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Respirometry and nutritional requirements of Zebu and dairy crossbred cattle at different levels of feeding and physiological status

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INTRODUCTION

Calorimetry is based on the laws of thermodynamics, in which "energy can neither be created nor destroyed, only transformed" and "the amount of energy released or absorbed in a system does not depend on the paths taken during its transformation, but only on the energy contained in reagents and in the final products" (Lavoisier, 1780). In indirect calorimetry, also known as respirometry, the gaseous exchange between the organism and the environment are measured. Once the oxygen consumption (O_2) and the production of carbon dioxide (CO_2) and methane (CH_4) are known, the energy losses by gas and heat are calculated. The Calorimetry and Metabolism Laboratory of the *Universidade Federal de Minas Gerais* (UFMG), located in Belo Horizonte, Minas Gerais, was the first laboratory to build respirometry chambers in Latin America. Since 2009, experiments have been carried out to evaluate energy metabolism and methane production by ruminants. The results obtained are expressed in net energy (NE), which can be net energy for maintenance (NEm), net energy for lactation (NE_l), net energy for weight gain (NEg) and net energy for pregnancy (NEpreg). Net energy is, in fact, what is used by the animal for maintenance and each productive function. The conversion factors of total digestible nutrients (TDN) into digestible energy (DE) and metabolizable energy (ME), the latter for every physiologic function or NE, are calculated. The values of *k* (conversion efficiency of ME into NE) for maintenance (*km*), milk production (k_l), gain or growth (*kg*), and pregnancy (*kpreg*) are determined.

OPEN-CIRCUIT RESPIROMETRY SYSTEM

In an open-circuit respirometry system, the animal is housed in a sealed chamber system that does not allow any gaseous exchange between the inside and outside air, except through the air circulation system. A mass flow meter adjusts airflow as a function of temperature, pressure and humidity, and the CO_2 concentration inside the chamber never exceeds 1%. During the 24-h measurements, the analyzer instrument (Sable®) takes readings of the concentrations of CO_2, CH_4, and O_2 in atmospheric air and the air coming out of the chamber every 5 min. These concentrations, multiplied by the volume of air that passes through the chamber during the time of measurement, allow for the calculation of how much O_2 was consumed and how much CO_2 and CH_4 were produced (Rodríguez et al., 2007).

A correction factor should be generated to adjust the readings, which should be within appropriate respiratory quotient values. The calibration of gas analyzers is performed whenever the equipment is used, and consists of injecting, at a constant flow rate, gases of known concentrations into the analysis system. Pure nitrogen is used to calibrate the analyzers to the zero value of gases concentration. Atmospheric air is used to calibrate O_2 analyzers, assuming that it presents constant O_2 concentration (20.948%) and gaseous mixtures of known concentrations: CO_2 at 5% diluted in nitrogen, and methane at 1%, also diluted in nitrogen.

RESPIROMETRY FOR DETERMINATION OF HEAT PRODUCTION

An apparent digestibility assay is performed immediately before every measurement in the respirometry chamber. Total feces are collected for 5 days and urine for 24 h. Then, the animal is confined for 24 h in the respirometry chamber. The procedures and system specifications have been described by Rodríguez et al. (2007). Heat production measurements are carried out with animals fed at production levels in accordance with the established treatment (maintenance, intermediate and *ad libitum* level), at the various physiological stages or after 48-h solid feed fasting. The volume (L/d) of O_2 consumed and CO_2 and CH_4 produced in 24 h, and urinary nitrogen excreted (UN, g/d) are used to estimate the heat production (HP) according to Brouwer's equation (1965): $HP\ (kcal) = (3.866 \times VO_2) + (1.200 \times VCO_2) - (0.518 \times VCH_4) - (1.431 \times UN)$. The ME in the diet is determined by subtracting the energy losses in the feces, urine, and methane from the gross energy intake (GEI). The energy loss in the form of methane is quantified by assuming a loss of the 9.45 kcal/L CH_4 produced (Brouwer, 1965). The concentrations of digestible energy (DE) and metabolizable energy (ME) in the diet, expressed in Mcal/kg DM, are obtained during the metabolic assay.

Measurement of gaseous exchange in the chamber is performed at least twice with each animal: once with the animal fed and once with the animal solid fasting of 48 h. Therefore, the heat production of the fed and fasted animal is known, the latter corresponding to the value of net energy required for the maintenance of the animal. The difference between the values obtained for the fed and fasting animal will correspond to the heat increment and, knowing the ME content of the diet, the NE value of the diet can be determined (Kleiber, 1975).

Some authors mention high values for the estimation of the NEm requirement from heat production in fasting. Thus, the regression of heat production in different diets, based on metabolizable energy intake, estimating the net requirement for maintenance by extrapolation, was also conducted in the experiments.

