Chapter 8

# Respirometry and Ruminant Nutrition

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Additional information is available at the end of the chapter

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**Abstract**

The gaseous exchange between an organism and the environment is measured by respirometry or indirect calorimetry. 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 can be calculated. Energy metabolism and methane production have been studied in the Calorimetry and Metabolism Laboratory of the Federal University of Minas Gerais, located in Belo Horizonte, Minas Gerais, Brazil. Animals used are mainly Zebu cattle and their crossbreeds that represent most beef and dairy cattle breed grazed on tropical pastures. System calibration and routine work are addressed in this text. The results obtained on respirometric chambers are expressed in net energy (NE), which can be net energy for maintenance ($NE_m$), lactation ($NE_L$), weight gain ($NE_g$), and pregnancy ($NE_p$). NE is, in fact, what is used by the animal for maintenance and each productive function. The values of $k$ (conversion efficiency of ME into NE) for maintenance ($k_m$), milk ($k_L$), weight gain or growth ($k_g$), and pregnancy ($k_p$) are determined. Thanks to the peculiarity of the respirometric technique, the same animal can be evaluated several times, in different physiological states and planes of nutrition.

**Keywords:** bovine, calorimetry, energy metabolism, gases, nutrient requirements

## 1. Introduction

Calorimetry is the process of measuring heat production in the body; it can be direct or indirect. In the first case, produced heat is measured by increasing ambient temperatures. Indirect calorimetry measures heat produced by the animal through the quantification of metabolism products, for example, the gas exchanges with the environment [2].

The Animal Metabolism and Calorimetry Laboratory (LAMACA), located at the Veterinary School of Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, is a pioneer in the construction of respirometric chambers in Latin America (**Figure 1**). The first experiment started in 2006 with small ruminants and since 2008, this kind of research has been carried out to evaluate the energy metabolism and the production of methane by cattle. The results obtained are expressed in net energy (NE), which can be net energy for maintenance ($NE_m$), net energy for milk production (NEL), net energy for weight gain ($NE_g$), and net energy for gestation (NEp). We can determine what was truly used by the animal in described productive functions. Conversion factors of total digestible nutrients (TDN) for digestible energy (DE) and metabolizable energy (ME) are calculated and the latter for each physiological function or NE. The *k* values are determined (conversion efficiency of ME in NE) for maintenance ($k_m$), milk yield ($k_L$), weight gain or growth ($k_g$) and gestation ($k_p$).

In this chapter, basic concepts of indirect calorimetry or respirometry are presented; some notes about the use of this methodology in the research into metabolism and nutrition of cattle in the laboratory are also included.

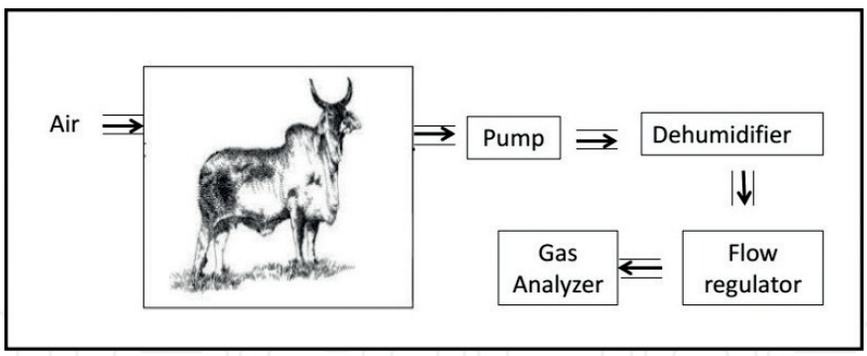


**Figure 1.** Respirometric chamber's design at LAMACA.

## 2. Calorimetry: concepts and basic principals

Several researches throughout history have energy as the focus of their study. In one of the first works, Leonardo Da Vinci, in his publication "Codex Atlanticus" postulated that where flame does not live no animal that breathes cannot live. Subsequently, Robert Boyle (1627–1691) concluded that both combustion and life necessitated a substance present in the air. The same observation relating "fire x life" was made by his contemporary, the scientist John Mayow (1643–1691), who built the first semi-quantitative "respirometer" and observed that by placing a candle and a mouse under a single flask, soon after the candle flame went out,

the animal died. In the next century, John Priestly (1733–1804) found evidence of the diversity of gases that compose atmospheric air (such as carbon dioxide and nitrogen) and observed that different chemical reactions could produce gases capable of sustaining life [3].Although such researchers have contributed brilliantly to the understanding of bioenergetics, the scientist Antonie Lavoisier (1743–1794) deserves special attention for the great importance of his discoveries. He discovered the existence and importance of the gas he named "*oxigène*" (oxygen). For Lavoisier, breathing was defined as a slow combustion process. His studies led to the creation of indirect calorimetry (which allows the evaluation of metabolic rates through oxygen consumption, changes according to exercise and diet), as well as direct calorimetry: when a mouse is surrounded by ice, heat production of the animal can be evaluated by the formation of water in the liquid state [4].

