Methane Production and Energy Partition in Sheep Fed Timothy Silage-or Hay-based Diets

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ABSTRAK

SANTOSO, B., B. MWENYA, C. SAR dan J. TAKAHASHI. 2007. Produksi metana dan partisi energi pada domba yang diberi pakan basal silase atau hay rumput timothy. *JITV* 12(1): 27-33.

Metana dihasilkan dari fermentasi anaerob karbohidrat dapat larut dan karbohidrat struktural oleh bakteri penghasil metana di dalam rumen ternak ruminansia. Metana yang hilang dari dalam rumen sekitar 7,22% dari energi yang dikonsumsi. Empat ekor domba Cheviot yang berfistula pada bagian rumen digunakan dalam rancangan *Crossover* untuk mengetahui produksi gas metana dan partisi energi pada domba yang diberi pakan basal silase atau *hay* rumput timothy. Pakan basal berupa campuran silase atau *hay* rumput timothy dan konsentrat (85 : 15, berdasarkan bahan kering (BK)). Variabel yang diukur adalah kecernaan nutrien, keseimbangan energi dan produksi metana. Kecernaan BK, bahan organik, protein kasar, NDF, ADF, selulosa dan hemiselulosa lebih tinggi secara signifikan (P<0,05) pada domba yang diberi pakan basal silase dibandingkan pakan basal *hay*. Domba yang diberi pakan basal silase menghasilkan energi yang hilang melalui urin, metana dan panas lebih tinggi (P<0,01), namun energi yang hilang melalui feses lebih rendah (P<0,05). Produksi metana yang diekspresikan dalam g kg^{-1} konsumsi BK dan g $hari^{-1}$ lebih rendah (P<0,01) pada domba yang diberi pakan basal hay dibandingkan silase. Terdapat korelasi yang erat antara produksi metana (g $hari^{-1}$) dan NDF tercerna (g $hari^{-1}$) (R^2 = 88,4%, P<0,001). Produksi metana yang diekspresikan sebagai g kg^{-1} NDF tercerna pada pakan basal silase lebih tinggi (P<0,05) dibandingkan *hay* (66,44 dibandingkan 62,70). Hasil penelitian ini menunjukkan bahwa produksi metana pada domba meningkat sejalan dengan peningkatan NDF tercerna.

Kata Kunci: Metan, Silase, *Hay*, NDF, Domba

ABSTRACT

SANTOSO, B., B. MWENYA, C. SAR and J. TAKAHASHI. 2007. Methane production and energy partition in sheep fed timothy silage- or hay-based diets. *JITV* 12(1): 27-33.

Methane is produced as a result of anaerobic fermentation of the soluble and structural carbohydrates by methanogens in the rumen of ruminant animals. Removal of methane from rumen represents a loss of approximately 7.22% of gross energy intake. Four ruminally fistulated Cheviot wethers were used in a crossover design to determine methane production and energy partition in sheep fed timothy silage- or hay-based diets. The experimental diets consisted of either timothy silage or timothy hay and a commercial concentrate (85:15, on DM basis). Variables measured were nutrients digestibility, energy balance and methane production. Apparent digestibilities of DM, OM, CP, NDF, ADF, cellulose and hemicellulose were significantly higher (P<0.05) on sheep fed silage-based diet than those fed hay-based diet. Sheep fed silage-based diet had greater (P<0.01) urinary energy loss, methane and heat production, but lower (P<0.05) fecal energy loss. Methane production, either expressed as g kg^{-1} dry matter intake or g day^{-1} was markedly lower (P<0.05) in hay-based diet as compared to silage-based diet. There was a strong relationship between methane production (g day^{-1}) and NDF digested (g day^{-1}) (R^2 = 88.4%, P<0.001). Methane production expressed as g kg^{-1} NDF digested in silage-based diet was higher (P<0.05) than in hay-based diet (66.44 vs 62.70). These results indicate that methane release by sheep increased with increasing NDF digested.