DATABASE

The database for measurements of respiratory exchanges includes a series of experiments performed in the Calorimetry and Metabolism Laboratory of UFMG, using respirometry chambers, since 2009. A total of 202 evaluations were included, and those that did not fit appropriately were discarded. The animals were Zebu (Nellore, Gyr, and Guzerat) and dairy crrossbred (F1 Holstein × Gyr). The forage used was *Tifton-85* hay (*Cynodon spp.*), corn silage (*Zea mays*), sorghum silage (*Sorghum bicolor*), and Tanzania grass silage (*Panicum maximum* Jacq cv. Tanzania) in forage:concentrate proportions ranging from 100:0 to 50:50. The concentrate was composed of ground corn, soybean meal, and mineral supplement. The animals were fed at maintenance, *ad libitum* and intermediate (moderate weight gain, 0.5 to 0.6 kg/d) levels. Table 6.1 describes the database used.

Table 6.1 - Database features used in the development and validation of methane production equations

Source	Degree/Year	n	Sex	Genetic Group	Breed[1]	Intake level
Ochoa, Sandra Lúcia Posada	PhD, 2010	5	Bulls	Zebu	Nellore	Maintenance Restricted[2] *Ad libitum*
Silva, Ricardo Reis	PhD, 2011	18	Non-pregnant females	Zebu, Dairy crossbred	Gyr Hol×Gyr Holstein	Maintenance
Lage, Helena Ferreira	Master, 2011	12	Non-pregnant females	Zebu, Dairy crossbred	Gyr Hol×Gyr Holstein	Maintenance
Fonseca, Marcelina Pereira da	Master, 2012	20	Bulls	Dairy crossbred	Hol×Gyr	*Ad libitum*
Ferreira, Alexandre Lima	PhD, 2014	15	Bulls	Dairy crossbred	Hol×Gyr	Maintenance Restricted[2] *Ad libitum*
Pancoti, Carlos Giovani	PhD, 2015	18	Non-pregnant females	Zebu, Dairy crossbred	Gyr Hol×Gyr Holstein	*Ad libitum*
Lage, Helena Ferreira	PhD, 2015	12	Pregnant females	Zebu, Dairy crossbred	Gyr HolxGyr	Restricted[2]
Carvalho, Pedro Henrique de Araújo	Master, 2016	12	Lactating cows	Zebu, Dairy crossbred	Gyr Hol×Gyr	Maintenance Restricted[2] *Ad libitum*
Souza, André Santos	PhD, 2016[1]	12	Non-pregnant females	Zebu	Nellore Guzerat	Maintenance Restricted[2] *Ad libitum*
Duque, Anna Carolinne Alvim	PhD, 2016	12	Non-pregnant females	Zebu	Guzerat	Maintenance Restricted[2] *Ad libitum*
Vivenza, Paolo Antônio Dutra	PhD, 2016	12	Lactating cows	Zebu, Dairy crossbred	Gyr Hol×Gyr	Maintenance Restricted[2] *Ad libitum*
Silva, Juliana Sávia	PhD, 2016	20	Bulls	Dairy crossbred	Hol×Gyr	Restricted[2] *Ad libitum*

[1] Hol×Gyr = F1 Holstein × Gyr animals

[2] Restricted = intermediate level of feeding between the *ad libitum* and maintenance intake.

The relationship among the dependent and independent variables was estimated used the statistical model below:

$$Y = B_0 + B_1X_{1ij} + b_0 + b_1X_{1ij} + B_2X_{2ij} + \ldots + B_nX_{nij} + e_{ij},$$

where B_0, B_1X_{1ij}, and B_2X_{2ij}, . . . , B_nX_{nij} are fixed effects (intercept and independent variable effects); b_0, is intercept, b_1, ee_{ij} *slope*, random effects of the experiments (i = 1...n studies and j = 1, ..., n_i value). The Minitab 16 program was used for statistical analyses. Multiple regression equations were developed using the unrestricted mixed model. To choose the variables for inclusion in the model, the stepwise regression and best subsets procedures were used. Each variable was tested for its random effects on the intercept, in order to choose the best fit based on the lowest RMSR (root mean square of the residual) and Mallows' CP. The presence of collinearities among the independent variables was evaluated. The equations that presented the best fit were selected.

Descriptive statistics (minimum, maximum, mean, median, standard error of the mean) for all variables, in the development of equations to predict methane production and energy partition, are shown in Table 6.2.

