Effects of *Aspergillus oryzae* Inclusion on Corn Silage Fermentation

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ABSTRACT : This study is aimed at evaluating the effect of *Aspergillus oryzae* fermentation extract (AFE) on corn silage fermentation characteristics. Trial included two groups of treatments, with or without AFE inclusion in corn ensilage. Sixty corn silage containers, including two treatments with thirty replicates each, were processed in a laboratory scale mini-silo of 21 cm radius by 45 cm height. Three replicate containers were opened and sampled for analysis at 0, 0.5, 1, 2, 3, 4, 6, 10, 18 and 34 days after being ensiled. One silage container from each treatment was installed with a remote controlled electronic thermometer to record the temperature changes. Analysis included silage temperature, pH, fermentation acids, the water-soluble carbohydrates and chemical compositions and the silage protein fractions. Results showed that on the first day, the temperature of the ensiled corn was slightly higher than room temperature, but returned to room temperature on the second day. The pH and concentrations of WSC, ADF, lignin and acetic acid in the AFE treated silage were significantly lower than the control groups ($p<0.05$). The lactic acid and crude protein on the other hand were significantly higher in the AFE treated silage as compared to the control ($p<0.05$) at the end of the ensilage period. The DM content was significantly higher ($p<0.05$) whereas the butyric acid content of the AFE treated silage was significantly lower ($p<0.05$) than the control at the end of the 34 day ensilage period. Titratable acid and buffering capacity in the corn silage were not significantly different between treatment groups ($p>0.05$). Ammonia N concentration in the AFE treated silage showed a trend of decrease ($p>0.05$). NPN and the protein fraction A in both groups increased during the conservation period, but fraction A in the AFE treated corn silage was significantly higher than the control silage ($p<0.05$). During the conservation period, the AFE treated corn silage showed a trend toward a decrease in fractions B_1, B_3 and C ($p<0.05$). The protein fraction B2 showed a trend toward increase in the control group and an inconsistent trend in the AFE treated silage during the ensiling period. The AFE treated silage showed a better Flieg score over the control silage (97 vs. 75) as calculated from the concentrations of lactic acid, acetic acid and butyric acid. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 11 : 1568-1579)*

Key Words : *Aspergillus Oryzae*, Fermentation Characteristics, Protein Fractionates, Corn Silage

INTRODUCTION

Silage is a method of forage preservation by means of stabilizing fermentation process through decreasing the pH that inhibits the growth of the hetero-bacterial activities of some plant endogenic enzymes. This decline in pH during silage fermentation is attributed to the increase in lactic acid production by lactic acid producing bacteria which consumes the soluble carbohydrates during ensilage (Bolsen et al., 1992). Since the population of lactic acid producing bacteria is low in the forage (Muck, 1988), adding enzymes and inoculation with homo-fermentative lactic acid bacteria to dominate the epiphytic bacteria and promote the break down of structural carbohydrates, hence releasing available sugars to the bacteria for lactic acid production. Inclusion of enzymes in forages with low soluble carbohydrates facilitates the decrease in pH (Chen et al., 1994; Fredeen and McQueen, 1993), acetic acid (Narasimhalu et al., 1992; Stokes, 1992) and the increase in lactic acid concentration (Stokes, 1992) of the silage. Inclusion of enzymes or inoculation with lactic acid producing bacteria in forage with high fermentative substrates, however, can still produce beneficial results (Chamberlain and Robertson, 1992; Chen et al., 1994; Stokes, 1992).

Inclusion of enzymes in ensiled forage or mixed forages decreases the contents of NDF and ADF (Chamberlain and Robertson, 1992; Selmer-Olsen et al., 1993). Sheperd and Kung (1996) demonstrated a decrease in NDF, ADF and hemicellulose in corn silage by adding enzymes. Autrey et al. (1975) also showed a 13% cellulose decrease in corn silage by adding cellulose-digesting enzymes. Stokes and Chen (1994) reported a decrease of NDF and cellulose content in corn silage with added enzymes. However, Chen et al. (1994) reported no positive response in cellulose digestion with enzyme-inoculated corn silage. Most research on the effect of AFE involved *in vitro* studies and *in vivo* trials on lactation performance. Limited information is available on the effect of AFE on the silage fermentation kinetics in ensiled corn. This study is therefore aimed at evaluating the effects of AFE on corn silage fermentation characteristics.

MATERIALS AND METHODS

This trial included two groups of treatments, with and

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without *Aspergillus oryzae* fermentation extract (AFE) inclusion in the ensiled process of corn. Biozyme Ltd., USA, provided the AFE with the product, Regular GX. The AFE, diluted with wheat middlings at a ratio of one to one hundred, was evenly spread and mixed over chopped corn forage at a rate of 10 pounds to each ton of forage corn. Corn forage, with or without AFE addition, was placed into sixty-two laboratory scale mini polyvinyl containers, 21 cm in radius by 45 cm in height and sealed after compression by a mechanical compressor. With equal numbers of replicates, both with and without AFE inoculation, each silage container contained 11 kg of corn forage. One silage container from each treatment was installed with a remote controlled electronic thermometer. The silage temperature readings were then recorded from the sealed container during the 34 day ensile period. Three replicate containers were opened and sampled at 0, 0.5, 1, 2, 3, 4, 6, 10, 18, 34 days after ensilage, respectively for chemical analysis and determination of the fermentation characteristics. Sample analysis included pH, titratable acidity, buffering capacity, neutral detergent fiber (NDF), acid detergent fiber (ADF), neutral detergent fiber insoluble nitrogen (NDFIN), acid detergent fiber insoluble nitrogen (ADFIN), crude protein, ammonia nitrogen, lactic acid, volatile fatty acids (VFA), water-soluble carbohydrates (WSC), dry matter (DM), ash, lignin, tungsten insoluble protein, and buffer insoluble protein, and various protein fractions (A, B and C fractions).

Silage pH was determined using a glass electrode after homogenization of 10 g of fresh silage with 100 ml of distilled water for 1 min in a Warring blender according to Chen et al. (1994). Titratable acidity which required milliequivalents of NaOH/100 g of dry matter to raise the current pH to 7.0, was determined by titration with 0.1 N NaOH after homogenization according to Chen et al. (1994). Buffering capacity was measured according to Bolsen et al. (1992). Water-soluble carbohydrate was determined by furfural reaction after aqueous extraction and sample dilution.

