Effects of Lactic Acid Bacteria and Cellulase on the Quality of Alfalfa Silage with Different Moisture Contents: Postprint

Authors: Zhong Shu, Zhang Xiaona, Yun-Gui Yang, Yang Yuxin

Date: 2017-10-23T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of moisture content and additives on the silage quality of alfalfa, in order to address the challenges associated with alfalfa silage and improve its quality. A two-factor (moisture content × additive) completely randomized design was employed, with moisture contents of 70% and 60%; additives were categorized into four groups: a control group without additives, and three treatment groups each with three levels, namely lactic acid bacteria group (LA group, 3, 6, 9 mg/kg), cellulase group (CE group, 25, 50, 100 mg/kg), and a combined lactic acid bacteria and cellulase group (LA×CE group, 3×25, 6×50, 9×100 mg/kg), with three replicates per group, totaling 20 groups. After 90 days of ensiling, the contents of crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), water-soluble carbohydrates (WSC), pH, and ammonia nitrogen/total nitrogen (AN/TN) were measured. The results showed that: compared with the control group, all LA groups significantly increased the WSC content of silage ($P<0.05$) and significantly decreased its pH ($P<0.05$); furthermore, the CP and WSC contents of silage in the 6 mg/kg LA group were higher than those in the 3 and 9 mg/kg LA groups, while its pH was lower. Compared with the control group, all CE groups significantly decreased the ADF content and pH of silage ($P<0.05$), and the ADF content and pH of silage in the 50 mg/kg CE group were lower than those in the 25 and 100 mg/kg CE groups. The CF and ADF contents and pH of silage in most LA×CE groups were lower than those in the LA and CE groups, while the WSC content was higher than that in both the LA and CE groups, and the CF, ADF, and WSC contents and pH of silage in the 6×50 mg/kg LA×CE group differed significantly from those in the LA and CE groups ($P<0.05$). Except for the 9 mg/kg LA group and 100 mg/kg CE group, the CP content of silage with 70% moisture content was significantly lower than that with 60% moisture content under the same additive ($P<0.05$); similarly, the CF and ADF contents of silage with 70% moisture content were significantly higher than those with 60% moisture content under the same additive (P<0.05). These results indicate that lactic acid bacteria, cellulase, and their combined addition all significantly improved the silage quality of alfalfa, with the combined addition of 6×50 mg/kg lactic acid bacteria and cellulase showing the best effect, and the silage effect being superior at 60% moisture content.

Full Text

Effects of Lactic Acid Bacteria and Cellulase on Alfalfa Silage Quality at Different Moisture Levels

ZHONG Shu, ZHANG Xiaona, YANG Yungui, *YANG Yuxin*
College of Animal Science and Technology, Northwest A&F University, Yangling 712100, China

Abstract

This study investigated the effects of moisture content and additives on alfalfa silage quality to address the challenges associated with alfalfa ensiling and improve silage quality. A two-factor (moisture × additive) completely randomized design was employed, with moisture levels of 70% and 60%. Additives were divided into four groups: a control group without additives, and three treatment groups each containing three levels—lactic acid bacteria (LA group: 3, 6, and 9 mg/kg), cellulase (CE group: 25, 50, and 100 mg/kg), and a combined lactic acid bacteria and cellulase group (LA×CE group: 3×25, 6×50, and 9×100 mg/kg). Each group had three replicates, totaling 20 groups. After 90 days of ensiling, crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), water-soluble carbohydrate (WSC) content, pH, and ammonia nitrogen/total nitrogen (AN/TN) were measured.

The results showed that compared with the control group, all LA groups significantly increased WSC content ($P < 0.05$) and significantly decreased pH ($P < 0.05$). The 6 mg/kg LA group exhibited higher CP and WSC content and lower pH than the 3 and 9 mg/kg LA groups. All CE groups significantly reduced ADF content and pH compared to the control ($P < 0.05$), with the 50 mg/kg CE group showing lower ADF content and pH than the 25 and 100 mg/kg CE groups. Most LA×CE groups demonstrated lower CF and ADF content and pH, and higher WSC content than individual LA and CE groups, with the 6×50 mg/kg LA×CE group showing significant differences in CF, ADF, WSC content, and pH compared to individual LA and CE groups ($P < 0.05$). Except for the 9 mg/kg LA group and 100 mg/kg CE group, CP content in silage at 70% moisture was significantly lower than at 60% moisture under the same additive treatment ($P < 0.05$). Similarly, CF and ADF content in silage at 70% moisture was significantly higher than at 60% moisture under the same additive ($P < 0.05$).

