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Ruminant Nutrition

Exogenous fibrolytic enzymes promoted energy and nitrogen utilization and decreased CH4 emission per unit dry matter intake of tan sheep grazed a typical steppe by enhancing nutrient digestibility on China loess plateau

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Abstract

Exogenous fibrolytic enzyme (**EFE**) products in ruminant nutrition may be an important alternative to meet the increased demands for animal products in the future with reduced environmental impacts. This study aimed to evaluate the dose–response of EFE supplementation on the nutrient digestibility, nitrogen and energy utilization, and methane (**CH_4**) emissions of Tan sheep grazed in summer and winter. A total of 20 Tan wether sheep with an initial body weight of 23.17 ± 0.24 kg were used in a randomized complete block design and categorized into two groups. Animals fed orally with 1 g of EFE (10,000 U/g) mixed with 30 mL of water using a drencher constituted the EFE group. For experimental accuracy, the control (**CON**) group was orally administered with 30 mL of normal saline daily before grazing. The following results were obtained: EFE in the diet increased dry matter intake (**DMI**) ($P < 0.05$), average daily gain (ADG) ($P < 0.05$), and digestibility ($P < 0.05$) compared with CON in summer and winter. DMI increased but ADG and digestibility decreased in winter compared with those in summer. Sheep fed with the EFE diet increased the concentrations of rumen ammonia nitrogen ($P < 0.05$) and total volatile fatty acids ($P > 0.05$), but reduced pH ($P > 0.05$), compared with CON in summer and winter. EFE increased nitrogen (**N**) intake, digestible N, retained N, and retained N/digestible N ($P < 0.05$) but reduced fecal N/N intake, urinary N/N intake, and excretion N/N intake in summer and winter ($P < 0.05$), compared with CON. Retained N/N intake was reduced and excretion N/N intake increased in winter relative to those in summer. In winter, gross energy (**GE**), manure E/GE, CH_4 emissions, CH_4/DMI, and CH_4/GE increased but digestion energy and metabolic energy decreased compared with those in summer. Sheep fed with the EFE diet had a greater GE intake than those fed with the CON diet ($P < 0.05$) but had lesser CH_4/DMI and CH_4E/GE ($P < 0.05$) than those fed with the CON diet in both summer and winter. In conclusion, EFE supplementation increased DMI, apparent digestibility, and N deposition rate. These effects were beneficial for animal production. The CH_4 emission per unit DMI of grazing Tan sheep was lesser and conducive for augmenting the environmental benefits.

Lay Summary

Globally, the supply–demand relationship between grassland and livestock is mainly mediated by the optimization of pasture management. The interaction between grassland and livestock is one of the fundamental drivers of grassland occurrence and development. Natural grassland yields and quality are affected by precipitation, heat, and grazing, and their dynamics vary seasonally with distinct peaks and troughs. The use of exogenous fibrolytic enzymes during troughs can improve the growth performance, digestion, and metabolism of grazing sheep. The exogenous fibrolytic enzyme supplement used in this research may aid in improving the health and overall productivity of grazing sheep.

Key words: digestibility, exogenous fibrolytic enzymes, methane emission, nitrogen and energy utilization

ABBREVIATIONS: ADF, acid detergent fiber; BW, body weight; Ca, calcium; CH_4, methane; CON, 30 mL of normal saline; CP, crude protein; DE, digestion energy; DMI, dry matter intake; EE, ether extract; EFE, mix 1 g of exogenous fibrolytic enzymes with 30 mL water; FE, fecal energy; FN, fecal nitrogen; ME, metabolic energy; NDF, neutral detergent fiber; NFC, non-fiber carbohydrates; NH_3-N, ammonia nitrogen; OM, organic matter; P, phosphorus; SF_6, sulfur hexafluoride; TP, total protein; TVFA, total volatile fatty acids; UE, urine energy; UN, urine nitrogen

Introduction

Grasslands are one of the world's largest terrestrial ecosystems and cover approximately 40% of the global land area (Hou et al., 2021; Török, 2021). These ecosystems are currently of high conservation concern because they provide as many as 33 vital ecosystem services. For example, grasslands are the primary source of meat and dairy products (Zhao et al., 2020). According to the United Nations, global livestock accounts for 14.5% of all anthropogenic greenhouse gas (**GHG**) emissions, which is an issue of grave concern (UN, 2019; Tommaso et al., 2022). The digestion and metabolism of grazing ruminants, including enteric CH_4 emission and excrement GHG emission, especially in sown pasture, have been studied adequately in Australia and New Zealand

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(Clark et al., 2010; Tomkins et al., 2011). Lack of information on the impacts of some environmental factors on natural pasture, especially their seasonal dynamics, is a key limitation in ensuring the sustainability of ruminant production in terms of energy expenditure. Moreover, the Intergovernmental Panel on Climate Change of the Food and Agriculture Organization of the United Nations has accurately evaluated the environmental footprint and food security situation and has imposed restrictions on CH_4 emissions, especially in the forecasted scenario of global climate change and increased world population (UN, 2019).

Given that most ruminants are raised under nutrition-poor conditions, optimizing the productivity of digestion and metabolism would be by far the most effective approach to reduce enteric CH_4 emissions (Berry and Crowley, 2012). Diverse CH_4 mitigation strategies for livestock, with most of them being nutrition-oriented, have been developed during the past 50 years. However, these strategies are primarily applied on animals with large intakes, high feed efficiencies, and with already low CH_4 yields or intensities in intensive feeding systems in temperate climates (Jarvis and Pain, 1994; Patiño et al., 2007). Their applicability to grazing systems, especially those in developing countries, is challenging given the technical, economic, animal welfare, and operational constraints. These strategies often fail to reach their mitigation potential in domestic ruminants (Thornton et al., 2009).

