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Methane Emission from Sheep Respiration and Sheepfolds During the Grazing Season in a Desert Grassland

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Abstract: Methane (CH_4) emissions from ruminants should be accounted for the natural grazed rangeland ecosystems when devising greenhouse gas budget inventory, in particular, their contribution to global warming. In this study, CH_4 emission from sheep respiration at different grazing intensities (light grazing, 0.75 sheep/ha, LG; moderate grazing, 1.50 sheep/ha, MG; and heavy grazing, 2.25 sheep/ha, HG) and in sheepfolds were evaluated in a desert grassland of Inner Mongolia. Results indicated that daily CH_4 emission from sheep was not significantly different between treatments. When CH_4 emission was expressed emission per 100g daily, there was a significant difference of LG *vs* HG and MG *vs* HG, with the values of 15.64g, 20.00g and 28.63g for LG, MG and HG, respectively, during the grazing season. There was no significant difference among CH_4 fluxes in sheepfolds (mean 39.0 ug m^{-2} h^{-1}). Considering CH_4 emissions from the grazing ecosystem, net CH_4 emissions from LG, MG and HG plots were -18.33, -1.91 and 21.19 g/ha/day, respectively. The digestibility of forage had a positive correlation with CH_4 emission expressed on daily and metabolic body weight basis. It is concluded that MG will improve the balance between CH_4 emission from grassland and grazing livestock in the desert grasslands of Inner Mongolia.

Keywords: CH_4 emission, desert grassland, grazing intensity, Inner Mongolia, sheep.

INTRODUCTION

Methane (CH_4) is long-lived in the atmosphere, with a perturbation lifetime of 12 years [1]. In addition to being a GHG, CH_4 also reacts in the atmosphere to produce ozone [2]. The Intergovernmental Panel on Climate Change [3] reported that global warming potential (GWP) weighted emissions of GHGs increased by approximately 70% from 1970 to 2004, including emissions of CH_4 which have risen by about 40%. Emission of CH_4 is responsible for nearly as much radiative forcing as all other non-CO_2 GHGs combined. Atmospheric concentrations of GHGs have risen by about 39% since the pre-industrial era, and CH_4 concentration has more than doubled during this period [4]. In the modern era, CH_4 production has been aggravated by large scale ruminant production, accounting for 18% of GHG emissions [5]. CH_4 emissions from ruminants are of major concern, especially due to their role in climate change [6,7] and their significant contribution to GHG inventories [8].

In recent decades, livestock numbers in Inner Mongolia have increased significantly. Sheep and goats are the primary grazing animals, accounting for more than 80% of the total. The total number of livestock was 112.6 million (heads, mid-year number) in 2012 [9]. Wang *et al.* [10] reported that grazing ecosystems are a source of CH_4 when the stocking rate is over 0.5 sheep-unit per ha during the growing season, and CH_4 emissions are exacerbated by grassland degradation. Shibata and Terada [11] identified factors affecting CH_4 production in ruminants, including level of intake, type and quality of feed and environmental temperature. GHG emissions from enteric fermentation and manure management of sheep grazing in Inner Mongolian steppe have been quantified and analyzed for lightly grazed, moderately grazed, and heavily grazed steppe [12]. Emissions from animal facilities primarily consist of animal respiration and enteric fermentation. In addition, emissions from manure storage are also a potential source of CH_4 [13].

The aim of our investigation was to directly determine the CH_4 flux in the grazing ecosystem of desert grassland in Inner Mongolia in relation to the grazing intensity and season by measuring the CH_4 emission from sheep expiration and from soil surface of sheepfold. The face mask method was used for the measurement of CH_4 emission from sheep respiration, and the static opaque chamber method was used for the measurement of CH_4 flux from sheepfolds. We expect find a suitable grazing intensity or grassland management mode to balance the environment demand (reduce CH_4 emission) and economic development (grazing for meat and so on).

