Additional Information

Table 2 contained an incorrect footnote. The correct version is supplied at: http://dx.doi.org/10.1017/S0021859615000556

Low-cost mobile open-circuit hood system for measuring gas exchange in small ruminants: From manual to automatic recording

SUMMARY

Improvements of a home-made mobile open-circuit respirometry system for the rapid determination of methane (CH_4) and carbon dioxide (CO_2) production, and oxygen (O_2) consumption and, thereafter, heat production (HP) for small ruminants are described and validated. Upgrades consisted of three main features: utilization of a head hood (replacing the previous face mask); use of computerized control system, data acquisition and recording for gases and air flux (replacing collecting bags for air sampling); and use of a gas cooler to remove the air sample moisture (replacing the chemical drier (silica gel) approach). Calibration factors were established injecting nitrogen (N_2) and CO_2 in the system into the head hood. Repetitive and consistent values for the calibration factor were obtained for O_2 and CO_2 which confirmed the absence of leaks and the good performance of the system. In addition, an experimental test with 12 Manchega female dry sheep was conducted to validate the system. Three diets based on cereal grain, fibrous by-products and alfalfa hay (ALH) were used with four sheep per diet. Metabolizable energy intake was close to metabolizable energy for maintenance. Average HP measured by indirect calorimetry (respiratory quotient (RQ) method) was close to the average HP determined from Carbon-Nitrogen balance (CN method) accounting for 443 and 426 kJ/kg$^{0.75}$ body weight (BW) per day, respectively. Fasting HP was determined by the RQ method with two sheep from the ALH diet accounting for 269 kJ/kg$^{0.75}$ BW per day. The head hood and computerized control, data acquisition and recording as well as the gas cooler improved the system by reducing the labour input without loss of functionality for measuring gas exchange and energy metabolism in small ruminants.

INTRODUCTION

Nowadays there is a great concern about gas production by ruminants in the scientific community. Many research institutions are building facilities for accurate measurement of such production, in many cases with small economic budgets.

Usually, indirect calorimeters are associated with high-cost facilities where respirometry chambers and equipment are allocated in a laboratory building. Any method developed to estimate energy metabolism needs to comply with various requirements: validity, reliability, acceptability, accuracy and cost (Lachica & Aguilera 2008). The first four requirements are accomplished in a calorimetry laboratory but with high economic cost. If the cost has to be low, one option is to build a mobile open-circuit respirometry system where costs can be reduced drastically, becoming an outdoor method. A detailed description of a home-made mobile open-circuit respirometry system was published previously (Fernández *et al.* 2012a): it showed that no recording apparatus was required to obtain the total average gas concentration during the measured period, by using non-diffusing gas bags for air collection and a face mask. Also, prior to gas analysis the air sampled was dried, passing through silica gel to absorb the moisture.

To obtain rapid response times on relatively short periods, a face mask enclosing only the mouth of animals or head hoods in connection with the open-circuit have been employed before, with good results (Brosh 2007). However, face masks prevent animals from eating during the measurements and extended use may cause discomfort and distress; therefore, the use of head hoods is preferred to masks for long-term measurements (Takahashi *et al*. 1999). Computerized system control, data acquisition and recording requires low labour input and has the advantage of producing, if required, minute-to-minute records of gas exchange. Also, with respect to the labour reduction, the use of a chemical drier (e.g. silica gel) to absorb moisture instead of a physics approach using a gas cooler is a disadvantage, since the drier needs to be replaced periodically.

The objective of the present study was to evaluate an improve system intended for use in energy metabolism studies: its construction, function and technical approach. It describes the improvements made on a previous home-made mobile open-circuit respirometry system that can also be used outdoors. Assitionally, an experimental test was performed with sheep using three different diets where heat production (HP) determined by indirect calorimetry (respiratory quotient (RQ) method) was compared with the estimated by carbon-nitrogen balance (CN method).