These findings indicate that lactic acid bacteria, cellulase, and their combination significantly improve alfalfa silage quality, with the 6×50 mg/kg combination showing the best results. Moreover, ensiling at 60% moisture produced superior quality compared to 70% moisture.

Keywords: alfalfa silage; moisture content; lactic acid bacteria; cellulase
CLC number: S816.5+3

Introduction

Alfalfa (*Medicago sativa* L.) is a high-yield, high-quality leguminous forage rich in protein, amino acids, vitamins, and minerals, with high energy value and excellent nutritional, feeding, and medicinal properties, leading to its widespread cultivation worldwide. Alfalfa production significantly influences the development of animal husbandry, and China has gradually begun to attach importance to the livestock industry, resulting in a certain scale of alfalfa production. In major alfalfa-producing regions of China such as the Northeast and North China, losses due to rain during harvest are substantial, making silage technology a common solution for alfalfa preservation. Silage is a conventional processing technique that maintains the nutrition of green forage, improves palatability and digestibility, and extends its availability period. However, alfalfa is difficult to ensile successfully due to its high protein content, low soluble carbohydrate and dry matter content, and high buffering capacity.

Silage additives can effectively address these issues, with common additives including fermentation promoters, inhibitors, and nutritional additives. As fermentation promoters, lactic acid bacteria facilitate rapid transition to lactic acid fermentation during early ensiling, promoting the conversion of glucose and other monosaccharides to lactic acid, which rapidly reduces pH while inhibiting protein hydrolysis, decreasing ammonia nitrogen, acetic acid, and butyric acid concentrations, and reducing yeast and mold growth. Tabacco et al. added *Lactobacillus buchneri* to corn silage and found it effectively inhibited the growth of harmful bacteria such as *Enterococcus* and *Klebsiella pneumoniae* while improving aerobic stability. Cellulase can decompose cellulose, hemicellulose, and lignin in alfalfa, degrading structural carbohydrates into water-soluble carbohydrates (WSC) usable by lactic acid bacteria, thereby increasing fermentation substrates for lactic acid and improving organic matter digestibility. Liu et al. reported that plant cell walls degraded by enzyme preparations can be utilized by rumen microorganisms, improving the dry matter digestibility of water-soluble vitamins and other organic compounds.

Moisture content also significantly affects silage quality. Shu et al. set three moisture levels (75.3%, 64.2%, and 54.6%) for *Hemarthria compressa* and found that medium and low moisture levels produced good silage quality with lower pH and ammonia nitrogen concentration and higher lactic acid bacteria counts. Zhang et al. ensiled king grass at high, semi-dry, and low moisture levels and found semi-dry silage produced the best quality. Both excessively high and low moisture levels are detrimental to silage fermentation; high moisture dilutes cellular sugars below concentrations required for lactic acid fermentation while promoting rapid fermentation by harmful bacteria, resulting in poor quality, whereas low moisture inhibits the activity of some lactic acid bacteria.

While additives have been extensively studied in gramineous silage, research on improving alfalfa silage quality has intensified in recent years with certain achievements. However, studies on the concentrations of the specific additives used in this experiment (lactic acid bacteria and cellulase) remain limited. Combining production practice, this experiment added lactic acid bacteria, cellulase, and their combination to alfalfa silage at different moisture levels. By measuring changes in nutritional and fermentation quality indicators, we aimed to elucidate quality variation patterns and identify optimal additive combinations, providing a basis for moisture conditions and additive dosages during alfalfa ensiling to enable stable and efficient production.

