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Effects of Different Carbohydrate Sources on Alfalfa Silage Quality at Different Ensiling Days

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Abstract: This study was evaluated the effects of different carbohydrate sources on the fermentation profiles, chemical compositions, and correlation of fermentation profiles and chemical compositions with water-soluble carbohydrate (WSC) of alfalfa (*Medicago sativa*) silage. Alfalfa was harvested at early flowering stage from the third cutting in September 2018, wilted to 32% dry matter (DM) and chopped into 1–2 cm pieces. Treatments included the addition of pectin (PEC), starch (STA; powdered corn), molasses (MOL), and fructose (FRU), as well as distilled water as a control (CON). Afterward, 300 g of prepared alfalfa was packed into polyethylene bags, vacuumed, and sealed, after which they were stored at room temperature for 1, 3, 7, 15, and 30 d. FRU and PEC additions resulted in desirable fermentation profiles and chemical compositions throughout the ensiling period. FRU and PEC rapidly decreased the pH and increased Fleig's point, exhibiting lower pH and higher Fleig's point from 3 d to the end of ensiling. Acetic acid (AA), propionic acid (PA) and ammonia nitrogen (AN) contents of FRU and PEC were lower at 30 d after ensiling. Higher lactic acid (LA) contents were found in FRU and PEC from 7 d to the end of ensiling and higher LA:AA ratios from 15 d to the end of ensiling. Butyric acid (BA) was not detected at any point during the ensiling period. Additives exhibited higher DM content from 7 to 30 d after ensiling. The WSC content decreased as the number of ensiling days increased and was stable from 15 d to the end of ensiling. PEC, STA, and FRU exhibited higher WSC than CON. FRU and PEC improved the fermentation quality throughout the ensiling period. Thus, FRU and PEC or related agricultural byproducts may offer alternative additives for improving the alfalfa silage fermentation profile.

Keywords: alfalfa; fructose; pectin; fermentation profile; chemical composition; water-soluble carbohydrate

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1. Introduction

Alfalfa (*Medicago sativa*) is an important protein source in ruminant livestock production [1,2] because of its high nutritional value, protein content, digestibility, and concentrations of vitamins and minerals. However, the nutritional value of alfalfa hay may be lost in the production, transport and storage processes. Thus, alfalfa is commonly processed into alfalfa silage in many regions. However, it has a low concentration of water-soluble carbohydrate (WSC) and dry matter (DM) and high buffering capacity [3–5], it is not easy to ensile successfully. Therefore, ensiled alfalfa is prone to decomposition, deterioration, and extensive proteolysis. Thus, it is necessary to apply additives (sugars, acids or microbial additives) or improve the DM by wilting or mixing with other high DM content forages to increase the fermentation quality of alfalfa silage.

Microbial additives are commonly applied to alfalfa to improve the quality of alfalfa silages in recent research. It is reported that lactic acid bacteria (LAB) and LAB mixing with cellulase could improve the quality of alfalfa silage [6–11]. Moreover, other organic acids, such as malic or citric acids, can effectively improve silage fermentation quality and inhibit proteolysis in alfalfa silage [12–14]. Certain carbohydrate additives are applied to alfalfa to improve silage fermentation quality. Sucrose and molasses are commonly applied to alfalfa to improve the quality of ensiling alfalfa. However, lots of crop products or byproducts containing high carbohydrate concentrations that have not been found to apply to alfalfa to improve alfalfa fermentation quality. Tian et al. [15] reported that dried jujube powder and *Lactobacillus plantarum* had positive effects on alfalfa fermentation and nutrition. Powdered corn is rich in starch and other nutrients and can be obtained worldwide. It may improve the fermentation quality of ensiled alfalfa. Powdered corn is rarely applied to alfalfa to improve the alfalfa quality. Fruit juice and honey is abundant in fructose and is produced by many fruit juice manufacturers. It not only can be applied to alfalfa to improve fermentation quality and promote utilization of waste. In addition, pectin is widely existed in citrus and apple peels and may be easily digested for ruminants to improve rumen fermentation [16,17]. Supplementation of alfalfa with molasses, orange pulp, and *Lactobacillus buchneri* were studied by Besharati et al. [18] to investigate the in vitro DM digestibility and gas production. Although few carbohydrates resources have already been applied to improve alfalfa fermentation quality, but the effects of adding these to alfalfa silages are not clear.

Thus, we hypothesized that different carbohydrates additives can improve the fermentation and nutritional quality while having no negative effects on alfalfa silage. Pectin, powdered corn, molasses, and fructose are rich in carbohydrates which may improve the alfalfa silage quality. However, it is unclear whether the different carbohydrate additives selected could improve the fermentation quality and nutritional composition of alfalfa silage. Therefore, the objective of the current study is to determine the changes in fermentation characteristics and chemical composition of alfalfa silage when adding different carbohydrate sources on different ensiling durations.

2. Materials and Methods

2.1. Silage Material and Ensiling

Alfalfa (Gannong No.4) was harvested at the early flowering stage from the third cutting in September 2018, in Yanchi, Ningxia, China. Alfalfa was planted for approximately two years without the application of fertilizers or herbicides throughout cultivation. Approximately 80 kg alfalfa was harvested with a sickle by hand, leaving a 5 cm stubble. Three duplicates were detected to evaluate the DM of alfalfa, the DM was 21%. After wilting for 1.5 d, the DM increased to 32%. Then, the alfalfa was immediately chopped into 1–2 cm pieces with a chopper (9Z-0.4, Jinniu Machinery Factory Rongyang, (Rongyang, Henan, China). Four additives were subsequently applied to the alfalfa, including pectin (PEC, 2% fresh matter (FM)) with 95% DM content; starch (STA, powdered corn, 2% FM) with 88% DM content; molasses (MOL, 2% FM) with 80% DM content; and fructose (FRU, 2% FM) with 95% DM content. In order to spray easily onto the prepared alfalfa, MOL and FRU were dissolved with 2% distilled water to account for FM allow for them to be easily sprayed onto the prepared alfalfa. To be consistent with those of MOL and FRU, the same volume of distilled water was used in the PEC and STA treatments. At the same time, the same volume of distilled water was sprayed onto prepared alfalfa without additives as control (CON), and 300 g of mixed alfalfa was weighed and packed into polyethylene bag, vacuumed with a vacuum sealer, and stored at room temperature until opening. Fifteen duplicates were prepared of each additive treatment, and three duplicates of each treatment (3 duplicates $\times$ 5 treatments) were opened and collected after 1, 3, 7, 15, and 30 d ensiling. A total of 75 bags were prepared in present study. Moreover, three duplicates were preserved before ensiling the material.

