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In Vivo Nutritive Value of Pangola Grass (*Digitaria eriantha*) Hay by a Novel Indirect Calorimeter with a Ventilated Hood in Thailand

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Abstract

A new animal calorimeter using a ventilated hood was constructed in Thailand. The recovery rates of our head cage were 96.5%, 97.0%, 95.7%, and 101.8%. The recovery rates of our head cage (95.7–101.8%) are reliable compared with Japanese whole-body chambers. The 99% CO_2 recovery time of our head cage was an average 14 min. It was shorter than whole-body chambers because of smaller effective volume and higher flow rate. Our head cage system excels at dynamic measurement of heat production. We started accumulating data concerning the energy value of Pangola grass (*Digitaria eriantha*) using 4 Brahman steers (body weight, 372.8 ± 34.4 kg, mean ± standard deviation; age, 3 years old). They were fed Pangola grass for 21 days; the grass was harvested approximately 45 days after cutting in several farms around Bangkok. Digestible energy and metabolizable energy of Pangola grass hay in our study were 10.28 and 7.99 MJ/kg dry matter. The average volume of methane production was 228.3 L/day and energy loss in the form of methane was 0.097 of the gross energy intake. These values are acceptable when compared with those obtained when low-quality tropical feed is provided to the steers.

Discipline: Animal industry
Additional key words: Brahman steers, digestible energy, metabolizable energy

Introduction

In 2005, Thailand had 478,836 head of dairy cattle and 7,796,272 head of beef cattle. Since the breed of cattle, climate conditions and available feed resources in Thailand differ from in the temperate zone, the nutrient requirements of the cattle and the nutritive value of feedstuffs in Thailand may not be the same as those recommended by NRC[14], ARC[2], Japanese Feeding Standard[3,4], and Standard Tables of Feed Composition in Japan[13]. However, the energy balance in cattle fed locally available feed has been measured only to a limited extent.

In order to study energy metabolism in cattle, Kawashima et al.[9] developed a respiration trial system using a ventilated flow-through method with a face mask in Thailand. Compared to the face mask system, the ventilated hood system is advantageous for conducting the gas exchange measurements throughout the day and even during eating; further, it is less stressful for the cattle. Therefore, in 2005, we constructed a new animal calorimeter using a ventilated hood and conducted a trial to esti-

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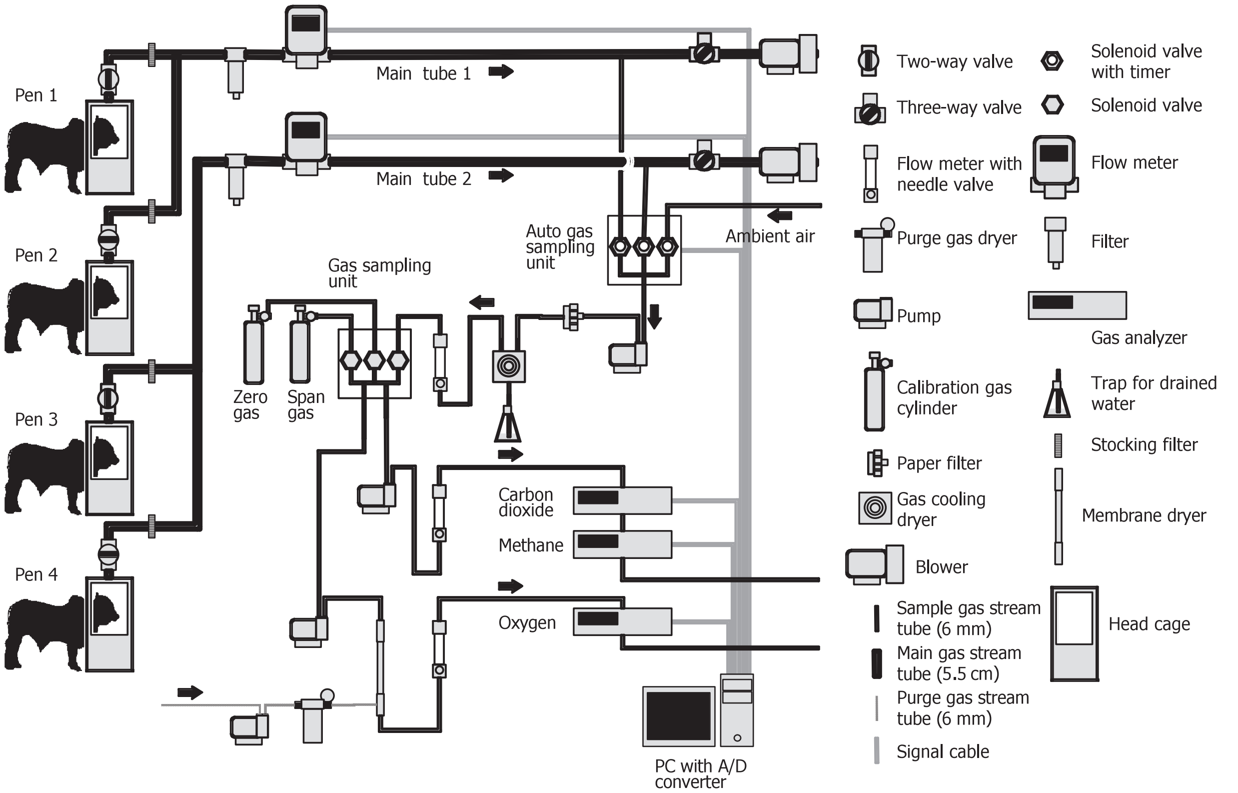