A fundamental advance in calorimetry development was the postulation of the first law of thermodynamics by the German Julius Robert von Mayer (1814–1878) in 1842, based on observations made by the Swiss chemist Germain Henry Hess (1802–1850). The first law, known as the "mass preservation law," tells us that energy can be transferred or transformed, but it cannot be destroyed or created. Later, in his work on the equivalence between work and heat, James Prescott Joule (1818–1889) eventually corroborated the concept proposed by Mayer in relation to energy conservation [5].

Still, in the nineteenth century, Berthelot (1827–1907) developed the adiabatic calorimetric pump. Its creation obeyed the principle of thermodynamics that energy is only transferred; therefore, the energy released in heat form during the combustion of an organic substance would be equivalent to the available gross energy in case of a food or loss by the organism, in case of excreta.

The development of bioenergetics concepts exploring the interrelationship between gas exchange and heat production had a significant advance with the work of Carl Von Voit, who used an open circuit respirometry apparatus developed by Max Von Pettenkofer (1818–1901). Other researchers (all Von Voit students) such as Henry Armsby, Wilbur Atwater, Oskar Kellner, and Max Rubner, using similar equipment, have developed work on energy metabolism [5].

Kellner and Köhler (1900), cited by [6], developed the "starch equivalent" concept, using a system based on foods net energy, in which foods energy value presented a relation to starch energy content, which has been used for many years in Europe and Russia, also serving as the basis for the development of later feeding systems. At the same time, Atwater and Bryant developed the physiological fuel values system to determine the metabolizable energy values of carbohydrates, fats and proteins—this energy value is corrected for the energy value of the excreted urea. Armsby (1903, 1907), also using respirometric calorimetry, developed the concept of net energy and defined the metabolizable energy (ME) as the net energy (or retained energy, RE) plus the food heat increment (HI) (ME = RE + HI).

It is noteworthy that the system proposed by Armsby at the beginning of the twentieth contains many of the principles used for the development of current net energy systems, such as [7, 6].

Another important advance in modern calorimetry, however, would only occur in 1965, with the publication of Brouwer's equation [8]. The equation (Eq. 1) allowed the calculation of the heat production.

Heat production

$$HP = (3.866 \times O_2) + (1.2 \times CO_2) - (0.518 \times CH_4) - (1.431 \times N) \quad (1)$$

where HP is the heat production; $O_2$ is the $O_2$ volume, L; $CO_2$ is the $CO_2$ volume, L; $CH_4$ is the $CH_4$ volume, L; N is the urinary nitrogen.

The food, feces, and urine crude energy are determined by caloric pump. Brouwer's equation allows the calculation of heat production by an animal, after evaluation of produced gases over time. A range of possibilities open up in the study of energy metabolism of animals, including food assessment and determination of nutrient requirements.

## 3. Open circuit respirometry system

LAMACA's respirometric chambers operate in open circuit system (**Figure 2**). The animal is housed in a chamber with a sealing that does not allow any gas exchange with the outside air, except by a proper air circulation system. Air tubing is coupled to a pump, which performs the renewal of air inside the chamber in a constant flow during the measurement, regulated by a mass flow meter, which corrects the airflow as a function of temperature, pressure, and humidity. According to [8], the flow control system represents a major limitation of this method, since the accuracy of this measurement is indispensable for the proper functioning of the system.

The air inside the chamber is continuously renewed by the constant input of external air. The input of fresh air into the chamber is possible due to the negative pressure created by the pump that promotes the suction of the internal air, thus allowing the entrance of external air. There is a renewal of the inside air that can be used for sampling and later evaluation by the gas analyzers. The internal negative pressure guarantees safety in the data acquisition because it prevents leakage of the air, which could constitute a source of errors in the analysis of the sampled gas.

**Figure 2.** Respirometric chambers for large animal (left) and small animal (right), presented by its designer, professor Norberto Mário Rodriguez.

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Air temperature and circulation inside the chamber are controlled. Air renewal is regulated by a mass flowmeter (model SABLE Flow-kit 500H). The flow rate is between 0.5 and 1 L/kg body weight/minute. The air leaving the chamber is piped to an outside area, and samples are pumped to gas analyzers. These are in the bypass system, that is, all are interconnected, allowing the passage of a single sample through all the analyzers. The gas analyzers used in this experiment come from the company SABLE SYSTEMS®, with the following models being used: TA-1B $O_2$ analyzer, CA-2A $CO_2$ analyzer, and MA-1 $CH_4$ analyzer.

