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Effects of Inoculants and Molasses on Silage Quality of Nettle (*Urtica cannabina*)

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Abstract: To preserve *Urtica cannabina* (nettle) as a high-quality forage all year round and reduce protein loss during ensiling, freshly-harvested plants were treated without additives (control) but with 0.5 mg kg^{-1} Lalsil Dry (LD) inoculant of Fresh Weight (FW), 4% molasses of FW (MS) or mixture of them (LD+MS) and then researchers assessed the chemical composition, *in vitro* digestibility and fermentative parameters of the products after 0, 3, 5, 15, 20 and 60 days of ensiling. The result showed that silage only treated with 0.5 mg kg^{-1} LD-inoculant produced very badly preserved silage. With 4% molasses or 4% molasses plus 0.5 mg kg^{-1} LD produced well-preserved silage. Importantly with 4% molasses plus 0.5 mg kg^{-1} LD considerably reduced protein loss during ensiling. Researchers recommend adding 4% molasses plus 0.5 mg kg^{-1} LD of FW to improve nettle silage. These results contribute information for conservation of nettles and forage king of this with high quality.

Key words: Nettle, Lalsil dry, molasses, silage quality, protein loss

INTRODUCTION

Nettle, *Urtica cannabina* L. is widely adaptable and extremely Winter-hardy. It is usually used medicinally (Bown, 1995) and in Inner Mongolia in China, it has traditionally used to feed animals for centuries (Khasbagan and Pei, 2000). Nettle is a nutrient-rich species: the protein and ash content are 344 g kg^{-1} Dry Matter (DM) and 257 g kg^{-1} DM (at vegetal period), respectively (Zhang *et al*., 2010a), it is rich in iron, calcium and magnesium and its extract has been found to contain all of the essential amino acids (Aotegen *et al*., 2007). Nettle is highly productive yielding up to 14 t DM/ha (Zhang and Zhao, 2008). These characteristics are comparable to alfalfa. Dried nettle mixed into cattle fodder boosts milk production in cows (Phillips and Foy, 1990) and nettle is used as medicinal forage for horses in Britain (Allison, 1996). Xu (1981) pointed out the herb for ruminants is believed to be completely non-toxic. Consequently, nettle could be a desirable alternative forage in areas where it is widely available.

However, nettel is a non-conventional forage and has received relatively little attention by the scientific community. Local farmers prepare the nettle by making hay or pulping it to soften the stinging hairs on the leaves and stems thus improving its palatability. This traditional way of preparing nettle has limitations (Zhang and Zhao, 2008). Ensiling may be an optimum method to preserve nettle forage. It could reduce nutritive loss during hay-making. Additionally, ensilage can eliminate the stinging hairs to improve palatability. However, like alfalfa, nettle is difficult to ensile successfully without additives. The research has found that the addition of 4% molasses before ensiling produced well-preserved nettle silage but when only applying LD-inoculant, regardless of high level (10-20 mg kg^{-1} fresh weight) produced badly preserved silages although their pH and lactic acid values significantly reduced compared to control silage (Zhang *et al*., 2010b). This implied that lactic bacterial inoculants was less effective as a silage additive to forage with a low sugar content improving inoculants efficiency lies in the application of inoculants and sugar together. However, little information is available on the fermentation characteristics and silage quality of nettle ensiled with inoculants plus sugar. This study was conducted to determine the effects of LD-inoculant plus molasses on the fermentation quality of nettle and to assess the chemical composition and *in vitro* digestibility in ensiled nettle. Researchers aimed to identify an efficient way to improved silage quality of nettles and to improve inoculants efficiency as silage additive.

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MATERIALS AND METHODS

Forage: The forage was collected in mid-July 2011 in Xilinguole, Inner Mongolia, China. The climate is semi-arid continental with a mean annual precipitation of 346 mm and a yearly average temperature of 2.0°C. More than 80% of the rainfall occurs between July and September, the main growth period of the local vegetation. There are about 90-115 frost-free days per year. The soil type of this area is a sandy chestnut soil (IUSS/ISRIC/FAO, 2006) and the natural vegetation is dominated by *Stipa grandis* and *Leymus chinensis*. Fresh nettle at the start of the flowering stage was manually collected using a hand sickle from three different patches where nettle dominates vegetation in the swards of this pasture.