Fig. 1. Schematic diagram of the ventilated hood-type respiration chamber system
The black arrows indicate the direction of airflow through the system.

mate the energy balance in the cattle. We also started accumulating data concerning the energy value of Pangola grass (*Digitaria eriantha*) using Brahman cattle as the first step.

Materials and methods

1. Calorimeter design and its components

The indirect calorimeter with ventilated hood was constructed at Khon Kaen Animal Nutrition Research and Development Center, Khon Kaen, Thailand. It comprises the following five components: (a) a digestion trial pen, (b) head cage, (c) gas sampling and analysis unit, (d) behaviour monitoring unit, and (e) data acquisition and processing unit. A detailed description of these components follows. A schematic diagram of the calorimeter used in this study is shown in Fig. 1.

2. Head cage

The head cage is installed in front of the digestion trial pen (Figs. 2 and 3). It is designed to be airtight, with the exception of an air inlet that has an adjustable “loose-fitting” collar. The position of the head cage and yoke are adjustable in order to permit animals of various sizes to be housed in it.

3. Gas sampling and analysis

<u>Flow meters</u>

Thermal mass flow meters are the simplest type of flow meter available; they are factory calibrated to measure the output flow rate under conditions of standard temperature and pressure (0°C and 760 mm Hg).

Company: Nippon Flow Cell Mfg. Co., Ltd., Tokyo, Japan
Type: FHW-N-S-O

<u>Blower</u>

The purpose of the blower is to pull the main airstream through the calorimetric system, i.e., from the inlet point at the loose-fitting collar of the ventilated hood through to the exhaust point.

Company: Hitachi, Ltd., Tokyo, Japan
Type: VB-620-DN

<u>Air filter and dryers</u>

Air filters and dryers are installed in the system to remove any dust particles and moisture prior to the transfer of the gas samples to the gas analysis system.

