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Chemical and Nutritional Changes in Sunflower Silage Associated With Molasses, Lactic Acid Bacteria and Enzyme Supplementation

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Abstract
This study was conducted to determine the effects of different silage additives on sunflower silage quality. The treatments were as follows: (1) control (C, no additive), (2) 5% molasses (M) (3) inoculation of lactic acid bacteria (LAB; 1.5 g/ton, a mixture of *Lactobacillus plantarum* and *Enterococcus faecium* applied at a rate of 6.00 log10 cfu LAB/g of fresh material) and (4) LAB+enzyme mixture 2 g/ton (LEN, *Lactobacillus plantarum* bacterium (6.00 log10 cfu/g) and cellulase (150000 CMCU/kg) and amylase (200000 SKB/kg) enzymes). Silage additives were mixed and stored in glass jars with 5 replicates for 90 days. There were no significant differences among the groups in terms of pH, ether extract (EE), acid detergent lignin (ADL) and hemicellulose (HEM). The water soluble carbohydrate (WSC) concentration was higher in the M group than those of other groups (P<0.01). Lactic and acetic acid concentration were lower in the LEN group than the other groups (P<0.05). In the LAB group, dry matter (DM), crude protein (CP), crude ash (CA) and fleig point (FP) contents were lower than those of other groups (P<0.01). In the M group, neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were lower and the total digestibility nutrient (TDN) and non-fiber carbohydrate (NFC) contents were higher than those of other groups (P<0.01). The crude cellulose (CC) contents of M and LEN groups were lower than that of C and LAB groups. In conclusion, addition of the bacterial inoculants (LAB and LEN) and molasses to sunflower crop before ensiling positively affected some quality traits.

Keywords*:* sunflower, inoculant, silage quality, volatile fatty acids

Ayçiçeği Silajlarına Melas, Laktik Asit Bakterisi ve Enzim İlavesinin Silajların Kimyasal ve Besin Madde Değişimi Üzerine Etkileri

Özet
Bu çalışma, farklı katkı maddelerinin ayçiçeği silajlarının kalitesi üzerine olan etkilerini belirlemek amacıyla yapılmıştır. Muameleler: (1) kontrol (K, katkı maddesi yok), (2) %5 melas ilavesi (M), (3) Laktik asit bakteri ilavesi (LAB; 1.5 g/ton, *Lactobacillus plantarum* ve *Enterococcus faecium* bakterisi içerir, 6.00 log10 cfu LAB/g içerir) ve (4) LAB+enzim karışımı 2 g/ton (LEN, *Lactobacillus plantarum* (6.00 log10 cfu/g) ve selülaz (150,000 CMCU/kg) ve amilaz (200,000 SKB/kg içerir). Silaj katımı maddeleri homojen biçimde ilave edilip 90 günlük fermantasyona bırakıldı. Gruplar arasında, pH, ham yağ (HY), asit deterjan lignin (ADL) ve hemiselüloz (HEM) bakımından farklılık bulunmadı. Suda çözünebilir karbonhidrat (SÇK) oranı M grubunda diğer gruplardan daha yüksek bulunmuştur (P<0.01). Laktik asit (LA) konsantrasyonu LEN grubunda (P<0.05) ve LAB grubunda kuru madde (KM), ham protein (HP), ham kül (HK) ve fleig puanı (FP) diğer gruplardan daha düşük bulunmuştur (P<0.01). Melas katılan grupta, nötral deterjan fiber (NDF) ve asit deterjan fiber (ADF) içeriği düşük; toplam besin madde sindirilebilirliği (TSBM) ile selüloz olmayan karbonhidrat içeriği diğer gruplardan daha yüksek olmuştur (P<0.01). Ham selüloz (HS) içeriği M ve LEN gruplarında K ve LAB gruplarından daha düşük bulunmuştur. Sonuç olarak, ayçiçeği silaj materyallerine bakteriyel inokulantların, (LAB ve LEN) ve melas ilavesi silaj kalitesini olumlu yönde etkilediği tespit edilmiştir.

