Effects of silage additives and varieties on fermentation quality, aerobic stability, and nutritive value of oat silage¹

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ABSTRACT: Oat is a main feed crop in high-altitude areas of western China, but few studies have been done on its silage making. The aim of this study was to evaluate the effect of silage additives on fermentation, aerobic stability, and nutritive value of different oat varieties (OV) grown in the Qinghai–Tibet Plateau of China. Two OV (*Avena sativa* L. cv. Longyan No.1 (OVL1) and *Avena sativa* L. cv. Longyan No.3 (OVL3)) were planted in a randomized complete block design, harvested at early dough stage with 32.6% and 34.1% DM, respectively. The fresh material was chopped to 2-cm length and treated with additives (0, Sila-Mix (MIX), Sila-Max (MAX) in a 2 × 3 factorial arrangement of treatments with three replicates. Both additives contained a mixture of lactic acid bacteria and supplied a final application rate of 2.5×10^8 of lactic acid bacteria per kg of fresh forage weight. After 60 d of ensiling, the number of lactic acid bacteria in treated silages was about 10-fold greater than the control and generally resulted in a lower pH and ammonia-nitrogen ($P < 0.001$), greater total acids and ratios of lactic acid/acetic acid ($P < 0.001$), and DM recovery ($P = 0.028$). Treatment with additives also decreased ($P < 0.001$) the number of yeasts, which resulted in marked ($P < 0.001$)

improvements in aerobic stability with the effect being greatest with MAX. Both additives improved ($P \leq 0.036$) the 48-h in situ DM digestion in OVL1, but not in OVL3 ($P \geq 0.052$). Treatment with both additives also increased ($P \leq 0.003$) NDF digestion in OVL1 while it was improved ($P < 0.001$) only by MAX in OVL3. In contrast, the additives did not affect ($P \geq 0.088$) in situ hemicellulose digestion in OVL1, but it was improved ($P = 0.048$) by MIX and further improved ($P = 0.002$) by MAX in OVL3. Treatment with MAX improved yields of digestible DM and digestible NDF in both varieties. Dry matter recovery was not affected ($P = 0.121$) by variety. Compared to CTRL, silage treated with MAX had a greater ($P = 0.015$) DM recovery (96.7% vs. 93.9%). Inoculation improved ($P < 0.001$) aerobic stability. The MAX was the most effective for both varieties, while MIX was intermediate and was more effective in OVL3 than OVL1 silage. The results also showed that in Qinghai–Tibet Plateau, compared to OVL1, OVL3 resulted in greater ($P \leq 0.002$) yields of digestible nutrients; specifically, treated with MAX improved silage fermentation efficiency, DM recovery, and provided excellent aerobic stability for feeding to ruminants.

Key words: additives, aerobic stability, fermentation, oat variety, silage

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INTRODUCTION

Oat (*Avena sativa*) is commonly grown as a forage crop in western China in altitudes between 2,000 and 3,500 m, especially in the Qinghai–Tibet Plateau (Zhang et al., 2015). Although oat-growing areas in the world have declined during the past 100 yr because of the shift from horse power to petroleum-powered mechanization in agriculture (Ren, 2013), oat has gained renewed interest in recent decades for both animal feed and human food especially in China. Specifically, in the Qinghai–Tibet Plateau, as much as a 15% increase was recently reported (Ren, 2013). Traditionally, oat is seeded in late spring and harvested in the fall to make hay for animals (Ren, 2013). However, frequent light rain during late summer and throughout autumn often makes it very difficult to produce high-quality oat hay (Qin et al., 2014). In recent years, there has been a growing interest in silage production in Qinghai–Tibet Plateau (Li et al., 2014; Zhang et al., 2015). Oat crops can be harvested later, at the milk or dough stage of maturity (Garnsworthy and Stokes, 1993; Qin et al., 2014), with a higher energy value because of increasing concentrations of starch.

A shift from a heterolactic to more homolactic acid fermentation via inoculation can result in a more efficient ensiling process that improves the recovery of nutrients and energy, and sometimes leads to better animal performance as indicated by increased milk yield, weight gain, and (or) feed intake (Meeske et al., 2002; Hashemzadeh-Cigari et al., 2014). Once exposed to air, silages can spoil rapidly due to metabolism of lactate-assimilating yeasts and different types of additives have also been used to improve silage aerobic stability (Kung et al., 2003). Attempts to improve both silage fermentation and aerobic stability have not been well studied with oat silages (Meeske et al., 2002; Nadeau, 2007; Keles et al., 2014). Accordingly, we hypothesized that application of additive could improve oat silage fermentation process by generally promoting a more efficient fermentation, and provide excellent aerobic stability for feeding to ruminants. Thus, the objectives of this study were to evaluate the effects of silage additives on the fermentation, nutritional quality, and aerobic stability of different oat varieties (OV) grown in the Qinghai–Tibet Plateau of China.

