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Effects of *Lactobacillis*, Formic Acid, Sucrose, and Cellulase on Oat Silage Quality

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Abstract

Oat is an annual small grain species that is highly valued for grain and forage uses. Forage utilization of oats in China is primarily as hay, since ensiling is difficult, due to low levels of fermentable carbohydrates and high crude fiber levels. However, when conserved as hay, the nutritive value is often low due to less than optimal weather during harvest. Thus, this study was conducted to evaluate the effectiveness of four additives for improving oat silage quality. Additives were lactic acid bacteria (0 mg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg), formic acid (0 ml/kg, 1 ml/kg, 5 ml/kg, 10 ml/kg), sucrose (0%, 1%, 2%, 4%) and cellulase (0 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg). Oat cultivar 'Danser' was harvested at the milk stage, chopped, and ensiled with one of four additives and compared with the check control. Lactic acid bacteria increased crude protein level, formic acid improved dry matter, sucrose reduced crude fiber content, and cellulase increased crude fat and ash content. Since lactic acid bacteria and sucrose both reduced the pH value and ammonia nitrogen ratio and increased the production of lactic acid, they are recommended as additives for ensiling oats.

Keywords: *Oat Silage; Lactobacillus; Formic Acid; Sucrose; Cellulase; Nutritional Value; Silage Quality*

Introduction

Oat (*Avena sativa* L.) belongs to the *Avena* genus in the *Poaceae* family (formerly *Gramineae*). Oats are divided into two types: covered oats (with a palea) and naked oats (with no palea) [1]. Oats planted in China are mainly naked oats and only a few areas are planted with common or covered oats. Oats are also classified as spring or winter oats. Spring oats do not require vernalization for stem elongation and heading and they have little winter survival potential in cold regions. Thus, spring oats are typically planted in the spring or early summer for a fall harvest.

Oats can have a high feeding value for livestock, but when harvested for hay, quality is often reduced due to weather conditions that are not conducive to curing hay. Silage has the advantage of not requiring drying weather and effectively preserving the nutritional value of forage [2,3]. However, compared to corn, oat forage had lower sugar, protein, and water content, higher crude fiber content and more difficult to ensile [4]. Therefore, providing additives is one approach to improving silage quality [5,6]. Few studies have evaluated the effectiveness of various additives to provide famers with options to increase oat silage quality. This experiment evaluated the effectiveness of lactic acid bacteria, formic acid, sucrose, and cellulase for increasing silage quality of 'Dancer,' a commonly used oat cultivar.

Materials and Methods

Location

The field experiment was conducted in the Grassland Science experimental area of the College of Animal Science and Technology of Northwest A&F University. The experimental area is located at the northern foot of the Qinling Mountains and west of the Weihe Plain, with an elevation of 454.8m, latitude 34°21‘ north, and longitude 108°10‘ east. The average annual temperature is 12 - 14°C, average annual precipitation is 622 mm and the frost-free period is 200 to 220 days. It belongs to a warm, temperate, semi-humid, and drought-prone climate.

Field planting

Oat cultivar 'Dancer' seed was provided by the Agricultural College of Northwest A&F University. Seeds were sown on March 20th, 2012 at 15 kg hm^{-2}, drilled with a row spacing of 20 cm. The soil of the experimental site is a deep, well drained, fine, loamy soil in the Lou series and the thermic typic haplustalf taxonomic class. It has a field water capacity of 23.6%, a clay content of 44.0%, and an average bulk density of 1.36 g/cm^3. The area had been previously planted to alfalfa. No herbicides, pesticides or fertilizers were used.

Experimental design

The experiment was arranged as a two-factor, completely randomized design, with additive type and additive concentration as factors.

Ensiling

Oat cultivar 'Dancer' was harvested at the milk stage on June 15th and whole plants were chopped into 2 - 3 cm pieces prior to ensiling. The types and concentrations of additives were lactic acid bacteria (0, 1mg / kg 5 mg/kg, 10 mg/kg), formic acid (0, 1mg / kg, 5 ml/kg, 10 ml/kg), sucrose (0,1%, 2%, 4%), and cellulase (0, 50mg / kg, 100 mg/kg, 150 mg/kg). The check treatment (CK) received no additive.

