Improvement of fermentation profile and structural carbohydrate compositions in mixed silages ensiled with fibrolytic enzymes, molasses and \textit{Lactobacillus plantarum} MTD-1

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\textbf{ABSTRACT}

In order to enhance the feed’s nutritive value and utilisation efficiency of local straws in Tibet, hulless-barley straw was conserved as silage by mixing with tall fescue and adding the tested additives and their combination. In experiment 1, hulless-barley straw was ensiled with four levels of tall fescue (0, 20, 40 or 60 of fresh weight, FW) in laboratory silos (1 L) for 60 days. The results suggested that 40\% hulless-barley straw inclusion of 60\% tall fescue was proper for further study. In experiment 2, a mixture of hulless-barley straw and tall fescue (40/60) were treated with (1) no additive (control), (2) fibrolytic enzymes (E, 0.1\% FW), (3) molasses (M, 4\% FW), (4) \textit{Lactobacillus plantarum} MTD-1 (Lp, 10\textsuperscript{6} cfu g\textsuperscript{-1} FW), (5) E + Lp and (6) M + Lp, respectively. Silos were opened after 7, 24, 45 and 60 days of ensiling respectively. The fermentation quality and structural carbohydrates were analysed. After 60 days of ensiling, all the additives except fibrolytic enzymes increased the lactic acid contents, and decreased the pH, ammonia nitrogen and butyric acid contents. All the treated silages had lower neutral detergent fibre, acid detergent fibre, hemicellulose and cellulose contents than that of control, especially in E + Lp and M + Lp treated silages. In conclusion, application of \textit{L. plantarum} together with fibrolytic enzymes or molasses could further improve the fermentation quality and structural carbohydrate compositions in comparison with using each of the three additives alone.

\textbf{HIGHLIGHTS}

- Enhancing the utilisation efficiency of local straws by mixed silage.
- Improving the feed’s nutritive value by reducing the crude fibre contents.

\textbf{Introduction}

Tibet is one of the five most important pastoral areas in China with 82.1 million ha of grassland (Gai et al. 2009). However, harsh climates limit the forage yield, resulting in malnutrition and poor production performance of livestock (Wang et al. 2017). Unreasonable feeding methods, such as using native grass as the only feed resource, overgrazing and so forth, also destroyed the grass production capacities (Qiu et al. 2014). In decade years, sustainable agriculture is gaining momentum, agricultural by-products are used to replace part of the native grass to compensate limited forage yield and constrain malnutrition and low performance. As a result, demand is increasing for efficient use of agricultural by-products due to economic and environmental concerns.

Large amounts of straws have been produced on the Tibetan Plateau and most of Tibetan herdsman feed straws to their cattle. Nevertheless, the Achilles heel of utilising straw is its high structural carbohydrate compositions (e.g. cellulose, hemicelluloses and lignin), resulting in low nutritional value and poor palatability for ruminant. Recently, ensiling straws with other palatable materials have been commonly used. Such ensiling could improve fermentation quality and produce a maintenance diet for ruminants (Ridla and Uchida 1994; Cao et al. 2011; Zhu et al. 2011). Tall fescue (\textit{Festuca arundinacea} L.) is a typically cultivated grass on the Tibetan Plateau due to its high water-soluble carbohydrate (WSC) and strong adaptive capacity. Therefore, it was assumed that ensiling straws with tall fescue could compensate fermentation substrate
for extensive fermentation. Optimum ratio was thus examined by mixing hulless-barley straw with tall fescue in varying proportions.