(1) Dust filter
Company: SMC Corporation, Tokyo, Japan
Type: AF60-10BD (holder), AF60P-060S (filter)

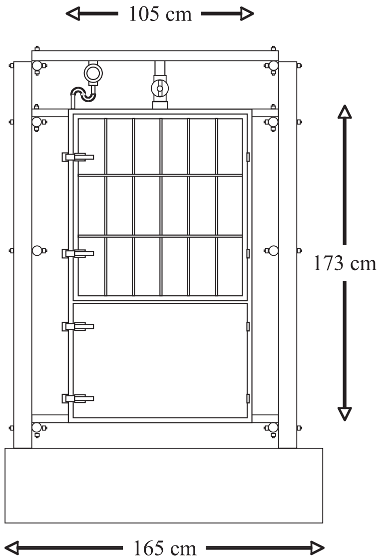

Fig. 2. Front view of the head cage and digestion stall

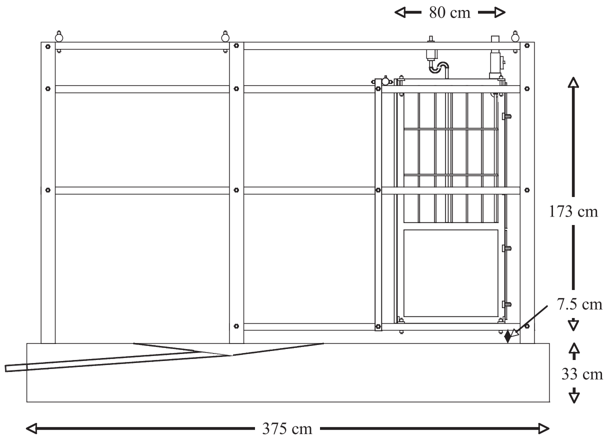

Fig. 3. Side view of the head cage and digestion stall

(2) Pump to pull the sample gas from the main airflow component into the gas analysis component
Company: ULVAC KIKO, Inc., Kanagawa, Japan
Type: DAL-10S, diaphragm-type dry vacuum pump

(3) Dryer with thermoelectric cooling and drying unit
Company: Soda Kogyo Co., Ltd., Osaka, Japan
Type: GCS-11A

(4) Dryers with a fluorine-containing polymer membrane
Object for drying: Sample gas
Company: Asahi Glass Engineering Co., Ltd., Chiba, Japan
Type: SUNSEP-W, SWF-M06-400
Object for drying: Purging gas
Company: Asahi Glass Engineering Co., Ltd., Chiba, Japan
Type: SUNSEP-W, SWC-01-150

(5) Pump for purging gas
Company: Oilless Rocking Piston Unit, GAST Mfg. Inc., Michigan, USA
Type: RAA-P103-EB

(6) Mist separator
Company: SMC Corporation, Tokyo, Japan
Type: AFM2000

Gas analyzers

The gas sample was analyzed at three positions (i.e., background air and two different head boxes) by using a set of gas analyzers. In this automated system, the gas sampling point switches at 90-s intervals among the three positions. The first 60 s are used to allow stabilization of the gas concentrations prior to measurement, while the final 30 s are for data acquisition.

(1) Oxygen analyzer (paramagnetic system)
Company: Servomex Group Ltd., East Sussex, England
Type: Servomex Xentra 4100
Analysis range: 19.000–21.000%

(2) Carbon dioxide analyzer (infrared system)
Company: Horiba Ltd., Kyoto, Japan
Type: VIA-510
Analysis range: 0–2%

(3) Methane analyzer (infrared system)
Company: Horiba Ltd., Kyoto, Japan
Type: VIA-510
Analysis range: 0–0.2%

(4) Pump for the oxygen analyzer
Pump to pull sample gas from the main airflow component into the gas analysis component
Company: Enomoto Micro Pump Mfg. Co., Ltd., Tokyo, Japan
Type: GA380-V-DA, diaphragm type dry vacuum pump

(5) Pump for the carbon dioxide and methane analyzer
Company: Iwaki Co. Ltd., Tokyo, Japan
Type: APN-085VX-1-07, diaphragm-type dry vacuum pump

(6) Dust filter for sample gas
Company: Whatman International Ltd., Kent, England.

Type: GF/A glass microfiber filters

4. Data acquisition and processing

An automated system should be established to enable 24-h data collection.