Materials and Methods

1.1 Experimental Materials

The alfalfa variety used was Golden Queen, planted on March 15, 2013, in the teaching and experimental area of grassland science at Northwest A&F University and harvested on May 7, 2015, at the budding stage when raw material quality is optimal. Samples with 70% moisture (fresh grass) and 60% moisture (air-dried for 10 h) were ensiled. The lactic acid bacteria (LA) additive from Kunshan Baishengyou Biotechnology Co., Ltd. contained *Lactobacillus bulgaricus* and *Lactobacillus plantarum* with 10 colony-forming units per gram. Cellulase (CE) from Sinopharm Chemical Reagent Co., Ltd. had a relative molecular mass of 52,000–61,000, primarily containing -glucanase, -glucosidase, and highly active xylanase with enzyme activity exceeding 15,000 U/g.

1.2 Experimental Design

A two-factor completely randomized design was employed. Factor one was moisture content with two levels (70% and 60%). Factor two was additive type with four groups: a control (CK) group with no additive, and three treatment groups each with three levels—lactic acid bacteria (LA group: 3, 6, and 9 mg/kg), cellulase (CE group: 25, 50, and 100 mg/kg), and a combined group (LA×CE group: 3×25, 6×50, and 9×100 mg/kg), totaling 20 groups with three replicates each.

Raw materials were chopped into 3–4 cm pieces using a knife. Different concentrations of additives were sprayed onto materials with different moisture contents, with the control group sprayed with distilled water. After thorough mixing, materials were packed into 500 mL plastic bottles and compacted. Bottles were sealed with an inner cap, wrapped with sealing film, covered with an outer cap, and sealed again with sealing film. A total of 60 bottles were ensiled for 90 days.

1.3 Measurement Indicators and Methods

1.3.1 Sensory Evaluation Using the German Agricultural Association (DLG) silage sensory evaluation standards and grades, silage was scored based on odor, texture, and color and rated as excellent (16–20 points), acceptable (10–15 points), moderate (5–9 points), or poor (0–4 points).

1.3.2 Index Determination Nutritional Indicators

Crude protein (CP) content: Samples (0.2000 g) were digested with 5 mL concentrated sulfuric acid at 350 °C for 50 min, followed by addition of hydrogen peroxide. After the solution became transparent, it was cooled, brought to volume, and nitrogen concentration was determined using an Italian SYSTEA continuous flow chemistry analyzer.

Crude fat (EE) content: Determined using Soxhlet extraction. Filter paper packets were placed in the extraction chamber, soaked in petroleum ether overnight, heated in a 50 °C water bath for 6–8 h with repeated extraction, then dried.

Crude fiber (CF) content: Samples placed in glass crucibles were digested sequentially with 1.25% sulfuric acid and 1.25% sodium hydroxide on an SLQ-6CF analyzer, then burned and dried.

Crude ash (Ash) content: Samples in crucibles were ashed in a KSW-4D-11-5 muffle furnace at 550 °C for 3 h.

Detergent fiber content: Determined using the Van Soest method on a DGX-9053BC-1 fiber analyzer (ANKOM). For neutral detergent fiber (NDF), samples in filter bags were placed on a tray and digested with neutral detergent, anhydrous sodium sulfite, and thermostable -amylase for 75 min, then rinsed twice with hot water and once with cold water (with -amylase added during the first rinse), soaked in acetone, dried, and weighed. For acid detergent fiber (ADF), the procedure was similar but used acid detergent for digestion without -amylase during rinsing. Relative feeding value (RFV) was calculated as: RFV = [(88.9 -0.779 × ADF) × 120/NDF]/1.29.

Fermentation Quality

pH: Ten grams of sample were placed in a 100 mL flask with 90 mL distilled water, mixed thoroughly, sealed with film, and left at 4 °C for 24 h. After filtering through four layers of gauze, pH was measured using an FE20K pH meter (METTLER TOLEDO, Zurich, Switzerland).

Ammonia nitrogen/total nitrogen (AN/TN): Determined using the phenol-hypochlorite colorimetric method. Total nitrogen was measured after digesting samples with concentrated sulfuric acid and perchloric acid at 380 °C for 3 h, cooling, and bringing to volume in a 100 mL volumetric flask. Two milliliters of supernatant were analyzed with an equal volume of distilled water on a SYSTEA continuous flow chemistry analyzer. Ammonia nitrogen was measured by shaking samples with KCl, filtering, taking 5 mL of filtrate in a 50 mL volumetric flask, adding alkaline phenol and sodium hypochlorite solutions, bringing to volume with KCl, standing for 1 h, and measuring absorbance at 560 nm on a UV-3100 spectrophotometer.

