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## Technical note

## Methods to estimate the energy expenditure of goats: From the lab to the field

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#### ABSTRACT

To asses the energy requirements of animals in open range has been an ambitious objective. Years of laboratory work have resulted in the development of an array of methods to estimate the energy expenditure (EE) on the base of calorimetry trials. Available and more usual methods with application in grazing conditions are: mobile indirect calorimetry (MIC), factorial method, heart rate (HR), and isotopic methods (DLW–double labelled water and CER–CO<sub>2</sub>-entry rate). This paper gives an overview of these methods, their potential and real use, and an account of EE values obtained, to our knowledge, by their application in grazing goats.

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#### 1. Introduction

Small ruminants, and particularly goats, well adapt to rather hard environmental conditions, such as those prevailing in arid and semi-arid lands. Energy is the main limiting factor in animal production and under certain circumstances, as those imposed under grazing conditions, this restriction may increase several fold, even being drastic if the animals are in arid or semi-arid lands where the shortage in vegetation and water imposes an extra energy drain to match their requirements. Energy availability is a key factor in the adaptation to environment, behaviour and feeding strategy of goats. It becomes of great importance to evaluate the additional energy expenditure (EE) and heat production (HP) due to grazing activity, and to provide accurate estimates of the overall EE on which to base patterns of management according to available herbage. There is little information in the literature concerning energy requirements of goats if we compare with other ruminant species and most studies to assess energy requirements have been performed at the laboratory even

\* Corresponding author. Tel.: +34 958 572757; fax: +34 958 572753. *E-mail address:* manuel.lachica@eez.csic.es (M. Lachica). when goat production has traditionally been in open range.

The assessment of energy requirements of animals in open range has been an ambitious objective. Under laboratory conditions it is easy and accurate and several methods are available, mainly calorimetry. Calorimeters restrict the free-living activities and the EE may be biased by this artificial environment. The attempts to escape from calorimeters promoted the development of some methods which allow to estimating the EE in free-living conditions, but, paradoxically, all of them need of the calorimetry for testing and calibration. Few investigators have applied them on farm animals under grazing conditions, and even less to assess EE in the free-living goat. None of the methods can be labelled as "ideal". An easy method in the lab becomes difficult in the field and even its use might not be advisable. The choice of one of them will depend of the available equipment, facilities and objectives.

# 2. Requisites to be complied by the out-door methods

Any method developed to estimate the EE needs complying with some requirements: validity, reliability, acceptability, accuracy and cost (Brockway, 1978; Schoeller





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and Racette, 1990). Validity means that the method has to measure whatever it has been designed for. Reliability implies that the method must work equally well with whatever source of alteration of animal's metabolic rate. Acceptability means that the animal tolerates the method, showing no sign of stress or discomfort and minimizing the time demands for a satisfactory response by the animal or protocol development by the researcher. Accuracy of the method should allow estimating the EE with a maximum error of  $\pm 10\%$ , even if this value may be too big for some purposes. Cost has to be equilibrated, but it will depend on several factors: when the price is high but facilitates a reduced labour cost, it will recoup the differential quickly if monitoring over any length of time is contemplated; if the equipment is reusable, this cost can be amortized over many experiments.

On the other hand, when surgery is necessary, it is advisable to ensure a fast and total recovery of the animal, with minimal interference in its regular activity. If the animal has to carry a device, it should be small, light, robust and as cheap as possible if we expect that damage is possible. Whatever type of device the animal has to carry, it may add some extra energy to the total energy cost. Even when this subject is important, the position and distribution of the device on the body and the visual impact for other animals are much more important than its weight (see review by Lachica and Aguilera, 2005a).

#### 3. Out-door methods

#### 3.1. Mobile indirect calorimetry (MIC)

This method, based on respiratory interchange, was widely used by Brody (1945) with domestic animals. Originally the method makes use of a mask or hood to fit the head of the animal and it is connected to portable respirometry equipment. Logically, in grazing trials hood and masks are difficult to use. It was Flatt et al. (1958) in cattle that use tracheal cannulation. A simple cannula fitted with twoway respiratory valve was used. Young and Webster (1963) used a re-entrant tracheal cannula, which did not by-pass the nasopharynx, so olfaction, communication and thermoregulation of the sheep were not disturbed. The portable respirometry equipment was placed on a little cart pulled by the sheep (Young and Corbett, 1972) and EE was measured. From the practical point of view, to pull the cart adds an extra energy cost and may involve some problems due to the nature of the terrain or some behaviour modifications. This method implies surgery that always adds some extra inherent problems to the animals and management.

A variant of the method was developed by Flatt et al. (1958). Grazing cows were fitted with a harness carrying a Douglas bag in which a portion of the expired air was accumulated. A similar system has been used in field goats by Shinde et al. (2002) to estimate the EE on semi-arid range during different seasons in India. Animals were fitted with a face mask and connected to a balloon. Later, expired-air was analysed for  $O_2$  in a Haldane gas analyser. The average EE obtained were 370, 948 and 1063 kJ kg<sup>-0.75</sup> per day for winter, summer and monsoon, respectively.

