CHAPTER 8

CONSTRUCTION AND OPERATION OF VENTILATED HOOD-TYPE RESPIRATION CALORIMETERS FOR *IN VIVO* MEASUREMENT OF METHANE PRODUCTION AND ENERGY PARTITION IN RUMINANTS

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INTRODUCTION

Indirect calorimetry refers to the methods for quantifying heat production from quantitative measurements of materials consumed and produced during metabolism. Most of these measurements involve respiratory gas exchange including enteric methane production. Calorimeters are typically classified according to the specific design of the apparatus (5). The three main types of indirect calorimeters are (i) confinement-type, (ii) closed-circuit and (iii) open-circuit systems. In confinement-type systems, animals are housed in completely sealed chambers and the changes in gas concentrations in the chamber measured. In closed-circuit systems the animals are similarly housed in completely sealed chambers. Water vapour and carbon dioxide produced by the animal is collected and measured using absorbers. Oxygen consumption is measured by the amount of oxygen required to maintain constant oxygen concentrations in the sealed system.

In open-circuit systems animals are housed so that they breathe into a one-way stream of air passing across the face or body. Airflow is typically measured at the

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outlet side of the animal cage and a sub-sample of air collected for analysis using an on-line gas analysis system. The ventilated hood-type calorimeter presented in this chapter is a type of open-circuit calorimeter where the animals head, rather than its whole body, is positioned within the head cage of the calorimeter.

The details reported in this chapter refer to the respiration calorimeter that was constructed as part of a JIRCAS (Japan International Research Centre for Agricultural Sciences) project at Khon Kaen Animal Nutrition Research and Development Centre (Department of Livestock Development, Thailand Government) in north-east Thailand. This calorimeter system replaced a former 'face mask'-based measurement system that formed the basis for the present 'head cage' calorimeter (2). The advantage of a ventilated hood system over whole body chamber calorimeters is that there are fewer components, which makes the system less complicated to construct and operate in a developing country where the availability of various components and expertise is often more difficult to access.

1. CALORIMETER DESIGN AND ITS COMPONENTS

The ventilated hood-type respiration calorimeter system constructed at Khon Kaen Animal Nutrition Research and Development Centre consists of five components, including (a) the digestion trial pen, (b) head cage, (c) gas sampling and analysis, (d) behaviour monitoring and (e) data acquisition and processing. These components are discussed in more detail below. A schematic diagram of the ventilated hood-type calorimeter discussed in this chapter is shown in Figure 1.

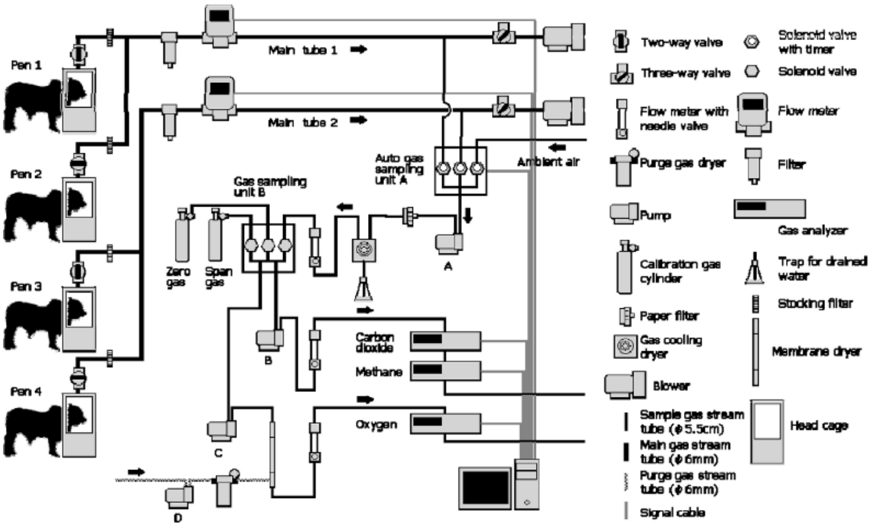

Figure 1. Schematic diagram of the ventilated hood-type respiration chamber system. The black arrows indicate the direction of air flow through the system

1.1. Digestion Trial Pen

The digestion trial pen is designed to house animals to enable accurate measurement of feed intake and excreta output to be made. The experimental apparatus is typical of other apparatus used in many animal nutrition laboratories around the world where classical feed digestibility studies are conducted. The pen also functions to position the animals head inside the head cage. Some photos of the digestion trial pen and head cage are shown in Figure 2.