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MATERIAL AND METHODS

Study Site Description

The study was conducted at an experimental site of the Inner Mongolia Academy of Agriculture and Animal Husbandry Research Station (41°47′17″N, 111°53′46″E). The site has an elevation of 1450 m and is in a temperate continental climate, characterized by a short growing season and long cold winter with a frost-free period of 175 days. January is the coldest month with an average temperature of -15.1°C while July is the warmest month with an average temperature of 19.6°C. Average annual precipitation is approximately 280 mm, of which nearly 75% falls during June through September. The grassland is dominated by *Stipa breviflora* Griseb., *Artemisia frigida* Willd., *Cleistogenes songorica* (Roshev.) Ohwi. The dominant soil types are Kastanozem (FAO soil classification) or Brown Chernozem (Canadian Soil Classification) with a loamy sand texture [14].

Measurement of CH_4 from Sheep Respiration

The area are used for a grazing experiment started in 2012 with four stocking rates (non-grazing, 0 sheep/ha, NG; light grazing, 0.75 sheep/ha, LG; moderate grazing, 1.50 sheep/ha, MG; and heavy grazing, 2.25 sheep/ha, HG) with three replications each (Table **1**). Sheep grazed from 6:30 am to 7:00 pm, and rested in sheepfolds from 7:00 pm to 6:30 am. The free water and mineral salt were offered for grazing sheep. Three grazed sheep (local Sunit breed) from each of the LG, MG, and HG areas were selected for the collection of gas examples from 5:30 to 6:30 am and 7:00 to 8:00 pm over three continuous days and for measurement of live weight gain at the middle of each month in the grazing season (July to October in 2012) (Table **2**).

Aboveground biomass and plant cover were measured at the mid of August.

The experimental sheep were acclimated to wearing a face mask which was a cylinder made of rubber material, and has a dimension 22 cm tall and 17 cm in diameter. The face mask system consisted of a face mask, gas bag and a gas flow device. Samples of breathing gas from the sheep were collected through the face mask into the gas bag. The average daily amount of CH_4 emitted from sheep was calculated by the volume of respired gas multiplied by CH_4 concentration. CH_4 emission from grazing sheep at grazing plots (g $ha^{-1}day^{-1}$) were calculated as the number of sheep in each grazing plots multiplied by daily CH_4 emissions, then divided by 4 ha (i.e., the grazing plot area). The experimental animals we used were with the approval of the Experimental Animal Committee of the Chinese Academy of Sciences, China.

Table 1. Characteristics of the investigated grazing areas: non-grazing (NG), light grazing (LG), moderate grazing (MG) and heavy grazing (HG).

Items	NG	LG	MG	HG
Plots size (ha)	4.0	4.0	4.0	4.0
Stocking rate (Sheep ha^{-1})	0.00	0.75	1.50	2.25
Sheep number (head)	0	3	6	9
Aboveground biomass(g DM m^{-2})	57.84	45.13	27.24	13.14
Plant cover(%)	21	22	19	18
Grazing period(Month)	-	6	6	6

Measurement of CH_4 Flux from Sheepfolds

Sheep from LG, MG, and HG grazing areas were enclosed in sheepfolds ($18m^2$) at night. Excrement was swept from the sheepfolds on the morning of the next day, which be usually used for fuel and fertilizer. CH_4 flux of sheepfolds was measured using the static opaque chamber method [15]. Three points in each sheepfold were designated for the collection of gas samples at the middle of each month (July to October in 2012) with measurement over three continuous days during the hours when sheep were not present in the sheepfold. During gas flux determination, a disposable syringe (100 ml) with a 3-way valve was used to collect 200 ml of chamber atmosphere into a sample gas bag at 10 min intervals over a 30 min period.

CH_4 concentration was analyzed using an automatic cavity ring-down spectrophotometer (Picarro G1301, Santa Clara, CA, USA). CH_4 fluxes of sheepfolds were calculated according to the following equation:

$$F = \frac{\rho \cdot V \cdot \Delta c}{A \cdot \Delta t}$$

where F is the flux (mg m^{-2} h^{-1}) of gas; ρ is the density of gas; $\Delta C/\Delta T$ is the slope of the linear regression for gas concentration gradient through time, negative values

Table 2. The effect of different grazing intensity on sheep weight in grazing period.

