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Nitrogen utilisation, energy utilisation and methane emissions of sheep grazing in two types of pasture

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ABSTRACT

Livestock grazing plays a significant role in maintaining grasslands and promoting animal production globally. To understand the livestock performance in sown pasture (**SP**) vs native pasture (**NP**) is important to ensure more effective grassland-livestock interactions with minimal environmental impact. A 2 (treatment) * 2 (period) Latin Square design experiment was conducted with 10 growing Hu sheep ♂ × thin-tailed Han sheep ♀ rams grazed perennially **SP** vs **NP** in an inland arid region of China. The objectives were to evaluate the effects of grazing management on nutrient digestibility, nitrogen (**N**) and energy utilisation and methane (**CH_4**) emission. The N intake, N retained and energy intake (gross energy (**GE**), and digestible and metabolisable energy) of sheep grazing in SP were significantly increased compared with those grazing in NP. There were significant linear relationships between DM intake (**DMI**) (g/kg BW or g/kg $BW^{0.75}$) or CH_4 (g/kg BW or g/kg $BW^{0.75}$) emissions and forage nutrient and GE concentrations within each grassland type. The linear regression analysis indicated that forage CP or ether extract concentration was a good predictor for DMI (g/kg BW or g/kg $BW^{0.75}$) (R^2 = 0.756 or 0.752), and CH_4 emission could be predicted using forage nutrient and GE concentrations (R^2 = 0.381–0.503). These results suggest that DMI and CH_4 emissions per unit metabolic BW were accurately predicted by multiple-factor combinations of forage nutrients, including ether extract and CP paired with GE. The present output could provide useful information for the development of sustainable sheep grazing systems in the inland arid regions of the world.

Implications

The livestock performance in sown pasture vs native pasture play important role in the grassland-livestock interactions across the world. There is increasing interest in developing mitigation strategies to reduce the environmental footprint of livestock production systems. The present study found that energy and nitrogen utilisation of sheep in sown pasture were significantly compared with those grazing in NP, and a range of models were developed for the prediction of methane emission. Our findings provide useful information on the integration of native pasture with sown pasture to improve grazing sheep productivity and mitigate the environmental footprint in the global arid and semiarid regions.

Introduction

The emissions of greenhouse gases (**GHGs**) from animal production account for about 60% of the total emissions from global agriculture (FAO, 2020). Methane (**CH_4**) from grazing ruminants accounts for 29% of the total global emissions from livestock agriculture, of which 26.4% of CH_4 emissions take place in the arid and semiarid regions (Clark, 2017). The sown pasture (**SP**) and native pasture (**NP**) provide approximately 50% of feeds to grazing livestock and directly support the livelihood of 1.3 billion people worldwide (IPCC, 2019). The primary goal of pasture management is to increase grazing efficiency with reduced environmental footprints (e.g., low CH_4 emissions and nitrogen (**N**) excretions) (Clark, 2013). Both sown and native grazing are standard practices of sheep production in the arid region of the Hexi Corridor of China and regions with a similar environment. However, there is little information available on the impact of grazing SP and NP on nutrient digestibility, energy and N metabolism, and CH_4 emissions of sheep in the global arid and semiarid regions.

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The total GHG emissions associated with meat and milk production under various production systems have been estimated using a whole-system modelling approach (e.g., life cycle analysis) with all emission sources accounted for within the farm gate (e.g., Yan et al., 2019). The most significant source of GHG emissions in the ruminant production sector is enteric CH_4 production (IPCC, 2006), which is affected by a range of animal and dietary factors, including dietary composition, management practices, and levels of productivity (Eckard and Clark, 2018). Dietary neutral detergent fibre (**NDF**) and ether extract (**EE**) concentrations are key variables for predicting enteric CH_4 emissions in different types of ruminants. Dietary fibre concentration is negatively related to the whole tract digestibility, while positively related to enteric CH_4 production. Therefore, improving forage digestibility for grazing livestock is an effective strategy to alleviate CH_4 per unit of meat or milk production (Moraes et al., 2014). The previous literature has indicated that lipid proportion, form, and type in diets had the potential to alter rumen fermentation and thus CH_4 emissions. For instance, Grainger and Beauchemin (2011) reported that CH_4 emissions from grazing livestock could be reduced by 4–5% with every increase of 1% forage lipid content. The IPCC (2006) recommends a standard CH_4 conversion factor (CH_4 energy as 6.5 ± 1% of gross energy intake (**GEI**)) for the quantification of CH_4 emissions from GEI for cattle and sheep. This factor was derived from experimental data with studies undertaken mainly in developed countries (e.g., New Zealand) (Clark et al., 2011). Since CH_4 emissions are influenced by several diet and animal factors, the direct adoption of the CH_4 emission factor of IPCC (2006) to calculate CH_4 emissions from grazing sheep in the inland arid region of China and elsewhere might result in great variations. Therefore, there is an urgent need to conduct research experiments to obtain scientific evidence on the effects of grazing local grasslands (e.g., SP vs NP) on nutrient utilisation and CH_4 emission of sheep.

It is estimated that about 1.6 billion people (approximately 22% of the world population) are living around the salinised regions of the world, and their gross domestic product (**GDP**) accounts for 18% of the world's GDP. Saline meadow is one of the dominant types of SP and NP that produces the most forage in the saline ecoregion, which occupies 63% of the areas of global terrestrial lands, and supplies approximately 50% of feed to local ruminants (Yimamu et al., 2015). The Hexi Corridor of China has various typical saline meadows and is one of the earliest places in the world to grow cultivated forage (Hou et al., 2021). The most widely distributed pastures in the region are SP and NP. The SP has high-quality forage with less species in the sward, while NP has medium-quality forage and contains more species which offers sheep the opportunity to choose forage species (Hilario et al., 2017). Therefore, in this study, we hypothesised (Fig. 1) that sheep grazing NP and SP would have similar DMI, nutrient digestibility, energy and N utilisation and CH_4 emission. To test this hypothesis, we conducted a Latin Square design experiment with sheep grazed SP vs NP in the Hexi Corridor, with the objectives: (1) to evaluate the effects of sheep grazed SP vs NP on feed intake and digestibility, N and energy utilisation, and enteric CH_4 emissions; and (2) to develop prediction equation for DMI and CH_4 emissions for grazing sheep.