Exogenous fibrolytic enzyme (EFE), a highly active probiotic that is mainly produced via bacterial and fungal fermentation, can hydrolyze cellulose with a complex structure into soluble carbohydrates (Zhang and Lynd, 2004). The fermentation of plant cell wall components with high fiber content produces a high ratio of acetic acid to propionic acid, thereby resulting in high methane emission, whereas the fermentation of soluble carbohydrates emits less methane (Vyas et al., 2014; Zhao et al., 2017). The addition of EFE can improve the growth performance of animals and increase the economic benefits (Titi and Lubbadeh, 2004; McSweeney and Denman, 2007; Azzaz et al., 2021). Therefore, we hypothesize that when used efficiently, EFE degrades the fibers in the forage and converts them into soluble carbohydrates, enhancing rumen fermentation parameters, thus allowing improvements in nutrient digestibility and animal performance and mitigating enteric CH_4 emission without affecting animal health (Figure 1).

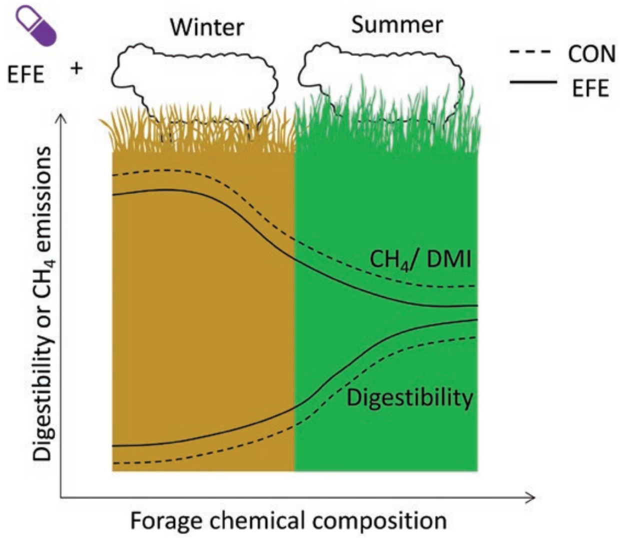

Figure 1. Digestibility and CH_4 emissions of Tan sheep grazed fed diets supplemented with EFE in summer and winter.

Materials and Methods

The protocol and experimental procedures were approved by the Animal Care and Use Committee of Lanzhou University (Protocol number: LZU 201805010).

Research area

A 138-day finishing study was conducted at the Huanxian Grassland Agriculture Research Station of Lanzhou University (37.12°N, 106.84°E, 1700 m a.s.l), China. The area has a typical continental monsoon climate (Ren et al., 2008). The average annual temperature is 8.4 °C. The annual rainfall is 266.2 mm, more than 70% of which is generally received from late June to September. The mean annual evaporation capacity is 1990 mm. The soil type is sandy loose (Li et al., 2021). In accordance with the *Comprehensive and Sequential Classification System of Grassland*, the rangeland is classified as a typical steppe (Ren et al., 2008). Major species include *Artemisia capillaris*, *Stipa bungeana*, *Lespedeza daurica*, *Heteropappus altaicus*, *Dodartia orientalis*, *Potentilla bifurca* L., and *Euphorbia esula*. Herbage starts to turn green in late March and begins to turn yellow in late September (Li et al., 2021). The chemical composition of the herbage in different seasons is listed in Table 1.

Animal management, experimental design, and sample collection

Two flatland areas separated by approximately 100 m were selected for summer and early winter grazing. Each area was divided into six plots comprising three replicates. Summer grazing extended for 90 days from early mid-June to early mid-September each year (10 days/paddock replicate, three grazing rotations). Winter grazing extended for 48 days from early mid-November to early January (8 days/paddock replicate, two grazing rotations). The grazing hours were 08:00–18:00 and 09:00–17:00 in summer and winter, respectively. During these periods, the animals were housed in a nearby overnight shelter. A density of 5.3 sheep/ha was considered reasonable on the basis of the local average grazing intensity (Li et al., 2021).

A total of 20 Tan wether sheep (average age = 6 months) with an initial body weight (**BW**) of 23.17 ± 0.24 kg (mean ± SE) were used in a randomized complete block design and categorized into two groups: the EFE group comprised animals fed orally with 1 g of EFEs (10,000 U/g) dry powder mixed with 30 mL of warm water using a drencher. For experimental accuracy, the CON group was orally fed with 30 mL of normal saline daily before grazing.

After feeding the sheep with EFE every day, the researchers went out into the pasture and followed the sheep to collect pasture samples. After 25 days of summer grazing (winter grazing for 11 days), the sheep were transferred to a separate metabolic cage for a 5-day digestibility trial, 5 days before the metabolic procedures, the animals will be placed in a separate metabolic cage after returning to pasture to prepare for the metabolic procedures. Fresh herbage was cut daily approximately 3 cm above the ground level. The herbage was stored at cryogenic temperatures until distribution, and the unutilized feed was disposed of within 2 days postharvest.

