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Full Length Research Paper

Characterization of carbohydrate fractions and fermentation quality in ensiled alfalfa treated with different additives

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This experiment was carried out to evaluate the effects of adding fast-sile (FS), previous fermented juice (PFJ), sucrose (S) or fast-sile + sucrose (FS + S) on the fermentation characteristics and carbohydrates fractions of alfalfa silages by the Cornell net carbohydrates and proteins systems (CNCPS). Silages quality were well preserved determined by pH, lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) and (NH_3-N, % of TN). Except for the silage with no addition of (CK), all other silages were well preserved. FS + S addition showed the lowest pH and contents of AA, PA, BA, and the highest contents of LA. The contents of WSC (Water soluble carbohydrate) in all alfalfa silages decreased with the extension of ensiling time, especially in the former 15 days and decreased sharply in the first 2 days. The content of sucrose in all alfalfa silages in the residual mono and disaccharides was highest, and the content of fructose was the least. The contents of all these sugars decreased sharply in the first 2 days. The content of hemicellulose decreased during ensiling, while no obvious change on content of cellulose. The content of ADL (acid detergent lignin) in alfalfa silages increased during ensiling. The content of starch in silages reduced rapidly in the former days, and then had not obvious change.

Key words: Carbohydrate fractions, alfalfa silage, additives, water soluble carbohydrate (WSC).

INTRODUCTION

It is well known that alfalfa is a forage crop with high nutritive value and is often a major component of diets for high-producing dairy cows (Schmidt et al., 2009; Albrecht et al., 2003). However, this forage crop is one of the most difficult forages to ensile due to its low fermentable carbohydrate and high buffering capacity (McAllister et al., 1998; Marshall et al., 1993). Therefore, it is necessary to use some additives to increase the supply of available carbohydrate substrates for the growth of lactic acid bacteria (LAB) or to inhibit the activity of aerobic bacteria and decrease the loss of water soluble carbohydrate (WSC) in the early stage of ensilage (Shao et al., 2003). Previous studies suggested that treatments with alfalfa silage additives, such as previous fermented juice (Wang et al., 2009; Ohshima et al., 1997), lactic acid bacteria (Schmidt et al., 2009; Tyrolova et al., 2008) and Sucrose (McDonald, 1991) could improve fermentation quality of alfalfa silage. However, there is limited information on carbohydrate fractions of alfalfa silage treated with different additives.

A lot of research on alfalfa silage carbohydrate fraction focused on the change of WSC content and organic acids contents of forage at ensiling. This is because the ensilage of forage depends on the natural fermentation,

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in which the epiphytic LAB could convert WSC into lactic acid (LA) under anaerobic conditions (Shao et al., 2003).

However, little information is known on the effect of different additives on fiber carbohydrates (FC) and non-fiber carbohydrates (NFC) during ensiling. Recent reports suggest that FC can also be substrates for microorganisms during ensiling (Yahaya et al., 2002; Mcdonald et al., 1991; Yahaya et al., 2000). The cellulose and hemicellulose contents of silage are decompounded into some simple carbohydrates by enzymes and micro-organism under the acidic environment. The simple carbohydrates can be utilized by microorganism (Uchida, 2000; Matsuoka et al., 1997; Mcdonald, 1981). To our knowledge, the Cornell Net Carbohydrates and proteins systems (CNCPS) carbohydrate fractionation has not been used to improve characterization of the quality of alfalfa silage and how silage chemical additives may affect the fractionation of carbohydrates in alfalfa silage.

The objectives of this study were to evaluate the effects of adding fast-sile (FS), previous fermented juice (PFJ), sucrose (S) or the combination of fast-sile + sucrose (FS + S) on the fermentation characteristics and FC fractions (hemicellulose, cellulose and acid detergent lignin (ADL)) and NFC fractions (organic acids, mono-and disaccharides and starch) of alfalfa silages by CNCPS.

MATERIALS AND METHODS

Ensiling materials

Alfalfa (*Medicago sativa* L) was planted at the Changping district of Beijing in 2001. The fertilizer of diammonium phosphate [$(NH_4)_2HPO_4$] was applied at a rate of 150 kg ha^{-1} and spray irrigation was carried out in middle of April and October. First, cut alfalfa was harvested at an early bloom stage and was chopped with a domestic cutter to 1 to 2 cm in length.

Additives preparation

The PFJ was prepared from alfalfa by mixing chopped herbage with two times weight of distilled water and macerating for 30 s using a high-speed blender. The macerated sample was filtered through double layers cheesecloth and aliquots of filtrate were collected in glass bottles to which glucose was added at 2 g/100 ml filtrate. These bottles were fitted with a fermentation gas trap and kept in an incubator for 72 h at 30°C. After 72 h of anaerobic incubation, the supernatant brown liquor was collected and considered as PFJ (Wang et al., 2009; Shao et al., 2003).

The FS was prepared as follows: the LAB (*Lactobacillus plantarum* and *Pediococcus acidilactici*, "fast-sile", Microferm Ltd., Malvern link, UK) in the solution was prepared on the day of ensiling. The other additives (S and FS+ S) in the solution were prepared on the day of ensiling.

Silage making

The harvested material was immediately chopped into about 1 to 2 cm length prior to treatments. The chopped forage was fully homogenized and was either untreated (control, CK) or treated with PFJ (0.2% FW), FS (5×10^5 CFU/g FW), S (1% FW), FS + S (5×10^5 CFU/g + 1% FW). An equal volume of distilled water was added to the CK. Additives were sprayed and thoroughly mixed with the chopped forage before packing into silos. About 500 g of alfalfa at each treatment were collected by quadruplicity and were immediately placed in separate polyethylene bags for silage (size: 240 × 300 mm, layer thickness: 0.65 mm) and then were exhausted, and sealed by automatic vacuum sealing machine. Mini-silos in triplicate were made for each of the treatments. Silage bags were stored in room temperature of 25°C and were followed by being analyzed at 2, 5, 10, 15 , 30 and 45 days of storage.

Chemical analysis

The chopped alfalfa was immediately collected for the determination of contents of dry matter (DM), mono and disaccharides (fructose, glucose and sucrose) compositions, WSC, neutral detergent fiber (NDF), acid detergent fiber (ADF), starch, ADL, cellulose (C) and hemicelluloses (HC). Triplicate silos from each treatment were opened after 2, 5, 10, 15, 30 and 45 days of ensiling and samples were frozen in sealed plastic bags at -20°C until analysis.