Gas reading in the analyzers occurs in 5 min cycles. At the beginning of each cycle, the circuit is automatically moved by the equipment to a piping, which is connected to an outside area outside and an air sample is collected. The external air sample (atmospheric), called "baseline," circulates throughout the circuit until the gaseous material is analyzed. The system is then shifted to a closed sampling loop and the air is sampled from the chamber interior and analyzed. The baseline and the gas sample pass continuously through the system for 5 min. The data reading occurs in the last 30 s (the first 4 min 30 s were for ensuring that there were no residuals from the samples). Animal oxygen consumption, methane, and carbon dioxide production are calculated by the difference between external air concentrations and the chamber air. Due to the gaseous nature of the material, the control of temperature, pressure, and humidity of the system is very important, since these factors are responsible for changes in the volumes of each gas evaluated in relation to the temperature and pressure normal conditions. The chamber is constructed of steel and has two opposing openings, one that allows the entrance and exit of the animal (larger door, 2 m length and 2.2 m height) and one for feeding, with minimum air displacement, in the front part, with an area of 0.75 $m^2$ (1 m long and 0.75 m high). On the sides, there are acrylic windows, sealed, which allow the visualization of the animal and the interior of the chamber, as well as another animal, placed parallel to the chamber, in a cage. The internal volume of the camera is 22.391 L.

Due to the complexity of this system, it is necessary to determine a correction factor for the whole system [9], in order to have a correlation between reading and actual gas concentrations.

## 4. Daily analyzer calibration

Gas analyzer calibration shall be performed whenever the equipment is used. Gases are injected in a constant flow and known concentrations. After stabilization, the read value is an adjustment to the actual value. Pure nitrogen is used to calibrate the analyzer for zero concentration, while atmospheric air is used to calibrate the $O_2$, $CO_2$, and methane analyzers. Atmospheric air $O_2$ concentration is 20.946%. The $CO_2$ and $CH_4$ have a known concentration because they are diluted in nitrogen (5 and 1%, respectively). Stabilization is inversely proportional to the gas aliquot directed to the devices. LAMACA uses 0.2 L/min flow [10], which requires approximately 5 min for values stabilization.

Atmospheric air or standard gas (21% diluted $O_2$ in nitrogen) were evaluated to carry out the $O_2$ analyzer calibration. The results for methane, carbon dioxide production, and oxygen consumption, as well as the animal heat production, were compared. All tests had best results with atmospheric air; then, we chose it for all analyses, with $O_2$ air concentration as constant.

## 5. Correct factor determination

Before starting any work, a correct factor must be determined to eliminate $CO_2$ and $O_2$ concentration effect, according to [8]. To determine the correction factors, the first activity to be performed is to check the chamber's sealing conditions, ensuring that no air is exchanged with the outside, except by the pump system.

The correct factor determination and use of the large animal chamber at LAMACA is described here. In this system, the pump that performs the air renewal was later allocated to the chamber generating a slight pressure inside the chamber, so that the external environment is well ventilated.

A negative pressure will be generated inside the chamber, which can be verified using a differential column manometer. This should be connected one point to the chamber at the endpoint and the other at an outside point. After a short time of operation of the flow, a gap can be seen between the two columns, indicating a considerable resistance for the external air to enter the chamber through another path than the pipe itself for its renewal. The total displacement of the water column (WC) is given by the sum of the elevation ($E$) of this on the side connected to the chamber and the lowering (L) on the side open to the environment. Usually, in a well-planned system, this total displacement reaches 0.5 cm.

After verification of the system seal, the quantity of each injected gas is calculated. The gases used were $CH_4$, $CO_2$, and $N_2$, with purity higher than 99.99%. These three gases were injected simultaneously, and the injection of methane and carbon dioxide resulted in an increase in their concentration inside the chamber, simulating what happens when the animal is housed. In turn, nitrogen injection resulted in all gases dilution, such as oxygen, which was reduced inside the chamber simulating the consumption by the animal. An important point of this step is determining the injected gas flow and air renewal flow. The determination of these values considers the achieved standard value. The established value was 200 L/min. Injected flow used for each gas ($CH_4$, $CO_2$, and $N_2$) aim to reach 0.04, 0.50, and 20.50% to $CH_4$, $CO_2$, and $O_2$, respectively. Calculations are as follows:

Methane and carbon dioxide flow

$$Fi = ((Cd \times Fr) - (Ca \times Fr))/(P/100) \quad (2)$$

where Fi is the injection flow (L/min); Cd is the desired gas concentration (%); Fr is the renewed air flow used (L/min); Ca is the atmospheric air concentration (%); P is the gas purity (%).

Nitrogen flow

$$Fi = \left(\left((Ca\,O_2 \times Fr)/Cd\,O_2\right) - Fr\right)/(P/100) \quad (3)$$

where Fi is the injection flow (L/min); $CaO_2$ is the oxygen atmospheric concentration (%); $CdO_2$ is the oxygen concentration desired (%); Fr is the renewed air flow used (L/min); P is the gas purity (%).

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After the injection flow determination, the manometers are assembled to the cylinders that are weighted in a 0.1 g accuracy balance. After this, all the cylinders are connected to the chamber by specific piping. Each one contains a flow meter for injection flows determination. The calibration process is started. When all the analyzers are calibrated, the readings are started. Desired injection flow for each gas is reached after the first reading cycle. The used injection time is approximately 4 h. The cylinder registers are closed and temperature and pressure inside the chamber are recorded hourly, after gas injection time is completed. All cylinders are weighed again. The cylinders with water condensation should be weighed the next day. After the initial and final cylinders weighing, we know how much gas was injected (g). One mole of any gas has 22.4 liters volume in normal temperature and pressure conditions. Each injected gas volume (L) can be calculated by dividing the weight (g) of the injected values of 1.2506, 1.9647, and 0.7162, respectively, for nitrogen, carbon dioxide, and methane.