Fresh silage was also homogenized with 0.1 N H_2SO_4 (12.5 g/125 ml) to determine the soluble components after residual particles were removed using a double filtration process. Dissolved silage extract was analyzed for lactic acid according to procedure No. 735 of the Sigma Chemical Co. Volatile fatty acids were analyzed according to Bolsen et al. (1992). Ammonia nitrogen was measured according to Chaney and Marbach (1962). The remaining silage was dried in a forced-draft oven at 55°C for 72 h to determine the dry matter content, ground to pass through a 1 mm screen and stored for chemical analysis. Ground and dried samples were analyzed for crude protein and ash according to AOAC (1984). NDF, ADF and lignin were analyzed according to Van Soest et al. (1991) using an automatic fiber analyzer (Fibertec System M. Tecator AB). Ground and dried silage samples were analyzed for NDFIN, ADFIN, buffered insoluble protein and tungsten insoluble protein according to Licitra et al. (1996).

Analysis of variance was done using the General Linear Models (GLM procedure) of statistical analysis system (SAS, 1985). Significant differences among treatments were determined by Duncan's New Multiple Range Test.

RESULTS AND DISCUSSION

Table 1 presents the effects of AFE inclusion on the

Table 1. Effects of AFE inclusion on the fermentation characteristics on corn silage at the end of the ensiled

Item	Control	AFE	SEM
Dry matter	18.79[b]	20.11[a]	0.19
Organic matter	93.39	93.00	0.31
NDF	67.09	66.63	0.65
ADF	38.20[a]	35.45[b]	0.67
CP	7.10[b]	9.09[a]	0.23
Lignin	3.26	3.24	0.03
Ash	6.61	7.00	0.31
pH	3.53	3.55	0.025
Buffering capacity	86.73	78.82	2.27
Titratable acidity	128.78	111.31	10.95
Water soluble carbohydrate	4.385[a]	3.327[b]	0.19
Lactic acid	1.524[b]	2.536[a]	0.21
Total VFA	1.432[a]	1.002[b]	0.20
Acetic acid	84.49	80.79	2.40
	(1.154)	(0.769)	
Propionic acid	9.16	9.53	0.26
	(0.155)	(0.112)	
Butyric acid	2.51[a]	1.06[b]	0.50
	(0.034)	(0.021)	
Iso-butyric acid	5.02[b]	6.89[a]	0.73
	(0.099)	(0.095)	
Iso-valeric acid	0.27	0.29	0.01
	(0.006)	(0.005)	
Ammonia-N	0.425	0.428	0.010
Protein fraction A	19.90[b]	43.32[a]	2.72
Protein faction B_1	6.36	5.40	3.44
Protein fraction B_2	41.91[a]	23.76[b]	4.15
Protein fraction B_3	26.84	20.82	3.48
Protein fraction C	4.98	6.67	1.49

[a,b] Means with different superscripts in the row differ ($p<0.05$).
The unit is % of dry matter except other specified.
Value of VFA is % of total VFA, whereas those within parenthesis is % of DM.
The value of protein fraction is % of crude protein.
The value of buffering capacity, titratable acid and water soluble carbohydrates are in meq/100 g DM.

fermentation characteristics of corn silage at the end of the ensiled period. These included DM, OM, NDF, ADF, CP, lignin, ash, pH, buffering capacity, titratable acidity, water soluble carbohydrate, lactic acid, various volatile fatty acids, amino-N, and protein fractionates. Figure 1 presents the effect of AFE inclusion on temperature, pH and titratable acid in corn silage during the ensiled period. Inclusion of AFE did not significantly influence temperature changes in the corn silage.

Silage temperature

The effects of AFE inclusion in the corn silage did not significantly influence silage temperature during the ensiled period. The temperature in the corn silage slightly increased from 32.2°C at the completion of the period for 5 h to 32.4°C, then gradually declined afterwards. The silage temperature was 1 to 6.3°C above the ambient temperature and returned to balance with the ambient temperature 24 hours after the completion of ensilage. From that point, the silage temperature followed the changes in ambient temperature. The short heating period in the corn silage reflected good silage fermentation with rapid filling, good packing and sealing which limited residue air inside the mini-silo and prevented the penetration of outside air.

Silage pH value

The pH value of the corn silage rapidly declined within the first two days from 5.3 at the start to 3.8 after being ensiled and became stable, that is the pH of the corn silage was below 3.63 from the third day onward. This silage pH was below the lowest range of pH 4.0-4.5 for grass. This corn silage reflected good corn preservation after 2 days of ensilage. Inclusion of AFE did not significantly influence the pH of the corn silage at the end of the ensiled period (table 1). It however, significantly decreased the pH value of the corn silage on the 6th (3.55 vs. 3.38), 10th (3.49 vs. 3.38), and 18th (3.35 vs. 3.29) days ($p<0.05$). This data agreed with the results of Kung et al. (1984) that a decreasing pH in grass silage, which included either mixed enzymes and lactic acid producing bacteria or enzymes alone. Sheperd et al. (1995) also demonstrated a sharp pH decline during the first 2 days of ensiled alfalfa silage.

Silage fermentation acids

Figure 2 presents the effects of AFE inclusion on the buffering capacity, lactic acid concentration and acetic acid concentration of corn silage. Figure 3 presents the effects of adding AFE on the concentration of total volatile fatty acids, molar percentage of propionic, butyric and iso-butyric acid in the corn silage during the ensiled period. The titratable acid and buffering capacity gradually increased during the first 2 days and then increased rapidly. The titratable acid increase from 10 to 144 meq/100 g of DM whereas buffering capacity increased from 11 to 81 meq/100 g of DM at the 4th day, and then reached stable conditions afterwards. The inclusion of AFE significantly decreased the titratable acid concentration in the corn silage on the 3rd and 10th day ($p<0.05$) where the control silage was significantly lower in titratable acid concentration on the 6th day of the ensiled period. The addition of AFE did not significantly influence buffering capacity during the ensiled period ($p>0.05$). Our data did not agree with the results of Stokes (1992), that inclusion of an enzyme mixture and inoculated bacteria depressed the buffering capacity and the titratable acid in silage. This probably is attributed to the different silage material used in the trial. He used timothy, alfalfa, and red clover mixed with grass forage in 50/50 ratios. He also used mixed cellulase enzymes, xylanase, cellobiase and glucose oxidase. These differences may result in different responses on buffering capacity and titratable acidity concentration in silage.

