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RESEARCH ARTICLE

The Effect of Fresh and Frozen Pre-Fermented Juice on the Fermentation Quality of Alfalfa Silage

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Summary

This study was carried out to examine the possible effect of fresh and frozen pre-fermented juice (PFJ) on the fermentation quality, nutritive value and *in vitro* gas production parameters of alfalfa (*Medicago sativa L.*) silage. Barley (B), wheat (W) and grass herbage (G) were used to prepare the PFJs. Both fresh (PFJ-B, PFJ-W and PFJ-G) and frozen (PFJ-B_F, PFJ-W_F and PFJ-G_F) PFJs were investigated. Frozen PFJs were prepared by freezing fresh PFJs at -22°C with 20% glycerol (v/v). Treatments of alfalfa silage consist of (1) control (untreated alfalfa silage); (2) silage treated with PFJ-B; (3) silage treated with PFJ-W; (4) silage treated with PFJ-G; (5) silage treated with PFJ-B_F; (6) silage treated with PFJ-W_F and (7) silage treated with PFJ-G_F. Each treatment had five replicate glass jars and replicates were ensiled in 1.0 L jars. Silages treated with fresh and frozen PFJs, regardless of plant material, had better fermentation quality than the control silage in terms of lower pH, butyric acid (BA) and ammonia nitrogen (NH_3-N) concentrations. Additionally, treated silages with fresh PFJs had higher lactic acid (LA) concentration ($P<0.05$) and *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME) content and gas production values ($P<0.05$) than control silage. According to results of this study, PFJ treatments increased the nutritive value, fermentation quality, IVOMD, ME content and gas production values of alfalfa silage.

Keywords: *Previously fermented juice, Alfalfa silage, Fermentation quality*

Taze ve Dondurulmuş Fermente Edilmiş Sıvının (PFJ) Yonca Silajının Fermentasyon Kalitesi Üzerine Etkisi

Özet

Bu çalışma, fermente edilmiş laktik asit sıvısının (PFJ) yonca silajının fermentasyon kalitesi, besinsel kompozisyonu ve *in vitro* gaz üretim değerleri üzerine etkisini araştırmak amacıyla yapılmıştır. PFJ'ler arpa (B), buğday (W) ve çayır otu (G) kullanılarak hazırlanmış ve hem taze olarak (PFJ-B, PFJ-W ve PFJ-G) hemde dondurulmuş olarak (PFJ-B_F, PFJ-W_F, ve PFJ-G_F) silaj materyaline ilave edilerek inkube edilmişlerdir. Muameleler (1) kontrol (katkısız yonca silajı); (2) PFJ-B katkılı; (3) PFJ-W katkılı; (4) PFJ-G katkılı; (5) PFJ-B_F katkılı; (6) PFJ-W_F katkılı ve (7) PFJ-G_F katkılı gruplardan oluşmuştur. Silajlar kontrol ve her bir muamele grubu için 5 tekerrür olacak şekilde 1 litrelik cam kavanozlarda hazırlanmışlardır. Dondurulmuş PFJ'ler -22°C'de %20 gliserol (v/v) ilave edilerek hazırlanmıştır. Genel olarak tüm taze ve dondurulmuş PFJ katkıları ile hazırlanan silajların fermantasyon kaliteleri kontrol silajından daha iyi bulunmuştur. PFJ katkıları ile hazırlanan silajların pH, bütirik asit (BA) ve silaj amonyak azotu (NH_3N) konsantrasyonları kontrol silajından düşük ($P<0.05$); laktik asit (LA) konsantrasyonu, *in vitro* organik madde sindirimi (İVOMS), metabolik enerji (ME) içeriği ve gaz üretim miktarları ise kontrol silajından yüksek ($P<0.05$) bulunmuştur. Sonuç olarak, PFJ ilavesinin yonca silajının fermentasyon kalitesi, İVOMS, ME ve gaz üretim değerlerini arttırdığı görülmüştür.