MATERIALS AND METHODS

Preparation of Whole Crop Oat Silage

A field experiment was conducted during 2015 at the experimental field station of Gansu

Agriculture University in Xiahe County, Gannan Tibetan Autonomous Region (Gansu, China), at 2,517 m altitude, with 489 mm annual precipitation. The soil pH was 7.82, containing 1.98% OM, 3.34 g/kg total N and 177 mg/kg available N, 46 mg/kg available P and 241 mg/kg of available K. A randomized complete block design was used with three blocks as replicates, each block contains two plots (4 m × 5 m per plot), with 0.5-m walkway in between. Two OV *A. sativa* L. cv. Longyan No.1 (OVL1) and *A. sativa* L. cv. Longyan No. 3 (OVL3) were used. Both varieties are widely grown in Qinghai–Tibet Plateau with similar maturity dates. The OVL1 is a bit shorter with higher grain yield, while OVL3 has higher biomass and disease resistance. The land was tilled using moldboard plough and the two varieties were planted in mid-April with a five-row planter with 0.20 m row spacing at a seeding rate of 150 kg/ha, with three replicates. The oat plants were harvested at early dough stage of maturity on August 5 at approximately 15 cm above the ground to measure fresh yield (t/ha), then mixed and chopped directly using a forage chopper (model 680, Juancheng Mechanical Equipment Co., Guangzhou, China) to 2-cm lengths. Samples were collected to determine initial DM and nutrient composition. For each replicate, chopped-oat forage was split into three lots treated with 1) no additive (control, CTRL), 2) Sila-Mix (MIX, Ralco Nutrition Inc., Marshall, MN), or 3) Sila-Max (MAX, Ralco Nutrition Inc.). Sila-Mix contained the following active ingredients: lactic acid, dried *Lactobacillus plantarum*, dried *Pediococcus acidilactici*, dried *Enterococcus faecium*, dried *Propionibacterium acidipropionici*, dried *Bacillus subtilis*, dried *Aspergillus niger*, fructo-oligosaccharide, starch, iron oxide, and cobalt-lactic; Sila-Max contained dried *L. plantarum*, dried *E. faecium*, *P. acidilactici*, dried *P. acidipropionici*, fructo-oligosaccharide, and starch. The additives were dissolved in 4 liter deionized water/t fresh forage and sprayed, supplied a final application rate of 2.5×10^8 of LAB per kg of fresh forage weight. The same amount of deionized water was also applied to the control. After thorough mixing for each treatment, 3 kg of forage was manually packed into three replicated laboratory silos (polyethylene bottles with screw caps, 5 liters capacity), nine silos for each treatment. Each silo had a packing density of 198 kg of DM/m³. The silos were weighed and stored in the laboratory at 25 ± 2 °C. After 60 d of storage, the silos were weighed, opened, and samples were collected for measurement of nutrient concentrations, nutrient digestibility, microbial testing, and the remaining forage material used for measurement of aerobic stability.

Chemical and Microbiological Analyses

Samples of forages collected before and after ensiling were analyzed for nutrient and microbiological composition. Twenty grams of forage were homogenized in 180 mL of distilled water for 1 min at high speed ($10,000 \times g$), and stored at 4 °C for 24 h. Water extracts were prepared by filtering the mix through double-layered cheese-cloth and filter paper (Xinhua Co, Hangzhou, China), the filtrate was subsampled for determination of pH by measuring with a glass-electrode pH meter (PHS-3C, Youke Instrument CO, Shanghai, China). Buffering capacity (BC) was determined by the method described by [Playne and McDonald \(1966\)](#). Ammonia-N ($\text{NH}_3\text{-N}$) was measured using the phenol-hypochlorite method according to the procedures of [Broderick and Kang \(1980\)](#), and lactic acid and VFA concentrations were determined by HPLC using a SB-AQ C_{18} column and G132B Ultraviolet fluorescence detector (1260 Infinity, Agilent Technologies). Total acid concentration was calculated by summing the concentrations of VFA and LA. Forage subsamples were dried in a forced-air oven at 65 °C for 48 h to determine DM ([Na et al., 2013](#)), ground to pass through a 2-mm screen using a grinding mill (SJP-500A Jinsui Mechanical Equipment Co., Yongkang, Zhejiang), and stored for later analyses. Crude ash (Ash) concentration was measured by placing a 1-g sample in a muffle furnace set at 500 °C for 5 h ([Zhang et al., 2015](#)). Water-soluble carbohydrate (WSC) concentrations were measured using a colorimetric assay based on the anthrone reaction ([Thomas, 1977](#)). Fermentation coefficient (FC) was calculated according to the formula: $\text{FC} = [\text{DM}\% + (8 \times \text{WSC}/\text{BC})]$ ([Weissbach and Honig, 1996](#)). The fiber fractions, NDF (using a heat stable amylase and sodium sulfite), and ADF were determined sequentially according to the methods of [Van Soest et al. \(1991\)](#). Hemicellulose was calculated as NDF minus ADF ([Jezierny et al., 2011](#)). Total nitrogen (TN) was determined by the Kjeldahl method ([AOAC, 2002](#), method 990.03), and CP was calculated by multiplying TN by 6.25. Silage quality was assessed by calculation of the V score based on concentrations of $\text{NH}_3\text{-N}$, TN, and VFA ([Cai, 2009](#)), where scores above 80 indicate a “good” fermentation, scores of 60 to 80 indicate a “fair” fermentation, and scores below 60 indicate a “poor” fermentation.

