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Full Length Research Paper

Ensiling and *in vitro* digestibility characteristics of *Ceratoides arborescens* treated with lactic acid bacteria inoculants and cellulase

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The effects of different additives, including lactic acid bacteria inoculants, acremonium cellulase and mixtures of lactic acid bacteria inoculants and acremonium cellulase on changes of nutritive components and *in vitro* digestibility of *Ceratoides arborescens* during ensiling were investigated. The silages of each treatment were prepared in plastic film bags, kept in an incubator at 30°C and bags were opened after 1, 3, 5, 10, 30 and 45 days at room temperature. The results show that addition of additives and ensiling time did not affect acid detergent lignin content during ensiling (*P*>0.05). With ensiling time, pH value decreased (*P*<0.05), whereas the concentrations of lactic acids, acetic acid, propionic acid, butyric acid and ammonia nitrogen increased (*P*<0.05). At 45 days of fermentation, the silages treated with the mixture of 50 mg lactic acid bacteria and 200 mg acremonium cellulase showed the lowest (*P*<0.05) pH value, neutral detergent fiber and acid detergent fiber contents, and the highest (*P*<0.05) *in vitro* digestibility of neutral detergent fiber and acid detergent fiber, water soluble carbohydrate and lactic acids concentrations compared to other treatments. Lactic acid bacteria can improve *C. arborescens* silage fermentation quality; cellulase had a positive effect on *in vitro* digestible of neutral detergent fiber. There was favorable interaction between the addition of lactic acid bacteria and cellulase on the silage fermentation quality and chemical composition of *C. arborescens*.

Key words: *Ceratoides arborescens*, additives, silage fermentation quality, *in vitro* NDF digestibility.

INTRODUCTION

There has been increasing interest in studying the characterization of shrubs quality in order to increase feed resource. Previous studies showed cultivation of shrubs in saline soils as good feed resources (Draz, 1983; Le Houerou, 1993). Some researchers point out that appropriate conservation of these shrubs could improve their palatability and nutritive value since feeding fresh materials hardly sustains the maintenance requirements of animals (El Shaer et al., 1990; Ben Salem and Nefzaoui, 1993). Ensiling is an alternative method to preserve nutritional value of forage. Extensive studies have been conducted to use the inoculants of lactic acid bacteria (LAB) to improve the fermentation quality of forage (Harrison et al., 1989; McAllister et al., 1998; Schmidt et al., 2009). Cellulase, which can decrease pH value and ammonia nitrogen content, whereas increase lactic acid content, was also widely

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Abbreviations: AA, Acetic acid; **ADL,** acid detergent lignin; **ADF,** acid detergent fiber; **AUS,** acremonium cellulase; **BA,** butyric acid; **LA,** lactic acetic; **LAB,** lactic acid bacteria; **CHO,** carbohydrates; **CP,** crude protein; **DM,** dry matter; **FM,** fresh matter; **NDF,** available neutral detergent fiber; **NFE,** nitrogen free extract; **NPN,** non-protein N; **NH3-N,** ammonia N; **PA,** propionic acid; **TN,** total protein; **WSC,** water soluble carbohydrate.

applied during ensiling (Broderick and Kang, 1980; Van Soest, 1991; Guo et al., 2008). Moreover, previous study suggested that enzymes combined with an inoculant can improve fermentation (Sheperd et al., 1995; Tengerdy et al., 1991).

Ceratoides arborescens (Losinsk.) is one of ceratoides species, mainly distributed in north temperate arid and semi arid areas, which usually contains 9.86% crude protein (CP) and 50.07% nitrogen free extract (NFE) on fresh matter (FM) basis. Especially in the winter period, *C. arborescens* still contain high crude protein, which is important for winter grazing (Yi et al., 2003). Therefore, we chose *C. arborescens* as the object of our study with the aim of investigating the feasibility of ensiling to preserve its nutritional value, and the effects of different additives, including LAB, cellulase and mixtures of LAB and cellulase, on changes of nutritive components and *in vitro* digestibility of *C. arborescens* during ensiling.

