Atatürk Üniv. Ziraat Fak. Derg.
39 (2), 201-207, 2008
ISSN : 1300-9036

Influence of microbial inoculation and molasses and their combination on fermentation characteristics and ruminal degradability of grass silages

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Geliş Tarihi : 19.02.2008

ABSTRACT. The aim of the current investigation was to determine the effect of applying a bacterial inoculant, molasses, and inoculant plus molasses combination on the fermentation dynamics of grass silages under laboratory conditions and ruminal degradabilities of dry matter (DM), acid detergent fiber (ADF) and neutral detergent fiber (NDF) for various incubation times. After harvesting, fresh grass forages containing 32% DM with or without additives were ensiled in 1-liter glass silos. Experimental groups were T0: no additive (control group), T1: microbial inoculation at 4.7 x 10^8 cfu kg^{-1} of silage DM, T2: addition of molasses at 5% of forage DM, and T3: a combination of additives T1 and T2. After 120 d ensiling, mini silos were opened and analyzed. It was found that DM's of molasses-added silage and molasses plus inoculant-added silage were higher than those of the other groups. Additives did not effected lactic acid concentration, and increased propionic acid while lowed silage pH. Molasses increased acetic acid concentrations. While not effected of additives on degradability 96h of DM and NDF in rumen of silages, molasses increased ADF degradability compared to control.

Key words: grass silage, additives, molasses, inoculant, quality, degradability

ÖZET : Bu çalışmanın amacı, bir bakteriyel inokulant, melas ve inokulant+melas kombinasyonunun laboratuvar şartlarında çayır silajının fermentasyon dinamiği ve değişik inkübasyon sürelerinde kuru madde (KM), asit detergent fiber (ADF) ve neutral detergent fiber (NDF)in rumende parçalanabilirliği üzerine etkilerini incelemektir. Biçimden sonra, % 32 kuru madde (KM) içeren taze çayır otu katkılı ve katkısız olarak 1 litrelik cam kavanozlarda silolanmıştır. Deneme grupları, TO: control, T1 : silaj kuru maddesinde 4.7 x 10^8 cfu kg mikrobiyal inokulant, T2: silaj kuru maddesinde % 5 melas, T3: T1 ve T2 katkılarının kombinasyonundan oluşturuldu. Silolamadan 120 gün sonra mini silolar açıldı ve analiz edildi. Silajlara melas ve melas + inokulant ilave edilen gruplardaki kuru madde miktarı diğer gruplardakinden yüksek bulundu. Katkılar silaj pH'sını düşürürken propiyonik asiti artırdı, laktik asit konsantrasyonunu etkilemedi. Katkılar rumende kuru madde (KM) ve neutral detergent fiber (NDF)'in 96 saatteki parçalanabilirliğini etkilemezken, melas katkılı silaj gruplarının rumende asit detergent fiber (ADF)'in parçalanabilirliğini control grubuna göre artırmıştır.

Anahtar Kelimeler : Çayır otu silajı, katkılar, inokulant, kalite, parçalanabilirlik

INTRODUCTION

Ensiling has been utilized for centuries in many places of the world. It is well known that silage has become one of the main methods of storing green nutritious fodder in times of plenty for feeding ruminants when feed is scarce. Some silage material contains inadequate numbers of viable homofermentative lactic acid bacteria (LAB) and low water-soluble carbohydrate (WSC). The most available inoculants consist of selected strains of homofermentative LAB, such as *Lactobacillus plantarum, Pediococcus,* and *Enterococcus* species (Filya et al., 2000).

In order to improve crop preservation and its feeding value various additives such as bacterial inoculant, molasses, etc. have been applied (Keady et al, 2000). Ensiling of poor quality of native grass pastures needs to adding the carbohydrate-rich by-products like molasses to promote the activity of epiphytic lactic acid bacteria. Molasses is a palatable source of fermentable carbohydrates which contains 79% WSC; 45 to 50% of which sucrose is the main component has been used as a fermentation stimulant for many years in silage. Also, it has relatively high concentrations of calcium, potassium and sulphur, but contains relatively little crude protein (Özen et al. 1993; Aksoy et al. 2000).

In the current study, ruminal degradabilities of DM, ADF and NDF of silages with or without additives were determined in various incubation periods. As known, acid detergent fiber (ADF) and Neutral detergent fiber (NDF) are most often used to as an important measurement characterize the nutritional value of forages. Generally, digestibility and intake are estimated from ADF and NDF analyses (Sarwar et al., 1992).