During the first four days of the ensiled period, the lactic acid concentration increased sharply from 0 to 1.6% of DM, and then slowly increased toward the end of the ensiled period. It reached a significantly higher lactic acid concentration in favor of the corn silage with the AFE inclusion at the end of the ensiled period (2.54 vs. 1.52% of DM) ($p<0.05$) (table 1). This trend was the opposite of the water-soluble carbohydrates, which decreased for the first two days, reaching significantly lower WSC in the corn silage with AFE addition (figure 4). This data agreed with both the results of Sheperd and Kung (1996) and Stokes (1992) that enzyme addition in the silage improved the degradation of structural carbohydrates, produced more available WSC, hence more substrates for lactic acid producing bacteria to proliferate and to produce lactic acid. The WSC in the silage was then utilized by the lactic acid producing bacteria, leading to a lower WSC concentration in the corn silage.

The concentration of acetic acid decreased from the 1st day to 18th day from 0.7 to 0.4% of DM and then sharply increased afterward during the ensiled period. Silage with added AFE significantly decreased the acetic acid concentration on the 34th day of the period (1.15 vs. 0.77% of DM) ($p<0.05$). Our results agreed with both the results of Narasimhalu et al. (1992) and Stokes (1992) that the concentration of acetic acid is depressed by enzyme inclusion in silage. The propionic acid concentration increased from 0.05 to 0.13% of DM at the end of the period. This showed a trend of depression of the propionic acid concentration by the addition of AFE in corn silage, but it did not reach a significant level except on the 3rd day of the period. The molar percentage of propionic acid also showed a similar trend as the propionic acid concentration and reached a

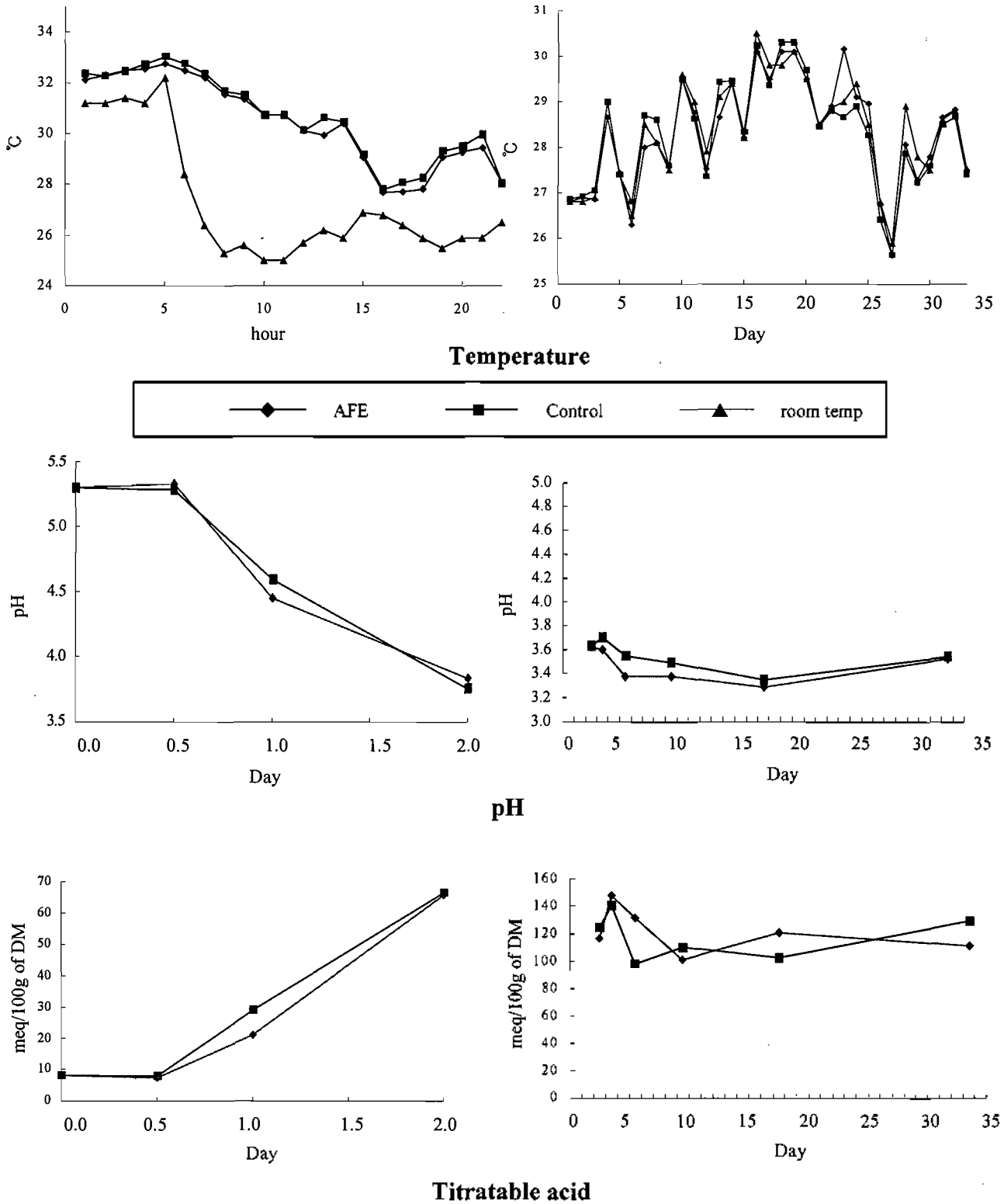

Figure 1. The effect of AFE inclusion on temperature, pH and titratable acid in corn silage
*: Means of the same column with the different superscript are significantly different ($p<0.05$).

significant level on the 10th day of ensilage ($p<0.05$). Both Bolsen et al. (1992) and Ruiz et al. (1992) suggested a decrease in propionic acid concentration by the inclusion of enzymes in silage. Butyric acid (concentration and molar %)

