Fermentation quality and microbial community of alfalfa and stylo silage mixed with Moringa oleifera leaves

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A R T I C L E   I N F O

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Microbial community
Mixed ensiling
Moringa oleifera

A B S T R A C T

The silage quality of alfalfa and stylo without or with 25%, 50% Moringa oleifera leaves (MOL) was investigated, and microbial community after ensiling was analysed. Results showed that the silage samples with MOL have lower butyric acid (0.50 vs 1.20, 0.60 vs 14.5 g/kg dry matter (DM) in alfalfa and stylo silage, respectively), ammonia-N (152 vs 262, 109 vs 180 g/kg total N) content and DM loss (7.71% vs 14.6%, 6.49% vs 18.9%). The addition of MOL also influenced the bacterial community distribution. The relative abundance of Enterobacter decreased from 58.6% to 30.5%, 17.4% to 9.1% in alfalfa and stylo silage when 50% MOL was added. Clostridium decreased from 23.5% to 0.2% in stylo silage, whereas Lactobacillus abundance increased from 30.4% to 49.9%, 41.8% to 86.0% in alfalfa and stylo silage, respectively. In conclusion, mixing with MOL could be a feasible way to improve the quality of alfalfa and stylo silage.

1. Introduction

Moringa oleifera Lam., originated from the Indian subcontinent, is a multipurpose tree growing almost worldwide. It could adapt to all types of soils and tolerate extreme temperatures (Zheng et al., 2016). Moringa oleifera yields a high amount of biomass ranging from 43 to 115 tons per hectare annually. Moreover, it contains 241–277 g crude protein (CP) (/kg dry matter, DM), with approximately 47% bypass protein, adequate amino acid profile and high level of vitamins A, B, and C (Kholif et al., 2016; Nouman et al., 2016). These nutritional traits along with high production and adaptability have turned Moringa a potential high quality food for human and feed source for livestock (Sriwichai et al., 2017). Many studies found providing M. oleifera for dairy cattle could improve feed utilization and milk production compared with traditional diets (Babiker et al., 2017; Kholif et al., 2018).

Moringa oleifera is a food of high nutritional value, but it also showed high antimicrobial and antioxidative activity attribute to the presence of unsaturated fatty acids, flavonoids, ascorbic acid, phenolics and carotenoids (Lee et al., 2017; Guillén-Román et al., 2018). Moringa oleifera leaf (MOL) extract could be used as a natural antimicrobial agent in food preservation (Jayawardana et al., 2015). Numerous scientific articles reported the extract of MOL could inhibit the growth of undesirable bacteria like Staphylococcus, Bacillus, Listeria, Escherichia (Ratschilivha et al., 2014), which are also frequently detected in silage (Yang et al., 2019). On the other hand, MOL are rich in polyphenols such as tannins (20.6 g/kg DM, Teixeira et al., 2014), which could restrict proteolysis in ensiled forage (Guo et al., 2008). Previous study demonstrated that M. oleifera could increase the nutritive value of the silage after mixing with other grass or sugarcane (Mendieta-Araica et al., 2009). However, little information about effects of MOL on microbial communities in silage is available. In addition, legume forages are not easy to ensile, undesirable microorganisms like Clostridia, Bacillus and Enterobacter are always lead to butyric acid accumulation and proteolysis during ensiling (Silva et al., 2016). Feeding silages of high butyric acid content will reduce dry matter intake and Clostridial endospores may even lead to clostridial contamination in milk. Furthermore, extensive non-protein N caused by proteolysis always results in inefficient N utilization. The economic loss and potential environmental pollution call for a better approach to minimize proteolysis in legumes silage.

Therefore, the main purpose of present study was to evaluate the effect on silage quality when MOL is introduced for ensiling alfalfa (Medicago sativa L.) and stylo (Stylosanthes guianensis Sw.). Particular attention was paid to bacterial communities, fermentation quality and
2. Materials and methods