Table 1. The chemical composition of the feeds used in the summer and winter

Item	Summer[1]	Winter
DM[2], %	94.60	95.20
OM, % DM	93.04	93.17
CP, % DM	14.86	5.48
NDF, % DM	53.06	76.44
ADF, % DM	32.41	50.42
NFC[3], % DM	22.19	8.78
EE, % DM	2.91	2.47
Ca, % DM	1.00	0.54
P, % DM	0.28	0.12
Ca: P	3.85	5.02
GE, MJ/ kg DM	17.70	17.42

[1]Summer grazing started in early mid-June and ended in early mid-September each year. Winter grazing ranged from early mid-November to early January. Pasture samples for chemical composition analysis were collected during this period.
[2]DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract; Ca, calcium; P, phosphorus; GE, gross energy.
[3]NFC, non-fiber carbohydrates [100 – (% NDF + % CP + % EE + % Ash)].

Feed stored for more than 1 day was reserved for topping up the herbage if fresh forage from the relevant day was in short supply. Total feces collection method was adopted in the metabolism test, residual forage, feces, and urine were collected twice daily, and approximately 100 g of representative fecal samples (Quartering method) were obtained for analysis. The fecal sample was dried at 105 °C for the measurement and analysis of basic nutrients and energy, and 10% sulfuric acid (**H_2SO_4**) was added to another sample to fix N. The pH of the urine was adjusted to <3 by adding 10% H_2SO_4. At the end of the digestibility trial, the animals were weighed and another round of grazing was started. Crude protein (**CP**) content was determined using the Kjeldahl nitrogen method (Yuen et al., 2010) and a semiautomatic Kjeldahl nitrogen determination instrument after furnace digestion was completed. Coarse ash (**Ash**) was determined using a muffle furnace (600 °C for 2 h; SX-G03173, Central Electric Furnace, China). The sample was first carbonized on an electric heating plate and then weighed after being ashed in a muffle furnace at 550 °C. Neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**) were determined using the filter bag method with an automatic fiber analyzer (Ankom Technology methods 5 and 6, respectively; ANKOM2000, ANKOM, USA). The gross energy (GE) of forage, fecal, and urine samples was determined using an automatic oxygen bomb calorimeter (PARR6400, PARR, USA). Ether extract (**EE**) was measured using an automatic fat meter (Ankom method 2; Ankom Technology; ANKOMXT15, ANKOM, USA). The animals were not given any supplementary feeding during the entire experimental period.

Rumen-fluid samples (20 mL) were extracted via an oral stomach tube before the morning. The tube was thoroughly cleaned using fresh warm water between sample collections, and 50 mL of the sample from each Tan sheep was always discarded to avoid contamination from saliva. The rumen-fluid pH was measured using a portable pH meter (Model 206-pH2, Testo, Germany), as previously described by Fan et al. (2020). The rumen-fluid samples were used for total volatile fatty acids (**TVFA**) testing and ammonia nitrogen (**NH_3-N**) concentration analysis. For the analysis of ruminal TVFA concentrations, the filtrate was thawed and centrifuged at 5,000 × *g* for 10 min and then analyzed by gas chromatography (GC-MS522; Wufeng Instruments, Shanghai, China) as described by Fan et al. (2020). The NH_3-N concentration was determined by colorimetry (UVVIS8500, Tianmei, Shanghai, China) as described by Chaney and Marbach (Dennis et al., 2013).

SF_6 tracer technique for CH_4 emission measurement

CH_4 samples were collected from the grazing sheep using the sulfur hexafluoride (**SF_6**) tracer technique. A permeation tube with an SF_6 content of 200 mg and a known SF_6 release rate was inserted into the reticulo-rumen of each sheep 5 d prior to the experiment. The device used to capture methane was removed before the animals were moved to individual digestion and metabolism cages where they remained until a new round of grazing trials was commenced. SF_6 was individually calibrated with 8 wk of serial weighing prior to the experiment (Patiño et al., 2007). In this experiment, the SF_6 permeation rates were 3.13 ± 0.23 mg SF_6/day. The head of each sheep was equipped with a 2.5 L gas collection tank for measuring the gas flow rate, and a similar device was used to measure the CH_4 and SF_6 of the atmosphere in the paddock. The collected gas was immediately analyzed using a Shimadzu gas chromatograph (GC-2014, Shimadzu Enterprise Management Co., Ltd., Japan). The detector of the GC analyzer included a FID-2014 hydrogen flame ionization detector and a TCD-2014 thermal conductivity detector. The main detection component of the FID-2014 hydrogen flame ionization detector was CH_4 (detection limit: $<5 \times 10^{-10}$ g/s; calibration range: 0.9999–1.0). The major detection component of the TCD-2014 thermal conductivity detector was SF_6 (sensitivity: >800 mV·mL/mg, calibration range: 0.9999–1.0). The injection method of the GC analyzer involved a six-port value automatic injection with N_2 of 99.999% purity as the carrier gas at a gas flow rate of 30 mL/min. Daily CH_4 emission was calculated from the SF_6 release rate in the breath sample and the CH_4/SF_6 concentration ratio was corrected for gas concentration.

$$CH_4\,(g/day) = CSF_6 \times [(CH_4)\,y - (CH_4)\,b]/[(SF_6)\,y - (SF_6)\,b]$$

Where CH_4 is the daily CH_4 production of the animal, CSF_6 is the SF_6 emission from the permeation tube, $[CH_4]$y and $[SF_6]$y are the gas concentrations in the collection canister, and $[CH_4]$b and $[SF_6]$b are the gas concentrations in the blank.