Material and method

Research area

The experiment was conducted at the Linze Grassland Agriculture Station of Lanzhou University (LGAS), Linze County, Gansu Province, China (at 39°42′N and 99°51′E–100°30′E, 1 390 m a.s.l.). The regional climate is classified as a temperate continental climate. The mean annual rainfall is 118.4 mm, and the mean annual average temperature is 7.7 °C. The NP in this region is comprised of a saline meadow grass that is widely developed in arid and semi-arid regions and is called the native oasis. Most of them have been cultivated on cropland for several thousand years, and the others are often used for grazing ruminant livestock. The dominant agricultural systems are intensively specialised crop and livestock production systems (Hou et al., 2008). Sheep and beef cattle are the primary livestock, while corn, spring wheat, and alfalfa are the dominant cultivated crops in the region.

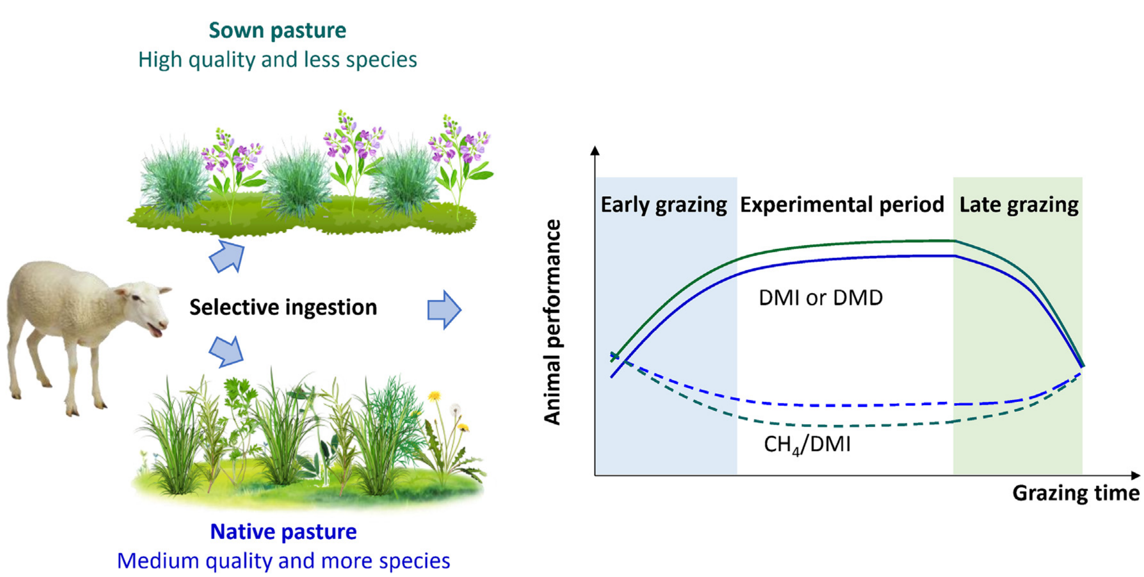

Fig. 1. Conceptual sketch of the relationship between sheep performance and sown pasture vs native pasture under rotational grazing. The figure showed the relationship between grazing time and DMI, DMD, and CH_4/DMI. (1) Early grazing: DMI, DMD and CH_4/DMI of livestock fluctuate greatly under the influence of grazing; (2) Experimental period (mid-grazing): if forage is sufficient, feed intake, digestibility and CH_4 emissions of grazing livestock are kept stable; (3) Late grazing: The amount of forage in the pasture increases with the grazing time, and the quality of the pasture also decreases, which has a greater impact on livestock (DMI, DMD or CH_4/DMI). Therefore, we choose the rotational grazing method to keep the forage enough, and carry out this study in the "Experiment period" period in the figure. Abbreviations: DMI = DM intake; DMD = DM digestibility.

Experimental design and animals

The experiment was organised in a 2 (pasture type) × 2 (period) Latin Square design to evaluate the effects of sheep grazed two types of pastures, including SP (a three-year mixed pasture of alfalfa (*Medicago sativa*) and smooth bromegrass (*Bromus inermis*)), and NP (Table 1). Ten 6-month-old Hu sheep ♂ × thin-tail Han sheep ♀ rams [BW, 22 ± 1 kg] with similar body conditions were selected. Before the commencement of the experiment, all animals were dewormed and disinfected. The 10 sheep were randomly assigned to two types of pastures (five sheep/treatment) in the first period. Each period lasted for 24 days, including 14 days of dietary adaptation and 5 days of methane measurement at pasture, and then 5 days of digestibility measurement in metabolic crates. The grazing was managed as a rotational grazing system with three grazing paddocks within each pasture type. Each paddock had a size of 50 m × 50 m and was managed for grazing for 8 days. Sheep grazing was carried out from 7 am to 7 pm each day and housed indoor during night with no supplement offered. Clean water was freely available at all time. Sheep were moved to the metabolic chamber to determine the feed digestibility and were weighed on the last day of each experimental period.