After the silos were opened and the contents were mixed thoroughly, 20 g of silage were diluted to 180 g with distilled water and macerated for 30 s at high speed in an organisation stamp mill (Waring™ -8010S, USA). This blend was filtered by four layers of cheesecloth and a qualitative filter paper. This filtrate was determined on the liquid phase using a glass-electrode pH meter (Leici PHS-3C, China) (Xu et al., 2007). An aliquot of 5 ml (250/L, w/v) trichloroacetic acid (TCA) was added to 20 ml of the filtrate to precipitate protein (Guo et al., 2007). After centrifugation (18,000 × *g*, 15 min, 4°C), the supernatant was analyzed for NH_3-N according to the methods of Broderick and Kang (1980) (Wang et al., 2009). Part of these filtrate were centrifuged at 6500 × *g* for 5 min. The silage filtrates were passed through 0.45 µm filter under pressure and the filtrates were stored at -20°C for chemical analyses of lactic, acetic, propionic and butyric acid.

The DM contents of alfalfa silage were determined by drying in forced-air oven at 60°C for 48 h (AOAC, 1999). After weighing, the dried sample was ground to pass a 1 mm screen with cyclone mill (Foss Cyclonetec 1093, USA) and analyzed for NDF (Van Soest et al., 1991), ADF (AOAC, 1999), ADL (Van Soest et al., 1991) by using automatic fiber analyzer (Ankom 2000i full, Ankom Technology Corporation, Macedon, NY, USA). C and HC contents were calculated by subtracting ADL from ADF and ADF from NDF, respectively. WSC in fresh alfalfa and silage was determined using the method of McDonald and Henderson (1964). LA, acetic acid (AA), propionic acid (PA) and butyric acid (BA) were determined by high performance liquid chromatography (HPLC, Shimadzu, Tokyo, Japan). The analytical conditions were as follows: Column, Shodex RSpak KC-811S-DVB gel C (8.0 mm × 30 cm, Shimadzu, Tokyo, Japan); Column temperature, 50°C; Detector, SPD-M10AVP; 450 nm; Mobile phase, 3 mM $HClO_4$; Flow rate, 1.0 ml/min; The injection volume, 5 µL (Xu et al., 2007). Mono and disaccharides compositions (fructose, glucose and sucrose) were determined by HPLC (Shao et al., 2002). The starch content of alfalfa silage was determined (Owens et al., 1999).

Statistical analysis

The statistical analysis included one-way of variance with different additives treatments and Duncan's multiple range tests using SPSS, the significance was declared at $p < 0.05$. The figures were

Table 1. Carbohydrate characteristics of fresh alfalfa forage before being ensiled.

DM (%)	WSC (%)	Starch (%)	Glucose (%)	Fructose (%)	Sucrose (%)	NDF (%)	ADF (%)	ADL (%)	C (%)	HC (%)
21.8	5.60	2.46	1.95	0.67	2.97	38.9	24.5	5.3	19.2	14.4

DM: Dry matter, WSC: water soluble carbohydrate, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, C: cellulose, HC: hemicellulose.

Table 2. Effects of additives treatment on fermentation quality of alfalfa silage at day 45.

Treatment	pH	LA (%, DM)	AA (%, DM)	PA (%, DM)	BA (%, DM)	NH3-N (% of TN)
C	6.20^{a}	0.37^{e}	4.06^{a}	1.30^{a}	5.97^{a}	24.98^{a}
PFJ	4.95^{b}	6.11^{c}	2.29^{c}	0.48^{b}	0.66^{b}	9.70^{b}
FS	5.08^{b}	5.71^{d}	2.37^{b}	0.52^{b}	0.67^{b}	10.02^{b}
S	4.47^{c}	11.13^{b}	1.95^{c}	0.21^{c}	0.62^{b}	8.57^{c}
FS+S	4.23^{d}	12.27^{a}	1.55^{d}	0.17^{c}	0.47^{b}	6.29^{d}

C: Control, PFJ: previous fermented juice, FS: fast-sile, S: sucrose, FS+S: fast-sile + sucrose, LA: lactic acid, AA: acetic acid, PA: propionic acid, BA: butyric acid. Values followed by different letters in the same column show significant differences at $p < 0.05$.

made by sigma plot 10.0.

RESULTS

Carbohydrate characteristics of fresh forage

Table 1 shows the characteristics of the initial forage. It contained 21.8% for DM, 5.60% for WSC, 2.46% for starch, 1.95% for glucose, 0.67% for fructose, 2.97% for sucrose, 38.9% for NDF, 24.5% for ADF, 5.3% for ADL, 19.2% for cellulose and 14.4% for hemicelluloses.

Fermentation quality of alfalfa silage

The effects of additives treatments on the fermentation characteristics of alfalfa silages are presented in Table 2. All alfalfa silages of additive treatments, regardless of the added quantity, were well preserved, with AA, BA and PA contents being lower ($p < 0.05$), and production of LA higher ($p < 0.05$) than those of the control (CK) silage. The most effective increase of LA content was observed in the FS + S treated alfalfa silage ($p < 0.05$). However, silages treated with FS and PFJ was not as effective as silages treated with S and FS + S in producing of LA and reduction of AA and PA. There were no significant differences in BA content among all silages of additives treatments ($p > 0.05$).

Lower pH and NH_3-N content were observed in all additives treated silages ($p < 0.05$). Silage treated with FS + S had the lowest pH and NH_3-N content. There was no difference in pH and NH_3-N content between PFJ and FS ($p > 0.05$).

Changes in NDF, ADF and ADL during ensiling

The contents of NDF, ADF and ADL in alfalfa silages were affected by additive and ensiling time (Table 3). Among all silages, the contents of NDF, ADF and ADL were increasing during ensiling. However, the contents of NDF, ADF and ADL in control increased more rapidly compared to those of additives treatments during ensiling. After 45 days, the contents of NDF, ADF and ADL in control were higher than those of additives treatments ($p > 0.05$).

WSC content of the 45-day alfalfa silages

Figure 1 shows the change of WSC in alfalfa silages during ensiling. The WSC contents of original materials were 5.60% (Table 1). Initial alfalfa WSC was 5.60% declining to less than 0.8% in the untreated silage. The WSC contents of all silages reduced rapidly in the former 2 days. The WSC content in FS/PFJ treated silages dropped more rapidly compared to those of S and FS + S (Figure 1). After 2 days of ensiling, the WSC content of FS + S treatment was the highest in all silages. The WSC in the first 15 days dropped more rapidly than those in the later 30 days, but all silages were stable after about 15 days, and silages treated with additives have higher WSC content than CK after 45 days (Figure 1).