2.1. Raw materials and silage preparation

*Moringa oleifera* leaves, alfalfa (GEA) and stylo (CIAT 184) were cultivated at the experimental field of South China Agricultural University (Guangzhou, China) and applied with no herbicides and fertilizers. Legumes were harvested at full bloom in second cutting, using a sickle by hand and leaving a 5 cm stubble. In trial 1, alfalfa and MOL were harvested in June 2018, and were mixed at ratios of 100: 0 (M), 75: 25 (M25), 50: 50 (M50) after chopping to 1–2 cm by hand with a paper cutter. In trial 2, stylo and MOL were harvested in July 2018, and were mixed at ratios of 100: 0 (S), 75: 25 (S25), 50: 50 (S50), respectively. After that, the materials (without wilting, approximately 180 g) were packed into plastic silo bags (20 × 30 cm; Dongguan Bojia Packaging Co. Ltd, Dongguan, China) which were vacuumed and sealed by vacuum sealer (Lvye DZ280; Dongguan Yijian Packaging Machinery Co. Ltd, Dongguan, China). A total of 18 bags (2 forages × 3 treatments × 3 repeats) were made and kept at room temperature (25–32 °C). The silage bags were opened to determine fermentation quality, chemical composition, bacteria communities after 60 days of ensiling.

2.2. Analysis of microbial population, organic acid and chemical composition

The bags were opened in a clean bench, the samples (20 g) were immediately blended with 180 mL sterilized saline water (8.5 g/L NaCl), and serially diluted. The number of lactic acid bacteria, coliform bacteria, yeasts and molds were incubated and counted using Man, Table 1

<table>
<thead>
<tr>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>alfalfa</td>
<td>M. oleifera leaves</td>
</tr>
<tr>
<td>Dry matter (g/kg FM)</td>
<td>263 ± 3.0</td>
</tr>
<tr>
<td>Crude protein (g/kg DM)</td>
<td>183 ± 7.7</td>
</tr>
<tr>
<td>Neutral detergent fiber (g/kg DM)</td>
<td>386 ± 11.6</td>
</tr>
<tr>
<td>Acid detergent fiber (g/kg DM)</td>
<td>278 ± 9.9</td>
</tr>
<tr>
<td>Water soluble carbohydrate (g/kg DM)</td>
<td>43.4 ± 1.6</td>
</tr>
<tr>
<td>Lactic acid bacteria (Log10 CFU/g FM)</td>
<td>5.60 ± 0.16</td>
</tr>
<tr>
<td>Yeasts (Log10 CFU/g FM)</td>
<td>3.79 ± 0.10</td>
</tr>
<tr>
<td>Moulds (Log10 CFU/g FM)</td>
<td>3.91 ± 0.13</td>
</tr>
<tr>
<td>Coliform bacteria (Log10 CFU/g FM)</td>
<td>6.72 ± 0.57</td>
</tr>
</tbody>
</table>

FM, fresh matter; DM, dry matter; CFU, colony forming units.

Table 2

<table>
<thead>
<tr>
<th>Trial 1</th>
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</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>M-25</td>
</tr>
<tr>
<td>Dry matter (g/kg FM)</td>
<td>233</td>
</tr>
<tr>
<td>Dry matter loss (%)</td>
<td>14.6</td>
</tr>
<tr>
<td>pH</td>
<td>5.12</td>
</tr>
<tr>
<td>Lactic acid (g/kg DM)</td>
<td>44.8</td>
</tr>
<tr>
<td>Acetic acid (g/kg DM)</td>
<td>7.56</td>
</tr>
<tr>
<td>Butyric acid (g/kg FM)</td>
<td>&lt; 2.00</td>
</tr>
<tr>
<td>Short-chain fatty acid (g/kg DM)</td>
<td>&lt; 2.00</td>
</tr>
<tr>
<td>Coliform bacteria (Log10 CFU/g FM)</td>
<td>&lt; 2.00</td>
</tr>
</tbody>
</table>

FM, fresh matter; DM, dry matter; CFU, colony forming units; ND, not detected; SEM, standard error of means; M, alfalfa; M-25, alfalfa: *M. oleifera* leaves at 75: 25; M-50, alfalfa: *M. oleifera* leaves at 50: 50; S, stylo; S-25, stylo: *M. oleifera* leaves at 75: 25; S-50, stylo: *M. oleifera* leaves at 50: 50.