Animal measurements

After the dietary adaptation in each period, the sulphur hexafluoride (**SF_6**) tracer gas technique (Deighton et al., 2013) was used to measure enteric CH_4 emissions from sheep for 5 days. Nine days prior to the first gas measurement, each sheep was administered with a single permeation tube 9 days before the first gas measurement and tube contained 2.5 g SF_6 with an SF_6 release rate of 3.1 35 ± 0.364 mg/d. The breath sample of each sheep was collected with a pre-evacuated and v-shaped canister (2.5 L) which was placed in the neck of sheep. The ambient CH_4 and SF_6 concentrations were also measured using the same technique.

Immediately after the completion of the SF_6 measurement, all sheep were transferred to individual metabolic crates for a five-day digestibility trial. Sheep were allowed free access to water and offered fresh forage ad libitum, which was mowed daily from the paddock in the morning and representative forage samples were collected to analyse the quality and DM of forage. The residual forage, faeces, and urine were collected twice every day. The DM content of the residual forage was measured by drying at 105 °C for 24 h. After the last day of collection, the faeces and urine samples of each sheep in the 5d were thoroughly mixed separately and representative samples were taken for analysis. Urine was collected into a container with added acid (10% sulfuric acid (**H_2SO_4**), v/v) to ensure the pH was below 3. Two faeces samples were taken daily, one for the oven DM measurement (105 °C) combined with 10% H_2SO_4 (v/v) to prevent N loss, and the another was frozen at −20 °C for later chemical analysis.

Table 1
Composition of sown pasture and native pasture grazed by sheep.

Items	Pasture composition (%)	
	SP	NP
Medicago sativa	68.8	
Bromus inermis	31.2	
Agrostis clavate		35.85
Phragmites communis		34.49
Triglochin palustre		6.56
Juncus articulates		4.49
Typha orientalis		3.37
Ranunculus japonicus		1.24
Plantago minuta		0.61
Others		13.59

Abbreviations: SP = Sown pasture; NP = Native pasture.

Grazed forage intake measurement

The method of Undi et al. (2008) was used to estimate grazed DM intake. Ten grazing cages were placed in each grazing area in a "W" shape at the beginning of each grazing period. Forage inside and outside the cages were clipped from 0.5 m × 0.5 m quadrats at 0, 14 and 24 days, and representative samples were taken for each experiment treatment. The DM content of the forage was measured by drying at 105 °C for 24 h. Forage for nutrient analysis were dried to constant weight at 60 °C. The forage consumed by grazing sheep was calculated using the following equation (Undi et al., 2008):

$$\mathrm{DMI}\ (\mathrm{kg\ d^{-1}}) = \frac{[\mathrm{DM\ inside\ cage}\ (\mathrm{kg\ ha^{-1}}) - \mathrm{DM\ outside\ cage}\ (\mathrm{kg\ ha^{-1}})] \times \mathrm{area\ (ha)}}{\mathrm{number\ of\ grazing\ days} \times \mathrm{sheep\ numbers}}$$

Chemical analysis

The forage and faeces were dried at 60 °C until constant weight, and passed through a 0.5 mm sieve. Later, the samples were used for chemical analysis following the AOAC (2002) method. The NDF and acid detergent fibre (**ADF**) were analysed by a semi-automatic fiber analyser (A2000i, ANKOM Instrument Co., Ltd, USA). Total N (including urinary N) was analysed by the Kjeldahl nitrogen analyser according to the Kjeldahl method (Model K9840; Hanon Instruments, China); **EE** was leached by petroleum ether through an automatic ether extract analyser (XT-15, ANKOM, Macedon, NY, USA). Organic matter (**OM**) was measured by electric furnace carbonisation and muffle furnace ashing (500 °C) for six hours. The total energy (including urine energy (**UE**)) was analysed in an A bomb calorimeter (6400, PARR Instrument Co, USA).

After collection, gas samples were immediately analysed for CH_4 and SF_6 concentrations using a SHIMADZU Gas Chromatograph analyser (GC-2014, Shimadzu Enterprise Management Co., Ltd., Japan) (Johnson et al., 2007). The analyser was equipped with an FID-2014 hydrogen flame ionisation detector for the CH_4 measurement (detection limit: $\leq 5 \times 10^{-10}$ g/s; calibration range: 0.9999–1) and a TCD-2014 thermal conductivity detector for the SF_6 measurement (sensitivity: ≥800 mV·ml/mg, calibration range: 0.9999–1). The analyser used the automatic injection of the six-port value (flow rate = 30 ml/min), using N_2 gas (purity = 99.999%) as the carrier gas. Daily CH_4 emissions were calculated from the SF_6 release rate in the permeation tube and the CH_4/SF_6 concentration ratio in the breath samples after correction for the ambient gas concentrations. Chromatographic analyses were performed after calibration with three gas standards; low (9.98 ppt SF6 and 9.98 ppm CH_4), medium (151.9 ppt SF6 and 100.9 ppm CH_4) and high (308 ppt SF6 and 308 ppm CH_4) supplied by Dalian Special Gases CO., Ltd (Dalian, Liaoning, China).

Statistical analyses

All data were analysed following the 2 × 2 Latin square design and using independent sample *T*-tests in SPSS statistical software (20.0, Inst., Chicago, Illinois, USA). The relationships between N intake and N outputs and energy intake and energy outputs were analysed using a general linear model in SPSS, with grassland type as a fixed factor. The REG program of SAS software was used to establish the correlation between forage chemical composition and nutrient digestibility and energy parameters, with the normality of residuals used for the test. Prediction equations for DMI and

CH_4 emission based on forage nutritional quality (e.g., NDF, EE, OM, and CP) were developed using the step-by-step multiple linear regression analysis technique (backwards elimination method). The linear regression model used was as follows:

$$Y = a + a_1X_1 + b_2x_2 + b_3x_3 + \ldots + b_nx_n.$$

Results

Nutritional composition of the two types of pastures

The DM of NP was 2.41% higher in than that of SP ($P < 0.05$) (Table 2). The nutrient composition (CP, NDF, and ADF) of SP were higher than NP ($P < 0.01$), while EE content in NP was higher than SP ($P < 0.01$).

