Journal of Animal and Feed Sciences, 20, 2011, 449–460

Effect of an inoculant and enzymes on fermentation quality and nutritive value of erect milkvetch (*Astragalus adsurgens* Pall.) silages*

Ch. Xu[1], H. Wang[1], F. Yang[2] and Zh. Yu[2,3]

China Agricultural University,
[1]College of Engineering
Beijing 100083, P.R. China
[2]College of Animal Science and Technology
Beijing 100193, P.R. China

(Received 6 January 2011; revised version 30 July 2011; accepted 9 August 2011)

ABSTRACT

The objective of this experiment was to determine the ensiling characteristics, microbial changes and *in vitro* degradation of erect milkvetch (*Astragalus adsurgens* Pall.) treated with different additives. Erect milkvetch was treated with distilled water (control), inoculant (I, lactic acid bacteria), enzymes (E, fibrolytic enzymes) and inoculant + enzymes (I + E) prior to ensiling. Three bag silos were used and opened after 1, 3, 5, 15, 30 and 45 days for chemical analysis and microbial measurement. For all the silages, there was a rapid decline in pH during the first 5 days of ensiling. Compared with the control, all added treatments (I, E and I + E) resulted in higher ($P<0.05$) lactic acid concentration at all ensiling periods. The crude protein content was higher, and neutral detergent fibre, acid detergent fibre and sulphuric acid lignin were lower for all treatments compared with the control ($P<0.05$). Treatments of enzymes (E, I + E) can also significantly improve *in vitro* dry matter digestibility by 10.9 and 13.6% and *in vitro* neutral detergent fibre digestibility by 19.8 and 21.7%, respectively. Compared with the control, treatments I and I + E increased *in vitro* crude protein digestibility ($P<0.05$). These results indicated that the addition of additives can improve both erect milkvetch silage fermentation quality and *in vitro* digestibility to some extent.

KEY WORDS: erect milkvetch (*Astragalus adsurgens* Pall.), additives, *in vitro* digestibility, silage fermentation

* Supported by 'Twelve Five-Year' National Scientific and Technological, Projects '2011BAD17B02'
[3] Corresponding author: e-mail: xucc@cau.edu.cn

INTRODUCTION

Erect milkvetch (*Astragalus adsurgens* Pall.) is used in China as forage and is widely cultivated in diverse environments in arid and semiarid areas of northern China. The planting area has reached 0.6 million hectare (Bai et al., 2009a). In spite of its extensive adaptability, erect milkvetch contains approximately 14 to 17% crude protein (CP), 40% neutral detergent fibre and 2% ether extract on dry matter (DM) basis, respectively (Liu et al., 2000; Bai et al., 2009b). Thus, it is an alternative feed source for animals.

Currently, haymaking is a major conservation method for erect milkvetch. However, this method needs to delay harvest until a mature stage of relatively high DM content is reached, resulting in a reduced digestibility. This fact coupled with an uncertainty of the weather during haymaking often led to a product of variable composition, and usually of low nutritional value (McDonald et al., 1991). Consequently, ensiling may be an appropriate method to preserve the high nutritive value of erect milkvetch. Ensiling is a preservation method that is based on lactic acid bacteria (LAB) converting water-soluble carbohydrates (WSC) into organic acids, mainly lactic acid, under anaerobic conditions. As a result, pH decreases and increase in the concentration of non-dissociated organic acid inhibits the microorganism that causes silage spoilage (McDonald et al., 1991). However, erect milkvetch contains a small amount of WSC and has a high buffering capacity that make it difficult to be ensiled (Bai et al., 2009b). Many studies have indicated that inclusion of inoculants and enzymes can significantly improve fermentation quality of silage (Guan et al., 2002; Hassanat et al., 2007; Xu et al., 2008; Schmidt et al., 2009).