The next step is the evaluation of $CO_2$ concentration inside the chamber after the complete injection process. At the beginning of the injection, gas concentration will increase. At the end of the injection, gas concentration reaches maximum value. Then, the $CO_2$ value starts to reduce. Further values are discarded after $CO_2$ concentration stabilizes at minimum values. Time from the beginning of gas injection and $CO_2$ stabilization is considered for the correction factor calculation.

The first point for measuring injected volumes of each gas by the analyzer is the determination of the initial, final, and average concentrations of methane, carbon dioxide, and oxygen in atmospheric air and in the air leaving the chamber. The air volume present inside the humidity-free chamber and in normal conditions is determined. Dry air volume in the initial normal conditions (Vsi) and the dry air volume in final normal conditions (Vsf), according to [9], are calculated as follows:

Vsi and Vsf

$$VSi\ or\ Vsf\ =\ (VC) \times (273/(273 + T)) \times \left(\left(P - P_{H_2O}\right)/760\right) \tag{4}$$

where Vs is the dry air size inside the chamber at normal conditions in the beginning or by the end of measurement (L); V is the inside chamber size; T is the beginning or end temperature (°C); P is the beginning or end environment pressure (mmHg); $P_{H_2O}$ is the beginning or end partial pressure (mmHg); the correction factor for methane and $CO_2$ was calculated according to Eq. (4).

$CH_4$ and $CO_2$ correct factor.

$$F\ =\ \left(V_{inj}\right)/\left[(Cs \times Vt/100) - \left\{(Ce/100) \times \left[Vt - \left(V_{CH_{4i}} + V_{CO_{2i}} + V_{N_{2i}}\right)\right]\right\} + \left\{\left[Cf \times Vsf/100\right] - \left[Ci \times Vsi/100\right]\right\}\right] \tag{5}$$

where F is the correct factor for $CH_4$ or $CO_2$; $V_{inj}$ is the injected gas size (L); Cs is the average gas concentration in air leaving the chamber (%); Vt is the total size in air through in the system (flow L × minutes); Ce is the gas average concentration at atmospheric air that is entering the chamber (%); $V_{CH_{4i}}$ is the injected $CH_4$ (L); $V_{CO_{2i}}$ is the injected $CO_2$ (L); $V_{N_{2i}}$ is the injected $N_2$ (L); Cf is the gas final concentration at last reading (%); Vsf is the gas final size inside chamber

corrected for normal conditions (L); Ci is the gas initial concentration at first reading (%); Vsi is the initial air size in chamber corrected for normal conditions (L).

Then, the calculations are performed to determine the correction factor for oxygen. The oxygen correction factor is a function of the carbon dioxide concentration ($FO_2 \times CO_2$). In the analysis systems used—paramagnetic sensor for oxygen and infrared for methane and carbon dioxide—there is interference of the concentration of $CO_2$ in the reading of $O_2$ concentration (Eq. 6). A gas mixture containing known $CO_2$, $O_2$, and $N_2$ concentrations has its concentrations measured several times in normal conditions and with the use of the $CO_2$ absorber, located before the analyzers. Several repetitions are observed with the $CO_2$ concentrations, so the effect of $CO_2$ (present or not) on $O_2$ concentration (with and without the use of absorber) can be known.

$O_2$ and $CO_2$ correction factor

$$FO_2 \times CO_2 = \left(CO_2 ab - CO_2 sab\right)/C_{CO_2} \tag{6}$$

where $FO_2 \times CO_2$ is the $O_2$ correction factor in function of $CO_2$; $CO_2$ ab is the $O_2$ concentration with absorber (%); $CO_2$ sab is the $O_2$ concentration without absorber (%); $C_{CO_2}$ is the $CO_2$ concentration utilized (%).

Eq. 7 determines the correct factor for oxygen.

$$F = \left((Ca/100) \times Vt - \left(V_{CH_{4i}} + VCO_2\, O_2\, i + V_{N_{2i}}\right)\right) - \left(\left(\left(Cf + \left(FO_2 \times CO_2 \times CfCO_2\right)\right) \times Vsf\right)/100 - \left(\left(Ci + \left(FO_2 \times CO_2 \times CiCO_2\right)\right) \times Vsi\right)/100\right) \tag{7}$$

where F is the $O_2$ correction factor; Ca is the $O_2$ average concentration at atmospheric air coming inside the chamber (%); Vt is the air total size through the system (flow, L × min); $V_{CH_{4i}}$ is the $CH_4$ (L) injected; $V_{CO2i}$ is the $CO_2$ (L) injected; $V_{N_{2i}}$ is the $N_2$ (L) injected; Cf is the $O_2$ final concentration, at last reading (%); $FO_2 \times CO_2$ is the $O_2$ concentration correct factor in function of $CO_2$; $CfCO_2$ is the $CO_2$ final concentration, at last reading (%); Vsf is the chamber air size correction for normal conditions (L); Ci is the $O_2$ initial concentration, at first reading (%); $CiCO_2$ is the $CO_2$ initial concentration, at first reading (%); Vsi is the chamber air initial size correction for normal conditions (L).