DM intake, methane emissions

There was no significant ($P = 0.987$) difference in the DMI between SP and NP (Table 3). Compared with SP, sheep grazed NP reduced the daily CH_4 emissions by 12.66% ($P < 0.001$). Based on metabolic weight ($kg^{0.75}$), CH_4 emissions in NP were lower by 1.80% than in SP ($P < 0.001$). There was no difference in CH_4 emissions as a proportion of DMI ($P = 0.726$) or GE ($P = 0.982$) between the two sheep groups. The CH_4 energy output as a proportion of DE intake ($P < 0.01$) and ME intake ($P < 0.001$) in SP were lower by 9.09 and 14.29% than in NP, respectively.

Nutrient digestibility

The DMD of SP was not significantly different from that of NP (Table 4). In addition, there was no significant difference in digestibility of NDF, ADF, or CP between SP and NP, although DMD in SP tended to be higher than that in NP ($P = 0.054$). The SP had higher digestibility of EE and OM than NP ($P < 0.05$).

Nitrogen utilisation

Compared with NP, N intake of sheep grazing SP was increased by 14.29% ($P < 0.01$) (Table 5). The faecal N ($P < 0.01$), urinary N ($P < 0.05$), and N retained ($P < 0.001$) of sheep grazed SP were higher by 12.07%, 10.00%, and 28.00% than that of grazed NP. Urinary N/N intake of NP was higher by 5.71% than in SP ($P < 0.001$), while N retained/N intake in NP was lower by 10.53% than in SP ($P < 0.01$).

Energy utilisation

Based on the metabolic weight ($kg^{0.75}$), GE intake, UE output, digestible energy (**DE**) intake, and metabolisable energy (**ME**) intakes in SP were higher by11.49, 40, 31.36, and 27.00% than those in NP ($P < 0.001$), while faecal energy (**FE**) output was lower by 24.44% in SP than in NP ($P < 0.001$), respectively (Table 6). The ratio of DE to GE and that of ME to GE in SP was higher by 17.65 and 14.04% than that of NP ($P < 0.001$), respectively. The CH_4 energy

Table 2
Nutrient composition of sown pasture and native pasture grazed by sheep (n = 20).

Index	SP	NP	SEM	*P*
DM (%)	39.5	40.45	0.310	0.012
Organic matter (%DM)	86.3	89.94	0.404	<0.001
CP (%DM)	22.66	10.58	0.570	<0.001
Ether extract (%DM)	1.82	4.40	0.119	<0.001
NDF (%DM)	36.12	59.84	1.661	<0.001
ADF (%DM)	29.08	40.05	1.054	<0.001

Abbreviations: SP = Sown pasture; NP = Native pasture.

Table 3
DM intake and CH_4 emissions of sheep grazed sown pasture and native pasture (n = 20).

Index	SP	NP	SEM	*P*
DM intake, kg/day	1.10	0.99	0.019	0.987
DM intake/$BW^{0.75}$, kg/kg	0.11	0.10	0.004	0.843
CH_4/$BW^{0.75}$, g/kg	3.89	3.00	0.128	<0.001
CH_4/DMI, g/kg	26.02	25.47	1.536	0.726
CH_4 energy/GE, MJ/MJ	0.08	0.08	0.0005	0.982
CH_4 energy/DE, MJ/MJ	0.10	0.12	0.0012	0.001
CH_4 energy/ME, MJ/MJ	0.12	0.14	0.017	<0.001

Abbreviations: SP = Sown pasture; NP = Native pasture; DMI = DM intake; GE = Gross energy; DE = Digestible energy; ME = Metabolisable energy.

Table 4
Nutrient digestibility of sown pasture and native pasture when fed to sheep (n = 20).

Index	SP	NP	SEM	*P*
DM digestibility	65.13	63.23	1.905	0.054
NDF	50.23	49.31	0.364	0.390
ADF	39.49	42.92	2.181	0.475
CP	61.24	64.36	2.593	0.283
Ether extract	44.20	40.29	0.660	0.037
Organic matter	67.32	53.75	6.072	0.038

Abbreviations: SP = Sown pasture; NP = Native pasture.

Table 5
Nitrogen intake and utilisation by sheep fed sown pasture or native pasture (n = 20).

N index	SP	NP	SEM	*P*
N intake, g/kg $BW^{0.75}$	1.52	1.33	0.056	0.003
Faecal N, g/ kg $BW^{0.75}$	0.65	0.58	0.025	0.006
Urinary N, g/ kg $BW^{0.75}$	0.55	0.50	0.021	0.034
N Retained, g/ kg $BW^{0.75}$	0.32	0.25	0.014	<0.001
Faecal N/ N intake, kg/kg	0.42	0.44	0.002	0.163
Urinary N/N intake, kg/kg	0.35	0.37	0.003	<0.001
N Retained/ N intake, kg/kg	0.21	0.19	0.006	0.002

Abbreviations: SP = Sown pasture; NP = Native pasture.

Table 6
Energy intake and utilisation by sheep fed sown pasture and native pasture (n = 20).