MATERIALS AND METHODS

Silage preparation and treatment

C. arborescens harvested at an early bloom stage from experimental plots at the Chifeng City, Inner Mongolia Linxi County (E 118° 04', N 43° 36', elevation: 800 m), leaving a stubble of 20 cm, was chopped with a domestic cutter to 2 cm lengths. The chopped forage was either untreated (control) or treated with lactic acid bacteria inoculants (LAB, *"LaLsIL Dry"*: Lactobacillus>6×10^{10} cfu/g, Pediococcus>2×10^{10} cfu/g, manufactured by Lallemand, Montréal, Québec, Canada) or acremonium cellulase (AUS, *Acremmoocium* and *Trichioderma* were mixed by the ratio of 1:2 to form a hybrid product, both enzyme activity>20 000 IU/g, manufactured by Snow Brand Seed Co. Ltd., Sapporo, Japan) or a combination of LAB and AUS.

The LAB in solution was applied by adding 5 ml of solution, containing three levels: 5, 10 and 50 mg per 1 kg of chopped *C. arborescens*. The AUS was also applied by adding at 5 ml of solution, containing three levels: at a rate of 20, 40 and 200 mg per 1 kg of chopped forage. LAB + AUS were applied by adding 5 mg + 20 mg, 10 mg + 40 mg, 50 mg + 200 mg per 1 kg of chopped forage. Additives were sprayed in a fine mist and thoroughly mixed with chopped forage before packing into silos. Sterilized distilled water was added (5 ml) in control silages to achieve the same water content. Small-scale system of silage fermentation in triplicate was made for each treatment following the method previously described (Xu et al., 2008). Briefly, 150 g chopped forage was packed into plastic film bags (Hiryu KN type, 180 mm × 260 mm; Asahikasei, Tokyo, Japan), and the bags were sealed with a vacuum sealer (BH 950; Matsushita, Tokyo, Japan). Silos per treatment were prepared and stored in a room (20 to 25°C) until further analysis.

Analytical procedures

Triplicate silos from each treatment were opened after 1, 3, 5, 7, 10, 30 and 45 days of ensiling and immediately frozen at –80°C in sealed plastic bags until further chemical analysis. The un-ensilaged forage was taken immediately after forage was chopped. Samples of fresh forage and the remaining ensiled forage from each silo were dried in a forced-air oven at 65°C for 48 h and the DM content calculated. Ground samples (1 mm) were analyzed for Kjeldahl N (AOAC, 1990, 954.01). Crude protein was calculated as Kjeldahl N × 6.25. Non protein nitrogen (NPN) was analyzed as described by Licitra et al. (1996). Water soluble carbohydrate (WSC) in fresh forage and silage was determined using the method of McDonald and Henderson (1964). A total of 20 g of each silage sample was homogenized in a blender with 180 ml of distilled water for 1 min and then filtered through four layers of cheesecloth and filter paper. The pH of the homogenized water extracts was determined immediately by a microprocessor pH meter S-3C, a subsample of the extract was frozen -20°C before further analysis.

Prior to lactic acetic (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA) analysis, the samples were centrifuged for 15 min at 10 000 × g and filtered with the dialyzer of 0.45 μM, then analyzed by High Performance Liquid Chromatography (HPLC) (KC-811column, Shodex; Shimadzu; Japan; oven temperatures were 25°C; flow was 1 ml/min; SPD 210 nm) by the methods described by Xu (2006). An aliquot of 5 ml (250 g/L, w/v) trichloroacetic acid was added to 20 ml of the filtrate to precipitate protein (Guo et al., 2008). After centrifugation (18,000 × *g*, 15 min, 4°C), the supernatant was analyzed for NH3-N by methods of Broderick and Kang (1980). Acid detergent lignin (ADL) and acid detergent fiber (ADF) were analyzed according to methods 973.18 of AOAC (1990), whereas neutral detergent lignin (NDF) was according to Van Soest et al. (1991) with the addition of 4 ml of α-amylase and no sodium sulfite. Dried silages are milled to pass through a 1 mm screen, and then digested by rumen microbes for 48 h, followed by hydrolysis with a pepsin–HCl (200 mg of pepsin in 2 L of 0.004 mol/l HCl, pH 2.4) solution for another 48 h. The rumen liquor was sampled from two ruminally cannulated cows (680 kg, lactation period, 4 years old) that were fed a maintenance energy diet (concentrate: roughage was 40:60, CP% DM was 10.80%, NDF% DM was 61.58%, ADF% DM was 29.56%). After such treatment, *in vitro* digestibility of NDF and ADF were measured using the method of Tilley and Terry (1963).