Anahtar Kelimeler: Ayçiçeği, inokulant, silaj kalitesi, uçucu yağ asitleri

Introduction

In ruminant nutrition, roughages are cheap, good nutrient sources and essential for good rumen function. Silage production has some advantages in the dairy farms. However, to provide superior quality silage, appropriate plant, appropriate harvest time, enough in easy water soluble carbohydrates (WSC), then good ensiling and fermentation are necessary.

Corn silage production is very common in dairy or beef cattle and sheep and goat farms. However, sunflower silage is not common as corn silage in these farms. For corn production, it is necessary better soil properties, high climatic temperature and more irrigation compared to sunflower plant (Gonçalves et al., 1999). As an alternative, sunflower has good ability for drought tolerance, resistance to cold and heat, adaptability to different climatic conditions high dry matter (DM) yields, and relative independence of latitude, altitude and photoperiod (Tomich, 1999). Sunflower silage has higher concentration of protein and fat compared to corn (Gregoire, 1999) and sorghum (Demirel et al., 2006) silages. However, when it late harvest, due to increase fiber content of sunflower silage, lower silage quality and digestibility of material in ruminants (Demirel et al., 2006; Ozduven et al., 2009).

Silage additives have been using commonly in many farms for improve silage fermentation and quality. There are many commercial bacterial inoculants, enzyme and organic acids and their combinations (Meeske and Basson 1998; Sucu and Filya, 2006). However, although many experiments were done in corn silage (Aksu et al., 2004; Baytok et al., 2005; Filya et al., 2006) the lesser experiments done with sunflower related to less known material (Denek et al., 2004; Ozduven et al., 2009).

The biological inoculants as silage additive are producing via *Lactobacillus* bacteria (LAB) and they can be stabilized as silage additives and generally they increase lactic acid concentration and reduce pH, acetic acid, butyric acid and ammonia-nitrogen levels in silage (Aksu et.al., 2004). Last decade, there are many effort to produce new strain of homo and hetero fermentative bacterial inoculants by the commercial companies as an alternative to former inoculants and new combinations with enzymes. On the other hand, commercial biologic silage inoculants may be costly to farmers and some conditions may not reliable due to inactivated microbial organisms (Weinberg and Muck 1996). Molasses is a by-product of sugar production factories and it can be used to get water soluble carbohydrate source in silage (Nkosi et al., 2010).

The aim of this study was to evaluate the effect of molasses and biological silage additives on sunflower silage quality traits.

Material and Methods

Sunflower plant material was obtained from Erciyes University Agricultural Research Field without any treatment and harvested at the late flower stage of maturity (31.10 ± 0.81% DM) at August17, 2013. The whole plants were chopped about 2 to 4 cm and ensiled in 1 kg capacity glass jars with 5 replications. The chopped fresh materials were filled tightly in order to avoid oxygen. The ensiled jars were stored at room temperature (20°C ± 3°C) for 90 days. The treatment groups were as follows: (1) control (C, no additive), (2) 5% molasses (M) (3) inoculation of lactic acid bacteria (LAB; 1.5 g/ton, a mixture of LAB consisting of

Lactobacillus plantarum and *Enterococcus faecium* applied at a rate of 6.00 log10 cfu LAB/g of fresh material, Pioneer 1174, USA), and 4: LAB+enzyme mixture 2 g/ton(Inoculant and enzyme mixture (LEN, *Lactobacillus plantarum* bacterium (6.00 log10 cfu/g) and cellulase (150000 CMCU/kg) and amylase (200000 SKB/kg) enzymes, Silaid WS^{TM}, Global Nutritech Co., USA). The molasses, LAB and LEN were dissolved in 20ml water and sprayed on the chopped sunflower fresh materials.