Change in starch, mono and disaccharides during ensiling

Figure 2 shows the change of starch content in alfalfa silages throughout the ensiling period. The starch

Table 3. Effects of additives treatment on the contents of NDF, ADF and ADL in alfalfa silage.

Store time (d)	Treatment	NDF	ADF	ADL
		(% of DM)		
2	CK	39.5	25.6	5.7
	PFJ	39.3	25.2	5.7
	FS	39.7	25.7	5.9
	S	39.5	25.5	5.7
	FS + S	39.3	25.3	5.7
5	CK	40.0^{a}	26.2	6.5^{a}
	PFJ	38.9^{b}	25.5	5.8^{b}
	FS	39.1^{b}	25.4	5.8^{b}
	S	39.1^{b}	25.3	5.9^{b}
	FS + S	38.8^{c}	25.3	5.8^{b}
10	CK	40.0^{a}	26.3	6.7
	PFJ	39.1^{b}	26.2	6.9
	FS	39.5^{b}	26.0	6.7
	S	39.3^{b}	26.0	6.6
	FS + S	39.0^{b}	25.7	6.3
15	CK	40.4	27.1^{a}	7.5^{a}
	PFJ	39.6	26.6^{b}	7.1^{ab}
	FS	39.6	26.5^{b}	6.9^{b}
	S	39.6	26.3^{b}	6.8^{b}
	FS + S	39.0	25.8^{c}	6.6^{b}
30	CK	41.0^{a}	28.1^{a}	7.9^{a}
	PFJ	39.7^{b}	26.8^{b}	7.6^{b}
	FS	39.8^{b}	27.1^{b}	7.4^{b}
	S	39.5^{b}	26.6^{b}	7.7^{a}
	FS + S	39.1^{b}	26.5^{b}	7.3^{b}
45	CK	41.1^{a}	28.9^{a}	8.2^{a}
	PFJ	39.7^{b}	27.5^{b}	7.6^{b}
	FS	39.8^{b}	27.2^{b}	7.6^{b}
	S	39.7^{b}	27.0^{bc}	7.3^{b}
	FS + S	39.3^{b}	26.9^{c}	7.4^{b}

CK: Control, PFJ: previous fermented juice, FS: fast-sile, S: sucrose, FS + S: fast-sile + sucrose. Values followed by different letters in the same column show significant differences at $P < 0.05$.

contents in all silages were decreasing during ensiling. The starch contents of silages reduced rapidly in the former 15 days. But all silages were stable after about 15 days and silages treated with additives have lower starch content than CK after 45 days (Figure 2).

The Figures 3, 4 and 5 shows the residual mono and disaccharides (glucose, fructose, sucrose) in alfalfa silages during ensiling. The glucose, fructose and sucrose contents of original materials were 1.95, 0.67 and 2.97%, respectively. The contents of three sugars reduced rapidly within the former 5 days. The trends of the glucose of alfalfa silages were big fluctuation, but the fundamental trend was coming down and the control showed a less change relatively. The contents of fructose decreased rapidly after ensiling, whereas the change was small after 15 days. All alfalfa silages contained less

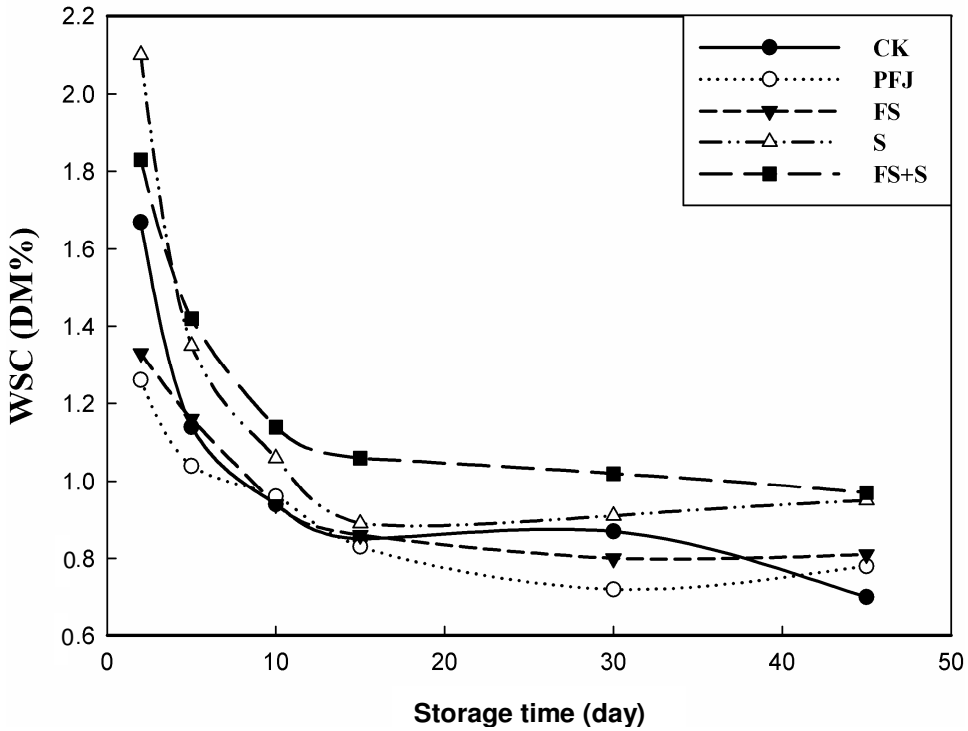

Figure 1. Change in WSC content during fermentation of alfalfa forage. CK; control, PFJ; previous fermented juice, FS; fast-sile, S; sucrose, FS+S; fast-sile + sucrose, WSC; water soluble carbohydrate.

