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Short communication

Heat production determined by the RQ and CN methods, fasting heat production and effect of the energy intake on substrates oxidation of indigenous Manchega sheep

C. Fernández[a,*], M.C. López[a], M. Lachica[b]

[a] Institute for Animal Science and Technology, Universidad Politécnica de Valencia, 46022 Valencia, Spain
[b] Department of Physiology and Biochemistry of Animal Nutrition, Estación Experimental del Zaidín (CSIC), Camino del Jueves s/n, 18100 Armilla, Granada, Spain

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ABSTRACT

Heat production (HP) from CH_4 and CO_2 production, and O_2 consumption was determined by the respiratory quotient (RQ) method and by the C–N balance (CN method). Twelve dry Manchegas sheep were fed with a diet constituted by alfalfa hay and barley grain at three levels, approximately 1, 1.5 and 2× metabolizable energy for maintenance (MEm). Later, the sheep were fed close to maintenance and HP determined by indirect calorimetry after 3 d fasting. The sheep were allocated to metabolism cages. After 10 d of adaptation, feed intake, and total fecal and urine output were recorded daily during 5 d, as well as the body weight (BW) at the beginning and end of the sampling period. Gas exchange measurements were recorded by a mobile open-circuit respirometry system. Average HP measured by RQ method was in agreement with the HP determined by CN method accounting for 410 and 407 kJ/kg$^{0.75}$ BW/d, respectively. Fasting HP was 268 kJ/kg$^{0.75}$ BW. Proportion of energy associated to the oxidation of protein (OXP), carbohydrate (OXCHO) and fat (OXF) was calculated. In fasting condition most of HP associated to substrates oxidation (HPx) was due to OXF (0.931) and less to OXP (0.037) and OXCHO (0.032). OXP was increased with the feeding level and was greater (0.193) than in fasting. OXCHO was increased with the feeding level until a stable level (0.507).

1. Introduction

There is a lack of information about energy metabolism in Spanish breeds with only one published paper which deals about fasting metabolism and energy requirements for maintenance of Segureña sheep (Aguilera et al., 1986). Manchega breed is one of the most important in Spain; from a census of 1.63 million pure sheep more than 9% is Manchega (FEAGAS, 2009). It is mainly used for making cheese with and elevated productivity (250 L milk/sheep/yr; 4–4.5 L/kg of fresh cheese), demand and profits.

Abbreviations: ADFom, acid detergent fiber exclusive of residual ash; aNDFom, neutral detergent fiber exclusive of residual ash; BW, body weight; CO_2x, CO_2 production from oxidation; CP, crude protein; DM, dry matter; GE, gross energy; HP, heat production; HPx, heat production associated to substrates oxidation; Lignin(sa), lignin determined by solubilization of cellulose with sulfuric acid; MEI, metabolizable energy intake; MEm, metabolizable energy for maintenance; OXCHO, oxidized carbohydrate; OXF, oxidized fat; OXP, oxidized protein; RE, retained energy; RE_{fat}, retained energy in fat; $RE_{protein}$, retained energy in protein; RQ, respiratory quotient.

* Corresponding author. Tel.: +34 963 877 007; fax: +34 963 877 439.
E-mail address: cjfernandez@dca.upv.es (C. Fernández).

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Most studies to determine heat production (HP) have used indirect methods. One of them entails measurements of O_2 consumption, CH_4 and CO_2 production and urine-N (RQ method); another uses the C–N balance (CN method) to calculate the retained energy (RE), assuming that all energy is retained either as fat or protein. Then, HP is calculated as the difference between metabolizable energy intake (MEI) and RE. Very similar values for HP have been obtained with both methods (Blaxter, 1967), although lightly greater values were obtained with the RQ method.

Fasting HP reduces the effect of the diet on HP to a minimum. The agreement with published data can be considered as an index of reliability and, tentatively, it could be used to estimate an approximate value of metabolizable energy requirement for maintenance (MEm) from the published data of its efficiency.

The CO_2 production is derived from nutrient oxidation and rumen fermentation. The separation between these two components is necessary to calculate the substrates oxidation and its contribution to the total HP (HPx). Production of volatile fatty acids from carbohydrate fermentation is followed by CO_2 and CH_4 production. Fahey and Berger (1988) demonstrated a ratio CO_2/CH_4 of 1.7/1 for high forage diets.