Key Words : Methane, Silage, Hay, Neutral detergent fiber, Sheep

INTRODUCTION

Methane (CH_4) is produced as a result of anaerobic fermentation of the soluble and structural carbohydrates by methanogens in the rumen of ruminant animals, which is released into the environment by eructation. In total of 1137 datasets, PELCHEN and PETERS (1998) noted that CH_4 release from sheep averaged 7.22% of gross energy (GE) intake or equivalent to 30.9 l day^{-1}. Recently, CH_4 production by ruminants has also received attention due to its contribution to global warming. Previous report (MOSS, 1993) indicated that population of ruminants in the world is responsible for 12 to 15% of total atmospheric CH_4 emissions.

A number of investigators have shown that the production of CH_4 could be influenced by nature of carbohydrate digested such as hemicellulose, cellulose and soluble residue, (MOE and TYRELL, 1979;

TAKAHASHI, 2001; SANTOSO *et al*., 2003). Further, MOSS (1994) revealed that digestible acid detergent fiber (ADF), cellulose and hemicellulose are important variables influencing CH_4 production in the rumen, whereas ESTERMANN *et al*. (2002) observed a strong relationship between CH_4 production and digestible neutral detergent fiber (NDF) for cows and calves. In contrast, CH_4 production expressed as mmol per gram of apparently digested NDF increased with decreasing concentration of NDF in the feeds (HINDRICHSEN *et al*., 2003).

Few studies comparing diets of different type but similar fiber fractions content, on CH_4 production have been condusted. Therefore, the objective of the present study was to determine CH_4 production and energy partition in sheep fed timothy silage- or hay-based diets. Aspect of apparent digestibility was also investigated to assess any relationship with CH_4 release.

MATERIALS AND METHODS

Design, animals and diets

Four Cheviot wethers with an average body weight (BW) of 54.5 ± 5.4 kg each fitted with a ruminal cannula, were kept in ventilated head cages equipped with containers for urine and faeces collections, and used in a crossover experimental design. Animals were fed in two equal meals (08.00 and 16.00 h) at the feeding level of 55 g dry matter (DM)/kgBW$^{0.75}$ per day. The experimental diets consisted of either silage or hay and a commercial concentrate (85:15, on DM basis). Both silage and hay were prepared from early first-cut timothy (*Phleum pratense*). Fresh water and a mineral lick containing Fe 1232; Cu 150; Co 25; Zn 500; I 50; Se 15 and Na 382 mg/kg were freely available. Each experimental period lasted 13 days, comprising 8 days of dietary adaptation followed by 5 days of digestibility measurement, which included 2 consecutive days for CH_4 measurement.

Digestibility measurement

Representative samples of the silage, hay and concentrate were taken before the experiment. Refusals if any, were removed daily before morning feeding. Total collections of faeces and urine were conducted for a 5-day period. Faeces output was weighed daily, pooled per treatment on a 5-day basis, sampled (10%) and stored frozen for subsequent analyses. Urine was collected into buckets containing 100 to 150 ml of 10% H_2SO_4. About 5% of urine sample was taken, stored at –20°C until analysis of gross energy (GE). Feed and faeces samples were dried at 60°C for 72 h in a forced-air oven and then ground through a 1-mm mesh sieve and analyzed for DM, ash, ether extract (EE) and N according to the methods of AOAC (1990). Crude protein (CP) was calculated by multiplying N × 6.25. Silage DM was determined by freeze-drying to a constant weight. Concentrations of NDF, ADF and acid detergent lignin (ADL) were determined using procedures described by VAN SOEST *et al*. (1991). For concentrate, an alpha amylase was used prior to NDF analysis. Hemicellulose was calculated as NDF–ADF, whereas cellulose was calculated as ADF–ADL. Soluble CP was assayed using the borate-phosphate buffer method (KRISHNAMOORTHY *et al*., 1982) with minor modifications. An adiabatic automatic bomb calorimeter (Shimadzu, CA-4P, Japan) was used to determine GE content of samples. Body weight of animals was weighed at the beginning and the end of each period.