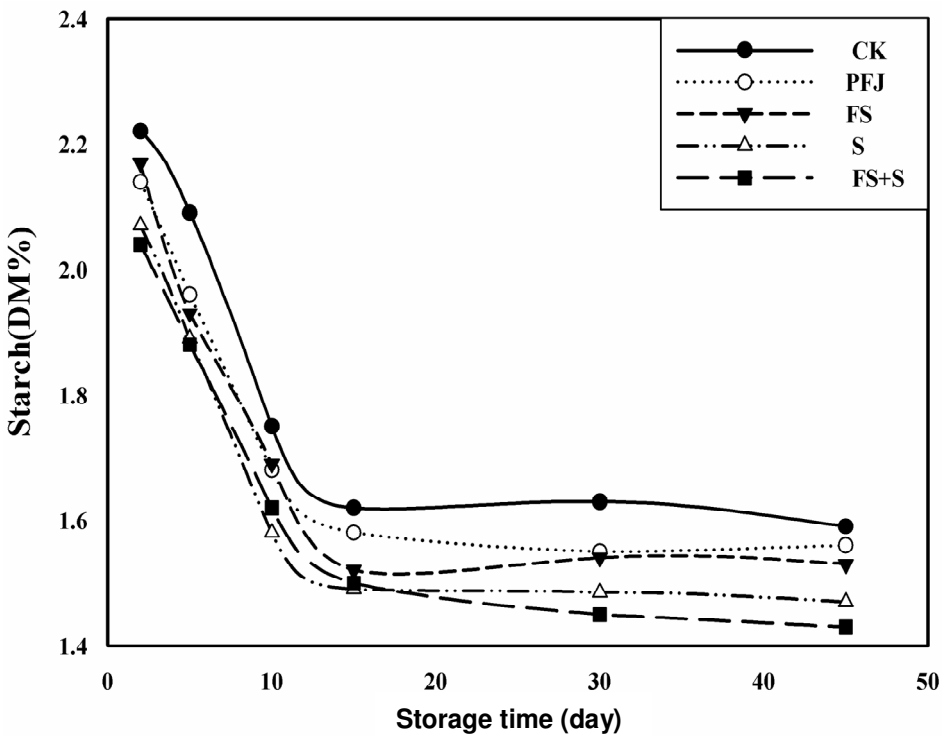

Figure 2. Change in Starch content during fermentation of alfalfa forage. CK; control, PFJ; previous fermented juice, FS; fast-sile, S; sucrose, FS+S; fast-sile + sucrose.

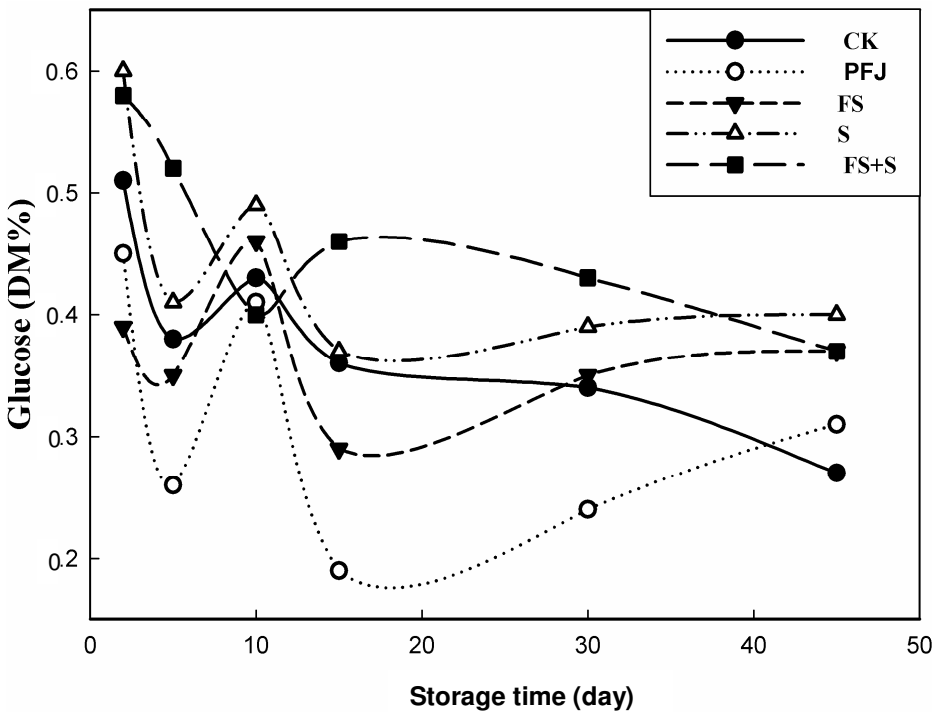

Figure 3. Change in glucose content during fermentation of alfalfa forage. CK; control, PFJ; previous fermented juice, FS; fast-sile, S; sucrose, FS+S; fast-sile + sucrose.

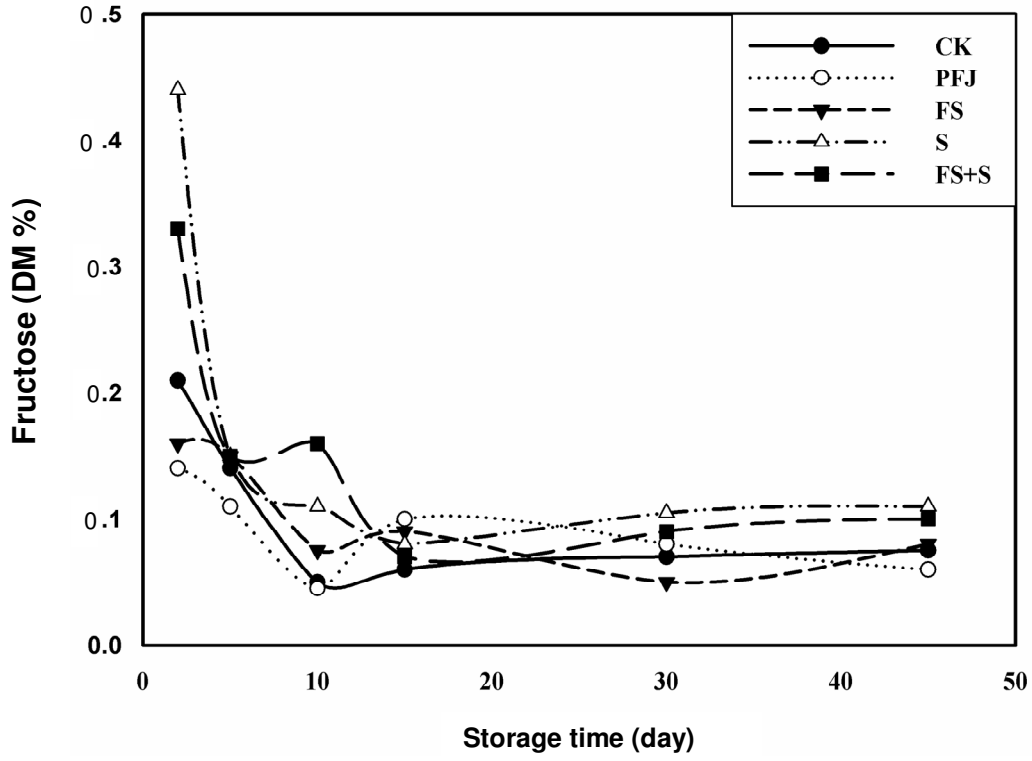

Figure 4. Change in fructose content during fermentation of alfalfa forage. CK; control, PFJ; previous fermented juice, FS; fast-sile, S; sucrose, FS+S; fast-sile + sucrose.