1.2. Head Cage

The head cage is installed at the front of the digestion trial pen. A technical drawing of the head cage used at Khon Kaen Animal Nutrition Research and Development Centre

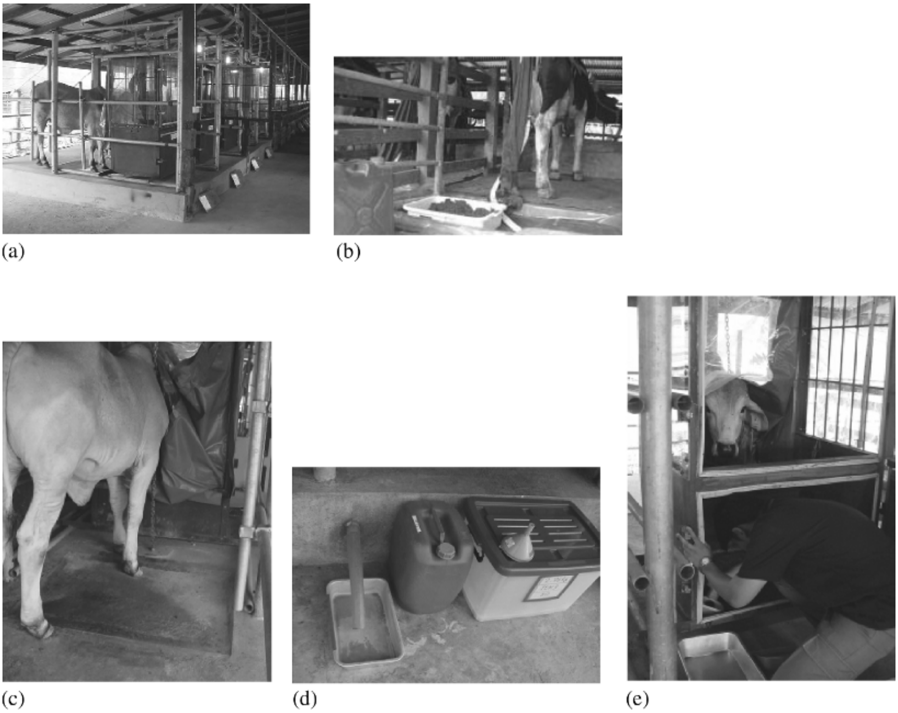

Figure 2. Photos of the digestion trial pen and head cage at Khon Kaen Animal Nutrition Research and Development Centre
(a) Digestion trial pen and head cage;
(b) Faecal and urine collection from a cow;
(c) Urine collection pan on floor for male cattle;
(d) Urine tube, bottle and box for faecal collection from male cattle;
(e) Collection of feed refusals from head cage through door

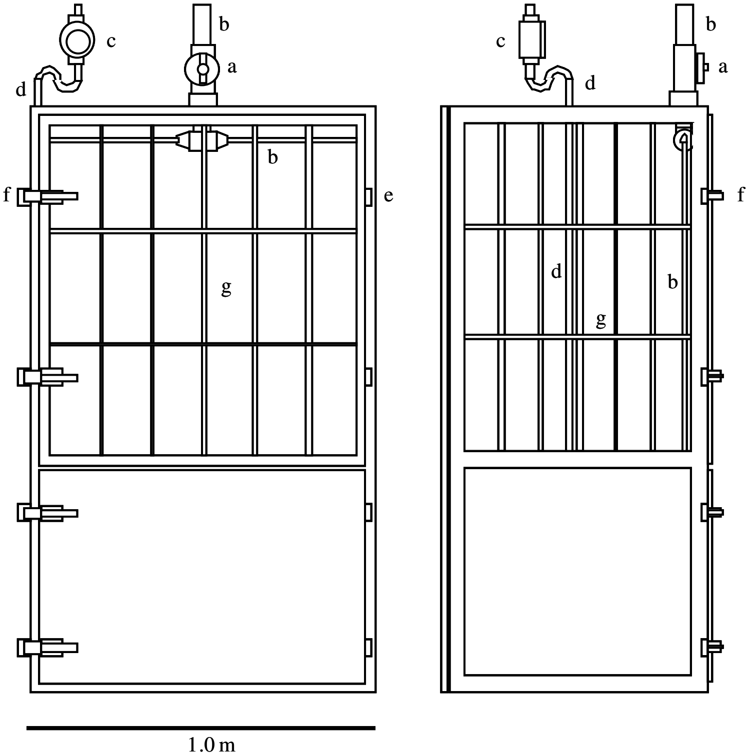

Figure 3. Diagram showing the front (left) and side (right) view of the head cage. (a) two-way valve; (b) gas outlet pipe; (c) water gauge; (d) water pipe; (e) hinge; (f) lock; (g) acrylic panel