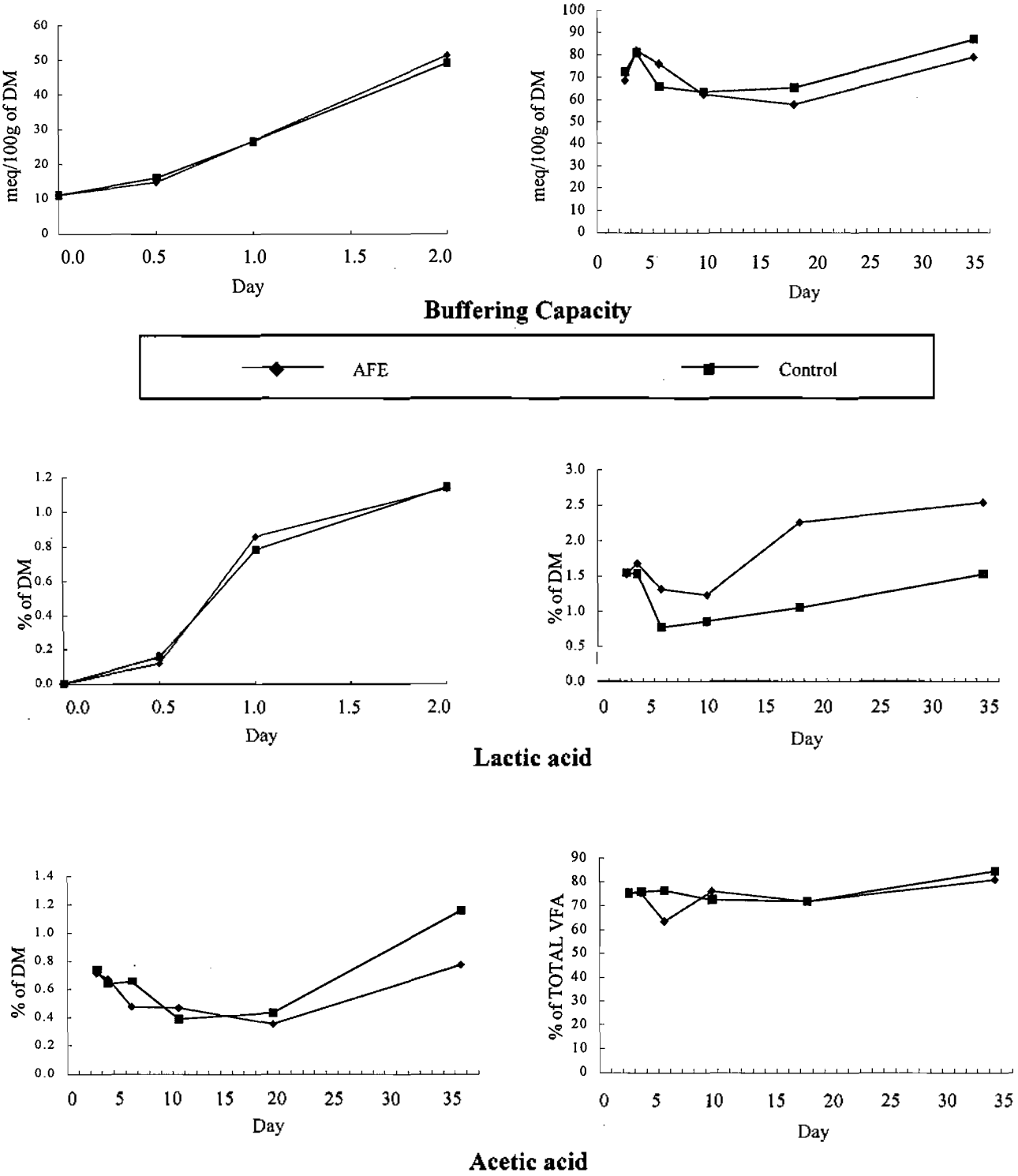

Figure 2. The effects of AFE inclusion on buffering capacity, lactic acid concentration and acetic acid concentration of corn silages. *: Means of the same column with the different superscript are significantly different ($p<0.05$).

gradually decreased during the ensiled period. Where the molar % of butyric acid reached a significantly lower in corn silage with AFE inclusion over the corn silage without AFE inclusion at the end of the ensiled period (1.06 vs. 2.51% of VFA) ($p<0.05$). The concentration of iso-butyric acid showed a trend of gradual decrease toward the 18th day from 0.13 to 0.05% of DM and then increased toward the end of the ensiled period (0.1% of DM). Inclusion of AFE also significantly influenced the iso-butyric acid molar percentage with a higher percentage in the AFE added