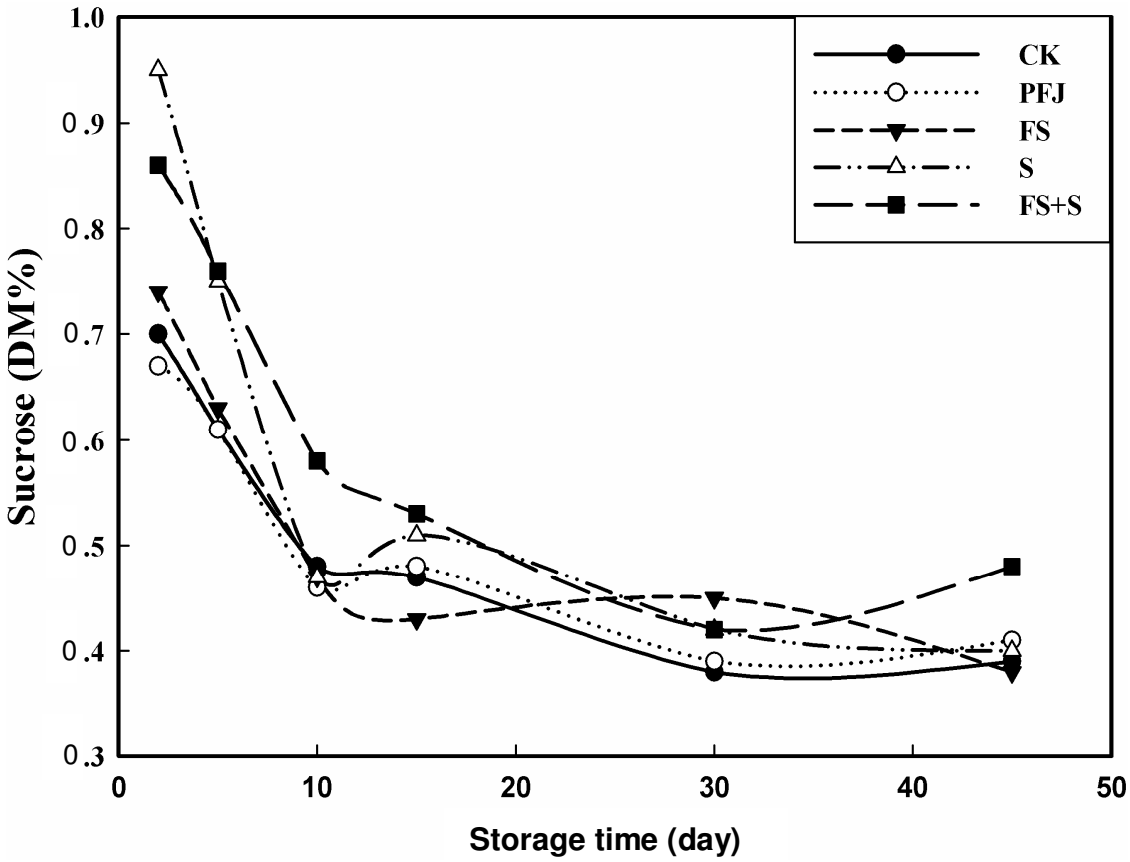

Figure 5. Change in sucrose content during fermentation of alfalfa forage. CK; control, PFJ; previous fermented juice, FS; fast-sile, S; sucrose, FS+S; fast-sile + sucrose.

fructose content after 15 days. The contents of fructose in silages treated with FS+ S and S were higher than those of others throughout the ensiling period. The trends of the sucrose of alfalfa silages decreased slowly during ensiling. The FS + S treatment had higher sucrose content during ensiling after 45 days.

Hemicellulose and cellulose contents of the 45 days alfalfa silages

The hemicellulose contents in all silages decreased during ensiling (Figure 6). The hemicellulose contents in silages treated with additives dropped more rapidly compared to CK in the 10 days period. Hemicellulose content in control and FS silages dropped more rapidly compared to silages treated with other additives after 30 days.

The change of cellulose contents in all alfalfa silages was relatively small during ensiling (Figure 7). The contents of cellulose in all treatments dropped slowly in the former 10 days. All alfalfa silages increased after 30 days and the cellulose contents in CK silages increased more rapidly as compared to those treated with additives.

DISCUSSION

Ensiling materials

In the present experiment, content of WSC in all pre-ensiled alfalfa forages (5.6% DM, Table 1) was lower than the 6 to 7% content recommended theoretical requirement to achieve well preserved fermentation (Wang et al., 2009; Smith, 1962). Thus the alfalfa without additives was not adequate for producing good quality silages.

Fermentation quality of alfalfa silage

Well preserved alfalfa silage is characterized by lower pH, greater LA content, lower contents of AA, PA, BA and NH_3-N (Zhang et al., 2009; Muck and kung, 1997). In this study, after 45 days of ensiling, alfalfa silages treated with all additives properly improved the silage fermentation