#### 3.2. Factorial method

It is based on the first law of thermodynamics. Most of the energy required by the grazing animal is due to standing, eating and locomotion. The contributions of other activities are usually considered negligible. The EE of each of these activities is quantified by calorimetry, and then the total extra energy daily expended is calculated by summation. The efficiency of utilization of metabolizable energy (ME) for maintenance ( $k_m$ ) is accepted to be close to 0.70. That means that the increment in the animal's energy requirements due to its physical activity will be given by the expression  $\Delta ME = \Delta HP/0.70$ . Graham (1964), applying the factorial method, reported that a sheep of 50 kg spent 42% more energy on the pasture than a caged animal.

The energy cost of locomotion is relatively well defined in domestic goats. Horizontal energy cost has been reported to range from 3.35 to  $3.63 \text{ J kg}^{-1} \text{ BW m}^{-1}$ . The energy cost of vertical ascent and the energy recovered on descent have been found to be 31.7 and  $13.2 \text{ J kg}^{-1} \text{ BW m}^{-1}$ , respectively. The energy cost of eating in goats, expressed as  $\text{ J kg}^{-1} \text{ BW g}^{-1} \text{ DM}$ , ranged from 1.8 to 12.0 for concentrates and forages, respectively; and from 44.3 to 118.3 for forages and concentrates, respectively, when expressed as  $\text{ J kg}^{-1} \text{ BW min}^{-1}$  (see review by Lachica and Aguilera, 2005b).

The accuracy in the determination of the energy costs would have no value if the assessment of the distance travelled or food ingested in the field would not have a similar level of accuracy. Several devices and techniques have been developed. Some of them are carried out by the observer and others, by the animals. The named "rangemeters", pedometers, GPS (Global Position System) and some others have been used to estimate distance travelled (see review by Lachica and Aguilera, 2005a). Total faeces collection with faecal bags, oral markers (Cr2O3, n-alkanes, internal markers, etc.), visual control, rumination/chewing monitors, etc. are used to estimate total intake directly or through the measurement of the time spent eating (see review by Mayes and Dove, 2000). Nowadays the currently technology has qualified the factorial method as a powerful tool to estimate EE in open-range. Prieto et al. (1992) observed in free ranging goats that the annual mean increase in EE above maintenance due to the act of eating attained 8.8% of their total HP. In goats, EE associated with locomotion was studied in two semi-arid areas of Spain by Lachica et al. (1997) and Lachica et al. (1999) which reported increases in HP expressed as annual mean above maintenance of 10.8 and 26.3%, respectively.

#### 3.3. Heart rate (HR)

The HR can be an indirect estimator of the EE, on the basis of observed correlations between heart rate and  $O_2$  consumption (VO<sub>2</sub>) and consequently HP, under the controlled, confined conditions of a calorimeter. However, there are some unsatisfactory points to have in mind. The HRs change independent of physical activity in response to emotional factors and stress. This implies an increase in HR without a proportional increase in  $O_2$  consumption. Also, there is an important intra individual variation. Webster (1967) and Brockway and McEwan (1969) obtained in sheep acceptable linear relationships between HR and VO<sub>2</sub> but not in all the animals. These facts emphasize the requirement of an "individual calibration" in the laboratory under similar conditions than in the field. The method has experienced a new renaissance as data retrieval is supported by new sensing, monitoring and data transmission technology. Lately, the development of biotelemetry permits the implantation of transmitters inside the animals. The signals used are generally carried by radio waves, although they may be transmitted by other systems. This technology eliminates the so-called "handling artefact" which may increase heart rate for many minutes. When telemetry devices are totally implanted the labour involved in data collection is reduced and the data can be collected from animals for long periods of time without the continuous presence of observers. This leads to lower cost/data point collected. This methodology has been used to estimate EE in open range by grazing goats at three stocking rates; the increase over MEm  $(438 \text{ kJ} \text{ kg}^{-0.75} \text{ per day})$  ranged from 13 to 46% (Animut et al., 2005) for medium and high stocking rates, respectively.

#### 3.4. Isotope dilution

Two isotope dilution methods have been proposed to estimate  $CO_2$  production and subsequently to assess total EE of animals in free-living conditions: double labelled water (DLW;  $^{2(3)}H_2$ <sup>18</sup>O) and CO<sub>2</sub>-entry rate (CER; NaH<sup>13(14)</sup>CO<sub>3</sub>). The estimated CO<sub>2</sub> production has to be related to EE (HP) assuming a value for the respiratory quotient (RQ). This could be considered a weakness of the methods. However, examination of RQ values in grazing animals suggests that RQ differences would be unlikely to cause large bias in estimates of EE in the field whenever relationships of CO<sub>2</sub> production with entry rate had been established in fed animals. The variation in the energy equivalent of CO<sub>2</sub> would be less than 10%.

