ORIGINAL ARTICLE

Effect of applying lactic acid bacteria and propionic acid on fermentation quality and aerobic stability of oats-common vetch mixed silage on the Tibetan plateau

Jie ZHANG,¹ Gang GUO,¹ Lei CHEN,¹ Junfeng LI,¹ Xianjun YUAN,¹ Chengqun YU,² Masataka SHIMOJO³ and Tao SHAO¹

¹Institute of Ensiling and Processing of Grass, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, ²Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, China and ³Department of Animal and Marine Bioresource Sciences, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

ABSTRACT

The objective of this study was to evaluate effects of lactic acid bacteria and propionic acid on the fermentation quality and aerobic stability of oats-common vetch mixed silage by using a small-scale fermentation system on the Tibetan plateau. (i) An inoculant (*Lactobacillus plantarum*) (L) or (ii) propionic acid (P) or (iii) inoculant + propionic acid (PL) were used as additives. After fermenting for 60 days, silos were opened and the aerobic stability was tested for the following 15 days. The results showed that all silages were well preserved with low pH and NH₃-N, and high lactic acid content and V-scores. L and PL silages showed higher ($P < 0.05$) lactic acid and crude protein content than the control silage. P silage inhibited lactic acid production. Under aerobic conditions, L silage had similar yeast counts as the control silage ($> 10^5$ cfu/g fresh matter (FM)); however, it numerically reduced aerobic stability for 6 h. P and PL silages showed fewer yeasts ($< 10^5$ cfu/g FM) ($P < 0.05$) and markedly improved the aerobic stability (> 360 h). The result suggested that PL is the best additive as it could not only improved fermentation quality, but also aerobic stability of oats-common vetch mixed silage on the Tibetan plateau.

Key words: aerobic stability, fermentation quality, lactic acid bacteria, oat-common vetch mixture, propionic acid.

INTRODUCTION

The Tibetan plateau occupies 2.5 million km² (approximately 25% of the P.R. China). About 70% is high-altitude, cold, alpine rangeland (Cao *et al.* 2011), an average altitude of over 4000 m where it is regarded as the highest unique territorial unit in the world. Seldom crops are suitable for growing in the highland due to inherently extreme and unstable climate and natural environment, particularly facing frosts from November to early April. Therefore, forage production is limited and the shortage of feedstuffs has resulted in big seasonal body weight variations, low milk production and low fertility of animals. As a result, the development of animal husbandry is restricted in Tibet. Yak farmers have long been storing forage as hay for winter supplementary feed. However, dry matter (DM) and nutritive value losses often occur during the stage of hay making. In recent years, there has been growing interest in silage production in Tibet, mainly

made from cereal–legume mixtures for production feeding purposes in beef, dairy and lamb enterprises. Ensiling could not only facilitate year-round fodder provision and avoid nutrition loss (Titterton & Maasdorp 1997), but also decrease DM and nutritive value losses associated with hay making (Kaiser & Curl 1987).

Oats (*Avena sativa* L.) and common vetch (*Vicia sativa* L.) can be cultivated successfully on the Tibetan plateau due to hardy drought-tolerant characteristics. Farmers widely use the practice of intercropping to plant oats and common vetch in Tibet, as in other countries

Correspondence: Tao Shao, Institute of Ensiling and Processing of Grass, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China. (Email: taoshaolan@163.com)

Received 8 December 2013; accepted for publication 28 August 2014.

(Assefa & Ledin 2001; Lauriault & Kirksey 2004). In this way, year-to-year variation in the nutritive value of whole-crop cereal silage due to changing ear-to-straw ratio is likely to be less. Moreover, there is a reduced need to apply N fertilizer through the use of N-fixating legumes. The legumes component is sought to improve yield and crude protein (CP) content of the final forage (Lunnan 1989). Furthermore, silage of high quality can be made by ensiling mixtures of legumes and cereal crops (Kennelly & Weinberg 2003). Such silages are also suggested to improve rumen microbial yields and supply balanced protein and energy for rumen microbe growth (Adesogan & Salawu 2004).

Lactic acid bacteria (LAB) containing homofermentative bacteria have become popular silage additives as they are non-corrosive and easy to use. Natural epiphytic LABs are usually found in low numbers in fresh crops (Weinberg *et al.* 1993). Homofermentative bacteria reduce nutrient losses in ensiled forages by increasing the rate of lactate production and acidification. However, use of such inoculants can predispose silages to aerobic deterioration because they result in relatively greater levels of residual water-soluble carbohydrates (WSC) and lactate, which are used as growth substrates by spoilage-causing yeasts and molds (Huisden *et al.* 2009).

