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ORIGINAL ARTICLE

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Effects of forage composition and growing season on methane emission from sheep in the Inner Mongolia steppe of China

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Abstract Understanding the effects of dietary composition on methane (CH_4) production of sheep can help us to understand grassland degradation resulting in an increase of CH_4 emission from ruminant livestock and its resulting significance affecting CH_4 source/sink in the grazing ecosystem. The objective of this study was to investigate the effect of forage composition in the diet of sheep in July and August on CH_4 production by sheep in the Inner Mongolia steppe. The four diet treatments were: (1) *Leymus chinensis* and *Cleistogenes squarrosa* (LC), (2) *Leymus chinensis*, *Cleistogenes squarrosa* and concentrate supplementation (LCC), (3) *Artemisia frigida* and *Cleistogenes squarrosa* (AC), and (4) *Artemisia frigida*, *Cleistogenes squarrosa* and concentrate supplementation (ACC). CH_4 production was significantly lower in July than in August (31.4 and 36.2 g per sheep-unit per day, respectively). The daily average CH_4 production per unit of digestive dry matter (DM), organic matter (OM) and neutral detergent fiber (NDF) increased by 10.9, 11.2 and 42.1% for the AC diet compared with the LC diet, respectively. Although concentrate supplementation in both the AC and LC diets increased total CH_4 production per sheep per day, it improved sheep productivity and decreased CH_4 production by 14.8, 12.5 and 14.8% per unit of DM, OM and NDF digested by the sheep, respectively. Our results suggested that in degraded grassland CH_4 emission from sheep was increased and concentrate supplementation increased diet use efficiency. Sheep-grazing ecosystem seems to be a source of CH_4 when the stocking rate is over 0.5 sheep-units ha^{-1} during the growing season in the Inner Mongolia steppe.

Keywords Methane emission · Sheep · Face mask · Plant composition · Seasonal influence · Inner Mongolia steppe of China

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Introduction

Methane (CH_4) is an important greenhouse gas (GHG) that affects the earth's energy balance and global climate change because of its radiation-forcing properties (Cicerone and Oremland 1988; Houghton et al. 1996; Harper et al. 1999). Annual CH_4 emission is approximately 535 Tg worldwide (Kroeze et al. 1999) with 375 Tg CH_4 coming from anthropogenic activities (Houghton et al. 1996; Kroeze et al. 1999). Subak et al. (1993) and Kroeze et al. (1999) suggested that approximately 20% of total CH_4 emission from anthropogenic activities is produced by animals. However, uncertainty in the amount of GHG emission is mainly due to a lack of data from different sources or sinks, especially from animals and their excrement (Janssen 1999; Boeckx and Cleemput 2001; Monteny et al. 2001). Therefore, the effect of anthropogenic activity on global warming is still not completely understood (Lin and Li 1998; Boeckx and Cleemput 2001; Monteny et al. 2001; Rossi et al. 2001). Methane is an unavoidable and inefficient product of rumen fermentation. The loss of CH_4 from ruminant livestock is a problem for efficient livestock nutrition as well as for GHG emissions. The amount of CH_4 emission is related to the level of feed intake, dietary composition and type of carbohydrate in the diet, especially with dietary components that affect fermentation types in the rumen (Johnson and Johnson 1995; McAllister et al. 1996).

Leymus chinensis is a dominant species of the climax grassland community in the Inner Mongolia steppe (Chen and Wang 2000), but it is replaced by *Cleistogenes squarrosa* under moderate grazing, which, in turn, is replaced finally by *Artemisia frigida* under heavy grazing with associated soil infertility (Li 1989; Wang et al. 1998; Chen et al. 2002). Although some studies have shown that the soil–vegetation system is a feeble sink for CH_4 (Du et al. 1998; Wang et al. 2003) in the region, the contribution of grazing animals under a grazing ecosystem to the change in forage composition of the plant community affecting CH_4 emission from the animals was not considered in these studies. On the other hand, there is a difference between traditional and current grazing management systems. The traditional grazing system is nomadic, and livestock depend mainly on selecting herbage of grassland to maintain and grow in the forage growing season. However, the current system is to limit the grazing style in certain forage growing seasons in order to protect grassland. Therefore, to improve production during the limited grazing periods, the diet of livestock has to supplemented with concentrate, which has a great influence on CH_4 emission from the livestock (Johnson and Johnson 1995). The objective of this study was to determine whether grassland degradation results in increased CH_4 emission from ruminant livestock in the Inner Mongolia steppe of China and what the key factor is that affects CH_4 source or sink in the grazing ecosystem using a face mask technique.