WSC content: Determined using the anthrone colorimetric method. A glucose standard curve was prepared. Samples were boiled for 10 min, cooled, filtered, and brought to volume. After mixing, anthrone was added and absorbance was measured at 620 nm.

1.4 Data Processing and Statistical Analysis

Experimental data were processed and statistically analyzed using Excel 2010, with results expressed as mean ± standard deviation (X ± SD). SPSS 20.0 was used for one-way ANOVA, with Duncan' s multiple comparison test applied among groups. $P < 0.05$ was considered statistically significant.

Results

2.1 Characteristics of Alfalfa Raw Material

The silage raw material was alfalfa harvested at the budding stage. The characteristics of the alfalfa raw material are shown in . The raw material contained relatively high CP (19.94%), CF (27.95%), and low EE (1.77%). NDF and ADF contents were 41.96% and 32.95%, respectively, with an RFV of 140.34.

2.2 Effects of Different Moisture and Additive Concentrations on Nutritional Value of Alfalfa Silage

As shown in , at 70% moisture, the 6 mg/kg LA group, 50 mg/kg CE group, and 6×50 mg/kg LA×CE group significantly increased CP and EE content and significantly decreased CF content compared to the CK group ($P < 0.05$). The 6 mg/kg LA group showed significantly higher CP and EE content and lower CF content than the 3 and 9 mg/kg LA groups ($P < 0.05$). The 50 mg/kg CE group had significantly higher CP content than the 25 mg/kg CE group ($P < 0.05$) and significantly lower CF content than both the 25 and 100 mg/kg CE groups ($P < 0.05$). The 6×50 mg/kg LA×CE group exhibited significantly higher CP content and lower CF content than other LA×CE groups ($P < 0.05$).

At 60% moisture, the 6×50 mg/kg group significantly increased CP content and decreased CF content compared to the CK group ($P < 0.05$), while also significantly increasing EE content compared to all other groups ($P < 0.05$). The 6 mg/kg LA group showed significantly higher CP content than the 3 and 9 mg/kg LA groups ($P < 0.05$), though CF content did not differ significantly among these three groups ($P > 0.05$). The 50 mg/kg CE group had significantly higher CP content than the 25 and 50 mg/kg CE groups ($P < 0.05$) and significantly lower CF content than the 25 mg/kg CE group ($P < 0.05$). The 6×50 mg/kg LA×CE group demonstrated significantly higher CP and EE content than other LA×CE groups ($P < 0.05$).

Comparing the same additive across moisture levels, CP content in silage at 60% moisture was significantly higher than at 70% moisture for all additives except the 9 mg/kg LA and 100 mg/kg CE groups ($P < 0.05$). CF content at 60% moisture was significantly lower than at 70% moisture for all additives except the 6 mg/kg LA group ($P < 0.05$). EE content at 60% moisture was significantly higher than at 70% moisture for the 3 mg/kg LA group and the 6×50 and 9×100 mg/kg LA×CE groups ($P < 0.05$), with no significant differences observed in other groups ($P > 0.05$). Ash content showed no clear pattern. Moisture, additive, and moisture × additive interactions all significantly affected CP, CF, EE, and Ash content ($P < 0.05$).

2.3 Effects of Different Moisture and Additive Concentrations on Detergent Fiber Content and RFV

As shown in , NDF and ADF contents in alfalfa accurately reflect the actual utilization of feed by livestock. At 70% moisture, all CE and LA×CE groups and the 6 mg/kg LA group showed significantly lower NDF and ADF content and significantly higher RFV than the CK group ($P < 0.05$). The 3 and 6 mg/kg LA groups had significantly lower ADF content than the 9 mg/kg LA group ($P < 0.05$). The 50 mg/kg CE group exhibited significantly lower ADF content than the 25 and 100 mg/kg CE groups ($P < 0.05$).