Microbiological testing was performed by taking 20 g of material and blending with 180 mL of sterilized water and serially diluting. Lactic acid bacteria were measured by plating serial dilutions

on de Man, Rogosa, Sharpe agar (HongzhouBaisi Biotechnology Co., Ltd., Hangzhou, China) incubated at 30 °C for 72 h under anaerobic conditions (Anaerobic box; Yiheng Technical co., Ltd, Shanghai, China). Aerobic bacteria were measured by plating serial dilutions using Nutrient Agar (HongzhouBaisi Biotechnology Co., Ltd.) incubated aerobically for 72 h at 30 °C ([Chen et al., 2013](#)). Molds and yeasts counts were determined by plating serial dilutions on Rose Bengal Agar (HongzhouBaisi Biotechnology Co., Ltd.) incubated aerobically for 72 h at 28 °C. All colonies were counted as viable numbers of microorganisms, from those serial dilution plates that contained between a minimum of 30 and a maximum of 300 colonies.

Aerobic Stability of Silages

After 60 d of ensiling, silages were subsampled for various analyses and the remaining material was returned to the silo and a temperature sensor attached to a temperature recording meter (MDL1048A, Shanghai Tianhe Automation Instrument Co., Shanghai, China) was placed in the center of the silage mass. The silos were kept at ambient temperature (25 ± 2 °C), and temperature was recorded every 10 min. Aerobic stability was defined as the time required for the silage temperature to increase by 2 °C ([Weinberg, 2008](#)).

In Situ Nutrient Digestibility

Six sheep (F2 of Merino $\times$ local breed) averaging 53.7 ± 1.8 kg BW with a ruminal cannula were used to measure the ruminal disappearance of nutrients. Animals were cared for according to the Chinese Standards for the use and care of research animals ([Lu and Xie, 1991](#)). These sheep were fed a 30:70 concentrate to roughage ration (concentrate + oat silage, $1.4 \text{ kg} \cdot \text{head}^{-1} \cdot \text{d}^{-1}$), with 12.6 MJ/d metabolizable energy, 153 g/d CP, 5.0 g/d calcium, and 2.3 g/d total phosphorous. All sheep had free access to fresh water and mineral blocks.

Dry matter, NDF, ADF, and hemicellulose disappearance from nylon bags (Ruitong Biotech Co., Ltd., Gansu, China) suspended in the rumen were measured according to the standardized procedures of [Harazim and Pavelek \(1999\)](#). For three replicates of each treatment (no additive control, MIX, and MAX), two samples were taken from each replicate. Five gram of silage (randomly selected and hand cut to 5–6 mm) for each sample was weighed and sealed in each nylon bag (4 cm $\times$ 5 cm) with 38 μm pore

size, totaling six bags for each treatment. Bags were placed into the rumens of the six sheep and incubated for 48 h. Upon removal from the rumen, bags were rinsed immediately under cold tap-water with subsequent washing in a tub with 38 °C water until the rinse water was clear. Residues were lyophilized, weighed, ground using a ball mill (Mixer Mill MM 400, Retsch, Germany) to pass 1-mm screen, thoroughly mixed and analyzed for nutrient composition as previously described.

Nutrient Yields

Yield of DM (t/ha) was calculated according to fresh yield and DM content, yield of NDF (t/ha) was determined by DM yield and NDF concentration. Yields of digestible DM (**DDM**, t/ha) and digestible NDF (**DNDF**, t/ha) were calculated according to DM and NDF yields and their digestibility, respectively.

Statistical Analyses

The counts of microbiological values were log-transformed (in $\log_{10}$ of colony-forming units per gram material) before statistical analysis and presented as log values. All data were subjected to least squares analysis of variance using the PROC GLM procedure of SAS (SAS Institute Inc., Cary, NC) for a randomized complete block design with a factorial arrangement of treatments (two OV by three treatments [**ADD**]). Fixed effects were replication, OV, **ADD**, and the interaction of OV $\times$ **ADD**. When significant differences were detected, the least squares means were separated by Fischer's Least Significant Difference (Steel and Torrie, 1980) using the PDIFF statement and statistical significance was declared at $P \leq 0.05$.

RESULTS

The chemical and microbiological compositions of fresh oat forages are shown in Table 1. The two OV were similar ($P \geq 0.060$) in concentrations of DM, WSC, ADF, hemicellulose, ash, BC, FC, and pH; however, the CP and NDF concentration were 16.1% and 4.3%, respectively, greater ($P \leq 0.018$) for OVL3 than OVL1. The microbiological data at harvest showed similar ($P \geq 0.087$) numbers of LAB, aerobic bacteria, and molds on the OV. However, numbers of yeasts were greater ($P = 0.016$) in OVL3 (5.00 $\log_{10}$ cfu/g FM) than in OVL1 (4.34 $\log_{10}$ cfu/g FM).

The DM and nutrient composition of silages after 60 d of ensiling are presented in Table 2.