Energy index	SP	NP	SEM	*P*
GE, MJ/kg $BW^{0.75}$	1.94	1.74	0.140	<0.001
FE, MJ/kg $BW^{0.75}$	0.45	0.56	0.030	<0.001
UE, MJ/kg $BW^{0.75}$	0.07	0.05	0.012	<0.001
DE, MJ/kg $BW^{0.75}$	1.55	1.18	0.020	<0.001
ME, MJ/kg $BW^{0.75}$	1.27	1.00	0.140	<0.001
DE/GE, MJ/MJ	0.80	0.68	0.010	<0.001
ME/GE, MJ/MJ	0.65	0.57	0.003	<0.001
ME/DE, MJ/MJ	0.82	0.85	0.007	<0.001
CH_4 energy, MJ	1.56	1.38	0.120	0.001

Abbreviations: SP = Sown pasture; NP = Native pasture; GE = Gross energy; FE = Faecal energy; UE = Urinary energy; DE = Digestible energy; ME = Metabolisable energy.

emission of sheep grazed NP was 11.54% lower than those grazed SP ($P < 0.01$).

Relationships between nitrogen intake and outputs and between energy intake and outputs

Data indicated linear relationships between N intake and urinary N, faecal N, and N retained (Fig. 2). The N intake of sheep grazing on perennial pasture was positively correlated with urinary N ($P < 0.01$), faecal N ($P < 0.001$) and N retained ($P < 0.05$). There was no significant positive correlation between GE and DE, ME, FE, UE, or CH_4 energy of the two grassland types ($P > 0.05$) (Fig. 3).

Relationships between forage chemical composition and nutrient digestibility, energy parameters, and methane emissions

The relationships between forage ADF ($P = 0.180$; $P = 0.193$) and OM ($P = 0.087$; $P = 0.134$) content and DMI per unit BW or meta-

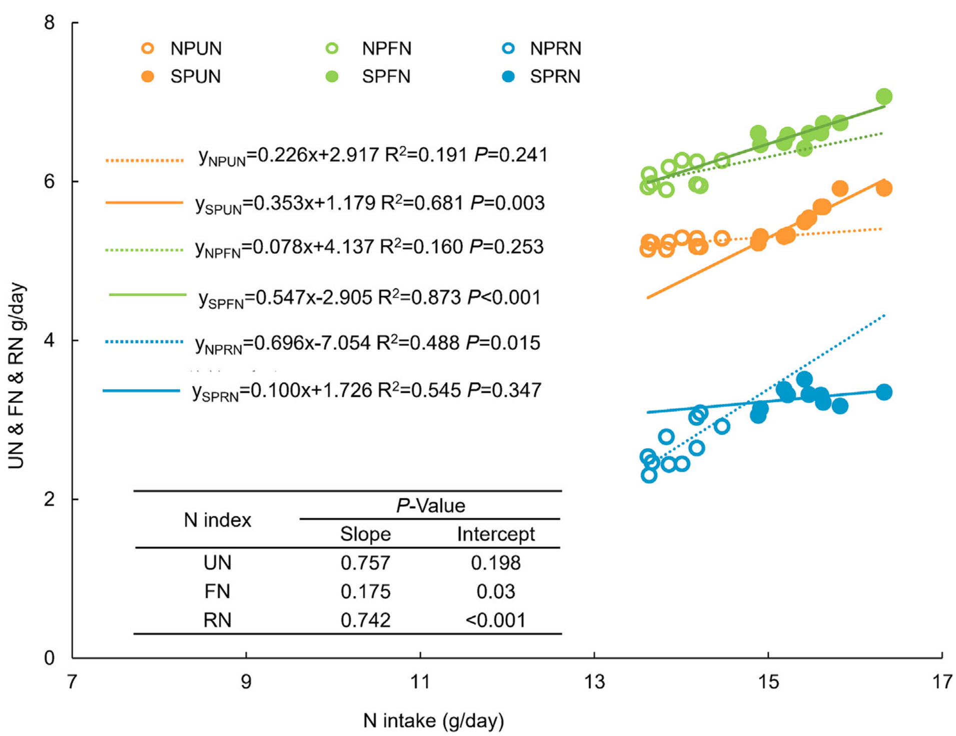

Fig. 2. The relationship between N intake and UN (orange) and FN (green) outputs or RN (blue) by sheep eating SP (closed circles) and NP (open circles). Circles and lines of the same colour represented the same indicators for both pastures (closed circles for SP; open circles for NP). Summary of linear regressions between N intake and N index in the table: differences in slope and intercept of linear regression equations corresponding to the same indicators of two pastures. Abbreviations: N = Nitrogen; UN = Urinary N; FN = faecal N; RN = N retained; SP = sown pasture; NP = native pasture.