Treatment: Nettle was ensiled using five treatments: without additives (control) with 0.5 mg LD-inoculant per kg fresh weight (LD) with 4% beet molasses of fresh weight (MS) mixture of and (LD+MS). The LD inoculant (Lallemand, Montreal, Quebec, Canada) contained *Lactobacillus buchneri* (NCIMB 40788, $>6\times10^{10}$ cfu g^{-1}), *Pediococcus acidilactici* (CNCM MA 18/5M, $>2\times10^{10}$ cfu g^{-1}) and cellulase and hemicellulose enzymes (enzyme activity >20,000 UI g^{-1}).

Ensiling procedure: Fresh forage (no wilting) was chopped into about 2 cm pieces with a forage cutter (Harqin Machinery, Chifeng, China). The chopped forage was mixed thoroughly and either treated with each of the additives or was untreated (control). The LD inoculant in solution was prepared on the day of experiment and applied in 3 mL of water to provide $>10^8$ cfu of lactic acid bacteria per kg of fresh forage. For silage production, forage was compacted into glass bottle silos each with a capacity of 1 L. There were 15 silage replicates per treatment. All bottles were stored indoors at 25°C for 60 days. Three silo bottles for each treatment were opened on 3, 5, 15, 20 and 60 days post-ensiling, respectively and samples were frozen at -20°C for later analysis.

Silage sample preparation: After thawing, 10 g of each silage sample was weighed out and homogenized in 90 mL distilled water and then stored overnight at 4°C. The silage extract filtered through four layers of gauge and pH was immediately measured using a pH meter (B-212 Twin, Horiba, Japan). The extracts were centrifuged at 12,000x g at 4°C for 10 min, the supernatants were subdivided into aliquots in 10 mL plastic vials (to avoid repeated cycles of thawing and freezing throughout experiment) and the vials were stored at -20°C for subsequent determination of organic acids and ammonia Nitrogen (NH_3-N).

The samples were dried in a forced-air oven at 65°C to a constant mass. Dried samples were ground with a Wiley mill (SM100, Retsch GmbH, Haan, Germany) and passed through a 1 mm for chemical analysis.

Chemical analyses: Dry matter of samples was analyzed according to AOAC (1990 Method 934.01). Crude protein was determined with a RAPID N-III Analyzer (Elementar Co., Germany). Neutral (NDF) and Acid Detergent Fiber (ADF), inclusive of residual ash were determined with a Fibertec System 2010 (Tecator, Hoganas, Sweden) using reagents described by Van Soest *et al.* (1991) without pre-treatment with amylase but with sodium sulfite added to the neutral detergent. NPN (Non Protein Nitrogen) was measured according to the method of Licitra *et al.* (1996) and Acid Detergent Insoluble Nitrogen (ADIN) was measured using the method of Goering and Van Soet (1970) on the ADF residue. Water-Soluble Carbohydrate (WSC) was evaluated using the method of McDonald and Henderson (1964). NH_3-N was measured by the method of Broderick and Kang (1980). Organic acid content was detected on a High-Powered Liquid Chromatography (HPLC) System (Shimadzu 10A, Tokyo, Japan).

In vitro digestibility of DM (IVDMD) for silage samples were determined using the Tilley and Terry (1963) procedure. The following experiment was approved by the Animal Welfare Committee of China Academy of Agricultural Sciences. The rumen liquor samples were hand-collected from three rumen-fistulated castrated male sheep that had been fed a forage-based diet of grass silage, wheat stalk, alfalfa hay and chopped maize at twice daily. Rumen fluid was combined and filtered through a four-layer gauze to provide the inocula for determination. All incubation vessels were continuously incubated at 39°C for 48 h. *In vitro* digestibility of NDF and ADF were calculated as the difference between the amounts of each that were incubated (on a DM basis) versus those of the residues after incubation. The NDF and ADF in the silage residues were measured as described above.