is shown in Figure 3. This head cage is designed to be air-tight, with the exception of the air inlet, which is an adjustable ‘loose-fitting’ collar. A rear view of the collar can be seen in Figure 2. The position of the head cage and yoke are adjustable in order to permit animals of various sizes to be housed in the digestion trial pen.

The head cage incorporates the following components: an air-tight outer wall constructed with acrylic plastic, a water supply for the animal, a feed bin and outlet to main stream of air. The front of the cage has a door that permits easy access for feeding and collecting feed residues. The amount of water consumption can be measured with an in-line water gauge fitted to the mains water supply. The main air streams from two head cages are combined to one air stream so that only two head boxes of the four head cages can be measured at any time (Fig. 1). These air streams are changed manually with a ball valve positioned close to the head cage.

1.3. Gas Sampling and Analysis

1.3.1. Flow meters

Flow meters are an essential component of the calorimeter and underpin the accuracy of the whole measurement system. Thermal mass flow meters are the simplest type of flow meter available, and are calibrated in the factory to output measurements of flow rates at standard temperature and pressure. In recent times, thermal flow meters have included a 'hot-wire'-type air mass flow sensor.

1.3.2. Blower

The purpose of the blower is to move the main air stream though the calorimeter system, from the inlet point at the loose fitting collar of the ventilated hood through to the exhaust point. Industrial blowers are typically used, and positioned at the exhaust point. It is important to position the exhaust point of the blower outside the barn in order not to contaminate the inlet air. Air flow rate through this main air stream can be adjusted using a 3-way valve in the main line immediately preceding the blower. The flow rate through the main air stream is set at a value determined by animal size, feed intake and production level. In the respiration calorimeter used at Khon Kaen Animal Nutrition Research and Development Centre, a flow rate of 450 L/min is used for a 300–400 kg liveweight American Brahman steer fed at 1–2 times maintenance energy requirement.

1.3.3. Air filter and dryers

Air filters and dryers are installed in the system to remove dust particles and moisture before gas samples are sent to the gas analysis system. In the main air stream both coarse 'stocking' filters and fine filters (filtration rating 5 μm) are positioned near the outlet of the ventilated hood preceding the flow meter. A paper filter and a dehumidifier are used in the gas stream being sampled to remove any dust particles and moisture. Dust and moisture in air sampled for gas analysis leads to unstable measurements of gas concentration, and may damage the analytical equipment permanently.

1.3.4. Gas analysers

One set of gas analysers is used to measure sampled gas from three positions (i.e. background air and two different head boxes). An automated system involving solenoids was established to permit the gas sampling point to alternate between the three positions. In this automated system the gas sampling point is switched at 90 second intervals between the three positions (Fig. 4). The first 60 seconds is used to allow stable gas concentrations to stabilize before measurement, while the final 30 seconds are for data acquisition.

There is another separate gas sampling line that connects the calibration gases (span and zero calibration) directly to the gas analysers. During calibration of the gas analysers the flow rate to the gas analysers is controlled with a needle valve. Infrared gas analysers are used for measurement of carbon dioxide and methane gas concentrations. The oxygen analyser used in this system is a paramagnetic analyser. The response of the oxygen analyser is slower than infrared analysers and hence is the primary limit to the responsiveness of the whole calorimeter system.