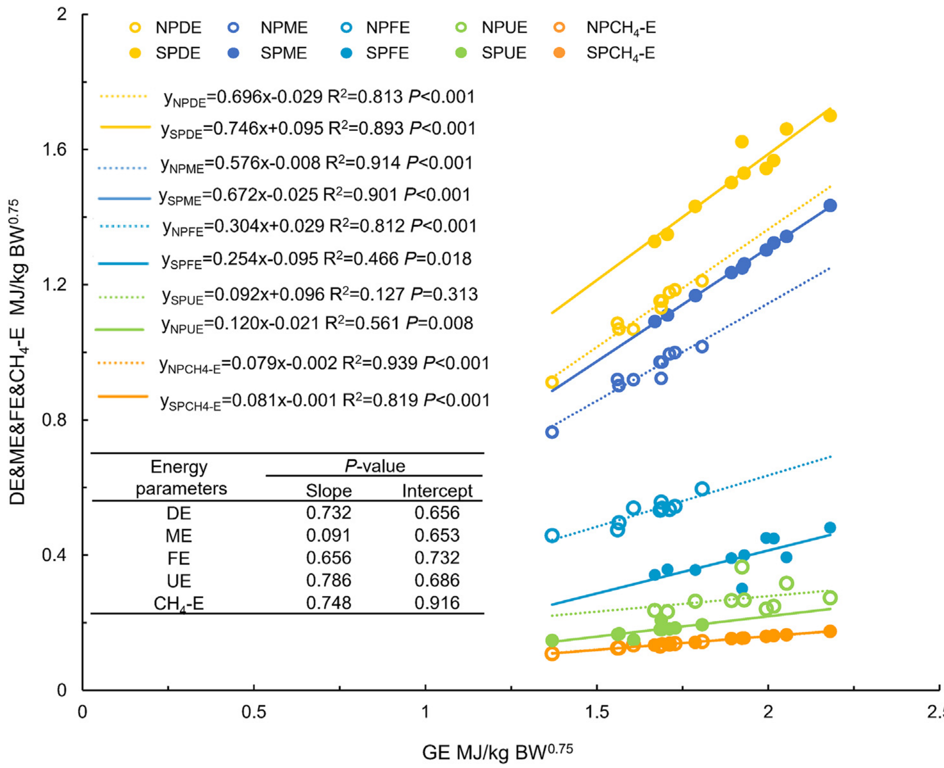

Fig. 3. The relationship between GE and DE (yellow), ME (dark blue), FE (blue), UE (green), or CH_4-E (orange) by sheep sown pasture (SP; closed circles) and native pasture (NP; open circles). Circles and lines of the same colour represented the same indicators for both pastures (closed circles for SP; open circles for NP). Summary of linear regressions between GE and energy parameters in the table: differences in slope and intercept of linear regression equations corresponding to the same indicators of two pastures. Abbreviations: SP = Sown pasture; NP = Native pasture; GE = Gross energy; FE = Faecal energy; UE = Urinary energy; DE = Digestible energy; ME = Metabolisable energy; CH_4-E = CH_4 energy.

bolic BW were not significant. The CH_4 emissions were significantly related to NDF content of forage ($P < 0.05$) (Fig. 4). The NDF ($P < 0.05$), ADF ($P < 0.05$), and OM ($P < 0.05$) contents of forage exhibited a significant positive correlation with the digestibility of NDF content of the forages. Additionally, the digestibility of CP had also significant and positive linear relationship with the content of CP ($P < 0.05$), NDF ($P < 0.05$), and OM ($P < 0.05$) in the forage.

Equations for predicting DM intake and methane output

Linear prediction equations for DMI and CH_4 emissions based on the combined data obtained with sheep fed both SP and NP forage are presented in Table 7. All equations were significant, and each predictor had a significant effect on the relationship ($P < 0.05$). Forage CP concentration was a good predictor for DMI per kg metabolic BW (Eq. (1a), $R^2 = 0.718$). Adding forage NDF or EE concentration to Eq. (1a) significantly increased the prediction accuracy with R^2 increased to 0.732 and 0.756 (Eqs. (1b) or (1c)). Similar results were also obtained during the prediction of DMI per kg BW (Eqs. (2a)–(2c)). The linear relationship between CH_4 per kg BW and forage EE composition was relatively poor (Eq. (3a), $R^2 = 0.381$), and adding forage CP, ADF, and NDF concentrations to Eq. (3a) could only increase the R^2 to 0.438 (Eq. (3d)). A similar poor relationship for prediction of CH_4 per kg metabolic BW was also obtained, although the R^2 was slightly increased (e.g., Eq. (4f), $R^2 = 0.503$).

Discussion

Feed intake and nutrient digestibility

In the present study, sheep fed SP had a higher DMD than those offered with NP, and the difference was close to be significant ($P = 0.054$). A possible explanation is the higher fibre and less protein content of NP, which could produce negative effects on palatability, digestibility, and rumen filling (Askar et al., 2016). A previous study reported a positive correlation between CP contents and forage digestibility when the feed CP content was less than 21.9%, but a negative correlation between feed CP content and nutrient digestibility when forage CP level reached the threshold value (Yang et al., 2018). Since the optimal value of CP content is considered 20.6% of the DM content of forages (Stergiadis et al., 2015a), a quadratic fitting between the CP content and OM digestibility of forages was more suitable because of lower forage CP content (less than 20.6%) of the DM content in NP (Table 1). Forage of SP had a simple species composition, sufficient mass and high quality, while NP had higher forage diversity and lower forage quality (Tables 1 and 2). Sheep grazing of NP prefers to ingest high-quality forage or plant tissues, so as to improve the quality of forage actually eaten by sheep (Yang et al., 2020; Cuchillo-Hilario et al., 2017). The quality of forage ingested by livestock is closely related to its digestibility (Wang et al., 2017; Cui et al., 2019), therefore, digestibility of DMI, NDF, and ADF in sheep did not differ significantly between the two pastures, which is consistent with our hypothesis, possibly due to the fact that the actual forage quality ingested by the sheep was similar. Result from this study will help reducing the production pressure brought by livestock production on SP and enabling more effective use of NP, thus contributing to the more rational use of forage in livestock production.

The chemical composition of forages is considered as a feasible approach to predict DMI per under-unit BW (g/kg) and metabolic BW (g/kg) (Andueza et al., 2011). The nutrient content and GE of the forage were the most suitable parameters to predict DMI per under-unit BW (g/kg) and metabolic BW in NP, because forage CP affected rumen microbial protein synthesis and rumen fill (Abidi et al., 2021). When combining more independent variables