The purity of PEC (technical grade) was 65%, which was sourced from citrus, and it was purchased from Yuanye Biological (Shanghai, China). STA was purchased from Green Liangcang (Sanhe, Hebei, China). The sucrose concentration of MOL was 48% (provided by the manufacturer), and it was purchased from Rongxin Chemical Industry

(Jinan, Shandong, China). The purity of FRU was 99%, and it was purchased from Shanghai Zhanyun Chemical Co., Ltd. (Shanghai, China).

2.2. Fermentation Profile and Chemical Composition

At each ensiling day, 15 bags (3 duplicates × 5 treatments) were opened and 20 g of each sample was collected and mixed with 180 mL distilled water, homogenized with a blender for 60 s, and successively filtered with 4-layer nylon gauze and qualitative filter paper. The pH was determined immediately using a pH meter (Five Easy Plus FE28, Mettler Toledo Co., Ltd., Shanghai, China). The ammonia nitrogen (AN) concentration was determined according to the method of Broderick and Kang [19], and the organic acid concentrations, including lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA), were analyzed via HPLC, as previously described [20].

The alfalfa silage samples before and after ensiling were dried in an oven (GZX-9140MBE, Shanghai Boxun Co., Ltd., Shanghai, China) at 65 °C for 48 h for DM measurement. The dried samples were ground in a hammer mill (FZ102, Test Instrument, Tianjin, China) to pass through a 1 mm sieve for further chemical analysis. The crude protein (CP) was analyzed with an automatic Kjeldahl nitrogen apparatus (Kjeltec 2300 AutoAnalyzer, FOSS Analytical AB, Hoganas, Sweden) according to AOAC [21] standards. The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined as described by Van Soest et al. [22] in a fiber analyzer (A2000I, Ankom Technology, Macedon, NY, USA). Sodium sulfite and thermostable α-amylase were used in the NDF analysis, and the results were expressed as DM content. The WSC content was measured by the anthrone method [23].

2.3. Calculations

The quality of the alfalfa silage was evaluated by Fleig's point index, which was calculated as follows [24]:

Fleig's point = 220 + [(2 × %DM) − 15] − 40 × pH

The relative feed value (RFV) index was estimated as follows [25]:

RFV = (DDM × DMI)/1.29

DDM = Digestible dry matter = 88.9 − (0.779 × %ADF)

DMI = Dry matter intake (% of BW) = 120/(%NDF)

2.4. Statistical Analyses

The experiment was a completely randomized design with a 3 × 5 × 5 (3 duplicates, 5 additives and 5 ensiling days) factorial arrangement. The ANOVA procedure of SAS 9.4 was used to elucidate the additive effects at each ensiling day and effect of the ensiling time of each treatment silage on the fermentation characteristics and chemical composition. Post hoc mean comparisons were compared with Duncan's multiple comparisons. For the chemical profile and fermentation quality, a scheme of time-repeated measurements was used to repeat the fermentation periods among each treatment. The GLM procedure of SAS was used to elucidate the effects of the additives, ensiling time and their interactions for each parameter during the entire ensiling duration. Significance was declared at $p \leq 0.05$. The R software package was utilized for correlation analysis between fermentation profiles and chemical compositions of alfalfa silages.

3. Results

3.1. ChemicalCompositions of Alfalfa before Ensiling

The pH and AN contents of alfalfa before ensiling were 6.02 and 1.06 g/kg total nitrogen (TN), respectively. The DM content before ensiling was 318.5 g/kg, and the CP, NDF, ADF, and WSC contents before ensiling were 194.4, 377.8, 285.2, and 53.5 g/kg DM, respectively. Moreover, the Fleig's point and RFV were 27.91 and 164.3, respectively.

3.2. Fermentation Characteristics ofAlfalfa after Different Durations of Ensiling

The pH of alfalfa silages are shown in Figure 1. Significant effects were detected for additives, ensiling days and their interaction. The pH gradually decreased as the number of ensiling days increased for all treatments. All of the different carbohydrate sources exhibited a significant effect on the pH of alfalfa silage from 3–30 d of ensiling (Table 1, $p \leq 0.01$). PEC and FRU treatments resulted in a lower pH than other treatments during 3–30 d after ensiling.