In order to further improve the fermentation quality of mixed silages, the tested additives and their combination are subsequently applied. Molasses is a by-product of sugar-cane and sugar-beet industries, and has a soluble carbohydrate content of about 650 g kg⁻¹ DM (dry matter) (McDonald et al. 1991). It has been extensively used as a fermentation substrate to stimulate lactic acid bacteria (LAB) growth and improve silage quality. Fibrolytic enzymes, such as xylanase and cellulose, are often added in silages to hydrolyse plant cell walls to provide more fermentable substrates for LAB growth during ensiling (Yu et al. 2011). Lactobacillus plantarum has been widely used to improve silage quality because of its wide fermentation substrates and lactic acid production efficiency. Adding LAB inoculants at ensiling could ensure rapid and vigorous fermentation, which leads to faster accumulation of lactic acid, lower pH during the early stages of ensiling and inhibition of the growth of undesirable bacteria (Cai et al. 1999). However, there is limited information regarding the effects of tested additives and their combination on straw-grass mixed silage, particularly in structural carbohydrate compositions.

This study aimed to screen an optimum ratio by mixing hulless-barley straw with tall fescue in different proportions, and then further evaluate the effects of adding fibrolytic enzymes, molasses and L. plantarum inoculant alone or combination of them on the fermentation profile and structural carbohydrates of mixed silage.

Materials and methods

**Experiment 1: screening of the optimal ensiling ratio of hulless-barley straw and tall fescue**

**Experimental design**

The hulless-barley and tall fescue were cultivated in the experimental field of Shigatse Grassland Station (Tibet, China: N 29°16’, E 88°51’, elevation 3836 m, annual mean temperature 6.5 °C and average annual precipitation 400 mm). The pH of the silty loam soil was 8.1, total N content of 0.54 g kg⁻¹, total potassium content of 5.75 g kg⁻¹, total P content of 0.46 g kg⁻¹. Hulless-barley straw (the residue remaining after grain harvest) and tall fescue (the heading stage) were harvested on 17 September 2011.

Four different mixtures were prepared on fresh weight (FW) basis (Table 2), as follows: (1) 100% hulless-barley straw; (2) 80% hulless-barley straw +20% tall fescue; (3) 60% hulless-barley straw +40% tall fescue; (4) 40% hulless-barley straw +60% tall fescue. Twenty bottles in total (4 ratios ×5 replicates =20) were prepared, and the silos for each treatment were opened after 60 days of ensiling. The materials were collected and chopped into approximately 2–3 cm lengths with a fodder chopper and immediately transported to the laboratory. According to the designed proportions, prepared hulless-barley straw and tall fescue were put into each plastic box. After thorough mixing, about 600 g of mixture was packed tightly into plastic laboratory silos (1 L capacity), followed by being sealed with a screw top and kept at ambient temperature (~22 °C).

**Chemical and microbiological analyses**

At sampling, each ensilage material or silage was put into an ethanol-disinfected plastic container and mixed uniformly, then we took three sub-samples from the total mixture.

The first sub-sample was analysed immediately for the DM content in a forced-draft oven to a constant weight drying at 65 °C for at least 48 h, and then ground to pass a 1 mm screen in a laboratory knife mill (FW100, Taïsite Instrument Co., Ltd., Tianjin, China) for later analysis. Total nitrogen (TN) was determined according to Krishnamoorthy et al. (1982), and crude protein (CP) content was calculated by multiplying TN by 6.25. The WSC content was determined according to Chen et al. (2014). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were analysed according to Van-Soest (1991). Hemicellulose is calculated as NDF minus ADF, and cellulose is calculated as ADF minus ADL. The results of NDF, ADF and ADL were expressed on DM basis including residual ash.

The second sub-sample was prepared according to Wang et al. (2017). The pH was measured immediately with a glass electrode pH metre (HANNA pH 211, Hanna Instruments Italia Srl, Villafranca Padovana, Italy). Ammonia nitrogen (NH₃–N) were determined according to Chen et al. (2014). Organic acid contents of the silage were analysed using the Agilent HPLC 1260 (Agilent Technologies, Inc., Berlin, Germany; column: Carbomix® H-NPS, Sepax Technologies, Inc., Santa Clara, CA, USA; detector: refractive index detector, Agilent Technologies, Inc. Berlin, Germany; eluent: 2.5 mmol L⁻¹ H₂SO₄, 0.5 mL min⁻¹; temperature: 55 °C).