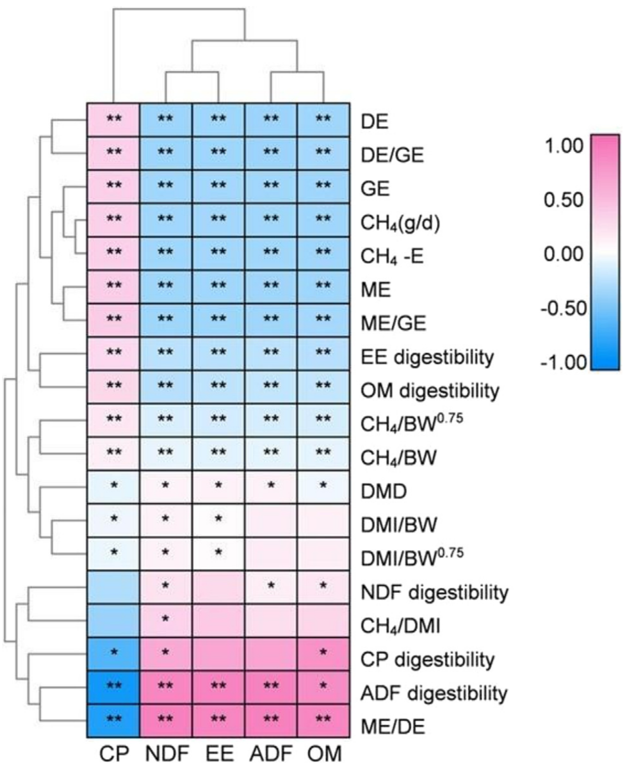

Fig. 4. Relationship among chemical composition, nutrient digestibility, energy parameters and CH_4 emission. At the bottom of the figure, CP, NDF, EE, ADF and OM are the nutrient content of forage. The spaces in the figure indicate that the linear relationship was not significant ($P > 0.05$). "**" means $P < 0.01$ and "*" means $P < 0.05$ in the figure. Abbreviations: DMD = DM digestibility; EE = Ether extract; OM = Organic matter; GE = Gross energy; DE = Digestible energy; ME = Metabolisable energy; CH_4-E = CH_4 energy.

such as forage CP and EE, or NDF to predict DMI per under-unit BW (g/kg) or metabolic BW (g/kg), the linear equation had higher fitness (R^2) than a single variable, which indicated that more nutrient factors contributed to DMI, which is consistent with findings from a previous study (Yang et al., 2018). However, EE was integrated into independent factors to predict the DMI per under-unit metabolic BW (g/kg) of yak grazing alpine meadows (Yang et al., 2018), which was different from our study because of differences in pasture species, environmental factors, animal species, gender, weight, and age of livestock, and so on (Andueza et al., 2011).

Nitrogen and energy utilisation

The excretion of faeces and urine is the primary process of N loss in ruminants (Waldrip et al. 2013). Sheep grazing on the two types of pastures had different N intake and excretion levels (Table 5), which was contrary to our hypothesis. The high N intake in SP was mainly due to the higher N concentration of the forage which was likely offered by the selective intake of higher N concentration components from the pasture, such as legumes and green leaves etc. (Boland et al., 2011). The faecal N/N intake of sheep grazing in NP was significantly higher than that of the SP, possibly because of the low N content and a low DMD of forage in NP (Zhao et al., 2015), and a decrease of faecal N excretion in NP indicated that more N were absorbed by the small intestine (Mingoti et al., 2016). Because most of the N in urine was urea, which was hydrolysed and volatilised into NH_3 faster in urine than in faeces (Todd et al., 2015), less of the N in urine returned to the pasture (Koenig and Beauchemin, 2018), and despite higher N in urine, sheep grazing in NP did not return N to the pasture more efficiently than those in SP. Previous study reported that the N intake of Tibetan sheep grazing in alpine meadows was less than 7% of forage DMI, there was a negative N balance (Abdelraheem et al., 2019). However, the threshold value in the present study was 10.14% for sheep grazing in SP, while the opposite was observed in NP. The possible reasons for these variations are possibly the significant differences between the sheep breeds, pasture types and environmental factors, and while consuming enough feed energy, sheep in our present study used N more effectively than the Tibetan sheep (Abdelraheem et al., 2019; Zhou et al., 2015).

The ratio of DE to GE and the ratio of ME to GE were positively correlated with CP content (Fig. 4), which is inconsistent with the relationship observed in dry cows in North Ireland (Stergiadis et al., 2015b), perhaps because of the proportional increase in energy utilisation as N intake increases (Zhao et al., 2017). Although the NDF and ADF content in SP was lower than that of NP, the DE and ME of SP were noticeably higher than NP (Table 6), because it has been reported that the NDF and ADF of native forage not only reduced the availability of energy required by microorganisms but also decreased the available nutrient content of the forage (Stergiadis et al., 2015a). The contents of NDF and ADF in forage were positively correlated with the ratio of ME to DE (Fig. 4), because the fibre (NDF and ADF) in forage had a greater impact on FE output than on UE (Stergiadis et al., 2015a). Additionally, the fibre content of forage was higher in NP because the stem content accounts for a larger proportion of the forage (Yang et al.,

Table 7
Linear prediction of DM intake and CH_4 emission of sheep using chemical composition parameters (n = 20).