Materials and methods

Location and site

The study was conducted from the middle of July to the end of August 2004, which represents summer and autumn, respectively, at the Inner Mongolia Grassland Ecosystem Research Station of the Institute of Botany, the Chinese Academy of Sciences in Xilinhot, located at 43°37′N, 116°43′E at an elevation of 1,000 m or more above sea level. The regional climate is continental. The 30-year average annual precipitation is approximately 350 mm (250–450 mm), with 60–70% of the total occurring from June to August. Annual mean temperature is −0.4°C, with 150–180 plant-growing days per year. Average monthly temperature is −21.5°C in January and 19°C in July. Minimum and maximum recorded temperatures are −43°C in 1981 and 35.1°C in 1973. *L. chinensis* and *Stipa grandis* grassland communities, which are the most widely distributed grassland types in the Eurasia steppe region (Chen and Wang, 2000), are taken over by a *C. squarrosa* and *A. frigida* community when the stocking rate is heavy and there is overgrazing (Li 1989; Wang et al. 1998).

Animals and diets

Sixteen 15-month-old Mongolian wethers, with a mean body weight (BW) of 38.4 kg (SD 2.7 kg) with a rumen cannula of 25 mm diameter, were divided randomly into four groups. Each group received one of four different diets assigned randomly in the middle of July and the end of August. The dietary treatments on the basis of fresh weight were: (1) 80% *L. chinensis* and 20% *C. squarrosa* (LC), (2) 80% *L. chinensis* and 20% *C. squarrosa* supplemented with 300 g concentrate (LCC), (3) 80% *A. frigida* and 20% *C. squarrosa* (AC), and (4) 80% *A. frigida* and 20% *C. squarrosa* supplemented with 300 g of concentrate (ACC). Each diet was given to the four sheep as two equal meals daily at 0800 and 1500 hours. Water was provided at 1100 and 1600 hours for each sheep during the experimental periods. Fresh forage was chopped to lengths of approximately 5–10 cm before feeding. The concentrate mixture consisted of 60% maize, 14% wheat bran, 12% soybean meal, 10% distillery residue and 4% mineral/vitamin mixture. The mineral/vitamin mixture consisted of salt 35%, bone meal 25%, Na_2SO_4 13%, $CuSO_4 \bullet 7H_2O$ 0.002%, $ZnSO_4$ 0.015%, $MnSO_4 \bullet 5H_2O$ 0.007%, 5000 IU vitamin A, 1,000 IU vitamin D, 20 IU vitamin E, and carrier 26.8%.

Measurements

During each experimental period, the digestibility for each diet was measured using four sheep in metabolism cages. The experimental duration was 15 days; feces were collected and weighed once daily for the last 7 days. A tenth part was dried at 65°C in an oven for 48 h. At the end of the collection period, fecal samples were mixed into one sample per sheep for subsequent chemical analysis. Daily samples of fresh forage consumed, concentrate and feed residues were collected and dried at 65°C in an oven for 48 h during the experimental period. They were pooled at the end of the experiments and milled through a 1-mm mesh sieve prior to chemical analysis.

On the 9th and 11th day of each experimental period, rumen fluid samples were taken via a rumen cannula using a flexible stomach tube and a manual vacuum pump 6 h after the morning feeding. The pH was measured immediately in fresh liquor using a portable digital pH meter with a combination electrode (Leici 733 automatic acid meter, Shanghai, China), and the liquor then filtered through four layers of gauze. The filtrate was acidified with phosphoric acid and stored at −20°C for measuring NH_3-N and volatile fatty acid (VFA) concentration after centrifuging at 4,000 rpm for 10 min.

All sheep in the study were acclimated to wearing a face mask after feeding in the preliminary experimental period. From the 12th to 14th day, the concentration and amount of CH_4 emissions from the sheep were measured using the face mask technique. Briefly, the face mask system consisted of a face mask, gas bag and a gas flow meter, which measured the gas volume. The face mask had two reverse gas shut-off valves that ensured gas flowed only in one direction. The gas bag (120×70× 70 cm) was connected to the facemask and the gas flow meter by flexible leak-free tubes. Samples of expired gas from the sheep were collected through the face mask into the gas bag, drawing gas from the bag through the gas flow meter. The volume of expired gas per unit of time was recorded by the gas flow meter. The gas samples, measuring average daily dynamic change in CH_4 emission from the 16 sheep, were collected at 2-h intervals during two experiments. The daily total CH_4 emission from the sheep was calculated based on the product of the expired gas volume and the concentration of CH_4. The gas samples were analyzed for CH_4 concentration using gas chromatography (HP5890 series, Institute for MCR, Beijing, China) in the laboratory.