In Tibet, agricultural areas are far from pastoral areas, long distance transportation is essential, and yak farmers do not know how to feed silages scientifically. During long-distance transport and stages between opening silos and feeding, silages are often exposed to air for a long time, thus it is susceptible to aerobic deterioration. Propionic acid has the greatest antimicrobial activity among the short-chain fatty acids (Woolford 1975); it has been used as a forage preservative for many years when used at high rates (1.0–3.0%) on a DM basis and is deemed to be an effective inhibitor of aerobic deterioration of silage (Woolford 1984). The antimicrobial effect of propionic acid is enhanced as pH declines (Woolford 1975), making it an ideal candidate to improve the aerobic stability of silage when pH is low. Few studies have investigated the effect of additives on ensiling characteristics and aerobic stability of oat-common vetch mixed silage on the Tibetan plateau.

The objective of the present experiment was to study the effect of LAB and propionic acid on the fermentation quality and aerobic stability of oats-common vetch mixed silage on the Tibetan plateau.

MATERIAL AND METHODS

Silage preparation

Oats and common vetch were intercropped with ratio based on sowing rate (137 kg/ha) on 18 May 2012 in the experimental field of the Grassland Station of Rikaze (29.27°N,

88.88°E, Tibet, China); the ratio is regular in Tibet. The pH of the soil was 8.1, total N content of 5.4 g/kg, total potassium content of 13.0 g/kg, total P content of 0.7 g/kg. Oats were harvested at the milk stage (318 g DM/kg fresh weight) and common vetch harvested at podding stage (296 g DM/kg fresh weight). Crops were cut at approximately 5 cm above the ground by hand using a sickle. Legume plants were separated from oats plants and sampled as whole plants for microbiological analyses. Forage was chopped with a conventional forage harvester to a length of 2–3 cm and sampled to determine the DM content and chemical composition. The heights of herbage of oats and common vetch in the stand at harvest were 83 and 57 cm respectively, and the proportion of oats and common vetch in the intercrops on a fresh matter (FM) basis was 3:7. Mixed forage (309 g DM/kg fresh weight) was ensiled with four different treatments: no additive (control); lactic acid bacteria (*Lactobacillus plantarum*) addition at 10^6 colony-forming units (cfu)/g (L); propionic acid addition at 0.4% (P); and 10^6 cfu/g LAB + 0.4% propionic acid addition (PL) on a FM basis of mixed forage. Additives were diluted with deionized water and applied with a hand-held sprayer, and then forage samples were stirred manually. A similar quantity of deionized water was sprayed on the control forage. From each treatment of forage mixture, samples of 760 g were packed into a laboratory silo (polyethylene bottle, 1 L capacity), followed by being sealed with a screw top and plastic tapes, and then kept at ambient temperature. The silos for each treatment were opened on day 60 after ensiling, and then subjected to an aerobic stability test for 15 days. Triplicate silos were made for each treatment on each sampling day.

Chemical and microbiological analyses

Fresh forage and unfermented mixed forage were analyzed for chemical and microbiological composition. To measure fermentation indices, 35 g of each mixed silage was blended with 75 mL of deionized water extracted at 4°C for 24 h. Then, the extracts were filtered through two layers of cheesecloth and a filter paper (Xinhua Co, Hangzhou, China). The filtrates were used for determining pH, buffering capacity (BC), ammonia-N ($\text{NH}_3\text{-N}$), lactic acid (LA) and volatile fatty acids (VFAs) contents. The pH of the silage was measured with a glass electrode pH meter (HANNA pH 211, Hanna Instruments Italia Srl, Villafranca Padovana, Italy). Buffering capacity was determined by the hydrochloric acid-sodium hydroxide method of Playne and McDonald (1966). The DM content of unensiled forage samples and silage samples were determined by drying the samples at 65°C for 48 h to a constant mass, and then ground to pass through a 2 mm screen for later analysis. Crude ash (Ash) was determined by placing samples in a muffle furnace set at 500°C for 5 h. The WSC were determined by colorimetric analysis after reaction with anthrone reagent (Thomas 1977). The fermentation coefficient (FC) of each forage was predicted according to the formula: $\text{FC} = \text{DM}\% + 8\text{WSC}/\text{BC}$ (Weissbach & Honig 1996). $\text{NH}_3\text{-N}$ was determined using the phenol-hypochlorite reaction method of Broderick and Kang (1980). The methods of Van Soest *et al.* (1991) were used for neutral detergent fiber (NDF) and acid detergent fiber (ADF) analysis and the analyses were not sequential. Amylase and sodium sulfite were used in the NDF analysis and the results were expressed on a DM basis inclusive of ash. Total nitrogen (TN) was analyzed by the Kjeldahl procedure (Krishnamoorthy *et al.* 1982), CP was determined as the TN multiplied by 6.25. Ether extract (EE) was determined according to Horii *et al.*

(1971). The LA was determined by the method of Barker and Summerson (1941). VFAs were determined with gas chromatography (Shimadzu GC-17A, Shimadzu, Kyoto, Japan, with 30 m × 0.25 mm (df 0.25 µm) capillary column, acid-modified poly (ethylene glycol) phase, GADA-24107, Sigma-Aldrich Co., Tokyo, Japan; condition: column temperature 130°C, injection temperature 220°C). To assess the quality of the silage, we calculated the V-score from the NH₃-N/TN and VFA concentrations (Takahashi *et al.* 2005).