The objective of the present study was to determine the effects of molasses and microbial inoculation and their combination on ensiling

characteristics, chemical composition and ruminal degradability of grass silage nutrients.

MATERIAL AND METHODS

Forage production and ensiling

Cool season gramineae grasses are widespread in Eastern Anatolia Region of Turkey. Plant communities of Erzurum meadows (longitude: 41.3, latitude: 39.9 and altitude: 1850m) were dominated by meadow barley (*Hordeum violaceum*), Kentucky bluegrass (*Poa pratensis*), and subdominated were by meadow foxtail (*Alopecurus pratensis*), red clover (*Trifolium pratense*), and meadow fescue (*Festuca pratensis*) (Gökkuş and Koç, 1996; Çomaklı et al., 2004). The forage used as a silage material was harvested in anthesis (full blossom) cutting stage from the grasslands belonging to Research and Application Farm of Atatürk University. At the beginning of the trial, the fresh forage had 32% DM, 6.99% CP, %9.11 Ash, 36.06% ADF and 45.05% NDF on dry matter basis. The samples of the swaths were chopped using a rotary guillotine (chop-length of 30 mm). Inoculation of fresh silage material was carried out according to manufacturer prospectus. 5 mg bacterial inoculum powder (SHC500, $3x10^{10}$ cfu g^{-1}) was dissolved in 1 ml water, and then it was sprayed into the 1 kg fresh forage material using spray-applicators. Microbial inoculant (S.H.C.500 INOCULANT, contained $3x10^{10}$ cfu g^{-1} LAB) consisted of *Pediococcus acidilactici*, *Propionobacterium shermanii*, *Lactobacillus plantarum, Enterococcus faecium*, *Aspergillus niger* and *Bacillus subtilis*; together with the enzymes *amylase, cellulase* and *hemicellulase*. Additionally, molasses was added at a rate of 50g per kg of forage DM (5% of forage DM), and then molasses-added forages were thoroughly mixed. Experimental groups were T0: no additive (control group), T1: microbial inoculation (containing LAB, at $3x10^{10}$ cfu g^{-1}) at 4.7 x 10^8 cfu/ kg of forage DM, T2: addition of molasses at 5% of forage DM, and T3: a combination of additives T1 and T2. The treatments were ensiled in 1-liter glass jars equipped with a lid that enables gas release. The jars were stored at ambient temperature of 20±2°C approximately for 120 days. Ten 1-liter mini silos were filled for each treatment, and at the end of the ensiling, five jars from each treatment were opened, mixed and sampled, and then these samples were frozen at -20°C for future chemical analysis. These samples was analyzed for organic acids, rumen degradability, ADF and NDF levels.

Chemical Analysis

Four treatment groups were evaluated in an experiment as a completely randomized design with 5 replicates for dry matter (DM), crude protein (CP), ash and pH. Five silo jars from per treatment were opened and sampled for chemical and ruminal degradability analysis after ensiling (stored -20°C). Dry matter, ash and CP contents of silages were defined to the procedure of Association of Official Analytical Chemists (AOAC, 1990). ADF and NDF were analyzed according to method of Van Soest and Robertson (1979). To determine the fermentation products, a 20-g sample of silage was taken from each silo jar and homogenized in 100 ml distilled water and filtrated, and then the filtrate was used for pH (Polan et al., 1998), lactic acid, acetic acid and propionic acid measurements. pH analysis was determined by digital pH meter in triplicate. Short-chain fatty acid analysis of silages was accomplished by using gas chromatograph (Shimadzu GC-14B) according to the Leventini et al. (1990).

On the other hand, degradability of DM, ADF and NDF was expressed as digestibility coefficient (g/kg) in related tables. To determine the degradabilities of these parameters, four ruminally cannulated Awassi rams about two years of age (weighing 40-42 kg) were used. Animals were kept in individual pens and fed a diet to meet their maintenance requirements (Ørskov and McDonald, 1979). A good quality dried alfalfa hay and commercial concentrate was offered to meet 1.25x maintenance requirements. As daily forage and concentrate fed was given 600 g from each one to rams. The concentrate had 88% DM, 16% CP, 10% CF and 2500 kcal ME per kg. Animals were fed twice daily with one half of ration at 7:30 h and the other half at 16:00h. Also, water consumption was ad-libitum.