Chemical analyses

Dry matter (DM), crude ash, crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the methods of the Chinese Technical Committee for Feed Industry Standardization and the Chinese Association of Feed Industry (1996, 2000). The NH_3-N concentration was determined by the phenol-hypochlorite method using spectrophotometric determination as described by Broderick and Kang (1980). VFA was analyzed with gas chromatography (SP-501, Institute for MCR, Beijing, China) as outlined by Qin (1982).

Statistics analyses

All data in the experiments were analyzed as a 4×2 cross-over arrangement with the general linear model (GLM) using commercially available statistical software (SPSS version11.5 for Windows, 2003). Multiple comparisons among treatment means were performed with the Duncan method. Differences among experimental treatments were declared to be significant at the 0.05 level throughout the study. Correlation coefficients of variance were calculated with Pearson's method, and the test of significance was two-tailed.

Results

Nutrient composition of diets and forage

Nutrient compositions of forage and diet are shown in Tables 1 and 2. Generally, the chemical composition of forage was not significantly affected by the growing season. Both the LCC and ACC diets (300 g added concentrate) contained slightly higher levels of CP and lower levels of NDF than the LC and AC diets in July and August. The other nutrient components were not significantly different between diet types and growing seasons.

Diet intake, apparent digestibility and gain weight

Table 3 shows a significant difference ($P < 0.05$) in feed intake per day between the diet treatments. Daily DM, OM, CP and NDF intakes by sheep on the LCC and ACC diets were significantly greater than by those on the LC and AC diets due to the concentrate supplementation in the diets, and higher for the LC than for the AC diet and for the LCC than for the ACC diet except for CP intake. Seasonal effects on feed intake and interactions ($P < 0.05$) between diet and growing season were observed. While sheep were housed in individual metabolic cages, their DM intake averaged 1.42 kg/day in July and 1.23 kg/day in August. Digestible DM, OM and NDF intakes of sheep were greater with the LCC and ACC diets than with the LC and AC diets, and with the LC than with the AC diet and with the LCC than with the ACC diet ($P < 0.05$). There was a higher digestible CP intake with the AC than with the LC diet due to a higher CP content in the AC diet (Table 4). Sheep had a higher apparent digestibility of DM and OM with the LCC than with the LC diet and with the ACC than with the AC diet. Higher digestibility of DM and OM was also observed with the LC than with the AC and with the LCC than with the ACC diet. Similarly, there was a higher apparent digestibility of NDF with the LC and LCC than with the AC and ACC diets, but there was no significant difference between the LC and LCC diets or between the AC and ACC diets during the experimental period. There was no significant difference ($P > 0.05$) in the digestibility of CP between the diet treatments. Generally, the apparent digestibility of DM and OM was greater in August than in July. The average daily weight gains (DWG) of sheep on the LCC

Table 1 Chemical composition of forage and concentrate of the diet used in the experiment. Values are percent of DM

Item	*Leymus chinensis*	*Artemisia frigida*	*Cleistogenes squarrosa*	Concentrate
July				
OM	95.4	93.6	94.7	89.5
CP	9.1	10.2	7.7	13.2
NDF	43.1	38.5	44.2	32.6
ADF	23.7	18.6	25.1	20.4
August				
OM	94.1	92.7	93.9	89.5
CP	9.0	10.0	7.0	13.2
NDF	44.7	40.3	45.7	32.6
ADF	25.3	20.4	25.8	20.4

DM Dry matter, *OM* organic matter, *CP* crude protein, *NDF* neutral detergent fiber, *ADF* acid detergent fiber

Table 2 Ingredient and chemical compositions of the diets used in the experiments. Values are percent of DM

