ORIGINAL ARTICLE

The effects of replacement of whole-plant corn with oat and common vetch on the fermentation quality, chemical composition and aerobic stability of total mixed ration silage in Tibet

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ABSTRACT

The objective of this experiment was to evaluate the effects of replacement of whole-plant corn with oat and common vetch on the fermentation quality, chemical composition and aerobic stability of total mixed ration (TMR) silage in Tibet. Four TMR that varied in the forage sources on dry matter basis were used: (i) 52% whole-plant corn (Control); (ii) 43% oat + 12% common vetch (OC3.6); (iii) 38% oat + 18% common vetch (OC2.2); and (iv) 33% oat + 23% common vetch (OC1.5). Silos were opened on day 45 and then subjected to an aerobic stability test for 12 days. The results showed that all silages were well preserved with low pH and $\text{NH}_3\text{-N}$, and high lactic acid and V-scores. With the increasing proportion of common vetch, crude protein, ether extract increased ($P < 0.05$), and neutral detergent fiber and acid detergent fiber decreased ($P < 0.05$). Under aerobic conditions, treated silages were more stable than the control silage as indicated by lower ($P < 0.05$) pH and yeast population. It was concluded that replacement of whole-plant corn with oat and common vetch had no unfavorable effects on the fermentation quality and improved crude protein content and aerobic stability of TMR silage. OC2.2 silage was the best among three treated TMR silages.

Key words: aerobic stability, common vetch, fermentation quality, oat, whole-plant corn.

INTRODUCTION

The Tibetan plateau located in southwest China with an average altitude of over 4000 m (Duan *et al.* 2008), is regarded as the highest unique territorial unit in the world. The development of animal husbandry in Tibet is relatively backward. On the Tibetan Plateau, dairy cows are mainly bred by yak farmers in the pastoral areas and provide > 90% of milk consumption for the local people. Shortage of feedstuffs is due to seasonal changes throughout the year and yak farmers do not know how to reasonably match roughage and concentrate to feed dairy cows, which results in the fluctuation in production performance of dairy cows. In recent years, total mixed ration (TMR) silage has been widely applied to feeding dairy cows in Tibet, particularly containing whole-plant corn as roughage. TMR silage application can not only facilitate year-round fodder provision and improve nutritive value of forage, but also make up for the inadequacy of feeding

roughage and concentrate separately. However, the cultivated areas for whole-plant corn are limited and with low yields, and local whole-plant corn resources cannot meet the needs of the roughage. Thus, whole-plant corn demand is mainly supplied by importing from inland China. This adds an extra cost to feeding.

Oat (*Avena sativa*) and common vetch (*Vicia sativa*) are the main cultivated forage in Tibet. Both of them have a long planting history and are suitable for growth in Tibet due to hardy drought-tolerant characteristics. Oat and common vetch contain high crude protein (CP) concentrations. However, common vetch

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is seldom used as material to make silage in Tibet as legumes are often considered hard to ensile well due to low fermentable carbohydrate content in combination with a high buffering capacity (BC) (McDonald *et al.* 1991; Buxton & O'Kiely 2003). Because of the chemical composition of whole-crops cereal, mainly in high fermentable carbohydrates, silage of high quality can be made by ensiling mixtures of legumes and cereal crops (Kennelly & Weinberg 2003). Moreover, a mixture of whole-crop cereals and legume silage is likely to have a higher nutritive value as compared with silage produced from separated crops due to higher CP concentration (Lunnan 1989) and nutrient degradability in the rumen (Mustafa *et al.* 2000; Salawu *et al.* 2001), and ruminants tend to eat more of mixed forage than sole forage rations (Huhtanen *et al.* 2007). Such silages are also suggested to improve rumen microbial yields and supply balanced protein and energy for rumen microbe growth (Adesogan & Salawu 2004). Thus, we replaced whole-plant corn with oat and common vetch in combination with a concentrate to make TMR silage. Using this method can solve the problem of roughage restriction and improve nutritive value of forage in Tibet. In previous studies, oat and common vetch were ensiled mainly as single crops (Mustafa *et al.* 2002; Shao *et al.* 2005). However, there is no information on effects of replacing of whole-plant corn with oat and common vetch on fermentation quality, aerobic stability and nutritive value of TMR in Tibet.

The objective of this study was to determine effects of replacement of whole-plant corn with oat and common vetch on the fermentation quality, chemical composition and aerobic stability of total mixed ration in Tibet.