The mixed samples (10 g) were blended with 90 mL of sterilized water, and serially diluted in sterilized water. Enumeration of yeasts and LAB was done from the fresh forage and silages (day 60). The number of LAB were measured by plate count on Lactobacilli de Man, Rogosa, Sharpe (MRS) agar incubated at 30°C for 48 h under anaerobic conditions (Anaerobic box; YIHENG Technical co., Ltd, Shanghai, China). Yeasts were counted on potato dextrose agar (Sincere Biotech co., Ltd, Shanghai, China) and incubated for 48 h at 30°C. Colonies were counted as viable numbers of microorganisms (cfu/g of FM) from plates containing a minimum of 30 and a maximum of 300 colonies. All the microbiological data were log transformed.

Aerobic stability test

After a silo was once thoroughly emptied, half (by weight) of the content was put into a polythene bottle (0.5 L capacity) without compaction. The top was left uncovered and a conventional alcohol thermometer was placed in the centre of the silage. The bottles were kept in a room maintained at 25°C, and the temperature was monitored every 12 h. Spoilage was considered to have occurred if the difference between silage and surrounding air reached 2°C (Weinberg 2008). A total of 48 silos were opened at 60 days and then subjected to aerobic deterioration test for 15 days. Triplicate silages of each treatment were removed at 0, 5, 10 and 15 days after aerobic exposure, and the silages were sampled to determine pH values, LA, VFAS and WSC levels as well as yeast counts. These wet samples were stored at -20°C, except for the samples used to determine the microorganisms, which were evaluated immediately.

Statistical analyses

Analyses were performed using the general linear model procedure of SAS (SAS Institute Inc., Cary, NC, USA). Data

on chemical and microbiological composition of fresh and ensiled mixture of oats and common vetch were subjected to one-way analysis of variance (ANOVA). In aerobic conditions, experiments were carried out according to a randomized factorial design, the data were subjected to two-way ANOVA with treated silages and deterioration period as two factors. Differences among means were tested using Tukey's test. Differences were considered significant when probability was less than 0.05.

RESULTS

Chemical composition of materials

The chemical composition and microbial counts of oats, common vetch and their mixture before ensiling are presented in Table 1. The DM content of the mixture was 309 g/kg fresh weight. The WSC content was 164 g/kg DM. The BC and CP contents of the mixture were 306 mEq/kg DM and 13.5% DM respectively. The numbers of epiphytic LAB on the mixture were more than 1.0×10^5 cfu/g FM and yeasts were 1.0×10^4 cfu/g FM.

Fermentation quality of mixture silage after 60 days of ensiling

The fermentation quality and the microbial composition of mixed silages after 60 days of ensiling are presented in Table 2. All mixed silages showed low pH value, which was below 4.0, and PL silage had the lowest pH. L silage had the highest LA content followed by PL silage and then the control and P silages. In contrast, treatment with additives resulted in a marked decrease ($P < 0.05$) in the contents of acetic acid, and PL silage had the lowest acetic acid content. Lactic acid/acetic acid ratio was significantly higher ($P < 0.05$) in L and PL silages than that in the control silage, and PL silage had the highest LA /acetic acid content. P and PL silages showed higher ($P < 0.05$) propionic acid contents as compared with the control

Table 1 Chemical and microbial composition of herbage before being ensiled

Items	Oat	Common vetch	Mixture†
DM (%)	31.7	28.6	30.9
pH	6.17	5.68	5.79
WSC (g/kg DM)	183	113	164
Buffering capacity (mEq/kg DM)	245	463	306
Fermentation coefficient‡	37.7	30.6	35.2
Crude protein (% DM)	11.3	19.4	13.5
Ether extract (% DM)	4.96	4.44	4.85
Ash (% DM)	6.55	7.00	6.69
Neutral detergent fiber (% DM)	48.9	40.9	46.7
Acid detergent fiber (% DM)	25.1	21.0	24.0
Lactic acid bacteria (log ₁₀ cfu/g FM)	6.38	3.05	5.41
Yeast (log ₁₀ cfu/g FM)	4.94	3.76	4.60

†Mixture: 70% oat and 30% common vetch on a fresh matter basis of mixture. ‡Fermentation coefficient = DM% + 8WSC/BC. DM, dry matter; FM, fresh matter; WSC, water soluble carbohydrate.

