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A respiration-metabolism chamber system for measuring gas emission and nutrient digestibility in small ruminant animals¤

Sistema de cámaras respiro-metabólicas para medición de gases y digestibilidad de nutrientes en pequeños rumiantes

Um sistema de câmara de respiração e metabolismo para medição de emissões de gás e digestibilidade de nutrientes em pequenos ruminantes

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Summary

A novel chamber system was developed to measure gas emission from small ruminants and to measure nutrient digestibility simultaneously. Animal behavior can also be easily observed in this system through transparent panels. The system is composed of respiration-metabolism chambers, gas sampling and analysis units, and a data acquisition unit. A recovery test was performed using standard methane (CH_4) gas. Values for recovery rate of CH_4 gas ranged from 96.7 to 99.6% in 8 replications, and the mean value was 98.1% (coefficient of variation = 1.3%). A preliminary animal experiment was also conducted using 4 Korean native black goats with a mean body weight of 23.5 kg. The goats consumed a 50:50 mixture of forage and concentrate, and daily feed allowance was 2% body weight. Dry matter digestibility was 70.9%, and the mean CH_4 gas production was 11.6 g/day that was calculated to be 24.7 g/kg dry matter intake. Using this system, researchers can accurately and efficiently conduct various experiments measuring methane emission, nutrient digestibility, or both in small ruminant animals including goats, sheep, and deer.

Key words: *digestibility, methane, respiration-metabolism chamber system, small ruminant*.

Resumen

Se ha desarrollado un sistema de cámaras para medir simultáneamente la emisión de gases de pequeños rumiantes y la digestibilidad de nutrientes dietarios. Gracias a que las cámaras cuentan con paneles transparentes, también se puede observar fácilmente el comportamiento animal. El sistema completo se compone de varias cámaras para respirometría y metabolismo, unidades de muestreo y análisis de gas,

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así como de una unidad de adquisición de datos. Tras una prueba de recuperación con metano (CH_4), se observó que la tasa de recuperación de CH_4 fluctuó entre 96.7 y 99.6% en 8 repeticiones, y el valor medio fue del 98,1% (coeficiente de variación = 1,3%). Un experimento preliminar con animales se llevó también a cabo con 4 cabras Coreanas negras nativas de 23.5 kg de peso. Las cabras consumieron una mezcla 50:50 de forraje y concentrado, con un suministro diario de alimento equivalente al 2% del peso corporal. La digestibilidad de la materia seca fue del 70.9%, y la producción media de CH_4 fue del 11.6 g / día, correspondiente a 24.7 g / kg de consumo de materia seca. Mediante este sistema se pueden realizar con precisión y eficiencia diversos experimentos de medición de emisiones de metano, digestibilidad de nutrientes, o ambos, en pequeños rumiantes tales como cabras, ovejas y ciervos.

Palabras clave: *digestibilidad, metano, sistema de cámaras de respiración y metabolismo, pequeños rumiantes.*

Resumo

Um curioso sistema de câmara foi desenvolvido para a medição simultânea de emissões de gás para pequenos ruminantes e a digestibilidade de nutrientes. O comportamento dos animais pode ser facilmente observado no sistema a traves de panéis transparentes. O sistema está composto de câmaras de respiração e metabolismos, unidades de amostragem análises de gás e uma unidade de aquisição de datas. Um teste de recuperação foi desenvolvido usando um gás metano estandardizado (CH_4). Os valores de recuperação de CH_4 variou entre 96.7 a 99.6% em 8 replicações, e a media foi de 98.1% (coeficiente de variação = 1.3%). Um experimento preliminar foi realizado utilizando 4 cabras pretas nativas coreanas com uma media de peso corporal de 23.5 kg. As cabras consumiram uma mistura de forragem e concentrado 50:50, e um suplemento do 2% do peso corporal. A digestibilidade da matéria seca foi de 70.9%, e a media da produção de gás CH_4 foi de 11.6 g/dia a qual foi calculada por 24.7 g/kg do consumo de matéria seca. Usando este sistema, pesquisas com exactidão e eficiência podem ser realizadas para a medição de emissões de metano, digestibilidade de nutrientes, ou ambas em pequenos ruminantes incluindo cabras, ovelhas e veados.