MATERIALS AND METHODS

Silage preparation

Whole-plant corn, oat, common vetch were cultivated in the experimental field of the Grassland Station of Rikaze (29.27°N, 88.88°E, 3836 m a.s.l. Tibet, China); whole-plant corn was harvested at the one-half milk line stage (227 g

DM/kg fresh weight); oat was harvested at the heading stage (318 g DM/kg fresh weight) and common vetch was harvested at the podding stage (296 g DM/kg fresh weight). Forage was chopped with a conventional forage harvester to lengths of 2–3 cm. The chemical composition of ingredients for TMR is shown in Table 1. The formulation and chemical composition of TMR are shown in Table 2. The control TMR is a regular ration formulation in Tibet, and it contains low CP content. We aimed to maximize the use of the common vetch, the proportion of common vetch increasing gradually in the treated TMR. The CP was kept at the same level in the treated TMR. Experimental treatments included four TMR that were varied in the forage sources on a DM basis: (i) 52% whole-plant corn (Control); (ii) 43% oat + 12% common vetch (OC3.6; oat: common vetch = 3.6:1); (iii) 38% oat + 18% common vetch (OC2.2; oat: common vetch = 2.2:1); and (iv) 33% oat + 23% common vetch (OC1.5; oat: common vetch = 1.5:1). From each treatment of the TMR mixture, samples of 190 g on a fresh matter base were packed into a laboratory silo (polyethylene bottle, 250 mL 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 at 45 days after ensiling, and then subjected to an aerobic stability test for 12 days. Three replicates per treatment on each sampling day were used for silage quality and aerobic stability analysis.

Chemical and microbiological analyses

Fresh forage, unensiled TMR and TMR silage were analyzed for chemical and microbiological composition. To measure fermentation indices, 35 g of each TMR silage was blended with 75 mL of deionized water extracted at 4°C for 24 h. Then, the extracts were filtered through 2 layers of cheese-cloth and a filter paper (Xinhua Co., Hangzhou, China). The filtrates were used for determining pH, BC, ammonia nitrogen (NH₃-N), lactic acid and volatile fatty acids (VFAs) concentrations. The pH of the silage was measured with a glass electrode pH meter (HANNA pH 211; Hanna Instruments Italia Srl, Villafrance Padovana, Italy). Buffering capacity was determined by the hydrochloric acid-sodium hydroxide method of Playne and McDonald (1966). Ammonia nitrogen was determined using the phenol-hypochlorite reaction method of Broderick and Kang (1980). The lactic acid was determined by the method of Barker and Summerson (1941). Volatile fatty acids were determined with gas chromatography (Shimadzu GC-17A, Kyoto, Japan, with 30 m × 0.25 mm (film thickness 0.25 µm) capillary column, acid-modified poly (ethylene glycol) phase, GADA-24107,

Table 1 Chemical composition of whole-plant corn, oat, common vetch and mixed concentrate in total mixed ration

	Whole plant corn	Oat	Common vetch	Mixed concentrate†
DM (%)	22.7	31.8	29.6	91.6
CP (% DM)	5.10	11.3	19.4	22.5
EE (% DM)	5.50	4.96	4.44	8.10
aNDFom (% DM)	46.7	43.5	35.7	34.2
ADFom (% DM)	21.0	21.2	16.6	9.31
Hemicellulose (% DM)	25.7	22.3	19.1	24.9
Ash (% DM)	4.52	6.55	7.00	9.09
NFC‡	38.2	33.7	33.5	26.1

†Mixed concentrate: 7.5% Crack corn, 20% rape cake meal, 20% cotton seed, 27.5% distillers dried grains with solubles, 20% wheat bran, 5% vitamin-mineral. ‡NFC (non-fibrous carbohydrate) = 100-CP-aNDFom-EE-Ash. ADFom, acid detergent fiber; aNDFom, neutral detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract.

