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Silage preparation and fermentation quality of natural grasses treated with lactic acid bacteria and cellulase in meadow steppe and typical steppe

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Objective: In order to improve fermentation quality of natural grasses, their silage preparation and fermentation quality in meadow steppe (MS) and typical steppe (TS) were studied.
Methods: The small-scale silages and round bale silages of mixed natural grasses in both steppes were prepared using the commercial lactic acid bacteria (LAB) inoculants Chikuso-1 (CH, *Lactobacillus plantarum*) and cellulase enzyme (AC, *Acremonium cellulase*) as additives.
Results: MS and TS contained 33 and 9 species of natural grasses, respectively. *Stipa baicalensis* in MS and *Stipa grandi* in TS were the dominant grasses with the highest dry matter (DM) yield. The crude protein (CP), neutral detergent fiber and water-soluble carbohydrate of the mixed natural grasses in both steppes were 8.02% to 9.03%, 66.75% to 69.47%, and 2.02% to 2.20% on a DM basis, respectively. All silages treated with LAB and cellulase were well preserved with lower pH, butyric acid and ammonia-N content, and higher lactic acid and CP content than those of control in four kinds of silages. Compared with CH- or AC-treated silages, the CH+ AC-treated silages had higher lactic acid content.
Conclusion: The results confirmed that combination with LAB and cellulase may result in beneficial effects by improving the natural grass silage fermentation in both grasslands.

Keywords: Meadow Steppe; Natural Grass; Silage Fermentation; Typical Steppe

INTRODUCTION

Meadow steppe (MS) and typical steppe (TS) are important natural steppes that are widely distributed in temperate semi-arid continental climate region and the northern hemisphere boreal and temperate. In China, they are distributed in the northeast, Inner Mongolia, Xinjiang, Qinghai and Tibet Plateau. These steppes play an important role in animal production. However, both steppes are limited in their hay production due to the cold, dry climate, regardless of water use [1]. They occupy about 400.02 million km^2 but can only support 13.62 million tons of hay, which provides about only 42% of the animal feed needed in the winter and spring. Local farmers usually begin storing grass in mid-August. During harvest and storage, dry matter (DM) and crude protein (CP) will be lost [2]. Previously, interest has shifted toward natural grass silage as a main feed source for ruminant animals. Not only can it be prepared ahead of time, in late July to early August, which preserves nutritional value, but it can also extend the retention time, facilitating fodder provision throughout the year, regardless of the weather [3].

It is usually difficult to prepare a good silage fermentation from natural grasses because of their lower moisture, water-soluble carbohydrate (WSC) content and lactic acid bacteria (LAB) counts, as well as their higher lactate buffering capacity [4]. Some studies have tried to solve the problem of poor fermentation by using silage additives, such as LAB and cellulase [5,6], which are widely used for silage preparation. The cellulase can enhance fiber degradation and increase WSC content as a substrate for LAB [7], which can convert WSC into lactic acid [8,9]. As a result, the silage pH is reduced and the forage well preserved. However, limited information is available on the preparation and fermentation of natural grass silage treated with microbiological additives in the both grasslands. The present study examined the grassland population, DM yield, fermentation quality, and chemical composition of natural grasses in MS and TS environments. To improve fermentation quality, small-scale silages and round bale silages of mixed natural grasses in both steppes were prepared using LAB inoculant and cellulase enzyme.

MATERIALS AND METHODS

Grassland population and yield analysis

Natural grasses were harvested at full-bloom stage from Hulunbuir MS (48.27°N, 119.44°E), and Xilingol TS (43.46°N, 115.13°E), Inner Mongolia, China on 24 July 2014. Grasses were harvested in three clipping grasslands with sample lines at 500 m length within the fenced exclosure. According to the specific locations, total 10 sample plots in every 50 m were set with signed global positioning system data on each sample line, we took the quadrat with 1 m×1 m to determine the grass species, and three replicated gradients were used to eliminated the random error. Species density was calculated by dividing the number of individuals in the quadrat [10]. Species cover was determined as the proportion (0% to 1%, 1% to 5%, 5% to 10%, 10% to 20%, 20% to 40%, 40% to 60%, 60% to 100%) of the quadrat covered by its canopy [11]. The biomass production of species was determined by the average weight of all quadrates, and the grasses were oven-dried at 65°C for 48 h to estimate the DM biomass [12].