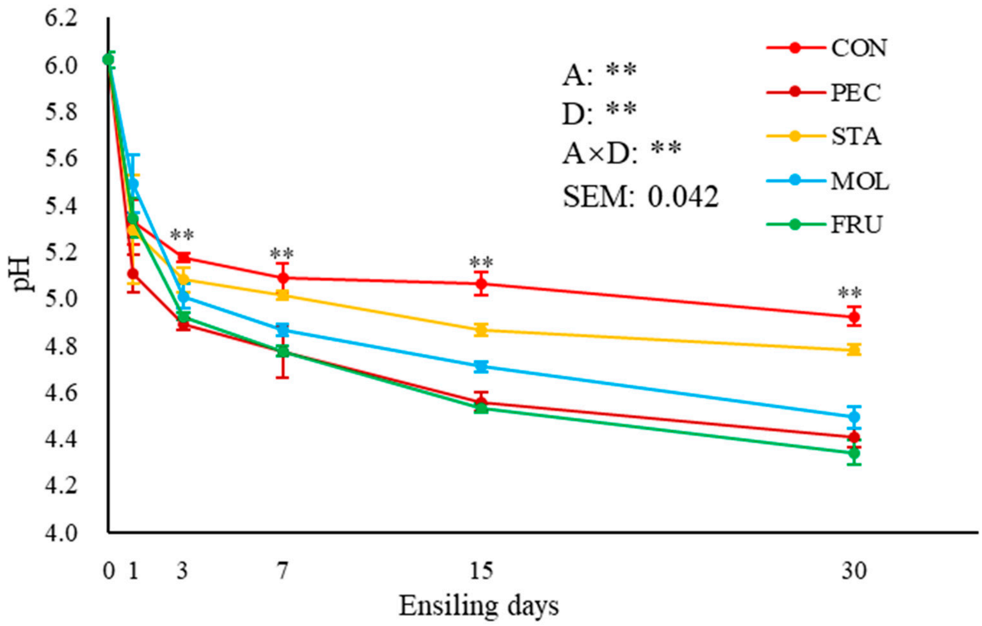

Figure 1. Effect of additives on pH as a function of the number of alfalfa ensiling days. CON, control; PEC, pectin; STA, starch; MOL, molasses; FRU, fructose. A denotes significance of additives; D, denotes significance of ensiling days; A × D denotes the interactive effect between additives and ensiling days. SEM, standard error of means. The asterisks (** $p \leq 0.01$) indicate the significance of additives and ensiling days and their interaction effects, and the difference at the specified ensiling day.

Table 1. Statistical analysis for the fermentation profile variables of alfalfa silage treated with different carbohydrate sources at different ensiling period.

Items	Ensiling Day				
	1	3	7	15	30
pH	0.11	<0.01	<0.01	<0.01	<0.01
LA	<0.01	0.05	0.01	<0.01	0.01
AA	0.28	0.16	0.18	0.97	0.26
PA	<0.01	<0.01	<0.01	0.22	0.01
LA:AA ratio	<0.01	0.15	0.88	0.53	<0.01
AN	0.44	<0.01	0.72	<0.01	<0.01
Fleig's point	0.11	<0.01	<0.01	<0.01	<0.01

Note: LA, lactic acid; AA, acetic acid; PA, propionic acid. AN, ammonia nitrogen.

Additives exhibited an effect on the LA concentration for each ensiling day (Table 1, $p \leq 0.05$). The LA content increased gradually at the first three days after ensiling, then decreased at 7 d after ensiling, except for in the PEC treatment. PEC significantly exhibited higher LA content at 7, 15, and 30 d after ensiling compared with other treatments. The CON and STA treatments had lower LA contents than the other treatments at 7, 15, and 30 d.

Additives were not significant in terms of the AA content at each ensiling day, but the ensiling days and their interaction were significant (Table 2). At the last ensiling day, FRU and PEC exhibited a lower AA content than the other treatments but was not significant (Table 1, $p > 0.05$).

Table 2. The dynamics of organic acid concentrations of silage alfalfa during the entire ensiling period.

Items [1]	Additives	Ensiling Days					SEM	*p*-Value		
		1	3	7	15	30		A	D	A × D [2]
LA (g/kg DM)	CON	6.45 ^{cB}	41.58 ^{aAB}	24.66 ^{bBC}	29.02 ^{bC}	44.26 ^{aC}	1.151	<0.001	<0.001	<0.001
	PEC	6.93 ^{dB}	32.49 ^{cB}	35.24 ^{bcA}	41.28 ^{bA}	58.32 ^{aA}				
	STA	24.37 ^{cA}	45.19 ^{aA}	23.00 ^{cC}	32.50 ^{bBC}	45.39 ^{aC}				
	MOL	24.88 ^{dA}	41.33 ^{bAB}	29.32 ^{cdAB}	36.81 ^{cbAB}	50.35 ^{aBC}				
	FRU	19.35 ^{dA}	46.04 ^{abA}	32.43 ^{cA}	40.71 ^{bA}	52.47 ^{aAB}				
AA (g/kg DM)	CON	4.17 ^{b}	21.63 ^{a}	12.58 ^{ab}	14.02 ^{a}	20.85 ^{a}	0.706	0.607	<0.001	0.033
	PEC	8.07	17.14	18.30	14.67	18.76				
	STA	7.58 ^{c}	27.21 ^{a}	12.02 ^{c}	13.97 ^{bc}	20.46 ^{ab}				
	MOL	7.84 ^{c}	17.99 ^{ab}	16.18 ^{ab}	15.47 ^{b}	20.37 ^{a}				
	FRU	5.28 ^{b}	16.78 ^{a}	18.18 ^{a}	15.60 ^{a}	15.88 ^{a}				
PA (g/kg DM)	CON	0.00 ^{cB}	1.08 ^{cB}	1.76 ^{cC}	5.30 ^{b}	12.70 ^{aAB}	0.522	<0.001	<0.001	0.087
	PEC	0.00 ^{cB}	0.36 ^{cB}	3.70 ^{bB}	4.98 ^{b}	10.69 ^{aBC}				
	STA	0.00 ^{dB}	1.02 ^{cdB}	2.73 ^{cBC}	6.22 ^{b}	12.75 ^{aAB}				
	MOL	2.58 ^{dA}	3.65 ^{dA}	5.09 ^{cA}	8.15 ^{b}	14.95 ^{aA}				
	FRU	0.00 ^{dB}	0.00 ^{dB}	2.27 ^{cC}	5.61 ^{b}	9.13 ^{aC}				
LA:AA ratio	CON	1.87 ^{B}	1.98	2.06	2.21	2.15 ^{B}	0.082	<0.001	<0.001	<0.001
	PEC	0.97 ^{cB}	1.91 ^{b}	2.01 ^{b}	2.90 ^{a}	3.12 ^{aA}				
	STA	3.25 ^{aA}	1.74 ^{b}	1.92 ^{b}	2.42 ^{b}	2.24 ^{bB}				
	MOL	3.18 ^{aA}	2.40 ^{b}	1.81 ^{b}	2.38 ^{b}	2.50 ^{abB}				
	FRU	3.67 ^{aA}	2.79 ^{b}	1.82 ^{c}	2.68 ^{b}	3.31 ^{aA}				

Note: No butyric acid was detected in any treatments during the ensiling duration. [1] DM, dry matter. SEM, standard error of means. LA, lactic acid; AA, acetic acid; PA, propionic acid. CON, PEC, STA, MOL, and FRU, respectively, represent control, pectin, starch, molasses, and fructose. $^{A–C}$ Means within a column with no common superscript differ ($p < 0.05$). $^{a–d}$ Means within a row with no common superscript differ ($p < 0.05$). [2] A, D, and A × D denote the significance of additives, ensiling days and their interactive effects, respectively.