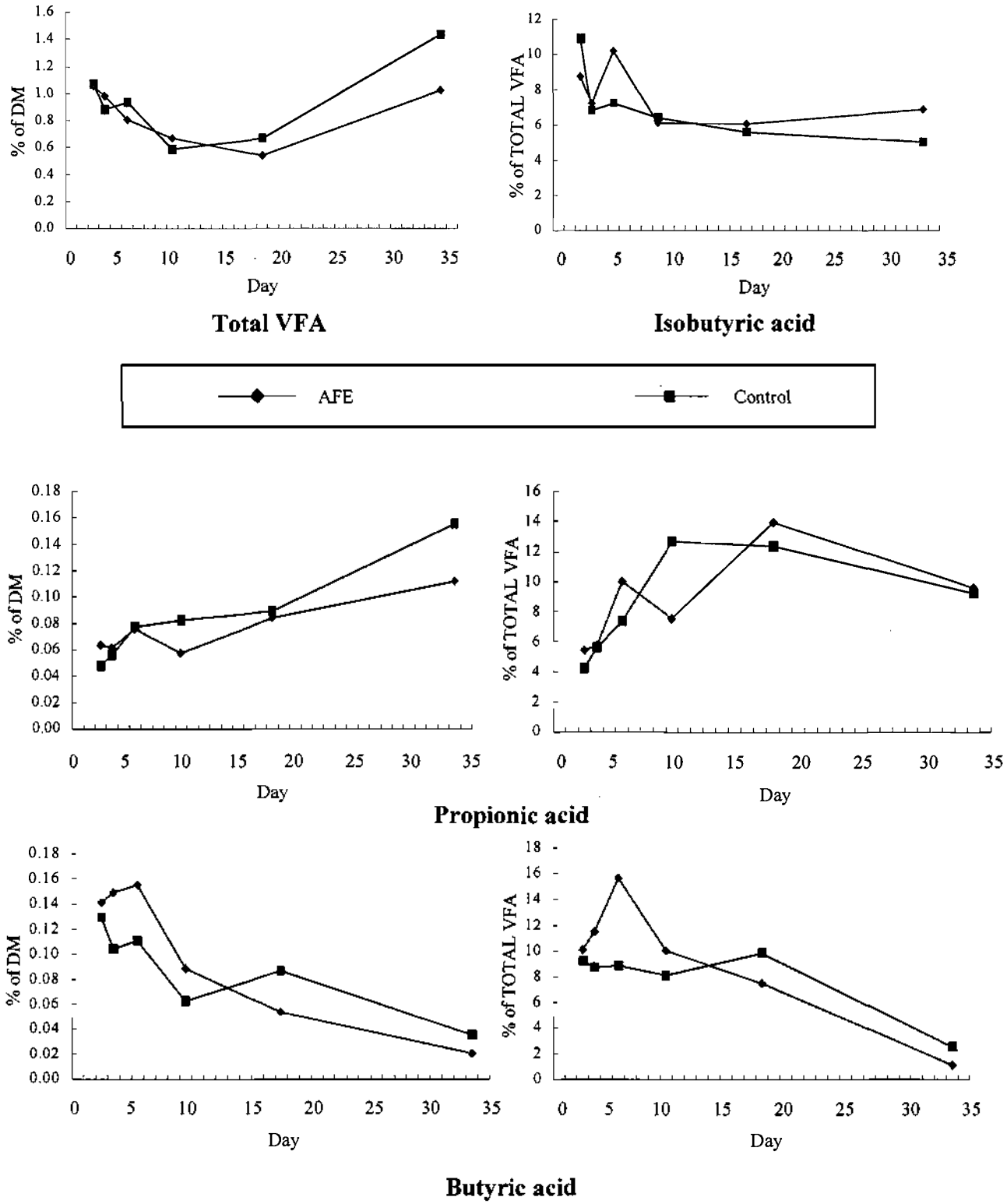

Figure 3. The effects of AFE on concentration of total volatile fatty acid, molar percentage of iso-butyric acid, concentration and molar percentage of propionic, and concentration and molar percentage of butyric in the corn silage during the ensiled period. *: Means of the same column with the different superscript are significantly different ($p<0.05$).

silage (6.89 vs. 5.02% of DM) at the end of the period ($p<0.05$). The total VFA concentration also showed a similar trend as iso-butyric acid which gradually decreased toward the 18th day of ensilage (1.06 to 0.6% of DM) and

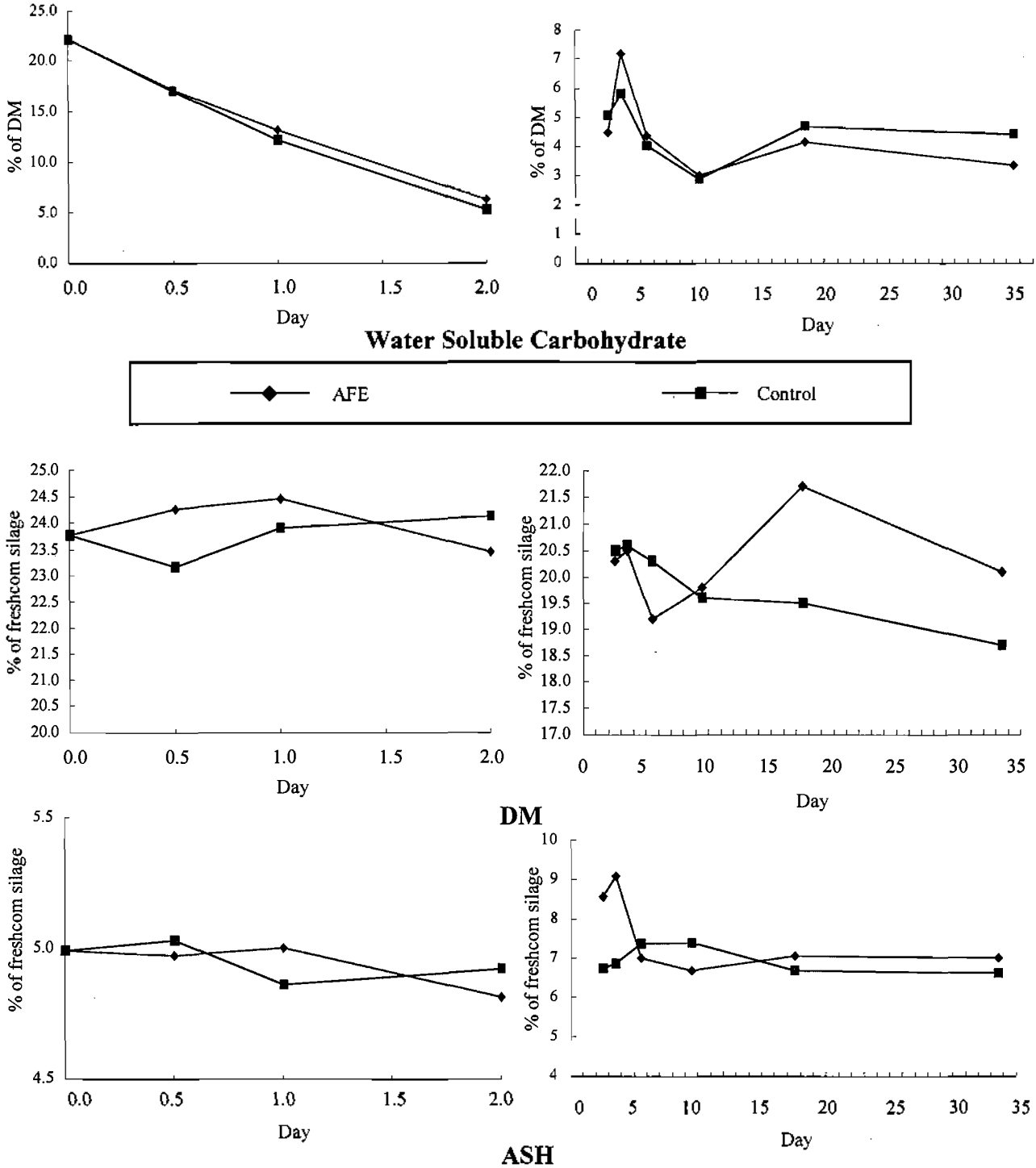

Figure 4. The effects of AFE inclusion on the content of water soluble carbohydrate, dry matter and ash in the corn silages.

