KSÜ Tarım ve Doğa Derg 24 (3): 671-678, 2021
KSU J. Agric Nat 24 (3): 671-678, 2021
https://doi.org/10.18016/ksutarimdoga.vi.777031

Effects of *Lactobacillus Buchneri* Inoculation and Fresh Whey Addition on Alfalfa Silage Quality and Fermentation Properties

Maghsoud BESHARATI[1], Valiollah PALANGI[2], Mojtaba NEKOO[3], Tugay AYAŞAN[4]
[1,3]University of Tabriz, Ahar Faculty of Agriculture and Natural Resources, Department of Animal Science, 51666, Tabriz, Iran, [2]Department of Animal Science, Agricultural Faculty, Ataturk University, 25240, Erzurum, Turkey, [4]Osmaniye Korkut Ata University, Kadirli Academy of Applied Sciences, Osmaniye, Turkey
[1]https://orcid.org/0000-0002-5233-6425, [2]https://orcid.org/0000-0001-6470-2608, [3]https://orcid.org/0000-0001-9290-2578
[4]https://orcid.org/0000-0001-7397-6483
✉: m_besharati@hotmail.com

ABSTRACT
This study was aimed to determine the effects of different levels of fresh whey and *Lactobacillus buchneri* inoculation on the nutrient properties and aerobic stability of alfalfa silage. Experiment was conducted with 8 treatments and 3 replicates for 90 days. Treatments included wilted alfalfa (control), wilted alfalfa + bacterial inoculant (AB), wilted alfalfa +30 g whey/kg (AW1), wilted alfalfa + 30 g whey + bacterial inoculant (AW1B), wilted alfalfa + 60 g whey per kg (AW2), wilted alfalfa + 60 g whey per kg + bacterial inoculant (AW2B), wilted alfalfa + 90 g whey per kg (AW3) and wilted alfalfa + 90 g whey + bacterial inoculant (AW3B). Results showed that, the pH values of treatments were found less than 4.86 but the control pH was higher in comparison with other treatments ($P<0.05$). The addition of fresh whey and complementary bacterial supplement to alfalfa caused a significant increase in total fatty acids. There was no significant difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF) in all treatments compared to control group. The least resistance to erosion was related to wilted alfalfa + bacterial additive (AB), and the highest amount was related to wilted alfalfa + 60 g whey (AW2: $P<0.05$). The results showed that fresh whey and bacterial inoculation with the fermentable carbohydrates leads to rapid reduction of pH, limiting the proliferation of yeasts, increased aerobic stability and improved silage quality.

Research Article

Article History
Received : 04.08.2020
Accepted : 16.09.2020

Keywords
Alfalfa silage
Fresh whey
Lactobacillus buchneri
Aerobic stability
Silage quality

Lactobacillus Buchneri Aşılaması ve Taze Peynir Altı Suyu İlavesinin Yonca Silajı Kalitesi ile Fermantasyon Özellikleri Üzerine Etkileri

ÖZET
Bu çalışmada, farklı seviyelerde taze peynir altı suyu ve *Lactobacillus buchneri* aşılamasının yonca silajının besin özellikleri ve aerobik stabilite üzerindeki etkilerinin belirlenmesi amaçlanmıştır. Deneme, 90 gün boyunca 8 muamele ve 3 tekerrür ile gerçekleştirilmiştir. Denemede şu gruplar oluşturulmuştur: Pörsütülmüş yonca (kontrol), Pörsütülmüş yonca + bakteri aşılaması (AB), Pörsütülmüş yonca +30 g taze peynir altı suyu / kg (SYP), Pörsütülmüş yonca + 30 g taze peynir altı suyu + bakteri aşılaması (SYP1B), Pörsütülmüş yonca + 60 g/kg taze peynir altı suyu (SYP2), Pörsütülmüş yonca + 60 g/kg peynir altı suyu + bakteri aşılaması (SYP2B), Pörsütülmüş yonca + 90 g/kg peynir altı suyu (SYP3) ve Pörsütülmüş yonca + 90 g taze peynir altı suyu + bakteri aşılaması (SYP3B). Araştırma sonunda, muamelelerin pH değerlerinin 4.86'nın altında olduğu, ancak kontrol pH'ının diğer uygulamalara kıyasla daha yüksek olduğu saptanmıştır ($P<0.05$). Yoncaya taze peynir altı suyu ve bakteri kültürü eklenmesinin, toplam yağ asitlerinin ortadan kaldırılmasında önemli bir artışa yol açtığı görülmüştür ($P<0.05$). Tüm muamelelerde kontrol grubuna göre nötr deterjan lif (NDF) ve asit deterjan lif (ADF) bakımından önemli bir fark bulunmamıştır. Erozyona karşı en az

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 04.08.2020
Kabul Tarihi : 16.09.2020

Anahtar Kelimeler
Yonca silajı
Peynir altı suyu
Lactobacillus buchneri
Aerobik kararlılık
Silaj kalitesi

direnç solmuş yonca + bakteri aşılaması ile; en yüksek miktar ise solmuş yonca + 60 g peynir altı suyu olan grupta saptanmıştır (P<0.05). Araştırma sonucunda, mayalanabilir karbonhidratlarla taze peynir altı suyu ve bakteri aşılamasının, pH'ların hızlı bir şekilde azalmasına, mayaların çoğalmasına, artan aerobik stabilitenin ve silaj kalitesinin artmasına neden olduğu sonucuna varılmıştır.