Table 1. Chemical and microbial composition of two oat varieties before ensiling

Item	OVL1 ¹	OVL3 ²	SE
DM, %	32.6	34.1	0.89
DM, t/ha	13.6 ^b	15.8 ^a	0.13
CP, %	9.2 ^b	10.7 ^a	0.03
WSC, ³ %	19.9	18.9	0.63
NDF, %	53.7 ^b	56.0 ^a	0.58
ADF, %	30.9	32.8	1.19
Hemicellulose, %	22.8	23.1	0.78
Ash, ⁴ %	5.8	6.0	0.10
Buffering capacity, mEq/kg DM	245.40	249.40	2.55
Fermentation coefficient ⁵	39.10	40.20	1.04
pH	6.04	6.01	0.01
Lactic acid bacteria, $\log_{10}$ cfu/g FM ⁶	4.45	4.27	0.12
Aerobic bacteria, $\log_{10}$ cfu/g FM	7.68	7.76	0.15
Molds, $\log_{10}$ cfu/g FM	4.66	4.41	0.09
Yeasts, $\log_{10}$ cfu/g FM	4.34 ^b	5.00 ^a	0.17

^{a,b}Means in rows with unlike superscript differ, $P < 0.05$.

¹*A. sativa* L. cv. Longyan No. 1.

²*A. sativa* L. cv. Longyan No. 3.

³Water soluble carbohydrates.

⁴Crude ash.

⁵Fermentation coefficient = DM% + [8 $\times$ (water-soluble carbohydrates/buffering capacity)].

⁶Fresh matter.

Dry matter concentration was greater ($P < 0.001$) for OVL3 silages (average of 33.5%) than OVL1 (average of 31.2%) but not affected ($P = 0.051$) by **ADD**. The concentration of CP was not different ($P = 0.214$) between OV but it was highest ($P \leq 0.014$) for silages treated with MAX (average of 10.1%), intermediate in silages treated with MIX (average of 9.0%), and lowest CTRL (average of 7.8%). An interaction of OV $\times$ **ADD** was detected ($P \leq 0.036$) for concentrations of WSC, NDF, and hemicellulose. Inoculation with MIX and MAX resulted in greater ($P < 0.001$) concentrations of WSC for the OVL3 than CTRL but had no effect ($P \geq 0.145$) in OVL1. In OVL3 silages, treatment with MIX and MAX progressively reduced ($P \leq 0.009$) the concentrations of NDF (51.1% and 47.4%, respectively) when compared to the control (54.2%). In contrast, the NDF was similar ($P \geq 0.163$) among all treatments for OVL1. The concentration of ADF was similar ($P = 0.284$) for both OV (29.7% for OVL1 and 30.5% for OVL3), but the addition of MAX and MIX reduced ($P < 0.001$) ADF concentration (MAX > MIX > CTRL). Hemicellulose concentrations were unaffected ($P \geq 0.103$) by inoculation in OVL1 silages but was significantly lower in OVL3-MAX than in OVL3-CTRL. The concentration of ash was greater ($P = 0.003$) in OVL3 (6.75%) than OVL1 (6.14%) and was also greater ($P \leq 0.030$) in

Table 2. Chemical composition of two oat varieties treated with silage additives after 60 d of ensiling

Nutrient	OVL1 ¹			OVL3 ²			SE	<i>P</i> value		
	CTRL ³	MIX ⁴	MAX ⁵	CTRL	MIX	MAX		OV ⁶	ADD ⁷	OV × ADD ⁸
DM, %	30.3	31.0	32.3	33.2	33.4	33.9	0.51	<0.001	0.051	0.452
CP, %	8.34	9.26	9.82	7.25	8.75	10.3	0.34	0.214	<0.001	0.090
WSC, ⁹ %	4.48 ^{bc}	4.28 ^c	4.85 ^b	4.26 ^c	5.83 ^a	6.14 ^a	0.17	<0.001	<0.001	<0.001
NDF, %	49.5 ^{bc}	49.3 ^{bc}	48.0 ^c	54.2 ^a	51.1 ^b	47.4 ^c	0.71	0.005	<0.001	0.010
ADF, %	29.7	27.8	25.7	30.5	29.5	27.2	0.54	0.081	<0.001	0.748
Hemicellulose, %	19.8 ^b	21.5 ^{ab}	22.3 ^{ab}	23.7 ^a	21.6 ^{ab}	20.3 ^b	1.00	0.454	0.904	0.036
Ash, ¹⁰ %	5.84	6.33	6.24	6.26	6.90	7.08	0.14	<0.001	0.001	0.330

^{a-c}Means in rows with unlike superscripts differ, $P < 0.05$.

¹*A. sativa* L. cv. Longyan No. 1.

²*A. sativa* L. cv. Longyan No. 3.

³Control.

⁴Treated with Sila-Mix (Ralco Nutrition Inc., Marshall, MN).

⁵Treated with Sila-Max (Ralco Nutrition Inc.).

⁶Main effect of oat variety.

⁷Main effect of additive treatment.

⁸Interaction of oat variety × additive treatment.

⁹Water-soluble carbohydrates.

