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Research Article

The Effects of Formic Acid or Formic Acid Plus Molasses Additives on the Fermentation Quality and DM and ADF Degradabilities of Grass Silage*

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Abstract: This experiment was carried out to evaluate the effects of formic acid and formic acid plus molasses additives on the grass silage fermentation characteristics and degradability of dry matter (DM) and acid-detergent fibre (ADF). Grasses were cut in early (experiment I), middle (experiment II) and late maturity (experiment III). The additive treatments in experiments I, II and III were: 1) Untreated; 2) 0.5% Formic acid (FA); 3) FA+2% molasses; 4) FA+4% molasses; and 5) FA+6% molasses. The silages were ensiled in jar silos (1 L) and DM and ADF degradability were determined by using the nylon bag technique in the rumens of four fistulated sheep. Increasing formic acid plus molasses decreased the ADF and neutral detergent fibre (NDF) content of silages as compared with FA and untreated silages in experiments I, II and III ($p < 0.001$). Formic acid plus molasses silages were well preserved with low pH (4.62, 4.51, 4.54), high lactic (30.62, 32.20, 28.54 g kg^{-1} DM) and acetic acid (19.21, 21.46, 20.65 g kg^{-1} DM) and low butyric acid (3.81, 2.19, 1.43 g kg^{-1} DM) as compared with untreated silages (pH: 5.12, 5.35, 5.36; lactic acid: 20.70, 21.47, 15.21g kg^{-1} DM; acetic acid: 14.32, 15.88, 11.28 g kg^{-1} DM; butyric acid: 4.50, 3.47, 6.68 g kg^{-1} DM in experiments I, II and III, respectively).

However, silage fermentation quality was not at the optimal level. The FA silages were moderately preserved with more restricted fermentation than the formic acid plus molasses and untreated groups. In experiments I, II and III, significant differences were noticed between untreated and other silages in respect of DM degradability. ADF degradability was not affected by the silage treatments at 4, 16 and 24 h, but was significantly ($p < 0.01$ or $p < 0.001$) affected at other times.

Key Words: Grass silage, formic acid, molasses, silage fermentation, degradability

Çayır Silajının Fermentasyon Kalitesi ve Rumende Kuru Madde ve ADF Yıkılabilirliği Üzerine Formik Asit veya Formik Asit+Melas Katkılarının Etkileri

Özet: Araştırma, çayır silajına formik asit ve formik asit+melas katılmasının silajın fermentasyon özellikleri, kuru madde (KM) ve acid-detergant fibre (ADF)'nin yıkılabilirliği üzerine etkisini belirlemek amacıyla yapıldı. Çayır otları erken (Deneme I), orta (Deneme II) ve geç (Deneme III) olmak üzere üç farklı vejetasyon döneminde biçildi. Her üç denemede: 1) Kontrol 2) % 0.5 Formik asit (FA) 3) FA+% 2Melas(M) 4) FA+% 4 M 5) FA+% 6 M katkılı gruplar oluşturuldu. Silajlar 1 kg'lık cam kavanozlarda inkubasyona bırakıldı. KM ve ADF'nin yıkılabilirliği rumen fistülü açılmış dört koyunda naylon kese tekniği kullanılarak belirlendi. Formik asitle birlikte artan oranlarda melas katılmasının silajın ADF ve NDF içeriğini formik asit ve kontrol grubuna göre düşürdüğü ($p<0.001$) belirlendi. Formik asit+melas katkılı gruplarda silajın kalitesinin kontrol grubuna (Deneme I, II, ve III'de sırasıyla; pH: 5.12, 5.35, ve 5.36; laktik asit: 20.70, 21.47 ve 15.21g kg^{-1} KM; asetik asit: 14.32, 15.88 ve 11.28 g kg^{-1} KM; butirik asit: 4.50, 3.47 ve 6.68 g kg^{-1} KM) göre daha iyi olduğu belirlendi. Bununla birlikte silajın fermentasyon kalitesinin optimal seviyede olmadığı görüldü. Formik asit katkısı silajın fermentasyonunu sınırlandırarak formik asit+melaslı gruplara göre orta kalitede silaj (pH: 4.62, 4.51, 4.54; laktik asit: 30.62, 32.20, 28.54 g kg^{-1} KM; asetik asit: 19.21, 21.46, 20.65 g kg^{-1} KM; bütirik asit: 3.81, 2.19, 1.43 g kg^{-1} KM) oluşturdu. Her üç denemede de melas katılan gruplarda asetik asit içeriğinin arttığı belirlendi. KM yıkılabilirliği bakımından gruplar arasında önemli farklılıklar gözlendi. ADF'nin yıkılabilirliğinin 4, 16 ve 24. saatlerde katkılardan etkilenmediği; diğer saatlerde önemli ölçüde ($p<0.01$ veya $p<0.001$) etkilendiği görüldü.

Anahtar Sözcükler: Çayır silajı, formik asit, melas, silaj fermentasyonu, yıkılabilirlik

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Introduction

Successful silage fermentation depends on achieving both anaerobic conditions and a low pH. The low pH, which is usually accomplished through the fermentation of sugars in the crop to lactic acid by lactic acid bacteria (LAB), decreases plant enzyme activity and prevents the proliferation of detrimental anaerobic microorganisms, especially clostridia and enterobacteria (1).

Mineral acids affect silage fermentation simply via reductions in pH. The mechanism of the action of formic acid becomes more complex as its concentrations are increased, since this is accompanied by a shift from species antimicrobial effects to more general antimicrobial effects, including inhibition of the LAB themselves (2).