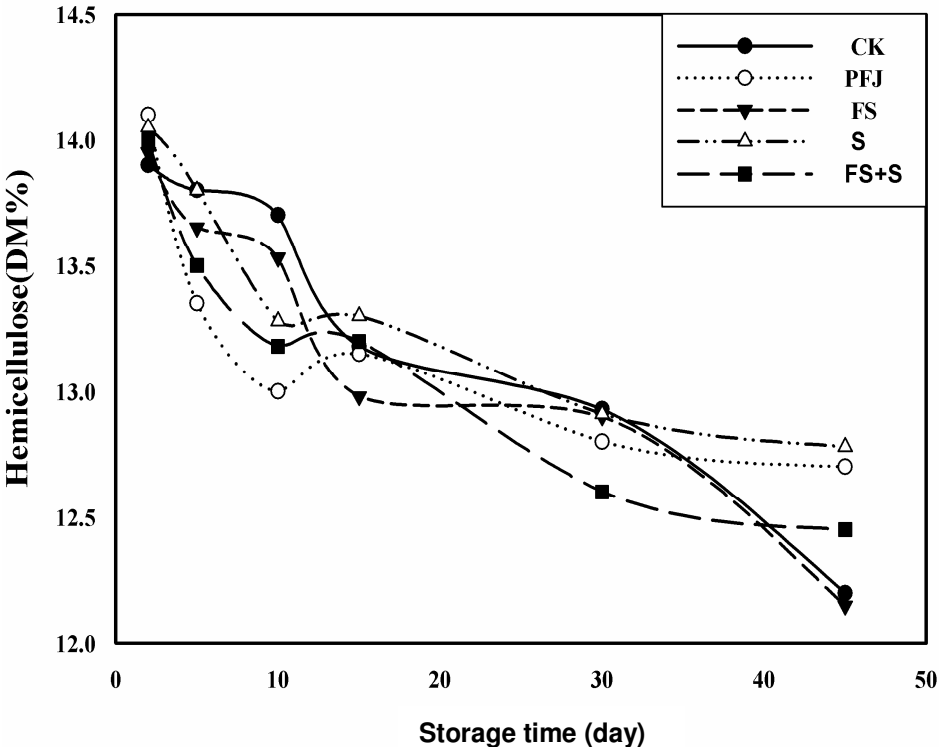

Figure 6. Change in hemicellulose content during fermentation of alfalfa forage. CK; control, PFJ; previous fermented juice, FS; fast-sile, S; sucrose, FS+S; fast-sile + sucrose.

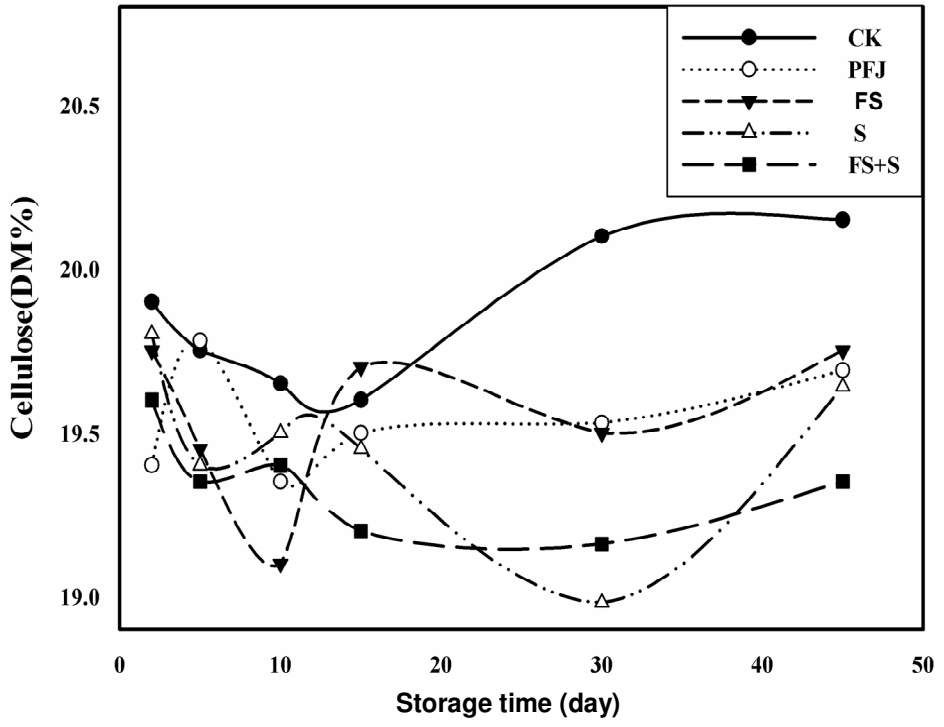

Figure 7. Change in cellulose content during fermentation of alfalfa forage CK; control, PFJ; previous fermented juice, FS; fast-sile, S; sucrose, FS+S; fast-sile + sucrose.

quality with markedly lower contents of AA, PA, BA and NH_3-N and markedly higher LA content as compared with the control silage ($p < 0.05$). This suggested that all additives in this study depressed the growth of clostridia or other aerobic bacteria (yeast and mould), lowered pH and the contents of acetic acid, propionic acid, butyric acid and NH_3-N, improved lactic acid content. This was contrarily reflected in the fermentation quality of control silage, it contained 6.20 for pH and 0.37% (DM) for LA, 4.06% (DM) for AA, 1.30% (DM) for PA, 5.97% (DM) for BA and 24.98% (TN) for NH_3-N. Our results for fermentation agree with the previous reports (Wang et al., 2009; Schmidt et al., 2009; Tyrolova et al., 2008; Zhang et al., 2009; Guo et al., 2009; Ohshima et al., 1997). However, silages treated with FS and PFJ were not as effective as silages treated with S and FS + S in producing of LA and reduction of AA and PA. This may be explained as follows: The addition of S increasing fermentable carbohydrate contents and improved ensiling process with the result of increasing the consumption of enough WSC, the enough fermentation substrates could allow the LA bacteria to produce more LA and also accelerate pH decline (Shao et al., 2003).

Change in NFC fractions during ensiling

Carbohydrates drive the efficiency of the ensiling process, during the ensiling process, increasing NFC (non-fiber carbohydrates) increases the rate of fermentation, which increases the preservation of the ensiled nutrients (Downing and Gamroth, 2007; Wollford, 1984). In this study, the extent of loss of WSC was similar to those of other studies (Hassanat et al., 2007; Lunden et al., 1990). The results shows that the WSC contents of all alfalfa silages reduced rapidly in the former 2 days, but the WSC contents of all silages were stable after about 15 days. Alfalfa silages treated with additives has higher WSC content than CK after 45 days. Differences in residual WSC concentration between silages treated with additives and untreated silages (CK) are likely due to increased contents of fermentable carbohydrate and population of LAB (Hassanat et al., 2007).

The residual mono and disaccharides mainly includes sucrose, glucose and fructose during ensiling (Shao et al., 2003). The metabolic activity of the three sugars during the former 5 days was remarkably intensive; after 5 days, large amounts of the three sugars were fermented. The rapid disappearance of the soluble sugars is in agreement with previous results (Lunden et al., 1990; Seale et al., 1986; Lindgren et al., 1985). Our results shows that the trends of the glucose of alfalfa silages were big fluctuation, but the fundamental trend was coming down and the control showed a less change relatively. This may be explained as follows: The big molecule infusibility carbohydrates were broken up by microorganism and enzyme under the acidity environment, which included starch, cell wall and so on (Ohyama et al., 1975), so the trend of glucose in alfalfa silages during ensiling showed the big fluctuation.

Previous studies suggested that starch concentrations were less likely to decline during fermentation because most lactic acid bacteria were not able to utilize it directly (McDonald et al., 1991). Other studies, however, did not show similar results (Melvin, 1965; Muck, 1990).