Table 2 Fermentation characteristics and microbial composition of four mixed silages after 60 days of ensiling

Item	Treatments				SEM
	Control	L	P	PL	
pH	3.98a	3.83b	3.86ab	3.78c	0.02
Lactic acid (g/kg DM)	71.9b	100.7a	49.4c	98.6a	6.41
Acetic acid (g/kg DM)	18.7a	11.6c	15.1b	9.6d	1.06
Lactic acid /acetic acid	3.85c	8.69b	3.27c	10.28a	0.92
Propionic acid (g/kg DM)	5.18c	0.65d	12.81b	16.29a	1.86
Butyric acid (g/kg DM)	1.50a	0.81b	0.61b	0.81b	0.10
VFAS (g/kg DM)	25.4b	13.1c	28.5a	26.7b	1.84
WSC (g/kg DM)	40.3bc	35.9c	52.2a	48.0ab	2.16
NH ₃ -N (g/kg TN)	81.0a	70.9b	58.7c	49.5d	3.64
Lactic acid bacteria (log ₁₀ cfu/g FM)	6.30b	7.74a	5.71c	6.72b	0.14
Yeasts (log ₁₀ cfu/g FM)	< 2.00	< 2.00	< 2.00	< 2.00	0.00
V-score	86	94	91	93	1.03

Values in the same row (a–d) with different following letters are significantly different ($P < 0.05$). DM, dry matter; L, lactic acid bacteria (*Lactobacillus plantarum*); P, propionic acid; PL = lactic acid bacteria + propionic acid; SEM, standard error of the mean; TN, total nitrogen; WSC, water soluble carbohydrate; VFAS, volatile fatty acids.

Table 3 Chemical composition of four mixed silages after 60 days of ensiling

Item	Treatments				SEM
	Control	L	P	PL	
DM (%)	30.16a	28.18b	30.44a	28.94ab	0.33
Crude protein (% DM)	13.75b	14.86a	14.54a	14.99a	0.15
Ether extract (% DM)	5.71b	6.45a	5.67b	6.50a	0.14
Neutral detergent fiber (% DM)	45.20a	43.50b	45.52a	43.29b	3.06
Acid detergent fiber (% DM)	22.22a	21.85a	21.66a	20.14b	2.49
Ash (% DM)	7.17a	7.37a	7.14a	7.73a	0.04

DM, dry matter; L, lactic acid bacteria (*Lactobacillus plantarum*); P, propionic acid; PL = lactic acid bacteria + propionic acid; SEM, standard error of the mean. Values in the same row (a–d) with different following letters are significantly different ($P < 0.05$).

silage, while only small amounts of propionic acid were found in the L silage. The contents of butyric acid in the treated silages were significantly lower than that in the control silage ($P < 0.05$). The residual WSC content in P silage was significantly higher ($P < 0.05$) than that in the control silage, the residual WSC content in PL silage numerically higher than that in the control silage, and L had the lowest residual WSC content. The NH₃-N/TN ratio of treated silages were lower ($P < 0.05$) than that of the control silage, and lowest NH₃-N/TN ratio was found in PL silage (49.53 g/kg DM). The counts of LAB in L silage were as high as 10^7 cfu/g, which was higher ($P < 0.05$) than that in the control silage, while the counts of LAB in P silage were lower ($P < 0.05$) than that in the control silage. Yeasts populations in all mixed silages were reduced to below the detectable level ($<10^2$ cfu/g FM).

Chemical composition of four mixed silages after 60 days of ensiling is presented in Table 3. In addition, DM of L silage was lower ($P < 0.05$) than the control and P silages, while EE of L and PL silages were higher ($P < 0.05$) than that of the control or P silages. Silages treated with L and PL had lower ($P < 0.05$) contents of NDF as compared with the control and P silages, and silages treated with PL had lower ($P < 0.05$) content of ADF than that of other silages. No notable differences

Table 4 Effect of additives on the aerobic stability (2°C above the ambient temperature) of four mixed silages after exposure to air

Item	Treatment				SEM
	Control	L	P	PL	
Aerobic stability (h)	212b	206b	> 360a	> 360a	22.73

Values in the same row (a–d) with different following letters are significantly different ($P < 0.05$). L, lactic acid bacteria (*Lactobacillus plantarum*); P, propionic acid; PL = lactic acid bacteria + propionic acid; SEM, standard error of the mean.

occurred in Ash, while CP increased ($P < 0.05$) with addition of additives.

Effects of LAB and propionic acid application on aerobic stability

The aerobic stability of the mixture silages is presented in Table 4. The duration of aerobic stability of the silages ranged from 206 to > 360 h. The silage treated with L was the first to spoil, numerically reduced at 6 h as compared with the control silage. Treatment with P and PL markedly improved ($P < 0.05$) the aerobic stability of the silages. Both of them did not deteriorated by the end of the 15 days temperature-monitoring period.

Table 5 Chemical and microbial composition of four mixed silages after exposure to air