Statistical analysis

All data from the summer and winter experiments were separately analyzed using an analysis of covariance model of SPSS Statistics 27 (IBM) to determine the fixed effect of dietary treatment (CON and EFE). dry matter intake (DMI), apparent digestibility, rumen fermentation parameters, nitrogen utilization, and energy utilization were presented as the average of the three values of metabolic cage tests in summer and winter. The differences between means were significant at $P < 0.05$.

Results

DMI and growth performance

DMI and growth performance are presented in Table 2. EFE in the diet increased DMI (1.22 vs. 1.09 kg/day for summer and 1.32 vs. 1.22 kg/day for winter, $P < 0.05$) compared with

Table 2. Growth performance of Tan sheep grazed fed diets supplemented with EFE in summer and winter (n = 20)

Item[1]	Summer[2]				Winter			
	Treatment[3]		SEM[4]	*P*-value	Treatment		SEM	*P*-value
	CON	EFE			CON	EFE		
DMI, kg/d	1.09	1.21	0.028	0.013	1.22	1.32	0.021	0.018
Initial BW, kg	22.96	23.42	0.314	0.483	34.38	34.98	0.579	0.618
Final BW, kg	32.02	34.89	0.522	0.003	30.68	32.34	0.498	0.097
ADG, g/d	100.64	127.45	6.283	0.029	-77.07	-55.03	12.245	0.384

[1]DMI, dry matter intake; IBW, initial body weight; FBW, final body weight; ADG, average daily gain;.
[2]Summer grazing from early mid-June and ended in early mid-September each year, winter grazing from early mid-November to early January.
[3]CON, 30 mL of normal saliney; EFE, mix 1 g of exogenous fibrolytic enzymes with 30 mL water.
[4]SEM = treatment standard error of the mean.

Table 3. Apparent digestibility of Tan sheep grazed fed diets supplemented with EFE in summer and winter (n = 20)

Item[1]	Summer[2]				Winter			
	Treatment[3]		SEM[4]	*P*-value	Treatment		SEM	*P*-value
	CON	EFE			CON	EFE		
DM	60.68	65.98	0.733	<0.001	55.11	60.91	0.984	0.001
CP	68.24	72.64	0.936	0.014	59.47	65.38	1.613	0.065
NDF	52.14	62.95	1.064	<0.001	52.49	56.05	0.997	0.002
ADF	48.50	57.30	1.237	<0.001	50.94	54.59	1.177	0.025
EE	55.03	64.31	1.632	<0.001	56.85	62.45	1.088	0.349

[1]DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; EE, ether extract.
[2]Summer grazing from early mid-June and ended in early mid-September each year, winter grazing from early mid-November to early January.
[3]CON, 30 mL of normal saliney; EFE, mix 1 g of exogenous fibrolytic enzymes with 30 mL water.
[4]SEM = treatment standard error of the mean.

CON. In summer, EFE increased the final BW and average daily gain (**ADG**) of Tan sheep ($P < 0.05$). In winter, EFE increased the final BW ($P = 0.097$). However, ADG was negatively correlated with DMI ($P = 0.384$).

Apparent digestibility

Apparent digestibility data are furnished in Table 3. The digestibility of the nutrients decreased in winter relative to that in summer. In summer, the apparent digestibility values of DM, CP, NDF, ADF, and EE were greater in sheep fed with the EFE diet than in those fed with the control diet ($P < 0.05$). In winter, EFE increased the digestibility of DM, NDF, and ADF ($P < 0.05$) but had no effects on the digestibility of CP ($P = 0.065$) and EE ($P = 0.349$).

Rumen fermentation parameters

The rumen fermentation parameters for Tan sheep are presented in Table 4. In summer, EFE increased the concentrations of rumen NH_3-N and the proportion of isobutyrate ($P < 0.05$), but reduced the proportion of butyrate ($P < 0.05$). In winter, EFE increased the concentrations of rumen NH_3-N and the proportion of propionate ($P < 0.05$). However, sheep fed with the EFE diet had less pH and the concentrations of rumen TVFA ($P > 0.05$). In addition, acetate: propionate ratio increased in summer and tended to acetate fermentation, while decreased in winter and tended to propionate fermentation.

Nitrogen utilization

The effects of dietary treatment on the utilization of N are given in Table 5. In summer, EFE increased N intake, digestible N, retained N, retained N/N intake, retained N/digestible N ($P < 0.05$) but reduced fecal N/N intake, urinary N/N intake, and excretion N/N intake ($P < 0.05$). In winter, EFE increased N intake, digestible N, retained N, retained N/N intake, retained N/digestible N ($P < 0.05$) but reduced urinary N/N intake and excretion N/N intake ($P < 0.05$). Moreover, N Intake, digestible N/N intake, and retained N/intake N reduced and excretion N/N intake increased in winter relative to those in summer.

Energy utilization

The energy utilization of sheep is presented in Table 6. GE, manure E, manure E/GE, CH_4 emissions, CH_4E, CH_4/DMI, and CH_4E/GE increased but digestion energy (**DE**) and metabolic energy (**ME**) decreased in winter relative to those in summer. In summer, sheep under the EFE treatment exhibited greater GE and urinary E output than those under the CON ($P < 0.05$) but CH_4E/GE was decreased. In winter, sheep fed with the EFE diet had greater GE, DE, and ME than those fed with the CON diet ($P < 0.05$). However, sheep fed with the EFE diet had less urinary E/GE, CH_4/DMI, and CH_4E/ GE than those fed with the control diet ($P < 0.05$). DE/GE, ME/DE, CH_4 emissions, and CH_4E did not differ between treatments in summer and winter ($P > 0.05$).

