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Research Article

The Effects of Formic Acid, Molasses and Inoculant as Silage Additives on Corn Silage Composition and Ruminal Fermentation Characteristics in Sheep*

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Abstract: The objective of this study was to determine the effects of formic acid, molasses, and microbial inoculant (homofermentative lactic acid bacteria) as silage additives on silage quality and ruminal fermentation characteristics. Silages with or without formic acid (0.5%), molasses (5%), or microbial inoculant (10 g/t) were fed to ruminally cannulated, 1.5 year-old Kıvırcık x Morkaraman sheep.

Silage treated with molasses had significantly greater DM and CP concentrations compared with other groups ($P < 0.05$). pH values did not significantly differ among treatments ($P > 0.05$). Lactic acid concentrations were significantly higher in silages treated with enzyme or molasses compared with others ($P < 0.05$). While acetic acid concentration was the highest in silage treated with acid, it was the lowest in silage treated with molasses ($P < 0.05$).

Silage NH_3-N concentration was the highest in silage treated with molasses, but the lowest in silage treated with acid ($P < 0.05$). Post-feeding ruminal total organic acid concentrations were significantly greater in sheep fed silages with additive than the control ($P < 0.05$). While percentages of acetic acid were greater, percentages of butyric acids were less in the rumen fluid of sheep fed silage without additive compared with the rumen fluid of sheep fed silage treated with silage additives. However, percentages of propionic acid did not differ among treatments.

Key Words: Corn silage, formic acid, molasses, bacterial inoculant, ruminal fermentation

Formik Asit, Melas ve İnokulant Katkılı Mısır Silajının Bileşimi ve Koyunlarda Ruminal Fermantasyon Üzerine Etkisi

Özet: Bu araştırma formik asit, melas ve mikrobiyal inokülant (homofermentatif laktik asit bakterileri) katkılı mısır silajlarının kalitelerini ve koyunlarda rumen fermantasyonuna etkilerini incelemek amacıyla yapıldı. Katkısız ve formik asit (% 0,5), melas (% 5) ve inokulant (10 g/ton) katkılı silajlar rumen kanüllü 1,5 yaşlı Kıvırcık x Morkaraman koyunlara yedirildi.

Melas katkılı silajlarda KM ve HP içerikleri diğer gruplara göre yüksek bulundu ($P < 0,05$). Muameleler arasında silaj pH'sı bakımından farklılık bulunmadı. Laktik asit düzeyi enzim ve melas katkılı gruplarda diğer gruplara göre daha yüksek belirlendi ($P < 0,05$). Asetik asit düzeyi en yüksek asit katkılı, en düşük melas katkılı grupta belirlendi ($P < 0,05$). Silajları tüketen tokluların yemleme öncesi ve sonrası rumen sıvısı organik asit miktarlarında katkılı silajlar lehinde farklılık gözlenirken ($P < 0,05$); katkılı silaj tüketen toklularda genel olarak asetik asit miktarı daha düşük, bütirik asit miktarı daha yüksek tespit edildi. Muameleler arasında propiyonik asit bakımından farklılığa rastlanmadı.

Anahtar Sözcükler: Mısır silajı, formik asit, melas, bakteri inokulantı, ruminal fermantasyon