In our research, the starch contents of silages reduced rapidly in the former 15 days. But all silages were stable after about 15 days. Our results for the change of starch content during ensiling agree with Muck (1990) report. Muck (1990) noted that the rate of starch hydrolysis in the silage was proportional to the starch content at the beginning of ensiling and decreased linearly with time (Muck, 1990; Owen et al., 2002). Alfalfa silages treated with additives have lower starch content than CK after 45 days. This can explain that additives improve silage fermentation process and produce enough lactic acid bacteria that hydrate starch in silage.

Change in FC fractions during ensiling

Little information is known on losses of FC (fiber carbohydrates, mainly include hemicellulose, cellulose and ADL) during silage fermentation .Recent reports suggest that fiber carbohydrates can be substrates for microorganisms during ensiling (Yahaya et al., 2002; Mcdonald et al., 1991; Yahaya et al., 2000).

Previous studies have shown a much large variation (that is, 11.4 to 54.4%) in hemicelluloses loss during ensiling (Yahaya et al., 2002; Yahaya et al., 2001; McDonald et al., 1960, 1962, 1991; Butler and Bailey, 1973) and no clear reason for this wide variation has been suggested. The hemicelluloses content in this study dropped in all alfalfa silages during ensiling. The hemicelluloses content in all pre-ensiled alfalfa forages was 19.2% (Table 1), after 45 days, the hemicelluloses contents in all silages were between 12% and 13% (DM) and the hemicelluloses loss in this study was between 6.2% and 13% (DM). Matsuoka (1997) pointed out the effect of enzyme in silage materials, the microorganism and the acidity environment were main reasons that the hemicellulose was broken up in silage (Matsuoka et al., 1997).

The hemicellulose contents in silages treated with additives dropped more rapidly compared to CK in the 10 day period. This may be explained as follows: Adding FS and PFJ to the silage increases the number of the microorganism, so the reducing extent of hemicellulose in the silage is obvious in the initial period; using S and FS + S in silage promoted the acidity environment which could improve the breaking up of hemicelluloses.

The change of cellulose contents in all alfalfa silages

was relatively small during ensiling, compared to that for hemicelluloses. The result agrees with previous studies (Yahaya et al., 2002; Yahaya et al., 2001; Morrison, 1979). According to Yahaya (2002) viewpoint, one possible reason for variability in cellulose degradation during ensiling could be due to its structural features which exist in two forms: One lignified and protected and the other free from the effect of lignin and the rate of hydrolysis depends largely upon its lignifications.

In this study, the contents of ADL of alfalfa silages increased during ensiling. After 45 days, the contents of ADL in control were higher than those of additives treatments ($p > 0.05$). There is very little information available on ADL change during ensilage.

Conclusions

All additives in this study depressed the growth of clostridia or other aerobic bacteria (yeast and mould), lowered pH and the contents of AA, PA, BA and NH_3-N, improved LA content. However, silages treated with FS and PFJ were not as effective as S and FS + S treated silages in producing of LA and reduction of AA and PA.

The contents of WSC, residual mono and disaccharides (glucose, fructose and sucrose), starch and hemicellulose in all silages dropped as ensiling advanced, especially in initial period, the reducing extent was very obvious. However, alfalfa silages treated with additives have higher WSC content, lower starch content, lower hemicelluloses content than untreated silages after 45 days. The change of cellulose contents in all alfalfa silages was relatively small during ensiling. The contents of ADL of all alfalfa silages increased during ensiling. After 45 days, the contents of ADL in control were higher than those of additives treatments ($p > 0.05$).

Abbreviations

AA, Acetic acid; **ADF,** acid detergent fiber; **ADL,** acid detergent lignin; **BA,** butyric acid; **CHO,** carbohydrate; **CK,** control; **FS,** Fast-Sile; **PFJ,** previous fermented juice; **S,** sucrose; **FS+S,** fast-sile + sucrose; **LA,** lactic acid; **PA,** propionic acid; **WSC,** water soluble carbohydrate; **C,** Cellulose; **HC,** hemicelluloses; **NDF,** neutral detergent fiber; **NRC,** National Research Council; **NDSC,** neutral detergent soluble carbohydrate; **FC,** fiber carbohydrates; **NFC,** non-fiber carbohydrates; **DM,** dry matter; **HPLC,** high-performance liquid chromatography.

REFERENCES

Albrecht AT, Beauchemin KA (2003). Alfalfa and other perennial legume silage. In Silage Science and Technology, pp. 633-655.

AOAC (1999). Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Gaithersburg MD, USA.

Broderick GA,Kang JH (1980). Automated simultaneous determination of ammonia and total amino acid in ruminal fluid and in vitro media. J. Dairy Sci., 63: 64-75

Butler GW, Bailey RW (1973). Criteria for assessing the efficiency of the Fermentation process.In:Butler GW,Bailey RW (Eds.). Chemistry and Boochemistry of herbage,Vol.3.Academic Press, New York, pp. 33-80.

Downing T, Gamroth M (2007). Nonstructural carbohydrates in cool-season grasses Oregon State University Extension Service.

Fox DG, Tedeschi LO, Tylutki TP, Russell JB, Van Amburgh ME, Chase LE, Pell AN, Overton TR (2004). The Cornell net carbohydrate and protein system model for evaluating herd nutrition and nutrient excretion. Anim, Feed Sci. Technol., 112: 29-78.

Guo XS, Ding WR, Han JG, Zhou H (2008). Characterization of protein and amino acids in ensiled alfalfa treated with different chemical additives. Anim, Feed Sci. Technol., 142: 89-98.

Guo XS, Zhou H, Zhang YJ (2007). Changes in distribution of N and Plant enzymatic activity during ensiling of alfalfa treated with different additives .Grass Forage Sci., 62: 35-43.

Hall MB (2000). Neutral detergent-soluble carbohydrates: nutritional relevance and analysis, a laboratory manual.2000.

Hassanat F, Mustafa AF, Seguin P (2007). Effects of inoculation on ensiling characteristics, chemical composition and aerobic stability of regular and brown midrib millet silages.Animal Feed Science and Technology.139:125-140

Lanzas C, Sniffen CJ, Seo S, Tedeschi LO, Fox DG (2007). A revised CNCPS feed carbohydrate fractionation scheme for formulating rations for ruminants. Anim. Feed Sci. Technol., 136(3-4): 167-190.

Linoren S, Linvall P, Kaspersson, Kartzoe A, Rydbehg E (1983). Effects of inoculants. grain andformic acid on silage fermentation. Swedish J. Agric. Res., 13: 91-100.

Lunden PK, Lindgren S (1990). The influence of the carbohydrate fraction and additives on silages quality. Grass Forage Sci., 45: 223–233.