then increased toward the end of the ensiled period. It however, reached a significantly lower concentration in the corn silage with AFE addition as compared to that without inclusion (1.43 vs. 1.00% of DM) ($p<0.05$). The Flieg score, calculated from the concentration of lactic acid, acetic acid and butyric acid was 97 versus 75 for the corn silage with and without AFE inclusion, respectively. It appears that the inclusion of AFE considerably improved the quality of the corn silage.

Water-soluble carbohydrates and chemical compositions

Figure 4 presents the effects of AFE inclusion on the

content of water soluble carbohydrates, dry matter and ash in the corn silage, whereas figure 5 presents the effects of AFE inclusion on the crude protein, NDF and ADF content in the corn silage.

The water-soluble carbohydrates (WSC) decreased from 22.2 to 5.7% of DM for the first two days after being ensiled, reaching significantly lower WSC in the corn silage with AFE addition (4.4 vs. 3.3% of DM) over the control silage at the end of ensiled period. The WSC was converted into lactic acid during the silage fermentation as lactic acid increased at the end of fermentation. Inclusion of AFE apparently improved lactic acid production, hence significantly decreasing WSC and producing significantly more lactic acid in the silage with AFE inclusion as compared to the silage without AFE inclusion.

The dry matter content of the corn silage did not demonstrate a big change during the ensiled period. The AFE inclusion, however, significantly contained more DM as compared to the control silage without AFE inclusion at the end of the ensiled period (20.1 vs. 18.8%) ($p<0.05$). High DM content at the end of silage fermentation indicated efficient silage fermentation due to the inclusion of AFE. Bolsen et al. (1992) suggested that an increase in silage DM would occur with the addition of enzymes to the silage. On the other hand, Buchanan-Smith and Yao (1981) suggested that DM content in the silage influenced the fermentation characteristics, i.e., pH, and ammonia-N and lactic acid concentration. Ash content increased from 5.0% at day 0 to the 3rd day and reached a significantly higher content in the silage with AFE inclusion on the 3rd (8.6 vs. 6.7%) and 4th day (9.1 vs. 6.9%) ($p<0.05$), indicating that a large amount of organic matter was lost during the 3rd and 4th day of silage fermentation. AFE inclusion in the corn silage significantly sped up the rate of fermentation during the early stages of fermentation.

Inclusion of AFE in corn silage improved fiber degradation during silage fermentation. At the end of the ensilage, NDF was 67.1% in the control vs. 66.8% in the AFE group, or 85.3% in the control vs. 78.6% in the AFE group after adjustment for the DM changes. The content of ADF at the end of the ensiled period were significantly lower in the silage with AFE inclusion of 35.5% as compared to that without inclusion of 38.2% ($p<0.05$), or 41.9% in AFE silage compared to 48.5% in control group after the adjustment in the DM change. Much research has shown an improvement in cell wall degradation by enzyme inclusion during fermentation in corn silage (Sheperd and Kung, 1996; Stokes, 1992), hence, the decrease in ADF and lignin content in corn silage.

Silage protein parameters

Figure 6 and 7 present the effects of AFE inclusion in the silage on the ammonia-N and protein fractions. The CP content gradually increased in the AFE inclusion corn silage where the control silage tended to have more fluctuation in the CP content during the ensiled period. Corn silage with added AFE contained significantly less CP on the 10th (8.34 vs. 9.47%) and 18th (8.32 vs. 9.49%) day, but contained significantly more CP on the 34th (9.09 vs. 7.10%) day during the ensiled period as compared to the control silage ($p<0.05$). The concentration of ammonia-N in the corn silage decreased gradually on the first two days from 0.38 to an average of 0.29% of DM, then increased gradually from 10th day onward. This showed a trend toward lower ammonia-N in the AFE added corn silage on the 18th day (0.33 vs. 0.48% DM), which reflected lower protein degradation due to the inclusion of AFE after 10 days of silage fermentation. Kung et al. (1984) reported significantly lower ammonia-N in alfalfa silage inoculated with lactic acid producing bacteria. This may be due to the effect of enzyme depression on protein hydrolysis during the ensilage period.

A gradual increase in the protein fraction A, NPN and true protein (TP), and a significantly higher content of protein fraction A on the 34th day was demonstrated by AFE inclusion (43.3 vs. 19.9% of CP) ($p<0.05$). This may be attributed to the promotion of silage fermentation and protein degradation by the AFE inclusion, hence the decrease in the percentage of protein fractions other than fraction A.

The protein fraction B_1 and B_3, that is true soluble protein (TSP) and the portion that is insoluble in neutral detergent but soluble in acid detergent, decreased gradually during the ensiled period. Since protein fraction B_1 is a rapid soluble true protein, it would be degraded rapidly from 15.7% to an average of 3.3% in CP during the first 4 days of silage fermentation. The fraction B_1 increased from the 4th day to the 10th day of silage fermentation. This may be attributed to the decrease in CP and fraction C content on the 10th day of silage fermentation, implicating that the protein was degraded rapidly during this stage of fermentation (4th-10th day). The degradation of protein fraction B_3, a slow degradable fraction, showed a similar trend as the degradation of protein fraction B_1. The increase in protein fraction B_3 degradation may be attributed to the increase of NDF and ADF degradation during the same stage of silage fermentation. The degradation of protein fraction B_2, a readily degradable fraction, showed a similar trend as the degradation of protein fraction B_1 and B_3, It however, showed a significantly higher fraction of B_2 in the silage with AFE included (41.9 vs. 23.8% of CP) as compared to the control. It appears that the heat produced during fermentation did not adversely affect the quality of the silage protein.