Items	LG	MG	HG	p Value	SEM
Initial live weight(kg)	38.6^a	38.8^a	38.9^a	0.63	0.1
Live weight in July (kg)	41.6^a	41.1^b	40.1^c	<0.01	0.2
Live weight in August (kg)	44.5^a	43.7^b	42.1^c	<0.01	0.4
Live weight in September (kg)	49.1^a	48.0^b	44.9^c	<0.01	0.5
Live weight in October (kg)	51.8^a	50.0^b	46.0^c	<0.01	0.6
Average daily gain weight (g/d)	109.2^a	94.2^b	60.8^c	<0.01	4.8

LG, light grazing; MG, moderate grazing; HG, heavy grazing. SEM, standard error of mean. Different superscript lowercase letters of a row indicate significant differences among treatments at $P < 0.05$.

indicating CH_4 uptake into the soil from the atmosphere; V and A are volume (m^3) and base area (m^2) of the hood, respectively.

Determination of *In Vitro* Dry Matter Digestibility of Forage

Forage samples were collected from each grazing plot at the middle of each month (July to October in 2012), dried at 65ºC in an oven for 24 h, and milled through a 1-mm mesh sieve prior to analysis. The 50 ml rumen liquor samples were taken from three experimental sheep *via* rumen cannulae using a flexible stomach tube before feeding in the morning, and stored in the vacuum flask at 39 ºC in the laboratory until they were put in a 39 ºC water bath after having been filtered through 4 layers of gauze, while entering CO_2 gas to the anaerobic state. A certain amount of buffer, rumen liquor and 0.5g forage sample filled in the ANKOM F57 fiber bag were put in an *in vitro* simulation incubator DAISY II at 39 ºC for 48 h.

$$\text{Digestibility (\%)} = 100 - \{[m_2 - (m_1 \times C_1)] \times 100 / m\}$$

where m is the forage sample (g), m_1 is fiber bag (g), m_2 is fiber bag and residue (g), and C_1 is the correction coefficient of an empty bag.

Statistical Analysis

The CH_4 emissions from sheep respiration were analyzed using a repeated measures mixed model with grazing intensity, month and grazing intensity×month as fixed effects, sheep as a random and repeating effect with the measurements obtained on one sheep at different times using the MIXED procedure of SPSS. The model providing the best-fit covariance structure included compound symmetry. The statistical model used was as follows:

$$y_{ijk} = \mu + T_i + S_{ij} + M_k + (T \times M)_{ik} + e_{ijk}$$

where y_{ijk} is the response on month k (k = 1-4) for sheep j (j = 1-3) in treatment group i (i = 1-3); μ is the overall mean; T_i is the fixed effect of treatment i; S_{ij} the random effect of sheep j in treatment i; M_k is the fixed effect of month k; $(T\times M)_{ik}$ is the fixed interaction effect of treatment i with month k; e_{ijk} is the random error for month k for sheep j in treatment group i. Significant differences in treatment means were determined using the Tukey's test with the level of significance takes as $P < 0.05$.

The CH_4 flux data from sheepfolds in different grazing months were analyzed by one-way analysis of variance (ANOVA-GLM) in SPSS. Correlations between CH_4 emissions and forage digestibility were calculated using Pearson's method with a two-tailed significance test.

RESULTS

CH_4 Emissions from Sheep Respiration

The effects of three grazing intensities on CH_4 emissions from sheep respiration were shown in Table **3**. CH_4 emissions were 17.23, 18.30 and 18.78 g/head/day at LG, MG and HG, respectively. The mean CH_4 emission at HG with 30.73 g/100g daily weight gain (DWG) was significantly higher ($P<0.05$) than at LG and MG (Fig. **1**), which were 15.79 and 19.46 g/100g DWG, respectively. CH_4 emissions per day and metabolic body weight did not differ significantly ($P>0.05$). CH_4 emissions from sheep in the LG, MG and HG plots were 12.92, 27.45 and 42.26 g/ha/day, respectively (Table **4**). The seasonal effects on CH_4 emissions from sheep respiration were not significantly different ($P>0.05$) expressed on per day, DWG, or metabolic body weight basis. Relative peak values were observed in September. The max daily CH_4 emission reached 23.77 g/head/day (Table **3**). There was no interaction between grazing intensity and month on CH_4 emissions from sheep respiration.