Table 6.2 - Descriptive statistics of the variables: methane production (CH_4), dry matter intake (DMI), dry matter intake per metabolic body weight ($DMI/BW^{0.75}$), body weight (BW), neutral detergent fiber intake (NDFI), neutral detergent fiber intake per metabolic body weight ($NDFI/BW^{0.75}$), digestible neutral detergent fiber intake (dNDF), gross energy intake (GEI), digestible energy intake (DEI), metabolizable energy intake (MEI), and gross energy of methane ($GECH_4$) of Zebu (n = 95) and dairy crossbred (n = 107) cattle

Variables	Minimum	Maximum	Mean	Median	MSE
CH_4, L/d	73.9	313	165	122	4.60
DMI, kg/d	2.92	13.4	6.08	5.70	0.21
DMI, $g/BW^{0.75}$	41.0	214	96.5	94.3	2.30
BW, kg	180	683	366	381	9.70
NDFI, kg/d[1]	1.27	9.21	3.18	3.84	0.11
NDFI, $g/BW^{0.75}$	16.3	72.4	38.6	40.6	1.20
dNDF, kg/d	0.70	4.39	1.94	1.78	0.08
GEI, Mcal/d	12.8	89.1	38.4	32.5	1.47
DEI, Mcal/d	9.10	62.8	27.6	24.5	1.17
MEI, Mcal/d	8.05	53.4	23.4	20.3	0.98
$GECH_4$, Mcal/d	0.70	6.58	2.31	1.83	0.09

[1] NDF = neutral detergent fiber corrected for ash and protein.

RESULTS

Animal, genetic group, sex, and physiological status were evaluated and presented no significant effect on methane production. On the other hand, significance was verified for the effect of study, which was considered in the development of the following equations. The database from specific experiments was deleted when it did not fit well with the models being developed. Equations for estimating the production of methane, shown in Table 6.3, were obtained using the variables selected by the stepwise and best subsets procedures. The same variables also provided the solution of the fixed effects of regression equations for predicting the daily production of methane (CH_4), expressed in L/d, and the respective coefficients of determination (R^2).

Evaluating the parameters obtained from the regressions, the adjusted coefficients of determination (R^2) were high and the RMSR values were relatively low. When analyzed as a fixed effect in the regression model, the dry matter intake (DMI) explained 87.7% of the variation in methane production, there being no improvement in the predictive model with the inclusion of other predictive variables. The same occurred with the GEI. Additionally, the quadratic effect for DMI was tested and, despite its significance ($P < 0.001$), there was no improvement in the fit of the regression model, suggesting the use of a simpler model. In Figure 6.1, methane production is verified as a function of DMI.

Table 6.3 - Fixed effects of regression equations based on variables: dry matter intake (DMI), gross energy intake (GEI), crude protein content in the diet (CP), and proportion of forage in the diet (F)

Equations		1	2	3
Intercept	Estimate	37.52	30.87	-439.0
	SE	4.773	5.238	199.2
	P-value	<0.001	<0.001	0.030
DMI (kg/d)	Estimate	19.33	---	21.71
	SE	0.7629	---	1.528
	P-value	<0.001	---	<0.001
GEI (Mcal/d)	Estimate	---	4.777	---
	SE	---	0.1969	---
	P-value	---	<0.001	---
CP (g/kg)	Estimate	---	---	1.155
	SE	---	---	0.445
	P-value	---	---	0.011
F (%)[1]	Estimate	---	---	417.3
	SE	---	---	189.1
	P-value	---	---	0.030
RQMR (L/d)		17.25	17.89	17.79
R^2		0.877	0.867	0.806

[1] F (%) = proportion of forage in the diet, expressed on a scale from 0 to 1.

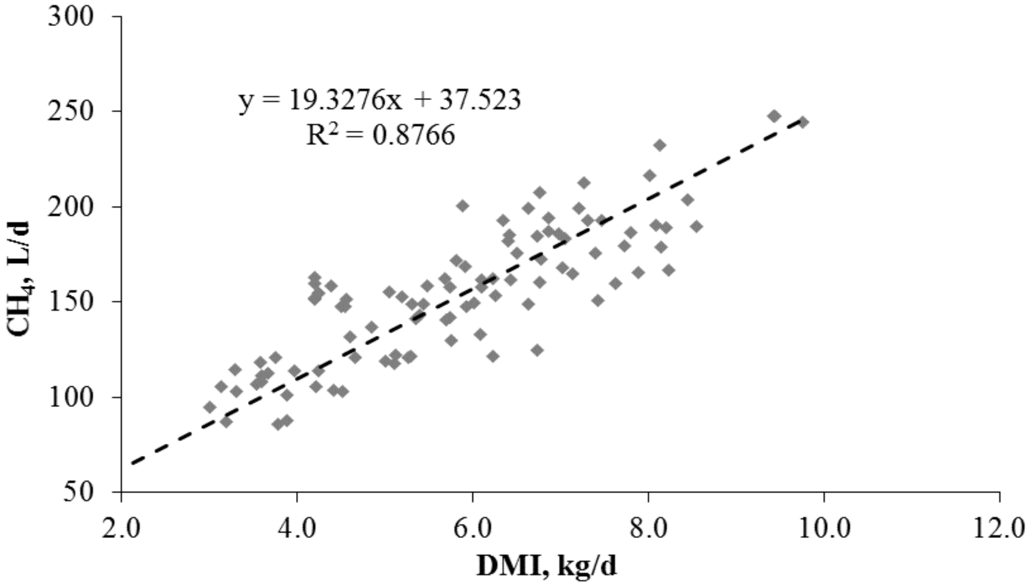

Figure 6.1 - Relationship between daily production methane (CH_4) and dry matter intake (DMI). The points represent the evaluations considered for the development model (n = 125).