Additives and ensiling days exhibited a significant effect on the PA content, but no interactive effect (Table 2). The MOL treatment exhibited a higher PA content during the entire ensiling period, and exhibited a significant effect at 1, 3, 7, and 30 d (Table 1, $p \leq 0.01$). On the last day of ensiling, FRU and PEC exhibited a lower PA content than other treatments.

The LA:AA ratio of all treatments were significantly different at 1 and 30 d after ensiling (Table 1, $p \leq 0.01$). At the first 7 d ensiling, LA:AA ratio of STA and MOL were dropped rapidly and, thereafter, increased slowly, reaching a value similar to that of CON (Table 2). The LA:AA ratio of CON did not significantly change throughout the ensiling period. However, the LA:AA ratio of PEC increased gradually and was similar to that of FRU on 15 and the last ensiling day.

Additives, ensiling days and their interaction exerted significant effects on the AN content of alfalfa silage (Figure 2). The AN content increased gradually as the ensiling time increased, and only MOL treatment decreased slightly at 15 d ensiling. Additives had a significant effect at 3, 15, and 30 d ensiling (Table 1, $p \leq 0.01$). FRU exhibited lower AN content at 15 and 30 d ensiling compared to that of the other treatments. The AN contents of CON and STA were higher than those of the other treatments on 15 and 30 d ensiling.

In addition, additives, ensiling days and their interaction exerted significant effects on the Fleig's point of alfalfa silage (Figure 3). Additives exhibited significant effects at 3, 7, 15, and 30 d after ensiling (Table 1, $p \leq 0.01$). The PEC and FRU treatments of alfalfa silage significantly increased Fleig's point compared with other treatments. CON exhibited lower Fleig's point than the other treatments throughout the ensiling period.

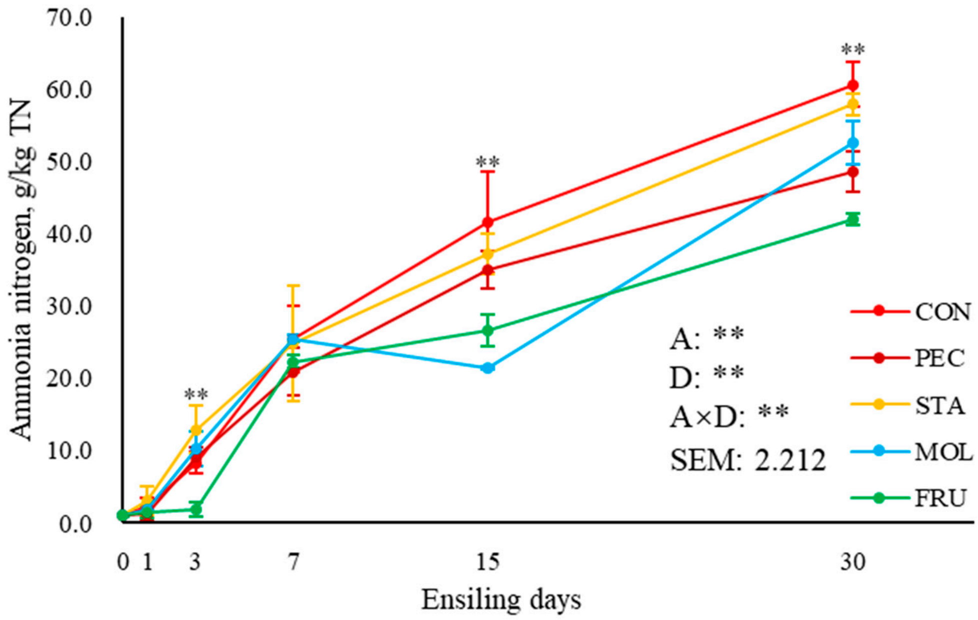

Figure 2. Effect of additives on the ammonia nitrogen (AN) content during ensiling of alfalfa. TN, total nitrogen; CON, control; PEC, pectin; STA, starch; MOL, molasses; FRU, fructose. A denotes significance of additives; D, denotes significance of ensiling days; A $\times$ D denotes the interactive effect between additives and ensiling days. SEM, standard error of means. The asterisks (** $p \leq 0.01$) indicate the significance of additives and ensiling days and their interaction effects, and the difference at the specified ensiling day.

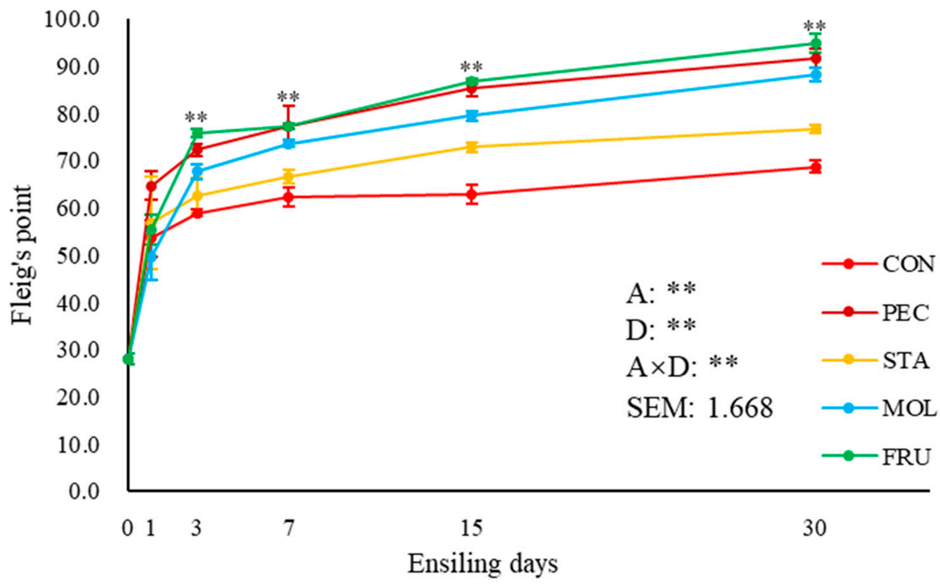

Figure 3. Effect of additives on the Fleig's point during ensiling of alfalfa. CON, control; PEC, pectin; STA, starch; MOL, molasses; FRU, fructose. A denotes significance of additives; D, denotes significance of ensiling days; A $\times$ D denotes the interactive effect between additives and ensiling days. SEM, standard error of means. The asterisks (** $p \leq 0.01$) indicate the significance of additives and ensiling days and their interaction effects, and the difference at the specified ensiling day.