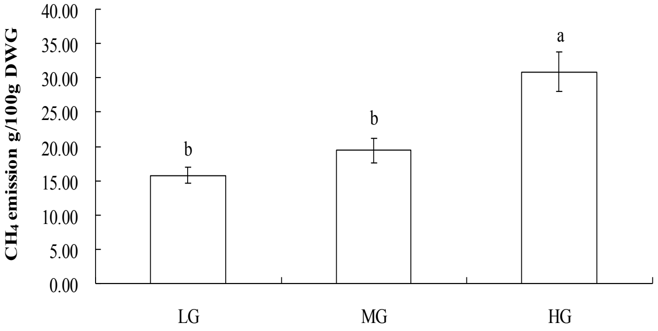

Fig. (1). CH_4 emission expressed on g/100g DWG under LG, MG and HG during the grazing season. Different lowercase letters indicate significant differences among treatments at $P < 0.05$. Error bars stand for standard error.

CH_4 Flux from Sheepfolds

The CH_4 flux from sheepfolds were not significantly different ($P>0.05$) during the grazing period, at 31.8, 48.7, 39.3 and 40.2 ug m^{-2} h^{-1} in July, August, September and October, respectively. The overall average CH_4 flux of sheepfolds was 39.0 ug m^{-2} h^{-1}.

Net CH_4 Emissions from the Grazing Ecosystem

The main influencing factors on CH_4 emissions or uptake from the grazing ecosystem were sheep respiration or enteric fermentation, the uptake of grassland soil and the interference from urine and dung. Tang *et al.* [16] reported that soil CH_4 flux varied during the growing season, and Jiang *et al.* [17] studied the contribution of urine and dung patches from grazing sheep to CH_4 fluxes in the desert grassland. Our results indicate that CH_4 emissions from urine and dung were much lower than from sheep respiration and soil uptake over a grazing plot with a large area. Combining sheep respiration with soil uptake, the net CH_4 emissions in LG, MG and HG plots were -18.33, -1.91 and 21.19 g/ha/day, respectively, during the grazing season (Table **4**).

Correlation of CH_4 Emissions and Forage Digestibility

There were positive correlations between the digestibility of forage and CH_4 emission (Fig. **2**). Digestibility had a significant ($P<0.05$) correlation with daily CH_4 emissions and CH_4 emissions per kilogram MBW. However, the correlation between digestibility and CH_4 emission per 100g DWG was not significant ($P>0.05$).

Table 3. The effect of different grazing intensity on CH_4 emission from sheep respiration in grazing period of 2012.

Item	July			August			September			October			SEM	p Value		
	LG	MG	HG	LG	MG	HG	LG	MG	HG	LG	MG	HG		GI	M	GI×M
g/head/day	15.67	14.37	17.49	16.53	17.30	15.87	21.16	22.57	23.77	15.55	18.94	17.98	1.41	0.934	0.632	0.999
g/kg MBW	0.90	0.84	1.05	0.95	1.01	0.96	1.21	1.31	1.43	0.89	1.10	1.08	0.08	0.857	0.623	0.999
g/100g DWG	14.35	15.26	28.76	15.14	18.36	26.11	19.38	23.97	39.09	14.24	20.11	29.58	1.73	0.015	0.504	0.995

LG, light grazing; MG, moderate grazing; HG, heavy grazing; GI, grazing intensity; M, month; GI×M is interaction between GI and M; SEM, standard error of mean; MBW, metabolic body weight; DWG, daily weight gains; significance of differences among experimental data is tested at the 0.05 level.

Table 4. Data on CH_4 emissions from the grazing ecosystem in an Inner Mongolian desert grassland.