Statistical analysis

Analysis of variance was used to test the effect of additives, time of ensiling, and the additive × time interaction using the univariate procedure of the Statistical Package for the Social Science (SPSS 17.0, SPSS, Inc., Chicago, IL). When the F test indicated a significant ($P<0.05$) additive effect, means separations were conducted using a least significant difference test. A probability of $P<0.05$ was used to denote significance unless otherwise indicated. Parameters were plotted when additive × time interactions were significant ($P<0.05$) to aid in the interpretation of results. The effects of control and treatments on fermentation quality and chemical composition were analyzed by ANOVA procedure.

RESULTS

Fermentation quality

As shown in Table 1, except AUS1 and AUS2 treatments, the other silages had lower pH value than control throughout the entire fermentation ($P<0.05$). All silages treated with mixture of LAB and AUS had higher LA content than the control and the concentration of NH3-N in all treated silages was lower than the control ($P<0.05$) during ensiling process. The pH value decreased ($P<0.05$) with ensiling time, whereas the concentration of LA, AA, PA, BA and NH3-N increased ($P<0.05$).

After 45 days of fermentation, AUS3 and LAB3+AUS3 treated silages had lowest pH value ($P<0.05$) during all

Table 1. Effects of additives and time on fermentation characteristics of *C. arborescens* silage during ensiling.

Treatment	DM[d]	pH	LA[e]	AA	PA	BA	NH3-N[f]
			%DM				%TN[j]
Additive							
Control	35.75^{c}	5.31^{a}	0.95^{e}	0.78^{b}	0.014	0.60^{d}	4.45^{a}
LAB1[a]	37.16^{a}	5.04^{bc}	1.03^{e}	0.66^{b}	0.016	1.07^{c}	4.12^{bc}
LAB2	37.23^{a}	5.09^{bc}	1.09^{de}	0.63^{b}	0.012	1.19^{bc}	4.14^{b}
LAB3	36.88^{ab}	5.01^{c}	1.14^{de}	0.62^{b}	0.022	1.28^{bc}	3.57^{e}
AUS1[b]	37.02^{ab}	5.29^{a}	1.00^{e}	0.64^{b}	0.015	1.14^{c}	3.90^{bcd}
AUS 2	35.81^{c}	5.36^{a}	1.11^{de}	0.80^{b}	0.017	0.77^{d}	4.01^{bcd}
AUS 3	36.38^{bc}	5.14^{b}	1.34^{ed}	0.84^{b}	0.016	0.78^{d}	3.51^{e}
LAB1+AUS1	33.91^{d}	4.99^{c}	1.51^{c}	0.81^{b}	0.019	1.43^{b}	3.73^{de}
LAB2+AUS2	34.47^{d}	4.89^{d}	1.87^{b}	1.13^{a}	0.014	1.68^{a}	3.81^{cde}
LAB3+AUS3	33.98^{d}	4.54^{e}	2.58^{a}	1.22^{a}	0.017	1.69a	4.12^{bc}
SEM	0.330	0.047	0.123	0.144	0.006	0.115	0.143
Time(days of ensiling)							
1	36.43^{a}	6.06^{a}	0.17^{d}	0.31^{d}	0.005^{b}	0.06^{d}	2.50^{d}
3	36.17^{ab}	5.32^{b}	0.92^{c}	0.65^{bc}	0.008^{b}	0.61^{c}	2.72^{d}
5	35.52^{c}	4.95^{c}	1.33^{b}	0.52^{cd}	0.005^{b}	1.16^{b}	3.16^{c}
10	35.70^{bc}	4.92^{c}	1.91^{a}	0.65^{bc}	0.030^{a}	1.75^{a}	4.79^{b}
30	35.47^{c}	4.55^{d}	1.93^{a}	0.87^{b}	0.022^{a}	1.65^{a}	4.86^{b}
45	35.87^{bc}	4.60^{d}	1.91^{a}	1.88^{a}	0.026^{a}	1.75^{a}	5.59^{a}
SEM	0.256	0.036	0.095	0.112	0.005	0.089	0.111
Significance							
Additive	**	**	**	**	NS	**	**
Time(T)	**	**	**	**	**	**	**
A*T[c]	**	**	**	NS	**	**	**