¹⁰Crude ash.

MIX (6.62%) and MAX (6.66%) than in CTRL (6.05%).

The fermentation characteristics and microbial composition of oat silages after 60 d of ensiling are shown in Table 3. Interactions were detected between OV and ADD for pH ($P = 0.021$), lactic acid, acetic acid, propionic acid, butyric acid, ratio of lactic to acetic, and total acids ($P < 0.001$). The pH was highest in OVL1-CTRL (4.88) followed by OVL3-CTRL (4.70) in silages.

Treatment with MIX resulted in lower ($P \leq 0.009$) pH (4.22 for OVL3 and 4.48 for OVL1) compared to CTRL but it was even lowest ($P \leq 0.001$) in MAX (3.90 for OVL3 and 3.85 for OVL1). In addition, the effect of MIX was greater ($P = 0.002$) in OVL3 (4.22 for MIX and 4.7 for CTRL) than OVL1 (4.48 for MIX and 4.88 for CTRL).

Treatment with MIX and MAX resulted in significantly greater ($P < 0.001$) concentrations of lactic acid than CTRL (Table 3); between the two varieties, OVL3 had better ($P < 0.001$) lactic acid formation (6.06% for MIX and 7.10% for MAX, respectively) than OVL1 (5.03% and 5.96%, respectively). Compared to OVL1-CTRL (0.86%) and OVL3-CTRL (1.23%), concentrations of acetic acid were lower ($P < 0.001$) in additive-treated silages (range 0.27% to 0.75%), but the effect of MAX was greater ($P = 0.001$) for OVL3 (0.27%) than for OVL1 (0.43%). Additives also significantly reduced ($P < 0.001$) propionic acid concentrations in oat silages; treatment with MIX and MAX were more effective ($P < 0.001$) in lowering propionic

acid in OVL1 than in OVL3. Concentrations of butyric acid were also decreased ($P < 0.001$) by additive treatments. Meanwhile, adding MAX and MIX significantly increased ($P \leq 0.001$) the ratio of lactic acid to acetic acid in oat silage (range 7.55 to 26.20) compared with the CTRL (range 2.42 to 3.71). All treated silages had higher ($P < 0.001$) concentrations of total acids (6.07% to 7.44%) than CTRL (4.84% to 5.17%), the highest value (7.44%) was obtained in OVL3-MAX treatment. Additive treatments decreased ($P < 0.001$) $\text{NH}_3\text{-N}$ concentrations remarkably (Table 3), and MAX was more effective (average of 3.89% of TN, $P < 0.001$) than MIX (average of 5.12% of TN).

Numbers of LAB were unaffected ($P = 0.355$) by OV, but significantly improved ($P < 0.001$) by ADD (average of 6.57 $\log_{10}\text{cfu/g}$ FM for CTRL and 7.72 $\log_{10}\text{cfu/g}$ FM for ADD). No distinct difference ($P \geq 0.197$) was observed between MAX and MIX treatments. In contrast, there was an OV × ADD interaction ($P = 0.001$) for numbers of aerobic bacteria. Control of OVL3 had the highest numbers of aerobic bacteria (3.61 $\log_{10}\text{cfu/g}$ FM), followed by OVL1-CTRL (2.94 $\log_{10}\text{cfu/g}$ FM), and treatment with MAX resulted in the lowest ($P \leq 0.003$) values for both OV (2.09 $\log_{10}\text{cfu/g}$ FM for OVL1 and 2.13 $\log_{10}\text{cfu/g}$ FM for OVL3). Oat variety did not affect ($P = 0.415$) numbers of molds, but ADD significantly reduced ($P = 0.013$) molds. Additives also decreased ($P < 0.001$) numbers of yeasts in both OV, MIX and MAX. They were equally effective in their reduction of yeasts in

Table 3. Fermentation characteristics, microbial composition, V score, and DM recovery of two oat varieties treated with silage additives after 60 d of ensiling