Item	Treatments	Days after unloading				Mean	Analysis of variance			SEM
		0	5	10	15		T	D	T × D	
pH	Control	3.99c	3.86c	4.60b	5.43a	4.47a	**	**	**	0.19
	L	3.83c	3.72c	4.39b	5.20a	4.29B				0.18
	P	3.86a	3.78a	3.77a	3.76a	3.79D				0.02
	PL	3.78b	3.81b	3.91b	4.25a	3.94C				0.06
Lactic acid (g/kg DM)	Control	71.86a	70.90ab	63.52ab	39.44b	61.43B	**	**	**	4.15
	L	100.69a	98.72a	73.86ab	51.40b	81.17A				6.20
	P	49.35a	54.31a	60.48a	52.60a	54.18B				1.39
	PL	98.64a	90.69a	93.14a	69.02b	87.87A				5.95
WSC (g/kg DM)	Control	40.29a	35.08a	25.69b	26.05b	31.78B	**	**	**	1.96
	L	35.91a	32.81a	30.51a	22.89c	30.53B				1.53
	P	52.21a	45.71a	38.54b	34.55b	42.75A				2.05
	PL	47.97a	44.75ab	40.96b	29.12c	40.70A				2.30
VFAs (g/kg DM)	Control	25.40a	28.75a	18.74b	9.15c	20.51C	**	**	**	2.26
	L	13.06b	21.15a	16.12ab	11.00b	15.33D				1.22
	P	28.49bc	38.00a	30.35b	23.37c	30.05A				1.79
	PL	26.71a	26.74a	24.95a	23.40a	25.45B				0.73
Yeasts (log ₁₀ cfu/g FM)	Control	2.00b	8.46a	8.75a	8.58a	6.95A	**	**	**	0.86
	L	2.00c	7.12c	8.09b	8.50a	6.42C				0.49
	P	2.00c	3.53b	4.27a	4.60a	3.60D				0.31
	PL	2.00c	4.60b	4.60b	4.74a	3.98B				0.64

** $P < 0.01$. Values in the same row (a–d) or in the same column (A–D) with different following letters are significantly different ($P < 0.05$). D, effect of deterioration period; DM, dry matter; L, lactic acid bacteria (*Lactobacillus plantarum*); P, propionic acid; PL = lactic acid bacteria + propionic acid; SEM, standard error of the mean; T, effect of treatment; VFAs, volatile fatty acids; WSC, water soluble carbohydrate.

Chemical and microbial compositions of four mixed silages after exposure to air are presented in Table 5. Yeast numbers were $> 1 \times 10^7$ cfu/g in the control and L silages, while yeast numbers were still $< 1 \times 10^5$ cfu/g in P and PL silages after aerobic exposure for 15 days. The LA content began to decrease at the beginning of the deterioration test in control silage and reached the lowest value at 15 days, quickly raising the pH from 3.99 to 5.43. Similar with the control silage, the LA content decreased gradually in L silage and reached the lowest value in the treated silages at the end of the aerobic stability test; the pH rose from 3.83 to 5.20. The LA content decreased significantly ($P < 0.05$) in PL silage at 15 days, and the pH increased significantly ($P < 0.05$) at 15 days. The LA content peaked until 10 days and then decreased in P silage. The pH was thus maintained as low as 4.25 until aerobic exposure was terminated after 15 days. The VFAS content decreased gradually in the control silage, the VFAS content in L silage increased significantly ($P < 0.05$) during the first 5 days of aerobic exposure, and then decreased, while the VFAS contents in P and PL silages did not change significantly. The WSC contents decreased gradually in all silages with time of aerobic exposure. After aerobic exposure, the L silage had the lowest WSC content and P silage had the highest WSC content.

DISCUSSION

Chemical composition of materials

The DM content of a crop influences strongly the rate and extent of the fermentation result; a clostridial fer-

mentation and subsequent poor acceptance of the silage by the animals might result from a low DM content with low sugar content at ensiling (Fraser *et al.* 2000). For proper ensiling, a material must have high content of WSC and adequate LAB prior to ensiling (Weinberg 2008). Well-fermented silages are produced from forages in which FCs are greater than 35, but are dependent on the nature of the epiphytic LAB number; a minimum number of LAB to suppress clostridial activity is suggested to be 1×10^5 cfu/g FM of herbage (Weissbach & Honig 1996). In the present study, the WSC, CP and organic matter contents of oats and common vetch were higher than that reported in the literature for oats and common vetch (Shao *et al.* 2005; Pursiainen & Tuori 2008), which might have resulted from the large diurnal differences in temperature and long hours of sunshine on the Tibetan plateau. Temperature and sunshine affect plant nutrient accumulation; high temperatures and long hours of sunshine favor photosynthesis to produce organic matter; low temperature at night can suppress forage respiration to decrease the content of organic matter degradation. Four mixture forage before ensiling contained high WSC content, proper DM content, FC was 35 and LAB numbers were more than 1×10^5 cfu/g FM, which is critical for a successful fermentation.

Effects of LAB and propionic acid application on fermentation quality

After ensiling for 60 days, the pH of the four mixture silages reduced to 4.2 or less, which was