The third sub-sample (10 g) was mixed with 90 mL sterilised saline solution (0.85%). The LAB count was
determined according to Liu et al. (2012). The microbial data were transformed to log10 and are presented on a fresh weight basis.

**Experiment 2: effect of different additives on the fermentation quality and structural carbohydrates of hulless-barley straw-grass mixed silages**

**Experimental design**

The hulless-barley and tall fescue were cultivated in the same experimental field as experiment 1 (Shigatse Grassland Station, Tibet). Hulless-barley straw (the residue remaining after grain harvest) and tall fescue (the heading stage) were harvested on 25 September 2012.

According to the result of experiment 1, the mixed silages inclusion of 40% hulless-barley straw performed best. Thus, hulless-barley straw and tall fescue were mixed in the proportions of 40/60 based on a fresh weight in experiment 2. The mixture was divided into equal portions for application of six treatments: (1) control without additive, defined as treatment C; (2) fibrolytic enzymes (0.1% FW, Cornzyme liquid, comprises cellulase and xylanase, Finn feeds International Co. Finland), treatment E; (3) molasses (4% FW, industrial by-product and obtained from a private company at Shigatse, chemical compositions are shown in Table 1), treatment M; (4) L. plantarum MTD-1 (6 log cfu g⁻¹ FW, a commercial LAB inoculant, Ecosyl. Products. Inc. Madison, WI, USA), treatment Lp; (5) fibrolytic enzymes+L. plantarum, treatment E+Lp; (6) molasses+L. plantarum, treatment M+Lp. One hundred twenty bottles in total (6 additive treatments ×4 sample time ×5 replicates =120) were prepared, and five replicates for each treatment were opened on 7, 24, 45 and 60 days of ensiling, respectively.

The silage making, sampling and chemical analysis of raw materials and silages were performed for the experiment 1, except that the silos were opened at different days after ensiling in the experiment 2.

**Statistical analyses**

Analyses were performed using the general linear model (GLM) procedure of Statistical Analysis System (SAS). Data on the fermentation quality of mixed silage in experiment 1 and the structural carbohydrates in experiment 2 were analysed by one-way analysis of variance (ANOVA). Data on the fermentation characteristics of mixed silage in experiment 2 was analysed by two-way ANOVA using the GLM procedure of SAS according to the model for a factorial treatment design as follows:

\[ Y_{ij} = \mu + l_i + T_j + (l \times T)_{ij} + e_{ij} \]

where \( Y_{ij} \) is the dependent variable; \( \mu \) is overall mean; \( l_i \) is the effect of additives; \( T_j \) is the effect of ensiling days; \( (l \times T)_{ij} \) is the effect of interaction between additives and ensiling days and \( e_{ij} \) is the residual error term. Statistical differences among means were determined using Tukey’s multiple comparison. Differences were considered significant at \( p < .05 \).

### Table 1. Chemical (g kg⁻¹ DM) and microbial (log cfu g⁻¹ FW) composition of tall fescue and hulless-barley straw.

| Items                  | Experiment 1 | Experiment 2 |
|------------------------|--------------|--------------|
| DM, g kg⁻¹ FW          | Tall fescue  | Hulless-barley straw |
| 269                    | 317          | 522          |
| CP                     | 62.40        | 83.50        |
| WSC                    | 264          | 210          |
| NDF                    | 553          | 522          |
| ADF                    | 302          | 297          |
| Hemicellulose          | 231          | 225          |
| Cellulose              | 267          | 262          |
| LAB                    | 4.89         | 2.78         |

DM: dry matter; FW: fresh weight; CP: crude protein; WSC: water soluble carbohydrates; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; LAB: lactic acid bacteria; cfu: colony forming unit.