To Cite : Besharati, M., Palangi, V., Nekoo M., Ayaşan, T. 2021. Effects of Lactobacillus Buchneri Inoculation and Fresh Whey Addition on Alfalfa Silage Quality and Fermentation Properties. KSU J. Agric Nat 24 (3): 671-678. https://doi.org/10.18016/ksutarimdoga.vi.777031.

INTRODUCTION

Ensiling is a momentous procedure in tropical regions to make good use of components with the highest nutritive value (Besharati et al., 2020a; He et al., 2020; Ren et al., 2020;). Alfalfa is viewed as a forage with high nutritive value, low water soluble carbohydrates, high buffering capacity and highly degradable crude protein (NRC, 2001; Schmidt et al., 2009; Jiang et al., 2020; Palangi et al., 2020). McDonald et al. (1991) investigated that, quickly reduction of silage pH, causes to decrease on clostridia growth, minimize the proteolysis and heterolytic fermentation, and silage palatability could worsen. Therefore, to solve this problems, the methods of adding sugar substances (such as fermentative sugars, molasses, whey, juice waste), or cultivating alfalfa with wheat (such as barley, corn, cluster corn, silage of a mixture of pulp or beet leaves, or corn with alfalfa), use fermented supplements (such as organic acids, fibrolytic enzyme or minerals) were recommended (Besharati et al., 2020b; Besharati et al., 2020c; Calabro et al., 2020; Rinne et al., 2020;).

According to Daniels et al. (1983), whey is a cheap by-product obtained from cheese production enterprises; straw and bran are added to silage. De Lurdes et al. (1998) reported that dehydrated whey and molasses are used as additives to support fish by-products (Ventura-Canseco et al., 2012). Cheese whey is a valuable source of lactose so it could be used as a silage additive (El-Shewy, 2016; Yüksel et al., 2019; Mariotti et al., 2020).

Exposure of silage to the air at the time of feeding causes erosion of the silage, yeasts that are able to metabolize lactic acid; are the first cause of corruption with increases the pH (Kleinschmit and Kung, 2000), which is also a stimulant for the growth of other harmful microorganisms in the silage (Besharati et al., 2017; Mariotti et al., 2020), ultimately reduces livestock production due to feed depreciation or poisoning. The use of Lactobacillus buchneri has been reported to improve the aerobic stability of silage (Kleinschmit and Kung, 2000). Lactobacillus buchneri, by converting lactic acid to acetic acid, increased the resistance of silage to aerobic erosion (Besharati et al., 2017; Schumcher et al., 2019).

According to the latest statistics, in Iran about 2.000,000 tons of fresh whey is produced annually, which is containing approximately 227.000 tons of dry matter. The use of this material in livestock feeding, will solve the environmental problems, and on the other hand, it will be economically useful by reducing the price of feeds that needed for livestock. Therefore, fresh whey can be used as valuable waste and also, because of the good source of fermentable of carbohydrates (lactose), which can be used to ensiling alfalfa in order that increases the quality.

The hypothesis of this study was that in bacterial inoculation with the fermentable carbohydrates causes to improve the silage quality. The purpose of this experiment was to investigate the effects of adding Lactobacillus buchneri and fresh whey to alfalfa silage on the chemical characteristics and fermentation properties.

MATERIALS and METHODS

The experiment was conducted in the Arasbaran area in West-North of Iran. The alfalfa was produced during the second cut growing season in June 2016 at the Ahar town (39°05’10” latitude N. 47°33’10” longitude E) in East Azarbaijan Province. Second crop alfalfa was collected and wilted for 24 h at comfortable ambient temperature. The wilted samples were cut into pieces to achieve approximately 2 cm theoretical length of cut. The fresh whey was also prepared from Factory in Tabriz and used as needed in experimental treatments.

Experimental treatments were Wilted Alfalfa (without additives, control), Wilted Alfalfa + bacterial inoculant (AB), Wilted Alfalfa +30 g whey/kg (AW1), Wilted Alfalfa + 30 g whey + bacterial inoculant (AW1B), Wilted Alfalfa + 60 g whey per kg (AW2), Wilted Alfalfa + 60 g whey per Kg + bacterial inoculant (AW2B), Wilted Alfalfa + 90 g whey per kg (AW3) and Wilted Alfalfa + 90 g whey + bacterial inoculant (AW3B). Bacterial additive used at level of 3×108 cfu per g of alfalfa wet weight. Bacterial additive, after dissolved in water, sprayed onto the treatments and for control only water was sprayed. Treatments were ensiled in laboratory mini silos (triplicate) for 90 d at ambient temperature (15 to 18 ^{o}C) in a room.