Silage were prepared in plastic bottles. The bottles were 10 cm in diameter and 15 cm tall, with a wall thickness of 0.3 cm, and a volume of 1 liter. Additives were sprinkled or sprayed onto the silage material and mixed thoroughly. The prepared oat forage was packed into plastic bottles in layers and compacted with a wooden rolling pin with a diameter of about 4 cm, being especially careful to compact at the edge of the bottles. After filling and compacting, bottles were double sealed with sealing membrane and bottle caps to ensure they were air-tight.

After packing and sealing, bottles were placed in a cool and dark place in the laboratory and stored for 60 days at ambient temperature (20°C). After 60 days, bottles were opened, and the silages were prepared for analyses.

Sensory evaluation index and method

Sensory evaluation was conducted according to the German Agricultural Society (DIG) silage sensory evaluation standards and rating method [7] which involved comparing the odor, structure, and color. Odor was divided into 5 grades, given 0 to 14 points; structure was divided into 4 grades, given 0 to 2 points; and color was divided into 3 grades, given 0 to 2 points. After adding scores of these three factors, final scores were grouped into 4 grades: excellent (16 - 20), very good (10 - 15), good (4 - 9), and poor (0 - 3).

Silage quality

Conventional nutrient analysis methods were used to determine dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), crude Ash (ASH), nitrogen-free extract (NFE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ammoniacal nitrogen

(NH_3-N). Methods were based on the feed analysis and feed quality detection technology reported by Yang Sheng [8]. The water soluble carbohydrate (WSC) content was determined by anthrone colorimetry. Lactic acid and acetic acid were determined by gas chromatography.

Statistical analyses

All data were initially analyzed with the Microsoft Office Excel 2010 software, and expressed in the form of x ± sd. SPSS v. 17.0 (SPSS Inc. Chicago, IL) was used for analysis of variance and Duncan's Multiple Range test was used to compare means declared significant ($P < 0.05$ throughout, unless otherwise indicated).

Results

Sensory evaluation

Sensory evaluation information is shown in table 1. *Lactobacillus* and sucrose additives had highest scores, but were not significantly different from the check.

Cultivar	Additives	Sensory evaluation*
Dancer	CK	17.33 ± 1.53^{abc}
	Lactobacillus	19.67 ± 0.58^{a}
	Formic acid	17.00 ± 2.00^{abc}
	Sucrose	19.33 ± 1.15^{ab}
	Cellulase	16.00 ± 0.58^{bc}

Table 1: *Sensory evaluation of 'Dancer' oat silage treated with four additives (Lactobacillus, formic acid, sucrose, and cellulase).*

Sensory evaluation was conducted according to the German Agricultural Society (DIG) silage sensory evaluation standards and rating method (Alexander., et al. 1998).

Effects of additives on the proximate analysis components of oat silage

Proximate analysis components of the prepared silage are shown in table 2. In many cases, the four additives significantly altered the composition of the silage compared to the check and to each other.

The addition of *Lactobacillus* increased the CP and EE content of oat silage and reduced the CF and ash content. No significant differences were observed for the three concentration levels but the 10 mg/kg application had the most favorable values for all factors except NFE for which there was no consistent trend.

Formic acid significantly increased the CP and EE values ($P < 0.05$) compared to the check. The 5 ml/kg concentration produced the most favorable composition.

Sucrose increased CP and reduced the percent moisture, CF, and ash content. The amount of change decreased with increased concentrations. There was no consistent trend for NFE. The 2% sucrose treatment provided the greatest benefit for CP, EE, and CF values.

All three levels of cellulase significantly increased the CP and EE content ($P < 0.05$). Most effective was the 50 mg/kg cellulase concentration in which the CP content was significantly higher than that of the check and all other treatments. In addition, this treatment resulted in significantly lower moisture content and CF (P < 0.05), although there was no significant difference in the effect of the three concentration levels (P > 0.05).