### Table 2. Chemical (g kg⁻¹ DM) and microbial (log cfu g⁻¹ FW) compositions of mixed substrates.

| Ratios of hulless-barley straw and tall fescue | Items | 100/0 | 80/20 | 60/40 | 40/60 |
|------------------------------------------------|------|------|------|------|------|
| DM, g kg⁻¹ FW                                  | 386  | 372  | 358  | 345  |
| CP                                              | 70.70| 73.30| 75.80| 78.40|
| WSC                                             | 79.50| 105.60| 131.70| 157.80|
| NDF                                             | 672  | 642  | 612  | 582  |
| ADF                                             | 387  | 369  | 351  | 333  |
| ADL                                             | 70.30| 63.20| 56.10| 48.90|
| Hemicellulose                                   | 285  | 273  | 261  | 249  |
| Cellulose                                       | 317  | 306  | 295  | 284  |
| LAB                                             | 3.21 | 3.48 | 3.75 | 4.01 |

DM: dry matter; FW: fresh weight; CP: crude protein; WSC: water soluble carbohydrates; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; LAB: lactic acid bacteria; cfu: colony forming unit.
Results

Experiment 1: Screening of the optimal ensiling ratio of hulless-barley straw and tall fescue

The fermentation quality of mixed silage after 60 days of ensiling is illustrated in Table 3. The increase of the tall fescue ratios increased WSC, lactic acid (LA) contents and ratios of lactic acid to acetic acid (LA/AA), and decreased the pH, DM, acetic acid (AA) and NH3-N contents. The mixed silages containing 60% tall fescue had significantly \( (p < .05) \) higher LA, WSC contents and ratio of LA/AA, and significantly \( (p < .05) \) lower pH and NH3-N contents than that of silage made of hullless-barley straw alone. Among all the mixed silages, propionic acid (PA) contents were detected in small amounts and there was no significant difference \( (p > .05) \) in butyric acid (BA) contents.

Experiment 2: Fermentation characteristics of mixed silages added with different additives

According to the result of experiment 1, the mixed silages inclusion of 40% hulless-barley straw performed best. Thus, the 40% hulless-barley straw and 60% tall fescue was ensiled with different additives to further improve the fermentation quality in experiment 2.

The fermentation quality of mixed silage, represented as dynamics change in chemical composition, is presented in Tables 4 and 5. The effect of additive treatment or ensiling days on fermentation characteristics was evident \( (p < .01) \), except the effect of additive treatment on LA and PA contents \( (p > .05) \). The interaction between additive treatment and ensiling days significantly influenced the silage pH, LA and BA contents \( (p < .01) \), NH3-N content \( (p < .05) \), whereas failed to effect on the silage DM, WSC, AA, PA contents and ratio of LA/AA \( (p > .05) \).

As shown in Tables 4 and 5, the DM and residual WSC contents in M and M + Lp silages were significantly \( (p < .05) \) higher than that of other silages during the ensiling. All the treated silages had significantly \( (p < .05) \) lower NH3-N and BA contents, and significantly \( (p < .05) \) higher residual WSC contents than control at the end of ensiling. Compared to M or E silages, Lp silages had significantly \( (p < .05) \) higher ratios of LA/AA. Compared to E silage, M silage had significantly \( (p < .05) \) higher LA contents and lower NH3-N and BA contents. After 60 days of ensiling, Lp, E + Lp and M + Lp treatments significantly \( (p < .05) \) increased LA contents and ratios of LA/AA, and significantly \( (p < .05) \) decreased pH and AA contents compared to the control and E treatment. E + Lp and M + Lp treatments significantly \( (p < .05) \) reduced NH3-N contents than E, M and Lp treatment alone.