(1) Data acquisition boards

Company: Keithley Instruments Inc., Ohio, USA

Type: KPCI-3107

(2) Software

Company: Capital Equipment, Massachusetts, USA

Type: TestPoint v5.0

5. Recovery tests

Recovery tests were performed for the final validation of the system. The procedures are described in full detail elsewhere[12]. Pure carbon dioxide was introduced into the head cage from a gas cylinder that was positioned on a gravimetric balance. The injection rate was measured gravimetrically. Subsequently, the test gas was measured using the gas analysis system, and the measurements were compared with the gas introduction rate. The introduction rate was similar to the expected production rate estimated from animals to be used for subsequent measurements in the system.

6. Effective volume and 99% CO_2 recovery time

Variation of CO_2 gas volume after infusion can be shown as the following equation[7].

$$\ln(\mathrm{CoutCO_2}\,(t) - \mathrm{CinCO_2}\,(t)) = \ln(\mathrm{CoutCO_2}\,(0) - \mathrm{CinCO_2}\,(0)) - t/T \qquad (1)$$

Variables are as follows: V_H, net volume of the system (L); F, flow rate (L/min); $\mathrm{CinCO_2}$ (t), CO_2 concentration of the inlet gas (= ambient air) at time t (%); $\mathrm{CoutCO_2}$ (t); CO_2 concentration of the outlet gas at time t (%); $\mathrm{CoutCO_2}$ (0), CO_2 concentration of the outlet gas at stop the injection of CO_2 gas (%); and T, time constant (V_H / F).

This equation means the logarithm of the concentration difference of CO_2 between outlet gas and ambient air will decrease linearly with time. We substituted the data and calculated the time constant (T) and finally net volume of the system (V_H).

When the concentration difference of CO_2 in Eq. (1) becomes 1%, we defined that time (t) as the 99% CO_2 recovery time.

7. Calibration of gas analyzers with certified standard gases

At the beginning of each measurement day, the gas analyzers were calibrated against certified gases (Takachiho Chemical Industrial Co., Ltd., Tokyo, Japan) with known gas concentrations.

8. Energy partition calculations

The heat production was determined using the Brouwer's equation[5].

9. Animals and experimental design

Four Brahman steers (body weight, 372.8 ± 34.4 kg, mean ± standard deviation; age, 3 years old) were fed Pangola grass for 21 days; the grass was harvested approximately 45 days after cutting in several farms around Bangkok. All animal-related procedures were in accordance with the Guide for the Care and Use of Experimental Animals[6]. Their diets comprised only Pangola grass, and the amount of feed corresponded to 1.2% of the body weight of a steer. The steers were fed equal amounts twice daily at 0930 and 1630 h outdoors. Water and mineral blocks (L.P. Blocks No.2, L.P. Feed Tech, Co., Ltd., Bangkok, Thailand) containing, per kg: Ca, 37 g; P, 3.5 g; Mg, 4,950 mg; Fe, 1,600 mg; Cu, 300 mg; Co, 45 mg; Zn, 320 mg; Mn, 210 mg; I, 125 mg; Se, 12 mg; Na, 362 g; and Cl, 590 g were made continuously available.

The experimental period was 21 days with the first 14 days comprising the adaptation period and the remaining 7 days comprising the test period. A balance trial was conducted to investigate whole tract digestibility by collecting total feces and urine over a 7-day test period. The steers were fed on the ground floor in a cage, and the feces and urine were collected manually. Ambient temperature and relative humidity were not conditioned and their average values were 27.7°C (minimum to maximum, 23.3 to 36.6°C) and 82.7% (minimum to maximum, 41.0 to 99.0%) during the experiment, respectively.