3.3. Chemical Composition of Alfalfa after Different Ensiling Days

Additives, ensiling day and their interaction exerted significant effects on the DM, CP, ADF (Table 3), WSC contents (Figure 4), and RFV (Figure 5) of the alfalfa silage, but no interactive effect was observed for the NDF (Table 3). Additives exhibited a significant effect on the DM content at 1, 3, 7 15, and 30 d after ensiling (Table 4, $p \leq 0.01$). CON exhibited a lower DM content than the other treatments throughout the ensiling duration. At 7 and 15 d after ensiling, FRU exhibited lower CP content than the other treatments (Table 4, $p \leq 0.01$). There were no differences among all treatments of CP content on the last ensiling day. FRU treatment exhibited lower NDF and ADF contents than the other treatments on 3 d after ensiling (Table 4, $p \leq 0.01$). Additives exhibited significant effect on the WSC content of alfalfa silages on 1, 3, 7, and 30 d after ensiling (Table 4, $p \leq 0.01$). The WSC content of all treatments decreased gradually as the number of ensiling days increased, except for those of MOL and FRU, which increased at 3 d after ensiling. The WSC content of all treatments stabilized from 15 d until the end of the ensiling period. Only at 3 d, RFV exhibited differences among treatments (Table 4, $p \leq 0.01$). CON exhibited a lower RFV compared with the other treatments throughout the ensiling process.

Table 3. The chemical composition of alfalfa before and after ensiling.

Items [1]	Additives	Ensiling Days						SEM	*p*-Value		
		0	1	3	7	15	30		A	D	A × D [2]
	CON	318.5 ^{a}	308.0 ^{bB}	304.5 ^{cC}	303.8 ^{cB}	301.9 ^{cB}	303.5 ^{cB}				
	PEC	318.5 ^{ab}	319.8 ^{aA}	314.2 ^{bcB}	315.5 ^{abcA}	313.2 ^{cA}	314.5 ^{bcA}				
DM(g/kg)	STA	318.5 ^{a}	318.3 ^{aA}	303.9 ^{bB}	311.2 ^{aA}	312.3 ^{bA}	314.7 ^{abA}	0.849	<0.001	<0.001	<0.001
	MOL	318.5 ^{ab}	320.5 ^{aA}	315.5 ^{bB}	316.0 ^{abA}	314.1 ^{bA}	314.0 ^{bA}				
	FRU	318.5 ^{bc}	320.6 ^{bA}	338.9 ^{aA}	316.3 ^{cA}	315.0 ^{cA}	317.0 ^{bcA}				
	CON	194.4 ^{b}	193.6 ^{b}	201.1 ^{a}	205.2 ^{aA}	201.2 ^{aA}	196.3 ^{b}				
	PEC	194.4 ^{ab}	191.0 ^{b}	198.1 ^{a}	198.0 ^{aAB}	194.1 ^{abBC}	196.4 ^{a}				
CP(g/kg DM)	STA	194.4 ^{b}	190.5 ^{b}	200.6 ^{a}	195.1 ^{bB}	192.5 ^{bC}	194.4 ^{b}	0.648	<0.001	<0.001	0.010
	MOL	194.4	190.7	198.9	199.6 ^{AB}	196.6 ^{B}	198.3				
	FRU	194.4	182.6	194.9	183.6 ^{C}	191.8 ^{C}	195.1				
	CON	377.8 ^{ab}	386.4 ^{a}	373.6 ^{abA}	354.6 ^{b}	381.8 ^{ab}	389.3 ^{a}				
	PEC	377.8	364.6	348.6 ^{B}	347.1	377.8	370.7				
NDF(g/kg DM)	STA	377.8 ^{a}	368.5 ^{ab}	349.0 ^{bB}	346.4 ^{b}	368.0 ^{ab}	391.3 ^{a}	2.283	0.002	<0.001	0.218
	MOL	377.8 ^{a}	355.0 ^{bc}	355.8 ^{bcB}	346.0 ^{c}	370.8 ^{ab}	364.8 ^{abc}				
	FRU	377.8 ^{a}	355.1 ^{ab}	319.7 ^{cC}	347.7 ^{b}	375.0 ^{a}	373.9 ^{a}				
	CON	285.2	290.3	288.3 ^{A}	277.7	282.2	292.1				
	PEC	285.2	274.1	266.1 ^{B}	269.8	280.8	278.5				
ADF(g/kg DM)	STA	285.2	278.9	273.9 ^{B}	263.5	276.3	288.3	1.614	<0.001	<0.001	0.002
	MOL	285.2	270.3	277.5 ^{AB}	270.2	277.3	277.9				
	FRU	285.2 ^{a}	272.2 ^{a}	229.8 ^{bC}	267.5 ^{a}	274.0 ^{a}	284.5 ^{a}				

Note: [1] DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber. SEM, standard error of means. CON, PEC, STA, MOL and FRU, respectively, represent control, pectin, starch, molasses and fructose. $^{A–C}$ Means within a column with no common superscript differ ($p < 0.05$). $^{a–c}$ Means within a row with no common superscript differ ($p < 0.05$). [2] A, D, and A × D denote the significance of additives, ensiling days and their interactive effects, respectively.

Table 4. Statistical analysis for the chemical composition variables of alfalfa silage treated with different carbohydrate sources at different ensiling period.