Table 3

<table>
<thead>
<tr>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>M-25</td>
</tr>
<tr>
<td>Crude protein (g/kg DM)</td>
<td>179</td>
</tr>
<tr>
<td>Nonprotein-N (g/kg TN)</td>
<td>687</td>
</tr>
<tr>
<td>Free amino acid (g/kg TN)</td>
<td>10.7</td>
</tr>
<tr>
<td>Ammonia-N (g/kg TN)</td>
<td>262</td>
</tr>
<tr>
<td>Neutral detergent fiber (g/kg DM)</td>
<td>424</td>
</tr>
<tr>
<td>Acid detergent fiber (g/kg DM)</td>
<td>323</td>
</tr>
</tbody>
</table>

DM, dry matter; TN, total N; SEM, standard error of means; M, alfalfa; M-25, alfalfa: *M. oleifera* leaves at 75: 25; M-50, alfalfa: *M. oleifera* leaves at 50: 50; S, stylo; S-25, stylo: *M. oleifera* leaves at 75: 25; S-50, stylo: *M. oleifera* leaves at 50: 50.

nitrogen distribution of silages.

2. Materials and methods

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2.2. Analysis of microbial population, organic acid and chemical composition

The bags were opened in a clean bench, the samples (20 g) were immediately blended with 180 mL sterilized saline water (8.5 g/L NaCl), and serially diluted. The number of lactic acid bacteria, coliform bacteria, yeasts and molds were incubated and counted using Man,
Rogosa, Sharpe (MRS) agar, Violet Red Bile agar, Rose Bengal agar, respectively (Wang et al., 2018).

Twenty grams of each silage sample was mixed with 180 mL distilled water and stored at 4 °C for 18 h, and then filtered. The pH of this filtrate was measured by a glass electrode pH meter (PHS-3C, INESA Scientific Instrument Co., Ltd, Shanghai, China). The concentration of organic acids (lactic acid, acetic acid, propionic acid and butyric acid) was measured using high performance liquid chromatography (HPLC) (column, Shodex RSpak KC-811S-DVB gel C (8.0 mm × 30 cm; Shimadzu, Tokyo, Japan); oven temperature, 50 °C; mobile phase, 3 mmol/L HClO₄; flowrate, 1.0 mL/min; injection volume, 5 μL; and detector, SPD-M10AVP) (Zhang et al., 2017).

About 100 g samples were analysed for DM content by oven drying at 65 °C for 48 h and then ground to pass a 1 mm screen by a laboratory knife mill (FW100, Taiste Instrument Co., Ltd., Tianjin, China). Crude protein (CP) was analysed using the Kjeldahl nitrogen analyser (Kjeltec 2300 Auto-Analyser, FOSS Analytical AB, Hoganas, Sweden) according to the methods of Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were measured without use of heat-stable amylase and sodium sulphite by an A220 Fiber Analyser (ANKOM Technology Corp., Macedon, NY, USA) according to the method of Van Soest et al. (1991). The concentration of ammonia-N was determined by the method of Broderick and Kang (1980). Protein fractions including nonprotein-N, and free amino acid were determined according to the method of Licitra et al. (1996).

2.3. Microbial diversity analysis

DNA extraction was performed according to Liu et al. (2019). Samples (10 g) were mixed with 90 mL of sterile 0.85% NaCl solution with vigorous shaking at 120 r/m for 2 h. The mixture was filtered through four layers cheesecloth and the filtrate was centrifuged at 10,000 r/m for 10 min at 4 °C. The deposit was resuspended in 1 mL of sterile 0.85% NaCl solution and the microbial pellets were obtained by centrifugation at 12,000 r/m for 10 min at 4 °C. Total DNA was extracted using the E.Z.N.A. stool DNA Kit (Omega Biotek, Norcross, GA, U.S.) according to the manufacturer’s protocols. The PCR reactions were conducted in a 50 μL mixture (100 ng of template DNA, 5 μL of 2.5 mM dNTPs, 1.5 μL of each primer (5 μM), 1 μL of KOD Polymerase, and 5 μL of 10 × KOD Buffer). According to Wang et al. (2018), the 16S rDNA V3-V4 regions were amplified using primers 341F (CCTACGGGNGGC-WGCAG) and 806R (GGACTACHVGGGTATCTAAT).
After purification and quantification, the PCR products were sequenced using Illumina platform (Guangzhou Gene Denovo Co. Ltd., Guangzhou, China). The raw sequences were selected according to Wang et al. (2018). Paired-end clean reads were merged as raw tags using FLSAH (v 1.2.11) with a minimum overlap of 10 bp and mismatch error rates of 2%. Noisy sequences filtering and data processing were performed using the QIIME (v 1.9.1). Clean tags were searched against the reference database (http://drive5.com/uchime/uchime_download.html) to perform reference-based chimera checking using UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html). Chimeric sequences were removed and the effective tags with 0.97 identities were clustered into operational taxonomic units (OTU) using UPARSE pipeline. Taxonomy assignment of representative sequences was performed using Ribosome Database Project (RDP) classifier (Version 2.2). Finally, functional genes of the bacterial communities were predicted using Tax4Fun (Xie et al., 2018). The sequences data reported in this study was archived in the Sequence Read Archive (SRA) with the accession number SRP161634.