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Introduction

The major goal in silage making is to preserve silage material with minimum nutrient loss. In order to achieve this goal, growth of lactic acid bacteria should be stimulated. Especially, formic acid is widely used to accomplish this target. While molasses is commonly used to provide readily available energy for lactic acid fermentation, bacterial inoculant is used to establish a desirable microbial flora in silage. Addition of formic acid to silage material has been reported to have generally positive effects on fermentation (1,2). It was reported that molasses stimulates silage fermentation (3), but it is not able to prevent proteolysis enough due to slow reduction in pH with molasses addition. Bacterial inoculants have positive effects on pH and lactic acid levels, an indication of good fermentation (4,5). When major bacterial population is lactic acid bacteria, fermentation products are mainly lactic acid, and acetic acid and ethanol at low levels. This type of silage increases dry matter intake (6,7), dry matter and organic matter digestibilities, and thus increases animal performance in ruminants (8,9). However, when animals are fed silage-based diets, metabolism of lactic acid is so fast that it is converted into acetic acid within 25 min. When lactic acid is used as energy source by rumen microbes, it enters into cell by active transport, which requires twofold energy; thus, it is not a good source of energy for rumen microbes. Thereby, silages high in lactic acid content may result in low microbial protein synthesis in the rumen (10). Formic acid as silage additive has anti-bacterial effect on many bacteria spp., including lactic acid bacteria; thus, addition of formic acid into silage results in limited fermentation and reduction in organic acid content of silage. This type of silage contains a greater amount of water soluble carbohydrate, which is a better source of energy for rumen microbe than lactic acid (11).

The objective of this study was to determine the effects of formic acid, molasses, and bacterial inoculant as silage additives on silage quality and ruminal fermentation characteristics.

Materials and Methods

Corn hybrid (Tareks 644®) was harvested by a one-row forage harvester at dough stage of kernel maturity. Four different silages were prepared form chopped forage. Silage treatments included control (no additives) 5% molasses, 0.5% formic acid, and 10 g/t inoculant (maize-all® obtained from Alltech). As recommend by the manufacturer, inoculant was added at $1.0x10^{11}$ cfu/g of fresh forage. Silages were prepared (quadriplicate) in approximately 150-l capacity plastic barrels with tight lids.

Ensiling were performed by stamping as much of chopped plant material into the barrels as possible. By this action most of the air was excluded. After ensiling, each barrel was sealed off tightly with a lid. The lids were poked with a pin to get rid of gas pressure that built up during the initial phase of ensiling and then the barrels were set upside down. The barrels were then stored for 60 d in a dark room with a temperature ranging between 20 to 25 °C. After 60 d ensiling, samples were taken to different locations of opened barrel during feeding trail. From this material, sub-samples were taken for determinations of dry matter, pH, organic acids, and chemical composition of silages.

Four rumen fistulated Morkaraman x Kıvırcık lambs, weighing 35 ± 1.2 kg, were used in metabolism trail. The experiment was carried out using 4 x 4 Latin square designs with 14-day adaptation and 1-day sampling periods. The animals were offered 20% cottonseed meal and 80% corn silage with or without treatment with silage additives, ad libitum intake.

During the experiment, all animals were housed in metabolism cages and fed twice daily at 08:00 and 20:00 h. Drinking water and vitamin-mineral block were always available.

Forty-milliliter samples of rumen fluid were removed at 0, 2, 4, 6, 8 and 10 h post-feeding via the rumen cannula by suction pump, acidified with HCl and stored for organic acid and NH_3-N analysis. Twenty milliliters of rumen fluid was used for NH_3-N concentrations using the distillation of Kjeldahl procedure. The remaining 20 ml was analyzed for volatile fatty acid concentrations using gas chromatography (Shimadzu, GC-14B) as described by Leventini et al. (12).

DM, ash, and CP contents of feed were determined following the procedure of Association of Official Analytical Chemists (13). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the method of Van Soest and Robertson (14).

The pH of each sample was determined in triplicate using approximately 25 g wet ensilage added to 100 ml

of distilled water. After hydration for 10 min using blender, the pH was determined using digital pH meter (15). The filtrate were filtered through filter paper, centrifuged and stored for organic acid analysis. All silage organic acids analysis was accomplished by using gas chromatograph (Shimadzu, GC-14B) as described by Leventini et al. (12).

Statistical analysis of data

All data were subjected to analysis of variance using General Linear Model procedure of SAS (16). Mean treatment differences were determined by Duncan's multiple range tests with a level of statistical significance of 5% (17).

Results

Chemical compositions of silages are presented in Table 1. The concentrations of DM, NDF, ADF, and CP were affected by molasses treatment.

pH, organic acids and NH_3-N concentrations of silages are presented in Table 2. While addition of formic acid into silage increased, addition of molasses decreased acetic acid concentration of silages.