Flieg point calculation: The Flieg points of silages were calculated by the following equation (Kilic, 1984):

$$\text{Flieg point} = 220 + (2\times \text{DM\%-15}) - 40\times \text{pH}$$

where flieg point values between 85 and 100 indicate high quality fermentation of ensiled forages, values between 60 and 80 means good quality and value below 20 are a sign of failed fermentation (Ziaei and Molaei, 2010).

Statistical analysis: Data on fermentation quality (including daily changes in pH and lactic acid values) were analyzed using the Mixed Model procedure of SAS (2000) Software V. 8. The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = The effect of i treatment in j silage replicate
μ = The overall mean
T_i = The treatment effect
e_{ij} = The residual error

A General Linear Model (GLM) procedure of SAS was used to evaluate the effects of each treatment on fermentation characteristics, chemical composition and digestibility of ensiled nettle at 60 days and the differences among means of treatments were compared by Duncan's multiple range test. Differences were considered significant when $p \leq 0.05$.

RESULTS

Fermentation quality from 0-60 days: The silage fermentation process was significantly accelerated by the MS and LD+MS treatments being much more effective than the control and LD treatments. The pH in the MS and LD+MS treatments fell fast at the first day of ensiling whereas in the control and LD treatments it fell slowly throughout the ensiling process (Fig. 1).

The concentrations of lactic acid in the MS and LD+MS treatments began to increase quickly after five days of ensiling and continued to accumulate gradually over time they ultimately exceeded the concentrations in the control and LD treatments (Fig. 2). The LD treatment was not substantively different from the control.

Fermentation quality after 60 days of ensiling: After 60 days of ensiling, silage treated with molasses or molasses plus LD-inoculant was well preserved. The processed fresh nettle had an initial pH of 8.40 and after 60 days of ensiling the pH of the control was 6.60 while that of the MS and LD+MS treatments fell to 4.15 and 4.01, respectively (Table 1). The pH fell much more slowly in the LD treatment and there was no significant difference between the LD and control treatments. More NH_3-N was observed in control silage (60.4 g kg^{-1} total N) compared to all the additive-treated silages ($p = 0.012$) and NH_3-N in the MS and LD+MS silages was significantly lower than that in the LD silage. WSC content considerably increased ($p<0.001$) in the MS and LD+MS silages but that was similar in the LD silage to the control.

Table 1: Effects of additives on the fermentation characteristics of nettle ensiled for 60 days

	Silage treatment					
Effects	Control	LD	MS	LD+MS	SEM	p-values
PH	6.66^{a}	6.10^{a}	4.15^{b}	4.01^{b}	0.179	<0.001
NH_3-N (g kg^{-1} TN)	60.40^{a}	54.80^{a}	27.20^{b}	24.60^{b}	4.400	0.012
LA (g kg^{-1} DM)	20.50^{c}	52.00^{b}	96.80^{a}	110.00^{a}	9.580	<0.001
AA (g kg^{-1} DM)	24.70^{a}	23.50^{a}	15.00^{b}	13.90^{b}	1.010	0.045
PA (g kg^{-1} DM)	1.37	2.31	1.34	1.89	0.405	0.253
BA (g kg^{-1} DM)	2.02^{a}	1. 9^{a}	0.78^{b}	0.69^{b}	0.092	0.006
WSC (g kg^{-1} DM)	2.10^{cd}	5.80^{c}	22.10^{b}	30.20^{a}	1.150	<0.001
Flieg point	-17.60^{c}	9.40^{b}	87.40^{a}	93.00^{a}	3.330	<0.001

Means on the same line with the same or no superscript are not significantly different; NH_3-N: ammonia nitrogen; TN: Total Nitrogen; LA: Lactic Acid; AA: Acetic Acid; PA: Propionic Acid; BA: Butyric Acid; WSC: Water-Soluble Carbohydrate; SEM: Standard Error of the pooled Means