2.4. Statistical analyses

The statistical analyses were performed using SAS 9.3 software (SAS Institute Inc., Cary, NC, USA). The effects of different treatments were evaluated using one-way analysis of variance, with Duncan’s multiple range tests. Differences were considered significant when P < 0.05. The data of high throughput sequencing analysed using the OmicShare tools, a free online platform for data analysis (http://www.omicshare.com/tools).

3. Results and discussion

3.1. Characteristics of fresh material before ensiling

The chemical composition and microbial population of fresh material are shown in Table 1. The DM contents of the four materials were 263, 245, 225, 216 g/kg, respectively. The CP content of MOL (264, 253 g/kg DM in two trials, respectively) was comparable with the data reported by Zheng et al. (2016) but higher than our previous report (Wang et al., 2018). The relatively high CP content and low fiber content (257 and 245, 176 and 167 g/kg DM for NDF, ADF, respectively) suggest MOL could be potentially used as quality protein source for animals. The CP content of alfalfa (183 g/kg DM) was lower than the value reported by Yang et al. (2019), while the CP content of stylo (152 g/kg DM) was a slightly higher than that determined by Liu et al. (2012). These differences might because the forage quality could be influenced by factors like climate, fertilization (Van Soest et al., 1978) and harvest time (Zhang et al., 2016). The chemical composition of the silage material, especially the WSC content, is an important factor involved in assessing fermentation quality. The WSC content of alfalfa and stylo (43.4, 15.3 g/kg DM, respectively) is much lower than 60–70 g kg⁻¹ DM, the theoretical requirement to obtain well preserved silage (Smith, 1962). It could explain the poor quality of alfalfa and stylo. The WSC content of MOL (99.0, 75.3 g/kg DM, respectively in two trials), is higher than alfalfa and stylo. It indicates mixing MOL might be helpful to achieve high quality alfalfa and stylo silage.

3.2. Quality of alfalfa and stylo silage

Organic acids contents, pH and microbial population of alfalfa or stylo silage with MOL are shown in Table 2. Butyric acid content and DM loss of alfalfa or stylo silage significantly (P < 0.01) decreased after mixing with MOL. Butyric acid content is decreased to lower than 5 g/kg DM in stylo silage with MOL. Butyric acid is undesirable in silage because it reduces the intake of livestock if more than 5 g/kg DM. Moreover, conversion of lactic acid to butyric acid is one of the most wasteful anaerobic fermentations in silage, producing a 51% loss of DM and 18% loss of gross energy (Muck, 2010). Therefore, the decrease of butyric acid content and DM loss might because MOL inhibited the metabolism of Clostridia. On the other hand, DM loss occurred during ensiling always due to the metabolism of yeasts, which utilizes soluble carbohydrates and produce ethanol (Avila et al., 2014). Dry matter loss of the two silages decreased after mixing MOL. It might because MOL decreased activities of yeasts. Rathshilivha et al. (2014) reported MOL extract had moderate inhibitory activity against Candida albicans and...
Cryptococcus neoformans.