Items [1]	Ensiling Day				
	1	3	7	15	30
DM	<0.01	<0.01	<0.01	<0.01	<0.01
CP	0.08	0.33	0.01	<0.01	0.18
NDF	0.23	<0.01	0.94	0.56	0.37
ADF	0.29	<0.01	0.69	0.69	0.43
WSC	<0.01	<0.01	<0.01	0.07	<0.01
RFV	0.26	<0.01	0.90	0.69	0.37

Note: [1] DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water-soluble carbohydrates; RFV, relative feed value.

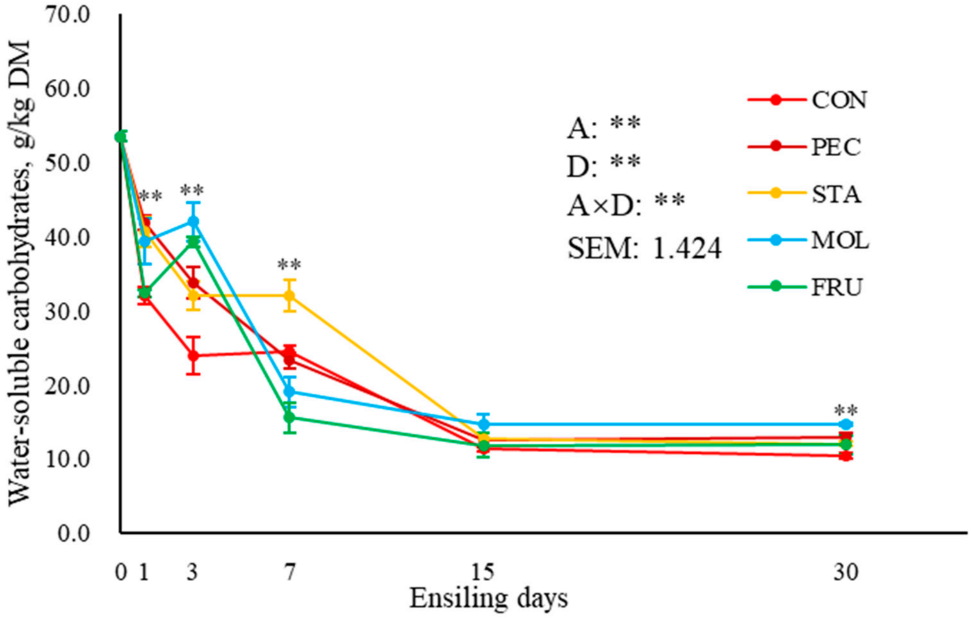

Figure 4. Effect of additives on the water-soluble carbohydrates (WSC) content during ensiling of alfalfa. DM, dry matter; CON, control; PEC, pectin; STA, starch; MOL, molasses; FRU, fructose. A denotes significance of additives; D, denotes significance of ensiling days; A × D denotes the interactive effect between additives and ensiling days. SEM, standard error of means. The asterisks (** $p \leq 0.01$) indicate the significance of additives and ensiling days and their interaction effects, and the difference at the specified ensiling day.

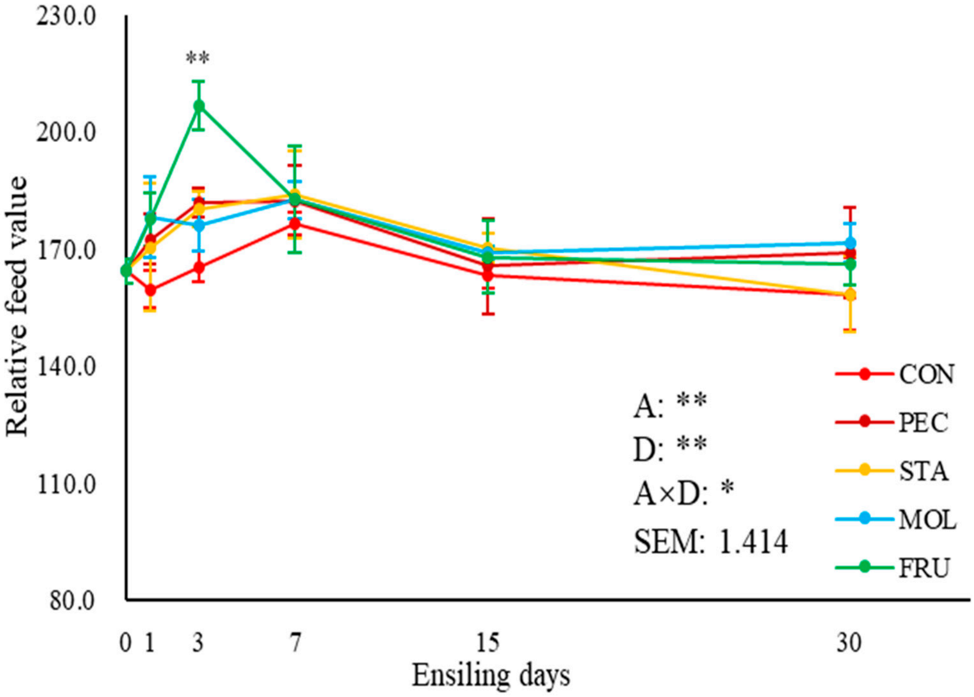

Figure 5. Effect of additives on the relative feed value (RFV) during ensiling of alfalfa. CON, control; PEC, pectin; STA, starch; MOL, molasses; FRU, fructose. A denotes significance of additives; D, denotes significance of ensiling days; A × D denotes the interactive effect between additives and ensiling days. SEM, standard error of means. The asterisks (* $p \leq 0.05$; ** $p \leq 0.01$) indicate the significance of additives and ensiling days and their interaction effects, and the difference at the specified ensiling day.

3.4. Analysis of Correlation between WSC and the Fermentation Profiles and Chemical Compositions

To determine the relationship between WSC content and fermentation profile and chemical compositions, Spearman correlation analysis was performed. Throughout the ensiling process, pH was positively correlated with the WSC content and negatively correlated with the PA and AN contents and Fleig's point (Figure 6). In other words, the pH decreased as the WSC content decreased. However, at 30 d of ensiling, the WSC content was positively correlated with Fleig's point and negatively correlated with the pH (Figure 7). The DM content and RFV tended to positively correlate with the WSC content, and the NDF and ADF contents tended to negatively correlate with the WSC content.