Silage preparation

The grasses in both steppes were harvested at full-bloom stage. Silages were prepared using small-scale fermentation and round bale system. A commercial LAB inoculant Chikuso-1 (CH, *Lactobacillus plantarum*, Snow Brand Seed Co., Ltd, Sapporo, Japan) and a commercial cellulase enzyme (AC, acremonium cellulase, Meiji Seika Pharma Co., Ltd, Tokyo, Japan) were used as silage additives. AC is produced from *Acremonium cellulolyticus*, the main composition are glucanase and pectinase, carboxymethylcellulase activity is 7,350 U/g. The LAB were inoculated at 20 mg/kg as 1.00×10^5 colony forming unit (cfu)/g on a fresh matter (FM) basis. AC was added at 10 mg/kg of FM. Silage treatments were designed as control, CH, AC, and CH+AC. The LAB and cellulase were diluted with deionized water, and the additive solution was sprated using an electronic sprayer (Solo, 417, Hamburg, Germany) for addition of round bale silage. For small-scale silage preparation, a hand-held sprayer (SX-MD16E-2, Shixia Holding Co., Ltd, TaiZhou, China) was used for addition. The same amount of deionized water was sprayed on the control treatment. The small-scale silages were prepared by using polyethylene jars (1L capacity, Changgan Co., Ltd, Huizhou, China). Grasses were cut into 10 mm length by using a chopper machine (130DX, ARS Co., Ltd, Osaka, Japan), and were mixed well with or without LAB and cellulase, maximum 1 kg of grasses were packed into the jars. Round bale silages were made using a Rollant round baler (375 RC, Harsewinkel, Germany). The natural grasses in the field were cut and packed continuously into the baler, and these bales were produced with a maximum weight of 200 kg, and approximately 1.20 m diameter and 1.20 m length. These bales were transported to storage place and four layers of polypropylene films (0.03 mm, the DOW Chemical Company, Hayward, CA, USA) were immediately wrapped by using a round bale wrapper (SW5000, Vermeer Manufacturing Co., Ltd, Pella, IA, USA). These bales and jars were stored in outdoor and indoor at temperature 20°C to 26°C. Three replicates per treatments were opened at 60 days of ensiling, fermentation quality and chemical composition were analyzed.

Chemical analysis

The fermentation products of silage were analyzed by using cold-water extract as described by Cai [13]. Silage (10 g) was blended with 90 mL deionized water and kept in a refrigerator at 4°C for 24 h [14]. The pH was measured with a glass electrode pH meter (STARTER 100/B, OHAUS, Shanghai, China), the ammonia-N content was analyzed by using steam distillation of the filtrates [13], the concentration of organic acid were measured by high performance liquid chromatography methods as described by Cai [13]. The DM content of the samples were oven dried at 65°C for 48 h, CP and organic matter (OM) were analyzed by Horwitz and Latimer [15] method. The content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined as described by Van Soest et al [16]. The WSC content was determined as described by Thomas [17].

Statistical analyses

Statistical analyses of chemical composition and silage fermentation were performed by one-way analysis of variance using the general linear model procedure of SAS version 9.1 (SAS Institute Inc, 2003). The differences between means were assessed by Tukey's multiple comparison tests at a significant level of $p<0.05$ [18].

RESULTS

Grassland population and yield of natural grasses

Grassland population and DM yield of MS and TS are shown in Table 1. Based on the DM yield, *Stipa Baicalensis*, *Leymus chinensis*, *Serratula centauroides*, *Achnatherum sibiricum*, and *Cleistogenes squarrosa* were the dominant grasses in MS. The DM yield at a high level of order were 163.29 kg/hm^2 for *Stipa Baicalensis* (10.66%, proportion of total DM yields), 107.23 for