MOL also decreased \( P < 0.01 \) pH, acetic acid content and increased \( P < 0.01 \) lactic acid content, LAB count of stylo silage. It should be noted that lactic acid in stylo silage was undetected when ensiled alone but increased to 16.3 g/kg DM when ensiled with 50% MOL (Table 2). It suggests that mixing MOL could improve fermentation quality of stylo silage. As is known, legume forages like alfalfa and stylo are difficult to ensile due to low WSC content and high buffering capacity. It is a common method to ensile these forages with high WSC content forages (Ni et al., 2018). Then more organic acids were produced and better fermentation quality was obtained (Zhang et al., 2017). However, contents of short-chain fatty acids in alfalfa and stylo silage with MOL were not increased in the present study. It indicates that MOL produced positive effects on the two silages might not just because of high WSC content, but also might because of antimicrobial agent. Moreover, more organic acids, especially lactic acid will be produced and lower pH will be occurred when silage was mixed with high WSC content forages. Subsequently, the number of LAB will be decreased as many LAB strains have low tolerance to pH below 4.0 (Ohmomo et al., 2002). In the present study, mixing MOL had no effect on LAB count in alfalfa silage and increased \( P < 0.01 \) in stylo silage. It indicates that mixing MOL might produce positive effects on growth of LAB.

As shown in Table 3, nonprotein-N in alfalfa silage mixed with MOL decreased \( P < 0.01 \), it indicates ensiling alfalfa with MOL might improve the utilization of silage-N. Because the efficiency of rumen microbial-N synthesis could be improved by supplementing silage with protein-N rather than nonprotein-N (Pahlow et al., 2003). The accumulation of ammonia-N in silage is typically caused by synthetic effect of plant protease activity and microbial activity. At pH 5.0 to 6.0, both clostridia and plant proteolytic enzymes are active. The relatively high ammonia-N contents might be explained by pH values (4.78–5.33) of these silages. The content of ammonia-N in silage is always indicative of protein breakdown (Pahlow et al., 2003). Addition MOL also decreased \( P < 0.01 \) ammonia-N content of alfalfa or stylo silage (Table 3). It might because MOL inhibited the growth and proteolytic activity of microorganisms like Clostridia. On the other hand, tannins in MOL could restrict proteolysis in ensiled forage (Guo et al., 2008). Therefore, MOL could be used as alternative treatment of proteolysis in legume silages. However, ammonia-N contents in MOL treated silages (> 109 g/kg TN) were still relatively high. Maybe the concentrated MOL extract could be added to decrease ammonia-N contents drastically.
3.3. Microbial community of alfalfa and stylo silage

As shown in Fig. 1, the result of principal coordinate analysis based on UniFrac (unweighted) distances clearly reflected the variance of the microbial community. Silage samples of alfalfa and stylo ensiled alone were separated from the samples treated with MOL, which suggested that MOL had an impact on microbial community. Distinctions among bacterial communities in silages mixed with two ratios of MOL were also very clear. Similar results have been reported by Ni et al. (2018), who found mixed ensiling had an impact on microbial community. The variation of microbial community might explain the difference in silage quality (Ni et al., 2018).