Table 4. Rumen fermentation parameters of Tan sheep grazed fed diets supplemented with EFE in summer and winter (n = 20)

Item[1]	Summer[2]				Winter			
	Treatment[3]		SEM[4]	*P*-value	Treatment		SEM	*P*-value
	CON	EFE			CON	EFE		
pH	6.80	6.67	0.032	0.053	6.89	6.78	0.040	0.180
NH_3-N (mg/dL)	6.46	11.43	0.659	<0.001	5.80	7.41	0.242	<0.001
TVFA (mmol/L)	53.90	55.43	0.972	0.451	42.14	43.14	1.136	0.674
Acetate (%)	67.79	68.81	0.636	0.440	72.18	73.67	0.677	0.287
Propionate (%)	14.90	14.62	0.093	0.138	14.70	15.37	0.116	0.001
Butyrate (%)	7.57	5.18	0.456	0.004	4.70	5.00	0.356	0.688
Isobutyrate (%)	4.50	5.38	0.199	0.022	3.61	2.42	0.313	0.053
Valerate (%)	2.09	2.15	0.150	0.849	1.47	1.24	0.113	0.328
Isovalerate (%)	3.14	3.85	0.250	0.165	3.33	2.29	0.274	0.056
Acetate/ Propionate	4.55	4.71	0.066	0.236	4.91	4.79	0.051	0.268

[1]NH_3-N, ammonia nitrogen; TVFA, total volatile fatty acids.
[2]Summer grazing from early mid-June and ended in early mid-September each year, winter grazing from early mid-November to early January.
[3]CON, 30 mL of normal saliney; EFE, mix 1 g of exogenous fibrolytic enzymes with 30 mL water.
[4]SEM = treatment standard error of the mean.

Table 5. Nitrogen utilization of Tan sheep grazed fed diets supplemented with EFE in summer and winter (n = 20)

Item[1]	Summer[2]				Winter			
	Treatment[3]		SEM[4]	*P*-value	Treatment		SEM	*P*-value
	CON	EFE			CON	EFE		
N intake, g/d	16.64	19.85	0.514	<0.001	11.91	13.60	0.395	0.028
Fecal N, g/d	5.28	5.43	0.126	0.371	4.83	4.71	0.170	0.081
Fecal N/ N intake, %	31.73	27.36	0.968	0.009	40.55	34.63	1.256	0.053
Urinary N, g/d	5.58	5.85	0.158	0.402	4.65	4.73	0.113	0.734
Urinary N/ N intake, %	33.51	29.58	0.872	0.020	38.98	35.18	0.888	0.028
Excretion N, g/d	10.86	11.28	0.256	0.336	9.48	9.44	0.279	0.770
Excretion N/ N intake, %	65.26	56.83	1.688	0.006	79.60	60.41	2.099	0.036
Digestible N, g/d	11.36	14.41	0.485	0.001	7.08	8.89	0.343	0.015
Retained N, g/d	5.78	8.57	0.454	0.001	2.43	4.16	0.343	0.022
Retained N/ N intake, %	34.74	43.17	1.688	0.006	20.40	39.59	2.099	0.036
Retained N/ DN, %	50.88	59.47	1.919	0.009	34.32	46.79	3.151	0.047

[1]N, nitrogen; DN, digestible nitrogen: Intake N—Fecal N; Retained N: DN—Urinary N.
[2]Summer grazing from early mid-June and ended in early mid-September each year, winter grazing from early mid-November to early January.
[3]CON, 30 mL of normal saliney; EFE, mix 1 g of exogenous fibrolytic enzymes with 30 mL water.
[4]SEM = treatment standard error of the mean.

Nitrogen and energy cycles in pasture land

EFE increased the GE and N intake of sheep grazed throughout the grazing land, energy intake in winter relative to that in summer, and total N intake in summer relative to that in winter. Notably, CH_4 emissions increased in winter ($P < 0.05$). Although the supplemental EFE increased CH_4 emissions, the results were not significantly different compared with the control group. This increase was negligible across the grazing land because of the increase in the ADG of the animals (Figure 2A). Our path analysis (Figure 2B) showed a clear positive relationship between EFE and digestibility and energy utilization, whereas nitrogen utilization was inversely related to EFE and winter. Chemical composition, energy utilization, and methane emission were positively correlated in winter. Digestibility promoted nitrogen utilization and energy utilization. Digestibility and nitrogen utilization jointly promoted the growth performance of sheep. However, methane emission was inversely related to energy utilization and negatively affected the growth performance of sheep.

Discussion

Natural grassland exhibits seasonal characteristics that affect the utilization of land resources and the growth of herbage, thus leading to seasonal variations in the nutritional value of herbage (Kleinebecker et al., 2011; Shan et al., 2011). In other words, during herbage growth, plant metabolism is accelerated and photosynthesis promotes the rapid deposition of organic material, such as coarse proteins. When winter arrives, temperatures fall and the lignification degree of

Table 6. Energy utilization of Tan sheep grazed fed diets supplemented with EFE in summer and winter (n = 20)