Anahtar sözcükler: *Laktik asit sıvısı, Yonca silajı, Fermantasyon kalitesi*

INTRODUCTION

Legumes are difficult to ensile successfully without an additive due to their low sugar content and high buffering capacity [1]. This is especially true for alfalfa (*Medicago sativa L.*). Various additives, such as bacterial inoculants, acidifiers,

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formaldehyde and tannic acid have been tested to improve the ensiling properties of alfalfa silage, but reports of their efficacy are inconsistent [2]. Previous studies suggested that the inoculation with lactic acid bacteria (LAB) at ensiling could improve the fermentation of legume silage by accelerating the production of lactic acid (LA) to cause occurrence of acidic conditions rapidly [3]. Beck [4] summarized several studies and indicated that the use of *Lactobacillus plantarum* cultures and sugar as an additive considerably improved silage quality and decreased silage spoilage loss. The possible beneficial effect of LAB inoculants depends on its composition, concentration and the properties of the crop being ensiled. Recently, it has been demonstrated that silage lactic acid concentration and fermentation quality have improved in all cases in which pre-fermented juices (PFJs) were added to the ensilage of lucerne [5,6]. Ohshima *et al.*[7-9] reported that the PFJ could often increase lactic acid concentration and decrease NH_3-N concentration in alfalfa silage even when the addition of commercial LAB was ineffective. Pre-fermented juice was prepared by culturing microorganism adherent to the alfalfa materials before ensiling silage material and the grown microorganisms were used as a starter of silage fermentation. Ohshima *et al.*[8] suggested that the PFJ preparation material made from the same silage crop might be more efficient for improving the quality of silage than those made from other crops. In contrast, Denek *et al.*[5] reported that silage treated with fresh and frozen molasses-based PFJs, regardless of plant material, had better fermentation quality than control silage in terms of lower pH, butyric acid and ammonia nitrogen concentrations, as well as higher lactic acid concentration and *in vitro* organic matter digestibility. PFJ materials other than alfalfa herbage might not be always available for preparing PFJ when the ensiling alfalfa. In these cases, Denek *et al.*[5] suggested that previously prepared and frozen molasses based PFJ could be used as an inoculum source. Therefore, this study was designed to evaluate the effect of fresh and frozen PFJs prepared using different herbages on the fermentation quality, *in vitro* organic matter digestibility and gas production values of alfalfa silage.

MATERIAL and METHODS

Preparation of PFJs

The PFJs were prepared using barley (B; *Hordeum vulgare*), wheat (W; *Triticum drum*) and grass herbage (G; *Bromus inermis*) harvested at the flag emerges; at least 3 nodes were visible above the soil surface from similar fields for PFJ herbage sources. These plant materials were harvested at 26 and 31 March 2010. PFJ was prepared according to the method described by Masuko *et al.*[10]. For this purpose, 200 g plant material was macerated with 1000 ml distilled water and 2 min in a high-speed blender. The macerate was filtered through two layers of cheesecloth and aliquots of filtrate were collected in glass bottles to which sucrose was added at 3 g per 100 ml (w/v) filtrate. These bottles were fitted with a gas trap and kept in an incubator for 48 h at 30°C. After precipitation, the supernatant brown liquor (i.e., PFJ) was collected on considered as pre-fermented juice (PFJ). PFJ-B (barley based), PFJ-W (wheat based) and PFJ-G (grass based) were used as the fresh inoculant source; PFJ-$B_{F:(frozen)}$, PFJ-W_F and PFJ-G_F were also used as the frozen inoculant source. Frozen PFJs were prepared by freezing PFJs at -22°C with 20% glycerol (v/v) one week (2 days incubation and 5 days frozen), while fresh PFJs prepared 2 days before ensiling. The number of LAB (colony-forming units, CFU/ml) in the PFJs was counted by using GYP-$CaCO_3$ agar plates which were incubated for 5 days at 35°C [11].

Silage Preparation and Treatments

Alfalfa (*Medicago sativa L.*), at 280 g/kg dry matter (DM) content of fresh material, was used as silage material and harvested at the early bloom stage with standard field equipment (mower-conditioner, forage harvester) from private farm near the Harran University at 2 April 2010. The chopped alfalfa (1-3 cm) was weighed, sprayed with the appropriate PFJ solution (or distilled water for the control) with a plant sprayer (one sprayer for each treatment), mixed by hand and then placed into the 1.0 L anaerobic glass bottle laboratory silos by hand compressing to a final density of 600 g/L. Disposable gloves were used for harvesting and handling then changing gloves after each treatment to prevent cross-contamination. The PFJs and water were applied at a rate of 5 ml/kg (v/w) fresh material. Experimental silage treatments consisted of five replicates for each treatment including (1) control; (2) silage treated with PFJ-B; (3) silage treated with PFJ-W; (4) silage treated with PFJ-G; (5) silage treated with PFJ-B_F; (6) silage treated with PFJ-W_F and (7) silage treated with PFJ-G_F. The silos were stored for 45 days at room temperature (~22°C).