Table 2 Ingredient and chemical composition of total mixed ration

Item	Treatments				SEM
	Control	OC3.6	OC2.2	OC1.5	
Ingredient composition (% DM)					
Whole-crop corn	52				
Oat		43	38	33	
Common vetch		12	18	23	
Mixed concentrate‡	48	45	44	44	
Chemical composition (% DM)					
DM	35.4C	44.6A	43.8B	43.6B	1.17
CP	13.8B	17.4A	17.7A	18.1A	0.531
EE	6.79A	6.34A	6.26A	6.21A	0.092
aNDFom	41.1A	38.5A	38.1A	37.4A	0.567
ADFom	15.6A	15.3A	15.2A	14.8A	0.272
Hemicellulose	25.6A	23.2B	22.9B	22.6B	0.391
Ash	6.75B	7.78A	7.77A	7.75A	0.142
NFC‡	31.6A	30.0A	30.2A	30.5A	0.415

†Mixed concentrate: 7.5% Crack corn, 20% rape cake meal, 20% cotton seed, 27.5% distillers dried grains with solubles, 20% wheat bran, 5% vitamin-mineral. ‡NFC (non-fibrous carbohydrate) = 100-CP-aNDFom-EE-Ash. Control = 52% whole-plant corn and 48% concentrate, OC3.6 = 43% oat + 12% common vetch + 45% concentrate, OC2.2 = 38% oat + 18% common vetch + 44% concentrate, OC1.5 = 33% oat + 23% common vetch + 44% concentrate. Values in the same row (A–D) with different following letters are significantly different ($P < 0.05$). ADFom, acid detergent fiber; aNDFom, neutral detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; SEM, standard error of the mean.

Sigma-Aldrich Co., Kyoto, Japan; condition: column temperature 130°C, injection temperature 220°C). DM content of unsiled forage samples and TMR silage samples were determined by drying the samples at 60°C for 48 h to a constant mass, which was corrected for loss of volatiles using the equation of Porter and Murray (2001), and then ground to pass through a 2 mm screen for later analysis. DM recovery of 45 days TMR silages was estimated by comparing the product of forage mass and forage DM contents before and after ensiling for each silo. Crude ash (Ash) was determined by placing samples in a muffle furnace set at 500°C for 5 h. The water-soluble carbohydrates (WSC) were determined by colorimetric after-reaction with anthrone reagent (Thomas 1977). The methods of Van Soest *et al.* (1991) were used for neutral detergent fiber (aNDFom) and acid detergent fiber (ADFom) analysis and the analyses were not sequential. Amylase and sodium sulfite were used in the aNDFom analysis and the results were expressed on a DM basis without residual ash. Hemicellulose = aNDFom – ADFom. 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) content was determined according to Horii *et al.* (1971). Non-fibrous carbohydrate (NFC) was calculated by the formula: $NFC = 100 - CP - aNDFom - EE - Ash$ (NRC 2001). To assess the quality of the silage, we calculated the V-score from the $NH_3-N/Total\ N$ and VFA concentrations (Takahashi *et al.* 2005).

The TMR samples (10 g) were blended with 90 mL of sterilized water, and serially diluted in sterilized water. Enumeration of yeasts and lactic acid bacteria (LAB) was done from the fresh TMR and silages (day 45). 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), incubated for 48 h at 30°C. Colonies were counted as viable numbers of microorganisms (cfu/g of fresh matter (FM))

from plates containing a minimum of 30 and a maximum of 300 colonies. All the microbiological data were $\log_{10}$ transformed.

Aerobic stability test

The climate and natural environment are inherently unstable with large differences in temperature between day and night on the Tibetan plateau. The annual average air temperature was 2.38°C with extremes of a 25.28°C high and a –32.58°C low, and without an absolute frost-free period. During the experiment, the average daytime air temperature was 16.8°C and it was –5.6°C at night. We kept the silos in the environmental temperature and it is difficult to control the temperature change. Measuring the temperature change of silages could not properly reflect the aerobic stability. Thus we used the following method to determine aerobic stability of TMR silages. The aerobic stability was defined as the number of hours that the pH value of the silage remained stable before rising more than 0.5 above the initial pH value (Zhang & Kumai 2000; Weinberg *et al.* 2008). Aerobic stability was measured by placing representatively sampled (disturbed) forage from each silo into plastic bags within an open-top polystyrene box. A total of 48 silos were opened at 45 day and then subjected to aerobic deterioration test for 12 days. During the aerobic exposure stage, triplicate silages of each treatment were sampled on 0, 6, 9 and 12 days to determine pH values, lactic acid content, 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 (SAS Institute, Cary, NC, USA). Data on chemical and microbiological composition of fresh and ensiled TMR were subjected to one-way analysis of variance (ANOVA). In aerobic conditions, experiments were carried out according

Table 3 Chemical and microbial composition of four total mixed ration mixtures before being ensiled