However, the literature contains little information on the chemical composition and silage fermentation quality of erect milkvetch. Liu et al. (2000) reported the chemical composition of erect milkvetch during different phenophases and Bai et al. (2009b) studied the effect of addition of formic acid and sucrose on fermentation quality, but there is no published study on the nutritive value of erect milkvetch silage. The objective of this study was to assess the effect of inclusion of inoculant, enzymes and their mixture during ensiling on the fermentation quality and *in vitro* degradation of silage from erect milkvetch forage.

MATERIAL AND METHODS

Forage, silage preparation and treatment

Erect milkvetch (*Astragalus adsurgens* Pall.) for ensiling was harvested at a bud stage from experimental plots at the China Agricultural University, Beijing,

in August with a domestic cutter. Triplicate forage samples were taken (200 g each) immediately after harvest and dried by heated air for dry matter (DM) and other chemical analyses. The rest of the harvested forage was chopped into approximately 1 to 2 cm theoretical lengths for ensiling experiment. The chopped erect milkvetch was manually mixed and then divided into four equal portions to subject the following treatments: 1. distilled water (Control); 2. inoculant (LaLsIL Dry, Medipharm, Des Moines, USA) containing *Lactobacillus plantarum* > 6×10^{10} colony forming units (CFU) per gram applied at a rate of 0.01 g/kg of fresh forage (I); 3. fibrolytic enzymes (*Acremonium* cellulose, Snow Brand Seed Ltd., Sapporo, Japan) applied at a rate of 0.033 g/kg of fresh forage (E); and 4. combination of I and E (I+E). The application rates for I and E followed the recommendations of the manufacturers. Inoculant, E or I+E was first mixed with a certain amount of water and solution was then sprayed on the corresponding chopped forage designated for each treatment using a single nosal sprayer. The silage control was added with the same amount of distilled water as the other three treatments.

Each of the above treated forage was mixed well with hand and then was packed into plastic film bags (Hiryu KN type, 200 × 300 mm; Asahikasei, Tokyo, Japan) with 200 g treated material per bag, which were sealed with a vacuum sealer (BH950, Matsushita, Tokyo, Japan) and stored at ambient temperature (20 to 25°C). According to the experiment design, 15 bag silos were prepared for each treatment and three bag silos per treatment were randomly opened on day 1, 3, 5, 15 and 45 of ensiling and the content was processed for quality assessment and laboratory analysis.

Chemical analysis

Fresh forage. Dried forage harvested for ensiling was ground to pass 1 mm screen with Wiley mill for compositional analysis. The DM and crude protein (CP) were analysed according to methods 934.01 and 990.03, respectively, of AOAC (1990). The neutral detergent fibre was determined with a heat stable amylase and expressed exclusive of residual ash (aNDF; Van Soest et al., 1991), and acid detergent fibre exclusive of residual ash (ADF; Robertson and Van Soest, 1981). The sulphuric acid lignin (Lignin (sa)) was determined by washing samples with 20N H_2SO_4 for 3 h (AOAC, 1990; method 973.18). The water-soluble carbohydrates (WSC) were estimated by the method of McDonald and Henderson (1964). Glucose, sucrose and fructose were determined by HPLC (Shimadzu, Tokyo, Japan). The analytical conditions were as follows: column, Shodex Sugar SC1011 (8.0 mm × 30 cm, Shoko, Tokyo, Japan); oven temperature, 80°C; mobile phase, water; detector, 1.0 ml/min; detector, Jasco RI-1530. Buffering capacity of erect milkvetch was measured by titration (Playne and McDonald, 1966) using the dried sample.

Silages. Ten grams of silage from each bag at opening was homogenized at room temperature (20-25°C) with 90 ml of sterilized distilled water for 30 pulses of 2 s, and then filtered through four layers of cheesecloth. The pH of the filtrate was measured using a glass electrode pH meter (pHS-3C, Shanghai, China). The organic acid contents were determined by HPLC (Xu et al., 2007). Ammonia-N (NH_3-N) was determined by steam distillation of the filtrates (Xu et al., 2003) and non-protein nitrogen (NPN) by the Kjeldahl method.