The amount of feed that was offered and refused as well as the output of feces and urine were recorded; further, the samples were collected daily during the collection period. Samples of orts and feces were collected daily. During the collection period, all the feces were collected in buckets daily in order to measure the digestibility and digestible energy (DE) intake. After measuring the total weight of the feces, a sample weighing 100 g/kg of the total weight was stored in a refrigerator at 5°C. Immediately after the collection period, the fecal samples collected during the 7-days test period were mixed well. Fresh total feces (1 kg) was dried at 60°C for at least 72 h in a forced air oven, ground through a 1-mm screen and stored until analysis. During the collection period, all urine was collected in plastic tanks containing 400−800 ml of 20% (vol/vol) H_2SO_4 daily. The weight of the urine was measured, and a sample that was 10 g/kg of the total

Table 1. Results of CO_2 recovery test of the head cages No. 1 to 4

	No. 1	No. 2	No. 3	No. 4
Duration of injection (min)	11.7 ± 0.6	13.3 ± 0.6	12.5 ± 0.7	10.7 ± 0.6
Amount of injected CO_2 (g)	121.3 ± 5.0	94.0 ± 15.9	91.5 ± 12.0	103.0 ± 21.4
Flow rate (L/min)	561.4 ± 0.4	570.0 ± 2.0	527.1 ± 0.1	536.6 ± 2.5
CO_2 recovery rate (%)	96.5 ± 1.0	97.0 ± 0.7	95.7 ± 1.9	101.8 ± 0.8
Net volume (kL)	1.77 ± 0.20	1.38 ± 0.06	1.61 ± 0.15	1.87 ± 0.05
CO_2 99% recovery time (min)	14.5 ± 1.6	11.1 ± 0.5	14.1 ± 1.3	16.1 ± 0.4

weight was stored in a refrigerator at 5°C during the collection period. Immediately after the collection period, the urine samples during the 7-day test period were mixed well and stored frozen at −20°C until analysis.

For 2 days during the test period, indirect calorimeters with a ventilated hood were used to measure methane and carbon dioxide production and oxygen consumption. A detailed description of the materials and methods used for the gas exchange measurements follows.

10. Chemical analysis

The dry matter (DM) of the feed was examined by drying the sample at 105°C for 24 h; after drying the fresh fecal samples at 60°C for 24 h, those pre-dried fecal samples were dried at 135°C for 2 h. The wet feces were used for the analysis of the total nitrogen (N) content. The feed samples were dried at 60°C for 24 h, and then were ground through a 1 mm screen prior to analysis. The crude protein (CP), ether extract (EE), crude ash[1], neutral detergent fiber (NDF) with a heat-stable amylase without the residual ash, and acid detergent fiber (ADF) without the residual ash[16] were determined. The gross energy (GE) of the sample was determined using an automatic adiabatic bomb calorimeter (CA-4PJ; Shimadzu, Kyoto, Japan). The urine samples for GE analysis were oven-dried (60°C, 48 h) prior to use.

Results and discussion

1. The recovery rates

The recovery rates of our head cages No. 1, 2, 3, and 4 were 96.5% ± 1.0%, 97.0% ± 2.7%, 95.7% ± 1.9%, and 101.8% ± 0.8% (mean ± standard deviation, Table 1), respectively. The coefficient of variation of the CO_2 recovery rate was within 0.8 to 2.0%. A newly constructed calorimeter system should achieve 95−105% recovery values prior to any measurements being performed. Itoh et al.[7] showed the recovery rates of whole-body respiratory chambers for cattle and goat were 91.2−104.2%. Iwasaki et al.[8] also reported the recovery rates of whole-body respiratory chambers for cattle, sheep, goat, and pig were 101−107%. Nonaka et al.[15] measured the recovery rates of whole-body respiratory chambers for cattle and they were 102.1−107.9%. The recovery rates of our head cage (95.7−101.8%) are reliable compared with these Japanese whole-body chambers.

2. Effective volume and 99% CO_2 recovery time

Effective volume includes the volume of head cage and pipe. Considering the physical volume of the head cage was 1.45 kL, estimated effective volumes of our head cages No. 1, 3 and 4 were appropriate results (Table 1). The coefficient of variation of the effective volume was within 3 to 11%.

The 99% CO_2 recovery times of whole-body respiratory chambers for large ruminants (flow rate 418 L/min and effective volume 8.2 kL)[7] were 91 min and 100−120 min (flow rate 450 L/min and effective volume 11.7 kL)[15] and our head cage was an average 14 min. The head cage 99% CO_2 recovery time was shorter than whole-body respiratory chambers because of smaller effective volume and higher flow rate. Our head cage system excels at dynamic measurement of heat production.