Analytical Procedures

Glass bottle laboratory silos were opened after 45 days of ensiling. The pH values and dry matter contents of the silages were immediately measured. Dry matter contents of the silages were determined by drying 20 g of the ensiled forage at 105°C for 24 h in a forced-air oven and then weighing it. A total of 25 g of fresh silage was macerated with 100 ml distilled water with a high-speed blender. The macerated silage samples were filtered through two layers of cheesecloth and the pH values of the filtrate were measured with a laboratory pH meter (Orion, Thermo Electron Corp., Kent, WA, USA). After pH determined, 10 ml filtrate was acidified with 0.1 ml 1 M HCl (v/v) and stored at -22°C for NH_3-N analysis. The NH_3-N content was analyzed according to Broderick and Kang [12] by the Kjeldahl method. Volatile fatty acids (VFA) were determined by gas chromatography (Hewlett Packard-6890 equipment, Palo Alto, CA, USA) [13]. Lactic acid was determined by high-performance liquid chromatography (HPLC) [14]. Dry matter contents of the silages were determined by drying 20 g of the ensiled forage at

60°C for 48-72 h in a forced-air oven and then weighing them. After weighing, the dried sample was ground through 1- mm screen in a Wiley mill and analyzed for crude protein (CP) content by the AOAC [15] method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content was analyzed according to methods described by Van Soest *et al.*[16]. The gas production values of the silage were determined through the method described by Menke *et al.*[17]. For this purpose, rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Animals were treated in accordance with the standards of the Harran University Animal Experimentation Local Ethics Committee (HRU-AELEC-06/2009) for the protection of animals used for experimental and other scientific purposes. Silage samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of Menke *et al.*[17]. Gas production was measured as the volume of gas in the calibrated syringes and was recorded before incubation (0) and 4, 8, 12, 24, 48, 72 and 96 h after incubation. Total gas values were corrected with a blank incubation that contained only rumen fluid. Cumulative gas production data were fitted to the model ($y = a + b(1 - \exp^{-ct})$) of Ørskov and McDonald [18] where *y* is the gas production (ml/g) at time *t*; *a* is the gas production from the immediately soluble fraction; *b* is the gas production from the insoluble fraction and *c* is the gas production rate constant for fraction *b*. The parameters *a*, *b* and *c* for each silage were calculated from the five incubations described earlier using a non-linear regression procedure.

The IVOMD (g/kg OM) and ME (MJ/kg DM) of silages were calculated using equations reported by Menke *et al.*[19].

Statistical Analysis

The statistical analysis of results included one-way analysis of variance and Tukey's multiple range test, which were applied to the results using Statistical Analysis System [20], and significance was declared at $P<0.05$ [21].

RESULTS

The pH and LAB counts of the PFJs are shown in *Table 1*. The pH values of the PFJs ranged from 3.67-3.97 and the LAB counts ranged from $8.0x10^7$- $1.9x10^9$ CFU/ml. The lowest pH (3.67) value and the highest LAB count ($1.9x10^9$ CFU/ml) were obtained from PFJ-G.

Nutritive Value of Silages

The effects of PFJs treatments on chemical composition, IVOMD and ME values of alfalfa silages are presented in *Table 2*. There were no significant differences in CP content among the control and other silages treated with PFJs. The control silage had a higher NDF (400 g/kg DM) and ADF (344 g/kg DM) contents compared to the silages treated with PFJs ($P<0.05$) only exception of PFJ-G. The control silage had lower IVOMD and ME values compared to the silages treated with PFJ-(B;W;G) ($P<0.05$) but not with frozen PFJs ($P>0.05$). The IVOMD of silages ranged from 581 to 620 g/kg OM and the

Table 1. *The average value of pH and LAB values of PFJs*
Tablo 1. *PFJ'lerin pH ve LAB değerleri*

PFJs	Origin	pH	LAB (cfu/ml)
PFJ-B	Fresh PFJ, prepared with Barley herbage	3.69	$1.4x10^9$
PFJ-W	Fresh PFJ, prepared with Wheat herbage	3.71	$1.5x10^9$
PFJ-G	Fresh PFJ, prepared with Grass herbage	3.67	$1.9x10^9$
PFJ-B_F	Frozen PFJ, prepared with Barley herbage	3,97	$8.0x10^7$
PFJ-W_F	Frozen PFJ, prepared with Wheat herbage	3.85	$2.9x10^8$
PFJ-G_F	Frozen PFJ, prepared with Grass herbage	3.89	$3.0x10^8$