The remaining of erect milkvetch silage in each bag was dried in an oven at 65°C for 48 h, ground to pass a 1 mm screen with a Wiley mill and analysed for DM, CP, aNDF, ADF, Lignin (sa) and WSC using the procedures as described above.

Microbial measurement

The numbers of microbes were counted from the plate count method (Cai et al., 1998). Colonies were counted from the plates at appropriate dilutions and the number of colony forming units (CFU) was expressed per gram of fresh forage.

In vitro digestibility determination

In vitro digestibilities of DM (IVDMD), aNDF (IVNDFD) and CP (IVCPD) were measured according to Tilley and Terry (1963). This is a two-stage *in vitro* technique in which ground silage samples were digested by mixed rumen microbes for 48 h, followed by hydrolysis with a pepsin-HCl solution (200 mg of pepsin in 2 l of 0.004 mol/l HCl, pH 2.4) for another 48 h. Two ruminally cannulated Holstein cows fed a maintenance energy diet (NRC, 1996) of grass silage without concentrates were used as rumen fluid donor for the *in vitro* digestibility determination.

Statistical analysis

Analysis of variance was used to test statistical significance of additives, time of ensiling, and the additive × time interaction using the PROC MIXED procedure of SAS (1990) with the model:

$$Y_{ij} = m + A_i + T_i + AT_{ij} + E_{ij}$$

where: Y_{ij} - the dependent variable for additive i and for time j; m - overall mean; A_i - additive effect, i - 1, 2, 3, 4 for erect milkvetch silages; T_j - time effect, j - 1, 2, 3, 4, 5 for erect milkvetch silages; AT_{ij} - additive × time interaction effect; E_{ij} - residual error.

When the F test indicated a significant (i.e. $P<0.05$) additive effect, means separations were conducted using a least significant difference test. Polynomial contrasts were used to test linear, quadratic and cubic effects of increasing fermentation time. 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 interpretation of results.

RESULTS

Chemical compositions of erect milkvetch

Chemical compositions of fresh erect milkvetch that was harvested for ensiling are present in Table 1. The fresh erect milkvetch had low levels of WSC, glucose, fructose and sucrose, and had a high buffering capacity.

Table 1. Chemical composition (g kg^{-1} DM) and buffering capacity of erect milkvetch

Items	Erect milkvetch
Dry matter, g/kg	290 ± 3.1
Crude potein	144 ± 2.6
Neutral detergent fibre	455 ± 4.7
Acid detergent fibre	303 ± 6.6
Lignin (sa)	81 ± 1.5
Water-soluble carbohydrates	47.3 ± 1.72
Sucrose	1.0 ± 0.06
Glucose	0.2 ± 0.10
Fructose	0.3 ± 0.06
Buffering capacity, meq/kg DM	507 ± 5.5

lignin (sa) - sulphuric acid lignin; means ± SD; n=3

Fermentation quality of silage

Silage pH was affected ($P<0.01$) by additive, ensiling time and there was an additive × ensiling time interaction (Table 2). Application of I, E and I + E reduced the pH of the silages with the treated silages having a sustained lower pH than control throughout the ensiling period. The pH of treated silages dropped more rapidly compared to that of control at the beginning of ensiling ($P<0.01$), but all silages were stable after about 5 days, and silages treated with I, E and I + E had lower ($P<0.05$) pH than control (Figure 1).

All silages treated with I, E and I + E, regardless of the added quantity, were well preserved, with propionic acid, butyric acid and NH_3-N concentrations being lower ($P<0.01$), and production of lactic acid higher ($P<0.01$) than those of the control silage (Table 2). Lactic acid (Figure 1), volatile fatty acid, NH_3-N and NPN

(Table 2) concentrations of treated and control silages increased (P<0.01) between day 1 to day 45 of ensiling. The WSC content in all silages decreased (P<0.01) from 4.73% DM in fresh forage to 0.68 to 0.84% DM by the end of ensiling.