Means within columns not sharing a common letter differ (P<0.05), *P<0.05, **P<0.01; NS, not significant. [a]LAB, lactic acid bacteria; [b]AUS, acremonium cellulose; [c]A×T, interaction between treatment and time; [d]DM, dry matter; [e]LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; [f]NH3-N, ammonia nitrogen; [j]TN, total nitrogen.

and mixture of two additives significantly reduced (P<0.05) the NH_3-N contents in the ensiled forage compared with that in the control silages. Silages treated with LAB3+AUS3 had the highest AA, PA and BA contents after 45 days of fermentation (Table 2).

Nutrient components and *in vitro* digestibility of NDF and ADF

Additives did not affect CP and NPN concentrations throughout the entire fermentation (P>0.05), whereas the concentration of CP decreased and NPN increased as time goes on (P<0.05). AUS2, AUS3 and LAB3+AUS3 treated silages had higher WSC content than the control throughout the entire fermentation (P<0.05). Compared with the control, AUS3 and all the mixture treatments decreased (P<0.05) the content of NDF and the content of ADF was decreased (P<0.05) by LAB3+AUS3 during ensiling. NDF concentration decreased after ensiling for 5 days and ADF decreased after ensiling 30 days (P<0.05). Also, additives and ensiling time did not affect ADL content (P>0.05) and the interaction between additive and time on ADL content was not significant (Table 3).

After 45 days fermentation, LAB3, AUS2, AUS3 and LAB3+AUS3 treatments conserved more (P<0.05) CP than the control, meanwhile LAB3, AUS3 and all the mixture treatments decreased (P<0.05) NPN concentration. WSC content of all the silages at day 45 did not differ from the un-ensilaged forage (P>0.05), which indicated that WSC was preserved well. Concentrations of NDF and ADF of all the silages at day 45 was lower (P<0.05) than un-ensilaged forage, and LAB3+AUS3 has the lowest concentration. All the AUS and mixture treatments could increase (P<0.05) *in vitro* digestibility of NDF compared with un-ensilaged forage, although, there was no difference (P>0.05) between different concentrations. AUS3, LAB2+AUS2 and LAB3+AUS3 treated silages had higher *in vitro* digestibility of ADF than control and un-ensilaged forage (P<0.05) (Table 4).

Table 2. Fermentation characteristics of *C. arborescens* silage on 45 days of ensiling.

Treatment	DM[c]	pH	LA[d]	AA	PA	BA	NH3-N[e]
			%DM				%TN[f]
Control	35.57	4.82^{a}	1.70^{d}	1.27^{c}	0.00^{b}	0.515^{e}	6.61^{a}
LAB1[a]	36.46	4.65^{bcd}	1.67^{d}	1.56^{bc}	0.01^{b}	1.66^{bcd}	6.16^{ab}
LAB2	36.63	4.69^{abc}	1.76^{c}	1.64^{bc}	0.00^{b}	1.89^{bc}	5.79^{abc}
LAB3	36.39	4.71^{abc}	1.77^{c}	1.39^{c}	0.00^{b}	1.82^{bc}	5.56^{bcd}
AUS1[b]	36.5	4.62^{cd}	1.62^{d}	1.34^{c}	0.00^{b}	1.80^{bc}	5.83^{abc}
AUS 2	37.05	4.53^{d}	1.99^{bcd}	1.86^{bc}	0.00^{b}	1.13^{d}	5.04^{de}
AUS 3	37.37	4.34^{e}	2.36^{bc}	2.21^{b}	0.01^{b}	1.27^{cd}	4.57^{e}
LAB1+AUS1	34.91	4.78^{ab}	2.22^{bcd}	2.06^{bc}	0.01^{b}	2.22^{ab}	5.24^{cde}
LAB2+AUS2	34.48	4.52^{d}	2.44^{b}	2.28^{b}	0.00^{b}	2.57^{a}	5.12^{cde}
LAB3+AUS3	33.41	4.33^{e}	3.36^{a}	3.17^{a}	0.02^{a}	2.64^{a}	5.44^{bc}
SEM	0.693	0.067	0.281	0.346	0.003	0.288	0.370
Significance	**	**	**	**	*	**	**

Means within columns not sharing a common letter differ (P<0.05), *P<0.05, **P<0.01. [a]LAB, Lactic acid bacteria; [b]AUS, acremonium cellulase; [c]DM, dry matter; [d]LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; [e]NH3-N, ammonia nitrogen; [f]TN, total nitrogen.