Additive	Concentration	Moisture (% FM)	CP (% DM)	EE (% DM)	CF (% DM)	Ash (% DM)	NFE (% DM)
Check		82.78 ± 0.99^{a}	14.75. ± 0.67^{e}	2.30 ± 0.22^{d}	34.19 ± 2.44^{a}	9.11 ± 0.40^{a}	39.65 ± 4.88^{bcde}
Lactobacillus	1 mg/kg	82.18 ± 1.98^{ab}	16.44 ± 0.16^{bc}	2.93 ± 0.35^{abc}	33.40 ± 1.12^{ab}	8.50 ± 0.05^{cd}	42.43 ± 6.24^{abc}
	5 mg/kg	80.84 ± 0.16^{bcd}	16.30 ± 0.26^{bcd}	2.61 ± 0.15^{c}	32.84 ± 1.07^{abc}	8.41 ± 0.16^{cde}	39.83 ± 1.11^{bcde}
	10 mg/kg	80.35 ± 0.45^{cd}	16.70 ± 0.14^{bc}	2.76 ± 0.24^{bc}	31.03 ± 1.55^{bcd}	8.50 ± 0.08^{cd}	38.76 ± 0.33^{bcde}
Formic acid	1 ml/kg	80.85 ± 0.67^{bcd}	16.96 ± 0.41^{b}	3.18 ± 0.26^{ab}	31.16 ± 0.94^{bcd}	8.50 ± 0.36^{cd}	38.07 ± 0.92^{bcde}
	5 ml/kg	81.51 ± 0.43^{abc}	16.27 ± 0.28^{bcd}	2.56 ± 0.06^{c}	29.25 ± 0.39^{de}	8.68 ± 0.25^{bc}	43.24 ± 068ab
	10 ml/kg	80.04 ± 0.40^{cd}	16.01 ± 0.76^{cd}	2.47 ± 0.11^{c}	29.16 ± 0.37^{de}	8.29 ± 0.06^{de}	39.94 ± 0.92^{bcde}
Sucrose	1%	79.86 ± 0.89^{d}	16.10 ± 0.18^{bcd}	2.75 ± 0.04^{bc}	30.50 ± 0.88^{bcd}	8.19 ± 0.08^{de}	39.68 ± 0.30^{bcde}
	2%	77.65 ± 0.21^{e}	15.54 ± 0.47^{d}	2.70 ± 0.19^{bc}	27.21 ± 0.71^{ef}	8.08 ± 0.23^{e}	46.47 ± 0.39^{a}
	4%	76.62 ± 0.87^{e}	14.46 ± 0.52^{e}	2.53 ± 0.24^{c}	25.73 ± 1.50^{f}	7.10 ± 0.14^{f}	42.62 ± 0.83^{ab}
Cellulase	50 mg/kg	80.11 ± 0.20^{cd}	17.91 ± 0.40^{a}	2.97 ± 0.32^{abc}	30.46 ± 1.51^{bcd}	8.90 ± 0.11^{b}	36.93 ± 0.68^{e}
	100 mg/kg	80.31 ± 0.38^{cd}	16.90 ± 0.60^{bc}	2.81 ± 0.19^{bc}	30.01 ± 0.94^{bcd}	8.74 ± 0.12^{bc}	41.55 ± 0.12^{bcd}
	150 mg/kg	80.25 ± 0.10^{cd}	16.86 ± 0.73^{bc}	3.39 ± 0.08^{a}	30.84 ± 0.82^{bcd}	8.97 ± 0.25^{b}	37.80 ± 1.31^{de}

Table 2: *Effects of additives and their concentrations on the moisture [% fresh matter (FM)], crude protein (CP), ether extract (EE), crude fiber (CF), Ash, and nitrogen free extract (NFE) of 'Dancer' oat silage.*
Note: Values followed by different letter superscript in a column are significantly different at the 0.05 probability level.

Effects of additives on NDF and ADF fractions of oat silage

NDF and ADF percentages as affected by the four additives are shown in table 3.

Lactobacillus reduced the NDF and ADF values significantly (P < 0.05) compared with the check. Lowest NDF and ADF values were observed with the 10 mg/kg concentration, although concentration level values were not statistically different.

Formic acid significantly reduced NDF and ADF percentages (P < 0.05). Lowest NDF values were observed with the 5 ml/kg concentration and lowest ADF values were found with the 10 ml/kg concentration, although these differences were not significantly different.

Sucrose addition significantly decreased NDF and ADF values. Increasing sucrose concentrations significantly reduced NDF and ADF values further, with the 4% concentration having lowest values ($P < 0.05$).

Cellulase addition reduced NDF and ADF values. NDF was significantly reduced ($P < 0.05$) whereas ADF values were numerically reduced but not significantly different from the check. Concentration levels were not significantly different.