Table 2. Effects of additive treatment and time of ensiling on fermentation characteristics of erect milkvetch silages

Item	pH	Organic acids[1], g/kg DM				Proteolysis[2], g kg^{-1} TN		WSC[3]
		LA	AA	PA	BA	NH_3-N	NPN	g kg^{-1} DM
Additives[4]								
control	5.48^{a}	17.6^{d}	10.8^{c}	0.7^{a}	5.8^{a}	40.3^{a}	68.7^{a}	13.9
E	5.20^{b}	23.6^{e}	11.4^{b}	0.5^{b}	4.4^{c}	35.9^{b}	66.5^{b}	14.2
I	4.98^{c}	31.9^{b}	11.9^{a}	0.3^{c}	4.9^{b}	21.8^{d}	63.7^{b}	13.8
I+E	4.83^{d}	34.5^{a}	7.8^{d}	0.4^{d}	1.3^{d}	28.7^{c}	64.0^{b}	14.8
SEM	0.024	0.72	0.15	0.01	0.16	0.67	0.52	1.01
Ensiling time, days								
1	6.01^{a}	0.5^{e}	2.7^{d}	0.1^{c}	0.4^{d}	4.3^{e}	48.5^{e}	35.6^{a}
3	5.22^{b}	26.3^{d}	8.4^{c}	0.4^{b}	3.5^{c}	25.5^{d}	63.1^{d}	13.0^{b}
5	5.05^{c}	32.6^{c}	12.6^{b}	0.6^{a}	4.3^{b}	31.6^{c}	70.1^{c}	8.4^{c}
15	4.82^{d}	36.4^{b}	12.3^{b}	0.6^{a}	4.5^{b}	47.1^{b}	72.8^{b}	7.3^{d}
45	4.54^{e}	38.7^{a}	16.4^{a}	0.5^{b}	7.8^{a}	49.8^{a}	74.0^{a}	6.6^{e}
SEM	0.022	15.31	5.30	0.28	3.34	19.91	9.56	6.10
Prob[5]								
A	**	**	**	**	**	**	**	NS
T	**	**	**	**	**	**	**	**
A×T	**	**	NS	NS	NS	**	**	NS

[1] LA - lactic acid, AA - acetic acid, PA - propionic acid, BA - butyric acid

[2] TN - total nitrogen, NH_3-N - ammonia-N, NPN - non-protein nitrogen

[3] WSC - water-soluble carbohydrates

[4] E - enzymes, I - inoculant, I + E - enzymes + inoculant; SEM - standard error of the mean; n=3

[5] Prob - standard probability; A - effect of additives; T - effect of ensiling time; A×T - interaction effect between additive and time; * P<0.05, ** P<0.01; NS - not significant

[a - e] means in the same column with different superscripts differ (P<0.05)

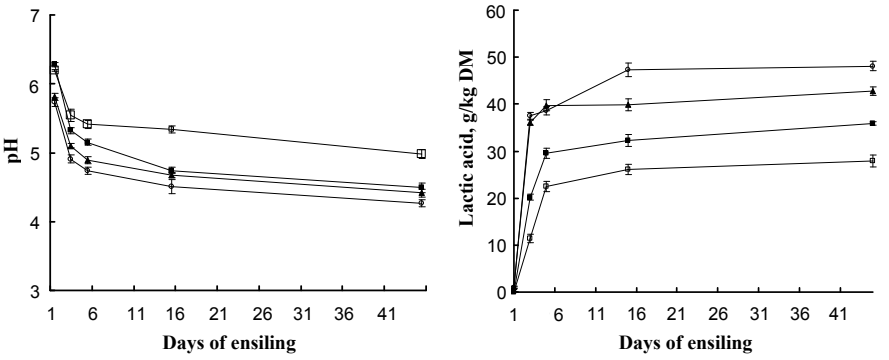

Figure 1. Changes in pH and lactic acid during ensiling of erect milkvetch treated with distilled water (□), inoculant (▲), enzymes (■) or inoculant + enzymes (○)