Table 2. *Effect of pre-fermented juice (PFJ) treatments on chemical composition, IVOMD and ME values of alfalfa silages*
Tablo 2. *PFJ muamelesinin yonca silajlarında kimyasal kompozisyon, IVOMD ve ME değerlerine etkisi*

Fresh Alfalfa Herbage	DM	CP	NDF	ADF	IVOMD	ME
	280	212	453	353	608	10.2
Treatments						
Control	252^{d}	231^{ab}	400^{a}	344^{a}	581^{c}	10.0^{b}
PFJ-B	269^{a}	236^{a}	348^{c}	319^{b}	620^{a}	10.5^{a}
PFJ-W	266^{ab}	236^{a}	382^{b}	320^{b}	617^{a}	10.4^{a}
PFJ-G	258^{bcd}	234^{ab}	383^{b}	335^{a}	610^{ab}	10.4^{a}
PFJ-B_F	257^{cd}	229^{ab}	349^{c}	313^{b}	603^{abc}	10.4^{a}
PFJ-W_F	269^{a}	227^{b}	350^{c}	296^{c}	593^{bc}	10.2^{ab}
PFJ-G_F	266^{ab}	233^{ab}	352^{c}	309^{bc}	607^{ab}	10.5^{a}
SEM	0.13	0.89	0.35	0.28	0.28	0.04
P-Value	***	*	***	***	***	**

*$^{a,b,c.}$ For each column, mean values with different letters are significant at $P<0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$, **SEM:** Standard error of the mean; **DM:** dry matter, (g/kg DM); **CP:** crude protein, (g/kg DM); **NDF:** Neutral detergent fibre, (g/kg DM), **ADF:** acid detergent fibre, (g/kg DM), **IVOMD:** in vitro organic matter digestibility, (g/kg OM); **ME:** metabolizable energy, (MJ/kg DM)*

highest IVOMD was obtained from the silages treated with PFJ-B. The ME values of silages ranged from 10.0 to 10.5 MJ/kg DM and ME values of the silage treated with PFJs were higher than control silage (P<0.05) with exception of PFJ-W_F (P>0.05).

Fermentation Quality of Silages

The effects of the PFJ treatments on fermentation characteristics of alfalfa silages are presented in *Table 3*. The PFJ treatments significantly decreased the pH values of the silages compared to the control (P<0.05). The pH values of silages ranged from 4.37 to 5.87 and highest pH value (5.87) obtained from control silage. In addition, there was an increase in LA levels and a decrease in NH_3-N levels of the silages treated with PFJs. Propionic acid (PA) was not detected in any of the silage treatments; on the other hand, butyric acid (BA) was only detected in control silages. Comparison of fresh and frozen PFJs was in consisted overall fermentation quality parameters.

Gas Production Parameters

The gas production values and estimated parameters are presented in the *Table 4*. Gas produced after 96 h incubation ranged from 214 to 234 ml/g DM. Fresh PFJs gave in considerably higher gas production values than control and frozen PFJs treatments at 96 h incubation (P<0.05). Gas production from the immediately soluble fraction *a* varied from - 4.1 to 1.2 ml. In addition, mean values of *a* fraction were negative for all silages, except the PFJ-B_F and PFJ-G_F treated silages. Gas volumes from the insoluble, but fermentable, *b* fraction ranged from 206 to 228 ml/g DM and the highest *b* fraction value (228 ml/g DM) determined from PFJ–B treated alfalfa silage. The rate of gas production *c* varied from 0.09 to 0.14 h and the highest gas production rates were obtained from frozen PFJ added alfalfa silages. The rate parameter values for time taken to produce 50 or 95% of the total gas production (T50 and T95, respectively) in PFJ treated silages were reduced compared to the control silage (P<0.05).