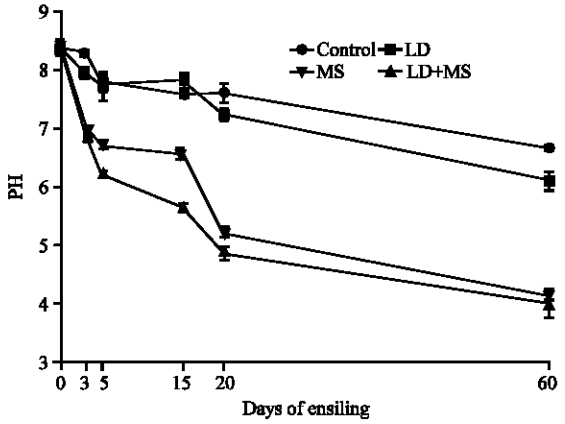

Fig. 1: Change in pH of nettle silage during fermentation process. Treatments and additives were control, no additive; LD: 0.5 mg LD per kg fresh weigh; MS: 4% molasses per fresh weigh; LD+MS: 4% molasses plus 0.5 mg kg^{-1} LD of fresh weigh. Error bars indicate the standard error of each individual mean from each ensiling time

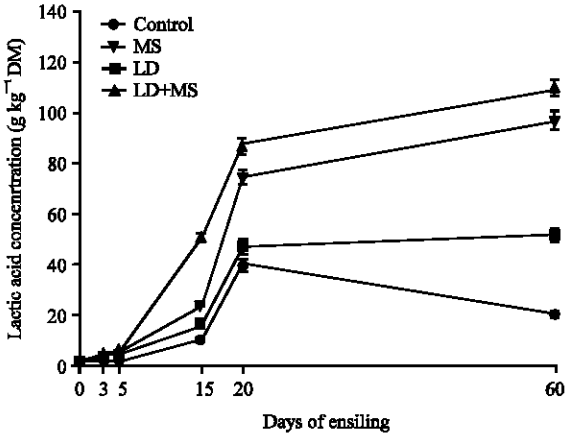

Fig. 2: Change in lactic acid content during fermentation of nettle silage. Treatments and additives were control, no additive; LD: 0.5 mg LD per kg fresh weigh; MS: 4% molasses per fresh weigh; LD+MS: 4% molasses plus 0.5 mg kg^{-1} LD of fresh weigh. Error bars indicate the standard error of each individual mean from each ensiling time

Table 2: Effects of treatments on the chemical composition and *in vitro* digestibility of nettle ensiled for 60 days

	Silage treatment					
Effects	Control	LD	MS	LD+MS	SEM	p-values
DM (g kg^{-1})	207^{b}	210^{b}	236^{a}	242^{a}	9.50	0.015
NDF (g kg^{-1} DM)	320^{a}	321^{a}	277^{b}	261^{c}	10.80	0.010
ADF (g kg^{-1} DM)	290^{a}	240^{b}	229^{b}	200^{bc}	7.30	0.006
CP (g kg^{-1} DM)	190	187	209	200	5.60	0.618
ADIN (g kg^{-1} DM)	11.8^{a}	8.8^{b}	9.2^{b}	7.2^{c}	0.13	0.001
NPN (g kg^{-1} DM)	110^{a}	71^{b}	68^{bc}	54^{c}	2.40	0.004
IVDMD (g kg^{-1})	672^{b}	660^{b}	738^{a}	740^{a}	34.10	0.020
IVNDFD (g kg^{-1})	349^{c}	360^{c}	452^{b}	503^{a}	40.60	<0.001
IVADFD (g kg^{-1})	310^{b}	327^{b}	405^{a}	435^{a}	30.00	<0.001

Means on the same line with the same no superscript are not significantly different; Abbreviations: DM: Dry Matter; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; CP: Crude Protein; ADIN: Acid Detergent Insoluble Nitrogen; NPN: Non Protein Nitrogen; IVDMD *in vitro* digestibility of dry matter; IVNDFD and IVADFD *in vitro* digestibility of neutral and acid detergent fibers; SEM: Standard Error of the pooled Means

The concentrations of lactic acid in the additive-treated silages were greater ($p<0.001$) compared to the control silage while the levels of acetic acid in those silages were significantly lower ($p = 0.045$) than the control silage. No significant differences were observed in propionic acid among the five treatments but butyric acid contents in the MS and LD+MS treatments were lower ($p = 0.006$) compared to the control and LD treatments. The Flieg points in the MS (87) and LD+MS (93) treatments were much higher ($p<0.001$) compared to the LD (9) and control (-18) treatments.