characteristic of well-preserved silages (Weinberg 2008). Ammonia-N production is related to CP degradation in all silages, which reveals the extent of proteolysis in silage (Wilkinson 2005) and well-preserved silages should have less than 100 g NH₃-N/kg TN (McDonald *et al.* 2002). In the present study all silages had values less than 81 g NH₃-N/kg TN, indicating that extensive protein proteolysis is unlikely to have occurred. Compared to the untreated silage, the LAB additive significantly increased the LA contents in the L and PL silages, which might be attributed to higher WSC content that could provide more substrates for LAB fermentation, thus allowing a rapid production of lactic acid by *L. plantarum*, and suppresses the buffering effect of legumes as suggested by Adesogan and Salawu (2002). Lower contents of acetic acid and higher LA/acetic acid ratio in L and PL silages as compared with untreated silage indicated a more homofermentative process. Muck and Kung (1997) concluded that microbial inoculation lowered the pH and improved the LA/acetic acid ratio in more than 60% of studies conducted between the years 1990 to 1995. In contrast, lower LA content in P silage might be attributed to inhibition of LAB activity by propionic acid. Britt *et al.* (1977) reported that LA contents of silages were decreased ($P < 0.01$) by addition of propionic acid, which suggests an inhibition of microbial activity. P silage also showed significantly ($P < 0.05$) lower acetic acid content as compared with the control silage. This might be explained as propionic acid application suppressed acetic acid producing bacteria activity during fermentation (Woelford 1975). The WSC are the main source of food for microorganisms during silage fermentation. Propionic acid is a potential antimycotic agent; during the early stage of ensiling, propionic acid could effectively inhibit the undesirable microorganisms activity, resulting in minimizing the consumption of WSC by undesirable microorganisms (Woelford 1975; Moon 1983). A higher butyric acid content was found in the control silage, which might be attributed to clostridial fermentation in the early stage of ensiling; this corresponds with higher NH₃-N content in the control silage.

Crude ash content increased a little throughout the experiment, which might be attributed to DM loss. Garcia *et al.* (1989) reported that the increase in ash content as percentage of DM is indicated by DM loss. Consistent with the findings of Bilal (2009), the CP of the treated mixed silages also increased, which might be attributed to efficient fermentation, preservation and stability of silage, reducing protein breakdown by plant enzymes and the growth of proteolytic microorganisms, the increase in CP content as percentage of DM might be also indicated by DM loss. The NDF contents of the L and PL silages were much lower than that of the control and P silages, which might have been a result of increased microbial respiration and

fermentation of the fiber fraction. This result is consistent with that of Mandebvu *et al.* (1999). Silage treated with PL had lower ($P < 0.05$) content of ADF than that of other silages and could be attributed to a combination of increased fermentation and acid hydrolysis of the fiber fraction which was consistent with results of Baytok *et al.* (2005). The numbers of yeasts in the final silages were very low, which is below the detectable level indicating strictly anaerobic conditions during ensiling. Similar findings have been reported by Pursiainen and Tuori (2008). The result showed that L and PL silages had better fermentation quality as indicated by higher LA and CP content, lower pH and acetic acid content than other silages.

Effects of LAB and propionic acid application on aerobic stability

When fermentation is completed and silage is exposed to air during feedout or storage (leaky silos, holes in bag silos, poorly packed silage), heating in the silo and feed bunk is usually initiated by yeasts. (Ranjit & Kung 2000). Changes occur in chemical and microbial composition, when the aerobically stable and yeast-contaminated silage is exposed to air. As the climate is inherently unstable and there are large differences in day and night temperatures on the Tibetan plateau, it is difficult to control the temperature change of silage in the open air. Measuring the temperature change of silages in the open air could not properly reflect their aerobic stability. In addition, there is no information on evaluating aerobic stability of oat-common vetch mixed silage by measuring the temperature change on the Tibetan plateau. Therefore, our assessment of the aerobic stability of oats-common vetch mixed silage was based on changes in sample temperature under laboratory conditions as well as changes in chemical composition and microbial populations with time of aerobic exposure in the open air.

According to the evidence that silages of greater levels of LA or those with more residual sugar contents were less stable when exposed to air (Huisden *et al.* 2009), it was anticipated that the control and L silages would deteriorate rapidly. The same result was observed in the control and L silages. The pH of the control and L silages increased gradually with the time of aerobic exposure which might be attributed to the decreasing of LA content. The pH was an indicator of aerobic deterioration of the silage because LA was consumed by yeasts during aerobic exposure, and then the silage becomes suitable for the growth of other undesirable microorganisms such as mold and aerobic bacteria (Basso *et al.* 2012). However, the opposite result was observed in P-treated mixed silage. The pH value of P silage did not change throughout the whole stage of aerobic exposure, which might be attributed to the increase of LA content. During the 15 days of exposure to air, LA content in P silage gradually

increased until 10 days and then decreased. This increase might be attributed to the volatilization and/or metabolism of propionic acid which resulted in lactate producers using the residual readily available carbohydrate during the normal fermentation periods. Yeasts have long been thought to be responsible for the aerobic deterioration of silage and silage containing more than 1×10^5 cfu of yeasts/g is prone to undergo aerobic deterioration once exposed to air (McDonald *et al.* 1991). Our results confirmed lower yeast numbers in P and PL silages as compared with the control and L silages on aerobic exposure for 5, 10 and 15 days. Yeast populations in P and PL silages were maintained below 10^5 cfu/g FM during the aerobic exposure. This may be due to propionic acid which is an antimicrobial agent, and high content of propionic acid could inhibit yeast growth, which is consistent with the findings of Huber and Soejono (1977). Thus, P and PL silage showed the greatest aerobic stability (> 360 h) of all the silages. L silage numerically reduced aerobic stability for 6 h as compared with the control silage which might be attributed to the lower content of VFAs as compared with the control silage during the aerobic exposure stage. Moon (1983) reported that VFAs could protect silage against aerobic yeasts and molds. The result of the aerobic stability test showed that P and PL silages were better for aerobic stability as indicated by longest aerobic stable hours, higher LA content, lower pH and yeasts numbers than other silages.