Table 3. Effects of additive and time on chemical composition of *C. arborescens* silage during ensiling.

Treatment	CP[e]	NPN	WSC[f]	NDF	ADF	ADL
	%DM[d]			%DM		
Additive						
Control	21.34^{ab}	26.70^{ab}	2.33^{c}	54.11^{a}	26.04^{b}	15.06
LAB1[a]	21.73^{a}	25.72^{b}	2.45^{c}	54.22^{a}	26.28^{b}	15.30
LAB2	21.65^{a}	26.61^{ab}	2.40^{c}	54.29^{a}	26.85^{ab}	16.11
LAB3	22.07^{a}	24.83^{b}	2.40^{c}	55.10^{a}	27.54^{a}	15.87
AUS1[b]	21.28^{ab}	27.96^{a}	2.18^{cd}	55.14^{a}	26.90^{ab}	16.62
AUS 2	21.09^{ab}	28.38^{a}	2.83^{b}	55.05^{a}	27.29^{a}	16.56
AUS 3	21.00^{ab}	27.26^{a}	2.81^{b}	53.60^{b}	26.45^{b}	17.39
LAB1+AUS1	20.32^{ab}	25.54^{b}	2.28^{cd}	53.46^{b}	25.43^{bc}	14.50
LAB2+AUS2	19.82^{b}	26.05^{ab}	2.38^{c}	53.70^{b}	25.94^{bc}	18.13
LAB3+AUS3	20.90^{ab}	24.59^{b}	3.21^{a}	48.96^{c}	24.14^{c}	15.41
SEM	0.940	1.447	0.344	4.006	1.115	4.043
Time(days of ensiling)						
1	22.76^{a}	15.05^{c}	1.93^{c}	60.43^{a}	27.51^{a}	18.65^{a}
3	21.88^{ab}	17.35^{c}	4.66^{a}	59.36^{a}	28.07^{a}	18.79^{a}
5	22.03^{ab}	23.07^{b}	3.07^{b}	55.16^{b}	26.11^{ab}	14.50^{b}
10	20.74^{abc}	28.96^{b}	2.27^{c}	52.06^{cd}	25.84^{ab}	15.47^{ab}
30	20.18^{bc}	34.94^{ab}	1.50^{d}	53.62^{c}	25.41^{b}	15.46^{ab}
45	19.13^{c}	38.79^{a}	1.72^{d}	50.56^{d}	24.77^{b}	13.70^{b}
SEM	0.856	0.936	0.412	3.025	1.089	2.111
Significance						
Additive(A)	NS	NS	**	**	**	NS
Time(T)	**	**	**	**	**	NS
A*T[c]	*	**	*	**	**	NS

Means within columns not sharing a common letter differ (P<0.05), *P<0.05, **P<0.01; NS, not significant. [a]LAB, Lactic acid bacteria; [b]AUS, acremonium cellulose; [c]A×T, interaction between treatment and time; NS, not significant; [d]DM, dry matter; [e]CP, crude protein; NPN, non-protein nitrogen; [f]WSC, water soluble carbohydrate; NDF, neutral detergent lignin; ADF, acid detergent fiber; ADL, acid detergent lignin.

Table 4. Chemical composition and *in vitro* digestibility of NDF and ADF of *C. arborescens* silage on 45 d of ensiling.