Palavras chave: *digestibilidade, metano*, *sistema de câmara de respiraçao e metabolismo, pequenos ruminantes.*

Introduction

Global heating is of great concern, and greenhouse gases are potentially major contributors to the climate changes. Methane (CH_4) is the second most important greenhouse gas next to carbon dioxide based on the quantity (Martin *et al*., 2008). The production of CH_4 from global ruminant livestock production is estimated to be approximately 80 million tonnes per year (Beauchemin *et al*., 2008) and is responsible for at least 15% of the total CH_4 from human activities including agriculture and industry (Moss *et al*., 1995).

Two major methods have been employed to measure CH_4 emission from animals: 1) the respiration chamber method (McGinn *et al*., 2004; Beauchemin and McGinn, 2005), and 2) the sulfur-hexafluoride tracer method (Swainson *et al*., 2007; Martin *et al*., 2008). The first method is generally used in the buildings, and the second is for grazing animals. When a large number of animals are employed for an experiment, the second method is more appropriate, but the chamber method is more frequently used due to the relatively low data variability (Klein and Wright, 2006; Suzuki *et al*., 2008).

While respiratory systems are relatively easily available for ruminants, systems for both quantifying gas emission and determining nutrient digestibility in small ruminant animals have been rarely documented (Iwasaki *et al*., 1982). The systems designed for large ruminant animals take large space and high cost, and they may be less efficient and potentially less accurate when used for small ruminant animals. Therefore, a novel chamber system was developed to measure CH_4 gas emission from small ruminant animals and to determine nutrient digestibility simultaneously. A recovery test was performed using standard CH_4 gas to verify full detection of gas, and a preliminary animal experiment was also conducted.

Materials and methods

The respiration-metabolism chamber system was installed in an environmentally controlled room (ambient temperature, 23 °C; relative humidity, 55%) at Konkuk University. The protocols for animal experiments were reviewed and approved by Institutional Animal Care and Use Committee at Konkuk University. The system is composed of 3 major parts: 1) respiration-metabolism chambers, 2) gas sampling and analysis units, and 3) a data acquisition unit (Figures 1 and 2).

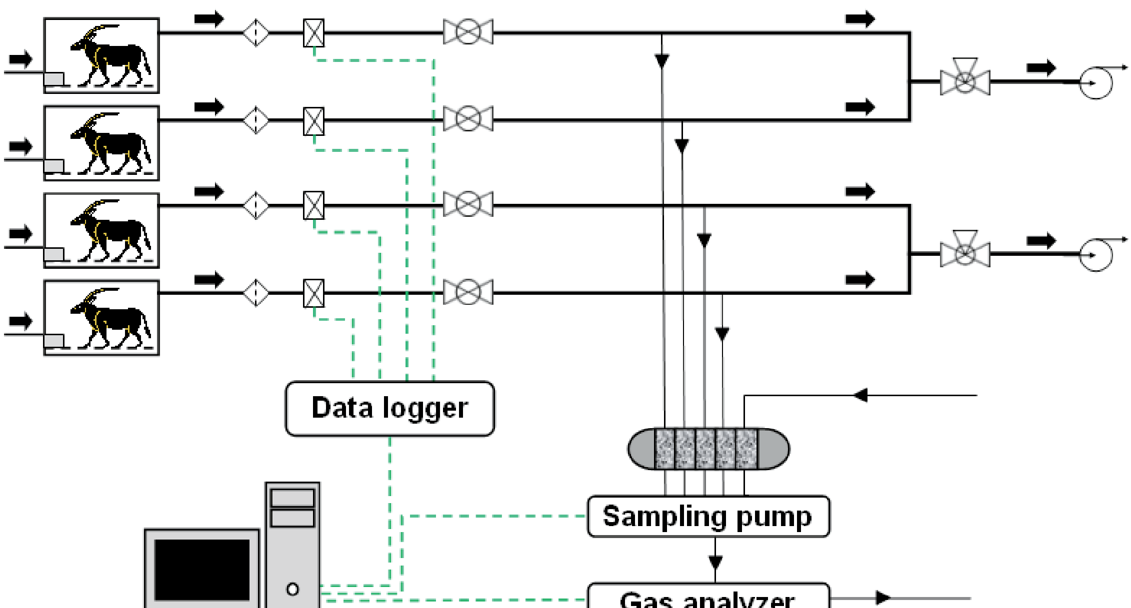

Figure 1. A schematic diagram of airflow through 4 chambers and the gas sampling system of the respiration chamber.
: dust filter; : flow meter; : 2-way valve; : 3-way valve; : blower; : dehumidifier.