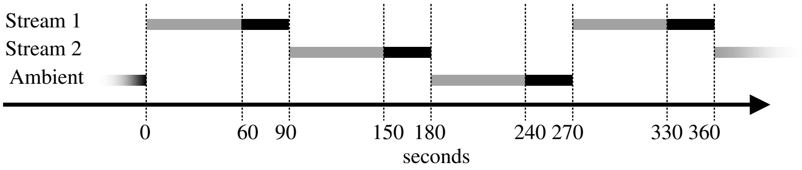

Figure 4. Order and timing of selection of sampling gas by auto gas analysis unit (■) and accumulation of gas concentration data by the data logging system (■)

1.4. Monitoring Animal Behaviour

Various other components can be installed in the system to monitor animal behaviour, which can assist greatly later on when trying to interpret the gas production data. The system in Khon Kaen includes a device for monitoring chewing activity of the animal and a video camera surveillance that is logged onto a separate computer that can be used for later reference. The halter with the mechanical switch positioned under the animals jaw for counting chewing activity and the web camera positioned in front of the head cage for recording cattle activity are shown in Figure 5 (a and b).

1.5. Data Acquisition and Processing

It is essential to have a system for collecting accurate data for accurate calculations. An automated system should be established to enable 24 hour data collection. Commercial systems are available (e.g. Testpoint, CEC, Bedford, NH, USA) or you can develop your own set of hardware and software. The essential components of the system include electronic devices (i.e. flow meters, gas analysers, selection

(a) (b)

Figure 5. Photos of animal behaviour monitoring equipment. (a) is the device used to monitor chewing activity and (b) is the web camera positioned in front of the head cage for recording cattle activity

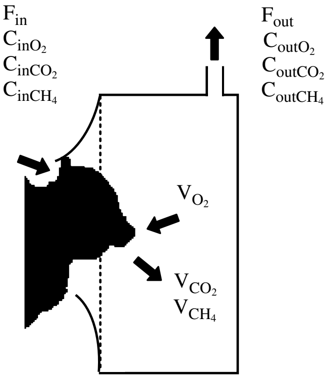

Figure 6. Schematic diagram illustrating the calculation of methane and carbon dioxide gas production and oxygen consumption. F_{in}, flow rate of inlet gas; C_{inO_2,CO_2,CH_4}, concentration of O_2, and CO_2 in inlet gas (ambient gas); F_{out}, flow rate of outlet gas; C_{outO_2,CO_2,CH_4} concentration of O_2, CO_2 and CH_4 in outlet gas; V_{O_2}, volume of O_2 consumed; V_{CO_2} and $_{CH_4}$, volume of CO_2 and CH_4 produced

of gas sampling points) termed A/D converters that are used to convert analog signal outputs from various electronic devices to digital signals that are sent to the computer and recorded. The software has functions of data accumulation and post experiment calculations.

A diagram showing the parameters required to calculate methane production and respiratory gas exchange from an animal is presented in Figure 6. The measurement parameters required include air flow out of the calorimeter, along with concentrations of methane, carbon dioxide and oxygen and the inlet and outlet point of the calorimeter.

It should also be noted that air flow into and out of an open circuit respiration calorimeter are not equal, because the total volume of gases (methane and carbon dioxide) produced is not equal to the volume of oxygen expenditure. In situations where you want to determine the difference between inflow and outflow of the system, you should compare calculated nitrogen gas in the ambient air and the head cage. Because nitrogen is not consumed or produced by cattle, the amount of nitrogen introduced into and coming out from the calorimeter is the same and, therefore, the flow rate into the calorimeter can be corrected by an adjustment of the measured outflow rate. This is particularly important in whole-body open circuit calorimeters where the volume of the animal cage is high, whereas the effects are negligible for the head cage calorimeter reported here. The procedures required for the measurements and calculations mentioned above are described in full detail by (4).

2. OPERATION OF THE SYSTEM

2.1. Instrumentation

The accuracy of the whole calorimeter system is only as accurate as the component instruments being used. It is therefore advisable to match the accuracy of the components at the time of purchase.

2.2. Recovery Tests

Recovery tests are the final validation of the system. The procedures used are described in full detail elsewhere (5). A newly constructed calorimeter system should achieve 95–105% recovery values before any measurements are made with the system. In essence, recovery tests are required to confirm that there are no leaks in the system and that the measurement system is accurately measuring emissions from animal. In the Khon Kaen laboratory, a pure gas source (i.e. carbon dioxide or nitrogen) is introduced into the head box from a gas cylinder positioned on a gravimetric balance. The injection of pure (99.99%) carbon dioxide gas during a recovery test is shown in Figure 7. The injection rate is measured gravimetrically. The test gas is then measured using the gas analysis system and the measurements compared to the gas introduction rate. An example of gas concentration measurements from a recovery test is shown in Figure 7.