Changes in microbial population during the ensiling period

As shown in Table 3, the number of LAB was affected (P<0.01) by additives and ensiling time with a significant additive × ensiling time interaction. Of all ensiling periods, the application of I, E and I + E significantly increased the number of LAB, especially for treatments I and I+E. For all treatments, the population of LAB increased numerically from days 1 to 5 post-ensiling and then decreased to day 45 post-ensiling.

Table 3. Effects of additive treatment and time of ensiling on microbial population of erect milkvetch silages (log_{10} CFU g^{-1} fresh material)

Item	Lactic acid bacteria	Yeast	Mold [1]
Additives[2]			
control	6.16[c]	3.05[a]	1.03
E	6.99[b]	2.99[ab]	nd
I	7.13[a]	2.95[b]	nd
I+E	7.24[a]	2.99[ab]	nd
SEM	0.035	0.025	-
Ensiling time, days			
1	5.22[e]	3.01[a]	nd
3	7.62[b]	3.07[a]	nd
5	8.01[a]	2.80[b]	nd
15	7.22[c]	3.05[a]	nd
45	6.52[d]	3.04[a]	1.03
SEM	0.040	0.028	-
Prob [3]			
A	**	NS	-
T	**	**	-
A×T	**	NS	-

[1] only detected in control silage at day 45 post- ensiling

[2] E - enzymes; I - inoculant; I + E - enzymes + inoculant; SEM - standard error of the mean; n=3

[3] Prob - standard probability; A - effect of additives; T - effect of ensiling time; A×T - interaction effect between additive and time; * P<0.05, ** P<0.01; NS - not significant

[a, b, c] means in the same column with different superscripts differ (P<0.05)

There were no differences in the number of yeast among the treatments. Of all the treatments, yeast tended to have lower numbers for day 5 post-ensiling. No mould was detected in the silages except for the control at day 45 post-ensiling.

Chemical composition and in vitro digestibility of the 45-day silages

Chemical composition of 45-day erect milkvetch silages was presented in Table 4. Similar DM content was seen in all silages ranging from 25.7 to 26.0%. However, CP content was higher, and aNDF, ADF and Lignin (sa) were lower

for all treatments as compared with control (P<0.05). Treatments of enzymes (E, I + E) can significantly improve IVDMD by 10.9 and 13.6% and IVNDFD by 19.8 and 21.7%, respectively. In contrast, the treatment I had no effect on IVNDFD (P<0.05). Compared with control silage, treatments I and I + E increased IVCPD (P<0.05), however, treatment E had no effect on IVCPD (P<0.05).

Table 4. Effects of additive treatments on nutrient compositions of 45-d erect milkvetch silages

Items [1]	Treatments [2]				SEM [3]	P
	control	E	I	I + E		
Dry matter, g/kg	259	257	259	260	0.1	0.119
Crude protein, g/kg DM	142^{d}	149^{c}	155^{b}	161^{a}	0.7	<0.001
aNDF, g/kg DM	467^{a}	448^{b}	451^{b}	407^{c}	2.1	<0.001
ADF, g/kg DM	315^{a}	297^{b}	303^{b}	272^{c}	1.5	<0.001
Lignin (sa), g/kg DM	86^{a}	76^{b}	77^{b}	74^{b}	0.5	0.006
WSC, g/kg DM	7.1^{ab}	7.6^{ab}	6.8^{b}	8.4^{a}	0.07	0.034
IVDMD, g/kg	530^{b}	588^{a}	594^{a}	602^{a}	9.3	<0.001
IVNDFD, g/kg	369^{b}	442^{a}	393^{b}	449^{a}	10.2	<0.001
IVCPD, g/kg	719^{b}	701^{b}	844^{a}	837^{a}	20.0	<0.001