Figure 2. A photo of the respiration-metabolism chamber system for small ruminant animals.

Respiration-metabolism chamber

Each chamber was made of 10-mm transparent acryl panels (for the large 3 chambers in Figure 2, length, 1.30 m; height, 1.00 m; width, 0.60 m; Figure 3). The transparent materials were used to facilitate visual observation of animal behavior. All panels except the front panel were sealed using silicone sealant. The front panel was fastened to the other panels using clamps. A hole of 15-mm diameter on the front panel enabled air inlets into the chamber. On the back panel, another hole was made to connect a 25-mm polyvinyl chloride (PVC) pipe for gas outlets. A screen floor was made of 10-mm thick wood panels. The width of each panel was 50 mm, and the wood panels were spaced by 15 mm as an animal can comfortably stand on and feces and urine can efficiently pass through. A water cup and a feeder were located on the top of the wood floor. A drawer-type container was placed below the wood floor to facilitate fecal collection. On the front bottom of the chamber, a 15-mm hole for urine outlet was located.

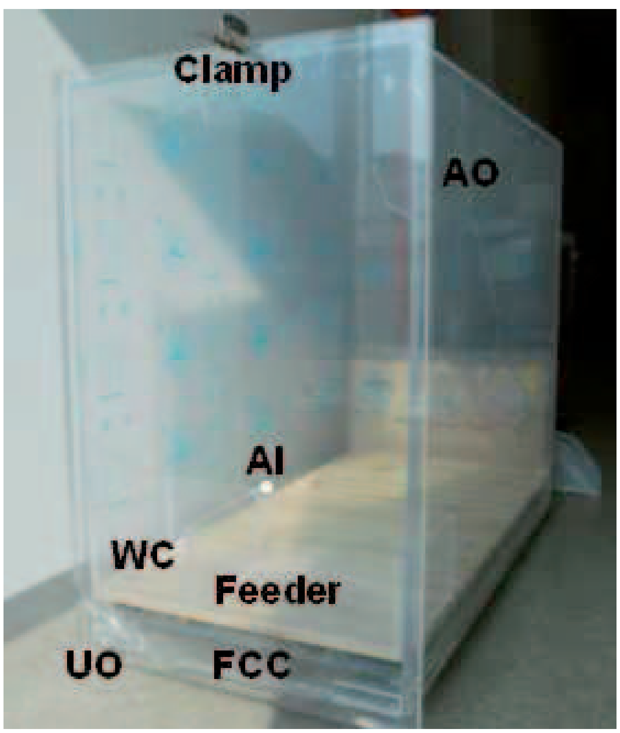

Figure 3. A chamber unit for the respiration-metabolism system. A chamber is made of 10-mm transparent acryl panels (1.30 m long, 1.00 m high, and 0.60 m wide for the 3 large chambers and 1.10 m long, 0.80 m high, and 0.60 m wide for the 1 small chamber demonstrated in Figure 2); AI: air inlet through a 15-mm diameter hole; AO: air outlet through a 25-mm polyvinyl chloride pipe; CF: chamber floor made of 10-mm thick and 50-mm width wood panels with 15-mm gaps between panels; FCC: fecal collection container; UO: urine outlet; WC: water cup.

Gas sampling and analysis unit

A ring blower (Model: GR40-610, Dongnam Engineering Co., Ansan, Korea) was equipped to draw air from 2 chambers through the PVC pipe at a rate of approximately 70 L/min (Figure 1). Based on our previous test, at this air flow rate, the carbon dioxide concentration in the chamber with a 25-kg goat did not exceed 0.5%, a suggested maximum concentration (Klein and Wright, 2006). The outlet air from a chamber was filtered through an air filter (Model: AF50, SMC Co., Tokyo, Japan) and the flow rate was measured using a standard temperature and pressure corrected-thermal mass flow meter (Model: GFM57, Aalborg Instruments & Controls Inc., Orangeburg, NY, USA). Gas samples were then drawn to a dehumidifier, composed of $CaSO_4$, and analyzed for CH_4 in the non-dispersive infrared gas analyzer with a measuring range of 0 to 2,000 ppm and repeatability of 0.5% (Model: VA-3000, Horiba Stec Co., Kyoto, Japan) through a 6-mm pipe. The sampling pump (Columbus Instruments, Columbus, OH, USA) was used to enable this air flow.