2.3. Measurement of Net Volume of the System

It is important to consider the effect of differences in gas concentration in the chamber throughout periods of gas measurement. To calculate this effect, it is essential to know the real volume of the respiration calorimeter system. Such a calculation is more important for whole-body respiration calorimeters than the hood-type system that we are discussing here, because the volume of the whole-body calorimeter is greater. Hence, the reaction time of the measurement system to changes in gas production by the animal is slower. The measurement of net volume of the system is used to consider the accuracy of the whole measurement system.

Values for recovery rate and net volume of the hood-type respiration calorimeters in Khon Kaen are provided in Table 1.

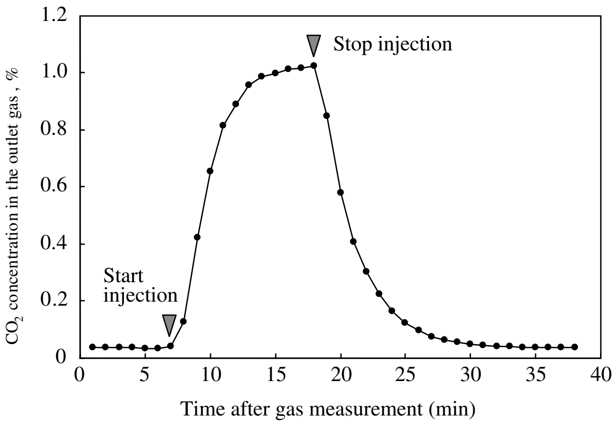

Figure 7. An example of the results obtained from an experiment where carbon dioxide concentration is measured in the outlet air during and after a period of carbon dioxide introduction into the calorimeter head cage

Table 1. Carbon Dioxide Recovery Rate and Net Volume of the Ventilated Hood-Type Respiration Calorimeter at Khon Kaen Animal Nutrition Research and Development Centre

	Recovery rate %	Net volume $\times 10^3$L
Head cage No.		
1	96.5 ± 1.0*	1.74 ± 0.12
2	97.0 ± 2.7	1.63 ± 0.09
3	95.7 ± 1.9	1.64 ± 0.10
4	101.8 ± 0.8	1.73 ± 0.07
Mean	97.8	1.70

* mean ± SD

2.4. Calibration of Gas Analysers with Certified Standard Gases

Calibrating the gas analysers with certified standard gases is an essential part of quality control for the measurement system and should be conducted at least on a daily basis during measurement periods. If measurement conditions are unstable then more frequent calibration is required. In the unit in Khon Kaen calibration is done with certified gases sourced from Japan.

2.5. Training Animals for Measurement and Experimental Design

It is important to train the animals to be used in the experiment and consider the experimental design carefully before making measurements with a respiration calorimeter. The respiration calorimeter is an artificial environment so all attempts should be made to ensure that behaviour of the animal when housed in the calorimeter, including feed intake, is representative of normal behaviour. At Khon Kaen Animal Nutrition Research and Development Centre, the training protocol for animals with no experience in experimental apparatus is two weeks housing in animal house pens, followed by one week housing in respiration calorimeter pens, then introduction to the head cage when the animal is sufficiently familiar. The most common difficulty is with individual animals that use their head and/or horns to damage the head cage.

Careful consideration needs to be made in the experimental design to make sure that the results can be analysed statistically using valid statistical procedures. An example of the order and timing of gas measurement and animal behaviour during a six day collection period is shown in Figure 8.

2.6. Calculating Energy Partition

A diagram of the partition of feed energy is provided in Figure 9. The full set of calculations for energy partition of ruminants is outlined elsewhere (3). In brief, energy of feed, feed refusal, faeces and urine are measured directly by bomb