Post-feeding ruminal organic acids levels are presented in Table 3. These parameters were variable at certain sampling times.

Discussion

The concentrations of DM, NDF, ADF, and CP were significantly different in silage treated with molasses compared with other groups ($P < 0.05$).

High DM content of silage treated with molasses may have resulted from the high DM content of molasses used, which is consistent with the results of Hinds et al. (18), and Lattemae et al. (19). Similarly, increased CP concentration may have caused by relatively higher CP content of molasses. There are conflicting data in literature about the effects of molasses on CP content of silage. Researchers reported that addition of molasses into silage increased (19,20), did not affect (21,22), or even decreased (23) CP content of silages. Both concentrations of NDF and ADF were significantly lower in silage treated with molasses compared with control or silage treated with formic acid ($P < 0.05$). These decreases in NDF and ADF concentrations may have resulted from increased cell wall digestion due to increased silage fermentation caused by addition of molasses (7,24).

Formic acid treatment did not alter lactic acid concentration of silage. Many researchers have reported that addition of formic acid into silage decreased silage lactic acid content by limiting silage fermentation (20,21); however, there are some data indicating that

Table 1. Chemical composition of silages with or without silage additives (% DM).

	Control	Acid	Inoculant	Molasses	SEM
DM	26.90^{b}	26.82^{b}	25.31^{b}	29.53^{a}	1.26
Ash	8.90	9.69	10.06	9.96	1.69
OM	91.10	90.31	89.94	90.04	1.69
NDF	61.89^{a}	60.91^{a}	55.31^{ab}	52.49^{b}	5.06
ADF	36.21^{a}	35.22^{a}	32.24^{ab}	29.21^{b}	3.23
CP	7.37 ^{b}	7.19 ^{b}	7.77^{b}	10.12^{a}	1.12

^{a,b}: Means with different superscript within same row significantly differ ($P < 0.05$).

Table 2. pH, organic acid and NH3-N concentrations of silages with or without silage additives.

	Control	Acid	Inoculant	Molasses	SEM
PH	3.77	3.96	3.86	4.03	0.33
Lactic acid	1.08^{b}	1.52^{b}	3.09^{a}	3.80^{a}	0.41
Acetic acid	1.26^{b}	3.22^{a}	1.70^{b}	0.91^{c}	0.55
Butyric acid	0	0.16	0.27	0.09	0.45
NH_3-N	1.06^{ab}	0.84^{b}	0.94^{ab}	1.38^{a}	0.37

^{a,b}: Means with different superscript within same row significantly differ ($P < 0.05$).

Table 3. Post-feeding ruminal organic acid and NH_3-N concentrations of sheep fed silages with or without silage additives.