Treatment	CP[c] (%DM[e])	NPN (%TN)	WSC[d]	NDF	ADF	ADL	NDF	ADF
			%DM				*in vitro* digestibility	
Un-ensilaged forage	21.71[a]	5.34[d]	2.13[ab]	63.51[a]	31.92[a]	12.46	35.55[c]	22.53[b]
Ensilaged forage								
control	18.05[c]	42.27[a]	1.79[ab]	49.95[bc]	26.07[bc]	14.41	37.28[bc]	23.23[b]
LAB1[a]	19.05[bc]	40.25[ab]	1.38[b]	48.15[bcd]	25.43[bc]	14.41	37.53[bc]	21.78[b]
LAB2	18.67[c]	42.46[a]	1.64[ab]	50.81[b]	25.65[bc]	13.85	38.33[bc]	22.79[b]
LAB3	20.27[b]	36.24[b]	1.34[b]	49.58[bc]	26.03[bc]	13.20	41.15[abc]	25.85[ab]
AUS1[b]	19.32[bc]	41.87[a]	1.81[ab]	50.29[bc]	26.73[b]	13.14	46.72[ab]	24.39[ab]
AUS 2	19.53[ab]	43.16[a]	1.62[ab]	45.68[de]	25.82[bc]	13.91	46.47[ab]	26.11[ab]
AUS 3	19.51[ab]	37.10[b]	1.69[ab]	48.32[cd]	26.91[b]	13.76	46.55[ab]	29.15[a]
LAB1+AUS1	18.53[c]	36.33[b]	1.57[ab]	50.80[bc]	26.39[bc]	14.05	46.28[ab]	26.20[ab]
LAB2+AUS2	18.96[bc]	34.61[bc]	2.04[ab]	51.28[b]	25.18[bc]	13.82	46.12[ab]	30.88[a]
LAB3+AUS3	19.41[ab]	33.66[c]	2.32[a]	43.27[e]	24.15[c]	13.07	48.24[a]	31.33[a]
SEM	0.658	3.392	0.333	1.414	1.076	0.813	4.104	6.734
Significance	*	**	NS	**	**	NS	**	NS

Means within columns not sharing a common letter differ ($P<0.05$), *$P<0.05$, **$P<0.01$; NS, not significant. [a]LAB, Lactic acid bacteria; [b]AUS, acremonium cellulose; [c]CP, crude protein; NPN, non-protein nitrogen; [d]WSC, water soluble carbohydrate; NDF, neutral detergent lignin; ADF, acid detergent fiber; ADL, acid detergent lignin; [e]DM, dry matter; TN, total nitrogen.

the treatments, which indicated that they were well preserved. LA content of LAB3+AUS3 treated silages was the highest ($P<0.05$). Adding LAB3, AUS2, AUS3.

DISCUSSION

The effect of lactic acid bacteria on *C. arborescens* silage

Many researchers (Harrison et al., 1989; McAllister et al., 1998; Schmidt et al., 2009) pointed out that using the inoculants of LAB can improve fermentation quality of forage. Since the additive "*LaLsIL Dry*" used in our study contained lactobacillus, inoculation with these strains probably resulted in propagation of lactic acid bacteria to inhibit growth of clostridia and aerobic bacteria, which improved silage quality (Wang et al., 2009). In this regard, differences between "*LaLsIL Dry*" and the control showed that silage treated with LAB2 and LAB3 had higher lactic acid content at 45 days ensiling ($P<0.05$, Table2). However, there was no difference between LAB2 and LAB3 treatment ($p>0.05$, Table 2), which may point out that the dosage of "*LaLsIL Dry*" in LAB2 treatment, 10 mg per 1 kg chopped *C. arborescens* was enough to improved lactic acid content.

Several findings indicate that microbial inoculants generally do not affect total N content, but their effect on the composition of silage N is more variable (McAllister et al., 1995; Mandebvu et al., 1999; Moshtaghi Nia and Wittenberg, 1999). In the current study, the CP and NPN concentrations of silage changed ($P<0.05$, Table 4) compared with the control only when increasing AUS up to 200 mg per 1kg of fresh forage. Moshtaghi Nia and Wittenberg (1999) and Hristov et al. (2000) found no significant differences in water-soluble carbohydrates or reducing sugars contents between inoculated and uninoculated whole-plant barley silages. However, Seale (1986) pointed that if microbial was inoculated directly *in silo* fermentation system through the homofermentative pathway, inoculated silages should have elevated nonstructural carbohydrate contents. Meanwhile, the observations of McAllister et al. (1995) were consistent with that of Seale (1986). Our observations showed all the "*LaLsIL Dry*" treatments could not increase WSC content. This variability in the effect of inoculants on water-soluble carbohydrates concentration may reflect differences in epiphytic LAB populations on the forage at the time of ensiling (Muck, 1990). All the "*LaLsIL Dry*" treatments could not affect NDF, ADF and ADL content and *in vitro* digestibility of NDF and ADF of *C. arborescens* silage compared with control on day 45 of ensiling, which could be mainly attributed to high content of lignin and NDF of un-ensilaged *C. arborescens* forage (Table 4). Hence, we concluded that adding lactic acid bacteria can improve *C. arborescens* silage fermentation

quality positively but not fibrous compounds and their digestibility was not markedly altered by "*LaLsIL Dry*".