Item	Treatments				SEM
	Control	OC3.6	OC2.2	OC1.5	
pH	5.29B	5.55A	5.54A	5.47A	0.033
WSC (% DM)	16.5A	11.2B	11.0B	10.8B	0.742
Buffering capacity (mE/kg DM)	231C	271B	285AB	296A	7.72
Lactic acid bacteria (log ₁₀ cfu/g FM)	5.56A	5.14A	5.52A	5.14A	0.091
Yeast (log ₁₀ cfu/g FM)	4.59A	3.54BC	2.96C	3.84AB	0.189

Control = 52% whole-plant corn and 48% concentrate, OC3.6 = 43% oat + 12% common vetch + 45% concentrate, OC2.2 = 38% oat + 18% common vetch + 44% concentrate, OC1.5 = 33% oat + 23% common vetch + 44% concentrate. Values in the same row (A–D) with different following letters are significantly different ($P < 0.05$).

DM, dry matter; FM, fresh matter; SEM, standard error of the mean; WSC, water soluble carbohydrate.

Table 4 Chemical and microbial composition of total mixed ration silages after 45 days of ensiling

Item	Treatments				SEM
	Control	OC3.6	OC2.2	OC1.5	
DM recovery (g/kg, FM)	91.7B	97.5A	97.7A	97.5A	0.772
pH	3.90B	4.22A	4.19A	4.14A	0.004
Lactic acid (% DM)	8.65A	6.81B	6.21B	6.79B	0.285
Acetic acid (% DM)	1.22A	1.07AB	1.04AB	0.826B	0.050
Lactic acid /Acetic acid	7.10AB	6.53B	5.99B	8.25A	0.029
Propionic acid (% DM)	0.017	0.014	0.021	0.027	0.002
Butyric acid (% DM)	0.019	ND	ND	ND	
VFAs (% DM)	1.26A	1.08B	1.06B	0.852C	0.044
WSC (% DM)	3.99C	5.39B	5.94A	5.55B	0.224
NH ₃ -N (% TN)	5.28AB	5.53A	5.17AB	4.79B	0.100
Lactic acid bacteria (log ₁₀ cfu/g FM)	6.30A	6.74A	6.71A	6.72A	0.014
Yeast (log ₁₀ cfu/g FM)	< 2.00	< 2.00	< 2.00	< 2.00	
V-score	97	96	97	98	0.023

Control = 52% whole-plant corn and 48% concentrate, OC3.6 = 43% oat + 12% common vetch + 45% concentrate, OC2.2 = 38% oat + 18% common vetch + 44% concentrate, OC1.5 = 33% oat + 23% common vetch + 44% concentrate. Values in the same row (A–D) with different following letters are significantly different ($P < 0.05$). DM, dry matter; ND, not detected; SEM, standard error of the mean; VFAs, volatile fatty acids; WSC, water soluble carbohydrate.

to completely randomized factorial design with TMR and deterioration period as two factors. The data were subjected to two-way analysis of variance. Statistical difference between means was determined by Tukey's multiple comparison. Differences were considered significant when probability was less than 0.05.

RESULTS

Chemical composition of materials and TMR before ensiling

As shown in Table 1, the contents of DM and CP of the whole-plant corn were less than those in the oat, common vetch and mixed concentrate. Levels of DM, CP, EE were highest in the mixed concentrate. Among the four materials, aNDFom and NFC were highest in the whole-plant corn.

In TMR before ensiling, no notable differences occurred in EE, aNDFom and ADFom, but DM, Ash and CP increased ($P < 0.05$), and hemicellulose ($P < 0.05$) decreased by replacement of whole-plant corn with oats and common vetch (Table 2). The pH and BC for the three treated TMR were higher ($P < 0.05$) than that for the control TMR and OC1.5

TMR had the highest ($P < 0.05$) BC. The WSC contents of the three treated TMR were lower ($P < 0.05$) than that of the control (Table 3). The LAB populations were not different among the four TMR, while the control TMR had higher ($P < 0.05$) yeast population than OC3.6 and OC 2.2 TMR.