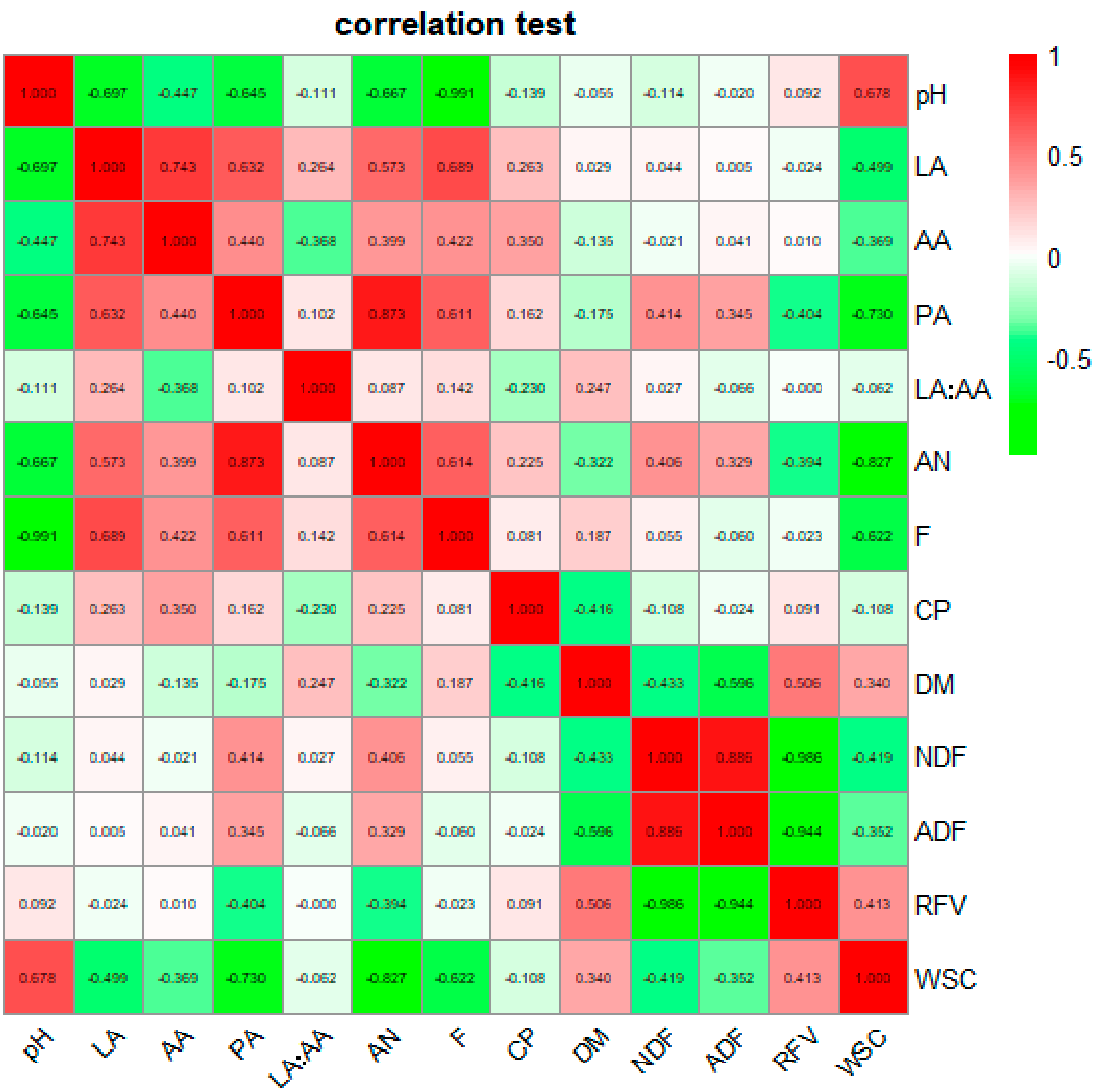

Figure 6. Pearson correlation between the fermentation and chemical indexes of alfalfa silages throughout the ensiling period. LA, lactic acid; AA, acetic acid; PA, propionic acid; LA:AA, lactic acid: acetic acid ratio; AN, ammonia nitrogen; F, Fleig's point; CP, crude protein; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; RFV, relative feed value; WSC, water-soluble carbohydrates. The number in each square represents the correlation extent; the color represents significant correlation ($p < 0.05$), the deeper the color of the square is, the more significant the correlation. The red color means a positive correlation, and the green color means a negative correlation.