The gas analyzer was calibrated 1 hour prior to the gas measurement. Zero gas of 99.99% N_2 gas (Uniongas Co., Yongin, Korea) and span gas of 1,002 ppm (v/v) CH_4 (Uniongas Co., Yongin, Korea) balanced with N_2 were used for calibration.

Data acquisition unit

Air flow rate data measured at the thermal mass flow meter were transferred to the data logger (Columbus Instruments, Columbus, OH, USA) and were saved in the computer every 4 minutes. The CH_4 concentrations in air samples were determined and saved in the computer every 10 seconds using software (Type: VA-3000, Horiba Stec Co., Kyoto, Japan).

Recovery test

To validate the detection of CH_4 gas in the chamber system, a recovery test was performed. In each test, the concentration of CH_4 gas from the chamber was measured for approximately 180 min. Standard CH_4 gas (1.67%, v/v) was released into the chamber at the rate of 1 L/min.

The gas release initiated at 30 min and terminated at 100 min. The basal concentration of CH_4 from the chamber was estimated based on the CH_4 gas emission from the chamber during the first 30 min. Based on the preliminary tests (data not shown), the concentration of CH_4 returned to the basal concentration approximately 50 min after the termination of gas injection, and thus, 80 min of further gas analysis after stopping gas injection was assumed to be sufficient. Recovery rate (%) was calculated using recovered amount of CH_4 gas and injected amount of CH_4 gas.

Animal experiment

Four Korean native black goats with a mean body weight (BW) of 23.5 kg (SD = 1.0) were employed in the preliminary animal experiment. The goats were individually housed in each chamber and acclimated to the chamber before the initiation of animal experiment. A mixed feed was prepared using 50% of forages (2 to 5 cm cuts of tall fescue hay) and 50% concentrates (ground corn and soybean meal) on the basis of dry matter (DM).

The experiment lasted 10 days. The initial 6 days were an adaptation period to the feed, and during the following 4 days gas emission was measured and total feces and urine were collected. The feed was provided at amounts of 2% BW based on DM once a day at 1100 h. Water was sufficiently provided to last at least 24 hours. Shortly after feeding, the CH_4 gas determination initiated and continued until 1.000 h of the next day. Methane concentrations of each chamber were recorded for 4 min, and every 3 consecutive cycles of measuring gas in 4 chambers methane gas in reference air was measured for 4-min. In each 4 min measurement, first 3 min was regarded as a settling period and last 1 min was used for data calculation. Feces and urine excreted were collected and water intake was recorded between 1000 and 1100 h.

Feces were dried in an oven for 24 hours at 60 °C. Ingredient and fecal samples were ground using a micro hammer mill (Culatti AG, Zurich, Switzerland) with a 1-mm screen. Samples were analyzed for DM by drying the samples for 2 hours at 135 °C (method 930.15; AOAC, 2005).

Results and Discussion

Recovery test

Values for recovery rate of CH_4 gas ranged from 96.7 to 99.6% in 8 observations, and the mean value was 98.1% with the coefficient of variation (CV) of 1.3% (Table 1). These results demonstrated that the present system is working very accurately and precisely. In the other respiratory system by Klein and Wright (2006), recovery rate of CH_4 gas was 101.1% but the variation was larger than in our test (CV = 4.5%; n = 6). The superior gas recovery rate of the present system compared with others may be attributed to the fact that this present system was designed for small ruminants, and thus, was smaller and than other systems designed for large animals. High accuracy of the gas analysis equipment may also be another positive contributor. An example of recovery test results was illustrated in figure 4.

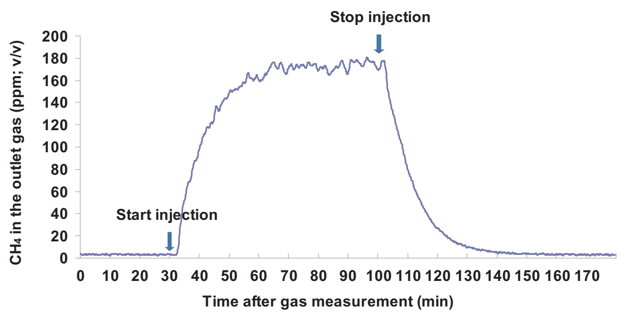

Figure 4. An example of methane recovery tests. The standard gas was released starting at 30 min and continued until 100 min. The basal concentration of CH_4 was calculated based on the data during the first 30 min. Recovery rate (%) was calculated as the ratio of the recovered amount of CH_4 gas to injected amount of CH_4.