Parameters		Equation	R^2	P	Code
$DMI/BW^{0.75}$, g/kg	=	$0.059_{(0.006)} + 0.003_{(0.0001)}$ CP	0.718	<0.001	1a
	=	$-0.003_{(0.001)} - 0.004_{(0.001)}$ CP $- 0.001_{(0.0004)}$ NDF	0.732	<0.001	1b
	=	$-0.017_{(0.003)} - 0.005_{(0.001)}$ CP $- 0.011_{(0.006)}$ EE	0.756	<0.001	1c
DMI/BW, g/kg	=	$-0.025_{(0.003)} + 0.001_{(0.0001)}$ CP	0.716	<0.001	2a
	=	$-0.002_{(0.0001)} + 0.002_{(0.001)}$CP $+ 0.0003_{(0.0002)}$NDF	0.728	<0.001	2b
	=	$-0.012_{(0.002)} + 0.003_{(0.001)}$ CP $+ 0.006_{(0.003)}$ EE	0.752	<0.001	2c
CH_4/BW, g/kg	=	$1.422_{(0.072)} - 0.076_{(0.021)}$ EE	0.381	0.002	3a
	=	$4.189_{(2.571)} - 0.015_{(0.005)}$ EE $- 0.023_{(0.002)}$ OM $- 0.022_{(0.013)}$ ADF	0.415	0.009	3b
	=	$4.399_{(2.581)} - 0.101_{(0.010)}$ EE $- 0.029_{(0.001)}$ OM $- 0.020_{(0.010)}$ ADF $+ 0.10_{(0.001)}$ NDF	0.414	0.016	3c
	=	$7.018_{(3.244)} - 0.164_{(0.019)}$ EE $- 0.049_{(0.033)}$ OM $- 0.020_{(0.013)}$ ADF $+ 0.004_{(0.001)}$ NDF $- 0.020_{(0.016)}$ CP	0.438	0.019	3d
$CH_4/BW^{0.75}$, g/kg	=	$8.19_{(5.82)} - 0.15_{(0.104)}$ EE $- 0.050_{(0.006)}$ OM	0.466	0.002	4a
	=	$1.048_{(0.702)} - 0.086_{(0.053)}$ EE $+ 0.154_{(0.077)}$ GE	0.475	0.002	4b
	=	$3.81_{(0.486)} - 0.420_{(0.219)}$ EE $- 0.023_{(0.003)}$ NDF	0.481	0.001	4c
	=	$4.26_{(0.166)} - 0.21_{(0.049)}$ EE	0.482	<0.001	4d
	=	$-9.443_{(1.028)} - 0.248_{(0.056)}$ GE	0.495	<0.001	4e
	=	$1.026_{(0.602)} - 0.315_{(0.222)}$ EE $+ 0.240_{(0.183)}$ GE $- 0.043_{(0.031)}$ CP	0.503	0.005	4f

Abbreviations: DMI = DM intake; EE = Ether extract; OM = Organic matter.

2018), which directly caused a decrease in carbohydrate utilisation and indirectly caused an increase in the FE despite the livestock's selective intake (Yang et al., 2020).

Enteric methane emissions and environmental footprints and mitigation of greenhouse gases

Enteric CH_4 emissions of livestock year-round grazing have seldom been studied in different types of pastures, while CH_4 emissions of some house-feeding livestock or grazing livestock with supplementation have been measured in pastures of Europe or northern America (Zhao et al., 2016). The ratio of CH_4 energy to GEI of house-feeding sheep in Europe was 6.2% (Zhao et al., 2016), that of grazing Holstein Friesian cows with supplementation was 7.5% (CH_4 energy/GEI) in the Andes in South America (Muoz et al., 2018), and the ratio of CH_4 energy to GE of Black Angus heifers was 6.9% in a mixture of alfalfa and grass in southern Saskatchewan (Chaves et al., 2006). The ratio of CH_4 energy to GEI of sheep was approximately 0.08 when grazing two types of pastures, which was higher than the 6.5 ± 1% reported by the IPCC (2006). This could be attributed to the difference in environmental factors, animal breeds, and dietary ingredients (Yan et al., 2010; Rushing et al., 2019), especially the higher ash content of forage in our research region (Liu et al., 2020). Thus, the emission factors of grazing experiments in typical ecoregions are critically important for the IPCC to accurately estimate the global emissions of pastures.

There was no significant difference in CH_4 emissions per DMI between the two types of pasture, because the DMI of sheep was similar between the two pastures, which accounted for most of the CH_4 emission variation. The feed ingredients only accounted for 20% of the CH_4 emission variation (Ellis et al., 2007; Hammond et al., 2009; Hammond et al., 2011), although ruminants grazing legumes such as alfalfa or red clover emitted less CH_4 than when grazing grass (Dini et al., 2012). The chemical composition of the forage was used to predict ruminant CH_4 emissions, which indicated that the R^2 of the regression equation was higher when the EE of forage was excluded from independent variables, although EE was negatively correlated with CH_4 emissions in some studies (Moraes et al., 2014). However, our results indicated that EE is an important variable to predict CH_4 per under-unit BW or metabolic BW (Table 7), possibly because the activity of methanogens was inhibited by EE (Patra and Yu, 2013). Whereas GE alone was used to predict CH_4 per unit metabolic BW, the R^2 (0.495) of the regressive equation was smaller than the chemical composition of forage (EE and CP) and its combination with GE (R^2 = 0.503) (Table 7). The perceived results might be because of the difference in carbohydrate, fat and protein in forage indirectly that led to inconsistent utilisation time of substrates by methanogens in the rumen (Moate et al., 2017; Alvarez-Hess et al., 2019).

Conclusion

In summary, the feed intake and DM digestibility of sheep were not significantly different within the two pasture types but N utilisation of SP was more efficient than NP. The EE and OM digestibility of grazing sheep in SP were higher than that in NP. DMI and CH_4 emissions per unit metabolic BW were accurately predicted by multiple-factor combinations of forage nutrients, including EE and CP paired with GE. The CH_4 conversion factor (CH_4 energy/GEI) of Hu sheep × thin-tailed Han sheep rams was 0.08 on the two types of pastures. The GE, DE, ME, and CH_4 of grazing sheep were higher in SP than in NP. Overall, these results provided significant information about sheep production in salinised meadows and can be used to establish appropriate grazing and management strategies. This research was expected to improve the grazing practices of ruminants by integrating SP and NP in order to harvest more metabolic energy and mitigate the environmental impact in arid region in terms of increasing the digestibility and utilisation of energy and N and reducing enteric CH_4 emissions and N excrement.

Ethics approval

The study and all animal procedures therein were approved by the ethics committee of Lanzhou University (Nos. 2010-1 and 2010-2).

Data and model availability statement

None of the data were deposited in an official repository. Data are confidential but available to reviewers upon request.

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Declaration of interest

The authors declare no competing financial interest.

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