At 60% moisture, all CE and LA×CE groups showed significantly higher RFV than the CK group ($P < 0.05$), while RFV in LA groups did not differ significantly from the CK group ($P > 0.05$). All additive groups significantly reduced ADF content compared to the CK group ($P < 0.05$), but only the 6×50 and 9×100 mg/kg LA×CE groups significantly reduced NDF content ($P < 0.05$). Additionally, the 3 mg/kg LA group had significantly lower ADF content than the 6 and 9 mg/kg LA groups ($P < 0.05$).

Comparing the same additive across moisture levels, ADF content in silage at 60% moisture was significantly lower than at 70% moisture for all additive groups except the CK group (where ADF was significantly lower at 70% moisture, $P < 0.05$) and the 9 mg/kg LA group (which showed no significant difference, $P > 0.05$). Additive groups at 60% moisture reduced NDF content by 0-2.1% and increased RFV by 2.69-16.57% compared to the CK group, while at 70% moisture, NDF content decreased by 3.3-8.05% and RFV increased by 10.25-31.68%. These results demonstrate that additives were more effective on NDF, ADF, and RFV at 70% moisture than at 60% moisture. Moisture, additive, and moisture × additive interactions all significantly affected NDF, ADF content, and RFV ($P < 0.05$).

2.4 Fermentation Quality Determination and Sensory Scores

As shown in , at 70% moisture, all additive groups significantly reduced silage pH compared to the CK group ($P < 0.05$). The 25 and 50 mg/kg CE groups and all LA×CE groups significantly reduced AN/TN ($P < 0.05$), while all LA and LA×CE groups significantly increased WSC content ($P < 0.05$). The 50 mg/kg CE group showed significantly lower pH than the 25 and 100 mg/kg CE groups ($P < 0.05$). The 6×50 mg/kg LA×CE group had significantly higher WSC content than other LA×CE groups ($P < 0.05$). The CK group received the lowest sensory score of 7 points (moderate grade), exhibiting strong butyric acid odor, poor leaf structure retention, and brown color. Additive groups scored similarly at 13-14 points (acceptable grade), showing strong sour odor, light yellow color, but poor stem-leaf structure retention.

At 60% moisture, all additive groups significantly reduced pH compared to the CK group ($P < 0.05$). All LA and LA×CE groups significantly increased WSC content ($P < 0.05$), and all LA×CE groups significantly reduced AN/TN ($P < 0.05$). The 50 mg/kg CE group showed significantly lower pH than the 25 and 100 mg/kg CE groups ($P < 0.05$). The 6×50 mg/kg LA×CE group had significantly lower AN/TN than other LA×CE groups ($P < 0.05$). The CK group scored 14 points (acceptable grade) with weak aroma and slight white color, while additive groups scored 17-18 points (excellent grade), showing strong aroma, color similar to raw material, but average stem-leaf structure retention.

Comparing the same additive across moisture levels, pH in silage at 60% moisture was significantly lower than at 70% moisture for all groups except the 3×25 and 6×50 mg/kg LA×CE groups ($P > 0.05$). The LA×CE groups reduced AN/TN by 1.19-2.07% and increased WSC content by 1.35-1.48% at 60% moisture, compared to reductions of 0.99-1.58% and increases of 0.52-1.04% at 70% moisture. Thus, additive effects on pH, AN/TN, and WSC content were superior at 60% moisture. Moisture significantly affected pH and WSC content ($P < 0.05$) but not AN/TN ($P > 0.05$), while additive and moisture × additive interactions significantly affected all three parameters ($P < 0.05$).

Discussion

Moisture content substantially influences silage effectiveness. This experiment compared alfalfa ensiled at 70% and 60% moisture, revealing that low-moisture silage showed significantly increased CP content and RFV, minimal change in EE content, and significantly decreased CF, NDF, and ADF content compared to high-moisture silage. High moisture promotes spoilage organism growth and nutrient loss through effluent, negatively affecting silage quality. However, semi-dry silage contains higher dry matter and soluble carbohydrates, increasing fermentation substrates and resulting in lower pH and higher lactic acid content. At 60% moisture, less nutrient loss occurred, with higher dry matter content than at 70% moisture without reaching physiological drought, preserving alfalfa nutrition more effectively and allowing additives to exert more pronounced beneficial effects. Yang et al. ensiled Italian ryegrass at 85%, 75%, and 65% moisture, finding that 65% moisture produced optimal results by concentrating WSC and other nutrients while inhibiting butyric acid bacteria, yeast, and enzymatic activity, significantly increasing CP content and decreasing CF content and AN/TN compared to 75% and 85% moisture. However, excessively low moisture during alfalfa ensiling reduces water activity, placing indigenous acid-producing bacteria under physiological drought and inhibiting acid accumulation, which is unfavorable for fermentation. Studies have also shown that air-dried alfalfa loses approximately 30% of its nutrients, with minimal differences in nutritional level and fermentation quality between direct ensiling of air-dried alfalfa and additive-treated silage.