It appears that inclusion of AFE in corn silage significantly decreased the pH and the concentration of WSC, ADF and acetic acid content ($p<0.05$), and significantly

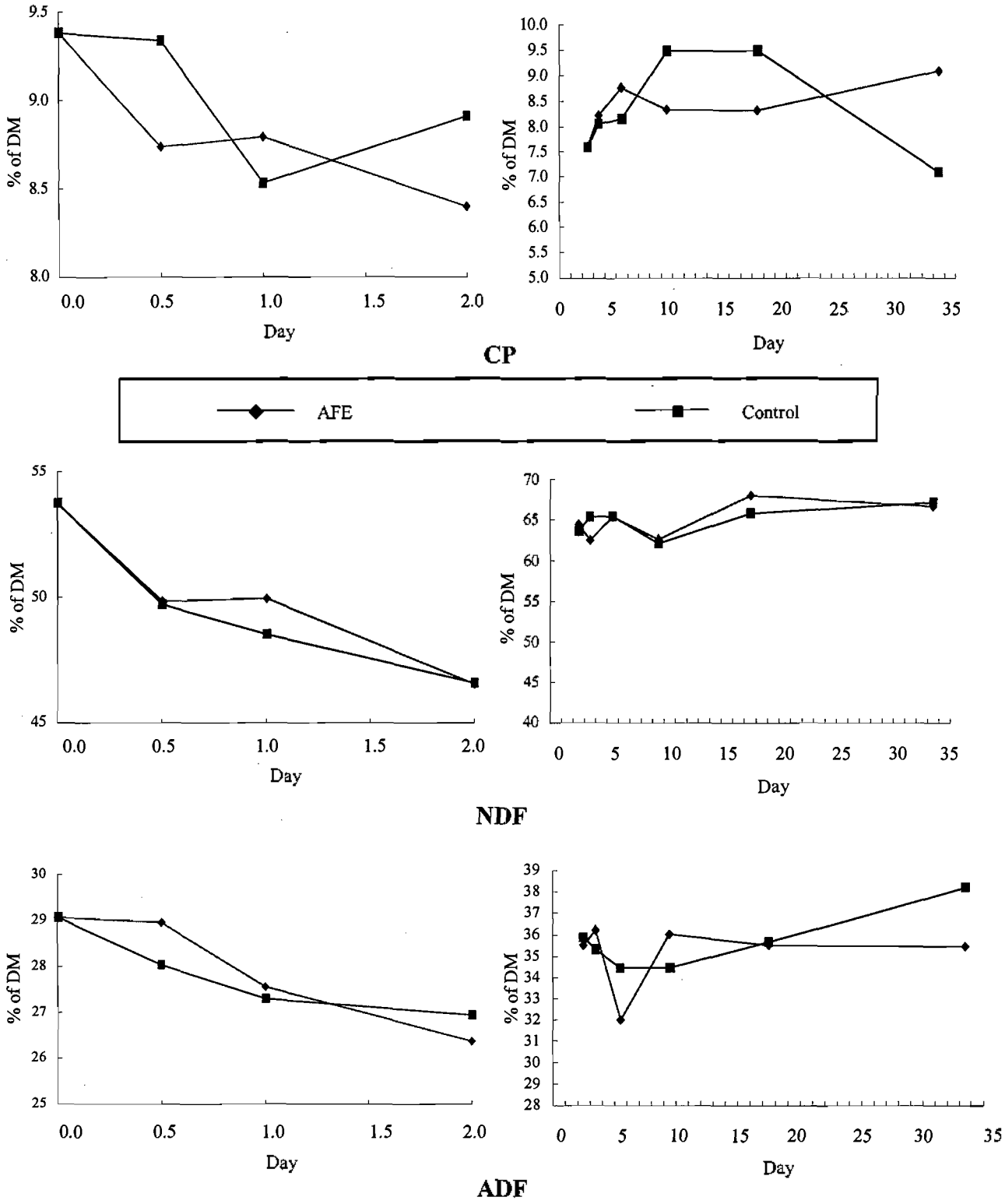

Figure 5. The effects of AFE inclusion on CP, NDF and ADF content in the corn silage.

increased the lactic acid and crude protein content ($p<0.05$) at the end of the ensilage period. AFE also significantly increased the DM content and significantly decreased the butyric acid content in corn silage ($p<0.05$). Inclusion of AFE also showed a better Flieg score over the control silage.