Some authors have corroborated this strong positive relationship, considering DMI as a dominant factor in methane production, independent of the diet consumed (Kriss 1930, Axelsson 1949, Shibata et al., 1993). Some equations have been developed relating methane production to dietary composition (Moe and Tyrell, 1979; Bratzler and Forbes, 1940), and to DE intake (DEI), GEI and the level of feeding (Blaxter and Clapperton, 1965). More recently, Ramin and Huhtanen (2013) have developed more complex equations associating variables like DMI, organic matter intake (OMI), ether extract intake (EEI), the ratio of non-fibrous carbohydrates:total carbohydrates (NFC: tCHO) and the organic matter digestibility (OMD). Their equations showed low RMSR values (21.0 – 21.1 L/d), attesting to the accuracy of the estimate. However, considering the greater ease of determination and greater availability of information regarding the DMI variable, Equation 1 (Figure 6.1) is recommended for predicting enteric methane production for cattle growing under tropical conditions.

In order to evaluate the relationships between the amount of energy lost as methane and the energy consumed as GE (Figure 6.2) and DE (Figure 6.3), regression analyses were conducted on these values. They were significant and their prediction errors were 0.546 and 0.532 Mcal, respectively.

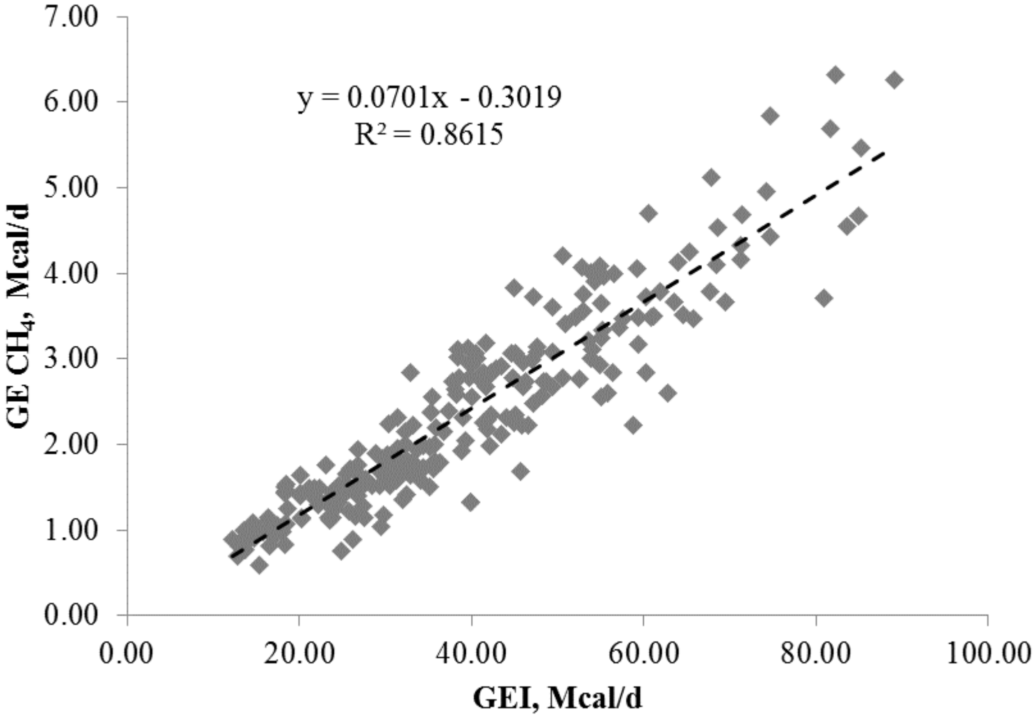

Figure 6.2 - Relationship between loss of gross energy as methane ($GECH_4$) and gross energy intake (GEI). The dots represent all evaluations contained in the database.