Structural carbohydrate contents of mixed silages added with different additives

The structural carbohydrate contents of mixed silage are illustrated in Table 6. After 60 days of ensiling, all the treated silages had significantly \( (p < .05) \) lower NDF, ADF, hemicellulose and cellulose contents than that of control, while there was no significant \( (p > .05) \) difference in ADL contents among all silages. E + Lp and M + Lp silages had significantly \( (p < .05) \) lower NDF, hemicellulose and cellulose contents than other treated silages. Compared with control, E, E + Lp and M + Lp treated silages had significantly \( (p < .05) \) lower ratios of NDF/ADL, ADF/ADL, hemicellulose/ADL and cellulose/ADL.

Discussion

In experiment 1, ensiling hulless-barley straw with tall fescue showed some improvement of fermentation quality after 60 days. Nevertheless, quality silage
Table 4. Effects of different additives on fermentation characteristics of mixed silages of hulless-barley straw and tall fescue (40/60) during ensiling.

| Items                      | Treatments | Significance |
|----------------------------|------------|--------------|
| DM, g kg⁻¹ FW              |            |              |
| DM                         |            |              |
| E                          |            |              |
| M                          |            |              |
| E + Lp                     |            |              |
| M + Lp                     |            |              |
| SEM                        |            |              |
| T                          |            |              |
| D                          |            |              |
| T × D                      |            |              |
| pH                         |            |              |
| pH                         |            |              |
| E                          |            |              |
| M                          |            |              |
| E + Lp                     |            |              |
| M + Lp                     |            |              |
| SEM                        |            |              |
| T                          |            |              |
| D                          |            |              |
| T × D                      |            |              |
| Lactic acid, g kg⁻¹ DM     |            |              |
| Lactic acid, g kg⁻¹ DM     |            |              |
| E                          |            |              |
| M                          |            |              |
| E + Lp                     |            |              |
| M + Lp                     |            |              |
| SEM                        |            |              |
| T                          |            |              |
| D                          |            |              |
| T × D                      |            |              |
| NH₃–N, g kg⁻¹ TN           |            |              |
| NH₃–N, g kg⁻¹ TN           |            |              |
| E                          |            |              |
| M                          |            |              |
| E + Lp                     |            |              |
| M + Lp                     |            |              |
| SEM                        |            |              |
| T                          |            |              |
| D                          |            |              |
| T × D                      |            |              |
| WSC, g kg⁻¹ DM             |            |              |
| WSC, g kg⁻¹ DM             |            |              |
| E                          |            |              |
| M                          |            |              |
| E + Lp                     |            |              |
| M + Lp                     |            |              |
| SEM                        |            |              |
| T                          |            |              |
| D                          |            |              |
| T × D                      |            |              |

Values in the same row with different superscript letters are significantly different (p < 0.05).

DM: dry matter; FW: fresh weight; NH₃–N: ammonia nitrogen; TN: total nitrogen; WSC: water soluble carbohydrates; C: control; E: fibrolytic enzymes; M: molasses; Lp: Lactobacillus plantarum MTD-1; E + Lp: fibrolytic enzymes + Lactobacillus plantarum MTD-1; M + Lp: molasses + Lactobacillus plantarum MTD-1; SEM: standard error of the mean; T: additive treatment; D: ensiling days; T × D: interaction of additive treatments and ensiling days. ** = p < 0.01; *= p < 0.05; NS = not significant (p > 0.05).

Table 5. Effects of different additives on organic acids and LA/AA ratios of mixed silages of hulless-barley straw and tall fescue (40/60) during ensiling.