## 6. Animal adaptation and taming

After system calibration, measures can begin. Small ruminant respirometric chambers methodology in LAMACA was published by [9]. Working with bovines, the system calibration process is hard since the chamber is big and so air circulation is complicated. Besides this, the species peculiarities have showed us that the adaption period must be longer, until the animal appears so calm that its behavior is similar inside and outside of the chamber. Since 2008, when investigations with bovines began on this lab, procedures have been adopted in order to get a similar inside and outside chamber dry matter intake, under normal conditions. This work is based on animal welfare assurance, with animal behavioral assessments and

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**Figure 3.** Animals used in experiments during rational taming (source: Personal archive).

monitoring of blood parameters that may indicate if something is wrong. Zebu and their crossbreeds—the focus of our research line—are more temperamental than taurine animals. Sometimes, they get angry and they always stay alert to external movements and sounds. The training we adopt is based on the principles of rational taming [10]. All animals are gradually presented to the experimental conditions that they will be subjected to. isolation, pain, sudden noise or fear situations make them stressed and should be avoided. Observation is done on each individual animal, and daily behavior is assessed as experimentation methodologies are introduced. Daily baths and brushings are used; and there is daily contact with undergraduate and graduate students, teachers and employees (**Figure 3**), always with a lot of care and patience. The basic principles are respect and communication in a language that the animal can understand. Fear, intimidation, or pain is never used. Nelore, Guzerá, Gyr and F1 – Holstein × Gyr animals were very afraid at the beginning of the work, but when they were presented to daily management, facilities and devices, they became calm and quiet.

## 7. Experimental routine

An apparent digestibility assay is performed immediately before every measurement in the respirometry chamber. Total stool is collected for 5 days and urine for 24 h. Then, the animal is confined for 24 h in the respirometry chamber. Temperature, pressure, and humidity are constant, with an automatic air conditioning. This way, the chamber is subjected to a continuous

flow of air so that the inlet points of the atmospheric air and the internal air outlet of the chamber are located on opposite sides. This results in a constant renewal of internal air, avoiding $CO_2$ concentration greater than 1% [11], cited by [9]. During the 24 h of measurements, analyzers (Sable brand) monitor carbon dioxide, oxygen, and methane concentrations every 5 min, alternately. Total air circulating throughout the chamber, air flow (in L/min) used, multiplied by the total measurement time (min) gives gas quantities entering and leaving the chamber. Therefore, by difference, carbon dioxide and methane output and consumed oxygen are used to determine animal heat production. The analyzers used in these experiments require a daily calibration to ensure read reliability. Calibration consists of adjusting the analyzer reading at the end of each 5 min cycle for each gas concentration range. At the end of each cycle, analyzers of each gas shows gas concentration similar to the cylinder concentration. In the $CO_2$ case, the concentration should range from 4.990 to 5.007 and for $CH_4$, the allowed range is from 0.997 to 1.003. In the case of $N_2$, all devices must have close to zero values with at least two decimal places. They can differ from each other only in the third decimal. For $O_2$, the reading indicated by the analyzer should be between 20.9450 and 20.9510. If the analyzers have performed right readings after three rounds (each round corresponds to the four 5 min cycles for each gas—$N_2$, $CO_2$, $CH_4$, and $O_2$) without adjustments, the equipment is calibrated.

Measurements start immediately after calibration. Mass flowmeter flow is adjusted according to the animal's live weight, as well as after ensuring air circulation and cooling systems are operating normally. Residual gas present inside the chamber must be added to the total volume of produced gases (carbon and methane) and consumed oxygen. Vsi and Vsf are determined by discounting animal volume multiplied by gases concentration at the beginning and at the end of the measurement, respectively. By subtracting the final and initial values, gas accumulated in the chamber (for carbon and methane) and consumed oxygen are obtained. These values are added to the values obtained previously, resulting in the final values of produced carbon dioxide, methane, and consumed oxygen, which are used to determine the animal heat production. Heat production measurements are carried out with fed animals at production levels in accordance with the established treatment (weight maintenance, intermediate and *ad libitum*), at the various physiological stages or after 48-h solid food fasting. The difference between the values of fed and fasting animal will be the caloric increment. Diet net and metabolizable energy content can be found [4].

Fasting heat production (FHP, kcal) corresponds to net energy requirements for maintenance. In the fed animal, it corresponds to the sum of the energy necessary for maintenance plus the caloric increment of feed consumed. PC is calculated by using an equation (Eq. 1) of [7]. Some authors mention high values for the estimation of the $NE_m$ requirement from heat production in fasting.