[1] aNDF - neutral detergent fibre assayed with a heat stable amylase and expressed inclusive of residual ash; ADF - acid detergent fibre expressed exclusive of residual ash; Lignin (sa) - sulphuric acid lignin; WSC - water soluble carbohydrates; IVDMD - *in vitro* dry matter digestibility; IVNDFD - *in vitro* neutral detergent fibre digestibility; IVCPD - *in vitro* crude protein digestibility

[2] E - enzymes; I - inoculant; I + E - inoculant + enzymes

[3] SEM - standard error of the mean; n=3

[a-d] means in the same row with different superscripts differ (P<0.05)

DISCUSSION

The WSC content (47.3 g/kg DM) of the pre-ensiled erect milkvetch was lower than the theoretical requirement number (60 to 70 g/kg DM) recommended for achieving well-preserved fermentation (Lunden Petterson and Lindgren, 1990), while the buffering capacity (507 meq kg^{-1} DM) was higher than that of typical forage grass (Woolford, 1984). Thus the erect milkvetch used was typical of legumes with a high buffering capacity and a low WSC content. Therefore, there is a need for better understanding of ensiling characteristics of erect milkvetch so that current technologies can be efficiently applied to the legume ensiling.

Silage pH is one of main criteria reflecting the extent of fermentation and quality of ensiled forages. As shown in Figure 1, for all treated silages, the pH decreased rapidly to below 5.0 during the first 5 days of fermentation except for treatment E that was about 4.3 to 4.8 during the last period of fermentation. However, it was found that the control silage reached a relatively high pH of over 5.3 during the entire periods. Furthermore, all the treated silages had a sustained lower pH than control throughout the ensiling period. Overall, all the treated silages (I, E and

I + E) accelerated decline in pH to a lower level than untreated erect milkvetch, especially for the treatments I+E and I.

Addition of LAB inoculants at ensiling ensured rapid and vigorous fermentation which resulted in faster accumulation of lactic acid, lower pH at earlier stages of ensiling and improved forage conservation. Many studies have shown the advantage of such inoculants (Hassanat et al., 2007; Xu et al., 2008; Schmidt et al., 2009). In the present study, treatments of both I and I + E exhibited positive effects on silage fermentation as indicated by lower pH and NH_3-N, and higher lactic acid concentration, than control silage. The additive 'LaLsIL Dry' used in the present study contained *Lactobacillus* which can improve silage quality by proliferation of LAB and consequently inhibition of growth of clostridia and aerobic bacteria.

A number of reports showed that the addition of LAB and enzyme to forage at ensiling resulted in reduction in structural polysaccharides and in increase in lactic acid content compared with untreated silages (Weinberg et al., 1993; Xu et al., 2008). In the present study, treatment I + E was well preserved as indicated by lower pH value, NH_3-N content and higher lactic acid content than others (Table 2; Figure 1). It is expected that other than the addition of LAB, cellulase increased WSC production which could be then used by LAB for primary lactic acid fermentation. Furthermore, treatment I had better effect than the E treated in this study.

Proteolysis is an inevitable consequence of the ensiling process. Up to 75% of forage true protein can be converted to NPN during the first days of ensiling process by the action of plant proteases (Hassanat et al., 2007) and microbial proteases (McDonald et al., 1991). Controlling of proteolysis is based on creating a low pH environment that is unsuitable for the action of plant and microbial proteases (McDonald et al., 1991). In the present study, treatments I and I + E had reduced NH_3-N content and lowered initial pH, while E treated silage had higher NH_3-N content and initial pH. These results agree with Mandebvu et al. (1999) who reported a similar result using bermudagrass treated with enzymes. The different trend of decline in pH for control, E, I and I + E silages may help to explain their effect on inhibiting proteolysis.