At the opening time, about 100 g from the top of each silo were throw away and the content of each silo were thoroughly mixed, and samples were randomly obtained for chemical analysis. For extraction of silage juice, 10 g of silage were homogenized with 100 ml of distilled water (22 °C) for 3 times (each time 30s) and strained through double layers of cheesecloth. The pH of silage was forthwith ascertained using pH-meter. Dry matter (DM) of silage samples were determined after being oven-dried for 72 h at 60 °C. After drying process, the samples were milled through a 1 mm screen using a Wiley mill. Organic matter (OM) was determined by incinerating it at 550°C for 5 h. The crude protein (CP) (1030 Micro Kjeltec Auto Analyzer, method 984.13; AOAC 2000), NDF and ADF (Van Soest et al., 1991) were analyzed by using the above methods. Concentration of total volatile fatty acids (VFA) of silages were measured according to the method of Filya (2003).

Aerobic stability was determined on all treatments. About 55 g of each replicate was placed in container and a type T thermocouple with approximately 5 cm into them. A double layer of sterile cheesecloth was placed over each container to prevent drying and contamination but allow penetration of air. Silage's temperature and ambient one were recorded manually every two hours until heating occurred. Aerobic stability was defined as the number of hours the silage remained stable before rising more than 2 °C above ambient temperature (Ranjit and Kung, 2000).

Data were analyzed as factorial arrangement of treatments in a completely randomized design (SAS, 2018) and treatment means were compared by the Duncan Multiple Range Test ($P< 0.05$).

RESULT and DISCUSSION

Characteristics and chemical composition of alfalfa and fresh whey with bacterial additive on silage are summarized throughly in Table.

Çizelge 1. Silolamadan önceki taze peynir altı suyu ile mikrobiyal katkılı solmuş yonca silajının kimyasal komposizyonu

Table 1. Chemical composition of wilted alfalfa silage with whey and microbial additive before ensiling

Item	DM	pH	NDF (% of DM)	ADF (% of DM)	CP (% of DM)
Wilted Alfalfa	25.63±0.45	7.01±0.11	41.25±0.37	33.12±0.24	16.00±0.16

DM: dry matter, NDF: neutral detergent fibre, ADF: acid detergent fibre, CP: crude protein.

Table 2 shows the effect of additives on chemical properties and their interaction in alfalfa silage. According to the reported results, the amount of dry matter in the bacterial inoculant treatments decreased significantly compared to the control ($P<0.05$), and fresh whey treatments in the treatment of 30 g fresh whey had a significant increase compared to the control treatment ($P <0.05$), but in the treatment of 60 g of water. The fresh whey was significantly less than the control treatment, and a significant increase was observed in the treatment of 90 g of fresh whey.

The amount of ash content in bacterial inoculant silages increased significantly compared to control, but compared to fresh whey treatments, 30, 60 and 90 g of fresh whey treatments were observed unreasonably. The amount of NDF, ADF and EE in bacterial inoculated treatments and fresh whey treatments at 30, 60 and 90 g levels were not significantly different from that of control ($P>0.05$). There was no significant difference in pH value in bacterial inoculant treatments, but in fresh whey treatments, the lowest amount of pH was observed for 90 g fresh cheeses and the highest amount was observed for control treatment. Also, 30 and 90 g fresh whey treatments were significantly reduced in terms of pH value compared to control ($P<0.05$).

The amount of ammonia nitrogen in bacterial inoculated treatments was increased significantly, but in fresh whey treatments at 30, 60 and 90 g, was decreased significantly compared to control. The total amount of total volatile fatty acids in the bacterial inoculant treatments increased significantly compared to the control, but in fresh whey treatments with 30 and 60 g were unreasonable, but in treatment containing 90 g fresh whey significant decrease was observed (Table 2).

The effect of fresh whey and bacterial inoculation on the nutritional value of alfalfa molded silage is given in Table 3. The results of this experiment showed that adding a carbohydrate source such as fresh whey to alfalfa silage reduced the pH of the silage compared to the control ($P<0.05$).

The results of this experiment were consistent with the results of other researchers (Asadi et al., 2004; Touqir et al., 2007; Hashemzadeh et al., 2011). The data of this experiment corresponded to the experimental data used for different levels of whey to alfalfa silage (grams per kilogram of dry matter) (Repetto et al., 2011). Other researchers reported the use of fresh whey in reducing the pH of feed materials from the beginning of the period. Bacterial additive also significantly decreased pH compared to control ($P<0.05$). In the experiment (Asadi et al. 2004), the effect of Lactobacillus plantarum additive on millet forage was studied and the results showed that the pH decreased in the treatment containing bacterial additive.

In experiment of Adesogan et al. (2004), the effect of bactericidal supplementation of pseudococcus pentoseto (105) and lactobacillus buccaneer (4 × 105) on Bermuda fodder was used. The pH of the whole

Table 2. The chemical composition of wilted alfalfa, fresh whey and bacterial inoculant silage after 90 d (DM% basis)
Çizelge 2. 90 günden sonraki taze peynir altı suyu ve bakteri inokulasyonlu solmuş yoncanın kimyasal komposizyonu (%KM bazında)