	July				August			
	LC	LCC	AC	ACC	LC	LCC	AC	ACC
Ingredient composition								
Leymus chinensis	78.4	66.1			81.5	67.4		
Artemisia frigida			77.4	64.7			82.9	69.4
Cleistogenes squarrosa	21.6	18.2	22.6	18.9	18.5	15.3	17.1	14.4
Concentrate		15.8		16.4		17.3		16.2
Chemical composition								
OM	95.2	94.3	93.8	93.1	94.1	93.3	92.9	92.4
CP	8.8	9.5	9.6	10.2	8.6	9.4	9.5	10.1
NDF	43.3	41.6	39.8	38.6	44.9	42.8	41.2	39.8
ADF	24.0	23.4	20.1	20.1	24.0	23.3	19.7	19.8

LC 80% *L. chinensis* and 20% *C. squarrosa*, *LCC* 80% *L. chinensis* and 20% *C. squarrosa* (fresh weight basis) supplemented with 300 g concentrate, *AC* 80% *A. frigida* and 20% *C. squarrosa*, *ACC* 80% *A. frigida* and 20% *C. squarrosa* supplemented with 300 g of concentrate

Table 3 Nutrient intake and digestibility in sheep under the different treatments

Measurement	July				August				SEM	*P* value		
	LC	LCC	AC	ACC	LC	LCC	AC	ACC		Diet	Season	Diet×period
Metabolic body weight[a]	15.38	15.76	15.22	15.18	15.70	15.55	15.71	14.72	0.95	0.33	0.90	0.61
DWG (g/d)	50.0	76.7	18.3	56.7	46.6	77.3	16.7	51.7	4.07	< 0.001	0.42	0.99
Intake (kg/day)												
DM	1.36	1.62	1.19	1.51	1.09	1.34	1.12	1.35	0.01	< 0.001	< 0.001	< 0.001
OM	1.30	1.53	1.13	1.42	1.05	1.28	1.07	1.28	0.01	< 0.001	< 0.001	< 0.001
CP	0.12	0.16	0.12	0.16	0.1	0.13	0.11	0.15	0.001	< 0.001	< 0.001	< 0.001
NDF	0.59	0.67	0.48	0.43	0.48	0.56	0.44	0.50	0.01	< 0.001	< 0.001	< 0.001
Apparent digestibility (%)[b]												
DM	62.6	64.9	57.2	61.3	63.8	66.1	61.7	63.2	0.72	< 0.001	0.006	0.32
OM	66.2	68.1	61.2	64.5	68.4	69.7	66.3	66.9	0.63	< 0.001	< 0.001	0.23
CP	56.7	58.6	54.5	56.1	54.4	57.2	56.4	55.0	0.79	0.10	0.27	0.29
NDF	51.5	53.0	41.7	43.3	55.6	56.4	47.6	46.1	0.84	< 0.001	< 0.001	0.61

DWG Daily weight gains, *SEM* standard error of mean

[a]Live body weight$^{0.75}$ kg

[b]Calculated as (Content in diet−content in feces)/content in diet ×100

Table 4 Daily nutrition digested and CH_4 emission from sheep under the different treatments

Item	July				August				SEM	*P* value		
	LC	LCC	AC	ACC	LC	LCC	AC	ACC		Diet	Season	Diet×period
Digested (kg/day)												
DM	0.85	1.05	0.68	0.93	0.70	0.88	0.69	0.85	0.01	< 0.001	< 0.001	< 0.001
OM	0.86	1.04	0.69	0.92	0.72	0.89	0.71	0.85	0.01	< 0.001	< 0.001	< 0.001
CP	0.07	0.09	0.07	0.09	0.05	0.08	0.07	0.08	0.001	< 0.001	< 0.001	< 0.001
NDF	0.30	0.36	0.19	0.25	0.27	0.31	0.21	0.23	0.01	< 0.001	0.01	< 0.001
CH_4 emission												
g/day	15.57	17.39	14.42	16.61	17.44	19.10	18.49	18.71	0.49	0.04	< 0.001	0.30
g/kg metabolic body weight	1.01	1.10	0.95	1.10	1.11	1.23	1.18	1.27	0.03	0.02	< 0.001	0.51
g/kg DM digested	18.29	16.52	21.24	17.93	25.11	21.61	26.90	21.93	0.74	< 0.001	< 0.001	0.61
g/kg OM digested	18.08	16.66	20.84	18.09	24.21	21.46	26.20	21.94	0.72	0.002	< 0.001	0.73
g/kg CP digested	227.40	191.23	217.83	187.17	320.22	250.82	284.26	228.98	7.95	< 0.001	< 0.001	0.18
g/kg NDF digested	51.59	48.89	76.50	66.75	65.36	60.70	89.65	80.81	2.63	< 0.001	< 0.001	0.99

and ACC diets were higher ($P < 0.05$) than of those on the LC and AC diets, and DWG was approximately threefold in sheep on the LC diet compared to those on the AC diet (Table 3).