Table 1. Dry matter yield of natural grasses in meadow steppe and typical steppe

Grass species	English name	Local name	DM yield (kg/hm^2)	Proportion of total DM yield (%)
Meadow steppe				
Stipa Baicalensis Roshev	Baical Needgrass	Bei Jiaerzhenmao	163.29±0.02	10.66
Leymus chinensis (Trin.) Tzvel.	China Leymus	Yang Cao	107.23±0.41	7.00
Serratula centauroides L.	Common Scawwort	Ma Huatou	93.77±0.02	6.12
Achnatherum sibiricum (L.) Keng	Siberian Jijigrass	Yu Mao	92.72±0.28	6.05
Cleistogenes squarrosa (Trin.) Keng	Scabrous Hideseedgrass	Cao Yinzica	85.33±0.79	5.57
Pulsatilla turczaninovii Kryl. et Serg.	Slenderleaf Pulsatilla	Xi Yebaitouweng	84.34±0.12	5.50
Carex tristachya Thunb.	Threespike Sedge	Tai Cao	74.36±0.03	4.86
Poa annua L.	Annual Bluegrass	Zao Shuhe	56.42±4.98	3.68
Melissilus ruthenicus (L.) Peschkova (*Trigonella ruthenica* L.)	Ruthenia Medic	Bian Xudou	52.58±0.55	3.43
Adenophora stenanthina (Ledeb.) Kitagawa	Longstyle Ladybell	Chang Zhushashen	52.22±0.44	3.41
Astragalus adsurgens Pall.	Erect Milkvetch	Xie JingHuangqi	52.17±0.15	3.41
Thalictrum aquilegifolium L. var. *sibiricum Regel et Tiling*	Siberia Golumbine Meadowrue	Tang Songcao	49.16±0.99	3.21
Artemisia frigida (Willd.) Bess	Fringed Sagebrush	Leng Hao	46.67±1.63	3.04
Iris ventricosa Pall.	Cystoidflower Swordflag	Nang Huayuanwei	44.74±3.55	2.92
Astragalus melilotoides Pall.	Sweetcloverlike Milkvetch	CaoMuxizhuanghuangqi	44.32±0.36	2.89
Potentilla acaulis L.	Stemless Cinquefoil	Xing Maoweilingcai	38.92±0.93	2.54
Oxytropis myriophylla (Pall.) DC.	Leafy Crazyweed	Duo Yejidou	38.25±1.27	2.50
Carex chinensis Retz.	China Sedge	Ri Yinjian	37.25±0.39	2.43
Bupleurum scorzonerifolium Willd.	Red Thorowax	Hong Chaihu	35.45±0.12	2.31
Koeleria cristata (L.) Pers.	Junegrass	Qia Cao	29.02±2.05	1.89
Ixeris polycephala Cass.	China Ixeris	Ku Maicai	28.34±0.12	1.85
Veronica incana L.	White Speedwell	Bai Popona	26.42±0.02	1.73
Gentiana dahurica Fisch.	Dahuria Gentian	Da Wulilongdan	24.39±0.44	1.59
Scorzonera Subacaulis (Regel.) Lipsch.	Low Serpentroot	Ai yacong	23.92±3.09	1.56
Cymbaria dahurica L.	Dahur Cymabria	Da Wulixinba	21.35±0.28	1.39
Sanguisorba officinalis L.	Garden Burnet	Di Yu	21.33±0.27	1.39
Scutellaria scordifolia Fisch. ex Schrenk.	Twinflower Skullcap	Bing Touhuangqin	20.35±0.13	1.33
Agropyron cristatum (L.) Gaertn.	Wheatgrass	Bing Cao	18.29±1.74	1.94
Heteropappus altaicus (Willd.) Novopokr	Altal Puppyflower	A Ertaigouwahua	16.33±1.36	1.07
Galium verum L.	Yellow Bedstraw	Peng Zicai	15.78±0.05	1.03
Silene conoidea L.	Conical Catchfly	Mai Pingcao	13.34±0.23	0.87
Allium tenuissimum L.	Thinleaf Leek	Xi Yejiu	12.07±0.06	0.79
Potentilla tanacetifolia Willd. ex Schlecht.	Tansyleaf Cinquefoil	Ju Yeweilingcai	11.29±1.52	0.74
Typical steppe				
Stipa grandis P. Smirn.	Larch Needlegrass	Da Zhenmao	1,147.04±0.19	61.27
Stipa krylovii Roshev	Altai Needlegrass	Ke Shizhenmao	428.66±0.76	22.90
Cleistogenes squarrosa (Trin.) Keng	Scabrous Hideseedgrass	Cao Yinzicao	122.17±0.04	6.53
Anemarrhena asphodeloides Bunge	Anemarrhena	Zhi Mu	79.96±1.49	4.27
Leymus chinensis (Trin.) Tzvel.	China Leymus	Yang Cao	47.84±0.46	2.56
Allium tenuissimum L.	Thinleaf Leek	Xi Yejiu	31.32±0.55	1.67
Agropyron cristatum (L.) Gaertn.	Wheatgrass	Bing Cao	12.25±0.04	0.65
Thalictrum aquilegifolium L. var. *sibiricum* Regel et Tiling	Siberia Golumbine Meadowrue	Tang Songcao	1.62±0.37	0.09
Artemisia scoparia Waldst. et Kit.	Virgate Sagebrush	Zhu Maohao	1.13±0.28	0.06

Data±standard deviation were the average of three sample lines and each line had 10 sample plots.

Leymus chinensis (7.00%), 93.77 for *Serratula centauroides* (6.12%), 92.72 for *Achnatherum sibiricum* (6.05%), while other grasses were below 93.00 kg/hm^2 in MS. On the other hand, *Stipa grandi*, *Stipa krylovii*, *Cleistogenes squarrosa*, *Anemarrhena asphodeloides* were the dominant grasses in TS, their DM yield at a high level of order were 1147.04 kg/hm^2 for *Stipa grandis* (61.27%), 428.66 for *Stipa krylovii* (22.90%), 122.17 for *Cleistogenes squarrosa* (6.53%), 79.96 for *Anemarrhena asphodeloides* (4.27%), while other grasses were below 47.84 kg/hm^2 in TS. The minimum DM yields were observed from *Potentilla tanacetifolia* (11.29%) in MS and from *Artemisia scoparia* (1.13%) in TS, respectively.

Chemical composition of natural grasses

Chemical composition of natural grasses in MS and TS are shown in Table 2. The DM of natural grasses were 29.45% to 67.94% in MS and were 23.09% to 47.92% in TS on a FM basis. In meadow steppe, the highest and the lowest moisture were found in *Adenophora stenanthina* at 65.30% and *Carex tristachya* at 34.37% of FM. In TS, the highest and the lowest moisture were found in *Thalictrum aquilegifolium* at 63.33% and *Agropyron cristatum* at 47.34% of FM. The OM of both steppes were similar ranging from 84.38% to 96.79% on a DM basis, their ether extract (EE) were 1.30% to 3.06% of DM. The CP of *Silene conoidea* was the lowest content at 3.93% in MS while other grasses were 7.12% to 12.77% of DM. The NDF and ADF were 37.04% to 69.86% of DM and 24.04% to 48.95% in MS, and were 44.79% to 73.33% and 37.64% to 52.19% of DM in TS, respectively.