Nutrients[1]	Bacterial Inoculation		SEM	fresh whey (g/kg fresh alfalfa weight)				SEM	P value		
	0	3×10^{8}cfu		0	30	60	90		Bacterial Inoculation	Fresh Whey	Interaction F×BI
DM	25.01^a±0.34	23.97^b±0.45	0.28	24.51^b±0.44	25.56^a±0.35	24.25^b±0.51	23.65^c±0.53	0.56	<.0001	<.0001	0.0813
Ash	10.66^b±0.12	11.7^a±0.17	0.48	10.91±0.66	11.08±0.54	11.58±0.71	11.16±0.58	0.96	0.0076	0.5700	0.3820
NDF	42.81±0.75	43.45±0.88	0.58	43.65±0.87	42.71±0.76	43.5±0.64	42.66±0.66	1.16	0.1449	0.2409	0.0585
ADF	34.25±0.66	34.1±0.63	0.50	34.81±0.45	33.88±0.48	34.06±0.50	33.93±0.51	1.00	0.6785	0.2534	<.0001
EE	12.93±0.21	13.36±0.45	0.42	13.23±0.89	13.35±0.87	13.08±0.84	12.93±0.88	0.84	0.1600	0.7694	0.0308
CP	14.2^a±0.05	14.03^b±0.07	0.011	13.83^b±0.09	14.44^a±0.08	13.75^b±0.08	14.43^a±0.09	0.02	0.0069	<.0001	<.0001
pH	4.72±0.04	4.75±0.04	0.053	5.01^a±0.11	4.70^b±0.09	4.68^b±0.09	4.56^c±0.12	0.10	0.3777	<.0001	0.0011
N-NH_3	67.66^b±0.54	72.16^a±0.48	1.54	89.00^a±1.08	76.33^b±0.96	58.83^c±0.84	55.50^d±0.90	3.08	0.0008	<.0001	0.0003
VFA	11.08^b±0.53	15.08^a±0.60	0.59	14.33^a±0.82	13.33^a±0.78	13.33^a±0.71	11.33^b±0.78	1.18	<.0001	0.0009	0.0007

[1]DM=Dry matter, NDF= Insoluble fiber in neutral detergent, ADF= Insoluble fiber in acid detergent, EE= Ether extract, CP= Crude protein; SEM: Standard error of means
a-c Differences between the averages indicated by different letters in the same line are important.

Table 3. The chemical composition of wilted alfalfa with whey and bacterial inoculant silage after 90 d (DM% basis)
Çizelge 3. 90 günden sonraki taze peynir altı suyu ve bakteri aşılamalı solmuş yoncanın kimyasal komposizyonu (%KM bazında)

	Silage treatment									
Nutrients	CON	AB	AW1	AWB1	AW2	AWB2	AW3	AWB3	SEM	P value
DM	25.41±0.33	23.60±0.54	26.16±0.32	24.95±	24.67±0.87	23.83±0.95	23.79±0.74	23.51±0.80	0.28	0.072
pH	5.16^a±0.05	4.86^b±0.04	4.61^{de}±0.04	4.80^{bc}±0.07	4.63^{ced}±0.07	4.73^{bcd}±0.04	4.49^e±0.07	4.63^{ced}±0.01	0.05	0.001
TVFA	12.00^c±0.39	16.66^a±0.50	12.66^b±0.46	14.00^b±0.44	9.66^c±0.65	17.00^a±0.77	10.00^c±0.41	12.66^b±0.40	0.59	0.001
NDF	44.30^{ab}±0.79	43.00^{ab}±0.55	42.06^c±0.22	43.36^{ab}±0.54	42.46^b±0.11	44.53^a±0.30	42.43^b±0.15	42.90^{ab}±0.40	0.58	0.050
ADF	34.56^{ab}±0.50	35.06^a±0.50	35.70^a±0.56	32.06^c±0.66	32.56^c±0.45	35.56^a±0.70	34.16^a±0.69	33.7^b±0.45	0.50	0.001
CP	13.81^b±0.13	13.82^b±0.16	15.40^a±0.10	13.50^c±0.20	14.25^b±0.16	13.81^b±0.14	14.53^b±0.14	15.47^a±0.10	0.10	0.001

CON: Wilted Alfalfa (Without additives). AB: Wilted Alfalfa + bacterial cfu 3×10 ^{8}g. AW1: Wilted Alfalfa +30 g/ kg wet weight whey. AWB1: Wilted Alfalfa + 30 gr of whey + bacterial 3×10 g cfu. AW2: Wilted Alfalfa + 60 grams of whey kg wet weight. AWB2: Wilted Alfalfa + 60 g whey +bacterial 3×10 g cfu. AW3: Wilted Alfalfa + 90 g whey kg wet weight. AWB3 Wilted Alfalfa + 90 g whey + bacterial 3×10 g cfu. SEM: standard error of means
Within a row, means followed by different letters differ (P< 0.05).

fermentation period was significantly lower in the bacterial inoculated treatment. In experiment of Nadeau et al. (2006), the effect of bacterial supplementation of Lactobacillus plantarum and Pseudococcus cerevisiae on alfalfa and archad grass with DM of 22 and 32% was observed that the bacterial additive in alfalfa containing cellulose caused a slight decrease but also a meaning it was in pH, but this was not observed in arched grass forage. Other studies have reported similar results (Curtis, 1996; Whiter and Kung, 2001; Nishino et al., 2004; Touqir et al., 2007; Hashemzadeh et al., 2011). In general, the reason for pH reduction in comparison with control treatment is the high soluble carbohydrate in other treatments and the availability of a higher nutrient for dominating the population of bacteria producing lactic acid and production of lactic acid.