Nutritive value after 60 days of ensiling: DM content in the three additive-treated silages significantly increased ($p = 0.015$) and NDF and ADF contents for the MS and LD+MS silages were significantly decreased ($p = 0.01$; $p = 0.006$) compared to the control silage (Table 2). No significant ($p = 0.618$) differences in CP contents were observed among those five treatments. The lowest contents of ADIN and NPN ($p = 0.001$; $p = 0.004$) were observed in LD+MS treatment. In comparison with control, ensiling with MS and LD+MS significantly ($p<0.05$) improved the digestibilities of the DM, NDF and ADF, however, the LD did not have this effect.

DISCUSSION

Effects of additives on fermentation quality of nettle silages: Generally, a critical sugar level, 60-120 g kg^{-1} DM for forage (Woolford, 1984) is required for proper fermentation. In this study, when 4% molasses was applied, acidification occurred at a faster rate and produced greater amounts of lactic acid. The pH with molasses was 4.15, highly suitable for fermentation and with molasses plus LD it was 4.01 which was even better. In the molasses- and molasses-plus LD-treated silages the NH_3-N (25-27 g kg^{-1} N) were lower than typical forage silages (40-100 g kg^{-1} N; Haigh, 1987), probably because nettle contains polyphenols (Jimoh *et al*., 2010) including tannin which could effectively prohibit crude protein degradation of alfalfa silage (Tabacco *et al*., 2006; Guo *et al*., 2007). For fermentative of feedstuffs, butyric acid content is indicative of degraded carbohydrates and lactic acid by saccharolytic clostridia and a rapid decrease in pH will reduce butyric acid concentration by limit the growth of undesirable bacteria (McDonald *et al*., 1991). From this point both MS and LD+MS treatments produced well preserved silages. Although, WSC are consumed by anaerobic microbes the WSC levels remained high in molasses- and molasses plus LD-treated silages mainly because molasses contains high sugar. Conversely because the LD-inoculant processed nettle lacked the available WSC for fermentation only in the LD treatment was the pH (6.10) above the critical value of 4.2 (McDonald *et al*., 1991) and thus inadequate to improve fermentation. According to the evaluation system, another useful tool to evaluate silage quality, reported by Ziaei and Molaei (2010) high fermentation quality was obtained in nettle silage treated with either molasses or molasses plus LD with these two treatments similar in quality.

Effects of additives on nutritive value of nettle silages: Addition of molasses or molasses plus LD to nettle silage resulted in increased DM, mainly because molasses has high DM. Because lower pH enhances hydrolysis of cell walls in nettle during ensiling (McDonald *et al*., 1991) the fiber fractions (NDF and ADF) in those silages declined significantly and their rapid decrease in pH would largely reduce fermentation losses by limit the breakdown of proteins into NPN by inactivating plant proteases and inhibiting the growth of undesirable bacteria (McDonald *et al*., 1991). Ensilage with molasses had low ADIN probably because of accelerating degradation of plant cell walls and reduction of proteins bound to the cell wall (Rinne *et al*., 1997). The molasses plus LD silage had the lowest ADIN because *Lactobacillus buchneri* in the LD-inoculant could inhibit heating damage of silage. This result confirmed the result of Zhang and Zhao (2008).

Ensilage with molasses or molasses plus LD resulted in high DM digestibility due to the highly digestible nature of molasses. The high fiber digestibility in the molasses or molasses plus LD processed silage considerably increased by the fermentation of some labile fibers and hemicelluloses as reported by Beuvink and Spoelstra (1994) and Repetto *et al*. (2011). Based on the above content both molasses and molasses plus LD treatments have produced well preserved silages, especially molasses plus LD treatment improves silage quality of nettle.

CONCLUSION

Researchers showed that ensiling nettle untreated or only with LD-inoculant produced very badly preserved silage. Molasses treatment produced well-preserved silage. Molasses plus LD treatment is similarly effective in ensiling and has more efficiency in reduction of the protein loss during ensiling. The recommended ratio is 4% molasses plus 0.5 mg kg^{-1} LD of FW.

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