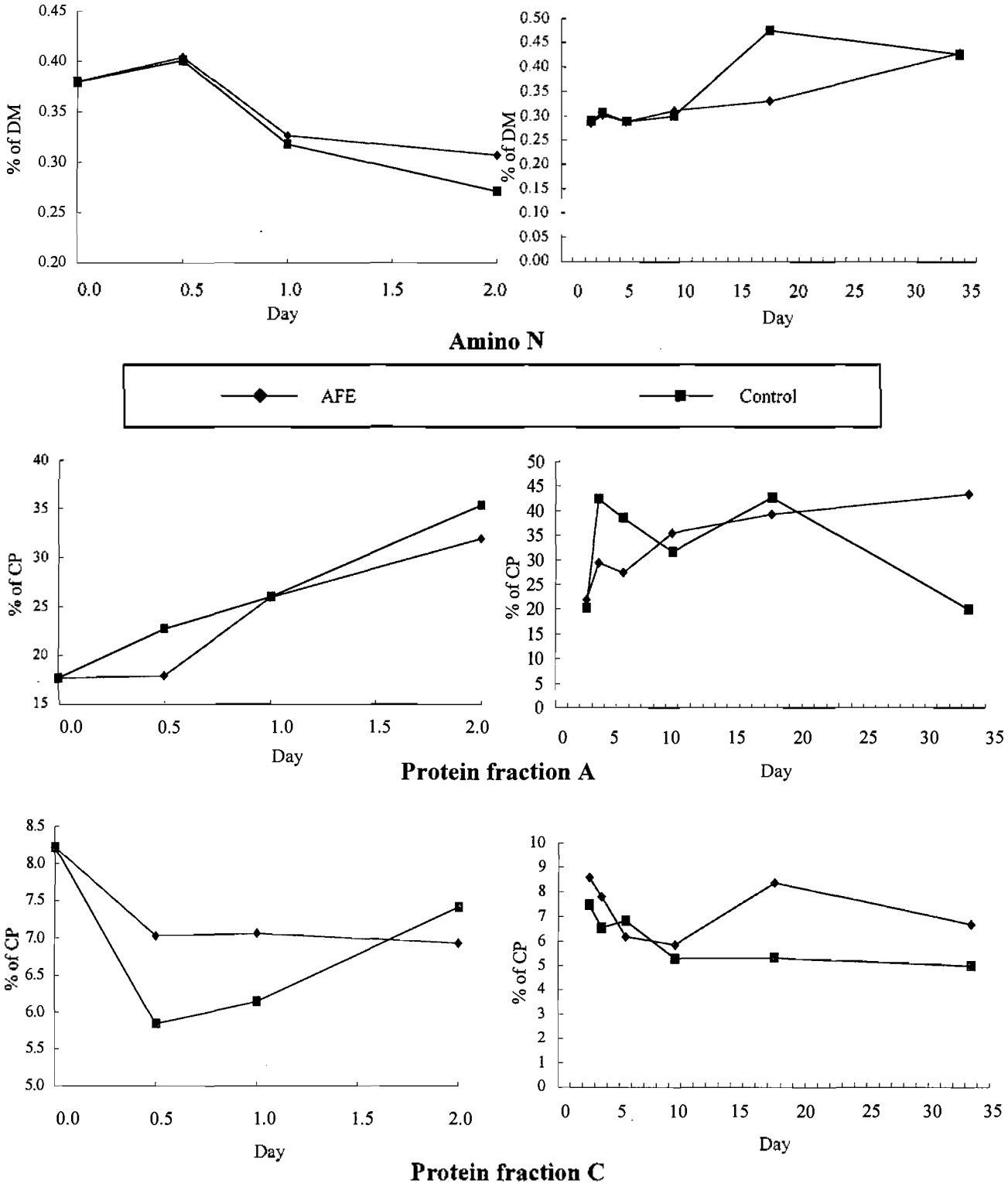

Figure 6. The effects of AFE inclusion in the silage on ammonia nitrogen, the protein fraction A, and the protein fraction C.

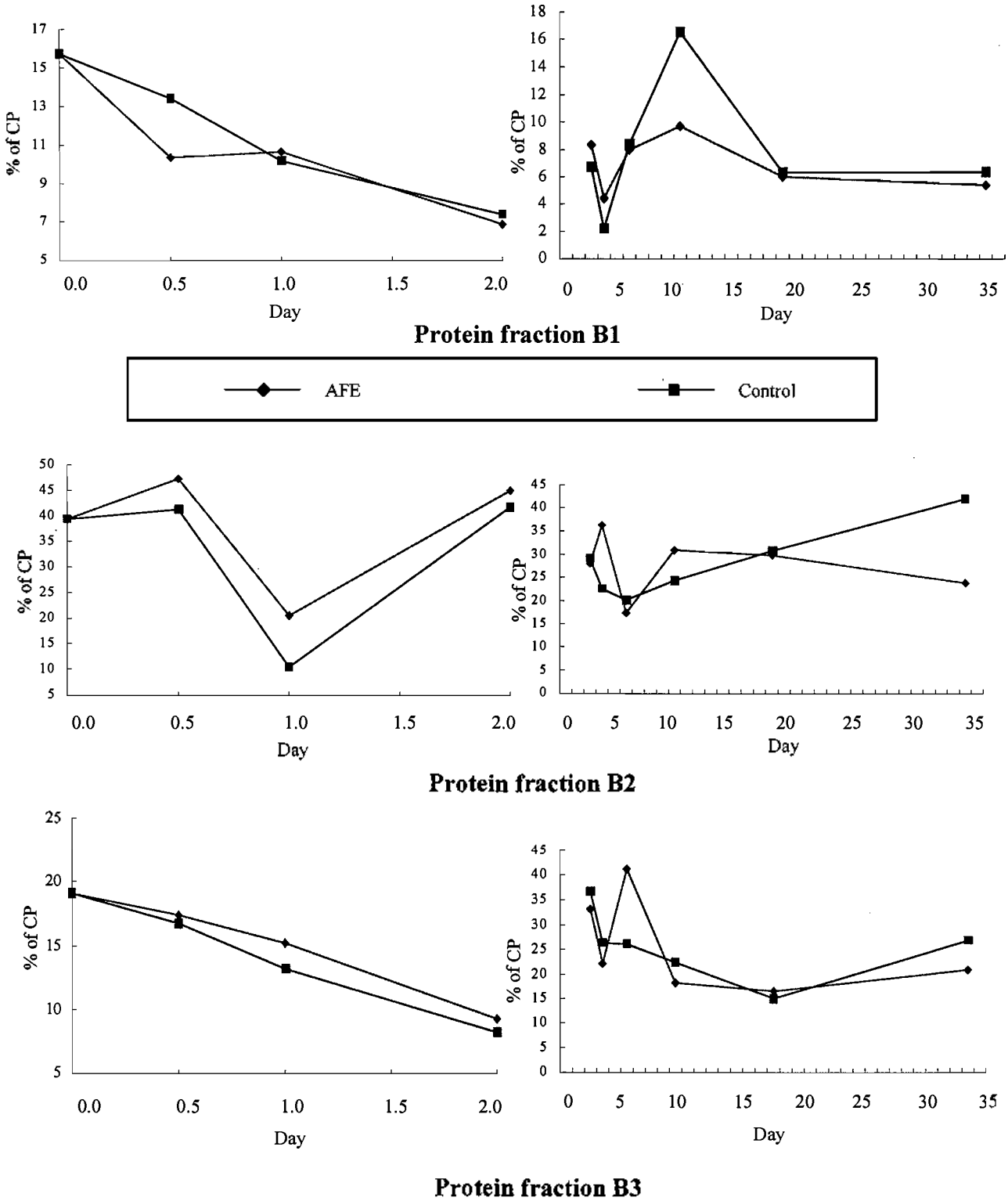

Figure 7. The effects of AFE inclusion in the silage on protein fraction B_1, the protein fraction B_2, and the protein fraction B_3.

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