Of all ensiling periods, population of LAB increased numerically from days 1 to 5 post-ensiling then decreased to day 45 post-ensiling. The decrease in LAB may be attributed to the low pH and lack of fermentable substrates which resulted in death of bacteria (McDonald et al., 1991). In addition, there were no differences for yeast among treatments.

Compared with the control silage, all treatments significantly decreased aNDF and ADF concentration, especially for treatment I + E (Table 4). It is expected that the use of enzymes as silage additives would degrade cell wall and subsequently improve the digestibility of silage fibre (McDonald et al., 1991). However, it is not common that treatment I resulted in a decrease in aNDF and ADF. This may

be attributed to degradation of crude fibre by enzymes from microbial secretion during the fermentation (Guan et al., 2002). In addition, Lignin (sa) concentration was decreased in all treated silages and there were no significant differences between treatments.

The IVDMD and IVNDFD were higher in silages treated with enzymes (E and I + E; Table 4). This is consistent with results reported by other researchers (Eun and Beauchemin, 2008). In addition, silages treated with I significantly increased IVDMD and IVCPD (Table 4). However, Filya et al. (2007) reported that the addition of I did not show significant effects on IVDMD of lucerne silage. From more than 60% of trials published between 1990 and 1995, Muck and Kung (1997) summarized that fermentation was improved (i.e. reduced pH, increased lactate to acetate ratio, or both) by inoculants, whereas DM digestibility (*in vitro* or *in vivo*) was increased in only 30% of the trials. Weinberg and Muck (1996), in their review, also reported instances in which fermentation was affected by a LAB inoculant, whereas digestibility was not.

CONCLUSIONS

Erect milkvetch is a typical legume with a high buffering capacity and a low water-soluble carbohydrates content. Silages treated with lactic acid bacteria (I), fibrolytic enzyme (E) and I + E were well preserved with high lactic acid content, and low pH, NH_3-N and NPN content compared with control silages ($P<0.05$). Treatments of enzymes (E, I + E) significantly improved silage *in vitro* dry matter and aNDF digestibility concentration of silages. In addition, treatments of I and I + E increased *in vitro* crude protein digestibility ($P<0.05$). These results indicated that the additives tested can improve the erect milkvetch silage fermentation quality and *in vitro* digestibility to some extent.

ACKNOWLEDGMENTS

Special thanks to Z. G. Weinberg (The Volcani Center, Israel) for revision of the manuscript.

REFERENCES

AOAC, 1990. Association of Official Analytical Chemists, Official Methods of Analysis. 15th Edition. Arlington, VA

Bai C., He X., Tang H., Shan B., Zhao L., 2009a. Spatial distribution of arbuscular mycorrhizal fungi, glomalin and soil enzymes under the canopy of *Astragalus adsurgens* Pall. in the Mu Us sandland, China. Soil Biol. Biochem. 41, 941-947

Bai C., Wang Y., Yu Z., 2009b. Effects of different additives on silage quality of erect milkvetch (in Chinese). Grassl. Turf. 2, 21-28

Cai Y., Benno Y., Ogawa M., Ohmomo S., Kumai S., Nakase T., 1998. Influence of Lactobacillus spp. from an inoculant and of Weissella and Leuconostoc spp. from forage crops on silage fermentation. Appl. Environ. Microbiol. 64, 2982-2987

Eun J.S., Beauchemin K.A., 2008. Relationship between enzymic activities and *in vitro* degradation of alfalfa hay and corn silage. Anim. Feed Sci. Tech. 145, 53-67

Filya I., Muck R.E., Contreras-Govea F.E., 2007. Inoculant effects on alfalfa silage: Fermentation products and nutritive value. J. Dairy Sci. 90, 5108-5114

Guan W.T., Ashbell G., Hen Y., Weinberg Z.G., 2002. The effect of microbial inoculants applied at ensiling on sorghum silage characteristics and aerobic stability. Agr. Sci. China 1, 1174-1179