Item	OVL1 ¹			OVL3 ²			SE	<i>P</i> value		
	CTRL ³	MIX ⁴	MAX ⁵	CTRL	MIX	MAX		OV ⁶	ADD ⁷	OV × ADD ⁸
pH	4.88 ^a	4.48 ^c	3.85 ^e	4.70 ^b	4.22 ^d	3.90 ^e	0.05	0.007	<0.001	0.021
Lactic acid, %	3.20 ^d	5.03 ^c	5.96 ^b	2.99 ^e	6.06 ^b	7.10 ^a	0.06	<0.001	<0.001	<0.001
Acetic acid, %	0.86 ^b	0.67 ^d	0.43 ^e	1.23 ^a	0.75 ^c	0.27 ^f	0.03	0.001	<0.001	<0.001
Propionic acid, %	1.03 ^a	0.38 ^c	0.26 ^d	0.55 ^b	0.10 ^e	0.07 ^e	0.01	<0.001	<0.001	<0.001
Butyric acid, %	0.07 ^a	ND ^c	ND ^c	0.07 ^a	0.02 ^b	ND ^c	0.002	0.001	<0.001	<0.001
Lactic acid/acetic acid	3.71 ^d	7.55 ^c	13.8 ^b	2.42 ^e	8.12 ^c	26.2 ^a	0.57	<0.001	<0.001	<0.001
Total acids, %	5.17 ^e	6.07 ^d	6.65 ^c	4.84 ^f	6.93 ^b	7.44 ^a	0.11	<0.001	<0.001	<0.001
NH ₃ -N, % of TN ¹⁰	7.77	5.50	3.85	7.47	4.74	3.93	0.18	0.039	<0.001	0.094
Lactic acid bacteria, log ₁₀ cfu/g FM ¹¹	6.34	7.67	7.83	6.80	7.54	7.85	0.15	0.355	<0.001	0.165
Aerobic bacteria, log ₁₀ cfu/g FM	2.94 ^b	2.41 ^d	2.09 ^e	3.61 ^a	2.70 ^c	2.13 ^e	0.06	<0.001	<0.001	0.001
Molds, log ₁₀ cfu/g FM	2.24	1.76	1.29	2.57	1.73	1.56	0.28	0.415	0.013	0.788
Yeasts, log ₁₀ cfu/g FM	2.33 ^a	1.66 ^b	1.21 ^d	2.51 ^a	1.41 ^c	1.40 ^c	0.06	0.399	<0.001	0.005
V score ¹²	78.6 ^e	92.5 ^c	96.3 ^b	79.3 ^d	92.8 ^c	98.9 ^a	0.19	<0.001	<0.001	<0.001
DM recovery, %	93.0	95.3	96.0	94.7	95.7	97.3	0.02	0.121	0.028	0.789

^{a-f}Means in rows with unlike superscripts differ, *P* < 0.05.

¹*A. sativa* L. cv. Longyan No. 1.

²*A. sativa* L. cv. Longyan No. 3.

³Control.

⁴Treated with Sila-Mix (Ralco Nutrition Inc., Marshall, MN).

⁵Treated with Sila-Max (Ralco Nutrition Inc.).

⁶Main effect of oat variety.

⁷Main effect of additive treatment.

⁸Interaction of oat variety × additive treatment.

⁹Ammonia-N.

¹⁰Total nitrogen.

¹¹Fresh matter.

¹²V scores above 80 indicate a “good” fermentation, scores of 60 to 80 indicate a “fair” fermentation, and scores below 60 indicate a “poor” fermentation.

OVL3, while MAX (1.21 log₁₀ cfu/g FM) was more effective (*P* < 0.001) than MIX (1.66 log₁₀ cfu/g FM) in OVL1. There was also a significant interaction of OV × ADD for V score, which was lowest for CTRL (range 78.6 to 79.3) and greatly improved (*P* < 0.001) by both additives with OVL3-MAX having the highest value. The recovery of DM was not affected (*P* = 0.121) by OV, but it was affected by ADD (*P* = 0.028). Compared to CTRL, silage treated with MAX had greater (*P* = 0.007) average DM recovery (96.7%) than CTRL (93.9%).

An OV × ADD interaction (*P* < 0.001) was detected for the aerobic stability of silages (Fig. 1). The OVL3 had greater (*P* = 0.015) aerobic stability than OVL1 even without additives (163 h vs. 227 h). When they were treated with MIX, both varieties improved (*P* ≤ 0.011) their aerobic stability, but OVL3 increased 90.31% (*P* < 0.001), while OVL1 only improved by 41.72% (*P* = 0.011) compared with the CTRL, indicating MIX was more effective

Figure 1. Aerobic stability of two oat varieties of control (CTRL) or treated with Sila-mix (MIX), or Sila-Max (MAX) after 60 d of ensiling and subsequent exposure to air. Main effects include oat variety (*P* < 0.001) and silage additive (*P* < 0.001), and there was an oat variety × silage additive interaction (*P* < 0.001). Bars with unlike letter differ (*P* < 0.05). SE = 36.08 (*n* = 3).

with OVL3. The MAX produced similar and highest effect ($P \leq 0.002$) on the two varieties (522 h for OVL1 and 521 h for OVL3).

Meanwhile, in situ digestibility was conducted with sheep (Table 4). An OV $\times$ ADD interaction ($P \leq 0.024$) was detected for the 48 h in situ digestibility of DM, NDF, and hemicellulose. In situ DM digestibility was highest for OVL1-MAX (60.0%) and OVL1-MIX (58.1%), and the lowest for OVL3-MIX (51.4%) with the remaining treatments being intermediate (range 53.9% to 55.4%). In contrast, the highest digestibility of NDF (55.9%) occurred for OVL3-MAX with the lowest value observed for OVL1-CTRL (41.5%). Oat variety and silage additive did not affect ($P \geq 0.106$) ADF disappearance. Digestion of hemicelluloses was considerably greater ($P \leq 0.023$) in OVL3 vs. OVL1 silages. Whereas inoculants did not affect ($P \geq 0.088$) hemicelluloses digestibility of OVL1 silages (62.4% and 61.1% for MIX and MAX, respectively) when compared with OVL1-CTRL (56.0%), both additives increased ($P \leq 0.048$) hemicellulose digestibility in OVL3 silages with response being greatest in OVL3-MAX (84.6%).