Additive	Concentration	NDF (%DM)	ADF (%DM)
Check		66.71 ± 5.14^{a}	41.37 ± 3.79^{a}
Lactobacillus	1 mg/kg	60.15 ± 3.42^{bc}	38.91 ± 3.36^{abc}
	5 mg/kg	61.62 ± 1.05^{b}	38.16 ± 1.02^{abc}
	10 mg/kg	60.13 ± 1.41^{bc}	36.89 ± 1.54^{bcd}
Formic acid	1 ml/kg	58.72 ± 1.49^{bc}	36.88 ± 1.00^{bcd}
	5 ml/kg	58.25 ± 2.11^{c}	37.27 ± 0.51^{bcd}
	10 ml/kg	58.76 ± 0.67^{bc}	35.88 ± 2.18^{cd}
Sucrose	1%	58.86 ± 0.97^{bc}	40.57 ± 5.47^{ab}
	2%	57.49 ± 1.69^{c}	37.55 ± 3.24^{bc}
	4%	54.30 ± 3.11^{d}	33.94 ± 3.97^{d}
Cellulase	50 mg/kg	58.04 ± 1.69^{c}	38.57 ± 1.56^{abc}
	100 mg/kg	58.99 ± 3.07^{bc}	38.73 ± 2.22^{abc}
	150 mg/kg	58.26 ± 1.28^{c}	38.18 ± 0.86^{abc}

Table 3: *Effects of additives and their concentrations on neutral detergent fiber (NDF) and acid detergent fiber (ADF) of oat silage.*
Note: Values followed by different letter superscript in a column are significantly different at the 0.05 probability level.

Effects of additive types and concentration on quality of oat silage

The effect of various concentrations of *Lactobacillus*, formic acid, sucrose, and cellulase on the quality of oat silage is shown in table 4.

Additive	Concentration	pH	AN/TN (%)	WSC (%DM)	LA (g/kg DM)	AA (g/kg DM)
Check		4.38 ± 0.75^{a}	12.64 ± 3.09^{a}	1.28 ± 0.29^{e}	36.13 ± 4.89^{bcd}	0.01 ± 0.00^{a}
Lactobacillus	1 mg/kg	4.11 ± 0.44^{b}	10.21 ± 1.06^{ab}	2.70 ± 0.60^{bcde}	46.79 ± 6.53^{abc}	0.13 ± 0.17^{a}
	5 mg/kg	3.49 ± 0.29^{cd}	4.53 ± 0.82^{bc}	3.46 ± 0.85^{bcde}	54.16 ± 3.60^{ab}	0.17 ± 0.02^{a}
	10 mg/kg	3.67 ± 0.15^{c}	5.66 ± 0.84^{abc}	2.28 ± 0.45^{cde}	59.89 ± 5.58^{a}	0.82 ± 1.02^{a}
Formic acid	1 ml/kg	3.18 ± 0.46^{de}	5.28 ± 0.96^{abc}	1.49 ± 0.47^{cde}	34.16 ± 4.46^{cde}	0.01 ± 0.00^{a}
	5 ml/kg	3.36 ± 0.23^{cde}	4.64 ± 0.31^{bc}	4.52 ± 0.69^{ab}	39.97 ± 2.77^{bcd}	0.00 ± 0.00^{a}
	10 ml/kg	2.96 ± 0.30^{e}	0.23 ± 0.00^{c}	6.38 ± 1.02^{a}	16.08 ± 2.00^{e}	0.00 ± 0.00^{a}
Sucrose	1%	3.19 ± 0.03^{cde}	3.14 ± 0.34^{bc}	4.79 ± 0.90^{ab}	36.59 ± 1.90^{bcd}	0.08 ± 0.09^{a}
	2%	3.05 ± 0.02^{de}	2.34 ± 0.50^{bc}	5.49 ± 0.96^{ab}	49.36 ± 3.37^{abc}	0.20 ± 0.05^{a}
	4%	3.05 ± 0.03^{de}	2.41 ± 0.41^{bc}	4.23 ± 0.81^{abcd}	41.74 ± 2.96^{abcd}	0.70 ± 0.99^{a}
Cellulase	50 mg/kg	3.42 ± 0.20^{cde}	4.25 ± 0.53^{bc}	4.47 ± 1.05^{ab}	27.14 ± 3.47^{de}	0.12 ± 0.03^{a}
	100 mg/kg	3.26 ± 0.03^{cde}	3.05 ± 0.66^{bc}	3.78 ± 0.78^{abcde}	37.01 ± 8.22^{bcd}	0.12 ± 0.04^{a}
	150 mg/kg	3.36 ± 0.12^{cde}	4.07 ± 0.41^{bc}	4.07 ± 0.69^{abcd}	23.46 ± 4.05^{de}	0.26 ± 0.15^{a}

Table 4: *Effects of additives and their concentrations on ammoniacal nitrogen to total nitrogen ratio (AN/TN), water soluble carbohydrates (WSC), lactic acid (LA) and acetic acid (AA) of oat silage.*
Note: Values followed by different letter superscript in a column are significantly different at the 0.05 probability level.