Item[1]	Summer[2]				Winter			
	Treatment[3]		SEM[4]	*P*-value	Treatment		SEM	*P*-value
	CON	EFE			CON	EFE		
Energy intake and output (MJ/d)								
GE	19.23	20.69	0.375	0.047	20.85	22.88	0.330	0.002
Fecal E	9.24	9.94	0.257	0.179	12.03	12.96	0.217	0.070
Urinary E	0.49	0.52	0.006	0.001	0.75	0.75	0.012	0.484
Manure E	9.73	10.47	0.261	0.165	12.78	13.71	0.220	0.081
DE	9.98	10.75	0.212	0.070	8.82	9.92	0.211	0.005
ME	8.25	8.97	0.196	0.062	6.54	7.63	0.215	0.004
Energy use, %								
DE/GE	52.04	51.97	0.725	0.965	42.31	43.36	0.645	0.292
Fecal E/GE	47.97	48.03	0.725	0.965	57.69	56.64	0.645	0.292
Urinary E/GE	2.55	2.53	0.029	0.738	3.60	3.30	0.079	0.008
Manure E/GE	50.56	50.51	0.724	0.976	61.29	59.93	0.669	0.189
ME/GE	42.98	43.38	0.716	0.787	31.35	33.32	0.722	0.081
Methane emissions and energy								
CH_4, g/d	22.64	22.72	0.428	0.929	27.72	27.89	0.448	0.682
CH_4E, MJ/d	1.25	1.25	0.024	0.929	1.53	1.54	0.025	0.694
CH_4/ DMI, g/ kg	21.16	18.72	0.686	0.074	22.86	21.17	0.502	0.030
CH_4E/ GE %	6.51	6.06	0.093	0.012	7.36	6.74	0.148	0.011

[1]GE, gross energy; E, energy; CH_4, methane; Manure E, Fecal E + Urinary E; DE, digestible energy: GE – Fecal E; ME, metabolic energy: DE – (Urinary E + CH_4E).
[2]Summer grazing from early mid-June and ended in early mid-September each year, winter grazing from early mid-November to early January.
[3]CON, 30 mL of normal saliney; EFE, mix 1 g of exogenous fibrolytic enzymes with 30 mL water.
[4]SEM = treatment standard error of the mean.

the herbage increases gradually; hence, NDF and ADF of the herbage are the highest (Pakeman, 2004; Otfinowski and Coffey, 2021). The NDF digestibility is, therefore, the lowest (El-Nomeary et al., 2021), and the NDF concentration significantly affects ADF digestibility and rumen pH. It is well-known that NDF and ADF contents are negatively correlated with DMI (Chen et al., 2022). EFE can enhance the digestibility of dietary ADF by increasing the proportion of NDF (Sujani and Seresinhe, 2015; Tirado et al., 2018). Bannink et al. (2012) showed that dietary supplementation with EFE can improve the fiber degradation rate by changing the type of rumen fermentation in dairy cows. Gómez-Vázquez et al. (2011) obtained the same results for beef cattle, and Meale et al. (2014) and Bernard et al. (2010) reported similar findings for dairy cows. In this study, EFE supplementation increased DMI, ADG, and nutrient digestibility in sheep. EFE is believed to be responsible for the increase in DMI in animals, which could be attributed to the release of sugars during hydrolysis prior to pasture intake. This effect augments the palatability of the diet (Alsersy et al., 2015) or the digestibility of nutrients, thus increasing rumen chyme output and reducing intestinal filling. Therefore, feed consumption is stimulated (Romero et al., 2015). Furthermore, EFE activates the secretion of endogenous enzymes (Beauchemin et al., 2003), which can compensate for the deficiency of microbial cellulase in herbivores. Simultaneously, EFE can improve the effects of other enzymes, such as amylase and pectinase, and work concertedly to improve the digestibility of fibrous foods (Zhang and Lynd, 2004). In addition, EFE maintains the integrity of villi and promotes the absorption of nutrients in the small intestine (Wang et al., 2019), which are beneficial for the growth of the animals.

The rumen pH reflects the internal rumen environment and is affected by several factors, including dietary structure, rumen circulation rate, and saliva secretion, while dynamic pH changes impact microbial activity, nutrient digestion, and absorption (Li et al., 2014; Tebbe et al., 2017). In this study, pH increased in winter, and EFE supplementation reduced pH levels. TVFA decreased in winter, and EFE supplementation increased their levels. Sheep grazing in grasslands in the dry grass season exhibited higher fiber and lower digestibility levels, prolonged chewing and rumination times, and generated saliva that entered the rumen and increased pH levels (Raffrenato et al., 2017). A lack of nitrogen sources in winter for grazing sheep decreased rumen microbial activity and quantity, such that rumen VFA levels were low. Another reason was the cold winter weather; metabolic rates were accelerated, low intestine absorption rates were increased, and rumen microorganisms were in short supply, thus reducing VFA levels. Supplemented EFE promoted fiber decomposition, while rumen propionic acid production was enhanced and increased glucose levels so that glucose was produced by gluconeogenesis from propionic acid. It is good for energy utilization (Zou et al., 2019). However, after grazing grass in green grass stages, carbohydrate fermentation in the rumen accelerated VFA production rates, while VFA uptake by the upper rumen crust was lower than production rates, which gradually increased VFA concentrations in the rumen and decreased pH levels (Li et al., 2015). EFE supplementation promoted plant fiber hydrolysis, and rumen fermentation