Selected yields of nutritive components are presented in Table 5. Yields of DM and DDM were greater ($P \leq 0.002$) for OVL3 (15.1 t/ha of DM and 8.12 t/ha of DDM) than OVL1 (13.0 t/ha of DM and 7.51 t/ha of DDM) silages. Silages treated with MIX had similar ($P \geq 0.330$) yields of DM/ha compared with CTRL, but MAX treatments (14.4 t/ha) out-yielded the CTRL (13.8 t/ha, $P \leq 0.016$). Silage treated with MAX (8.28 t/ha) produced more ($P \leq 0.042$) DDM than CTRL (7.54 t/ha) and MIX (7.64 t/ha). There was an interaction between OV $\times$ ADD ($P = 0.007$) because additives did not affect

($P \geq 0.267$) the NDF yield in OVL1; but for OVL3, MAX resulted in the lowest value. The OVL3-CTRL had much greater ($P < 0.001$) DNDF yield than OVL1-CTRL (3.94 vs. 2.58). Additives had different impact on DNDF yield of the two varieties. For OVL1, both additives increased DNDF yield (12.41% from MIX and 14.73% from MAX), while 2.79% reduction from MIX and 3.3% increase from MAX were obtained for OVL3.

DISCUSSION

Freshly harvested OVL3 was numerically greater in DM concentration than OVL1 suggesting it was slightly more mature at harvest, which was substantiated by it having greater concentrations of NDF. All other chemical components (except of CP) were similar between the two OV. Numbers of LAB on fresh OV were within the range (between 4 and 5 log₁₀ cfu/g of FM) of that previously reported in oat forage (Chen et al., 2016) and other similar small grain crops such as barley (Kung and Ranjit, 2001; Amanullah et al., 2014). The interactions between the DM content, concentration of WSC and BC can determine the success or failure of a silage fermentation. For example, relatively greater concentrations of WSC are required for successful silage fermentation in low compared to higher DM silages. Fermentation coefficients less than 35 would suggest too low concentrations of WSC or too low DM for a good fermentation (Weissbach and Honig, 1996). The OV used in the current study had high concentrations of WSC and a moderate BC resulted in an FC score of about 40, thus predicting successful fermentations.

Table 4. In situ DM, NDF, ADF, and hemicelluloses digestibility for two oat varieties treated with silage additives after 60 d of ensiling

Nutrient	OVL1 ¹			OVL3 ²			SE	<i>P</i> value		
	CTRL ³	MIX ⁴	MAX ⁵	CTRL	MIX	MAX		OV ⁶	ADD ⁷	OV $\times$ ADD ⁸
DM, %	55.4 ^b	58.1 ^a	60.0 ^a	53.9 ^{bc}	51.4 ^c	55.4 ^b	0.82	<0.001	0.005	0.024
NDF, %	41.5 ^d	45.6 ^c	45.9 ^c	48.4 ^b	49.6 ^b	55.9 ^a	0.78	<0.001	<0.001	0.008
ADF, %	31.8	32.4	32.9	30.8	31.8	34.6	1.10	0.977	0.106	0.423
Hemicellulose, %	56.0 ^d	62.4 ^d	61.1 ^d	71.4 ^c	74.0 ^b	84.6 ^a	2.84	<0.001	0.010	0.008

^{a-d}Means in rows with unlike superscripts differ, $P < 0.05$.

¹*A. sativa* L. cv. Longyan No. 1.

²*A. sativa* L. cv. Longyan No. 3.

³Control.

⁴Treated with Sila-Mix (Ralco Nutrition Inc., Marshall, MN).

⁵Treated with Sila-Max (Ralco Nutrition Inc.).

⁶Main effect of oat variety.

⁷Main effect of additive treatment.

⁸Interaction between OV $\times$ ADD.

Table 5. Yield of digestible nutrients for two oat varieties treated with silage additives after 60 d of ensiling

Nutrient	OVL1 ¹			OVL3 ²			SE	<i>P</i> value		
	CTRL ³	MIX ⁴	MAX ⁵	CTRL	MIX	MAX		OV ⁶	ADD ⁷	OV × ADD ⁸
DM, t/ha	12.6	12.9	13.4	15.0	15.1	15.3	0.23	<0.001	0.049	0.487
DDM, ⁹ t/ha	6.97	7.50	8.05	8.10	7.77	8.50	0.21	0.002	0.026	0.120
NDF, t/ha	6.22 ^c	6.36 ^c	6.44 ^c	8.14 ^a	7.72 ^a	7.28 ^b	0.12	<0.001	0.113	0.007
DNDF, ¹⁰ t/ha	2.58	2.90	2.96	3.94	3.83	4.07	0.09	<0.001	0.067	0.146

^{a-d}Means in rows with unlike superscripts differ, *P* < 0.05.

¹*A. sativa* L. cv. Longyan No. 1.

²*A. sativa* L. cv. Longyan No. 3.

³Control.

⁴Treated with Sila-Mix (Ralco Nutrition Inc., Marshall, MN).