Chemical Analyses

At the end of 90-day ensilage period, silage samples were taken for chemical and nutritional analyses. For pH measurements, 25 g of silage samples were taken into a beaker and 100 ml distilled water was added. Then the mixture was mixed in a blender for 5 minutes and resultant mixture was filtered through Whatman filter paper and pH measurements were performed in this filtrate (Akyildiz, 1986). The dry matter (DM, Method 934.01**)** content of the crops and silages was determined by drying the samples at 60°C for 72 h in an oven. Crude Ash (CA) was obtained after drying at 600°C for 4 h (AOAC, 2005, Method 942.05). Crude Protein (CP, Method 954.01), Crude Cellulose (CC, Method 978.10) and Ether Extract (EE, Method 920.39) were determined in accordance with the methods specified in AOAC (2005). Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were analyzed using the sodium sulphite addition method with residual ash (Van Soest et al., 1991). The difference between NDF and ADF values provides an estimate of hemicellulose (HEM). To determine water soluble carbohydrate (WSC) content, liquid extractions were prepared with 40 g silage. Samples were placed into a beaker, 360 ml distilled water was added and mixed in a blender. The resultant slurry filtered through Whatman 54 filter paper and then centrifuged. Samples were stored at -20 °C until the analyses. The WSC of samples were determined by phenol sulphuric acid method (Dubois et al., 1956).

The fleig point (FP) was calculated with the equation of FP = 220 + (2 x DM% - 15) - 40 x pH (Akyildiz, 1986). The Total Digestible Nutrients (TDN) were calculated according to the equation proposed by Chandler (1990), where TDN% = 105.2-0.68 x NDF%. The Non-Fiber Carbohydrates (NFC) were calculated by the equation proposed by Weiss et al., (1992): NFC% = 100 - (NDF% + CP% + EE% + CA%). Total carbohydrates (TC) were determined according to Sniffen et al., (1992) with the equation TC% = 100 - (CP% + EE% + CA%). The metabolic energy (ME) was calculated by the equation proposed by Robinson et al, (2004): ME = 14.03-(0.01386 x CF%)-(0.1018 x CA%)

The lactic acid (LA) content of silages were determined by Lepper's methods (Akyildiz, 1986) and acetic (Chem Service O-4), propionic (Chem Service O-25) and butyric acid (Chem Service O-5) were determined in a gas chromatograph (Shimadzu GC-2010+, Kyoto, Japan) with a capillary column (30 m × 0.25 mm × 0.25 µm, Restek) and with FID over a temperature range of 45–230°C.

Statistical analysis

Data were analyzed using the general linear model procedure of the SPSS program. Differences between reported means were determined using the Duncan's multiple range tests with a 5% level of probability. The results of statistical analysis were shown as mean values and standard error of the

means (SEM) in the tables.

Results and Discussion

The effect of molasses, lactic acid bacteria inoculant and enzyme supplementation on the sunflower silage pH, dry matter, crude protein, crude cellulose, crude ash and ether extract concentration are showed at Table 1. There were no statistically differences between the treatment groups in terms of pH, and ether extract values. Similar findings were observed that molasses addition to ryegrass (Islam et al., 2001) and LAB inoculant addition to corn silage (Filya et al., 2004) did not chance silage pH. Addition of 5% molasses caused an increase in DM content compared to LAB and LEN groups that result is related to its DM content. In the LAB group the DM was lower than those of other groups (P<0.01). Islam et al. (2001) and Gul et al. (2008) noted that molasses addition caused an increase in DM content of grass silages. However, Ozduven et al. (2009) reported that LAB and LEN inoculant addition did not affect DM content. Similar findings were reported by Meeske and Basson (1998) and Filya and Sucu (2006) LAB inoculant addition in corn silage did not affect DM of corn silage.