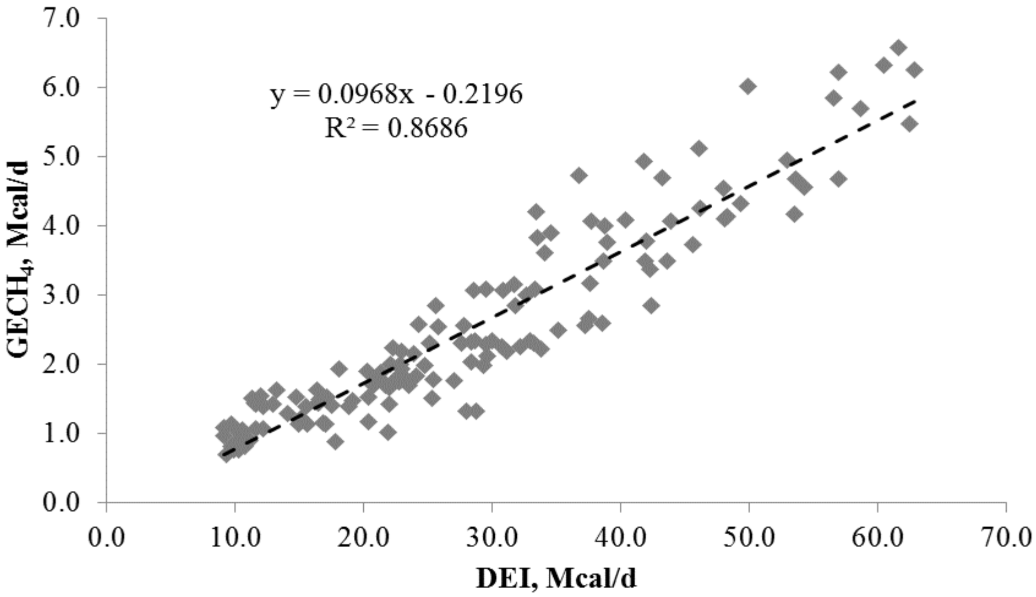

Figure 6.3 - Relationship between loss of gross energy as methane ($GECH_4$) and digestible energy intake (DEI). The dots represent all evaluations contained in the database.

Mcallister et al. (1996) mentioned the importance of nutrient availability for the ruminal microbiota as a main defining factor of the upper limit of production. Thus, when there is a lower efficiency of microbial growth, that is, a lower efficiency of microbial crude protein synthesis, there will be a low protein:energy relationship among the nutrients absorbed and consequently, greater methane production. Therefore, methane emission in relation to the productivity of the ruminant depends on rumen fermentation efficiency and feed conversion efficiency in animal products. Leng et al. (1993) claimed that cattle subjected to low-quality diets lost approximately 15% to 18% of DE in the form of methane, while those provided with balanced diets reduced their methane emission by approximately 7%.

Several studies have shown that when animal productivity is increased, there is a reduction in the proportion of methane produced per unit of product. According to the United States' Environmental Protection Agency (EPA, 2005), increasing livestock productivity to achieve lower methane emissions per unit of product is the most promising and cost-effective way to reduce emissions. Ferreira (2014) found moderate correlations (-0.49; $P = 0.03$) showing that the level of intake relative to maintenance was inversely related to methane production. Increasing the intake by one unit above maintenance resulted in a decrease of 0.73 percentage units of methane production (% GEI).

Moss (1994) claimed that, in low-quality forage, the addition of nutrients for microorganisms increases the efficiency of microbial growth because it increases the efficiency of the fermenting process in the rumen with a decrease in the methanogenic activity per unit of degraded carbohydrates. However, there is an increase in methane production per animal ranging from 8.4% to 12.3% of the GEI because more organic matter is fermented. It was found that the coefficient of the equation shown in Figure 6.2, represents 7% of GEI, and it is lower than the values suggested by the literature. Similarly, it was found that the coefficient of the equation in Figure 6.3 represents 9.68% of the DEI.

The results of NEm, efficiency of ME used for maintenance (*km*), weight gain (*kg*), pregnancy (*kpreg*), and milk (k_l) obtained in the different experiments are shown in Table 6.4.

Table 6.4 - Net energy requirement for maintenance (NEm) and efficiency of utilization of metabolizable energy for maintenance (*km*), weight gain (*kg*), pregnancy (*kpreg*), and lactation (k_l) of Zebu and crossbred cattle in different weight ranges and physiological states (status)

Growing

Reference	Category	*Status*	BW (kg)	Genetic group	NEm	*km*	*kg*
Ochoa	Zebu, bulls	Growing	200	Nellore	124[2]	0.65	0.23
					116[3]	0.60	
			300		94.0[2]	0.60	0.25
					92.0[3]	0.59	
			400		98.0[2]	0.70	0.40
					92.0[3]	0.65	
			450		83.0[2]	0.65	0.40
					84.0[3]	0.64	
Fonseca	Dairy crossbred, bulls	Growing	250	F1 HxG	-	-	0.27
Ferreira	Dairy crossbred, bulls	Growing	350	F1 HxG	108[2]	0.76	0.23
					74.6[3]	0.60	
Silva	Zebu, heifer	Growing	300	Gyr	88.0[2]	0.60	-
	Dairy crossbred, heifers			F1 HxG	95.6[2]	0.67	-
Pancoti	Zebu, heifer	Growing	400	Gyr	83.9[2]	-	-
	Dairy crossbred, heifers			F1 HxG	96.7[2]	-	-
Silva	Dairy crossbred, bulls	Growing, 0-60 days	30-60	F1 HxG	73.7[2]	0.67	0.45