Table 3. *Effect of pre-fermented juice (PFJ) treatments on fermentation characteristics of alfalfa silages*
Tablo 3. *PFJ ilavesinin yonca silajlarında fermentasyon kalitesi üzerine etkileri*

Treatments	pH	NH_3-N	LA	AA	PA	BA
		(g/kg TN)	(g/kg DM)			
Control	5.87^{a}	52.6^{a}	33.0^{d}	12.4^{d}	ND	18.8
PFJ-B	4.45^{d}	25.8^{cd}	71.5^{a}	17.1^{c}	ND	ND
PFJ-W	4.37^{e}	25.9^{cd}	75.1^{a}	22.7^{b}	ND	ND
PFJ-G	4.52^{c}	26.4^{cd}	56.0^{c}	33.7^{a}	ND	ND
PFJ-B_F	4.63^{b}	39.9^{b}	53.2^{c}	13.0^{d}	ND	ND
PFJ-W_F	4.48^{cd}	28.2^{c}	66.0^{b}	15.2^{cd}	ND	ND
PFJ-G_F	4.48^{cd}	28.0^{c}	65.0^{b}	17.4^{c}	ND	ND
SEM	0.08	1.74	2.78	1.21	ND	ND
P-Value	***	***	***	***	NS	NS

*^{a,b,c,d} For each column, mean values with different letters are significant at P<0.05; **NS:** P>0.05; *** P<0.001, **ND:** not detected, **SEM:** Standard error of the mean, **NH_3-N:** Silage ammonia nitrogen content, **LA:** Silage lactic acid content, **AA:** Silage acetic acid content, **PA:** Silage propionic acid content, **BA:** Silage butyric acid content*

Table 4. *In vitro gas production (ml/g DM) values and gas production parameters*
Tablo 4. *İn vitro gaz üretim değerleri (ml/g KM) ile gaz üretim parametreleri*

Treatments	Time (h)							Estimated Parameters				
	4	8	12	24	48	72	96	*a*	*b*	*c*	T_{50}	T_{95}
Control	48^{e}	112^{b}	147^{c}	180^{d}	202^{cd}	215^{ab}	216^{b}	-4.1^{c}	216^{ab}	0.09^{c}	7.79^{a}	33.65^{a}
PFJ-B	76^{cd}	140^{a}	169^{ab}	203^{a}	223^{a}	228^{a}	234^{a}	-0.8^{b}	228^{a}	0.11^{b}	6.25^{b}	27.01^{b}
PFJ-W	78^{bc}	140^{a}	172^{a}	200^{a}	222^{a}	228^{a}	234^{a}	-0.4^{b}	227^{a}	0.11^{b}	6.13^{b}	26.49^{b}
PFJ-G	68^{d}	133^{a}	163^{ab}	195^{ab}	218^{ab}	223^{a}	231^{a}	-1.2^{b}	225^{a}	0.10^{bc}	6.67^{b}	28.83^{b}
PFJ-B_F	84^{ab}	138^{a}	159^{b}	185^{bc}	191^{c}	211^{ab}	214^{b}	0.3^{ab}	208^{b}	0.14^{a}	4.89^{c}	21.12^{c}
PFJ-W_F	88^{a}	144^{a}	161^{ab}	191^{abc}	204^{bc}	213^{ab}	215^{b}	-1.3^{b}	206^{b}	0.13^{a}	5.15^{c}	22.28^{c}
PFJ-G_F	89^{a}	141^{a}	168^{ab}	193^{abc}	207^{abc}	215^{ab}	217^{b}	1.2^{a}	210^{b}	0.13^{a}	5.23^{c}	22.64^{c}
SEM	2.34	1.97	1.58	1.65	2.29	2.08	1.85	0.31	1.78	0.03	0.17	0.74
P-Value	***	***	***	***	***	***	***	***	***	***	***	***

*$^{a,b,c,d..}$ For each column, mean values with different letters are significant at P<0.05, *** P<0.001; **SEM:** Standard error of the mean, **a:** gas production from the immediately soluble fraction, ml; **b:** gas production from the insoluble fraction, ml; **c:** gas production rate constant for fraction (b), %; **T_{50}:** time taken to produce 50% of the total gas production, h; **T_{95}:** time taken to produce 95% of the total gas production, h*

DISCUSSION

The control silage had lower IVOMD compared with the silages treated with PFJs (P<0.05). This result was supported by some researchers [5,6,22] who reported that PFJ treatments increased the IVOMD of silages. Cao *et al.*[23] reported that the addition of PFJ to alfalfa improved the apparent digestibility of dry and organic matter in cows that were fed alfalfa silage. Many factors such as the type and properties of the plants to be ensiled, climatic conditions, epiphytic microflora, ensiling technique and the properties of the inoculant affect the success of silage inoculants [24]. Weinberg *et al.*[25] indicated that usage of LAB improved fermentation patterns in the legume silages helped to reduce DM losses and repressed undesirable microorganisms. In the current study, all PFJ treatments showed significantly positive effects on the fermentation quality alfalfa silage.