In the LAB group, crude protein and ash were lower than those of C, M and LEN groups (P<0.01). In the experiment LAB supplementation may have encouraged lactic acid bacteria growth and thus, bacteria used some protein and minerals to bacterial growth and improvement. These results can explain related to decreasing protein (Wilson and Wilkins, 1973) and ash (Mello et al., 2004) contents and increasing fermentable carbohydrate ratio of silages (Mehmet, 2006). Some experimental results showed that CP of sunflower silages may change between 7.90 and 9.86% (Ayasan and Karakozak, 2012) and also others determined that sunflower silage protein ratio may vary between 11.60-13.45% (Tan et al., 2015). Ozduven et al. (2009) and Koc et al. (2009) reported that LAB and enzyme mixture inoculants did not change crude protein, ash and ether extract of sunflower silages. The crude cellulose content of 5% M and LEN groups were lower than those of C and LAB groups (P<0.05). These results explained that molasses had less cellulose content and LEN had cellulase enzyme. In the M group, ADF and NDF values were lower than those of other groups (P<0.01). There were no significant differences among the groups in terms of HEM and ADL values. Islam et al. (2001) determined that molasses and bacterial inoculants reduced cellulose components in grass silages. However, Ozduven et al. (2009) and Koc et al. (2009) reported that LAB and enzyme mixture inoculants did not change crude cellulose, ADF, NDF, ADL, HEM contents of sunflower silages. As a biological material, effectiveness of silage additives and silage fermentation characteristic may vary according to ensiled material properties, ensiling techniques, stored conditions and properties inoculants (Kılıç, 1986).

Table 1. The effects of molasses, lactic acid bacteria (LAB) and LAB and enzyme mixture supplementation on the pH, and chemical composition of sunflower silage

	Treatment groups				SEM	P
Items	**C**	**M**	**LAB**	**LEN**		
pH	4.26	4.23	4.24	4.21	0.01	NS
Dry matter, %	34.74^{ab}	35.51^{a}	31.89^{c}	33.65^{b}	0.41	**
Crude Protein, % DM	7.94^{a}	7.96^{a}	6.33^{b}	7.27^{a}	0.20	**
Crude ash, % DM	13.63^{a}	12.72^{a}	10.05^{b}	13.72^{a}	0.46	**
Ether extract, % DM	4.53	4.35	5.36	4.58	0.20	NS
Crude cellulose, % DM	21.94^{a}	19.56^{b}	22.90^{a}	21.53^{b}	0.51	*
Neutral detergent fiber, % DM	39.62^{a}	35.70^{b}	42.14^{a}	39.82^{a}	0.73	**
Acid detergent fiber, % DM	34.27^{a}	30.81^{b}	36.41^{a}	34.92^{a}	0.64	**
Hemi-cellulose, % DM	5.35	4.89	5.73	4.9	0.28	NS
Acid detergent lignin, % DM	12.34	11.66	13.51	13.39	0.43	NS

$^{a,b,c:}$ Values with different superscript in a line differ significantly, C: control, M: 5% molasses, LAB: Lactic acid bacteria inoculant, LEN: enzyme and bacteria inoculant, DM: dry matter, P:probability, *:P<0.05, **:P<0.01, SEM: pooled standard error of means, NS: non significant

The effects of M, LAB and LEN supplementation on the WSC, lactic, acetic, propionic and butyric acid concentration of treatment groups are showed at Table 2. Molasses supplementation was increased WSC concentration compared to other groups. Also, in the LEN group's WSC concentration was higher than those of C and LAB groups (P<0.001). It is reported that WSC content of sunflower silage (Ozduven et al., 2009) increased by the LAB and enzyme inoculants addition. The lactic acid concentration in the LEN group was lower than those of other groups (P<0.05) and the acetic acid concentration was lower than M and LAB groups (P<0.05), however there was no statistical difference with C group. There was no difference between the treatment groups in terms of propionic acid concentrations (P>0.05). In silage samples, butyric acid was not detected. It is expected that silage additives such as LAB and LAB+enzyme mixture inoculants can increase WSC and lactic acid and decrease in pH, acetic, propionic and butyric acid ratio in silages. These additives may support to release fermentable sugars to produce more lactic acid in proportion to other products and lowered pH level (Kung et al., 1991). In the current experiment LA concentration did not change in LAB group and lowered with LEN addition. In contrast to these findings, Islam et al. (2001), Filya et al. (2004), Ozduven et al. (2009), and Koc et al. (2009) reported LAB and enzyme addition reduced LA concentration in silages.