Due to its low soluble carbohydrate content and high buffering capacity, alfalfa is difficult to ensile successfully. Additive use during ensiling can significantly improve fermentation quality. Lactic acid bacteria are crucial for successful silage, requiring populations of 10 CFU/g to rapidly reduce pH, inhibit harmful microorganisms, reduce nutrient consumption and decomposition, decrease toxic substance production such as amines, and ensure silage quality. Reports indicate that adding *Lactobacillus buchneri* under aerobic conditions can improve aerobic stability and prevent aerobic deterioration. In this experiment, lactic acid bacteria addition significantly reduced pH, likely by increasing indigenous lactic acid bacteria populations to dominate fermentation and inhibit harmful bacteria. However, AN/TN values in LA groups did not decrease significantly compared to the CK group, possibly because continued cellulase activity after lactic acid fermentation intensified protein degradation, reducing feed quality.

Cellulase degrades cellulose, hemicellulose, and lignin that are poorly digested by animals, hydrolyzes cell walls to release contents, increases fermentation substrates for lactic acid, improves organic matter digestibility, and enhances forage silage quality. In this experiment, cellulase addition effectively reduced ADF content and increased ADF digestibility in alfalfa silage, consistent with Zhang et al.' s findings. However, Sun et al. found that cellulase addition to *Lespedeza bicolor* and *Ceratoides latens* silage did not reduce ADF content compared to controls, possibly due to variations in plant species, physiological state, cellulase composition, and fermentation conditions. Li et al. added lactic acid bacteria and cellulase to rice straw silage and found limited cell wall decomposition by cellulase and minimal interaction with lactic acid bacteria on fermentation quality, likely related to differences in lignification degree and moisture content.

In this experiment, combined addition of lactic acid bacteria and cellulase reduced AN/TN, consistent with Hou et al.' s results. NDF and ADF content decreased significantly while RFV increased compared to the CK group, consistent with Ni et al.' s findings. Xu et al. ensiled smooth vetch and found that combined lactic acid bacteria and cellulase addition increased CP content and decreased NDF and ADF content, but pH did not differ significantly from the CK group, possibly due to different bacterial and enzyme types and dosages. In this experiment, combined additives improved fermentation quality more than single additives, though CP content increase and NDF content decrease were modest, possibly indicating some antagonism between the two additives. The 6×50 mg/kg LA×CE group showed optimal ensiling effects, while high concentrations of both additives resulted in higher pH and AN/TN and lower WSC content compared to lower concentrations, possibly because excessive lactic acid bacteria produced overly low pH that inhibited cellulase activity, making antagonism more pronounced. Tian et al. added combined lactic acid bacteria and cellulase to *Leymus chinensis* silage and found no improvement in CP and WSC content or reduction in AN/TN compared to single additives, possibly due to similar antagonistic effects. This experiment did not measure organic acids such as lactic, acetic, and butyric acids; including these measurements would provide more comprehensive reflection of alfalfa silage quality. Future research should examine multiple drying times to determine optimal moisture content for alfalfa ensiling to establish ideal conditions.

Conclusions

1. Adding lactic acid bacteria during alfalfa ensiling significantly increased WSC content and decreased pH, with 6 mg/kg being the optimal concentration. Adding cellulase significantly reduced ADF content and pH, with 50 mg/kg being the optimal concentration.
2. Combined addition of lactic acid bacteria and cellulase increased WSC content and decreased CF, ADF content, and pH compared to single additives, with 6×50 mg/kg being the optimal concentration.
3. Under the same additive treatment, silage at 60% moisture showed higher CP content and lower CF, ADF content, and pH than at 70% moisture. Therefore, ensiling at 60% moisture produced superior results compared to 70% moisture.