Hassanat F., Mustafa A.F., Seguin P., 2007. Effects of inoculation on ensiling characteristics, chemial composition and aerobic stability of regular and brown midrib millet silages. Anim. Feed Sci. Tech. 139, 125-140

Liu Y., Liu J., Liu J., 2000. Study on feed value of creeping type of *Astragalus adsurgens* Pall (in Chinese). Agric. Res. Arid Areas 18, 110-115

Lunden Petterson K., Lindgren S., 1990. The influence of the carbohydrate fraction and additives on silage quality. Grass Forage Sci. 45, 223-233

Mandebvu P., West J.W., Froetschel M.A., Hatfield R.D., Gates R.N., Hill G.M., 1999. Effect of enzyme or microbial treatment of bermudagrass forages before ensiling on cell wall composition, end products of silage fermentation and *in situ* digestion kinetics. Anim. Feed Sci. Tech. 77, 317-329

McDonald P., Henderson A.R., 1964. Determination of water-soluble carbohydrates in grass. J. Sci. Food Agr. 15, 395–398

McDonald P., Henderson A.R., Heron S.J.E., 1991. The Biochemistry of Silage. 2nd Edition. Chalcombe Publ., Cambrian Printers, Ltd. Merlow, Bucks. Aberystwyth, Wales (UK)

Muck R.E., Kung Jr.L., 1997. Effects of silage additives on ensiling. In: Proceedings of Silage: Field to Feedbunk. North American Conference. NRAES-99, pp. 187-199

NRC, 1996. Nutrient Requirements of Beef Cattle. 7th Edition. National Academy Press. Washington, DC

Playne M.J., McDonald P., 1996. The buffering constituents of herbage and silage. J. Sci. Food Agr. 17, 264-268

Robertson J.B., Van Soest P.J., 1981. The detergent system of analysis and its applications to human foods. In: W.P.T. James, O. Theander (Editors). The Analysis of Dietary Fiber in Food. Chapter 9. Marcel Dekker, New York, pp.123, 158

SAS, 1990. SAS User's Guide: Statistics, Version 6. SAS Institute Inc. 4th Edition. Cary, NC

Schmidt R.J., Hu W., Mills J.A., Kung Jr. L., 2009. The development of lactic acid bacteria and *Lactobacillus buchneri* and their effects on the fermentation of alfalfa silage. J. Dairy Sci. 92, 5005-5010

Tilley J.M.A., Terry R.A., 1963. A two stage technique for the *in vitro* digestion of forage crops. J. Brit. Grassl. Soc. 18, 104-111

Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597

Weinberg Z.G., Ashbell G., Azrieli A., Brukental, I., 1993. Ensiling peas, ryegrass, and wheat with additives of lactic acid bacteria (LAB) and cell wall degrading enzymes. Grass Forage Sci. 48, 70-78

Weinberg Z.G., Muck R.E., 1996. New trends in development and use of inoculants for silage. FEMS Microbiol. Rev. 19, 53-68

Woolford M.K. 1984. The Silage Fermentation. Marcel Dekker Inc. New York

Xu C., Cai Y., Fujita Y., Kawamoto H., Sato T., Masuda N., 2003. Chemical composition and nutritive value of tea grounds silage treated with lactic acid bacteria and acremonium cellulose (in Japanese). Nihon Chikusan Gakkaiho 74, 355-361

Xu C., Cai Y., Moriya N., Ogawa H., 2007. Nutritive value for ruminants of green tea grounds as a replacement of brewers' grains in totally mixed ration silage. Anim. Feed Sci. Tech. 138, 228-238

Xu C., Cai Y., Zhang J., Fukasawa M., Moriya N., 2008. Ensiling and subsequent ruminal degradation characteristics of barley tea grounds treated with contrasting additives. Anim. Feed Sci. Tech. 141, 367-374