## 8. Chamber measurements

At the first time, the animals pass through the chamber receiving the same diet provided in the digestibility assay. The power supply must only be provided when the equipment is ready to start reading. The chamber door will be closed and the reading will begin. Next day,

we stop readings and the animal is removed from the chamber. Sorts are weighed. Knowing dry matter intake inside the chamber allows the calculation of the caloric increment required for energy partition. It is essential that the animals maintain the feed intake observed in the apparent digestibility assay.

The second measurement is with a fasting animal. The animal is placed in the chamber after 48 h of fasting solid food and staying there until the next day (72 h). Water must be *ad libitum* all the time.

## 9. Energy partition, net energy requirement, and energy efficiency

Gross energy intake and feces gross energy are determined in an adiabatic calorimetric pump for digestible energy calculation. Metabolizable energy is calculated considering urine and methane. The quantification of energy losses in the form of methane will be done in the respirometric chamber. For each liter of methane, a value corresponding to 9.47 kcal should be attributed [7]. The metabolizability (q) of the diet will be calculated by the relation between metabolizable energy and gross energy ingested [7]. The efficiency of using metabolizable energy for different functions ($k_m$, $k_g$, $k_l$, and $k_p$) is the relation between the net energy and metabolizable energy.

In one study with cross-breed milk cattle, [12] evaluated heat production in fasting bulls fed different diets corresponding to 1, 1.5, and 2 times (1×, 1.5×, and 2×) the dry matter intake (DMI) for weight maintenance. $O_2$ consumption (L/kg $LW^{0.75}$) 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/kg $LW^{0.75}$, which represented a 36.4% increase in $O_2$ consumption as a function of eating. The 2× treatment provided the greatest ($P < 0.001$) $O_2$ consumption with values of 26.77 and 39.03 L/kg $PV^{0.75}$ for the animals under fasted and fed conditions, respectively. $CO_2$ production, similar to $O_2$ consumption, was greater for the 2× animals, which showed 21.2% and 37.6% higher 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 two × group (133.3 kcal/kg $LW^{0.75}$), compared with the other groups (112.1 and 107.9 kcal/kg $LW^{0.75}$, respectively), among those in which the FHP did not differ. The lowest $O_2$ consumption and $CO_2$ production that occurred with reduced intake are in line with the results obtained by [13], who indicated that the rates of oxygen consumption by organs like 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 [14].

A linear increase ($P < 0.001$) in FHP was seen in the present study with 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 diet becomes an issue of

semantics, as [15] reports, as the curvilinear relationship between retained energy and food intake may be explained by considering a decrease in the efficiency of use of the food 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 author's report increased $NE_m$ values when using the FHP [16, 12] 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 "$NE_m$ [3]" values described in **Figure 6**. It is noted that these "$NE_m$ [3]" values are less than those obtained by the FHP ($NE_m$ [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 $NE_m$, the $k_m$ found using the "$NE_m$ [3]" is different from the value obtained using the "$NE_m$ [2]."

## 10. Basal metabolism and maintenance

The metabolizable energy for maintenance is composed of two main components. The first is the basal metabolism, which corresponds to the minimum energy required to support the vital processes in a fasting healthy animal, in the post-absorptive state (48–144 h of fasting after feeding), performing the activity in the thermoneutral environment [17]. The second component associated with the requirement of metabolizable energy for maintenance involves several factors associated with the production of heat originated by the maintenance level, that is, by the heat increment, such as body temperature regulation, voluntary activity, digestion, nutrient absorption and assimilation, fermentation [19, 21].

The difference between basal metabolism and maintenance is that when in maintenance, the animal is not fasting [17]. The metabolizable energy requirement for maintenance (EMm) is defined as metabolizable energy intake (MEI), which corresponds exactly to the heat production, without any loss or gain of body reserves [19, 21]. This will occur when the retained energy equals zero (RE = 0) and the net energy for maintenance, although fundamentally important in net energy systems, cannot be directly determined by experimental techniques. So, it was stipulated that the net energy requirement for maintenance could be obtained by measuring the energy requirements of basal metabolism (EBM), which corresponds to the fasting heat production. At first, the net energy determination through the animal fasting heat production would not be appropriate, since this represents the requirements of ATP at the cellular level added to the heat produced in the formation of ATP by the mobilization of the body reserves. The most appropriate way to obtain the net energy for maintenance would be through the ratio ELm = EMB × $k_b$, where $k_b$ is the conversion efficiency of body reserves to useful energy in the form of ATP. However, the $k_b$ has minimal variation (as the contribution of body reserves to ATP generation varies very little in fasting animals with similar nutritional plains), thus making the energy required for basal metabolism and fasting heat production have a strong relationship [20, 22]. This justifies the use of fasting heat production as the value adopted for net maintenance energy.