Fermentation quality and aerobic stability

The four silages were well preserved, as indicated by low pH values and NH₃-N/TN ratio, high lactic acid contents as well as V-scores (Table 4). Although levels of propionic acid and V-scores did not differ, pH values, lactic acid contents, acetic acid contents, WSC contents and NH₃-N/TN ratio did ($P < 0.05$), with pH values ranging from 3.90 to 4.22. The lactic acid contents of the three treated silages were lower ($P < 0.05$) than that of control silage. The acetic acid content for the OC1.5 silage in particular was lower ($P < 0.05$) than that for the control silage. Lactic acid /acetic acid ratio was significantly higher in OC1.5 silage than that in OC3.6 and OC2.2 silages. Total VFA contents were lower in treated silages than that in the control silage

Table 5 Chemical composition of four total mixed ration silages after 45 days of ensiling

Item	Treatments				SEM
	Control	OC3.6	OC2.2	OC1.5	
DM (%)	32.7C	43.8A	42.9B	43.0B	1.39
CP (% DM)	14.2C	18.0B	19.4A	19.0A	0.631
EE (% DM)	7.71B	7.77B	8.78A	9.16A	0.202
aNDFom (% DM)	39.5A	31.5B	30.3B	30.3B	1.24
ADFom (% DM)	15.2A	13.6AB	13.0B	13.5AB	0.319
Hemicellulose (% DM)	24.3A	18.0B	17.4B	16.7B	1.08
Ash (% DM)	8.06A	8.07A	8.14A	8.13A	0.032
NFC (% DM)	30.5A	33.7A	33.4A	34.5A	0.628

NFC (non-fibrous carbohydrate) = 100-CP-aNDFom-EE-Ash; Control = 52% whole-plant corn and 48% concentrate, OC3.6 = 43% oat + 12% common vetch + 45% concentrate, OC2.2 = 38% oat + 18% common vetch + 44% concentrate, OC1.5 = 33% oat + 23% common vetch + 44% concentrate. Values in the same row (A–D) with different following letters are significantly different ($P < 0.05$). ADFom, acid detergent fiber; aNDFom, neutral detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; SEM, standard error of the mean.

Table 6 Chemical and microbial composition of four total mixed ration silages after exposure to air

Item	Treatments	Days after unloading				Mean	Analysis of variance			SEM
		0	6	9	12		T	D	T × D	
pH	Control	3.90D	4.28C	5.10B	7.07A	5.09a	**	**	**	0.37
	OC3.6	4.22A	4.19A	4.24A	4.23A	4.22c				0.009
	OC2.2	4.19A	4.24A	4.21A	4.18A	4.21c				0.009
	OC1.5	4.14B	4.32B	4.34B	4.77A	4.39b				0.072
Lactic acid (%DM)	Control	8.65A	6.70B	3.86C	1.55D	5.19c	**	**	**	0.818
	OC3.6	6.81A	6.61A	5.59AB	5.09B	6.02a				0.241
	OC2.2	6.21A	5.91A	5.66A	5.41A	5.80ab				0.197
	OC1.5	6.79A	5.68B	5.60B	3.83C	5.48bc				0.337
WSC (% DM)	Control	4.00A	3.46AB	2.92B	2.96B	3.33c	**	**	**	0.138
	OC3.6	5.41A	4.67B	3.98B	3.15C	4.30b				0.262
	OC2.2	5.94A	5.28A	4.48B	3.68C	4.85a				0.266
	OC1.5	5.58A	4.44B	4.15B	2.73C	4.23b				0.310
VFAs (% DM)	Control	1.26A	0.472B	0.290B	0.326B	0.586c	**	**	**	0.119
	OC3.6	1.09A	1.16A	0.880B	0.977A	1.03a				0.040
	OC2.2	1.06A	0.956A	0.735B	0.964A	0.929b				0.040
	OC1.5	0.855A	0.358B	0.225B	0.199B	0.409d				0.080
Yeasts (log ₁₀ cfu/g FM)	Control	2.00B	8.46A	8.75A	8.58A	6.95a	**	**	**	0.861
	OC3.6	2.00C	4.60B	4.60B	6.60A	4.45c				0.488
	OC2.2	2.00C	3.53B	4.27A	4.60A	3.60d				0.311
	OC1.5	2.00C	6.12B	6.42B	7.54A	5.52b				0.638

** $P < 0.01$. Control = 52% whole-plant corn and 48% concentrate, OC3.6 = 43% oat + 12% common vetch + 45% concentrate, OC2.2 = 38% oat + 18% common vetch + 44% concentrate, OC1.5 = 33% oat + 23% common vetch + 44% concentrate. 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; SEM, standard error of the mean; T, effect of treatment; VFAs, volatile fatty acids; WSC, water soluble carbohydrate.

and lowest in OC1.5 silage. The WSC contents of the three treated silages were higher ($P < 0.05$) than that of the control silage and highest in OC2.2 silage. NH₃-N/TN ratio was lowest in the OC1.5 silage. Butyric acids were found in trace quantities in treated silages.