Mature and pregnant

Reference	Category	*Status*	Body weight	Genetic group	NEm	*km*	*kpreg*
Lage[1]	Zebu, non-pregnant females	Mature	450	Gyr	76.8[2]	0.64	-
	Dairy crossbred, non-pregnant females			F1 HxG	92.0[2]	0.63	-
		Pregnancy (days)	Body weight	Genetic group	NEpreg (Mcal/d)	*km*	*kpreg*
	Zebu, pregnant females	180 days	450	Gyr	2.86	-	0.15
		210 days			2.33	-	
		240 days			1.62	-	
	Dairy crossbred, pregnant females	180 days	550	F1 HxG	2.70	-	
		210 days			2.71	-	
		240 days			2.88	-	

Lactation

Reference	Category	*Status*	Body weight	Genetic group	NEm	NE_l[4]	k_l[5]
Vivenza	Zebu, lactating cows	1st third of lactation	453	Gyr	79.1[3]	0.778	0.69
	Dairy crossbred, lactating cows	1st third of lactation	526	F1 HxG	88.3[3]	0.778	0.72

[1] Data from master's dissertation and PhD thesis; [2] Net energy requirement for maintenance (NEm[2]) obtained by fasting heat production (FHP); [3] Net energy requirement for maintenance (NEm[3]) obtained by extrapolation; [4] Net energy requirement for lactation (Mcal/kg milk); [5] Efficiency of utilization of metabolizable energy for lactation.

Ferreira (2014) using dairy crossbred cattle, evaluated heat production in fasting bulls fed different diets corresponding to 1, 1.5, and 2 times (1×, 1.5×, and 2×) the DMI for body weight maintenance. The O_2 consumption ($L/BW^{0.75}$) under fasted and fed conditions did not differ between animals at 1× and 1.5× the maintenance diet, providing mean values of 22.25 and 30.35 $L/BW^{0.75}$, which represented a 36.4% increase in O_2 consumption as a function of feeding. The 2× treatment provided the greatest ($P < 0.001$) O_2 consumption with values of 26.77 and 39.03 $L/BW^{0.75}$ for the animals under fasted and fed conditions, respectively. The CO_2 production, similar to O_2 consumption, was greater for the 2× animals, which presented 21.2% and 37.6% greater production ($P < 0.001$) than the animals in the 1× group, under fasted and fed conditions.

Fasting heat production (FHP) was greater ($P < 0.001$) for the 2× group (133.3 $kcal/BW^{0.75}$), compared with the other groups (112.1 and 107.9 $kcal/BW^{0.75}$, respectively), among those in which the FHP did not differ. The lowest O_2 consumption and CO_2 production that occurred with reduced intake agrees with the results obtained by Ferrell et al. (1986), who indicated that the rates of oxygen consumption by organs as the liver and kidneys, per gram of tissue or as a function of their mass, decreased in response to feeding at the maintenance level. The effect of diet on maintenance metabolism has been associated with variations in the tissue metabolic rate. The causes of these variations are associated with changes in the energy rates and costs of blood flow, of the entrance of oxygen into the liver and in nutrient transference in the intestinal lumen (CSIRO, 2007).

A linear increase ($P < 0.001$) in FHP was seen in the present study with the increased intake of DM. The highest values of FHP found, for the highest levels of feeding, reflect the increase in energy demands as a function of the productive condition of the animal. Calculating how much of this increase is due to the maintenance or weight gain becomes an issue of interpretation, as the ARC (1980) reports, as the curvilinear relationship between retained energy and feed intake may be explained by considering a decrease in the efficiency of use of the feed supplied above the constant maintenance level. It may also be explained by considering a constant efficiency and a progressive increase in the components analogous to the maintenance diet.

Some authors report increased NEm values when using the FHP. Ochoa (2010) and Ferreira (2014) constructed the regression equation obtained by the logarithm for heat production (HP) measured in the respirometry chamber, on different diets, as a function of MEI. The values found by the extrapolation for metabolizable energy intake equal to zero corresponded to the "NEm^3" values described in Table 6.4. It is noted that these "NEm^3" values are lower than those obtained by the FHP (NEm^2), and closer to those obtained in experiments with comparative slaughter. The studies are in an initial phase, and need to be expanded, since they may indicate the change of methodology adopted in the experiments using respirometry. Similar to the NEm, the k_m found using the "NEm^3" is different from the value obtained using the NEm^2.