For ruminal degradability analysis, approximately 3 g fresh silage samples were put into nylon bags (5x12 and 102 µm pores) two replicated from each samples, and then, they were incubated in the rumen of each animal in quadruplet for 4, 8, 16, 24, 48, 72 and 96 hours. Upon removal from rumen, the bags were immediately soaked in cold water (+3°C) and washed in a washing machine for 20 minutes. Then, these bags were dried for 72h at 65°C for residual dry matter determination.

Statistical Analyses

The analyses of variance were performed by the General Linear Model procedure of SAS (1998) for the completely randomized experimental design for the analyses of the effects of treatment on DM, CP, ash

contents, pH, acetic, propionic and lactic acid in silages. The mathematical model was: $y_{ij} = \mu + a_i + e_{ij}$. On the other hand, the analyses of ruminal degradability (DM, ADF, NDF) were conducted using 4x4 Latin square designs, and the model was: $y_{ijk} = \mu + a_i + b_j + (axb)_{ij} + e_{ijk}$. Where: y_{ij} and y_{ijk} is the observation of i treatment and j incubation period; μ: population mean; a_i: is the effect of i treatment; b_j: is the effect of j incubation period; e_{ij} and e_{ijk}: is the experimental error. Differences between means were determined by Duncan's multiple range test at a significance level of $P < 0.05$.

Results

Chemical composition of grass silages was presented in Table 1. Treatment had effect on DM concentration, pH value ($P<0.01$), ash and CP content ($P<0.05$). DM content increased by molasses, inoculant and molasses combination in T2 and T3 compare to control. Also, pH values in all treated silages were lower than that of the control group. Ash content of the silages was lower in only T3 silage than control. Crude protein content of the silages was high in T2 than those other groups, but It was obtained different in only T1 and T2 groups..

Table 1. Chemical composition of silages with or without additives.

Treatment / Parameters	T0	T1	T2	T3	± *SEM*	*Significance*
DM	340.3[b]	347.9[b]	364.6[a]	379.0[a]	4.9	**
pH	4.76[a]	3.89[b]	4.00[b]	3.93[b]	0.04	**
Ash, g/kg DM	111.4[a]	107.0[ab]	107.6[ab]	102.2[b]	1.7	*
CP, g/kg DM	76.1[ab]	71.8[b]	80.3[a]	75.1[ab]	1.7	*

(N= 5, for each treatment), *: P<0.05, **: P<0.01, DM: Dry matter; CP: crude protein; SEM: Standart error means, a,b: Means within a row with no common letters differ significantly at $P < 0.05$. T0: no additive (control group), T1: microbial inoculation at 4.7 x 10^8 colony forming units (cfu)/kg of silage DM, T2: addition of molasses at 5% of forage DM, and T3: a combination of additives T1 and T2.

In fermentation products, Table 2 showed that there was no statistically difference amongst silage groups in lactic acid concentration. On the other hand, the treatments significantly increased the propionic acid concentration ($P<0.01$).

Table 2. Organic acid concentrations of silages with or without additives (g/kg DM).

Treatment / Parameters	T0	T1	T2	T3	± *SEM*	*Significance*
Lactic acid	32.18	32.57	24.21	28.12	2.76	NS
Acetic acid	21.46[b]	22.71[b]	30.92[a]	23.41[b]	2.34	*
Propionic acid	21.88[b]	38.24[a]	34.37[a]	36.41[a]	3.55	**

(N= 5, for each treatment), *: P<0.05, **: P<0.01, NS: Nonsignificant, SEM: Standart error means, a,b: Means within a row with no common letters differ significantly at $P < 0.05$.

Acetic acid concentration was higher molasses-added silages than those other treatment groups.

Digestibility coefficients of silages are in Table 3.

Table 3. Ruminal degradability of DM of grass silages with or without additives (as digestibility coefficient).

Treatment / Incubation (h)	T0	T1	T2	T3	± *SEM*	*Significance*
4	0.6731	0.6651	0.6816	0.6845	0.0084	NS
8	0.7508[a]	0.6971[b]	0.7187[ab]	0.7423[a]	0.0146	*
16	0.7247	0.7340	0.7336	0.7199	0.0088	NS
24	0.7704[a]	0.7845[a]	0.7470[b]	0.7567[b]	0.0095	*
48	0.7718[b]	0.8132[a]	0.7857[b]	0.7795[b]	0.0096	*
72	0.8127	0.8057	0.8149	0.8009	0.0117	NS
96	0.8210	0.8514	0.8394	0.8468	0.0128	NS

(N= 5, for each treatment), *: P<0.05, NS: Nonsignificant, DM: Dry matter, SEM: Standart error means, a,b: Means within a row with no common letters differ significantly at $P< 0.05$.