## 11. Energy efficiency use: relationship between metabolizable and net energy

From energy partition in the animal, we can obtain values that indicate the efficiency of the animal in using the energy for maintenance and/or production. The terms that make this evaluation possible are known as the metabolizability (q) and energy efficiency of use (*k*). [7] defines "q" (the quality factor) as the portion of metabolizable energy contained in the gross energy ingested, and the constant "*k*", as the portion of the metabolizable energy retained as net energy directed to maintenance, weight gain, fetus and fetal attachments and milk. When the animal is fed at maintenance level, the letter "m" ($q_m$ and $k_m$) is added to such constants. Likewise, the terms $k_g$ are used for growth and weight gain, $k_l$ for milk production and $k_f$ for gestation.

$k_m$ was defined by [23] cited by [5], as the linear regression slope between negative energy retention, that is, energy loss, and ingested metabolizable energy. The efficiency of the use of metabolizable energy for gain ($k_g$), according to the same author, was defined as the slope of the linear regression between positive retained energy and metabolizable energy intake. When evaluating the nutritional requirements through the respirometric technique, the efficiencies of retention of the metabolizable energy are calculated as a function of the relation between the retained energy, that is, net energy, and the metabolizable energy, being $k_m = 1 - (PC_{alimentado} - PC_{jejum})/MEI$, where MEI corresponds to the ingested metabolizable energy [24].

The efficiency of using the metabolizable energy for maintenance is greater than that directed to the productive processes [1]. The various body functions of mammalian animals of the same species are more efficient in retention of metabolizable energy for maintenance, followed by lactation, weight gains, and reproduction functions. When comparing different species, the ruminant is known as the holder of the lowest net energy efficiency [25], which makes this field of research promising in the identification of components of the management of production systems that have a greater impact on the nutritional efficiency of these animals.

It is important to determine the energy efficiency use since several factors can influence them. The variable "q", for example, changes as a function of intake levels, and there are larger fecal losses with highest intake due to a higher passage rate and potentially digestible material escape. The digestible energy can decrease from 2.1 to 6.2% as energy consumption increases in relation to the maintenance level [26]. Urine energy losses tend to be constant, as well as losses due to methane production, ranging from about 5 to 12% in urine and 3 to 5% for methane [27].

## 12. Some results obtained with respirometry

Gyr, Nelore, Guzerá, Holstein, and F1 Holstein × Gyr animals (**Figure 4**) were evaluated at different physiological stages (growth, adult animal, weight gain, gestation, and lactation) and different nutritional levels (maintenance, intermediate, *ad libitum*). Animal breed, sex, and physiological state were evaluated and presented no significant effect on methane production. Dry matter intake (DMI) explained 87.7% of the variation in methane production;

**Figure 4.** F1 – Holstein × Gyr (left) and Gyr (right) heifers inside the respirometric chamber of the Metabolism and Calorimetry Laboratory of the Veterinary School of UFMG.

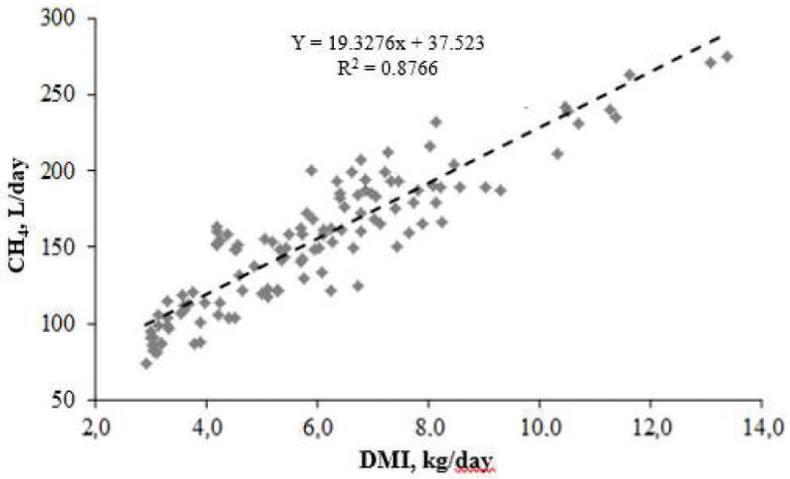


**Figure 5.** Relationship between daily production methane ($CH_4$) and dry matter intake (DMI). The points represent the evaluations considered for the development model (n = 125).

there is no improvement in the predictive model with the inclusion of other predictive variables (**Figure 5**). The same occurred with the GE intake (GEI). These data are published [1].

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 [28], increasing livestock productivity to achieve lower methane emissions per unit of product is the most promising and cost-effective way to reduce emissions. Moderate correlations were obtained (−0.49; $P = 0.03$) in the study by [12], 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).

In low-quality fodder, 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 [29]. However, there is an increase in methane production per animal ranging from 8.4 to 12.3% of the GEI because it is organic.