The amount of dry matter was not affected by the bacterial inoculation (P>0.05). Other studies also reported similar results with this study (Driehuis et al., 2001; Filya, 2003; Kung et al., 2003; Filya et al., 2007; Hashemzadeh et al., 2014).

According to Table 3, total volatile fatty acids in AB and AWB2 treatments increased significantly compared to control (P<0.05). In the experiment (Hashemzadeh et al. 2011), adding molasses as carbohydrates to alfalfa fodder increased total fatty acids relative to treatment was monitored. The addition of fresh whey with bacterial inoculation with lower levels of fresh whey cheese (AW1, AWB1) and higher levels (AW3, AWB3) was not significantly different, but significantly increased compared to the two treatments (AW2, AW3).

The results of this experiment were consistent with the results of other researchers (McAllister et al., 1998; Baytok et al., 2005; Kleinschmit et al., 2006). In an experiment with 14 types of bacterial inoculant on forage hay in China, all additives increased esophageal fatty acids (Filya et al., 2007). This increase in the total amount of volatile fatty acids can be attributed to the high population of lactic acid bacteria and the availability of carbohydrate source for the production of lactic acid and acetic acid in experimental treatments relative to the control treatment. Adding fresh whey with bacterial inoculant to alfalfa fodder resulted in a small increase in the amount of NDF, which was not statistically different (P>0.05) (Table 3). This slight increase in NDF levels can be attributed to higher acetic acid production due to higher soluble carbohydrates in the treatment. The bacterial inoculant in all four levels with fresh whey with different levels did not change the NDF compared to the control.

In addition, AWB2 had significant increase with other treatments. Many studies also reported similar results to the current test results (Gordon et al., 1999; Ranjit and Kung, 2000; Kung and Ranjit, 2001; Kızılşimşek et al., 2007; Hashemzadeh et al., 2014). A study was conducted to investigate 14 different types of bacterial inoculant on first-line hay fodder, in the 6 additive increases in NDF, 5 additives unchanged and 3 additives reductions in NDF levels (Filya et al., 2007). There was no significant difference between ADF and AW1, AW3, AWB2 and AB treatments. However, ADF levels in AWB1, AW2 and AWB3 treatments were significantly decreased in control silage (P<0.05) (Table 3).

In the study of the effect of *L. bouchneri* inoculation on corn silage, L. *bouchneri* reduced ADF at low levels of consumption (Ranjit and Kung, 2000). In another study, the same authors of the *L. bouchneri* inoculation at each of three levels reduced the ADF number in 100 days of barley, with a low level of *L. bouchneri* consumption at 5% level. Adding fresh whey and bacterial inoculant to alfalfa forage, in the amount of crude protein in alfalfa treatment with 30 g fresh whey (AW1) and in alfalfa treatments with 90 g fresh cheese and bacterial inoculation (AWB3), a significant increase compared to control (P<0.05). The rest of the treatments were not significantly different from each other (Table 3). The results of this experiment were perfectly consistent with the results of Takasi (1996) and Nkosi and Meeske (2010) which all of these studies reported adding fresh whey to the amount of crude protein. Adding 30 g of fresh whey and bacterial inoculant (AWB1) haylage reduced the amount of crude protein compared to control (P>0.05). Repetto et al. (2011) was reported that the effect of adding fresh whey on alfalfa fodder has a decrease in crude protein content with a rise in fresh whey.

Adding fresh whey to different levels of alfalfa silage reduced aerobic stability compared to control and other experimental treatments (Figure 1). The main reasons for accelerating the occurrence of aerobic agglomeration can be due to high levels of soluble carbohydrates and probably lactic acid and the absence of volatile fatty acids (Adesogan et al., 2004).

The level of aerobic stability in treatments (AWB3 and AW1, AW2, AWB2) increased significantly compared to control and AB treatment (P<0.05). The least resistance to corrosion was related to AB treatment and the highest amount was related to AW2 treatment (30 hours and 120 hours, respectively, in the vicinity of the air). The other main reason causing aerobic silage is the low amount of dry matter. Lindgren (1999) reported that the use of *L. buchneri* supplementation in silage materials improved the acetic acid and increased the aerobic stability during silo storage. Injes et al. (2001) examined three different experiments of the *L. buchneri* additive alone and in combination with *L. plantarum* on grass forage in the 90 and 180 days, and reported thet experimental treatments were improved aerobic stability of silage.

KSÜ Tarım ve Doğa Derg 24 (3): 671-678, 2021
KSU J. Agric Nat 24 (3): 671-678, 2021
https://doi.org/10.18016/ksutarimdoga.vi.777031