Effect of cellulase on *C. arborescens* silage

Reports showed that adding cellulase can decrease pH value and ammonia nitrogen content, whereas increase lactic acid content (Broderick and Kang, 1980; Van Soest, 1991; Guo et al., 2008). This was also confirmed by our results; AUS3 treatment has the best effect (Table 2). In addition, AUS3 treatment preserved more CP and decreased NPN content comparing with the control, which accorded with adding cellulose enzyme can reduce the decomposition of protein reported by Jaakkola and Huhtanen (1991). Muck and Kung (1997) pointed out that sugars released during enzymatic cell-wall hydrolysis provide additional substrates for desirable lactic acid bacteria to produce lactic acid. AUS3 treatment increased content of lactic acid, but AUS1 and AUS2 did not, which indicated that the dosage of cellulose in AUS3 treatment could improve *C. arborescens* silage quality. Increasing levels of cellulase did not affect WSC content compared with the control, which agreed with Adogla-Bessa and Owen (1995). Kung et al. (1991) and Tengerdy et al. (1991) found little or no effect of increased enzyme application on NDF and cellulose concentrations in alfalfa silage when mixtures of cellulase, hemicellulase, and pectinase were used. In our study, results of ADF and ADL concentrations were similar with theirs. Nevertheless, there was a rising trend of *in vitro* digestible of NDF and ADF of all the three application rates of cellulase, thus indicating that adding cellulase could affect *C. arborescens* silage chemical composition to some extent. In view of all the silages decreased contents of NDF and ADF, and increased *in vitro* digestible of NDF compared with un-ensilaged forage, we might regarded ensiling as a good way, making effectively use of *C. arborescens*. In conclusion, the enzyme treatment had a positive effect on *C. arborescens* silage fermentation, in particular on increasing *in vitro* digestible of NDF.

The effect of combine of lactic acid bacteria and cellulase on *C. arborescens* silage

After 45 days formation, LAB2+AUS2 and LAB3+AUS3 increased LA content and decreased the pH value (Table 2). This could because *C. arborescens* silage, treated with mixtures of lactic acid bacteria and cellulose, had a large number of LAB at the beginning of fermentation; in addition, cellulase increased WSC content during fermentation. The WSC was used by LAB, which could make lactic acid fermentation to be of priority (Xu et al., 2008). NH3-N and NPN content of all the mixture treatments decreased ($P<0.05$) compared with the control (Tables 2 and 4). In agreement with our results, previous study suggested that enzymes combined with an inoculant can improve fermentation and decrease proteolysis of silage (Sheperd et al., 1995; Tengerdy et al., 1991). Whereas, Stokes (1992) reported that the two silage additives were antagonistic when combined and did not improve silage fermentation, nutritional value, or animal performance, and activity of enzyme which could cause degradation of forage structural carbohydrates. There were significant differences in NDF degradation between treatments of AUS2 and LAB2+AUS2, AUS3 and LAB3+AUS3 (Table 4), which may be certified by the result of Nadeau's (1995) study, no effect of inoculants on cell-wall concentration. Thus, cell-wall degradation by the mixtures of lactic acid bacteria and cellulase may be related to their interaction in this experiment. LAB3+AUS3 treatment increased AA, PA and BA concentrations, which may cause silage aerobic stability reduced. Thus, combination of cellulase with lactic acid bacteria inoculants requires careful evaluation to avoid adverse effects.

Conclusion

Ensiling may be a good way of making effective use of *C. arborescens* to increase feed source in arid and semi-arid areas. It was observed that lactic acid bacteria can improve *C. arborescens* silage fermentation quality positively. Also, adding cellulase had a positive effect on *C. arborescens* silage fermentation, particularly on increasing *in vitro* digestibility of NDF. There was favorable interaction between lactic acid bacteria and cellulase on *C. arborescens* silage fermentation quality and chemical composition.

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