| Items                      | Treatments | Significance |
|----------------------------|------------|--------------|
| Acetic acid, g kg⁻¹ DM     |            |              |
| Acetic acid, g kg⁻¹ DM     |            |              |
| E                          |            |              |
| M                          |            |              |
| E + Lp                     |            |              |
| M + Lp                     |            |              |
| SEM                        |            |              |
| T                          |            |              |
| D                          |            |              |
| T × D                      |            |              |
| Propionic acid, g kg⁻¹ DM  |            |              |
| Propionic acid, g kg⁻¹ DM  |            |              |
| E                          |            |              |
| M                          |            |              |
| E + Lp                     |            |              |
| M + Lp                     |            |              |
| SEM                        |            |              |
| T                          |            |              |
| D                          |            |              |
| T × D                      |            |              |
| Butyric acid, g kg⁻¹ DM    |            |              |
| Butyric acid, g kg⁻¹ DM    |            |              |
| E                          |            |              |
| M                          |            |              |
| E + Lp                     |            |              |
| M + Lp                     |            |              |
| SEM                        |            |              |
| T                          |            |              |
| D                          |            |              |
| T × D                      |            |              |
| LA/AA                      |            |              |
| LA/AA                      |            |              |
| E                          |            |              |
| M                          |            |              |
| E + Lp                     |            |              |
| M + Lp                     |            |              |
| SEM                        |            |              |
| T                          |            |              |
| D                          |            |              |
| T × D                      |            |              |

Values in the same row with different superscript letters are significantly different (p < 0.05).

DM: dry matter; LA/AA: the ratio of lactic acid to acetic acid; C: control; E: fibrolytic enzymes; M: molasses; Lp: Lactobacillus plantarum MTD-1; E + Lp: fibrolytic enzymes + Lactobacillus plantarum MTD-1; M + Lp: molasses + Lactobacillus plantarum MTD-1; SEM: standard error of the mean; ND: not detected; T: additive treatment; D: ensiling days; T × D: interaction of additive treatments and ensiling days. ** = p < 0.01; *= p < 0.05; NS = not significant (p > 0.05).

should have a pH value of 4.2 or less and a value of less than 2 g kg⁻¹ DM for BA (McDonald et al. 1991). All the mixed silages in experiment 1 were not ideal, because they had higher pH values (>4.3) and BA contents (>2.1 g kg⁻¹ DM). Among all the silages, the mixed silages inclusion of 40% hulless-barley straw performed best. Thus, to enhance the fermentation quality of mixed silage, it is necessary to use different silage additives.

The WSC content, together with the activity of naturally occurring LAB, determines the rate of increase in LA and decline in pH during the early stages of ensiling, which is important for the production of stable silage. Hence, some additives which increased...
WSC contents and LAB numbers could result in good fermentation and preservation (McDonald et al. 1991). In experiment 2, our results suggested that applying fibrolytic enzymes, molasses or L. plantarum alone could improve fermentation characteristics of mixed silage.

In this study, L. plantarum was more efficient than molasses or fibrolytic enzymes alone in improving the silage quality. This may be related to the WSC contents and natural LAB population of mixed substrates. The WSC content (157.8 g kg\(^{-1}\) DM) of mixed substrates met the minimum requirement (60–70 g kg\(^{-1}\) DM) for successful fermentation, as judged by Zhang et al. (2016). Moreover, epiphytic LAB plays an important role in natural silage fermentation, and well-preserved silage required a minimum amount of lactobacilli (10\(^5\) cfu g\(^{-1}\) FW; Lin et al. 1992). However, the mixed substrates had a lower epiphytic LAB population (10\(^4\) cfu g\(^{-1}\) FW). Thus, the lack of effective epiphytic LAB was the main limiting factor for improving fermentation quality. Furthermore, the LAB inoculation in this study was L. plantarum, which could ferment a wide variety of substrates and quickly produce large amounts of lactic acid (McDonald et al. 1991). Therefore, the mixed silage inoculated with L. plantarum showed a better fermentation quality compared with the silages treated with molasses or fibrolytic enzymes alone.

Molasses alone or the combination of molasses and L. plantarum obviously increased DM contents than other silages during the whole ensiling, which may be due to the high DM content of the molasses; this finding was consistent with the study of Rezaei et al. (2009). Besides, molasses had better improvement efficiency in silage quality compared with fibrolytic enzymes. This discrepancy was apparently reflected in the initial fermentation days (7 day), which was the key to the success of silage making (Shao et al. 2007). This was because molasses could directly provide fermentation substrates for LAB growth, while fibrolytic enzymes need some time to degrade cell wall polysaccharides to fermentable sugar (Lynch et al. 2014). Accordingly, the improvement efficiency of these separate additives for mixed silages was in the following order: L. plantarum > molasses > fibrolytic enzymes.