Main bacterial communities in alfalfa and stylo silage are shown in Figs. 2 and 3. Lactobacillus (30.4%) and Enterobacter (58.6%) were the dominant microbes in alfalfa silage samples, while stylo silage samples were dominated by Lactobacillus (41.8%), Clostridium (23.5%) and Enterobacter (17.4%). During ensiling, the presence of enterobacteria is undesirable as they may compete with the LAB for nutrients and produce ammonia-N. The reduction of enterobacteria in silage reflects the combined presence of good ensiling conditions, the availability of nutrients and water, an efficient conversion of those nutrients to fermentation products and a low pH by LAB, and also moderate temperatures (Pahlow et al., 2003). Generally, aerobic microorganisms consume oxygen and consequently anaerobes like Lactobacillus grow and decrease pH of silage during the early stage of fermentation. Finally, undesirable microorganisms like Enterobacter are inhibited and Lactobacillus which are active and vigorous at low pH value dominate in the silage (Dunière et al., 2013). It is known alfalfa is difficult to ensile and pH is uneasy to decrease due to its high buffer capacity. In the present study, the relative abundance of Lactobacillus (30.4%) in alfalfa silage was far below it of Enterobacter (58.6%). It might because the pH of alfalfa silage is too high to inhibit the activities of Enterobacter. Similarly, Enterobacter were abundant (17.4%) and Lactobacillus were not dominant (41.8%) in stylo silage. Parvin et al. (2010) reported a shift of the bacterial communities from Enterobacter to Lactobacillus and Lactococcus after fermentation of whole corn silage. In our study, the relative abundance of Enterobacter decreased to 30.5% and 9.1% in 50% MOL mixed alfalfa silage and stylo silage, respectively. On the other hand, Lactobacillus became more and more abundant along with the increase of MOL in silage, especially in stylo silage (86% in 50% MOL mixed silage). It indicates mixing MOL might enhance fermentation quality of alfalfa and stylo silage by inhibiting undesirable microorganisms like Enterobacter and promoting profitable microorganisms like Lactobacillus. Furthermore, other major desirable LAB after ensiling, Leuconostoc, Pediococcus, Weissella, became more abundant in MOL mixed silages (Figs. 3 and 4). It is consistent with the increase of LAB count, which might explain the better fermentation quality of stylo silage mixed with MOL. It suggests that MOL and LAB might be added at the same time to improve fermentation quality of the two silages. Clostridia are considered undesirable in silage, as they may result in excessive protein degradation, DM loss and butyric acid production, which can promote the growth of less acid-tolerant spoilage microorganisms and result in reduced silage intake. Their spores have ability to survive in the gastrointestinal tract in dairy cows and their contamination in milk can lead to off-flavors and excessive gas formation in cheeses. Some species even produce an extremely pathogenic toxin. Their occurrence and transmission through the dairy chain always causes death of animals and humans (Dunière et al., 2013). The relative abundance of Clostridium in stylo silage is 23.5% and decreased obviously in silages mixed with MOL. It is consistent with the decrease of
ammonia-N and butyric acid content. It means the addition MOL might be an effective method to inhibit *Clostridia* in high moisture silage. Furthermore, Flythe and Russell (2004) found some *Clostridium* could produce large amounts of acetic acid apart from butyric acid. The decrease of acetic acid in alfalfa and stylo silage mixed with MOL might be explained by lower abundance of *Clostridium, Bacillus* spp., which can produce butyric acid, is another important spoilage microorganism in silage, milk and milk products. This undesirable genus was decreased by MOL addition, even though they were not abundant (< 1%) in silages in the present study.

Some genera like *Capriocriopducro*, *Leucostoc* and *Pseudomonas*, whose roles in silage have not been extensively studied, were affected by MOL mixing in alfalfa and stylo silage. *Capriocriopducro* was also observed in Italian ryegrass and corn stover mixed silage by Yan et al. (2019). Kong et al. (2018) reported *Capriocriopducro* could produce CO₂, ethanol, acetic acid and butyric acid under anaerobic condition. The relative abundance of *Capriocriopducro* in stylo silage was 6.2%, and decreased after MOL mixed. It may partly explain the change of DM loss, acetic acid and butyric acid in stylo silage with or without MOL. Some species in *Leucostoc* were always used as silage inoculants to improve fermentation quality (Adesogan et al., 2003). But Cai et al. (1998) reported one selected strain belong to *Leucostoc pseudomesenteroides* did not improve fermentation and may cause fermentation loss. Therefore, more studies are needed to investigate the role of *Leucostoc* in MOL mixed alfalfa silage. *Pseudomonas*, a dominant genus in cabbag waste silage (Ren et al., 2018), are undesirable microorganisms in silage due to its potential production of biogenic amines. The relative abundance of *Pseudomonas* decreased in alfalfa and stylo silage mixed with MOL. Flythe and Russell (2004) reported *Clostria* could ferment amino acids and produce ammonia. Therefore, the decrease of ammonia-N in alfalfa and stylo silage mixed with MOL might because MOL reduced the abundance and amino acid metabolism of *Clostridium* and *Enterobacter*. Apart from the decrease in the protein content and nutritional value of the silage, the decarboxylation of tryptophane, histidine and arginine will cause biogenic amines accumulation, which has negative effects on animal health (Dunière et al., 2013). These above phenomena suggest that MOL could be used to reduce metabolism of amino acid in silage.

4. Conclusions

This study revealed that mixed ensiling of alfalfa and stylo with MOL is useful to improve the fermentation quality and nutrition. Butyric acid, ammonia-N content and DM loss of alfalfa or stylo silage decreased after mixing with MOL. The abundance of *Clostridium* and *Enterobacter* decreased, whereas *Lactobacillus* abundance increased when MOL was added. These results indicated that mixing with MOL could be an alternative approach to improve the quality of high moisture alfalfa and stylo silage.

Acknowledgements

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