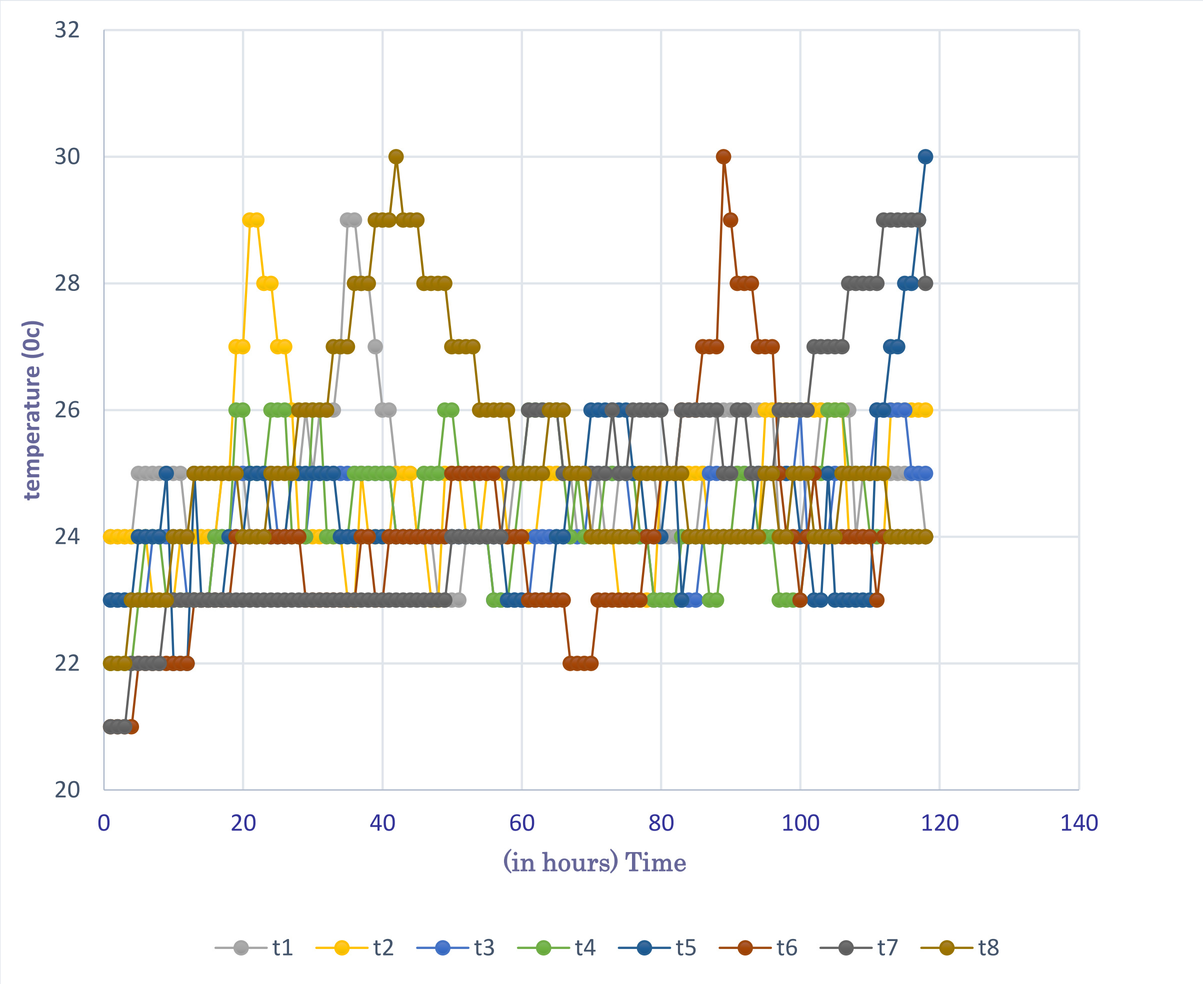

Figure 1. Effect of fresh whey and bacterial inoculation on aerobic stability
Şekil 1. Taze peynir altı suyu ve bakteri aşılamasının aerobik stabilite üzerine etkisi

T1 : Wilted Alfalfa (Wthout additives). T2: Wilted Alfalfa + bacterial cfu 3×10 ^{8}g . t3: Wilted Alfalfa +30 g/ kg wet weight whey. T4: Wilted Alfalfa + 30 gr of whey + bacterial 3×10 g cfu. T5: Wilted Alfalfa + 60 grams of whey kg wet weight. T6: Wilted Alfalfa + 60 g whey +bacterial 3×10 g cfu. T7: Wilted Alfalfa + 90 g whey kg wet weight. T8: Wilted Alfalfa + 90 g whey + bacterial 3×10 g cfu.
Within a row, means followed by different letters differ ($P < 0.05$).

In the experiment of Filya (2003), the effect of *L. Buchneri* alone and in combination with *L. plantarum* on wheat, corn and sorghum was evaluated. In all treatments, improvement of aerobic stability was reported. Pahlow and Zimmer (1985) found that bacterial inoculant leads to improve aerobic stability of forage silage.

Improving the stability of these silages to a special energy detoxification system, yeasts are attributed to the excessive concentration of organic acids inside the cell. Kautz (1998) reported that, *L. Buchneri* was used to produce lactic acid and acetic acid, which reduced the yeast and improved the aerobic stability step from 26 hours to 400 hours in silages. In general, the effect of *L. Buchneri* on the improvement of aerobic stability can be explained by increasing the amount of acetic acid produced by bacteria, as well as reducing the amount of carbohydrate remaining solution and lactic acid and lower ammonia nitrogen accumulation.