MATERIALS AND METHODS

Open-circuit respiration system

The system was made and set up for small ruminants (sheep and goats). Improvements made over a previous home-made mobile open-circuit respirometry system consisted of: (1) the substitution of a face mask by a head hood; (2) substitution of collecting bags for air sampling by a computerized control system, data acquisition and recording for gases and air flux; and (3) substitution of a

chemical drier (silica gel) approach for a gas cooler to remove moisture from the air sample. A detailed description of the home-made mobile open-circuit respirometry system was previously published previously (Fernández *et al.* 2012a). Briefly, the instrumentation was installed on a mobile cart to make the system portable (Fig. 1). Pipes, rotameter (DK800; ABB, Alzenau, Germany), flow meter (Thermal Mass Flowmeter Sensyflow VT-S; ABB Automation Products GmbH, Alzenau, Germany), air volume totalizer (Totalizer VT-S; ABB Automation Products GmbH, Alzenau, Germany), adjustable and precise membrane pump (ABB Automation Products GmbH, Alzenau, Germany), gas cooler (SCC-C; ABB, Alzenau, Germany) and fan (CST60; Soler Palau Inc., Parets del Vallès, Barcelona, Spain) were attached to the bottom of the mobile unit. A gas analyser unit for measurement of methane (CH_4), carbon dioxide (CO_2) and oxygen (O_2) (Easyflow 3020; ABB Automation Products GmbH, Alzenau, Germany) and a computer for system control, data acquisition and recording were on the upper part of the unit. All the analytical apparatus were acquired with digital output for computer connection. The respirometry system had two separate but linked sampling lines. The main line suctioned air across a head hood attached to a polyvinyk chloride corrugated tube (internal diameter 25 mm) equipped with an air filter to avoid atmospheric dust. A secondary line (internal diameter 5 mm), situated behind the mass flow meter, took a gas sample from the main line by means of the aforementioned membrane pump attached to the rotameter and connected to the gas analyser unit. The gas sample was filtered and dried through the gas cooler to remove moisture prior to analysis.

The head hood (Fig. 2) was made with 1.5 mm galvanized plate (530 mm long × 1160 mm high × 360 mm wide; volume = 219 litres). It was suspended on the front structure of the metabolic cage by two hooks placed on its rear side. The hood had a transparent acrylic window (bolted and glued with silicon on the edge) at the front (420 mm long × 530 mm high), and a drawer (500 mm long × 290 mm high × 350 mm wide) with a handle to open and place the animal food and water in a bucket. The drawer was locked by two lateral locks situated in its front side and main body of the head hood. A foam tape was placed on the edge of the drawer for an adequate seal. An opening (200 mm long × 520 mm high) was in the rear panel of the hood set up with a tightly woven nylon curtain (funnel shape) with a hole in the middle for the animal neck, fixed by four bolted platens and glued all around the opening edge. It was set up with a nylon drawstring through a fold edge to fit and tie it around the neck to avoid gas leaking. The animal was attached to the front structure of the metabolic cage by a necklace and chain so that it could freely stand or lie down. Atmospheric air entered into the head hood through a hole (internal diameter 20 mm) made in the top on the opposite side of the main suction line.

System control, data acquisition and recording were handled by a computer (Fujitsu Siemens; Lifebook Series, Pentium 4 laptop, Munich, Germany) under a LabVIEW (www.ni.com/labview) environment. The software to operate the system was created using National Instrument LabVIEW 7.1 (National Instruments, Austin, Texas, USA) running on Microsoft Windows XP. The system was capable of

recording data at intervals of 1 s, but intervals of one per minute were used in the present study. The gas analyser unit and flow meter was connected to the computer by a universal serial bus connector. A serial communication protocol was used with its programmable logic controllers (MODBUS Organization, Inc., Hopkinton, MA, USA; www.modbus.org) for connection between the analytical devices (analyser unit and flow meter) and the computer. The electronics prototyping platform Arduino (www.arduino.cc) was used to send data from the gas analyser unit to LabVIEW by RS-232 protocol in real time. The flow meter was monitored by a 10-bit analogue to digital converter (model DS2438; Maxim Integrated Products, Inc. Sunnyvale, CA, USA).