Characteristics of rumen fermentation

Supplements significantly reduced the pH values of the rumen ($P < 0.05$) for both the LCC and ACC diets, but dietary composition had no significant effect on rumen pH (Table 5). There were no significant differences among diets between July and August. The NH_3-N concentrations were higher ($P < 0.05$) for the LCC and ACC diets than for the LC and AC diets, but effects of season and an interaction between season and diet type were not observed. Although the proportion of the individual VFA and the ratio of acetic to propionic acids in rumen fluid were not significantly different among treatments ($P > 0.05$), concentrate supplementation in the diets decreased significantly the concentration of total VFA in ruminal fluid. Higher concentrations of total VFA ($P < 0.05$) were also observed for the AC and ACC diets than for the LC and LCC diets. In addition, total VFA concentrations were higher ($P < 0.05$) in July than in August.

CH_4 production

The peak average CH_4 production by sheep occurred 5–6 h after each meal (Fig. 1). Regardless of concentrate supplementation and dietary composition, there were higher ($P < 0.05$) CH_4 emissions per kg metabolic body weight (MB) in sheep on the LCC and ACC diets than in those on the LC and AC diets (Table 4). However, daily CH_4 productions per kg digestible DM and CP were significantly lower ($P < 0.05$) with the LCC than with the LC diet and with the ACC than with the AC diet. The CH_4 productions per kg digestible DM, OM and NDF were also significantly lower ($P < 0.05$) with the LC than with the AC diet. There was higher average CH_4 production in August than in July ($P < 0.05$). Average daily CH_4 production in July and August were 0.93 and 1.08 g kg^{-1} MB, 18.49 and 23.89 g kg^{-1} DM digested, 18.42 and 23.45 g kg^{-1} OM digested, 205.91 and 271.07 g kg^{-1} CP digested, and 60.93 and 74.13 g kg^{-1} NDF digested, respectively.

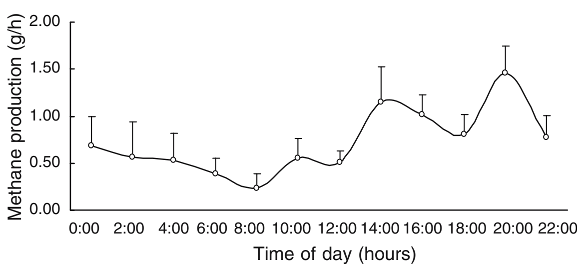

Fig. 1 Average daily dynamics of CH_4 production by sheep given restricted amounts of mixed diets at 0800 and 1500 hours. Each point represents the mean of 32 observations, derived from 16 sheep on 2 days, with their standard error represented by vertical bars

Correlation between variables and CH_4 production

Table 6 shows correlation coefficients of measured variances. MB had no significant effect on CH_4 production by sheep. There was a negative linear correlation between DWG and CH_4 production per unit of digestible DM, OM and NDF. The concentration of NH_3-N had a significantly ($P < 0.05$) positive correlation with CH_4 production per kilogram MB, whereas a negative correlation was found between total VFA in ruminal fluid and CH_4 production per kilogram MB. The digestibilities of DM and OM had a significantly positive correlation with CH_4 production per kg MB, but there was a significant negative correlation with CH_4 production per unit of digestible NDF. There were significant negative correlations between the digestibility of CP and CH_4 production per unit of digestible CP, and between the digestibility of NDF and CH_4 production per unit of digestible NDF.