Digestibility of dry matter was not affected by treatment in various (4, 16, 72, 96h) incubation times, except for 8, 24 and 48h. However, as the time goes digestibility coefficient belonging to silages also increased.

Table 4 and Table 5 demonstrates ADF and NDF degradability in rumen for a given incubation times.

Table 4, Ruminal degradability of ADF of grass silages with or without additives (as digestibility coefficient).

Treatment / Incubation (h)	T0	T1	T2	T3	±*SEM*	*Significance*
4	0.5280	0.5259	0.5254	0.5278	0.0048	NS
8	0.5239	0.5239	0.5357	0.5401	0.007	NS
16	0.5508	0.5424	0.534	0.5349	0.006	NS
24	0.5387	0.538	0.5324	0.5427	0.0059	NS
48	0.5477	0.5186	0.56	0.5533	0.015	NS
72	0.5497	0.5439	0.5338	0.5327	0.0081	NS
96	0.5399[b]	0.5419[ab]	0.5485[a]	0.5294[ab]	0.0058	*

(N= 5, for each treatment), *: P<0.05, NS: Nonsignificant, ADF: Acid detergent fiber; SEM: Standart error means, a,b: Means within a row with no common letters differ significantly at $P < 0.05$.

Table 5. Ruminal degradability of NDF of grass silages with or without additives (as digestibility coefficient).

Treatment / Incubation (h)	T0	T1	T2	T3	±*SEM*	*Significance*
4	0.7608 [a]	0.7479[ab]	0.7288 [b]	0.7337[ab]	0.0098	*
8	0.7022	0.7723	0.7558	0.7655	0.0259	NS
16	0.7751	0.7111	0.7785	0.7701	0.0218	NS
24	0.7964	0.7943	0.8093	0.8141	0.0134	NS
48	0.8068	0.8135	0.8201	0.8096	0.0138	NS
72	0.8065	0.7961	0.8083	0.8034	0.0085	NS
96	0.7930	0.7911	0.7641	0.7691	0.0232	NS

(N= 5, for each treatment), *: P<0.05, NS: Nonsignificant; NDF: Neutral detergent fiber; SEM: Standart error means, a,b: Means within a row with no common letters differ significantly at $P < 0.05$.

DISCUSSION

The degradability DM of feeds is one of important key variables for nutritive value in feeding systems. This parameter may be affected or limited by both diet fiber and the rate at which that fiber is digested in the rumen. With a few exceptions, additive treatment was beneficial for reducing pH, DM losses in silages (Patterson et al., 1998). In numerous experiments (Seale et al., 1986; Zahar et al., 2002), silages supplemented with molasses have been proven to be an effective silage additive in terms of promoting lactic fermentation, reducing silage pH, discouraging a clostridial fermentation and proteolysis, and generally decreasing organic matter losses. In the current experiment, dry matter was lower ($P<0.01$) in untreated (T0) and inoculated (T1) silages than those of molasses-treated silages (T2 and T3), which had similar DM values each other (Table 1). Molasses supplementation reduced pH and increased DM as compared with control. Hargreaves et al. (1984) reported that the dry matter of corn silage supplemented with molasses and ammonia was higher than that of the control. Also, Kurtoğlu (1998) observed that as fresh alfalfa fortified with molasses at 5% of DM at ensiling, silage dry matter significantly increased.

The highest pH value was in control group (4.76), whereas inoculated groups had lower pH (Table 1). The addition of LAB inoculants at ensiling is intended to ensure rapid and vigorous fermentation that results in faster accumulation of LA, lower pH values. Umana et al., (1991) showed that an inoculant containing homolactic bacteria enhanced the quality of bermudagrass silage when it was supplemented with molasses. It was observed that microbial inoculation lowered pH and improved the lactic acid: acetic acid ratio in more than 60% of studies conducted between 1990 to 1995 years (Muck and Kung, 1997). Baytok et al. (2005) noted that molasses stimulates silage fermentation, but it is not able to prevent enough proteolysis due to slow reduction in pH.