Conclusions

According to the results of our study, it was concluded that adding LAB increased the LA and CP content during ensiling, and had no effect on the aerobic stability of oats-common vetch mixed silage. Applying propionic acid decreased LA content during ensiling, and preserved more WSC which stimulated LA production during aerobic exposure stage, thus applying propionic acid significantly improved aerobic stability of oats-common vetch mixed silage. Applying LAB together with propionic acid could not only improve the fermentation quality, but also aerobic stability of oats-common vetch mixed silage on the Tibetan plateau.

ACKNOWLEDGMENTS

This work was supported by the grant for key techniques research in straw-grass mixed silage from Tibet (XZ20093ZD), National '12th Five-Year' Plan for Science and Technology: Comprehensive treatment key technology and demonstration project of the Degraded grassland in north Tibetan Plateau (2011BAC09B03) and Tibet innovation platform construction by Chinese Academy of Sciences 'construction and demonstration of agriculture and animal

husbandry combination technology to promote the income of farmers and herdsmen in Tibet'.

REFERENCES

- Adesogan AT, Salawu MB. 2002. The effect of different additives on the fermentation quality, aerobic stability and in vitro digestibility of pea/wheat bi-crop silages containing contrasting pea to wheat ratios. *Grass and Forage Science* **57**, 25–32.
- Adesogan AT, Salawu MB. 2004. Effect of applying formic acid, heterolactic bacteria or homolactic and heterolactic bacteria on the fermentation of bi-crops of peas and wheat. *Journal of the Science of Food and Agriculture* **84**, 983–992.
- Assefa G, Ledin I. 2001. Effect of variety, soil type and fertiliser on the establishment, growth, forage yield, quality and voluntary intake by cattle of oats and vetches cultivated in pure stands and mixtures. *Animal Feed Science and Technology* **92**, 95–111.
- Barker SB, Summerson WH. 1941. The colorimetric determination of lactic acid in biological material. *Journal of Biological Chemistry* **138**, 535–554.
- Basso FC, Bernardes TF, Roth APDTP, Lodo BN, Berchielli TT, Reis RA. 2012. Fermentation and aerobic stability of corn silage inoculated with *Lactobacillus buchneri*. *Revista Brasileira de Zootecnia* **41**, 1789–1794.
- Baytok E, Aksu T, Karsli MA, Muruz H. 2005. The effects of formic acid, molasses and inoculant as silage additives on corn silage composition and ruminal fermentation in sheep. *Turkish Journal of Veterinary and Animal Science* **29**, 469–474.
- Bilal MQ. 2009. Effect of molasses and corn as silage additives on the characteristics of mott dwarf elephant grass silage at different fermentation periods. *Parameters* **45**, 19–23.
- Britt DG, Huber JT, Rogers AL. 1977. Fungal growth and acid production during fermentation and re-fermentation of organic acid treated corn silages. *Journal of Dairy Science* **58**, 532–539.
- Broderick GA, Kang JH. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. *Journal of Dairy Science* **63**, 64–75.
- Cao J, Holden NM, Lü XT, Du G. 2011. The effect of grazing management on plant species richness on the Qinghai-Tibetan Plateau. *Grass and Forage Science* **66**, 333–336.
- Fraser MD, Fychan R, Jones R. 2000. Voluntary intake, digestibility and nitrogen utilization by sheep fed ensiled forage legumes. *Grass and Forage Science* **55**, 271–279.
- Garcia AD, Olson WG, Otterby DE, Linn JG, Hansen WP. 1989. Effects of temperature, moisture, and aeration on fermentation of alfalfa silage. *Journal of Dairy Science* **72**, 93–103.
- Horii S, Kurata Y, Hayashi Y, Tanabe S. 1971. Physicochemical analytical method for nutritional experiments. In: Morimoto H (ed.), *Animal Nutrition Testing Method*, 1st edn. pp. 280–298. Yokendo, Tokyo. (In Japanese)
- Huber JT, Soejono M. 1977. Organic acid treatment of high dry matter corn silage fed to lactating dairy cows. *Journal of Dairy Science* **59**, 2063–2070.
- Huisden CM, Adesogan AT, Kim SC, Ososanya T. 2009. Effect of applying molasses or inoculants containing homo-fermentative or heterofermentative bacteria at two rates