Chemical composition of mixed natural grasses in MS and TS are shown in Table 3. The DM contents of mixed grasses were similar levels ranging from 52.40% to 55.07%, and their OM

Table 2. Chemical composition of natural grasses in meadow and typical steppe

Grass species	DM (%)	OM (% DM)	CP (% DM)	EE (% DM)	NDF (% DM)	ADF (% DM)
Meadow steppe						
Stipa Baicalensis Roshev	57.94±0.08	95.37±0.17	8.76±0.19	2.55±0.05	69.86±0.53	41.34±0.57
Leymus chinensis (Trin.) Tzvel.	57.80±1.42	95.97±0.27	10.09±0.06	2.64±0.11	62.19±0.17	36.92±0.64
Serratula centauroides L.	34.64±1.46	94.37±0.59	10.09±0.06	2.31±0.13	38.02±1.88	25.31±1.20
Achnatherum sibiricum (L.) Keng	48.45±0.96	95.53±0.23	9.21±0.46	2.79±0.05	59.28±0.43	35.06±0.61
Cleistogenes squarrosa (Trin.) Keng	55.39±0.54	91.77±0.02	9.54±0.12	2.40±0.03	61.34±0.32	37.29±1.18
Pulsatilla turczaninovii Kryl. et Serg.	67.94±0.28	89.97±0.81	8.92±0.20	2.04±0.09	37.93±0.32	24.04±0.51
Carex tristachya Thunb.	57.61±0.27	94.17±0.29	9.73±0.09	2.74±0.03	58.03±0.15	39.17±0.60
Poa annua L.	52.04±0.30	92.74±0.27	7.12±0.02	1.93±0.06	58.33±0.95	35.17±0.55
Melissilus ruthenicus (L.) Peschkova Trigonella ruthenica L.)	45.34±0.40	94.14±0.27	11.73±0.04	2.61±0.09	47.39±0.02	31.25±0.48
Adenophora stenanthina (Ledeb.) Kitagawa	29.45±0.33	94.38±0.37	10.80±0.08	2.67±0.06	47.54±0.31	36.09±0.21
Astragalus melilotoides Pall.	36.51±0.05	95.91±0.17	12.08±0.02	1.87±0.02	59.73±0.17	48.95±0.29
Thalictrum aquilegifolium L. var. *sibiricum Regel et Tiling*	53.27±0.47	92.43±0.14	10.35±0.18	2.11±0.03	40.12±0.24	24.59±0.66
Artemisia frigida (Willd.) Bess	37.03±0.62	94.74±0.17	10.77±0.19	2.56±0.07	50.17±0.68	24.69±0.79
Iris ventricosa Pall.	37.64±0.23	93.27±0.01	9.12±0.05	2.77±0.24	53.23±1.02	40.17±1.11
Astragalus adsurgens Pall.	43.78±0.40	93.24±0.18	11.33±0.08	1.99±0.02	57.34±0.40	40.59±0.28
Potentilla acaulis L.	50.17±0.41	85.93±0.41	9.37±0.02	3.02±0.05	54.11±0.57	34.52±0.40
Oxytropis myriophylla (Pall.) DC.	51.19±0.21	93.52±0.20	12.77±0.40	1.78±0.01	52.09±0.21	31.27±0.36
Carex chinensis Retz.	39.32±0.40	93.48±0.69	8.89±0.50	2.12±0.33	57.33±0.40	48.20±0.47
Bupleurum scorzonerifolium Willd.	38.50±0.22	94.10±0.76	11.30±0.15	2.04±0.30	42.09±0.27	28.95±0.05
Koeleria cristata (L.) Pers.	60.00±0.59	95.77±0.12	9.02±0.19	2.03±0.05	62.35±0.89	37.14±0.53
Ixeris polycephala Cass.	31.20±0.40	89.73±0.25	10.75±0.30	2.81±0.05	56.82±0.30	49.03±0.69
Veronica incana L.	50.33±0.25	90.17±0.24	8.82±0.11	2.09±0.02	47.05±0.10	35.39±0.40
Gentiana dahurica Fisch.	44.29±0.43	88.82±0.24	10.67±0.37	3.02±0.06	43.28±0.43	37.35±0.39
Scorzonera Subacaulis (Regel.) Lipsch.	39.29±0.42	92.34±0.59	11.07±0.08	3.06±0.19	37.04±0.58	27.53±0.29
Cymbaria dahurica L.	41.93±0.05	88.89±1.51	9.56±0.07	1.54±0.03	43.98±0.02	31.27±0.41
Sanguisorba officinalis L.	33.27±0.14	89.53±0.32	9.13±0.31	2.74±0.09	52.39±3.25	37.09±0.79
Scutellaria scordifolia Fisch. ex Schrenk.	49.38±0.36	84.38±0.28	10.89±0.07	2.28±0.02	40.17±0.08	29.33±0.39
Agropyron cristatum (L.) Gaertn.	56.45±0.37	95.45±0.27	9.56±0.27	2.43±0.06	62.13±0.13	37.09±0.25
Heteropappus altaicus (Willd.) Novopokr	47.34±0.32	90.77±0.42	11.67±0.06	2.10±0.03	39.30±0.26	28.89±0.11
Galium verum L.	51.74±0.17	92.08±0.16	10.78±0.43	3.03±0.12	47.28±0.47	35.05±0.42
Silene conoidea L.	30.77±0.25	96.79±1.17	3.93±0.01	1.30±0.01	47.45±0.25	38.41±0.27
Allium tenuissimum L.	33.47±0.22	90.75±0.16	10.54±0.25	2.43±0.20	40.45±0.33	32.93±0.35
Potentilla tanacetifolia Willd. ex Schlecht.	31.18±0.16	89.73±0.16	9.37±0.05	2.72±0.05	52.09±1.29	33.33±0.44
Typical steppe						
Stipa grandis P. Smirn.	44.73±0.40	93.29±0.04	10.29±0.15	2.33±0.09	68.30±0.06	47.01±0.69
Stipa krylovii Roshev	47.32±0.39	92.77±0.09	9.56±0.27	2.73±0.08	73.33±0.38	52.19±0.07
Cleistogenes squarrosa (Trin.) Keng	41.35±0.07	93.06±0.41	9.05±0.23	2.14±0.11	67.23±0.47	43.92±0.07
Anemarrhena asphodeloides Bunge	47.92±0.42	93.23±0.03	10.12±0.05	3.05±0.29	69.92±0.06	40.35±0.16
Leymus chinensis (Trin.) Tzvel.	43.29±0.28	88.37±0.84	10.03±0.20	3.04±0.24	66.29±0.03	39.84±0.63
Allium tenuissimum L.	23.09±0.08	89.77±0.10	11.57±0.13	2.58±0.17	57.59±0.25	46.32±0.14
Agropyron cristatum (L.) Gaertn.	35.31±0.03	92.38±0.17	8.34±0.28	2.51±0.05	69.34±0.39	45.03±0.21
Thalictrum aquilegifolium L. var. *sibiricum* Regel et Tiling	30.10±0.11	85.43±0.02	10.29±0.16	3.02±0.05	50.32±0.60	37.64±0.63
Artemisia scoparia Waldst. et Kit.	45.05±0.19	94.75±0.33	8.35±0.25	2.34±0.06	44.79±0.14	38.83±0.75