Energy balance and CH4 production measurements

The energy values of feed, faeces and urine samples collected from the 5 day digestion trial were used to calculate energy balance. Methane and CO_2 production and O_2 consumption were measured by an open circuit respiratory system using a ventilated hood attached to each metabolism cage (TAKAHASHI *et al*., 1999) for 2 consecutive days. During each measurement, air was continuously sampled from ventilated hood and analysed for CH_4 and CO_2 concentrations with infrared analyser (Horiba VIA 300 and Horiba VIA 500, Japan), and for O_2 with a paramagnetic oxygen analyser (Shimadzu MAG-2, Japan). Data were taken and pooled into the computer from analyser through an interface at 1 min intervals, and standardized automatically at 0 °C, 101 kPa and zero water vapour pressure. The CH_4 gas volume was converted to energy and mass values using the conversion factors 9.45 kcal/l and 0.716 g/l respectively, whilst heat production was calculated according to Brouwer`s equation (BROUWER, 1965).

Statistical analysis

Analysis of variance was carried out using the GLM procedure of SAS version 6.12 (1996) as a crossover design. The model was: $Yijk = \mu + Si + Pj + Tk + Eijk$, where *Y*ijk represents an observation on the *i*th sheep, *S* (*i* = 1 to 4), at the *j*th period, *P* (*j* = 1 to 2), with the *k*th treatment, *T* (*k* = 1 to 2). The overall mean is expressed as μ, and the residual error is *E*ijk. Significant differences were assumed at $P<0.05$.

RESULTS AND DISCUSSION

Diet composition and apparent digestibility

The chemical composition of the silage, hay and concentrate is given in Table 1. The DM concentration of the silage-based diet differed drastically from that of the hay-based diet. The difference was probably the consequence of particle losses of material during hay making. Concentrations of OM, NDF, hemicellulose, NFC and GE were slightly higher in the hay-based diet, whereas those of CP, ADF, cellulose and EE were slightly higher in the silage-based diet. Silage-based diet had two times higher soluble crude protein concentration than that of hay-based diet.

Sheep fed silage-based diet showed markedly greater (P<0.05) apparent digestibilities of DM, OM, CP, NDF, ADF, cellulose and hemicellulose than those fed hay-based diet (Table 2). This is likely related to the relatively high concentration of fibrous carbohydrate fraction in silage-based diet which in turn causes slower rate of passage and longer retention time in the rumen, thus resulting in higher coefficient of apparent digestibility compared with hay-based diet. It has been clearly demonstrated that total mean retention time was longer for silage-based diet than for hay-based diet (UDÉN, 1984). This result is consistent with previous study by SHINGFIELD *et al*. (2002) that preservation of timothy as hay compared with silage tended to reduce diet digestibility.

The reason for higher digestibility of fibre fractions *i.e.* NDF, ADF, hemicellulose and cellulose observed in silage-based diet might be related to the higher fermentable fractions resulting from enzymatic action on cell wall of grass. In the present study, the higher NDF digestibility in silage-based diet confirms that fibres contained in forage based diet, particularly of NDF, is not the only constraint in digestibility of diet. Nevertheless forage preservation had significant (P<0.01) effect on CP digestibility. Higher digestibility of CP in silage-based diet compared with hay-based diet observed in this study may be due low soluble protein content. The results are in agreement with VERBIČ *et al*. (1999), who found higher effective protein degradability value in the rumen for silage compared with hay (855 *vs* 677 g/kg). They also reported silage had almost two times higher soluble protein fraction than that of hay.

Table 1. Chemical composition of experimental diets

	Silage	Hay	Concentrate[1]	Silage-based diet	Hay-based diet
Dry matter (%)	29.1	89.5	87.4	37.8	89.2
Percentage of DM					
Organic matter	90.8	93.2	93.2	91.2	93.2
Crude protein	13.3	11.0	24.1	15.0	13.0
Neutral detergent fiber	55.8	58.6	12.9	49.4	51.8
Acid detergent fiber	37.0	34.4	7.1	32.5	30.3
Hemicellulose	18.8	24.2	5.8	16.9	21.4
Cellulose	33.4	32.6	6.3	29.4	28.7
Ether extract	3.6	1.9	5.6	3.9	2.5
NFC[2]	18.1	21.7	50.6	23.0	26.0
Soluble CP (% of CP)	51.2	20.5	46.6	50.5	24.4
GE (kcal/kg DM)	4488.2	4592.5	4476.3	4486.2	4575.0