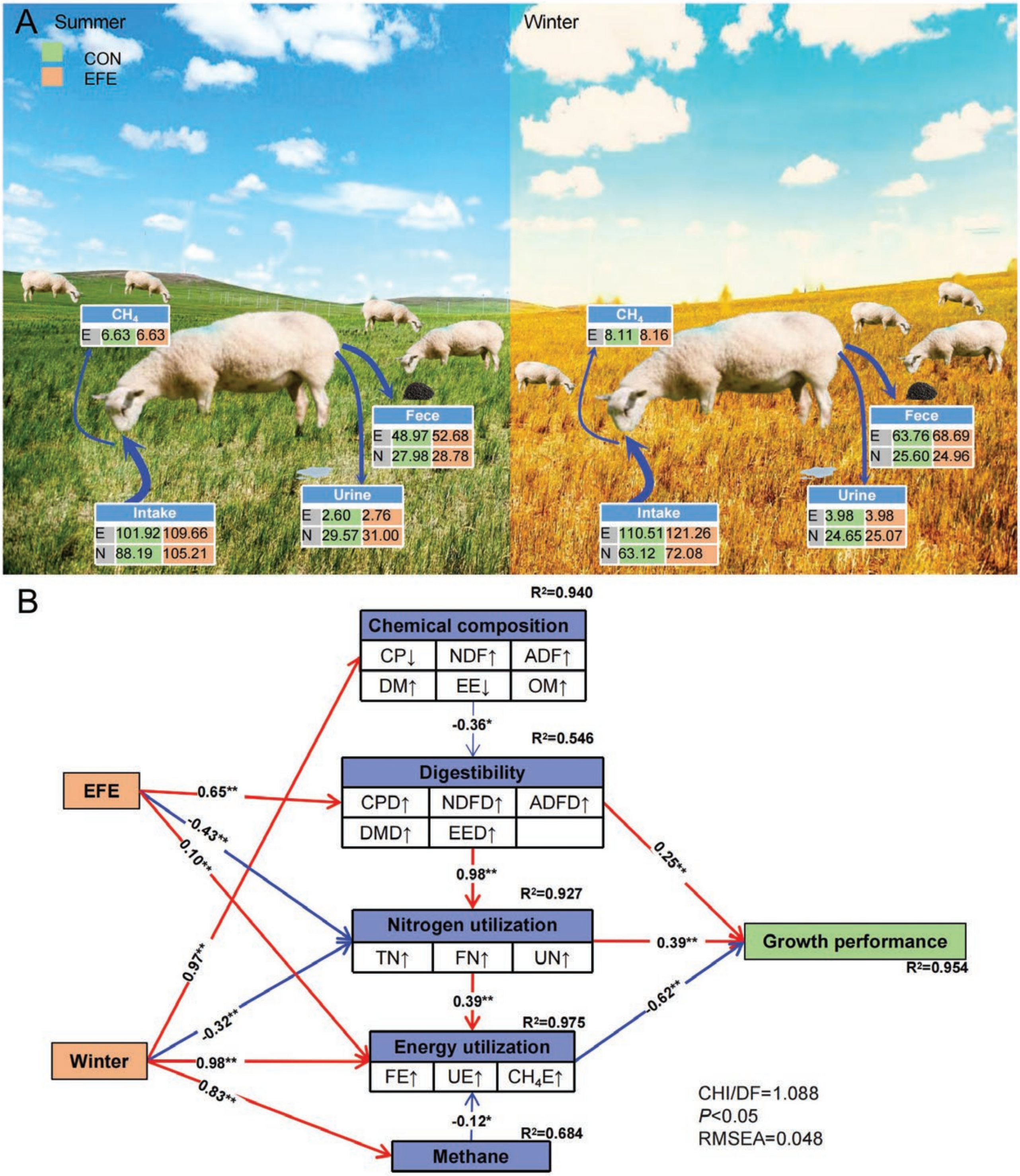

Figure 2. Relationship between the intake and output of grazing Tan sheep per hectare grassland (A). E, energy (MJ/ ha d^{-1}); N, nitrogen (g/ ha d^{-1}). The result of SEM relating EFE, winter, the chemical composition of the herbage, digestibility, nitrogen utilization, energy utilization, methane emission, and growth performance (B), Red and blue arrows indicate positive and negative regression/covariance coefficients, respectively. Arrows thickness and color intensity are proportional to the value of the regression/covariance coefficients, which are presented within the arrows.

increased organic acid levels, increased the concentrations of rumen TVFA in winter and acetate: propionate ratio in summer. Although there is no significant alter in the concentration of rumen acetate, it can still be argued that EFE can modify the rumen fermentation pattern and make the rumen fermentation type transition from acetate to propionate fermentation. This is one of the main mechanisms by which feed additives inhibit ruminal CH_4 production in ruminants (Li et al., 2015). This increased the digestibility and energy utilization of the animal.

Rumen ammonia nitrogen is the final protein decomposition product in feed, a protein synthesis precursor in rumen microorganisms, and its concentrations reflect dynamic changes in nitrogen decomposition and microbial utilization in the rumen (Grummer et al., 1984). In this study, EFE increased NH_3-N concentrations, which were higher in summer. NH_3-N is generally positively correlated with protein levels in animal intake (Fan et al., 2021) and may be due to winter forage biomass, the content of CP and nonstructural carbohydrates and structural carbohydrate ratio is low, not easy to degrade protein content increased, making livestock quality low intake of protein, and rumen microbial activity is abated. Finally, NH_3-N levels in rumen fluid decreased (Fan et al., 2021). However, supplemented EFE reduced fiber proportions in herbage, reduced non-degradable protein levels forming NH_3-N, and suggested that EFE addition increased nitrogen efficiency and promoted growth performance in Tan sheep with a lack of herbage resources.