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber.
Data±standard deviation are the average of three grasses samples.

Table 3. Chemical composition of mixed grasses in meadow and typical steppe

Material	DM (%)	OM (% DM)	CP (% DM)	EE (% DM)	NDF (% DM)	ADF (% DM)	WSC (% DM)
Meadow steppe							
Small-scale	52.49±0.35[b]	95.24±0.04[b]	8.91±0.02[b]	2.40±0.05[ab]	69.47±0.16[a]	47.29±1.27[a]	2.20±0.02[a]
Round bale	55.07±1.14[a]	95.27±0.08[b]	9.03±0.01[a]	2.48±0.09[a]	69.09±0.61[a]	47.06±0.66[a]	2.15±0.01[a]
Typical steppe							
Small-scale	53.00±0.44[ab]	95.69±0.03[a]	8.02±0.06[d]	2.13±0.09[c]	67.32±0.13[b]	43.47±0.94[b]	2.02±0.01[b]
Round bale	53.15±0.61[ab]	95.73±0.04[a]	8.13±0.02[c]	2.19±0.05[bc]	66.75±0.20[b]	43.33±0.98[b]	2.04±0.02[b]
SEM	0.70	0.03	0.03	0.07	0.33	0.99	0.02
p value	0.13	<0.001	<0.001	0.02	0.001	0.03	<0.001

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water-soluble carbohydrate.
Data±standard deviation are means of three grasses samples.
[a-d] Means±standard deviation within columns with different superscript letters differ (p<0.05).

were also similar with 95% of DM in both steppes. The CP of mixed grasses were 8.91% to 9.03% in MS and were 8.02% to 8.13% in TS. Their NDF and ADF were 69% and 47% of DM in MS, while they were lower more than about 2% and 4% of DM in TS. The WSC contents of mixed grasses in MS (2.15% to 2.20% of DM) were higher (p<0.05) than that in TS (2.02% to 2.04% of DM).

Fermentation quality of mixed grasses silage prepared without or with LAB and cellulase in MS and TS are shown in Table 4. The grasses (G), preparation methods (P), additives (A) and their interaction (G×P, G×A, A×P, and G×A×P) influenced (p<0.001) butyric acid, in addition the additives also influenced (p<0.001) pH, lactic acid, and ammonia-N content. The small-scale and round bale silages in both steppes were showed as similar fermentation results. After 60 days of ensiling, the CH, AC, and CH+AC treatments of small-scale silages and round bale silages in MS and TS were preserved with significantly (p<0.05) lower pH and ammonia-N content, and significantly (p<0.05) higher lactic acid content than those of control. Acetic acid were produced in all silages with 0.38% to 0.57% of FM. Only the control treatment in MS had detected butyric acid (0.16% to 0.25% of FM) and propionic acid (0.11% to 0.17% of FM).

Chemical composition of mixed natural grasses silage in MS and TS were shown in Table 5. The additives influenced (p<0.001) CP, EE, NDF, and ADF. The OM contents of all silages were similar levels raging 92.89% to 95.13% of DM. The CP contents of MS silages (8.13% to 8.92% of DM) were significantly (p<0.05) higher than in the TS silages (7.22% to 7.89% of DM). The CP contents of CH+AC-treated silages were significantly higher (p<0.05) than other treatments, and MS had higher (p<0.05) CP content in the four typies of silages. AC- and CH+AC- treated silages significantly (p<0.05) decreased NDF and ADF contents compared to the control or CH-treatment.

DUSICUSSION

Natural steppes, such as MS and TS, are important feed sources for livestock, and most of the local livestock in China are dependent on these environments. MS occur in the eastern part of the grassland belt, extending westward to the eastern edge of the Inner Mongolian Plateau, China [19,20]. It is reported that the structural species in MS was *Sibirian filifolium*, and the dominant grasses were *Stipa baicalensis* and *Leymus chinensis*. Our study found that the MS contained 33 species of natural grasses, dominated by *Stipa baicalensis* and *Leymus chinensis*. However, we did not observe the structural species *Sibirian filifolium* due to steppe environmental degradation [21].