Discussion

Forage type and CH_4 emission

The basal diet used in this study represented two types of grassland, a climax community and a degraded community. The different forages had different chemical compositions and physical morphologies, which greatly affected their palatability and daily intake by sheep in the region (Wang 2000, 2001). During the growing season, the *Artemisia* genus has strong volatile lipids such

Table 5 Ruminal fermentation characteristics of sheep under the different treatments

Parameter	July				August				SEM	*P*-value		
	LC	LCC	AC	ACC	LC	LCC	AC	ACC		Diet	Season	Diet×period
pH	6.78	6.31	6.77	6.59	6.84	6.17	7.02	6.26	0.07	< 0.001	0.55	0.03
NH_3-N (mmol/l)	3.95	4.14	2.60	5.07	3.54	4.93	2.66	5.61	0.23	< 0.001	0.15	0.07
Total VFA (mmol/l)	67.40	65.58	72.73	70.23	63.68	60.28	66.25	64.30	0.35	< 0.001	< 0.001	0.06
VFA proportions (%)												
Acetic	58.3	59.7	57.2	56.6	61.7	60.3	61.1	60.6	0.89	0.72	< 0.001	0.09
Propionic	19.6	20.2	20.3	19.4	21.5	19.4	21.2	20.6	0.93	0.24	0.06	0.10
Butyric	12.4	11.1	13.4	14.5	13.4	12.4	12.6	15.3	0.83	0.31	0.13	0.11
Acetic:propionic	2.97	2.95	2.81	2.91	2.87	3.10	2.88	2.94	0.56	0.29	0.07	0.13

VFA Volatile fatty acid

Table 6 Correlation coefficient calculated between measured variance and CH_4 production

Variance	CH_4 production				
	g kg^{-1} MB	g kg^{-1} DM digested	g kg^{-1} OM digested	g kg^{-1} CP digested	g kg^{-1} NDF digested
MB (kg)	−0.29	0.17	0.17	0.26	-0.04
DWG (g/day)	0.19	−0.54*	−0.53*	−0.34	−0.67*
pH	−0.37	0.32	0.28	0.32	0.24
NH_3-N (mmol/l)	0.46*	−0.33	−0.29	−0.34	0.24
Total VFA (mmol/l)	−0.57*	−0.23	−0.24	−0.41*	0.17
Digestibility (%)					
DM	0.41*	−0.22	−0.20	0.02	-0.51*
OM	0.43*	−0.06	−0.06	0.17	-0.43*
CP	0.17	−0.27	−0.25	−0.34*	−0.29
NDF	0.22	−0.02	−0.03	0.33	-0.57*

*$P < 0.05$

as monoterpenes, sesquiterpenes etc. which vary with growing season (Chen et al. 2000). These scents could result in a poor palatability to sheep, resulting in less daily DM intakes for the AC and ACC diets as compared with the LC and LCC diets in our study (Table 3). These unsaturated lipids have the function of defaunating protozoa (Chinese medicine compilation 1975; Xu et al. 2004). CH_4 production by ruminant animals is closely related to ruminal protozoa (Whitelaw et al. 1984; Broudiscou et al. 1990) because it has a symbiotic relationship with ruminal methanogens (Stumm et al. 1982). In this study, although we did not measure the population of protozoa, there was a lower CH_4 production per sheep on the AC diet than in sheep on the other diets (Table 4). Probably the unsaturated lipids of *A. frigida* inhibit growth of protozoa in the sheep rumen, which could result in a marked decrease in CH_4 production. The lower methane emission from sheep also could be associated with the poor palatability of the AC diet because there was a lower daily DM intake compared with the other diets (Table 3).

Dietary type, chemical composition and CH_4 emission

Our results indicated that added concentrate in diets increased daily CH_4 production per sheep or per kilogram MB, but decreased CH_4 production per unit of digestive DM, OM and NDF intake due to the higher nutritive level of the LCC and ACC diets than of the LC and AC diets. The higher nutritive level not only increased feed intake, but was also associated with a rapid DWG. These results indicated that although concentrate supplementation in a diet increases total daily CH_4 production by sheep because of an increase in digestive nutrient intake, it decreased CH_4 production per unit of digestive nutrients because it increased diet utilization efficiency. Therefore, total CH_4 production could decrease to get the same amount of animal products by improved dietary nutritive level. For example, the results here show that CH_4 production per 100 g of DWG of sheep on the LC, LCC, AC and ACC diets were 38.80, 24.72, 101.65 and 33.70 g, respectively. In addition, high feed intake leads to an increased weight of digesta and fractional passage rate in the rumen (Colucci et al. 1982; Kennedy and Milligan 1987). Okine et al. (1989) reported the CH_4 production is correlated negatively ($r = -0.53$) with the passage rate constant for ruminal particulate matter. Our results also indicated that a higher feed intake was associated with less CH_4 production per unit of digestible diet. Probably rapid passage rate decreased the rate of degradation and retention time of the diet in rumen perhaps influencing the methanogenic population (Angela et al. 1994). The average daily CH_4 production per unit of digestible DM, OM and NDF by sheep increased by 10.9, 11.2 and 42.1% for AC diet than for LC diet, respectively. These data imply that, based on per unit of digestive diet, a degraded *A. frigida* community not only decreased feed intake and digestibility, but also relatively increased CH_4 production by sheep compared with a climax *L. chinensis* community in Inner Mongolia steppe.