Nitrogen metabolism reflects the extent of dietary protein utilization, and the nitrogen utilization efficiency of ruminants depends on the balance between fermentable energy and degradable proteins in the rumen (Wilkinson and Lee, 2018). In this study, urine nitrogen ratios increased in winter relative to those in summer. The possible reason is that when winter arrives, sweat pores of the sheep tighten in response to the cold, which reduces the amount of perspiration on the skin. Therefore, excess water is passed only via the urine, which carries away a large amount of nitrogen. Furthermore, winter forage CP content decreases, which limits the tricarboxylic acid cycle, decreases the rates of energy synthesis and rumen microbial ammonia capture, and increases nitrogen excretion rates. These effects eventually decrease the nitrogen deposition rate and efficiency. Some studies have shown that carbohydrate supplementation can reduce rumen ammonia nitrogen concentration and urinary nitrogen excretion and improve nitrogen use efficiency (Ellis et al., 2011; Recktenwald et al., 2014; Stergiadis et al., 2015). These effects could be attributed to the fact that carbohydrates provide energy to rumen microorganisms for protein synthesis, improve the rate of ammonia capture by rumen microorganisms, and balance the ammonia production rate. Structural alterations caused by the EFE-hydrolyzed fiber matrix can stimulate the growth of rumen microbial population, increase the surface area of microbial adhesion, and improve the fiber degradation ability of the rumen (Giraldo et al., 2008). Endogenous enzymes produced by these bacteria, such as pectinase and glucanase, break down the crude fiber in the feed into simple carbohydrates that can be easily digested, absorbed, and used by sheep (Beauchemin et al., 2003). Additionally, coarse proteins in the herbage are degraded by rumen microorganisms into ammonia. A part of this ammonia is used for the synthesis of bacterial proteins by microorganisms, and the other part is absorbed via the rumen and enters the rumen nitrogen cycle. However, the addition of EFE likely plays a major role in the synthesis of microbial body proteins and does not have a significant effect on the nitrogen absorbed by the rumen.

Energy loss occurs primarily via increased fecal energy, urine energy, CH_4 energy, and body heat. Fecal energy accounts for approximately one-third of the total energy intake and is the largest component of energy loss (De et al., 2013). Cold stress in winter stimulates ruminants to increase their grazing time, reduce their water consumption, and increase their feed intake. These changes accelerate intestinal peristalsis and gastrointestinal dysfunction, and food is discharged from the body with the stool before it is sufficiently digested (Potts et al., 2017). During the grazing period, sheep cannot satisfy their own needs because the herbage is of low nutritional quality. Sheep cope with the cold by increasing their feed intake to maintain the normal activities of the body because the entire energy required for animal body activities ultimately comes from oxidation. Thus, tissue decomposition is increased and animal weight loss occurs in winter (Ding et al., 2014). The results of this work showed that manure E/GE was less in the EFE than in the control group, thereby suggesting that EFE may reduce manure energy loss. This effect is most likely a result of the reduction in the pH of the rumen and a significant increase in the VFA content, which must have been beneficial to the energy utilization of animals owing to the increased digestibility of crude fiber and CP. In summary, supplementary EFE improved the utilization rate of digestible energy and metabolized energy and reduced the energy loss of the pasture mainly by alleviating energy loss in the form of fecal and urine energy rather than by lowering gas energy and heat consumption (Deng et al., 2018).

CH_4 emissions from ruminants are affected by DMI and other factors. When forage nutrition quality is similar, DMI is positively correlated with CH_4 emissions (Patiño et al., 2008; Knapp et al., 2014). However, under conditions of significantly differing herbage masses, CH_4 emission is positively correlated with the NDF and ADF contents of the herbage. In other words, NDF and ADF are converted into large amounts of volatile fatty acids via microbial action, which provides a large amount of reduced H_2 for CH_4 production. Methanogens produce CH_4 by reducing CO_2 and H_2 in the rumen (Lyu et al., 2018). However, rumen degradation and fibro-lysozyme treatment can optimize this process and may reduce CH_4 production (Beauchemin et al., 2003). EFE enhanced the digestibility of fibers, especially NDF, which indicates that the fraction of NDF was reduced. When the proportion of NDF in the diet is decreased, the proportion of NFC increases. This effect modifies the dominant rumen colony and inhibits the growth of the protozoa. Meanwhile, the methanogens attach themselves to the surface of the protozoa, and a symbiotic relationship exists between these two microbial populations. When protozoan populations decrease, methanogens also decrease, and therefore, CH_4 emissions are reduced (Janssen et al., 2008; Belanche et al., 2014; Patra et al., 2017).

Conclusion

In grazing Tan sheep, EFE supplementation increased DMI, apparent digestibility, and N deposition rate. These effects were beneficial for animal production. The CH_4 emission per unit DMI of grazing Tan sheep was low and conducive to augmenting the environmental benefits. This conclusion confirms our hypothesis.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Author Contributions

Hairen Shi: Data analysis, Writing –original draft, Editing, and finalize manuscript for submission. **Pei Guo:** Supervision, Data analysis, Editing. **Jieyan Zhou:** Supervision, Drafting, Editing. **Zhen Wang:** Design study, Sampling. **Meiyue He:** Conceptualization. **Liyuan Shi and Xiaojuan Huang:** Data curation; Methodology. **Penghui Guo:** Formal analysis; Methodology. **Zhaoxia Guo and Yuwen Zhang:** Supervision. **Fujiang Hou:** Conceptualization, Writing—review & editing, Project administration, Funding acquisition.

Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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