Whole system calibration

The whole system was calibrated by injecting from a cylinder pure nitrogen (N_2) gas (0.9999) and CO_2 (0.9999) into the head hood (McLean & Tobin 1987), to produce an O_2 decrement and CO_2 increment. Calibration factors comparing the volume of gas injected and detected by the system were obtained. Total gas released was determined gravimetrically using a precision electronic scale (MOBBA mini-SP 0.2-30 kg). Sufficient gas was released (c. 375 and 177 g of N_2 and CO_2, respectively, during 6 h) to ensure accuracy in the measurement of the cylinder weights, and subsequently injected at the required flow rate to simulate the O_2 and CO_2 exchange produced by an animal in the system. Calibration factors were calculated according to Brockway *et al*. (1971).

Calculations

Methane and CO_2 production, and O_2 consumption, were calculated as described Aguilera & Prieto (1986) using the Haldane transformation, except that no theoretical values for atmospheric CH_4, CO_2 and O_2 concentrations were used. Before gas measurement, atmospheric air was sampled and the gas concentration values used as reference in calculations.

Experimental test

Animals and feeding

Experimental procedure was approved by the Committee on Animal Use and Care at the Polytechnic University of Valencia (Spain).

Twelve Manchega female dry sheep of similar body weight (58 ± 1.2 kg BW) were selected to determine the energy and C-N balances. Three diets were used based on cereal grain (CGR), fibrous by-products (FBP) and alfalfa hay (ALH) with four sheep per diet. Cereal straw was offered as forage in CGR and FBP diets, and the concentrate was mixed and pelleted along with the premix. The forage : concentrate (on dry matter (DM) basis) ratio of CGR and FBP diets was 30 : 70. Ingredients and chemical composition of the diets are shown in Table 1. The feeding level was close to metabolizable energy for maintenance (MEm). Sheep had free access to a vitamin-mineral block and water. Full daily ration was offered at 09.00 h.

Sheep were housed in a barn where temperature and relative humidity were recorded automatically every 15 min and averaged daily during the experimental trial; these ranged from 20.2 to 25.1 ºC and from 62 to 77%, respectively.

Chemical analysis

Feed and faeces were dried in a forced air oven at 55 ºC for 48 h and then ground to pass through a 1 mm screen. Urine was dried by lyophilization. Chemical analyses were conducted according to methods of AOAC (2000) for DM (no. 934.01), ash (no. 942.05), ether extract (no. 920.39) and crude protein (no. 968.06). Gross energy content was determined in an adiabatic bomb calorimeter (Gallenkamp Autobomb; Loughborough, UK). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) of diets were determined using filter bags and a fibre analyzer (A220; ANKOM Technologies, Fairport, NY, USA) following AOAC (2000) official methods (no. 973.18) according to Mertens (2002). Acid detergent lignin (ADL) was determined according to Robertson & Van Soest (1981). Carbon and N were analysed by Dumas principle (TruSpec CN; Leco Corporation, St. Joseph, MI, USA).

Energy and C-N balances, and heat production determination

Sheep were allocated on individual metabolism cages. After 12 days of adaptation to experimental conditions, feed intake, total faecal and urine output were recorded daily for each sheep over a 5-day period. Body weight was recorded at the beginning and end of experimental period. Representative samples of diet, faeces and urine were collected daily, stored at -20 ºC, and pooled for chemical analysis.

Metabolizable energy intake (MEI) was the difference between GE intake and energy losses via faeces, urine and CH_4 (with an energy equivalent value of 39.5388 kJ/l; Brouwer 1965).

After the energy and C-N balances, gas exchange was sequentially measured during 24 h/sheep/diet. Gas exchange was repeated with two sheep from ALH diet after 3-day fast to measure the fasting HP.

Daily HP (kJ), determined by the RQ method, was calculated according to Brouwer (1965) for O_2 consumption, CO_2 and CH_4 productions (l), and urine-N (g) as $HP = 16.18 \times O_2 + 5.02 \times CO_2 - 2.17 \times CH_4 - 5.99 \times$ urine-N. The RQ was calculated as the ratio of CO_2 produced : O_2 consumed. Retained energy (RE) was determined as the difference between MEI and HP.