Table 2. The effects of molasses, lactic acid bacteria (LAB) inoculants and enzyme supplementation on the water soluble carbohydrate (WSC), lactic, acetic, propionic and butyric acid concentration of sunflower silage

	Treatment groups				SEM	P
Items	**C**	**M**	**LAB**	**LEN**		
Water soluble carbohydrate, % DM	2.43^{c}	3.19^{a}	2.50^{c}	2.69^{b}	0.079	**
Lactic acid, % DM	5.51^{a}	5.84^{a}	4.97^{a}	2.87^{b}	0.427	*
Acetic acid, % DM	1.94^{ab}	2.64^{a}	2.71^{a}	0.56^{b}	0.327	*
Propionic acid, % DM	0.03	0.01	0.03	0.06	0.010	NS
Butyric acid, % DM	ND	ND	ND	ND	-	-

$^{a,b,c:}$ Values with different superscript in a line differ significantly, C: control, M: 5% molasses, LAB: Lactic acid bacterial inoculant, LEN: enzyme and bacterial inoculant, DM: dry matter, P: probability; ND: Not detected, *:P<0.05, **:P<0.01, SEM: pooled standard error of means, NS: non significant.

The effects of M, LAB inoculants and LEN supplementation on the TDN, OM, NFC, TC, ME and FP are given at Table 3. In the M group, TDN and NFC values were significantly higher than those of C, LAB and LEN groups (P<0.01 and P<0.05). The LAB supplementation increased the OM, TC and ME values statistically higher than those of C, M and LEN groups (P<0.01 and P<0.05). However, in the LAB group the FP was lower than those of other groups (P<0.01). No significant effects were determined between the C and LEN groups in terms of TDN, OM, NCF, TC, ME and FP. Molasses and LAB inoculants were increased energy value parameters. Islam et al (2001) reported that molasses and inoculant addition did not affect gross energy of silages. Feed value of ensiled feed is affected by variety of factors such as seed content that varies according to vegetation period, NDF content and digestibility, carbohydrate, fat and protein content of feed (Bal et al., 1997).

Table 3. The effects of molasses, lactic acid bacteria inoculants and enzyme supplementation on the total digestibility nutrients, organic matter, non-fiber carbohydrate, total carbohydrate, metabolizable energy and fleig point

	Treatment groups				SEM	P
Items	**C**	**M**	**LAB**	**LEN**		
Total digestibility nutrients, % DM	78.26^{b}	80.93^{a}	76.55^{b}	78.13^{b}	0.497	**
Organic matter, % DM	86.37^{b}	87.28^{b}	89.95^{a}	86.28^{b}	0.458	**
Non-fiber carbohydrate, % DM	34.28^{b}	39.28^{a}	36.12^{b}	34.61^{b}	0.659	*
Total carbohydrate, % DM	73.90^{b}	74.97^{b}	78.26^{a}	74.43^{b}	0.584	*
Metabolizable energy, Mcal/kg DM	12.34^{b}	12.47^{b}	12.69^{a}	12.34^{b}	0.044	**
Fleig point	104.18^{a}	107.03^{a}	99.28^{b}	103.80^{a}	0.881	**

$^{a \text{ and } b:}$ Values with different superscript in a line differ significantly, P:probability, *:P<0.05, **:P<0.01, SEM: pooled standard error of means, C: control, M: 5% molasses, LAB: Lactic acid bacteria inoculant, LEN: enzyme and bacteria inoculants, DM: dry matter.

Conclusion

In conclusion, addition of molasses increased DM and lowered ADF and NDF level in silage samples. Also, molasses inclusion increased WSC and LA concentration in silages. The LEN group (LAB+enzyme mixture) did not influence positively silage quality traits. The molasses and LAB inoculant addition positively affected silage TDN, OM, NFC, TC and ME levels. According to these results molasses and LAB can be preferred as a sunflower silage inoculant.

Attachments

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