CONCLUSION

The addition of fresh whey to alfalfa fodder resulted in a decrease in the final pH of the silage and an increase in total volatile fatty acids, insoluble fiber in acid detergent and, on the other hand, decreased the aerobic stability in control silage. The bacterial inoculation also improved the fermentation parameters and increased the overall quality of the silage. With regard to the above results, it can generally be concluded that the bacterial inoculation and whey alone and together produced a better fermentation quality and the use of a carbohydrate source such as whey as sources of bacterial populations. Bacterial inoculations are essential for the preparation of high-quality silage from alfalfa, due to the deficiencies of this valuable plant.

Conflict of interest

The authors declare no inflict of interest.

REFERENCES

Adesogan AT, Krueger N, Salawu MB, Dean DB, Staples CR 2004. The influence of treatment with dual purpose bacterial inoculants or soluble carbohydrates on the fermentation and aerobic stability of bermuda grass. Journal of Dairy Science 87: 3407-3416.

Asadi al-Mutati A, Alikhani M, Ghorbani GH, Sami AH 2004. Effect of different additives on the quality of millet silage fermentation under laboratory conditions. Journal of Agricultural Science and Technology 3: 149-161.

Baytok E, Aksu T, Karsli MA, Muruz H 2005. The effects of formic acid, molasses and inoculant as silage additives on corn silage composition and ruminal fermentation characteristics in sheep. Turkish Journal of Veterinary and Animal Sciences 29: 469-474.

Besharati M, Palangi V, Niazifar M, Nemati Z 2020a. Comparison study of flaxseed, cinnamon and lemon seed essential oils additives on quality and fermentation characteristics of lucerne silage. Acta Agriculturae Slovenica 115(2): 455-462.

Besharati M, Palangi V, Moaddab M, Nemati Z, Pliego A B, Salem A Z 2020b. Influence of cinnamon essential oil and monensin on ruminal biogas kinetics of waste pomegranate seeds as a biofriendly agriculture environment. Waste and Biomass Valorization, https://doi.org/10.1007/s12649-020-01167-2

Besharatı M, Karımı M, Taghïzadeh A, Nemati Z, Kaygısız A 2020c. Improve quality of alfalfa silage ensiled with orange pulp and bacterial additive. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi 23(6): 1669-1677. DOI: 10.18016/ksutarimdoga.vi.673623

Calabro PS, Fazzino F, Sidari R, Zema DA 2020. Optimization of orange peel waste ensiling for sustainable anaerobic digestion. Renewable Energy 154: 849-862.

Curtis JL 1996. Effect of variety on the forage yield, ensiling characteristics, and nutritive value of alfalfa, and effects of cutting, stage of maturity and silage additives on the preservation and nutritive value of alfalfa silage. A dissertation. Kansas State University. Department of Animal Science and Industry Collage of Agriculture.

Daniels LB, Smith LJ, Stallcup OT, Rakesa JM 1983. Nutritive value of ensiled broiler litter for cattle. Animal Feed Science and Technology 8 (1): 19-24.

De Lurdes M, Dapkeviius E, Batista I, Nout MJR, Rombouts FM, Houben JH 1998. Lipid and protein changes during the ensilage of blue whiting (*Micromesistius poutassou Risso*) by acid and biological methods. Food Chemistry 63: 97-102.

Driehuis F, Oude Elferink S, Van Wikselaar PG 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with Lactobacillus buchneri, with or without homofermentative lactic acid bacteria. Grass and Forage Science 56: 330-343.

Filya I 2003. The effect of lactobacillus buchneri and lactobacillus plantarum on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. Journal of Dairy Science 86: 3575-3581.

Filya I, Muck RE, Contreras-Govea FE 2007. Inoculant effects on alfalfa silage: fermentation products and nutritive value. Journal of Dairy Science 90: 5108-5114.

Gordon FJ, Dawson LER, Ferris CP, Steen RWJ, Kilpatrick DJ 1999. The influence of wilting and forage additive type on the energy utilization of grass silage by growing cattle. Animal Feed Science and Technology 79: 15-27.

Hashemzadeh-Cigari F, Khorvash M, Ghorbani GR, Ghasemi E, Taghizadeh A, Kargar S, Yang WZ 2014. Interactive effects of molasses by homofermentative and heterofermentative inoculants on fermentation quality, nitrogen fractionation, nutritive value and aerobic stability of wilted alfalfa (Medicago sativa L.) silage. Journal of Animal Physiology and Animal Nutrition 98: 290-299.

Hashemzadeh-Cigari F, Khorvash M, Ghorbani GR, Taghizadeh A 2011. The effects of wilting, molasses and inoculants on the fermentation quality and nutritive value of lucerne silage. South African Society Animal Science 41: 377-388.

He L, Wang CH, Xing Y, Zhou W, Pian R, Chen X, Zhang Q 2020. Ensiling characteristics, proteolysis and bacterial community of high moisture corn stalk and stylo silage prepared with Bauhinia variegate flower. Bioresource Technology 296: 122336.

Jiang D, Li B, Zheng M, Niu D, Zuo S, Xu CH 2020. Effects of pediococcus pentosaceus on fermentation, aerobic stability and microbial communities during ensiling and aerobic spoilage of total mixed ration silage containing alfalfa (Medicago sativa L.). Grassland Science 66: 215-224. . https://doi.org/10.1111/grs.12272

Kautz WP 1998. Advances in inoculants silage.Technical services, Milwaukee.