Retained energy (kJ) determined via the CN method, was calculated according to Brouwer (1965) from the C (g) and N (g) balance ($RE = 51.8 \times C - 19.4 \times N$). Heat production was calculated as the difference between MEI and RE.

Statistical analyses

Analysis of variance was conducted on the experimental data, with the animal as the experimental unit, to determine the treatment (diets) effect. The effects of the diet (based on CG, FBP and ALH) on energy and C-N balances were analysed using the PROC GLM of SAS (2001). The statistical model was $Y = \mu + (D) + \varepsilon$, where Y was

the dependent variable, μ the overall mean, D the fixed effects of diet and ε the random error. A Tukey multiple range test was used to ascertain the statistical significance of differences ($P < 0.05$). Mean values of HP obtained by RQ and CN method were compared by Fisher's least significant difference test, and significance was set at $P < 0.05$. Regression analysis (PROC REG of SAS (2001)) was used to establish the relationship between HP obtained by RQ and CN methods.

RESULTS

The average value for the calibration factor was 1.006 ± 0.0016 (standard desviation (SD); n = 5) and 0.992 ± 0.0092 (SD; n = 5) for O_2 and CO_2, respectively. The values were consistent, proving the absence of leaks and good performance of the whole system.

Table 2 shows the daily energy balance and HP determined by the RQ method, including the average MEI and the resultant RE. Although comparison among diets was not the main objective of the present study, MEI was similar for all three ($P < 0.05$); however, HP was higher ($P < 0.05$) for the ALH diet with respect to the other two. This was possibly as a result of the different nature and physical structure of the feed consumed (Lachica et al. 1997) since the period of eating was included in the 24 h gas exchange measurements.

Daily C-N balance, calculated RE and estimated HP by the CN method are also displayed in Table 2.

The RE determined with the CN method was greater than that calculated with the RQ method (95 v. 67 kJ/kg$^{0.75}$ BW per day, on average).

When daily HP (kJ/kg$^{0.75}$ BW) obtained with the RQ method was related to the CN method, the best fit corresponded to the following equation:

$$HP_{RQ\ method} = 1.07 \pm 0.018(S.E.)\ x\ HP_{CN\ method};$$
$$R^2 = 0.769;\ RMSE = 24.7;\ n = 12$$

Where S.E. is the standard error (SD), R^2 is the coefficient of determination and RMSE is the root-mean-square error. However, both methods have shown no significant differences for each diet, accounting for 431 v. 410 ($P = 0.987$), 404 v. 379 ($P = 0.269$) and 462 v. 425 ($P = 0.315$) kJ/kg$^{0.75}$ BW/day (CGR, FBP and ALH, respectively) for RQ v. CN methods.

The average RQ value for the three diets was 0.92 ± 0.031 (SD) and 0.66 ± 0.024 (SD) for fasting sheep. Fasting HP for the ALH diet was 269 ± 10.3 (SD) kJ/kg$^{0.75}$ BW/day.

DISCUSSION

An open-circuit respirometry system has the primary function of measuring gas exchange and then the determination of HP based on the animal's CH_4 and CO_2 production, and O_2 consumption. The accuracy of the gas exchange determination is further dependent on the ability of the system to measure gas composition and

importantly, the total volume of the air moved through the respirometry system (Fernandez *et al.* 2012a). To obtain a fast response of the system in a short time period, masks or hoods enclosing only the animal's face or head in connection with open circuits have been frequently employed with ruminants (Young *et al.* 1988; Takahashi & Young 1992; Puchala *et al.* 2007). An open-circuit system attached to a face mask presents the lowest cost and it is best suited and sufficiently accurate for fast response applications for measurement of gas exchange in animals for a short period of time (Fernandez *et al.* 2012a). However, the mask prevents animals from eating and may cause discomfort and distress, which can consequently affect the gas exchange. Also, the use of a face mask requires a very close contact with the staff involved in the study to ensure full adaptation of animals to the experimental conditions (Lachica & Aguilera 2005). To avoid this inconvenience, the head hood represented a better and cheaper alternative for measuring gas exchange for 24 h, with minimum disturbance for the animal (Kelly *et al.* 1994; Brosh 2007). The system was built for operational flexibility, which allows a variety of experimental arrangements. It can be connected not only to a head hood, but also to a mask or a respirometry chamber and is able to measure a broad animal range of body weights.