- on the fermentation and aerobic stability of corn silage. *Journal of Dairy Science* **92**, 690–697.
- Kaiser AG, Curll ML. 1987. Improving the efficiency of forage conservation from pastures. In: Wheeler JL, Pearson CJ, Robards GE (eds), *Temperate Pastures: Their Production, Use and Management*, pp. 397–411. CSIRO Publishing, Melbourne.
- Kennelly JJ, Weinberg ZG. 2003. Small grain silage. In: Buxton DR, Muck RE, Harrison JH (eds), *Silage Science and Technology*, pp. 749–779. ASA-CSSA-SSSA, Madison, WI.
- Krishnamoorthy U, Muscato TV, Sniffen CJ, Van Soest PJ. 1982. Nitrogen fractions in selected feedstuffs. *Journal of Dairy Science* **65**, 217–225.
- Lauriault LM, Kirksey RE. 2004. Yield and nutritive value of irrigated winter cereal forage grass-legume intercrops in the Southern High Plains, USA. *Agronomy Journal* **96**, 352–358.
- Lunnan T. 1989. Barley-pea mixtures for whole crop forage. Effects of different cultural practices on yield and quality. *Norwegian Journal of Agricultural Sciences* **3**, 57–71.
- Mandebvu P, West JW, Froetschel MA, Hatfield RD, Gates RN, Hill GM. 1999. Effect of enzyme or microbial treatment of bermudagrass forages before ensiling on cell wall composition, end products of silage fermentation and in situ digestion kinetics. *Animal Feed Science and Technology* **77**, 317–329.
- McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA. 2002. *Animal Nutrition*, 6th edn. Longman Scientific and Technical, Harlow.
- McDonald P, Henderson AR, Heron SJE. 1991. Microorganisms. In: McDonald P, Henderson AR, Heron SJE (eds), *The Biochemistry of Silage*, pp. 81–151. Chalcombe Publications, Marlow.
- Moon NJ. 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. *Journal of Applied Microbiology* **55**, 453–460.
- Muck RE, Kung Jr L. 1997. Effects of silage additives on ensiling. In: Muck RE, Kung Jr L (eds), *Proceedings of the Silage: Field to Feed Bunk*, pp. 187–199. NRAES-99. Northeast Regional Agricultural Engineering Service, Ithaca, NY.
- Playne MJ, McDonald P. 1966. The buffering constituents of herbage and silage. *Journal of Science Food and Agriculture* **17**, 264–266.
- Pursiainen P, Tuori M. 2008. Effect of ensiling field bean, field pea and common vetch in different proportions with whole-crop wheat using formic acid or an inoculant on fermentation characteristics. *Grass and Forage Science* **63**, 60–78.
- Ranjit NK, Kung JL. 2000. The effect of lactobacillus buchneri, lactobacillus plantarum, or a chemical preservative on the fermentation and aerobic stability of corn silage. *Journal of Dairy Science* **83**, 526–535.
- Shao T, Shimojo M, Wang T, Masuda Y. 2005. Effect of additives on the fermentation quality and residual mono- and di-saccharides compositions of Forage Oats (*Avena sativa* L.) and Italian Ryegrass (*Lolium multiflorum* Lam.) silages. *Asian Australasian Journal of Animal Sciences* **18**, 1582–1588.
- Takahashi T, Horiguchi K, Goto M. 2005. Effect of crushing unhulled rice and the addition of fermented juice of epiphytic lactic acid bacteria on the fermentation quality of whole crop rice silage, and its digestibility and rumen fermentation status in sheep. *Animal Science Journal* **76**, 353–358.
- Thomas TA. 1977. An automated procedure for the determination of soluble carbohydrates in herbage. *Journal of Science of the Food and Agriculture* **28**, 639–642.
- Titterton M, Maasdorp BV. 1997. Nutritional improvement of maize silage for dairying: mixed crop silages from sole and intercropped legumes and a long season variety of maize. 2. Ensilage. *Animal Feed Science and Technology* **69**, 263–270.
- Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597.
- Weinberg ZG. 2008. Preservation of forage crops by solid-state lactic acid fermentation-ensiling. In: Weinberg ZG (ed.), *Current Developments in Solid-State Fermentation*, pp. 443–467. Springer, New York.
- Weinberg ZG, Ashbell G, Azrieli A, Brukental I. 1993. Ensiling peas, ryegrass and wheat with additives of lactic acid bacteria (LAB) and cell wall degrading enzymes. *Grass and Forage Science* **48**, 70–78.
- Weissbach F, Honig H. 1996. On the anticipation and control of the run of fermentation in silage making from extensively grown forages. *Landbauforschung V lkenrode* **46**, 10–17.
- Wilkinson JM. 2005. Silage. Chapter 19 In: Wilkinson JM (ed.), *Analysis and Clinical Assessment of Silage*, pp. 198–208. Chalcombe Publications, Marlow.
- Woolford MK. 1975. Microbiological screening of straight chain fatty acids (C₁–C₁₂) as potential silage additives. *Journal of Science of the Food and Agriculture* **26**, 219–228.
- Woolford MK. 1984. Managing aerobic deterioration in silage. In: McCullough ME, Bolsen KK (eds), *Silage Management*, pp. 42–75. National Feed Ingredients Association, Silage Technology Division, West Des Moines, IA.