The efficiency of converting DE to ME is influenced by several factors, such as the rate of microbial growth in the rumen, production of methane, relationship between energy and protein in the diet, and efficiency of the use of metabolizable protein, among others. The ARC (1980) reports that the ME/DE relationship is approximately 0.82. The CSIRO (1990) and the NRC (2000) suggest a value between 0.81 and 0.80, respectively; whereas the AFRC (1993) uses values from 0.81 to 0.86. Higher relationships, from 0.89 to 0.92, were found by Hales et al. (2013). An analysis of the relationship between DE intake (DEI) and ME intake (MEI), determined from the metabolism trials in respirometry chambers, was conducted. The effect of author was significant, and was considered in the development of the plotted models (Figures 6.4 and 6.5).

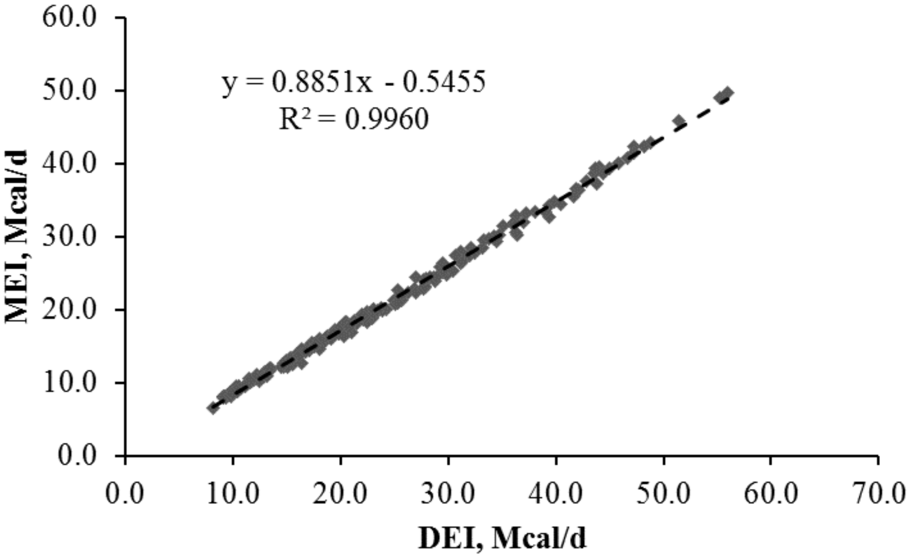

Figure 6.4 - Relationship between digestible energy intake (DEI) and metabolizable energy intake (MEI) expressed as Mcal/d.

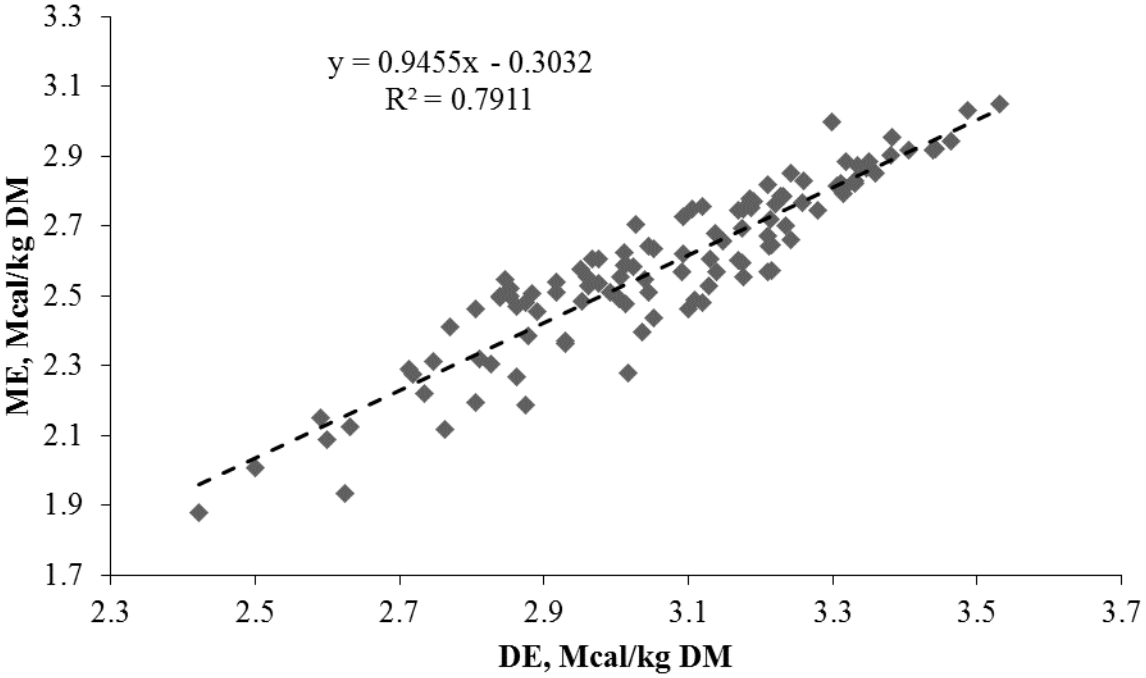

Figure 6.5 - Relationship between digestible energy and metabolizable energy expressed in Mcal per kg of dry matter.