In one study with cross-bred milk cattle, [12] 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 weight maintenance. $O_2$ consumption (L/kg $LW^{0.75}$) 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/kg $LW^{0.75}$, which represented a 36.4% increase in $O_2$ consumption as a function of eating. The 2× treatment provided the greatest ($P < 0.001$) $O_2$ consumption with values of 26.77 and 39.03 L/kg $PV^{0.75}$ for the animals under fasted and fed conditions, respectively. $CO_2$ production, similar to $O_2$ consumption, was greater for the 2× animals, which showed 21.2% and 37.6% higher 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/kg $LW^{0.75}$), compared with the other groups (112.1 and 107.9 kcal/kg $LW^{0.75}$, respectively), among those in which the FHP did not differ. The lowest $O_2$ consumption and $CO_2$ production that occurred with reduced intake are in line with the results obtained by [13], who indicated that the rates of oxygen consumption by organs like 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 [14].

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 diet becomes an issue of semantics. [15] reports that the curvilinear relationship between retained energy and food intake may be explained by considering a decrease in the efficiency of use of the food supplied above maintenance level.

Some authors report increased $NE_m$ values when using the FHP. [16, 13] 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. It is noted that the "$NE_m$ [3]" values obtained by this regression are smaller than those obtained by the FHP ($NE_m$ [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 $NE_m$, the $k_m$ by using the "$NE_m$ [3]" is different from the value obtained by using the "$NE_m$ [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. [15] reports that the ME/DE relationship is approximately 0.82. [14, 18] suggest a value between 0.81 and 0.80, respectively; whereas [7] uses values from 0.81 to 0.86. Higher relationships, from 0.89 to 0.92, were found by [30]. An analysis of the relationship between DE intake (DEI)

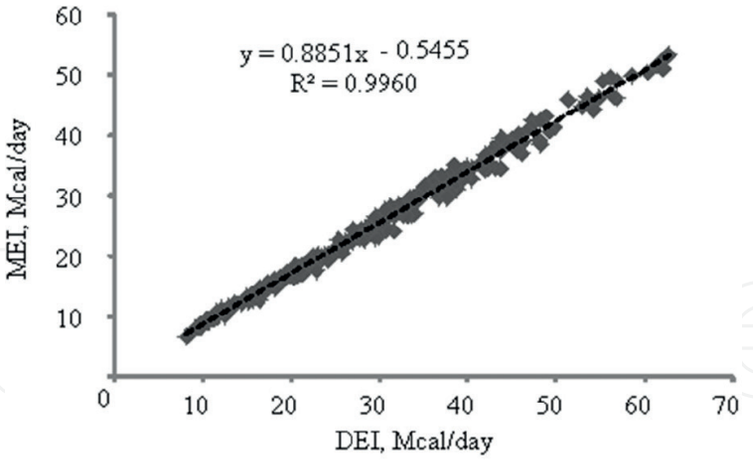


**Figure 6.** Relationship between digestible energy intake (DEI) and metabolizable energy intake (MEI) expressed as Mcal/day.

and ME intake (MEI), determined from the metabolism trials in respirometry chambers, was conducted (**Figure 6**).

The data presented show the high dependence of the MEI variable as a function of DEI. It is important to stress 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.

## 13. Maintenance and production nutrient requirements

Many experiments were already carried out at LAMACA. Nutrient requirements data are still scarce, but some observations can be done. When milk production increases, maintenance requirements in relation to total energy requirement decrease. Energy requirements for maintenance in relation to total energy requirement are 50:50, 32:68, 24:76 on 15, 30, and 45 L of milk/day cows, respectively, according to NRC. Zebu cows (like Gyr) and F1 cows have low to medium milk production. Maintenance requirements can mean a good part of total requirements of these cows. Some papers compared animals with different production potential (milk or weight gain) and showed that there is a positive correlation between production ability and maintenance. Dairy Zebu cows' data is still scarce. [13] compared slaughter technique and respirometry in male F1 – Holstein × Gyr on maintenance, intermediate and *ad libitum* energy intake or 1×, 1.5×, or 2× NEm. *Ad libitum* group had higher NEm (+29%). In this group, the heart, liver, kidneys, and gastrointestinal tract weight were 25, 22, 22, 31% bigger, respectively.

Energy requirement of Gyr, F1 – Holstein × Gyr, and Holstein heifers were studied. Gyr had lower maintenance requirement than Holstein, and F1 was intermediate. Gyr heifers were selected for milk production, but maintenance requirement did not increase at the same proportion. It showed us that Zebu cows require less energy for maintenance, so they can be more economic. We also noticed that younger animals have higher maintenance requirements.

Energy requirements for maintenance increased during lactation. It was expected since organs and visceral tissues are adapted to metabolize many nutrients during lactation. Dairy Gyr heifer had lower dry matter intake than the Holstein, probably because Gyr gastrointestinal tract is smaller. In this way, their $NE_m$ is smaller too.

## 14. Conclusions

Respirometry is an excellent technique that allows the evaluation of the same animal many times, from birth through life, at different physiological status.

Animal nutrition knowledge can be improved by using the respirometric technique that is presented as a technology complementary to comparative slaughter, since it allows the determination of both methane production as well as the efficiencies of energy use.

The determination of the nutritional energy requirements for bovines of different genetic groups and under different feeding conditions allows the appropriate adjustment of the formulation of feeds for each animal category.

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