Due to the different action modes of the tested additives on silage fermentation, the combination of both additives would overcome the limitation of single additive. When L. plantarum was applied together with fibrolytic enzymes or molasses, the fermentation quality was further improved compared with the silages treated with three kinds of additive alone. This was consistent with the reports of Lima et al. (2010), who found that homofermentative lactobacilli in combination with cellulases or molasses could enhance fermentation quality. It was suggested that LAB inoculant combined with fibrolytic enzymes or molasses had a synergistic effect on improving silage quality. This also conformed to our broad consensus concerning that adequate WSC content and homofermentative lactobacilli counts could rapidly promote LA production and reduce the silage pH, resulted in inhibition of undesirable microbial numbers and low NH\(_3\)-N contents during ensiling.

In the present experiment, lower NDF, ADF, hemicellulose and cellulose contents were observed in all treated silages compared with control, which was consistent with the studies of Sun et al. (2009) and Yu et al. (2011). However, the tested additives and their combination reduce the structural carbohydrates by different mechanisms. Fibrolytic enzymes, comprising cellulase and xylanase, could directly hydrolyse lignocellulose to mono-saccharides and release additional fermentable substrate. Potentially, as a result of fibre hydrolysis by exogenous fibrolytic enzymes, the liberation of additional soluble sugars would promote
both the rate and extent of lactic acid production during silage fermentation. Molasses and LAB inoculant could also conserve more fermentable substrate (WSC) in silages after 60 days of ensiling. Shao et al. (2007) concluded that adding LAB to silages could reserve more sugars, because LAB addition could ensure rapid and vigorous LA fermentation and faster reduction of silage pH at earlier stages of ensiling, depressing the loss WSC fermented by undesirable bacteria and resulting in more WSC being saved in silages.

In our study, M + Lp silage had the same levels of structural carbohydrate contents with E + Lp silage after 60 days of ensiling. M + Lp silage also maintained almost the lowest pH values (4.07–4.37) during the whole ensiling. Thus, we speculated that the cell wall degradation in M + Lp silage was mainly related to acid hydrolysis. The organic acids produced during ensiling could contribute to the hydrolysis of structural carbohydrates. Similarly, Aksu et al. (2006) found that molasses addition could significantly decrease the NDF and ADF contents probably due to acid hydrolysis of cell walls resultant from reduction in silage pH by lactic acid fermentation. Huisden et al. (2009) also suggested that concentrations of hemicellulose and NDF in corn silage decreased markedly because of the acid hydrolysis of cell wall fractions during the ensiling. The ratios of NDF/ADL, ADF/ADL, hemicellulose/ADL and cellulose/ADL could be used to reflect the changes of various structural carbohydrates (Desta et al. 2016). In our study, E, E + Lp and M + Lp treatments all obviously decreased the ratios of NDF/ADL, ADF/ADL, hemicellulose/ADL and cellulose/ADL compared with control. This clearly indicated that fibrolytic enzymes could effectively degrade structural carbohydrates during the ensiling, and the cell wall degradation occurred in M + Lp silage due to the acid hydrolysis.

Conclusions

Our results confirmed the known benefits of combining straw with grass. Moreover, to further improve the fermentation quality, the tested additives and their combination were applied to the binary mixtures before ensiling. The results showed that all the additives not only markedly improved fermentation quality, but also reduced the structural carbohydrate contents, especially in E + Lp and M + Lp treatments. Therefore, the addition of LAB inoculant combined with fibrolytic enzymes or molasses to mixed silage could be a feasible strategy towards solving the forage shortage.

Disclosure statement

No potential conflict of interest was reported by the authors. The authors alone are responsible for the content and writing of this paper.

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