The data presented high dependence of the MEI variable as a function of DEI (Figure 6.4, $R^2 = 0.99$). It is important to emphasize that, considering that in all experiments studied, the methane losses were measured in the respirometry chamber and were not estimated, the ME/DE ratio was always greater than 0.82.

Similarly, Galyean et al. (2016) proposed a model to predict the ME from the DE, in Mcal/kg of DM, based on their analysis of 23 studies published in several journals between 1975 and 2015. The prediction of the ME, using a linear model, showed a strong correlation with dietary components. However, the increase in the precision of the model with the inclusion of the crude protein (% CP), ether extract (% EE), and starch (%) variables was small and the authors recommended the use of a simple linear regression. The comparison between the proposed model (Figure 6.5) and the one suggested by Galyean et al. (2016) is shown in Figure 6.6.

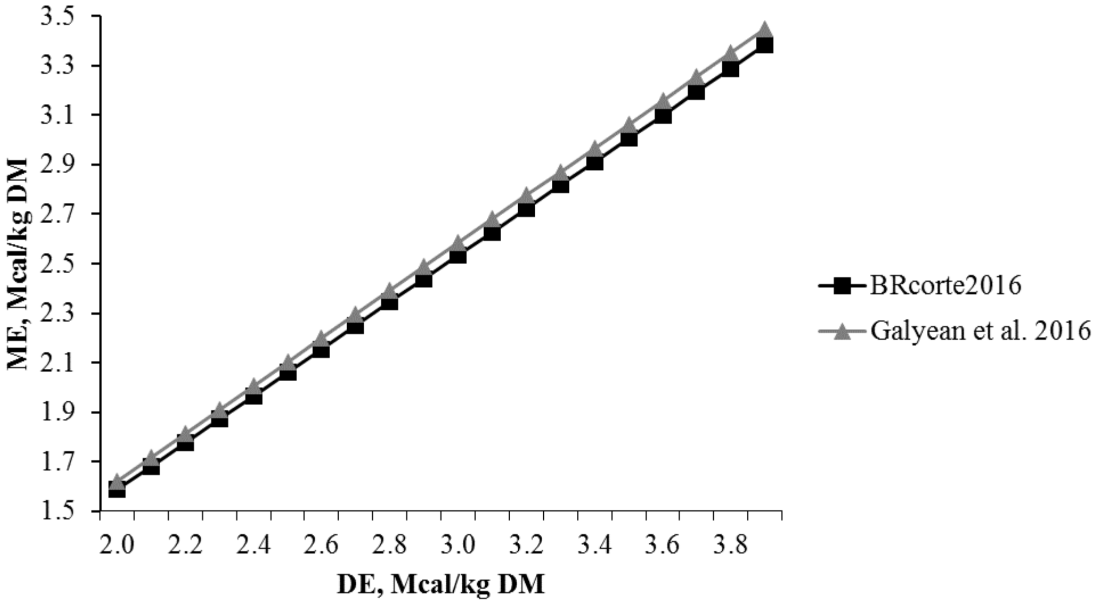

Figure 6.6 - Prediction of metabolizable energy (ME, Mcal/kg) from the digestible energy (DE, Mcal/kg) according to the model proposed by BR-CORTE 2016 and Galyean et al. (2016).

As may be seen in Figure 6.6, there is great similarity among the values predicted by the models. The efficiency of the ME conversion proposed by Galyean et al. (2016) is greater than the efficiency found when the conversion from DE to ME uses the model proposed in the present study (BR-CORTE, 2016, Figure 6.5). It is stressed that the national database contains a greater number of studies that used diets with lower energy density than that of Galyean et al. (2016). Therefore, the use of the simple linear model, proposed in Figure 6.4, is recommended in order to determine the ME from DE.

ACKNOWLEDGMENTS

The authors would like to thank the support provided by the *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG), *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), INCT of Animal Science, *Empresa de Pesquisa Agropecuária do Estado de Minas Gerais* (EPAMIG), *Escola de Veterinária da Universidade Federal de Minas Gerais* (UFMG), Santa Paula Farm – Curvelo (MG), Canoas Farm – Luz (MG), Conesol Agricultural Farm, Campos Altos (MG), and BR-Nova Sistemas Nutricionais.

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