The range of temperature during the experimental test implied that no HP associated with temperature stress was elicited. The diets used were typical of those in Eastern Spain for dry sheep to prepare the herd for a new production cycle.

Fasting HP was also measured in order to compare the present data with that from the literature, since fasting reduces the possible effect of the diet on HP to a minimum. The agreement with published data can be considered as further evidence of the system's reliability. Fasting HP was in agreement with the value obtained for the same sheep breed by using the previous non-improved mobile open-circuit respirometry system (268 kJ/kg$^{0.75}$ BW per day; Fernandez *et al.* 2012b) and similar to that obtained for Segureña sheep, also by indirect calorimetry (272 kJ/kg$^{0.75}$ BW per day; Aguilera *et al.* 1986). Freetly *et al*. (1995, 2002) found no differences for fasting HP between Suffolk and Texel ewes (318 kJ/kg$^{0.75}$ BW per day, on average) and between Finnsheep and Rambouliet ewes (297 kJ/kg$^{0.75}$ BW per day, on average) when compared at the same proportion of mature BW, and no differences were found in fasting HP (296 kJ/kg$^{0.75}$ BW per day, on average) with ewes of seven breeds differing in potential of production (Olthoff *et al*. 1989). It is well known that differences in fasting HP could be explained by the previous MEI of the animals. Fasting HP is typically determined after 3- day fast and one might expect less variation in energy expenditure than in the fed state. Distribution of body components (protein and fat) is important in determining fasting HP, especially with an *ad libitum* consumption before the fasting HP measurement. Tovar-Luna *et al*. (2010) determined fasting HP in lactating dairy goats after *ad libitum* consumption (0.98 kg DM/day) and subsequently after an intake close to maintenance (0.84 kg DM/day), reporting a 13% decrease for the latter.

The RQ for fed and fasting sheep were in close agreement with the regular values obtained under such circumstances (Kleiber 1972) and could also be used as evidence for reliability of the measurements.

The C-N balance (CN method) is frequently determined in association with indirect calorimetry measurements (Blaxter 1967) and it depends on measurements of C and N intake and their losses as urine, faeces and gases (CO_2 and CH_4). The CN method generally results in an overestimation of RE because C-N balance is usually overestimated due to evaporative and other losses in excreta (Just et al. 1982). Thus, the RQ method can be expected to systematically yield higher values for HP than the CN method. Both methods are partially dependent on each other and the close agreement between them may be considered as further evidence of the system's reliability (as shown by the regression analysis). Detailed descriptions of the errors on the estimation of RE (or HP) by the CN method have been given elsewhere (Blaxter 1967; Christensen et al. 1988). Discrepancies between both methods averaged 0.055 when expressed as percentage of the MEI, a rather satisfactory value taking into account the considerable value taking into account the considerable amount of technical and analytical work involved. Fernández et al. (2012b) obtained an average discrepancy of 0.005 in sheep using a face mask and the same diet at three different MEI (instead of the same MEI for the three diets in the present study), and Aguilera & Pietro (1986) of 0.018 in respirometry chambers and fed at about maintenance level. As an outdoor method, the present mobile open-circuit hood system complies with the accuracy requisite for determining gas exchange and hence HP (where the maximum acceptable error is 0.1; Lachica & Aguilera 2008).

