Jenkins (2003) defined ME_m requirement as the amount of ME that will exactly balance HP and results in no loss or gain of body energy reserves. The literature reports values (expressed in kJ kg^{-1} $EBW^{0.75}$ d^{-1}) of 470 and 522 for confined and grazing animals, respectively (Marcondes *et al.* 2010a), and 494 (Tedeschi *et al.* 2002), 501 (Ferrell and Jenkins 1998) and 469 (Chizzotti *et al.* 2008) on purebred and crossbred Zebu cattle. The values obtained in the present study, especially in Periods 1 and 2, are greater than those reports (Table 5), which was expected, once ME_m requirements were derived from NE_m requirements.

NE_g requirements A 115% increase in NE_g requirements was observed between Periods 1 and 2, and 58% between Periods 2 and 3. However, a 33% decrease was observed for Period 4 over the previous period (Table 5). The energy required for growth corresponds to the caloric value of tissues, which is a function of fat and protein accretion. Protein and water percentage decrease as animal weight increases, while fat and energy increase. Fat caloric value is higher than that of protein (39.2 kJ g^{-1} *vs.* 23.8 kJ g^{-1}).

The decrease in Period 4 is paradoxical, since maintenance requirements as a proportion of BW decreased with age, which should have reflected more energy invested in carcass growth, particularly in the form of fat deposition. However, the ADG during *ad libitum* feeding in Periods 1, 2, 3 and 4 (1 070, 1 109, 1 206, and 848 g $animal^{-1}$, respectively) explained the dynamics of NE_g requirement until Period 3 and its reduction for Period 4. The same trend was shown in the energy balance (RE) (Table 4).

While the NE_g values (kJ kg^{-1} $EBW^{0.75}$ d^{-1}) are within the range described by Lofgreen and Garrett (1968), it is interesting to discuss its variation, reflected in the dispersion of both k_g (Table 5) and energy density of feed in terms of NE_g (Table 6). According to the methodology proposed here to estimate NE_g requirements, it is clear that energy balance results (represented by RE=MEI–HP) were directly related to DMI in the chamber. Lower DMI generated lower MEI and, therefore, lower RE. Consequently, NE_g requirements were the result of just one experimental day and did not reflect the cumulative energy retention throughout the period they purport to represent, a non-existing issue in comparative slaughter where RE fully reflects animal performance during the evaluation period. This limitation to estimate NE_g requirement reflects the low relationship between the full period ADG (g d^{-1}) (*x*) and the estimated NE_g (kJ kg^{-1} $EBW^{0.75}$ d^{-1}) (*y*) according to the methodology suggested by Lofgreeen and Garret (1968). In the present study, the relation between these variables was very low (R^2=13%) (data not shown). The weight gain obtained on the day that NE_g is determined is not valid to relate both variables because this gain does not represent the entire period. Only two weighings — at the entry and exit of the chamber — can result in negative gains when energy balance is positive, indicating that factors related to DMI and water intake are compromising the results. A report by Blaxter (1967) supports this concept: Sheep subjected to a calorimetry assay for 4 d resulted in 604-g weight loss, even when food and water were available *ad libitum*. Further studies should make animals repeatedly go through the chamber with *ad libitum* feeding to estimate energy balance (RE), thus obtaining an average value that best represents the experimental period.

4.5. Metabolicity and efficiency of metabolizable energy utilization

Metabolicity is the relation between ME and GE (ARC 1980), while efficiency of ME utilization is defined as the increase in energy retention per unit increase in MEI (CSIRO 2007). The lower metabolicity in Period 1 compared with Periods 2 and 3 resulted from a lower DE (as a proportion of GE; Table 4).

According to ARC (1980), k_m and k_g may vary due to ME concentration and diet metabolicity, with greater variation for k_g than k_m. No difference was found in ME (*P*>0.05) between experimental periods (Table 6). The differences in metabolicity (Table 4) did not reflect the k_m and k_g values (Table 5). Marcondes *et al.* (2010a) did not evidence the relationship between k_m and dietary ME concentration. Johnson *et al.* (1998) also reported problems when using dietary energy density to predict k_g, reporting that the model is not appropriate when low-digestible feeds are used. While metabolicity fluctuation was not remarkable between experimental periods, lower k_g values were obtained during Periods 1 and 2, and higher in Periods 3 and 4, suggesting that efficiency of energy utilization was affected by other factors besides diet, presumably age.

Maintenance is a more energy-efficient process than growth (Garrett and Johnson 1983), which was demonstrated in this work. The k_m values observed in this study are within the ranges estimated by the NRC (1984; between 57.6 and 68.6%), Garrett and Johnson (1983; between 66.2 and 74.2%), and Ferrell and Jenkins (1998; between 65–69%). Using Nellore cattle, Marcondes *et al.* (2010a) reported 64% efficiency for steers and heifers, and 66% for bulls. For Zebu cows, Cárdenas-Medina *et al.* (2010) reported 62% efficiency.

The k_g values observed during the first two periods are lower than those reported by the NRC (1984) (between 29.6 and 47.3%). To understand the higher k_g values in the last two periods we should consider weight gain composition. As energy retention may be in the form of protein or fat, different percentages of each in the total RE correspond to different efficiency of energy utilization (Ferrell and Jenkins 1998). According to Garrett and Johnson (1983), net efficiency of ME for growth is lower when gain involves a high proportion of protein accretion, which has lower net efficiency than fat. Garrett (1980) reported between 10 and 40% efficiency of ME utilization for protein synthesis, and between 60 and 80% for fat synthesis. Similarly, the

CSIRO (2007) considers greater efficiency of ME utilization for fat (75%) than for protein deposition (45%). Considering the normal growth curve of cattle, it can be concluded that animals had a higher degree of physiological maturity in Periods 3 and 4, and therefore increased fat deposition and, which resulted in higher k_g.

4.6. Dietary energy density

Feed GE was lower than that indicated by CSIRO (2007) and AFRC (1993): 18.4 or 18.8 MJ kg^{-1} DM, respectively, but closer to the value reported by Cárdenas-Medina *et al.* (2010; (16.3±0.4) MJ kg^{-1}) (Table 6). The DE and ME densities had the same proportions in relation to GE density, as shown by the energy balance (Table 4). The NE concentration, either for maintenance or weight gain, corresponded to the ME density affected by the respective k_m and k_g.

5. Conclusion

The NE_m and NE_g requirements were influenced by age and possibly by the stress, nervousness and activity of animals into the respirometry chamber.

Considering that animal behavior affects HP, we suggest to include video recording of the animals in the respirometry chamber to register positional changes (time spent standing *vs*. lying down) for a better understanding of the results. Furthermore, we propose to reduce stress during HP assessments by allowing visual contact among animals.

Experimental procedures to estimate energy requirements for weight gain should improve to diminish variability. Records of BW and NE_g changes at various times within each period should be included in future studies. Precision associated with measurements taken at least during 4 d should be evaluated.

The NE_m requirements of *B. indicus* cattle were not lower than those reported for *B. taurus*. More studies should be conducted to simultaneously determine maintenance requirements in both species in order to see whether real differences exist between them or if our results had a systematic error associated with the methodology.

Restricted feeding in this experiment exceeded the minimum of 21 d suggested in the literature. A study relating HP with restricted feeding periods of varying length would show the minimum time required to reduce the metabolic rate associated with *ad libitum* feeding. This preliminary assessment could improve the precision associated with NE_m estimation by linear regression or FHP.

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