TS are located west of MS in the Inner Mongolian Plateau. Previous studies have reported the structural species as *Stipa grandi* and the dominant grasses as *Stipa grandi*, *Stipa krylovii*, and *Cleistogenes squarrosa* [22-24]. We found nine species of natural grasses, dominated by *Stipa grandi*, *Stipa krylovii*, *Cleistogenes squarrosa*, and *Anemarrhena asphodeloides*, which is consistent with previous studies [25-27]. The structural species had shifted from *Compositae* to *Gramineae*, which may lead to easier ensiling. The TS had fewer species than the MS, and the species of *Gramineae* accounted for a large percentage of total grasses, which could reduce the abundance of other grass species that have an uncertain value for ensiling [28].

Yield is the dominant factor affecting the quality of ensiling [20]. The DM yield of *Stipa baicalensis*, *Leymus chinensis*, *Serratula centauroides*, *Achnatherum sibiricum*, and *Cleistogenes squarrosa* accounted for 35.41% of the whole DM yield in MS, whereas *Stipa grandi* and *Stipa kryloviiin* accounted for 84.17% of the whole DM yield in TS.

Generally, natural grasses do not grow during the cold season because of the low winter temperatures. Therefore, it is necessary to preserve a feed supply to continuously feed ruminants during the cold season. Silage fermentation is considered the most effective technique for addressing the cold season feed shortage [29].

Silage is now the most common preserved feed for cattle production in many countries, including China [30]. Generally, farm silage is based on natural lactic acid fermentation, in which epiphytic LAB convert WSC into organic acid during the ensiling process. The epiphytic LAB population density has become an important factor in predicting whether to apply LAB in silage

Table 4. Fermentation quality of mixed grasses silage in meadow steppe and typical steppe[1)]

Silage	Treatment	DM (%)	pH	Lactic acid (% FM)	Acetic acid (% FM)	Propionic acid (% FM)	Butyric acid (% FM)	Ammonia-N (g/kg of FM)
Meadow steppe								
Small scale	Control	50.36±0.01	4.63±0.08[a]	0.56±0.01[fg]	0.38±0.01	0.11±0.08[b]	0.16±0.00	0.56±0.04[a]
	CH	50.60±1.45	4.25±0.13[cd]	0.74±0.00[de]	0.42±0.01	ND	ND	0.24±0.05[c]
	AC	50.21±1.10	4.32±0.26[c]	0.66±0.08[ef]	0.50±0.07	ND	ND	0.14±0.04[cde]
	CH+AC	51.21±0.09	4.13±0.06[de]	1.11±0.03[a]	0.55±0.02	ND	ND	0.12±0.02[de]
Round bale	Control	50.29±0.42	4.59±0.16[ab]	0.52±0.07[fg]	0.45±0.08	0.17±0.01[a]	0.25±0.01	0.54±0.03[a]
	CH	50.27±0.34	4.20±0.22[d]	0.90±0.02[bc]	0.44±0.03	ND	ND	0.19±0.03[cd]
	AC	51.32±0.24	4.27±0.14[cd]	0.73±0.13[de]	0.51±0.03	ND	ND	0.17±0.02[cd]
	CH+AC	51.50±0.31	4.16±0.17[de]	1.10±0.04[a]	0.50±0.02	ND	ND	0.14±0.01[cde]
Typical steppe								
Small scale	Control	51.13±0.49	4.45±0.16[b]	0.76±0.04[d]	0.47±0.02	ND	ND	0.40±0.04[b]
	CH	50.64±0.69	4.17±0.08[de]	0.97±0.02[b]	0.49±0.02	ND	ND	0.12±0.01[de]
	AC	50.04±0.49	4.23±0.09[d]	0.82±0.08[cd]	0.47±0.02	ND	ND	0.14±0.03[cde]
	CH+AC	51.40±0.40	4.07±0.05[e]	1.17±0.31[a]	0.57±0.01	ND	ND	0.07±0.03[e]
Round bale	Control	50.62±0.23	4.47±0.17[b]	0.51±0.05[g]	0.48±0.02	ND	ND	0.44±0.02[b]
	CH	50.72±0.23	4.13±0.04[de]	1.10±0.16[a]	0.55±0.01	ND	ND	0.14±0.01[cde]
	AC	50.34±0.38	4.19±0.03[d]	0.95±0.02[b]	0.50±0.07	ND	ND	0.11±0.01[de]
	CH+AC	50.92±0.26	4.08±0.04[e]	1.13±0.03[a]	0.53±0.06	ND	ND	0.12±0.02[de]
	SEM	0.5712	0.0778	0.0364	0.0391	0.0206	0.0016	0.0277
Grass means	Meadow steppe	50.76±0.25[a]	4.36±0.05[a]	0.78±0.05[b]	0.47±0.02[b]	0.04±0.01	0.05±0.02	0.27±0.04[a]
	Typical steppe	50.62±0.14[b]	4.21±0.21[b]	0.93±0.04[a]	0.51±0.01[a]	0	0	0.20±0.03[b]
PM means	Small scale	50.57±0.23[b]	4.27±0.05	0.85±0.04	0.49±0.01	0.01±0.01	0.02±0.01	0.23±0.04
	Round bale	50.80±0.14[a]	4.30±0.05	0.87±0.05	0.50±0.02	0.02±0.01	0.03±0.02	0.23±0.03
Additive means	Control	50.60±0.18	4.58±0.04[a]	0.59±0.04[d]	0.45±0.02	0.07±0.02	0.10±0.03	0.50±0.02[a]
	CH	50.56±0.36	4.19±0.05[bc]	0.93±0.04[b]	0.47±0.02	0	0	0.17±0.02[b]
	AC	50.57±0.34	4.27±0.19[b]	0.79±0.04[c]	0.50±0.02	0	0	0.14±0.01[bc]
	CH+AC	51.01±0.17	4.11±0.09[c]	1.12±0.01[a]	0.54±0.02	0	0	0.11±0.01[c]
Significance of main effects and interactions								
	Grass (G)	0.49	0.003	<0.001	0.07	<0.001	<0.001	<0.001
	PM (P)	0.39	0.46	0.44	0.55	<0.001	<0.001	0.56
	Additives (A)	0.52	<0.001	<0.001	0.02	<0.001	<0.001	<0.001
	G×P	0.57	0.97	0.14	0.73	<0.001	<0.001	0.88
	G×A	0.23	0.09	0.01	0.29	<0.001	<0.001	0.18
	A×P	0.43	0.99	<0.001	0.46	<0.001	<0.001	0.60
	G×A×P	0.77	0.27	0.07	0.73	<0.001	<0.001	0.58