Silage pH is one of the main factors that influence the extent of fermentation and silage quality of ensiled forage. The contribution of PFJ is to increase the concentration of LA to provide an acid condition as rapidly as possible [8]. There was an abundance of microorganisms in the PFJ before it was sprayed on to the alfalfa herbage. The mode of action of PFJ was likely similar to the lactic acid bacteria additives by providing large amounts of lactic acid bacteria to accelerate production of lactic acid and the resultant decline of pH [6]. However, PFJ benefits occurred in this study such that silage treated with PFJ had lower pH and NH_3-N content and higher lactic acid concentrations. This result can be explained due to PFJs probably contained a number of species lactic acid bacteria or inhibiting butyric acid fermentation [8,10]. The growth of proteolytic microbes is depressed by low pH [1]. Carpintero *et al.*[26] concluded that NH_3-N content of less than 45 g/kg or 11% of TN has been identified as characteristic of well-preserved silage. In the current study, the NH_3-N content of the silages treated with PFJs (23.0-39.9 g/kg TN) was lower than that of the control (52.6 g/kg TN). This indicated that the higher concentration of LA and lower pH values in the silages treated with PFJs than in the control can be explained due to inhibiting the activity of proteolysis by plant enzymes and clostridial bacterial activity during the early stage of ensiling [22]. Butyric acid was only detected in the control silage, which indicates that some clostridial bacterial activity had occurred in control silage [25]. This can be result of the low WSC amount of the initial alfalfa, which resulted in insufficient production of LA and pH decline; thus, it increases the activity of clostridial bacteria during ensiling. The beneficial effect of PFJs observed in the current study and our previous study [5] is because the PFJs probably caused intensive LAB fermentation. This could be linked to the LAB content of PFJs. In the current study, acetic acid values of PFJ-B, PFJ-W, PFJ-G and PFJ-G_F were higher than the control, PFJ-B_F and PFJ-W_F silages. Acetic acid found in the various silages serves as an antimycotic agent [27], thus presence of acetic acid might be advantageous for improving silage quality in this study.

Gas produced after 96 h of incubation was in agreement with the findings of Muck *et al.*[28], who reported that 96 h gas production values for alfalfa silages were 187-208 ml/g DM. Denek *et al.*[5] reported that frozen and fresh molasses-based PFJs produced more gas production at 96 h incubations than control alfalfa silages. On the contrary, frozen sucrose-based PFJ produced similar 96 h gas production with the control. Additionally, there were less variations among the control and the silages treated with PFJs in terms of gas production at all incubation times comparison with our previous study [5]. The result of gas parameters of control alfalfa silage obtained in the present experiment are supported by Kamalak *et al.*[29], who reported that the c value of alfalfa silage was 0.113 ml. In addition, mean values for the intercept *a* fraction were negative for all of the silages, except the silages treated with PFJ–B_F and PFJ-G_F. Negative intercepts are not consistent with the concept of *a* reflecting fermentation of the soluble fraction of the feed but are consistent with findings Blümmel and Becker [30] and Rodriguez *et al.*[31], who suggested that a lag phase could occur after the soluble part of the substrate had been consumed but before the fermentation of the cell walls had started. For the rate parameter values, the time taken to produce 50 or 95% of the total gas production (T_{50} and T_{95}, respectively) was improved compared to the control silage (P<0.05). In the application of frozen PFJ for preparing silages has been studied only in our previous study [5]. Present and our previous studies show that frozen PFJs have a potential inoculants source for alfalfa silage.

The results in this study showed that the ensiling of alfalfa by treating it with fresh or frozen PFJs increased silage fermentative quality and organic matter digestibility. It can be concluded that PFJs can be directly used as a good sources of LAB for alfalfa silage and it might be comparable to conventional silage additives and commercial LAB inoculants as a fermentative promoter. According to our current and previous studies [5], molasses or sucrose can be used source of energy during preparation PFJ without causing any major effect of alfalfa silage quality. More research is required to determine herbage type, herbage chemical composition and active population of desirable and undesirable microbes of herbage used for PFJ preparation to minimize inconsistent results in practice.

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