3. Energy value of Pangola grass hay

Digestible energy (DE) and ME of Pangola grass hay in our study were 10.28 and 7.99 MJ/kgDM. Kearl[10] demonstrated that the nutrients values of Pangola grass hay were lower, i.e., 9.58 MJ/kgDM of DE, 7.82 MJ/kgDM of ME, 52% of total digestible nutrients (TDN) and 6.9% of CP, than those obtained in our study (Tables 2 and 3). The nutrient contents of the Pangola grass used in our study (Table 3) were better than that of other tropical grasses as well as the feeds used in the above mentioned studies.

The energy balance in our study was negative (Table 4). Average body weight changed from 376.3 ± 17.6 kg at the beginning of experiment to 369.3 ± 17.1 kg at the end of the experiment. A diet of Pangola grass hay at an amount corresponding to 1.34% of body weight as DM cannot meet the general requirements for maintenance.

In our study, the average volume of methane produc-

Table 2. Dry matter, chemical composition and energy content of Pangola grass hay

DM	OM	CP	EE	NDF	ADF	ADL	GE
%	% of DM						KJ/gDM
90.1	92.5	9.5	1.5	74.6	42.3	5.0	19.16

DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, GE: gross energy.

Table 3. Dry matter intake, digestibility, TDN, and energy content of Pangola grass hay

		Mean*	S.D.**
DMI	(kgDM/d)	5.02	0.56
DMI/BW	(%)	1.34	0.03
Digestibility	(%)		
DM		58.1	2.0
OM		60.7	1.8
CP		53.7	2.0
EE		53.0	5.7
NDF		68.0	1.7
ADF		66.3	1.8
TDN content	(%)	57.7	1.8
DE content	(MJ/kgDM)	10.28	0.37
ME content	(MJ/kgDM)	7.99	0.53

*: Mean values of four animals. **: standard deviation.
DMI: dry matter intake, BW: body weight, DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, TDN: total digestible nutrient, DE: digestible energy, ME: metabolizable energy.

Table 4. Energy metabolisms in cattle fed Pangola grass hay

	Mean*	S.D.**
GE intake (KJ/MBS/d)	1,090.7	48.6
Energy loss (KJ/MBS/d)		
via		
Feces	483.7	26.8
Urine	28.7	9.0
Methane	106.0	2.0
Heat production	508.1	36.9
Energy retention (KJ/MBS/d)	−35.7	18.0
DE/GE	0.556	0.020
ME/GE	0.432	0.029
ME/DE	0.777	0.030
Urine/GE	0.027	0.010
Methane/GE	0.097	0.005
Heat production/GE	0.465	0.016
Methane production (L/d)	228.3	16.0
(L/DMI/d)	45.7	2.4
(L/NDFI/d)	57.3	2.9

*: Mean values of four animals. **: standard deviation.
GE: gross energy, MBS: metabolic body size (kg body weight$^{0.75}$), DE: digestible energy, ME: metabolizable energy, DMI: dry matter intake, NDFI: neutral detergent fiber intake.

tion was 228.3 L/day and energy loss in the form of methane was 0.097 of the GE intake (Table 4). This value is acceptable when compared with those obtained when low-quality tropical feed is provided to the steers[11]. For the tropical forage diets (i.e. Angleton grass and Rhodes grass) methane production (g/d) was linearly related to DM intake described by the equation $y = 41.5x - 36.2$ ($r = 0.99$, $P = 0.001$)[11]. Pangola grass hay in our study also agrees with that (DM intake 5 kg/d, methane production 163 g/d).

Conclusion

The ventilated hood-type respiration calorimeter is useful for the *in vivo* measurement of methane production and energy partition in ruminants. Such a system can be established in animal research laboratories in developing countries. In the future, we will conduct a lot of digestion and respiration trials with tropical cattle fed locally available feed, and continue to accumulate data for development of livestock industry and farmers in tropical areas.

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