After ensiling for 45 days, Ash in the control silage and treated silages were not notably different, but DM, CP, EE increased ($P < 0.05$) with addition of oats and common vetch (Table 5). The aNDFom, aADFom and hemicellulose decreased ($P < 0.05$) with addition of oats and common vetch. In addition, DM for OC3.6 silage were higher ($P < 0.05$) than that for OC2.2 and OC1.5 silages, while CP, EE for OC3.6 silage were lower ($P < 0.05$) than that for either OC2.2 or OC1.5 silages.

Yeast numbers were $>10^7$ cfu/g after aerobic exposure for 12 days in the control and OC1.5 silages (Table 6). While yeast numbers were still $<10^5$ cfu/g in OC2.2 silages, and $<10^7$ cfu/g in OC3.6 silage. The lactic acid contents started to decrease gradually at the beginning of the deterioration test and reached the lowest value at 12 days in the control and OC1.5 silages. The lactic acid content began to decrease significantly in OC3.6 silage at 12 days. The pH in the control and OC1.5 silages were raised from 3.90 to 7.07 and 4.14 to 4.77, respectively. The pH in OC2.2 and OC3.6 silages were unchanged, particularly the pH in OC2.2 silage maintained as low as 4.20 until aerobic exposure was terminated after 12 days. The VFA contents decreased gradually in the control and

OC1.5 silages, while the VFAs contents in OC3.6 and OC2.2 silages did not change significantly. The WSC contents decreased gradually in all silages with time of aerobic exposure. After aerobic exposure (day 12), OC1.5 silage had the lowest WSC content and OC 2.2 silage had the highest WSC content.

DISCUSSION

The rate and extent of the resulting fermentation was influenced strongly by the DM content of a crop, a clostridial fermentation and subsequent poor acceptance of the silage by the animals might result from a low DM content with a low sugar content at ensiling (Fraser *et al.* 2000). In the present study, fermentation quality of the control silage and treated silages were affected by the difference in DM content between control TMR and treated TMR. High DM content delays bacterial multiplication in grass silage. The effect of decreasing the moisture content on the activity of LAB and other microorganisms was reflected in the high residual amounts of soluble carbohydrate in treated silage. Other fermentation products, especially acetic and butyric acids were also relatively low in treated silage. This is consistent with the findings of Morgan *et al.* (1980). Although fermentation in treated silages was restricted as compared with that in the control silage, four TMR before ensiling contained high WSC content, proper DM content and LAB populations were more than 10^5 cfu/g FM, which is critical for a successful fermentation (Weinberg 2008). After ensiling for 45 days, the pH of the four TMR silages reduced to 4.2 or less, which was characteristic of well-preserved silages (Weinberg 2008). Ammonia nitrogen content 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 $\text{NH}_3\text{-N/kg}$ TN (McDonald *et al.* 2002). The $\text{NH}_3\text{-N}$ contents of four TMR silages were less than 56 g $\text{NH}_3\text{-N/kg}$ TN in the present study which was an indicator of well-preserved silages. The control TMR silage had lower pH value and higher lactic acid content than the treated TMR silages, which might be attributed to the control TMR having higher WSC content and lower BC than the treated TMR. Higher WSC content could provide more substrates for LAB fermentation, thus stimulating fermentation; lower BC of the control TMR silage made it less resistant to change in pH, which affected the final content decreasing in pH. In the present study, the acetic acid and total VFAs contents of treated silages decreased with increasing proportion of common vetch, which was inconsistent with the result reported by Pursiainen and Tuori (2008). They found acetic acid and total VFA contents increased with increasing the proportion of common vetch in the mixed silage of common vetch and wheat herbage. Moreover, the $\text{NH}_3\text{-N}$ content also

decreased gradually with increasing proportion of common vetch in treated silages, suggesting that legumes might have some chemical or biological effects on bacterial flora during ensiling. This might be attributed to treated silages being successful in partially made LAB dominating the microflora and enhancing metabolism to a more homofermentative process as indicated by high lactic acid content, lactic acid/acetic acid ratio and low $\text{NH}_3\text{-N}$ content throughout the whole fermented period. However, the mechanism underlying this phenomenon is not clear. It needs further research.