[1]Contained heat treated corn, maize, soybean and rye (52%), soybean oil (34%) corn gluten feed (8%) and alfalfa meal, molasses, $CaCO_3$ powder, Dicalcium phosphate, salt, malt, yeast, lactic acid bacteria, bacillus, streptococcus, vitamins and minerals (vitamin A, 34350 IU/kg; vitamin D3, 6870 IU/kg; vitamin E 46 mg/kg; Zn 229 mg/kg; Mn, 126 mg/kg; Fe, 69 mg/kg; Cu, 33 mg/kg; I, 2.6 mg/kg; Co, 0.8 mg/kg; Se, 0.46 mg/kg) (6%)

[2]NFC, non-fiber carbohydrate (OM – (NDF + CP + ether extract))

Table 2. Dry matter intake and apparent digestibility of diets in sheep fed silage-or hay-based diet

	Diets		SEM	P-value
	Silage	Hay		
DM intake (g/kg $BW^{0.75}$)	53.3	54.2	0.27	0.071
Digestibility (%):				
Dry matter	72.6	69.4	0.32	0.014
Organic matter	74.8	71.0	0.24	0.018
Crude protein	70.2	67.0	0.59	0.047
Neutral detergent fiber	63.3	58.8	0.37	0.013
Acid detergent fiber	58.4	55.4	0.49	0.049
Cellulose	65.8	59.5	0.49	0.012
Hemicellulose	67.0	64.1	2.64	0.519

SEM, standard error of the mean

Energy balance and CH_4 production

Table 3 summarizes the energy balance and CH_4 production of sheep given silage- or hay-based diets. Sheep fed hay-based diet had higher GE intake ($P<0.01$) and energy losses as faeces per unit GE intake ($P<0.05$) compared with those fed silage-based diet. The greater fecal energy loss is consistent with the reduced digestibility observed with the hay-based diet. Energy loss through urine per unit GE intake was significantly higher ($P<0.01$) in sheep given silage-based diet than in sheep given hay-based diet. This could be attributed to a greater volume of urine excretion that is presumably related to a higher soluble CP content in silage-based diet than in hay-based diet. Urinary energy loss as a proportion of GE intake in the present study was 5.03% and 3.17% for silage- and hay-based diets respectively, which is consistent with the 5% average for cattle and sheep observed by BLAXTER and WAIMNMAN (1964). Sheep fed on silage-based diet had significantly greater ($P<0.01$) energy losses in CH_4 and heat production as a proportion of GE intake compared with those fed on hay-based diet. This difference can be explained by the higher energy costs of eating, rumination and fermentation in sheep fed on silage-based diet as a result of lower NFC content compared

Table 3. Energy balance and methane production in sheep fed silage-or hay-based diet

	Diets		SEM	*P*-value
	Silage	Hay		
Energy balance:				
GE intake (kcal/kg $BW^{0.75}$/day)	239.4	248.0	0.51	0.007
Digestible energy (kcal/kg $BW^{0.75}$/day)	171.1	165.6	0.80	0.039
Metabolizable energy (kcal/kg $BW^{0.75}$/day)	141.3	141.9	0.16	0.658
Energy loss as:				
Feces (% of GE intake)	28.54	33.11	0.46	0.018
Urine (% of GE intake)	5.03	3.17	0.06	0.002
Methane (% of GE intake)	7.43	6.35	0.06	0.008
Heat production (% of GE intake)	49.22	46.01	0.07	0.001
Retained energy (kcal/kg $BW^{0.75}$/day)	23.4	27.8	0.94	0.080
Methane production:				
g/kg DMI	25.17	21.94	0.23	0.010
g/d	26.46	23.52	0.24	0.013
g/kg NDF digested	66.44	62.70	1.10	0.034

SEM, standard error of the mean

with hay-based diet (23.0 vs 26.0%). Earlier reports (ARMSTRONG and BLAXTER, 1957; CHANDRAMONI *et al*., 2000) showed that more energy is lost as heat production on high roughage diets due to increased energy cost of digestion. In the present study, metabolizable energy and retained energy values were similar (P>0.05) between sheep fed silage-based diet and sheep fed hay-based diet.