⁵Treated with Sila-Max (Ralco Nutrition Inc.).

⁶Main effect of oat variety.

⁷Main effect of additive treatment.

⁸Interaction between OV × ADD.

⁹Digestible DM.

¹⁰Digestible NDF.

Numbers of LAB in silages were greater in MIX and MAX silages than in CTRL silages by about 10-fold, which is indicative of a stimulated fermentation. Supportive of these numbers, treatment with MIX and MAX resulted in a more efficient ensiling process as characterized by generally having lower pH, higher concentrations of total acids, greater ratios of lactic acid/acetic acid, and lower concentrations of NH₃-N and butyric acid than CTRL silages. These changes in silage fermentation are often a result of a more homolactic acid type of fermentation (McDonald et al., 1991) and resulted in greater V scores for MIX and MAX silages (>90) than CTRL (<80). Any V scores above 80 (high of 100) are indicative of “good” silages (Cai, 2009), which was corroborated in improved DM recovery for the additive-treated silages compared to the control.

The numbers of yeasts in fresh forages declined substantially after ensiling for all treatments. It is not uncommon for this to happen because fermentation produces acids that have antifungal activity (e.g., acetic, propionic, and butyric acids; Qin et al., 2014). However, those yeasts that survive the ensiling process and that can metabolize lactic acid are often the initiators of spoilage when silage is exposed to air (Woolford, 1990). Relatively low numbers of yeasts (<2.51 log₁₀ cfu/g FM) were probably the reason that CTRL silages were stable for 163 and 227 h, in OVL1 and OVL3, respectively (Fig. 1). Similar findings were reported by Kung and Ranjit (2001) where aerobic stability was very long (about 377 h) in untreated barley silage (similar to oat silage) because numbers of yeasts were also very low (2.89 log₁₀ cfu of yeasts/g FM). In our

study, treatment with MIX and MAX improved aerobic stability, with MAX being more effective than MIX in both OV and MIX was more effective in OVL3 than OVL1. Although silage is seldom in a feed trough for more than 24 h, it can be exposed to air in the silo well before it is removed for actual feeding. For example, Muck and Pitt (1994) reported that even in a well-packed silo, air can penetrate up to a meter into the exposed feeding face. In addition, in silos that are not considered “oxygen limiting”, portions of silage in those structures can be chronically exposed to low levels of air for months before feed out. The reason(s) for improvements in aerobic stability by MIX and MAX in our study are unclear because these additives resulted in production of high concentrations of lactic acid and low concentrations of antifungal acids (e.g., acetic and butyric acids). Muck and Kung (1997) summarized the literature and reported that treatment with homolactic acid bacteria only improved the aerobic stability of silages about one third of the time in the studies that they reviewed. Their review also showed that aerobic stability was worse in studies with added homolactic acid bacteria about a third of the time. For example, Cai et al. (1999) inoculated forages with homolactic acid bacteria that improved silage fermentation but decreased aerobic stability. In addition, although MIX contained *Propionibacteria* that can produce propionic acid (a good antifungal compound), that acid was lower in the treated than control. One possible explanation for improved aerobic stability by MIX and MAX might be that they stimulated the production of other unknown, but active, antifungal compounds. For example, some LAB has been

shown to produce compounds other than organic acids that are capable of inhibiting yeasts in silages (Sjögren et al., 2003; Arasu et al., 2013). Arasu et al. (2013) isolated an antifungal compound from *L. plantarum* KCC-10 from forage silage and identified it as a phenolic-related antibiotic. They also measured its minimum inhibitory concentration against *Aspergillus clavatus*, *Aspergillus oryzae*, *Botrytis elliptica*, *Scytalidium vaccinii*, *Aspergillus fumigatus*, *A. niger*, and *Sterrhopterix fusca*.

Both MIX and MAX increased NDF digestion for OVL1 compared to CTRL, whereas only treatment with MAX improved NDF digestion in OVL3. In contrast, both additives improved hemicellulose digestion in OVL3 with the effect being more from MAX than MIX. Improvements in fiber digestion by the additives we used were impressive because Muck and Kung (1997) reported that bacterial inoculants improved fiber digestibility only about one third of the time. Although MIX contained Co that has improved ruminal fiber digestion in some studies (Zelenák et al., 1992; Lopez-Guisa and Satter, 1992), it did not appear that it was beneficial in our study because MIX did not improve NDF or hemicellulose digestion more than MAX.

Overall, we noted that the response to the MIX and MAX was often generally greater for OVL3 than OVL1, which indicates that OV and its intrinsic characteristics may have had an impact on the resulting responses during the ensiling process. Thus, some varieties may ensile better than others and this information would be important in the selection process of a silage additive.

CONCLUSION

The application of the additives used in this study improved the silage fermentation process of both OV by generally promoting a more efficient homolactic acid fermentation, increasing DM recovery, improving aerobic stability, and increasing digestion of NDF with Max being overall more effective than MIX. Specifically, OVL3 treated with MAX improved the efficiency of silage fermentation, the recovery of DM, and provided excellent aerobic stability for feeding to ruminants in Qinghai–Tibet Plateau.

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