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 indicates DM loss. The CP of the four TMR silages also increased, which might be attributed to efficient fermentation, preservation and stability of silages; different types of bacteria present in the medium could not perform their activity and they became the part of the medium (silages). These bacteria contain amounts of protein which are part of the structure of bacteria. This result is consistent with that of Bilal (2009). There were differences in aNDFom in the unensiled treated TMR compared with values of treated silages. The aNDFom and hemicellulose of the treated silages were much lower than the unensiled TMR. In contrast, the ADFom of the treated silages was similar to the unensiled treated TMR. Apparently, considerable loss of hemicellulose occurred in the ensiling process. This loss could be due to a combination of enzymatic and acid hydrolysis of the more digestible cell wall fractions during the fermentation which was consistent with results of Basso *et al.* (2012). Adding oat and common vetch had no unfavorable effect on the fermentation quality of TMR silage as indicated by high lactic acid content and low pH. In addition, the feeding value including CP and EE contents improved with the increasing proportion of common vetch in TMR silage in Tibet. OC2.2 and OC1.5 silages have better feeding value and fermentation quality than OC3.6 silage.

When exposed to air for 12 days (Table 6), according to the evidence that silages of greater levels of lactic acid or those with more residual sugar contents were less stable when exposed to air (Weinberg & Muck 1996), it was anticipated that the TMR silages would rapidly deteriorate. The same result was observed in the control silage. The pH of the control silage increased gradually with the time of aerobic exposure which might be attributed to decreasing lactic acid. The pH was an indicator of aerobic deterioration of the silage because the lactic acid was consumed by yeasts during aerobic exposure, and then the silage becomes suitable for the growth of other undesirable microorganisms such as molds and aerobic bacteria (Basso *et al.* 2012). Hara and Ohyama (1978) also reported

that if silage deteriorated, the content of lactic acid would be lowered and the pH raised. At 9 days, the pH in the control silage had already risen to 5.1, thus we deemed it as aerobic deterioration because it was more than 0.5 above the initial pH value (3.9). Similar with the control silage, the lactic acid content decreased gradually in OC1.5 silage and reached the lowest value at the end of the aerobic stability test, it was considered that OC1.5 silage had aerobic deterioration at about 12 days. However, the opposite result was observed in OC3.6 and OC2.2 silages. The pH value of OC3.6 and OC2.2 silages did not change throughout the whole stage of aerobic exposure. This might be explained by the fact that OC3.6 and OC2.2 silages had higher aerobic stability than the control and OC1.5 silages. The lactic acid content decreased a little in OC3.6 and OC2.2 silages with time of aerobic exposure. Therefore, the decreased contents of lactic acid in OC3.6 and OC2.2 silages were not enough to change the pH in our study. It was difficult to explain the result that treated silages prolonged the hours of aerobic stability but low numbers of yeasts in silages might be associated with enhanced stability (Nishino *et al.* 2003). Yeasts have long been considered to be responsible for the aerobic deterioration of silage and silages containing at least 1×10^5 cfu of yeasts/g are prone to undergo aerobic deterioration once exposed to air (McDonald *et al.* 1991). Our results confirmed lower yeast numbers in OC3.6 and OC 2.2 silages as compared with that in the control and OC1.5 silages after aerobic exposure for 6, 9 and 12 days. Another explanation for aerobic stability enhancing effect of treated silages might be attributed to better aerobic stability of legume silages. It is suggested to be the consequence of inherent factors (e.g. saponins) which are inhibitory to fungi (Weinberg *et al.* 1999; Adesogan & Salawu 2004). Yeast numbers were higher in OC1.5 silage than that in OC3.6 and OC2.2 silages, which might be attributed to the decreasing content of VFAs during aerobic exposure stage. Moon (1983) reported volatile fatty acids protected the silage against aerobic yeasts and molds. The result of aerobic stability test showed that OC2.2 silage is the best regarding aerobic stability as indicated by higher lactic acid content, lower pH and yeast numbers than other silages.

Conclusions

According to the results of our study, it was concluded that replacement of whole-plant corn with oats and common vetch had no unfavorable effect on fermentation quality, while improving CP content and aerobic stability of TMR silage in Tibet. We suggest that oats and common vetch can be potential sources of roughage of TMR silage in Tibet and OC2.2 TMR silage was the best among the three treated TMR silages. However, further research is needed to better

determine the methods for practical production and application.

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