Methane production as either g/kg DMI or g/day was significantly higher (P<0.05) in silage-based diet than in hay-based diet. These results suggest that longer retention times of feed in the rumen were associated with greater digestibility of cell wall and therefore greater CH_4 production (Table 2). The results support previous findings (MOE and TYRELL, 1979; HINDRICHSEN *et al*., 2003; SANTOSO *et al*., 2003) who found CH_4 production influenced by digestible structural carbohydrates such as NDF, ADF, hemicellulose and cellulose. The relationship between CH_4 production and fiber fractions digested is summarized in Table 4.

Result of linear regression indicated that NDF digested was a better predictor of CH_4 production than ADF, hemicellulose or cellulose digested. In this case, there was a positive and a significant relationship between CH_4 production (g/day) and NDF digested (g/day) (R^2 = 88.4%, P<0.001) (Figure 1).

In agreement with the present result, ESTERMANN *et al*. (2002) demonstrated that energy loss through CH_4 is closely and linearly related to intake of NDF and digestible NDF. In the present study, sheep fed on silage-based diet had a significantly higher (P<0.05) CH_4 production, expressed as g/kg NDF digested, compared with those fed on hay-based diet. The higher CH_4 production with the silage-based diet may be a result of increased fiber digestibility resulting from microbial activity through ensiling. This finding is also supported by greater fibrous carbohydrate fraction that is associated with longer retention time of feed in the rumen, consequently higher NDF digestibility and therefore higher CH_4 output. It is worth noting that although both experimental diets used have almost similar content of NDF but resulted in different CH_4 output. This result suggests that differences in fragility of forages particle should be considered as an important

Table 4. Linear regression to predict CH_4 production (g/day) from fiber fractions digested (g/day)

	SEM	R^2	*P*-value
CH_4 = 0.05 NDF digested + 7.4	0.58	0.88	0.001
CH_4 = 0.06 ADF digested + 11.9	0.86	0.75	0.005
CH_4 = 1.5 hemicellulose digested + 4.0	0.69	0.84	0.001
CH_4 = 0.06 cellulose digested + 13	0.88	0.74	0.006

SEM, standard error of the mean

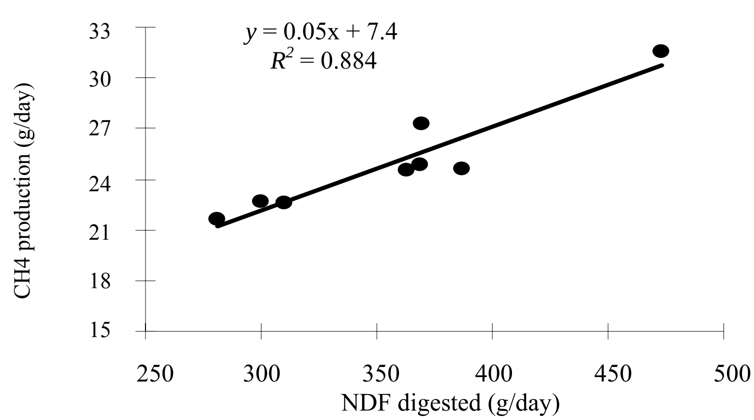

Figure. 1. Relationship between methane production (g/day) and NDF apparently digested (g/day)

factor that affecting rate of CH_4 production per unit of substrate fermented. The variation of CH_4 production within measurement day observed in this study was 2.4%, which is slightly lower than value of 3.6% reported by BLAXTER and CLAPPERTON (1965) for day-to-day variation in CH_4 production measured over 4 days from sheep given constant feed. In the present study, CH_4 production expressed as a percentage of GE intake is within the range cited by PELCHEN and PETERS (1998), but slightly higher than the 5.5 – 6.5% suggested losses by JOHNSON and WARD (1996) for cattle, sheep and goats.

CONCLUSION

Sheep fed silage-based diet had higher apparent digestibility of diet and CH_4 production than those fed hay-based diet. Methane production is greatly influenced by fiber fractions (mainly NDF) digestibility. There was a strong relationship between CH_4 production (g/day) and NDF digested (g/day) (R^2 = 88.4%, P<0.001).

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