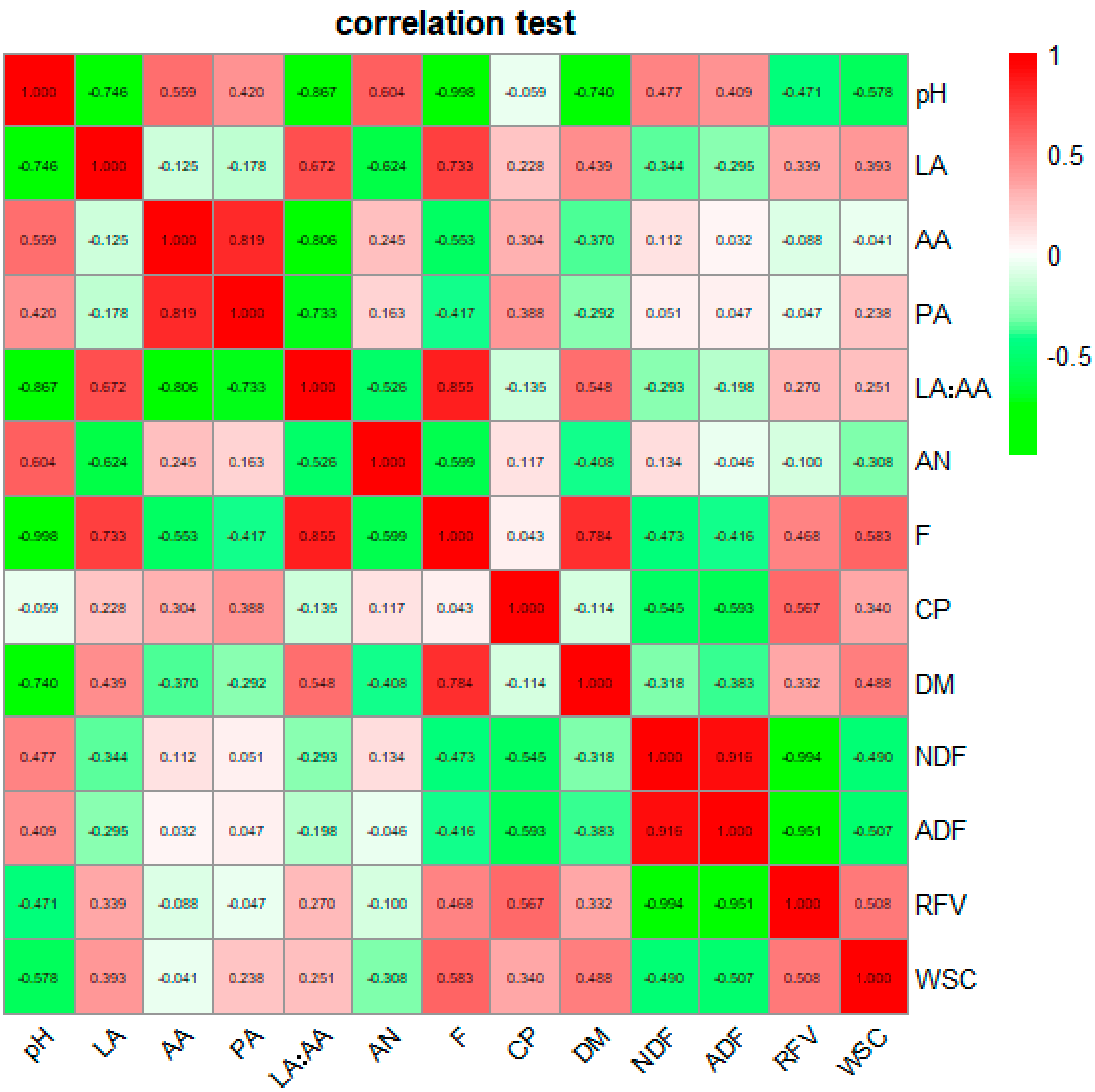

Figure 7. Pearson correlation between the fermentation and chemical indexes of alfalfa silages at 30 d of ensiling. LA, lactic acid; AA, acetic acid; PA, propionic acid; LA:AA, lactic acid: acetic acid ratio; AN, ammonia nitrogen; F, Fleig's point; CP, crude protein; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; RFV, relative feed value; WSC, water-soluble carbohydrates. The number in each square represents the correlation extent; the color represents significant correlation ($p < 0.05$), the deeper the color of the square is, the more significant the correlation. The red color means a positive correlation, and the green color means a negative correlation.

4. Discussion

Various additives have been applied to alfalfa to improve its fermentation quality [6–15]. In a recent study, the main additive *L. plantarum* was applied to alfalfa because few LAB are attached to alfalfa surfaces to support fermentation [2]. However, alfalfa also lacks of a WSC substrate that is sufficient to sustain the fermentation for LAB during the ensiling [26]. The main ingredient of MOL is sucrose, which is widely applied to alfalfa silage to improve fermentation quality [27,28]. In our research, MOL was not found to be the optimal additive to improve alfalfa silage fermentation. Compared with CON and STA, MOL exhibited a lower pH, and higher LA and Fleig's points during the ensiling period. The result was similar to that of Yuan et al. [29], who reported that alfalfa treated with MOL resulted in a lower pH than that of the control. It was also reported that adding MOL lowered the quality of both wilted and fresh lucerne silage [30]. However, MOL exhibited a higher pH than FRU and PEC and a lower LA content and Fleig's point during the ensiling period.

Li et al. [31] reported that the application of sucrose decreased the pH and AA content and increased the LA and ethanol contents of alfalfa silages, which resulted in high-quality alfalfa silage. It was revealed that lucerne treated with fresh or frozen MOL-based pre-fermented juice had an increased fermentation quality and organic matter digestibility [28]. Li et al. [31] also reported that the application of sucrose or organic acids improved the quality of alfalfa silage.

The FRU and PEC treatments exhibited lower pH than the other treatments from 3 d to the end of the ensiling period in the present study. This result was consistent with that of Wang et al. [32], who reported that the fermentation quality was improved of alfalfa silage upon the application of PEC and FRU treatments. One possible reason was that FRU and PEC increased the carbohydrate for LAB fermentation, and the lower pH condition inhibited the growth of *Clostridium* and *Escherichia coli*, which are not desirable for the fermentation process. It was reported that PEC degradation is an important factor for alfalfa fermentation because of the resulting diverse bacterial community [33], and the application of PEC and pectinase lowered the pH, AA, PA and AN contents and increased the LA content. *Leuconostoc* abundance was higher upon PEC treatment and the application of pectinase resulted in higher *Bacillus, Aeromonas,* and *Curvibacter* abundances. These results show that PEC and pectinase improved the fermentation quality of alfalfa silage. *L. plantarum* is typically applied to alfalfa to improve its fermentation quality. Zhao et al. [6] reported that *L. plantarum* ZZU203, which was selected because of its advanced structural carbohydrate metabolites, resulted in higher DM, LA, AA, and WSC concentrations, lower AN and NDF concentrations, and aerobic bacterial and *Clostridium* counts in silage. However, FRU and PEC treatments exhibited advanced effects on fermentation quality in the present study. FRU and PEC exist in fruit juice and in citrus and apple peels, respectively; therefore, it is possible to apply fruit juice and citrus or apple peels to alfalfa to improve its fermentation quality. These additives not only can improve fermentation quality but also may be one of high-quality forages to feed ruminants [16,33].

5. Conclusions

The addition of FRU and PEC effectively improved the fermentation quality of alfalfa silage, reduced the pH and PA and AN contents, as well as increased the contents of LA, LA:AA ratio, and Fleig's point at 60 d ensiling day. Thus, FRU and PEC or their related agricultural byproducts may offer alternative additives to improve the fermentation profile of alfalfa silage.

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