Animal experiment

In the animal experiment, all goats remained healthy and consumed all the daily feed allowance. Dry matter digestibility was 70.9% in goats fed a 50:50 mixture of forage and concentrate at 2% BW (Table 2). In our previous experiment, DM digestibility was 68.0% (unpublished data) that is reasonably close to the present results. Islam *et al*. (2000) conducted an experiment using goats with a mean BW of 24.9 kg and provided 0.49 kg of feed daily. They reported that DM digestibility was 74.7% in goats fed a mixture of 50% Italian ryegrass and 50% corn. Shibata *et al*. (1992)

reported that DM digestibility in goats fed forage and concentrate mixture with ratios of 70:30 and 30:70 was 64.9 and 72.6%, respectively. As DM digestibility can be affected by many factors, our data appeared to be within a reasonable range.

The CH_4 gas production was 24.7 g/kg DM intake on average (Table 2). In a similar study conducted by Islam *et al*. (2000), a CH_4 production of 23.5 g/kg DM intake was observed that was very comparable to our data. In other goat experiments, the CH_4 gas emission varied from 16.2 to 24.6 g/kg DM intake when using various types of feed and feed intake (Shibata *et al*., 1992; Puchala *et al*., 2005; Animut *et al*., 2008). It seems that CH_4 emission from goats may vary depending on many factors including feed type and feed intake.

The present novel respiration-metabolism chamber system is appropriate for measuring CH_4 gas emission from small ruminants. The present system also enables nutrient digestibility determination and visual observation of animal behavior.

Table 1. A methane (CH_4) recovery test of the respiration chamber system[1].

Item	Mean	Minimum	Maximum	CV[2] (%)
Injected CH_4 weight (g)	0.612	0.586	0.625	2.05
CH_4 in the outlet gas (ppm, v/v)				
Mean concentration	68.5	60.7	72.8	5.15
Basal concentration	3.49	2.56	4.08	14.0
Mean concentration above the basal[3]	65.1	56.7	69.5	5.82
Mean flow rate (L/min)	71.1	70.6	71.9	0.65
Test time (min)	182	172	205	5.31
Recovered CH_4 volume[4] (L)	0.840	0.817	0.848	1.23
Recovered CH_4 weight[5] (g)	0.600	0.584	0.606	1.23
CH_4 recovery rate[6] (%)	98.1	96.7	99.6	1.26

[1] Data were calculated using 8 observations.
[2] CV = coefficient of variation.
[3] Mean concentration above the basal (ppm) = Mean concentration (ppm) – Basal concentration (ppm).
[4] Recovered CH_4 volume (L) = Mean CH_4 concentration above the basal (ppm) × Mean flow rate (L/min) × Test time (min) × 10^{-6}.
[5] Recovered CH_4 weight (g) = Recovered CH_4 volume (L) × 16 (g/mol) ÷ 22.4 (L/mol), assuming that 1 mole of CH_4 gas is equivalent to 16 g and 22. 4 L.
[6] CH_4 recovery rate (%) = Recovered CH_4 weight (g) ÷ Injected CH_4 weight × 100%.

Table 2. Dry matter (DM) digestibility, water intake, urine output, and methane emission from Korean native black goats[1].

Item	Goat 1	Goat 2	Goat 3	Goat 4	Mean	CV[2] (%)
Body weight (kg)	22.3	23.6	23.3	24.8	23.5	4.4
DM intake (g/day)	446	472	466	496	470	4.4
Fecal DM output (g/day)	144	136	130	135	137	4.2
DM digestibility[3] (%)	67.6	71.1	72.0	72.7	70.9	3.2
Water intake (mL/day)	1,325	1,335	1,565	975	1,300	18.7
Urine output (mL/day)	660	568	695	300	556	32.2
CH_4 production						
CH_4 (g/day)	13.9	11.7	9.9	10.7	11.6	15.0
CH_4 (g/kg body weight$^{0.75}$)	1.35	1.09	0.93	0.96	1.09	17.7
CH_4 (g/kg DM intake)	31.2	24.8	21.2	21.6	24.7	18.6

[1] The goats were fed a 50:50 mixture of forage and concentrate at amounts of 2% body weight based on DM.
[2] CV = coefficient of variation.
[3] DM digestibility (%) = 100% – Fecal DM output (g/day) ÷ DM intake (g/day) × 100%.

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