	0 h	2 h	4 h	6 h	8 h	10 h
			Total Organic Acid Concentrations, mM			
Control	157.73 ^{b}	241.98 ^{a}	176.10 ^{b}	136.40 ^{c}	118.98 ^{b}	138.58 ^{b}
Acid	168.15 ^{ab}	217.38 ^{a}	200.70 ^{a}	207.55 ^{a}	189.28 ^{a}	180.47 ^{a}
Inoculant	133.13 ^{c}	143.40 ^{b}	159.48 ^{c}	190.58 ^{ab}	136.88 ^{b}	131.28 ^{b}
Molasses	144.30 ^{b}	210.10 ^{a}	185.65 ^{ab}	175.63 ^{b}	133.77 ^{b}	114.03 ^{b}
SEM	5.32	8.01	6.03	7.01	9.01	10.07
			Acetic Acid, mM/ 100 mM			
Control	63.49 ^{a}	61.26	62.90 ^{a}	64.94 ^{a}	64.96 ^{a}	56.92
Acid	59.01 ^{b}	61.24	47.75 ^{b}	51.79 ^{c}	54.95 ^{b}	58.13
Inoculant	58.13 ^{b}	62.94	55.81 ^{a}	54.32 ^{b}	64.29 ^{a}	60.83
Molasses	53.66 ^{c}	61.26	60.72 ^{a}	54.33 ^{b}	53.27 ^{b}	62.89
SEM	2.02	1.33	5.01	0.51	5.61	4.20
			Propionic Acid, mM/100 mM			
Control	18.62	20.28	19.73	19.61	19.29	22.11
Acid	20.10	19.40	23.31	22.21	19.78	19.11
Inoculant	19.23	18.26	21.03	22.05	19.28	19.67
Molasses	21.67	19.79	18.78	22.96	21.94	19.73
SEM	1.62	1.82	4.32	2.78	1.87	1.43
			Butyric Acid, mM/100 mM			
Control	17.90 ^{c}	22.40	17.36 ^{c}	15.45 ^{b}	15.75 ^{b}	20.97
Acid	20.89 ^{bc}	19.36	28.95 ^{a}	26.00 ^{a}	25.27 ^{a}	22.76
Inoculant	22.65 ^{ab}	18.81	23.17 ^{b}	23.63 ^{a}	16.43 ^{b}	19.51
Molasses	24.68 ^{a}	18.95	20.50 ^{bc}	22.72 ^{a}	24.80 ^{a}	17.39
SEM	2.06	4.37	2.41	1.91	3.01	3.33
			Rumen NH_3-N, mg/dl			
Control	13.47 ^{a}	15.57	12.21	12.85	12.10	12.93
Acid	10.91 ^{b}	12.88	11.14	9.59	10.14	9.33
Inoculant	7.72 ^{c}	12.15	11.29	10.31	9.92	10.37
Molasses	13.15 ^{ab}	14.20	10.42	10.34	10.55	11.22
SEM	1.01	2.62	1.88	2.61	1.82	2.48

^{a,b}: Means with different superscript within same column significantly differ ($P < 0.05$)

formic acid increases silage lactic acid concentrations (25,26). Inoculant and molasses treatments increased silage lactic acid levels, which are in agreement with the literature (27,28).

While addition of formic acid into silage increased, addition of molasses decreased silage acetic acid concentrations ($P < 0.05$).

Some researchers reported a decrease (20,21,27); others reported no changes in concentrations of acetic acid with addition of formic acid into silage. Silage treated with molasses had lower acetic acid concentrations compared with control ($P < 0.05$), which is in agreement with the results reported in the literature (27,29). Addition of molasses into silage have been reported to cause heterofermentative fermentation (30) or lactic acid produced during ensiling is further fermented into acetic acid, resulting in a higher acetic acid concentration with addition of molasses to silage (31).

Silage NH_3-N concentration, which reveals the extent of proteolysis in silage, was significantly ($P < 0.05$) lower in silage treated with formic acid, but numerically greater in silage treated with molasses compared with control.

While decrease in formic acid treated group can be explained with limited fermentation caused by formic acid, an increase can also be explained with increased fermentation in molasses treated group due to a higher soluble carbohydrate content of molasses.

Post-feeding total ruminal VFA concentrations and the percentage of acetic, propionic and butyric acids were variable in certain sampling time.

While percentages of acetic acid were greater, percentages of butyric acids were lower in the rumen fluid of sheep fed silage without additive compared with the rumen fluid of sheep fed silage treated with silage additives. However, percentages of propionic acid did not differ among treatments. It has been reported that the rumen fluid of animals fed forage based diets contained 60-70% acetic acid, 15-20% propionic acid, and 10-15% butyric acid (31), which support the results of the current study.

Post-feeding ruminal NH_3-N concentrations did not differ among sheep fed silages treated with different silage additives (Table 3). Sheep fed silages treated with different silage additives had ruminal NH_3-N concentrations at all sampling times in excess of 5 mg/100 ml, which has been reported to maximize microbial protein synthesis (31).

In conclusion, silage additives had no positive or negative effect on silage fermentation. However, effects of these additives on OM digestibility and microbial protein synthesis should further be studied to determine proper silage additive.

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