References

[1] Sun QY. Nutritional value of alfalfa and its application progress in meat rabbit production[J]. Chinese Rabbit Industry, 2012(7): 19–20.

[2] Li YP, Yang HM, Yang Z, et al. Nutritional value of alfalfa and its application in livestock and poultry production[J]. Feed Research, 2015(9): 14–18.

[3] Yang C, Wang ML. Alfalfa production and dairy development in China: grass-livestock integration is key to promoting development[J]. Chinese Journal of Animal Science, 2011, 47(16): 14–17, 21.

[4] Saricicek BZ, Kilic U. Effect of different additives on the nutrient composition, in vitro gas production and silage quality of alfalfa silage[J]. Asian Journal of Animal and Veterinary Advances, 2011, 6(6): 618–626.

[5] Shan GL, Chu XH, Chen G, et al. Discussion on alfalfa silage technology and its application[J]. Grassland and Livestock, 2011(7): 21–25.

[6] Tabacco E, Piano S, Revello-Chion A, et al. Effect of *Lactobacillus buchneri* LN4637 and *Lactobacillus buchneri* LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions[J]. Journal of Dairy Science, 2011, 94(11): 5589–5598.

[7] Zhang Q, Zhang WJ, Tian JP, et al. Research progress on lactic acid bacteria silage technology[J]. Pratacultural Science, 2014, 31(2): 328-333.

[8] Liu T, Wang TF, Luo K, et al. Research progress on alfalfa silage additives[J]. Feed Research, 2016(19): 12-14, 22.

[9] Liu ZY, Yu Z, Zhi JF, et al. Research progress on alfalfa silage[J]. Journal of Hebei Agricultural Sciences, 2013, 17(6): 62-65, 83.

[10] Shu SM, Yang CH, Tang ZS, et al. Effect of adding fermented green juice on silage quality of *Hemarthria compressa* with different moisture contents[J]. Grass-Feeding Livestock, 2011(4): 41-43.

[11] Zhang Y, Zhou HL, Liu GD, et al. Effect of different moisture contents on silage quality of king grass at different growth stages[J]. Journal of Livestock Ecology, 2013, 34(7): 39-43.

[12] Zhang YD, Yang JX, Wang ZW, et al. Research progress on physicochemical quality evaluation of silage[J]. Chinese Journal of Animal Science, 2016, 52(12): 37-42.

[13] Li GY, Chen YX, Lian HX, et al. Overview of evaluation index systems and determination methods for alfalfa silage quality[J]. Pratacultural Science, 2010, 27(8): 151-154.

[14] Zhang LY. Feed Analysis and Feed Quality Detection Technology[M]. 3rd ed. Beijing: China Agricultural University Press, 2007: 49-75.

[15] Yan XH, Xing Q, Wang JF, et al. Analysis and evaluation of nutritional value of natural hay fields in different regions of Xilingol grassland[J]. Grassland and Prataculture, 2015, 27(4): 40-44.

[16] Weatherburn MW. Phenol-hypochlorite reaction for determination of ammonia[J]. Analytical Chemistry, 1967, 39(8): 971-974.

[17] Owens VN, Albrecht KA, Muck RE, et al. Protein degradation and fermentation characteristics of red clover and alfalfa silage harvested with varying levels of total nonstructural carbohydrates[J]. Crop Science, 1999, 39(6): 1873-1880.

[18] Wan LQ, Li XL, He F. Effect of adding lactic acid bacteria and cellulase on alfalfa silage quality[J]. Pratacultural Science, 2011, 28(7): 1379-1383.

[19] Li P, Bai SJ, Yan JJ, et al. Effect of additives and moisture content on silage quality of *Elymus sibiricus*[J]. Acta Agrestia Sinica, 2013, 21(6): 1176-1181.

[20] Zhang XG, Han WX. Alfalfa semi-dry silage technology[J]. Animals Breeding and Feed, 2010(8): 78-80.

[21] Yang XL, Li JL, Yu Z, et al. Influence of moisture content on the silage quality of *Lolium multiflorum*[J]. Journal of Animal and Veterinary Advances, 2014, 13(12): 702-705.