The objective of this study was to determine under different feeding levels the HP *via* RQ and CN methods and their agreement, fasting HP and the contribution of protein (OXP), carbohydrates (OXCHO) and fat (OXF) oxidation to HPx in indigenous Manchega breed.

2. Materials and methods

2.1. Animals and feeding

The experimental procedure was approved by the Committee on Animal Use and Care at the Politechnic University of Valencia. Twelve mature Manchega female dry sheep of similar body weight (62.3 ± 2.3 kg BW) were used to determine gas exchange and C-N balances. Diet was a mix of 130 and 870 g/kg of barley grain and alfalfa hay, respectively. Its chemical composition was 897.9 g/kg for dry matter (DM); 310.5, 57.1, 471.0 and 145.9 g/kg DM for acid detergent fiber (ADFom), lignin(sa), neutral detergent fiber (aNDFom) and crude protein (CP), respectively; and 18.6 MJ/kg DM for gross energy (GE). Three levels of MEI were used with the same sheep corresponding approximately to 1, 1.5 and 2× MEm (374 kJ/kg$^{0.75}$ BW/d, Aguilera et al. (1986); treatments Low, Medium and High, respectively). Later, the sheep were fed close to maintenance level (15 d) for determining after 3 d fasting the HP by indirect calorimetry. Half daily ration was offered at 08:00 and 16:00 h, respectively. Sheep had free access to a mineral-vitamin block and water.

2.2. Experimental schedule and measurements

Sheep were allocated in individual metabolism cages. After 10 d of adaptation, feed intake, and fecal and urine output were recorded daily during 5 d, as well as BW at the beginning and end of the period. Representative samples of diet, feces and urine were collected daily, stored at −20 °C, and pooled for chemical analysis. Within the balance trial, gas exchange was measured for each treatment during 15 min/h per sheep and repeated each 3 h during 24 h (8 measures/d with 4 sheep/h) using a mobile open-circuit respirometry system (Fernandez et al., 2012) attached to the sheep by a face mask. When the gas exchange determinations were over, fasting HP was determined following identical schedule.

2.3. Calculations

MEI was calculated as the difference between GE intake and energy losses in feces, urine and CH_4 (CH_4 energy equivalent = 39.54 kJ/L; Brouwer, 1965).

HP (RQ method) was calculated according to Brouwer (1965) as HP (kJ) = 16.18×O_2 (L) + 5.02×CO_2 (L) − 2.17×CH_4 (L) − 5.99×urine N (Nur, g). RQ was determined as CO_2 produced/O_2 consumed. RE was calculated as MEI − HP.

In the CN method, C balance was the total amount of C retained in the body where the amount of C retained in fat was calculated by subtracting the amount of C retained in protein determined by N balance. Assuming an energy equivalent of 39.76 kJ/g and a content of 0.767 C for fat, and 23.86 kJ/g and 0.16 N and 0.52 C for protein, RE (kJ) in protein ($RE_{protein}$) and fat (RE_{fat}) was calculated, respectively, as $RE_{protein}$ = N balance (g)×6.25×23.86, and RE_{fat} = (C balance (g) − N balance (g)×6.25×0.52)×1.304×39.76. The RE (kJ) was calculated according to Brouwer (1965) as RE = $RE_{protein}$ + RE_{fat}.

The energy associated with the oxidation of protein (OXP), carbohydrate (OXCHO) and fat (OXF) was calculated by the method followed by Chwalibog et al. (1997). CO_2 production from oxidation (CO_2x) was calculated as $CO_2 - (1.7 \times CH_4)$ (high forage diet; Fahey and Berger, 1988). The calculations were carried as following: OXP = 6.25×Nur×18.42 (kJ/g), OXCHO = (−2.968×O_2 + 4.174×CO_2x − 2.446×Nur)×17.58 (kJ/g), and OXF = (1.719×O_2 − 1.719×CO_2x − 1.963×Nur)×39.76 (kJ/g). Then, HP from oxidation was HPx (kJ) = 16.18×O_2 + 5.02×CO_2x − 5.99×Nur. Gases were expressed in L/d and Nur in g/d.

2.4. Chemical analysis

It was conducted according to the methods of AOAC (2000) for DM (no. 934.01) and CP (no. 968.06). GE content was determined in an adiabatic bomb calorimeter. Acid detergent fiber (ADFom) and neutral detergent fiber (aNDFom) were