Addition of *Lactobacillus* significantly reduced the pH value of oat silage ($P < 0.05$). Increasing its concentration further reduced the pH, with the 10 mg/kg concentration significantly lower than the 1 mg/kg concentration. The 5 mg/kg concentration of *Lactobacillus* also significantly reduced the AN/TN value ($P < 0.05$). Water soluble carbohydrates (WSC) were increased nearly three-fold by the addition of *Lactobacillus*, but not significantly different ($P > 0.05$). The lactic acid (LA) content of oat silage was significantly increased by the 10 mg/kg concentration of *Lactobacillus*. Acetic acid values were not significantly altered by the addition of *Lactobacillus*.

The addition of formic acid significantly reduced the pH and AN/TN value ($P < 0.05$), but there were no significant differences among the three concentrations. Water soluble carbohydrate content was significantly higher than that of the check group when formic acid was applied at 5 or 10 ml/kg. The addition of 10 ml/kg of formic acid significantly decreased LA whereas the lower concentrations (1 and 5 ml/kg) did not decrease LA significantly.

Sucrose significantly reduced the pH and AN/TN value of oat silage ($P < 0.05$). Increasing sucrose concentrations did not further significantly affect these values. As expected, sucrose significantly increases the WSC percentage, but 2 and 4% concentrations were not different from the 1% concentration. Neither LA nor AA was significantly affected by sucrose addition.

Cellulase significantly reduced the pH value and AN/TN value of oat silage ($P < 0.05$), with no further significant decrease with increased concentrations. The WSC level was significantly increased by cellulase ($P < 0.05$), with no further significant decrease with increased concentrations. Cellulase treatment had no significant influence on LA or AA content.

Discussion

The four additives evaluated in this study had positive effects on the quality of 'Dancer' oat silage [9-13]. Determining which additive to choose and the concentration to apply will depend on the economics, convenience of application, and intended use for the silage. The following discussion provides key research findings to assist in those decisions.

Lactobacillus improved proximate analysis values, reduced fiber fractions, the AT/TN value, the pH, and increased the LA concentration of the silage. Most favorable values were found with the 5 or 10 mg/kg concentrations, although even the 1 mg/kg concentration resulted in improvements over the check.

Highest CP and EE values were found when formic acid was applied at 1 ml/kg. However, when the formic acid concentration was 5 ml/kg, the pH and AN/TN value were significantly reduced, the WSC was significantly increased, and the LA content was somewhat increased compared with the control. Thus, if formic acid is chosen as an additive for oat silage, the 5 ml/kg concentration is recommended.

Sucrose applied at 1% resulted in the highest CP and EE percentages, but most favorable pH, AN/TN, WSC, and LA values were observed at the 2% concentration. CF, NDF, and ADF values were best with the 4% level. Thus, the optimum sucrose concentration will depend on the economics and quality components most important in the livestock feeding operation.

When cellulase concentration was 50 mg/kg, all indices were excellent, including CP, EE and CF, NDF, and ADF. However, pH value, AN/TN, WSC, and LA were best with the 100 mg/kg concentration. As with sucrose, the optimum concentration of cellulase will depend on the quality component of most importance to the livestock enterprise.

Summary

Field-grown 'Dancer' oats was harvested at the milk stage, chopped into 2 - 3 cm pieces, and ensiled in 1L plastic bottles. Three concentrations of four additives, *Lactobacillus*, formic acid, sucrose, and cellulase, were evaluated to determine their influence on the quality of the silage. Silages were evaluated by sensory and laboratory analyses.

The four additives had positive effects on the silage quality. When *Lactobacillus* was applied at 5 mg/kg, most quality indicators were optimized. Formic acid applied at 5 ml/kg produced the most favorable values for pH, AN/TN value and percent WSC and LA content. The 2% sucrose treatment optimized pH, AN/TN, WSC, and LA values whereas fiber values were lowest with the 4% level. Cellulase concentration level had little affect on fiber levels but pH, AN/TN, WSC, and LA were best with the 100 mg/kg concentration.

Based on the improvement in numerous quality indicators, including both sensory and laboratory analyses, lactic acid bacteria and sucrose are recommended as good additive choices for ensiling oats. The choice of concentration will depend on what factors are of most importance in the livestock enterprise.

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