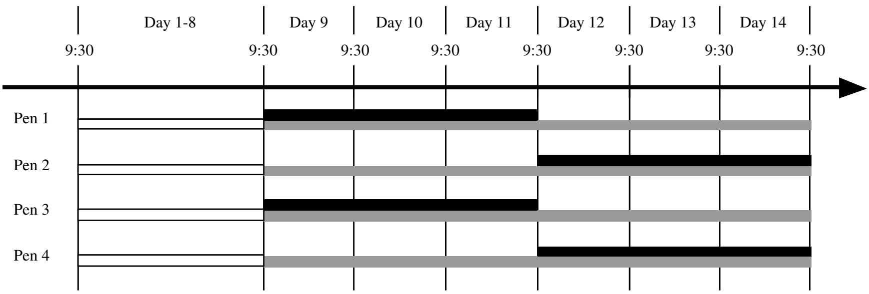

Figure 8. An example of the order and timing of gas measurement and collection of animal behaviour data (■) during a 6 day collection period (■)

calorimetry, and energy of methane and heat production are determined using the following relationships:

$$\text{Energy of methane (kJ)} = 39.5 \times V_{CH_4}$$

$$\text{Heat production (kJ)} = 16.18 \times V_{O_2} + 5.02 \times V_{CO_2} - 2.17 \times V_{CH_4} - 5.99 \times N$$

Where V_{O_2} is the volume of consumed oxygen, V_{CO_2} and V_{CH_4} are the volumes of carbon dioxide and methane produced in litres and N is the amount of nitrogen excreted in urine (g).

2.7. An Example of Typical Output Measurements

An example of typical results of a measurement run is presented in Figure 10, where methane and carbon dioxide production, oxygen consumption and chewing activity over a 24 hour period have been recorded for an American Brahman steer.

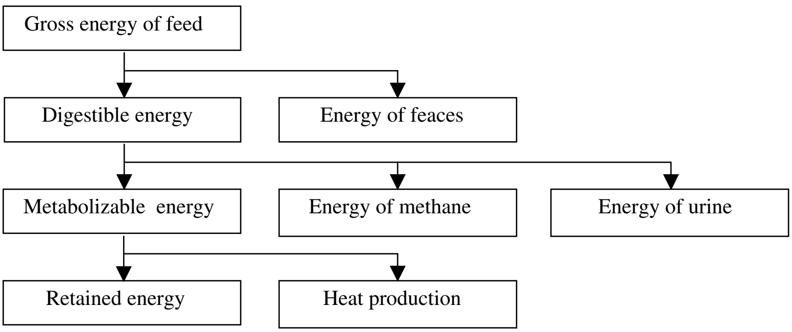

Figure 9. Schematic diagram energy partition of feed

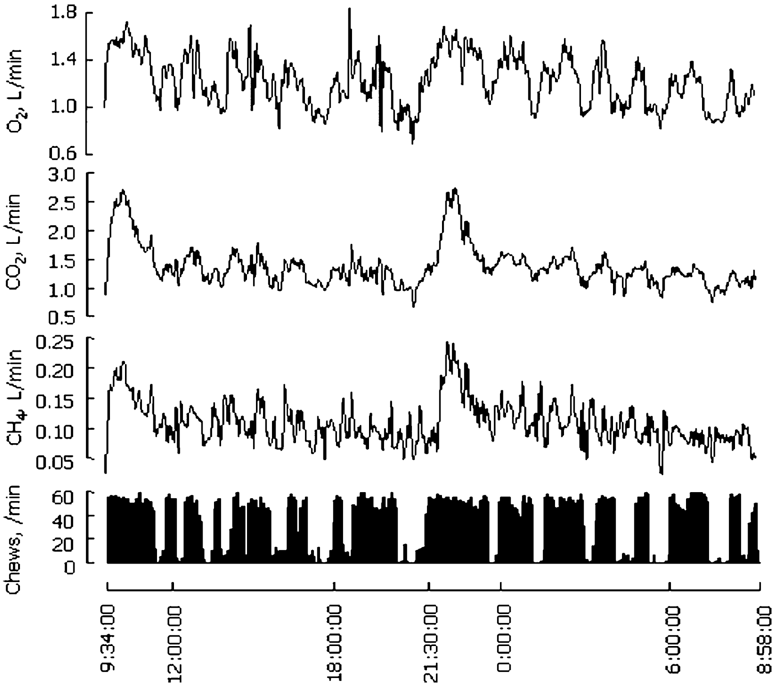

Figure 10. An example of methane and carbon dioxide production, oxygen consumption and chewing activity over a 24 hour period for an individual American Brahman steer

3. CONCLUSION

The ventilated hood-type respiration calorimeter is useful for *in vivo* measurement of methane production and energy partition in ruminants. Such a system can be established in animal research laboratories in developing countries. The need for trained expertise during construction and the initial stages of operation is considered essential.

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