DM, dry matter; FM, fresh matter; CH, Chikuso-1 inoculant, *Lactobacillus plantarum*, Snow Brand Seed Co. Ltd, Sapporo, Japan; ND, not detected; AC, Acremonium cellulase, Meiji Seika Pharma Co., Ltd, Tokyo, Japan; SEM, standard error of the mean; PM, preparation method of silage.
[1)] Silage was stored for 60 d; data are the average of three silage samples.
[a-g] Means ± standard deviation within columns with different superscript letters differ ($p<0.05$).

[31]. LAB population densities $\geq 10^5$ (cfu)/g FM usually result in silage that is well preserved [14]. WSC is also an important factor that influences the fermentation quality of silage [32]. A good silage needs a DM >5% WSC for lactic acid fermentation [33]. However, these mixed natural grasses have a relatively low WSC (Table 3). Furthermore, only a few epiphytic LAB are found on these materials [34], suggesting that silage fermentation may need to be improved using LAB inoculants or cellulase enzymes [14].

The LAB- and cellulase-treated silages in both steppes were well preserved, with significantly ($p<0.05$) lower pH and ammonia nitrogen content and significantly ($p<0.05$) higher lactic acid content than that of each control. These results are likely explained by the WSC content of the materials and by the numbers and physiological properties of epiphytic LAB. The low WSC content of the mixed grasses could hardly provide enough substrate for LAB fermentation. Added cellulase may degrade the cytoderm and increase the available sugars, thereby providing a substrate for lactic acid fermentation, which is consistent with what Sun et al. found for maize silage [35]. Furthermore, the population of epiphytic LAB is usually very low, and some lactic acid-producing cocci cannot not grow in a pH<4.5. During silage fermentation, the cocci grew rapidly only in the early stage. If the silage pH remained >4.0, then the growth of clostridia was not inhibited, and butyric acid fermentation occurred.

In MS, the control silages were of poor quality, with a high

Table 5. Chemical composition of mixed grasses silage in meadow and typical steppe[1)]