Madison WI, Marshall SA, Campbell CP, Buchanan-Smith JG (1993). Proteolysis and rumen degradability of alfalfa silages preserved with a microbial inoculant, spent sulfite liquor, formic acid or formaldehyde. Canadian J. Anim. Sci., 73(3): 559-570.

Matsuoka S, Branda LN, Fujita H (1997). Breakdown of Structural Carbohydrates during the Ensiling Process of Grasses Treated with Lactobacillus Inoculant and Cellulase Preparation and the Subsequent Effects on their *in vitro* Digestibilities [J]. Anim. Sci. Technol., 68(7): 661-667.

McAllister TA, Feniuk R, Mir Z, Mir P, Seling LB, Cheng KJ (1998). Inoculants of alfalfa silage: Effects on aerobic stability, digestibility and the growth performance of feedlot steers. Livest. Prod. Sci., 53: 171-181.

McDonald P (1981). The Biochemistry of Silage [M]. New York : John Wiley & Sons, Ltd.

McDonald P, Henderson AR, Heron SJE (1991). The Biochemistry of silage. 2nd ed. Cambrian Printers Ltd. Aberystwyth. pp.184-236.

McDonald P, Sterling AC, Henderson AR, Dewar WA, Stark GH, Macpherson HT, Reid AM, Slater J (1960). Studies on ensilage. Edin. Sch. Agric. Tech. Bull., 24: 1-83.

McDonald P, Stirling AC, Henderson AR, Whittenbury R (1962). Fermentation Studies in wet herbage. J. Sci. Food Agric., 13: 581-590

Melvin JF (1965). Variations in the carbohydrate content of lucerne and the effect on ensilage. Australian J. Agric. Res.,16: 951-959

Morrison IM (1979). Changes in the cell wall components of laboratory silages and the effect of various additives on these changes. J. Agric. Sci. Camb., 93: 581-586.

Muck RE (1990). Dry matter level effects on alfalfa silage quality. Fermentation products and starch hydrolysis.Transactions Am. Soc. Agric. Eng., 33: 1005-1009.

Muck RE, Kung L (1997). Effects of silage additives on ensiling in silage: field to feed bunk.NRAES-99. Northeast Regional Agricultural Engineering Service, Ithaca, pp. 187-199.

National Research Council (2001). Nutrient Requirements of Dairy

Cattle. 6*th* Rev. Ed. National Academy of Sciences, Washington, D.C.
Ohshima M, Cao L, Kimura E, Ohshima Y, Yokota H (1997). Influence of addition of previously fermented juice to alfalfa ensiled at different moisture contents. Grassland Sci., 43: 56-58.
Ohyama Y, Masaki S, Morichi (1973). Effects of temperature and glucose addition on the process of silage fermentation. Jap. J. Zootech. Sci., 44: 59-67.
Ohyama Y, Morichi T, Masaki S (1975). The effect of inoculation with *Lactobacillus Plantarum* and the addition of glucose at ensiling on the quality of aerated silages. J. Sci. Food Agric., 26: 1001-1008.
Owens VN, Albrecht KA, Muck RE and Duke SH (1999). Protein degradation and fermentation characteristics of red clover and alfalfa silage harvested with varying levels of total nonstructural carbohydrates. Crop Sci., 39: 1873-1880.
Schmidt RJ, Hu W, Mills JA, Kung 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.
Seale DR, Henderson AR, Pettersson KO, Lowe JF (1986).The effect of addition of sugar and inoculation with two commercial inocuiants on the fermeniaiion of lucerne silage in laboratory silos. Grass Forage Sci., 41: 61-70.
Shao T, Ohba N, Shimojo M and Masuda Y (2002). Dynamics of early fermentation of Italian ryegrass (*Lolium multiflorun* Lam.)silage. Asian-Aust. J. Anim. Sci., 15: 1606-1610.
Shao T, Ohba N, Shimojo M, Masuda Y (2003). Effects of addition of glucose and sorbic acid on the fermentation quality of guineagrass (*Panicum maximum* Jacq.) silages. J. Fac. Agric. Kyushu University, 47(2): 351-358.
Smith LH (1962). Theoretical carbohydrate requirements for alfalfa silage production. Agron. J., 54: 291-293.
Sniffen CJ, Oconnor JD, Van Soest PJ, Fox DG, Russell JB (1992). A net carbohydrate and protein system for evaluating dairy diets.2. Carbohydrate and protein availability. J. Anim. Sci., 70: 3562-3577.
Tyrolova Y, Vyborna A (2008). Effect of the stage of maturity on the leaf percentage of Lucerne and the effect of additives on silage characteristics. Czech J. Anim. Sci., 53(8): 330-335.
Uchida S (2000). The Advance of Silage Science [M]. Tokyo: Dairy Japan.
Van Soest PJ, Robertson JB, Lewis BA (1991). Methods for dietary fiber, neutral detergent fiber,and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.
Wang J, Wang JQ, Zhou H, Feng T (2009). Effects of addition of previously fermented juice prepared from alfalfa on fermentation quality and protein degradation of alfalfa silage. Anim. Feed Sci. Technol., 151: 280-290.
Woolford MK (1984). The Silage Fermentation. Marcel Dekker. Inc. NY, NY.
XU Chun-cheng, Cai Yi-min, Moriya Naoko, Ogawa Masuhiro (2007). Nutritive value for ruminants of green tea grounds as a replacement of brewers' grains in totally mixed ration silage. Anim. Feed Sci. Technol., 38: 228-238.
Yahaya MS, Kawai M, Takahashi J, Matsuoka S (2002). The effect of different moisture contents at ensiling on silo degradation and digestibility of structural carbohydrates of orchardgrass. Anim. Feed Sci. Technol., 101: 127-133.
Yahaya MS, Kimura A, Harai M, Kawai M, Takahashi J, Matsuoka S (2001). Effects of length of ensiling on silo degradation and digestibility of structural carbohydrates of lucerne and orchardgrass. Anim. Feed Sci. Technol., 92: 141-148.
Yahaya MS, Kimura A, Harai M, Kawai M, Takahashi J, Matsuoka S (2000).The breakdown of structural carbohydrates of lucerne and orchardgrass during different length of ensiling and its effects on nutritive value of silage. Aust. J. Anim. Sci., 13(Suppl. Asian), p. 153.
Zhang T, Li L, Wang XF, Zeng ZH, Hu YG, Cui ZJ (2009). Effects of *Lactobacillus buchneri* and *Lactobacillus plantarum* on fermentation,aerobic stability,bacteria diversity and ruminal degrability of alfalfa silage.