Item	LG	MG	HG
CH_4 emission from sheep respiration (g $ha^{-1}day^{-1}$)	12.92	27.45	42.26
CH_4 uptake by soil (g ha^{-1} day^{-1})[1]	31.25	29.36	21.07
Net CH_4 emission from the grazing ecosystem(g $ha^{-1}day^{-1}$)	-18.33	-1.91	21.19

[1] CH4 uptake by soil (g ha^{-1} day^{-1}): The data comes from Tang *et al.* [16].

DISCUSSION

CH_4 Emission from Sheep Respiration

The level of CH_4 emissions from livestock is positively correlated with live weight, dry matter intake, and feeding levels [18]. Daily CH_4 emissions from grazing sheep at LG, MG and HG in this study were lower than the results reported by Schönbach *et al.* [12] for a steppe ecosystem. The probable cause is that the typical steppe grassland had more available forage than desert grassland during the growing season. The second reason may be that stocking rates (LG, MG and HG) were lower in our experimental design. These two factors influenced dry matter intake by sheep, which was greater in the typical steppe study than in our experiment. Live weight also has an influence on CH_4 emissions from ruminants. Wang *et al.* [10] studied CH_4 production using sheep with a similar average initial live weight in the summer–autumn season, and produced results in a similar range to ours. The results of Pinares-Patiño *et al.* [19] are higher than ours, and average initial live weight of sheep was also higher in their study. Higher live weight means more forage demand, so more CH_4 production is inevitable. Monthly effects are also clearly evident. Our results show that CH_4 emissions had a peak in September. Kumar *et al.* [20] indicated that CH_4 emissions, enteric fermentation patterns, and change in methanogen population appear only with a higher level of roughage. Wang *et al.* [10] also showed that with improved dietary nutrition, total CH_4 production could decrease without decreasing the yield of animal products. As forage quality declines in October, forage utilization efficiency, palatability, forage supply and feed intake also decreased, which was accompanied by lower CH_4 emissions from grazing sheep. For the relationship between dietary digestibility and CH_4 emission, protein supplementation in the diets increased the nutrient digestibility and decreased significantly CH_4 production in