[22] Zhang JX, Qiao HX, Liu YT. Effect of moisture and additives on alfalfa silage quality[J]. Pratacultural Science, 2014, 31(4): 766-770.

[23] Zhang XP, Wan LQ, Li XL, et al. Effect of adding lactic acid bacteria and cellulase on alfalfa silage quality (brief report)[J]. Acta Prataculturae Sinica, 2007, 16(3): 139-143.

[24] Li GY, Chen JH, Zhang LJ. Application progress of additives in alfalfa silage[J]. Feed Research, 2014(7): 14-16.

[25] Heinl S, Wibberg D, Eikmeyer F, et al. Insights into the completely annotated genome of *Lactobacillus buchneri* CD034, a strain isolated from stable grass silage[J]. Journal of Biotechnology, 2012, 161(2): 153-166.

[26] Weinberg ZG, Ashbell G, Hen Y, et al. The effect of applying lactic acid bacteria at ensiling on aerobic stability of silages[J]. Journal of Applied Bacteriology, 1993, 75(6): 512-518.

[27] Xi XJ, Han LJ, Yuan SY, et al. Effect of adding lactic acid bacteria and cellulase on corn straw silage quality[J]. Journal of China Agricultural University, 2003, 8(2): 21-24.

[28] Zhang Y, Zhou HL, Liu GD, et al. Effect of fermented green juice and cellulase on king grass silage quality[J]. Pratacultural Science, 2013, 30(10): 1640-1647.

[29] Al-Ghazzewi FH, Tester RF. Efficacy of cellulase and mannanase hydrolysates of konjac glucomannan to promote the growth of lactic acid bacteria[J]. Journal of the Science of Food and Agriculture, 2012, 92(11): 2394-2396.

[30] Zhang JG, Liu QH, Hattori I, et al. Applying cellulases to improve the fermentation quality and nutritive value of roll bale silage made with rice straw[C]//Proceedings of International Conference on Agricultural Engineering. Zibo, China: IEEE, 2011: 972-974.

[31] Sun QZ, Gao FQ, Yu Z, et al. Fermentation quality and chemical composition of shrub silage treated with lactic acid bacteria inoculants and cellulase additives[J]. Animal Science Journal, 2012, 83(4): 305-309.

[32] Li LX, Wang YF, Zhang MJ, et al. Utilization and research of additives in silage feed[J]. Animal Husbandry and Feed Science, 2015, 36(4): 60-63.

[33] Li J, Gao LY, Shen YX. Effect of lactic acid bacteria and cellulase on rice straw silage quality[J]. Journal of Nanjing Agricultural University, 2008, 31(4): 86-90.

[34] Hou ML, Geng GT, Sun L, et al. Effects of formic acid, cellulase, and lactic acid bacteria agent on silage quality of typical steppe natural forage[J]. Chinese Journal of Animal Nutrition, 2015, 27(9): 2977-2986.

[35] Ni KK, Wang YP, Pang HL, et al. Effect of cellulase and lactic acid bacteria on fermentation quality and chemical composition of wheat straw silage[J]. American Journal of Plant Sciences, 2014, 5(13): 1877-1884.

[36] Xu R, Chen PF, Bai SJ, et al. Effect of lactic acid bacteria and cellulase on silage fermentation quality of smooth vetch[J]. Acta Agrestia Sinica, 2014, 22(2): 420-425.

[37] Ding WR, Yang FY, Guo XS, et al. Effect of adding lactic acid bacteria and cellulase on silage quality of *Lespedeza bicolor*[J]. Journal of Northwest A&F University (Natural Science Edition), 2008, 36(4): 8-14, 21.

[38] Sun ZH, Liu SM, Tayo GO, et al. Effects of cellulase or lactic acid bacteria on silage fermentation and in vitro gas production of several morphological fractions of maize stover[J]. Animal Feed Science and Technology, 2009, 152(3/4): 219-231.

[39] Tian JP, Yu YD, Yu Z, et al. Effects of lactic acid bacteria inoculants and cellulase on fermentation quality and in vitro digestibility of *Leymus chinensis* silage[J]. Grassland Science, 2014, 60(4): 199-205.

Note: Figure translations are in progress. See original paper for figures.