Silage	Treatment	Chemical composition (% of DM)				
		OM	CP	EE	NDF	ADF
Meadow steppe						
Small-scale	Control	93.56±0.04^{def}	8.18±0.22^{bc}	2.31±0.04^{a}	64.58±0.02^{ab}	43.59±0.17^{abc}
	CH	93.37±0.28^{ef}	8.85±0.02^{a}	2.24±0.03^{ab}	64.28±0.02^{ab}	42.74±0.57^{cde}
	AC	92.89±0.29^{f}	8.71±0.10^{a}	2.22±0.07^{ab}	62.29±0.01^{abcd}	40.65±0.16^{ef}
	CH+AC	92.90±0.09^{f}	8.92±0.03^{a}	2.19±0.07^{ab}	60.74±0.01^{cd}	40.12±0.05^{f}
Round bale	Control	94.61±0.26^{ab}	8.13±0.09^{bc}	2.33±0.04^{a}	64.40±0.01^{ab}	44.95±1.94^{ab}
	CH	94.40±0.51^{abcd}	8.16±0.01^{ab}	2.19±0.05^{ab}	63.76±0.01^{abc}	42.43±0.68^{cde}
	AC	94.53±0.01^{abc}	8.75±0.00^{a}	2.27±0.08^{a}	63.40±0.01^{abc}	42.14±0.07^{cde}
	CH+AC	95.13±0.42^{a}	8.92±0.13^{a}	2.12±0.02^{bc}	61.36±1.03^{bcd}	40.77±0.05^{e}
Typical steppe						
Small-scale	Control	93.65±0.45^{cdef}	7.22±0.64^{f}	2.11±0.02^{bc}	65.31±0.09^{a}	43.35±0.72^{abcd}
	CH	94.05±0.64^{bcde}	7.45±0.07^{def}	2.00±0.01^{cd}	63.18±0.19^{abc}	43.16±0.07^{bcd}
	AC	93.92±0.03^{bcde}	7.44±0.12^{def}	2.03±0.02^{cd}	61.23±0.01^{bcd}	41.38±0.58^{def}
	CH+AC	94.38±0.09^{abcd}	7.89±0.02^{cde}	1.95±0.03^{d}	61.04±0.06^{d}	40.81±0.18^{e}
Round bale	Control	93.83±0.32^{bcde}	7.34±0.15^{ef}	2.10±0.06^{bc}	65.54±0.01^{a}	45.35±0.24^{a}
	CH	94.47±0.33^{abc}	7.46±0.00^{def}	1.98±0.01^{cd}	65.47±0.01^{a}	43.48±0.78^{abcd}
	AC	94.10±0.56^{bcde}	7.49±0.21^{def}	2.04±0.03^{bc}	63.47±0.32^{abc}	42.64±0.23^{cde}
	CH+AC	94.59±0.38^{ab}	7.52±0.12^{d}	1.93±0.02^{e}	61.88±0.01^{cde}	41.32±0.03^{def}
	SEM	0.27	0.17	0.04	0.99	0.63
Grass means	Meadow steppe	93.88±0.19^{b}	8.59±0.09^{a}	2.24±0.02^{a}	63.25±0.49^{a}	42.23±0.40^{b}
	Typical steppe	94.15±0.09^{a}	7.60±0.09^{b}	2.02±0.02^{b}	63.06±0.44^{b}	42.49±0.36^{a}
PM means	Small scale	93.59±0.14^{b}	8.08±0.14	2.13±0.03	62.65±0.53^{b}	41.79±0.34^{b}
	Round bale	94.46±0.10^{a}	8.06±0.13	2.12±0.03	63.65±0.03^{a}	42.95±0.38^{a}
Additive means	Control	93.91±0.18^{b}	7.72±0.14^{b}	2.21±0.13^{a}	64.94±0.41^{a}	44.31±0.52^{a}
	CH	94.07±0.17^{a}	8.05±0.19^{ab}	2.10±0.04^{ab}	64.17±0.65^{a}	42.95±0.97^{b}
	AC	93.86±0.19^{b}	8.09±0.20^{ab}	2.14±0.04^{ab}	62.60±0.40^{b}	41.70±0.92^{c}
	CH+AC	94.25±0.29^{a}	8.41±0.19^{a}	2.05±0.04^{b}	60.90±0.43^{c}	40.51±0.10^{d}
Significance of main effects and interactions						
	Grass (G)	0.16	<0.001	<0.001	0.88	0.27
	PM (P)	<0.001	0.97	0.76	0.05	<0.001
	Additives (A)	0.12	<0.001	<0.001	<0.001	<0.001
	G×P	<0.001	0.18	0.67	0.27	0.31
	G×A	0.16	0.25	0.93	0.56	0.81
	A×P	0.30	0.77	0.79	0.61	0.26
	G×A×P	0.25	0.79	0.64	0.78	0.80

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; CH, Chikuso-1 inoculant, *Lactobacillus plantarum*, Snow Brand Seed Co. Ltd, Sapporo, Japan; AC, Acremonium cellulase, Meiji Seika Pharma Co., Ltd, Tokyo, Japan; SEM, standard error of the mean; PM, preparation method of silage.

[1)] Silage was stored for 60 d; data are the average of three silage samples.

[a-f] Means ± standard deviation within columns with different superscript letters differ ($p<0.05$).

butyric acid content. These results suggest that the inoculant strain Chikuso-1 used in this study is *Lactobacilli plnatarum*, as it can promote lactic acid fermentation as a homofermentative lactic bacteria and may grow in a low-pH environment [36,37]. Therefore, inoculating silage with these strains may result in beneficial effects by promoting the propagation of LAB and by inhibiting the growth of clostridia, as well as by decreasing ammonia nitrogen, which is an indicator of high-quality fermentation [38,39]. The combination of LAB and cellulase had a greater effect than did treatment with either one alone, showing that these additives promote each other to improve silage fermentation.

The CP content was higher in treatment groups than in controls, and the NDF and ADF contents were lower than those in the controls reported in studies of alfalfa silages [40,41]. The CP content was greatest in the CH+AC- silage, whereas there was no difference between CH- and AC- treatments, which is consistent with previous findings [35]. The lower NDF and ADF contents in the CH+AC- and AC- treatments were probably the result of cellulose-promoted degradation of fiber, which is consistent with the results of Colonbatto [9].

Small-scale fermentation systems were developed and used for LAB screening and silage preparation, because this method can be easy to control under different fermentation conditions [14]. In this study, the silages were prepared using small-scale fermentation and round bale systems. The results showed that small-scale silage values were slightly greater than those observed in round bale silage, because round bale silage with plastic film allows some air permeability [42]. The similarities between the two kinds of silage showed that small-scale fermentation can be used to test the fermentation quality of silage.

These results confirmed that the addition of LAB, cellulase, and their combination benefited silage fermentation by increasing lactic acid, decreasing butyric acid and ammonia nitrogen contents, and improving the silage quality of natural grasses from MS and TS environments.

CONCLUSION

MS and TS contained 33 and 9 species of natural grasses, *Stipa Baicalensis* and *Stipa grandi* were the dominant grasses with the highest DM yield in each steppe. Their mixed grasses in both steppes had 8.02% to 9.03% CP and 66.75% to 69.47% NDF of DM. LAB and cellulase, especially their combination could effectively improve fermentation quality of mixed grasses silage in both steppes.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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