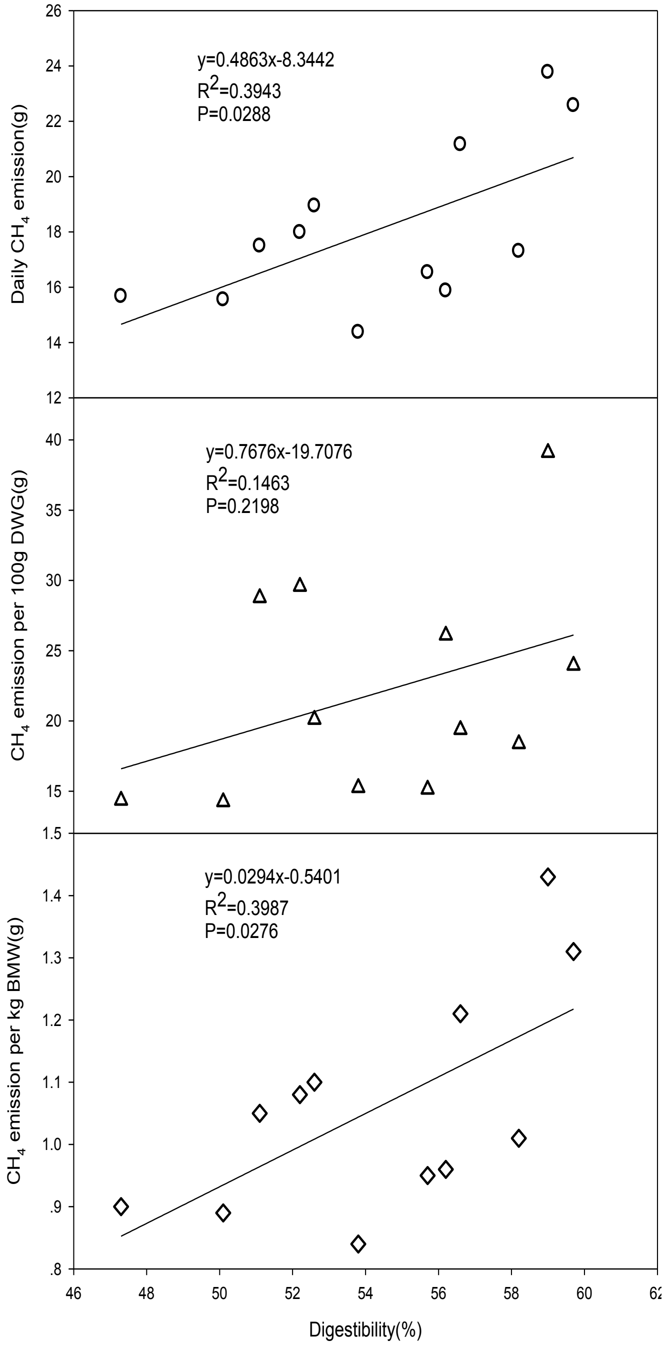

Fig. (2). Relationship of *in vitro* dry matter digestibility and CH_4 emission. MBW = metabolic body weight; DWG = daily weight gains.

rumen [21]. Wang *et al.* [10] studied that the digestibilities of DM and OM had a significantly positive correlation with CH_4 production per kg MB, which supports our results. Other factors are also likely to have an effect because of assessing CH_4 emission from enteric fermentation in any particular country requires a detailed description of the livestock population (species, age, and productivity categories), combined with information on the daily feed intake and the feed's conversion CH_4 rate [22]. In addition, some experiments for CH_4 production were measured in respiration chambers [23, 24] or tunnel system [25], but the possible effects of confinement in respiration chambers on the behavior and metabolism of wild ruminants are not known [26].

Schönbach *et al.* [12] estimated area-based CH_4 emissions from enteric fermentation that were significantly affected by grazing treatment. Owing to an increase in stocking rate, area-based enteric CH_4 emissions increased with grazing intensity, a result that is also shown in our data. Under light grazing the grazing system is a sink, but under heavier grazing the system may become a source of CH_4. We measured just one grazing season in 2012, and further experiments will be focused on the relationship between factors affecting CH_4 emissions from ruminants and the appropriate grazing intensity to meet ecological and economic management objectives. Continuous data for multiple grazing seasons should be used to analyze the effects of grazing intensity and season on CH_4 emissions from sheep and the effects of grazing intensity and season on the grazing system.

CH_4 Flux from Sheepfolds

Most previous studies have focused on the CH_4 flux from sheepfolds. Chen *et al.* [27] studied the mean CH_4 flux for winter and summer sheepfolds in the Baiyinxile region of Inner Mongolia. Our results show that CH_4 emission from sheepfolds was a CH_4 source during grazing season. However, our result was lower than the mean flux in summer sheepfolds in Chen *et al.* [27], because our measurement did not include the flux of new feces (which were cleaned out in our study) was the direct reason. Many studies had reported pronounced and short pulses of CH_4 emission immediately following application of fresh animal excreta [28, 29] and livestock can be an important source of CH_4 through direct enteric emissions and decomposition of excreta deposited on grassland [30, 31]. Chen *et al.* [27] also reported that the annual budget from sheepfolds in Inner Mongolia is mainly driven by emissions during the growing season, with most of these emissions occurring in the summer sheepfold. However, the small area of sheepfolds means that its effect on ecosystems is limited.

In conclusion, grazing intensity had an important influence on CH_4 emission from sheep respiration during the summer growing season. Higher grazing intensity increases total animal product yield, but also produces more CH_4 emissions. Gross livestock emissions have a large effect on the net CH_4 emissions of the grazing system. A moderate grazing intensity is an efficient method to maintain a certain number of livestock while also maintaining the CH_4 emission balance of the grazing ecosystem in Inner Mongolian desert grasslands. CH_4 fluxes from sheepfolds in different months of the grazing season are similar and have a negligible effect on net CH_4 balance at the regional scale. In addition, it should be noted that CH_4 emission monitoring equipment and temporal or spatial differences are the main limiting factors for this study.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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