Kizilsimsek M, Schmidt RJ, Kung Jr L 2007. Effects of a mixture of lactic acid bacteria applied as a freezedried or fresh culture on the fermentation of alfalfa silage. Journal of Dairy Science 90: 5698-5705.

Kleinschmit DH, Kung Jr L 2006. A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. Journal of Dairy Science 89: 4005-4013.

Kung Jr L, Ranjit NK 2001. The effect of lactobacillus buchneri and other additives on the fermentation and aerobic stability of barley silage. Journal of Dairy Science 84: 1149-1155.

Lindgren S 1999. Can HACCP principles be applied for silage safety? In: Proceeding of International Silage Conference 66: 51-66. Sweden.

Mariotti M, Fratini F, Cerri D, Andreuccetti V, Giglio R, Angeletti FGS, Turchi B 2020. Use of fresh scotta whey as an additive for alfalfa silage. Agronomy Journal 10: 365.

McAllister TA, Feniuk R, Mir Z, Mir P, Selinger LB, Cheng KJ 1998. Inoculants for alfalfa silage: Effects on aerobic stability, digestibility and the growth performance of feedlot steers. Livestock Production Science 53: 171-181.

McDonald P, Henderson AR, Heren SJE 1991. The biochemistry of silage. 2nd ed. Chalcombe Publ; Bucks, UK.

Nadeau EMG, Buxton DR, Russell JR, Allison MJ, Young JW 2006 Enzyme, bacterial inoculant, and formic acid effect on silage composition of orchard grass and alfalfa. Journal of Dairy Science 83: 1487-1502.

Nishino N, Wada H, Yoshida M, Shiota H 2004. Microbial counts, fermentation products, and aerobic stability of whole crop corn and a total mixed ration ensiled with and without inoculation of lactobacillus casei or lactobacillus buchneri. Journal of Dairy Science 87: 2563–2570.

Nkosi BD, Meeske R 2010. Effects of whey and molasses as silage additives on potato hash silage quality and growth performance of lambs. Journal of Dairy Science 40: 229-237.

NRC 2001. Nutrient Requirements of Beef Cattle (7th ed.). National Academy Press, Washington D.C., USA.

Palangi V, Macit M, Bayat AR 2020. Comparison of mathematical models describing disappearance curves from the in sacco technique for incubating lucerne hay cuts in the rumen. South African Journal of Animal Science 50(5): 719-725.

Pahlow G, Zimmer E 1985. Proceedings of the 15th International Grassland Congress, Kyoto. 877-897.

Ranjit NK, Kung Jr L 2000. The effect of lactobacillus buchneri, lactobacillus plantarum, or a chemical preservative on the fermentation and aerobic stability of corn silage. Journal of Dairy Science 83: 526–535.

Ren H, Feng Y, Pei J, Li J, Wang Z, Fu SH, Zheng Y, Li Z, Peng Z 2020. Effects of lactobacillus plantarum additive and temperature on the ensiling quality and microbial community dynamics of cauliflower leaf silages. Bioresource Technology 307: 123238.

Repetto JL, Echarri V, Aguerre M, Cajarville C 2011. Use of fresh cheese whey as an additive for lucerne silages: Effects on chemical composition, conservation quality and ruminal degradation of cell walls. Animal Feed Science and Technology 170: 160-164.

Rinne M, Winquist E, Pihlajaniemi V, Niemi P, Seppälä A, Siika-Aho M 2020. Fibrolytic enzyme treatment prior to ensiling increased press-juice and crude protein yield from grass silage. Bioresource Technology 299:122572.

SAS 2018. Statistical Analysis Systems. Version 9.4 SAS Institute, Cary, NC, USA.

Schmidt RJ, Hu W, Mills JA, Kung JL 2009. The development of lactic acid bacteria and Lactobacillus buchneri and their effects on the fermentation of alfalfa silage. Journal of Dairy Science 92(10): 5005-5010.

Schumcher LL, Viégas J, Pereira SN, Tonin TJ, Rocha LT, Bermudes R, Carpes RP 2019. Effect of the additives milk whey, Lactobacillus casei and l. Acidophillus on the quality of sorghum silage. Boletim De Indústria Animal Nova Odessa 76: 1-8.

Takasi M 1996. Use of fresh whey in the enrichment of wheat straw with urea. Final report of Kerman Natural Resources and Animal Resources Research Center. Animal Research Division.

Touqir NA, Ajmal Khan M, Sarwar M, Nisa M, Lee WS, Lee HJ, Kim HS 2007. Influence of varying dry matter and molasses levels on berseem and lucerne silage characteristics and their in-situ digestion kinetics in nili buffalo bulls. Asian-Australian Journal of Animal Science 20(6): 887-893.

Van Soest PJ, Robertson JB, Lewis BA 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74: 3583.

Whiter AG, Kung L 2001. The effect of a dry or liquid application of Lactobacillus plantarum MTD1 on the fermentation of alfalfa silage. Journal of Dairy Science 84: 2195–2202.

Yüksel M, Yüksel AK, Ürüşan H 2019. Peynir altı suyunun çeşitli özellikleri ve kullanım olanakları. Kahramanmaraş Sütçü İmam Üniversitesi Mühendislik Bilimleri Dergisi 22(3): 114-125.