Rumen environment and CH_4 emission

Ruminal pH and concentration of NH_3-N, VFA of rumen fluid were used to monitor rumen fermentation characteristics. Decreased pH values in the rumen due to concentrate supplementation in this study could be affected by the total ruminal VFA concentration. A significant positive correlation between the NH_3-N concentration of rumen liquor and the CP content of the diets was observed because NH_3-N is derived from protein degradation and NH_3 absorption as well as incorporation into microbial protein (Schmidt et al. 2001). VFA are the end-products of diet fermentation in the rumen, and are also the energy source for ruminant livestock (Orskov et al. 1990). The concentration and proportion of VFA are principally affected by the amount of dietary carbohydrate fermented in the rumen and reflect the level of fermentation (McDonald 1988). In the present experiments we only found a significant difference ($P < 0.05$) for total VFA concentration among treatments and a negative correlation between total VFA concentration and CH_4 production. Although with a concentrate supplementation in the LCC and ACC diets, the acetic proportion in all treatments was lower than 65%, which we regarded as propionate fermentation in the rumen (McDonald 1988). Therefore, experimental treatments did not change the type of rumen fermentation. Based on the data of in situ ruminal DM degradability, the degradability of the three forages (*L. chinensis*, *A. frigida* and *C. squarrosa*) was markedly different ($P < 0.05$) in different growing seasons, and there was a higher rapidly degradable DM in July than in August ($P < 0.05$) (unpublished). CH_4 production by sheep was significantly affected by forage maturity because the type of carbohydrate fermented influences CH_4 production due to the amount of dietary carbohydrate fermented and the available hydrogen supply (Johnson and Johnson, 1995). Moe and Tyrrell (1979) also reported that higher cell wall fiber results in higher CH_4 losses, and fermentation of soluble carbohydrate results in less CH_4 production.

Implication for grassland management

First, under heavy grazing, a dominant species of *L. chinensis* in the climax community can be finally replaced by *A. frigida*, changing the original community structure of the Inner Mongolia steppe. The degraded succession results in decreasing sheep productivity, thus increasing CH_4 emission per unit sheep production. Second, in view of the current management system that is concentrate supplementation of the diets in certain forage growing seasons, higher nutrient levels in a diet may decrease total CH_4 production based on the same amounts of animal products because it increases diet utilization efficiency. Third, Wang et al. (2000, 2003) have reported that the soil–vegetation system is a feeble sink of atmospheric CH_4 and that CH_4 flux is 2.89–6.53 g CH_4 ha^{-1} per day in the *L. chinensis* steppe community during the summer–autumn season. In this study, the average CH_4 production was 16.00–18.43 g per sheep per day using 38.4 kg as the average weight of a sheep in the summer–autumn season. Additionally, the grazing ecosystem would show an increase in its contribution to CH_4 emission because spots of feces and urine decrease the absorbency of the soil–vegetation to CH_4 (unpublished). Thus, if the present estimation of methane production is available and sheep select forage in pasture during the daytime, the grazing ecosystem of soil–plant–animal could be a CH_4 source when the stocking rates are over 0.5 sheep-unit ha^{-1}, which corresponds to 1.0 individual per ha, where 1 sheep-unit is a 50-kg ewe (live weight) with a lamb, during the growing season from May to September on the Inner Mongolia steppe. Therefore, a stocking rate would be a key factor controlling greenhouse gas emissions in the grazing ecosystem.

Conclusion

The study demonstrated that growing season has a significant effect on CH_4 production and a concentrate supplementation in the diet can increase productivity and decrease CH_4 production per unit of digestive diet. Grassland degradation resulted in a higher CH_4 emission from grazing ruminant livestock. The CH_4 source or sink will depend on the stocking rate in the grazing ecosystem and it could be a CH_4 source when the stocking rate is over 0.5 sheep-units ha^{-1} during the growing season in the region.

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