Most of kinetic studies in the past decades used radioactive tracers. Since the 1970s an increased awareness of the biohazards of radioactivity, the greater availability of stable isotopes and development of mass spectrometry techniques stimulated the use of stable isotopes. However, as they are naturally occurring in the body pool it is necessary to determine their natural abundance before the start of the tracer infusion.

#### 3.4.1. Double labelled water

The DLW method (Lifson et al., 1955) relies in an unique dose of  ${}^{2}\text{H}_{2}{}^{18}\text{O}$  to the animal, after which  ${}^{2}\text{H}$  is eliminated as H<sub>2</sub>O but  ${}^{18}\text{O}$  is removed also as CO<sub>2</sub>, so the different elimination rates between both isotopes is used to determine CO<sub>2</sub> production by sampling a body fluid. As the water pool in animals is very large, the method allows integrated values of EE of long periods of time (1–4 weeks). The natural abundances of  ${}^{2}\text{H}$  and  ${}^{18}\text{O}$  in body's organic compounds prior to the administration of doubly labelled water as tracer need to be measured, as well as any loss of  ${}^{2}\text{H}$  or  ${}^{18}\text{O}$  as products other than CO<sub>2</sub> and water (sequestration). Hydrogen can be lost from the body water as sequestered hydrogen bound to carbon in fat, protein and carbohydrate or as hydrogen in the exchangeable positions of compounds which are subsequently exported from the body. To a limited extent <sup>18</sup>O may also be sequestered and exported as exchangeable <sup>18</sup>O and this will partially offset any error due to <sup>2</sup>H loss. There are several correction (fractionation) factors to be considered as well as the total evaporative loss of water, a factor which may be an important cause of error when the method is used in hot climates where the evaporation loss can be important. Its use is not recommendable for short periods of time, and for labelling animals with the large size of a goat because of the high cost it implies.

This method has been used in goats by Junghans et al. (1997) at the laboratory, simultaneously with  $H^{13}CO_3^-$  and calorimetry for comparative purposes, with a good agreement; and by Toerien et al. (1999) in open range to estimate the EE by goat bucks during peak breeding season, obtaining an increase over the MEm (424 kJ kg<sup>-0.75</sup> per day) of 9%.

### 3.4.2. CO<sub>2</sub>-entry rate

The CER makes use of  $H^{13}CO_3^-$  which is infused into the animal at a constant rate, and equilibrium is established with endogenous bicarbonate. The entry rate of bicarbonate (production of  $CO_2$ ) can be calculated from the infusion rate and the measured dilution of the isotope at equilibrium in some body fluid, what can be performed by continuous collection or collecting multiple samples taken at appropriate intervals. It was developed by Young et al. (1969) using <sup>14</sup>C in sheep.

In a broad sense, two main problems arise when <sup>13</sup>Clabelled tracers are used: First, <sup>13</sup>C naturally contributes approximately 1.1% of the carbon pool. Furthermore, isotopic fractionation occurs within components of animal tissues. Specifically, differences in natural abundance of <sup>13</sup>C in fat, carbohydrate and protein, whenever different metabolic fuels are used during various physiological and nutritional conditions, will alter the enrichment in H<sup>13</sup>CO<sub>3</sub><sup>-</sup> produced. The variation in natural abundance of <sup>13</sup>C must be taken into account in the design and subsequent analysis involved in the tracer study. This disadvantage can become an advantage, i.e. in some traceability studies, when either the origin of the naturally labelled energy source or its contribution to dietary energy intake or energy balance is unknown. Concerning the second problem a serious drawback of CER technique concerns the sequestration of labelled carbon in body metabolites which prevents quantitative recovery of the isotope and results in overestimation of CO<sub>2</sub> production, so the isotope recovery needs to be quantified.

The CER method has been widely applied in goats with success. Even when the animals need to be fitted with a harness housing an infusion pump and bags containing the NaH<sup>13</sup>CO<sub>3</sub> infusate and for a continuous fluid sampling, this approach offers the possibility of relatively simple analytical procedures and lower cost in terms of isotope and mass spectrometry facilities. In a first step, the method was set up at the laboratory and later it was applied to grazing conditions (see review by Lachica and Aguilera, 2003). The impact of grazing activity and stocking rate were studied (see review by Lachica and Aguilera, 2005b). It was found an increase over maintenance HP of 52 and 23% for high and low stocking rate, respectively. Cold exposure, induced

by shearing, caused a drastic increase in HP (Lachica et al., 2007). Unshorn goats showed a HP value in agreement with the ME<sub>m</sub> (438 kJ kg<sup>-0.75</sup> per day). This fact can be considered as an index of the validity of the method. Whereas, shorn goats showed a value of  $1.98 \times ME_m$ .

#### 4. Conclusions

None of the above methods can be labelled as "ideal". Researchers must calibrate the pros and the cons and make the choice according with their research objectives. The spectacular development of the current technology qualifies the above methods as very powerful tools to estimate EE in open-range with minimal disturbance and improving animal welfare. Anyway, further investigation is needed to improve them and develop new ones for contributing to significant improvements in models for grazing systems, particularly, for goats.

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