CONCLUSIONS

The present study shows the improvements made on a home-made mobile open-circuit respiration system to measure CH_4, CO_2 and O_2 animal exchange and consequently HP. All sheep were easily adapted to the head hood for periods of 24 h with no sing of discomfort or stress. The low-cost system described is suitable and feasible to be adapted and used for studies of gas exchange and energy metabolism under a wide range of physical, physiological and nutritional situations with a considerable labour reduction.

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Table 1. *Ingredients (g/kg) and chemical composition (g/kg DM) of the three diets based on cereal grain (CGR), fibrous by-products (FBP) and alfalfa hay (ALH)*

	CGR	FCH	ALH
Ingredients			
Alfalfa hay	-	-	1000
Barley	430	53.3	-
Straw	306	303	-
Soymeal (440 g CP/kg)	187	121	-
Soya hulls	-	223	-
Gluten feed (180 g CP/kg)	-	209	-
Wheat bran	-	48.3	-
Lard	20.8	20.9	-
By-pass fat	6.4	12.7	-
Beet molasses	27.8	3.5	-
Calcium carbonate	8.9	1.9	-
Bicalcium phosphate	6.9	-	-
Sodium chloride	2.9	0.8	-
Premix*	2.8	2.8	-
Chemical composition			
DM	891	901	894
Ash	63.7	67.5	95.0
CP	169	158	152
EE	50.7	62.8	9.9
NDF	437	508	565
ADF	167	262	389
ADL	10.7	9.2	76
C	428	425	449

DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; C, carbon.

* Provided by NACOOP S.A. (Spain) to give (ppm or IU/kg of premix): Se, 40 ppm; I, 250 ppm; Co, 80 ppm; Cu, 3000 ppm; Fe, 6000 ppm; Zn, 23 400 ppm; Mn, 29 000 ppm; S, 60 000 ppm; Mg, 60 000 ppm; vitamin A, 2000 000 IU; vitamin D3, 400 000 IU; vitamin E, 2000 ppm; nicotinic acid, 10 000 ppm; choline, 20 300 ppm.

Table 2. *Daily energy (kJ/kg$^{0.75}$ BW) and Carbon-Nitrogen (g/kg$^{0.75}$ BW) balances, heat production (HP) and retained energy (RE) of female dry Manchega sheep (n = 12; 58 ± 1.2 kg BW on average; four sheep per diet) with the three offered diets based on cereal grain (CGR), fibrous by-products (FBP) and alfalfa hay (ALH) calculated by indirect calorimetry (RQ method) and Carbon-Nitrogen balance (CN method)*

	CGR	FCH	ALH	S.E.M.	*P* value
Gross energy intake (GEI)	785.5	801	1010	35.1	
Energy in faeces	212	214	445	34.0	
Energy in urine	20	26	34	4.2	
Energy in methane	40	51	58	2.8	
MEI	514	511	474	14.3	0.530
RQ method					
HP	431	404	462	15.5	0.046
RE*	83	107	11	17.8	0.041
CN method					
C intake	18	18	26	1.1	
C in faeces	4.2	4.4	11.1	0.98	
C in urine	0.72	0.50	0.80	0.054	
C in CO_2	10	10	11	0.3	
C in CH_4	0.72	0.92	1.05	0.051	
C retained	2.2	2.7	1.1	0.4	
N intake	1.1	1.1	1.4	0.05	
N faeces	0.29	0.33	0.50	0.031	
N urine	0.43	0.32	0.33	0.031	
N retained	0.42	0.43	0.56	0.056	
RE†	104	132	49	20.2	0.011
HP‡	410	379	425	14.4	0.011

S.E.M., standard error of mean; degrees of freedom=2; MEI, metabolizable energy intake; RQ, respirometry quotient; CO_2, carbon dioxide; CH_4, methane; C, carbon; N, nitrogen.

* Calculated as RE = MEI - HP.

† Calculated as RE = 51.8 × C retained - 19.4 × N retained.

‡ Calculated as HP = MEI - RE†.

Fig. 1. Whole mobile open-circuit respirometry system. Head hood attached to the front structure of a double metabolic cage with an animal on it.

Fig. 2. From left to right: front, lateral and rear side of the head hood.