The additives statistically affected ash concentration of silages ($P<0.05$). Whereas control group had highest ash content, this value was lower in molasses and inoculant-added silages (especially, in T3). Castle and Watson (1985) observed that ash contents of grass silages fortified with molasses at level of 1.0, 2.0 and 3.0% on DM basis were 8.8, 8.6 and 9.4%, respectively. Ash contents of the current study were higher than the findings of researchers mentioned above.

Crude protein was higher T2 than T1, and it was found higher in silage which treated with molasses alone. Similarly, Adesogan et al. (2004) found that untreated silages had lower CP concentrations than inoculated and molasses-added silages. This differences to appear in dry matter, ash and crude protein can be arise from silage fermentation or itself of native grass pastures.

The production of lactic acid did not differ amongst silages. The treatment had significant effect on propionic acid concentration ($P<0.01$). Control group (T0) had the lowest propionic acid as compared with the other silages. These results are consistent with previous findings (Meeske and Basson, 1998; Nousiainen et al., 2003) in lactic and acetic acid concentrations. Acetic acid level increased in molasses-treated group. This may be arisen from fermentation of pentose sugars, which come in to existence depending on degradability of hemicellulose by homofermentative LAB inoculants. Additives were to cause a homofermentative fermentation.

Nadeau et al. (2000) and Zahiroddinia et al. (2004) indicated that mixtures of inoculants and enzymes, where utilized, have also been employed on grass silages and, in some instances, have resulted in considerable improvement in grass silage quality. Keller et al. (1994) supplemented with enzyme-inoculant mixture to alfalfa forage (18% of DM) and observed that the supplementation increased pH, lactic acid and acetic acid levels in comparision control silage.

Digestible organic matter content in dry matter of grass silage is essential measurement in the formulation of ruminant rations (Beever and Mould, 2000; Yan and Agnew, 2004). Ruminal degradability of silage dry matters increases as incubation time goes in the rumen (Table 3). The degradability amongst treatment groups was significantly different at 8, 24 and 48 h of incubation period ($P<0.05$). This result agrees with the findings of Demirel et al. (2003). Gallop et al. (2005) obtained constructive effect on dry matter degradability of these additives, other Kung and Muck (1997) did not obtained. The highest degradability (at 48h) was obtained from inoculated group (T1), perhaps due to live inoculant. But dry matter degradability did not differ at 72 and 96h. Because, Gollop et al. (2005) and Weinberg et al. (2004) reported that LAB in inoculum are able to survive up to 48 or 96 h in rumen. Some data suggests that certain microbial inoculants can increase fiber digestion. Decreases in fiber content may be due to partial acid hydrolysis of hemicellulose (Kung and Muck, 1997). On the other hand, Keady

(1996) concluded that molasses treatment improved silage preservation, but did not significantly alter the silage digestibility or animal performance although silage DM intake was improved.

CP concentration of silages did not affect the DM degradability in all incubation times, whereas there is in the literature some evidence of significant relationships between CP content and DM degradability in forages. In a study, the indicated that (Gül, 2000) crude protein level did not effect the degradability dry matter of silage, on the other hand it was reported that (Hoffman et al. 1993) a positive relationship of DM degradability with CP concentration and a negative relationship with dietary fibrous fraction (Von Keyserlinngk et al. 1996). Umana et al. (1991) found that molasses-added and inoculated groups alone had higher degradability of DM compared with control.

While ADF is important because of relating to the ability of an animal to digest the forage, NDF is the best indicator of how much forage an animal will eat. It was determined that there was significant difference amongst groups at 96 h ($P<0.05$) in ADF digestibility. ADF degradability at 96h was higher in molasses-added (54.48%) silages than those control silage (53.99%). This state can be arise from a positive effect on silage fermentation of molasses. At 4h, NDF was higher degraded (76.08%) in control group than those of T2. At 72 and 96h did not obtain different in among groups. Chen et al. (1994) reported that addition of enzyme and inoculant combination to straw silage increased NDF degradability.

CONCLUSION

In this study, addition of molasses, bacterial inoculant or their combination to fresh silage material had a positive effect on silage pH, but did not effect of lactic acid concentration. Molasses-added did increase of DM, pH, acetic acid, propionic acid concentration and at 96 h ADF degradability compare to control. On the other hand it was not positive effect on the other parameters. As a result, in this study arrived result to happen a positive effect on silage parameters of alone molasses-added of pasture silage.

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