Formic acid has long been known to be an efficient additive (3). Formic acid is an effective preservative, especially in conditions where ensiled grass is low in dry matter content and water soluble carbohydrate (WSC) (4). For most of the products available, variable rates of addition are recommended, usually from 2 to 5 L^{-1} fresh crop (5). A variable rate of application recognises that factors such as herbage species and stage of growth have marked effects on the buffering capacity of the crop (6).

The use of formic acid has been found to reduce pH, lactic acid, acetic acid and butyric acid in different kinds of silage as compared to untreated silages (7-9). When comparing untreated silages with formic acid silages, no differences were observed in digestibility (10-12). It has been reported that the digestibility of grass silage has improved after formic acid treatment (13,14).

Molasses and formic acid may be added in an appropriate mixture to take advantage of the positive effects of both additives on silage fermentation. Molasses enriches the fresh material with carbohydrate and fills the gaseous pores, thereby reducing the influx of oxygen in the silage. Formic acid ensures a rapid pH-reduction and simultaneously inhibits microbial activity (15).

The type of additive used can also influence the amount of fermentation end products. Using molasses as an additive increases the amount of fermentation end products, due to the fermentation of the available sugars (lactic acid and acetic acid) while the addition of formic acid reduces the formation of these products due to the reduced activity of microorganisms as a result of an increase in acidity (16).

The purpose of the present experiments was to study the effects of formic acid and molasses as grass silage additives on the fermentation quality and dry matter (DM) and acid-detergent fibre (ADF) degradabilities in sheep.

Materials and Methods

Materials

Plant material: The grass used in the experiment was obtained from Altındere-TİGEM. After determining the grass field of the experiment, the grass was cut in a circular path at a height of 5-10 cm with a grass-cutter in an area of 2.5 m^2. The grass was cut on June 8, 18 and 29, in the pre-bloom, half-bloom and full-bloom stages in early, middle and late maturity, respectively. The forages were dominated by grasses (> 96%). The grass was cut into approximately 1 cm lengths before ensiling. The grass for silage-making was ensiled directly. The mean composition of the grass ensiled in experiments I, II and III is given in Table 1.

Table 1. Chemical composition of the early, middle and late cut grasses.

Experiment	Exp. I	Exp. II	Exp. III
Cutting Maturity	Early cut	Mid cut	Late cut
Cutting Date	June 8	June 18	June 29
Dry Matter, g kg^{-1}	250.70	280.30	330.60
Composition of dry matter (g kg^{-1})			
Ash	80.09	80.02	70.34
Crude Protein	120.19	110.26	90.25
Crude Fat	20.55	20.98	20.87
Crude Fibre	270.02	280.74	300.50
Acid Detergant Fibre (ADF)	380.14	400.10	420.04
Neutral Detergant Fibre (NDF)	570.00	600.47	620.26

Silos: Jars (1 L capacity; holding approximately 850 g grass) were used as silos. After filling and packing down with a metal ramrod, the silos were sealed with a metal lid.

Methods

Treatments: There were five treatments: untreated (control), formic acid (FA) (0.5%), FA (0.5%) + 2% molasses, FA (0.5%) + 4% molasses, and FA (0.5%) + 6% molasses fresh grass. Each jar silo had five replicates. Molasses was heated before application to reduce viscosity, and added by using watering cans. The silos were stored at room temperature (19 ºC) and were opened 120 days after filling. Silage samples for pH were analysed immediately. Samples for chemical analyses were kept frozen.

Silage Degradability: Ruminal DM and ADF degradability were determined by incubating samples for 4, 8, 16, 24 and 48 h by using the in sacco nylon bag technique in the rumens of four fistulated sheep.

Chemical analysis: Silages were analysed for DM and crude protein (CP) according to AOAC methods (17), and for ADF and neutral-detergant fibre (NDF) according to the method of Georing and Van Soest (18). Silage pH was determined with a glass electrode after homogenization of 10 g of fresh silage with 100 ml of distilled water for 1 min in a blender (19). Liquidised silage extracts were analysed for lactic acid and VFA by HPLC.

Statistical methods: Silages were examined statistically by analysis of variance, using a 3 x 4 factorial design: three harvesting methods and four additives (untreated (control) and 2, 4 and 6% molasses). The pooled model $Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$, where μ = overall mean, a_i = effect of stage of maturity (Experiment), b_j = effect of additives, $(ab)_{ij}$ = interaction of stage of maturity and additives and e_{ijk} = error. All statistical analyses were carried out by using the Statistical Analyses System, (SAS) (20).

Results

Silage Composition

Silage composition changed with maturity (Table 2), particularly with regard to the DM, ADF and NDF

Table 2. Chemical composition of the grass silages.

Additives (A)	In Dry Matter, g/kg							
	PH	DM, g/kg	CP	ADF	NDF	Lactic Acid	Acetic Acid	Butyric Acid
				Experiment (E) I				
Untreated	5.12	264.69	116.61	314.51	642.18	20.70	14.32	4.50
FA	4.62	274.40	136.62	310.49	632.18	30.62	19.21	3.81
FA + 2%M	4.47	280.52	146.28	306.36	633.98	33.98	20.88	1.85
FA + 4%M	4.43	266.56	144.49	294.54	612.20	38.32	22.56	1.87
FA + 6%M	4.30	272.56	154.56	290.62	608.82	43.42	26.17	1.70
				Experiment (E) II				
Untreated	5.35	278.61	122.51	328.52	658.85	21.47	15.88	3.47
FA	4.51	302.40	132.47	320.24	634.44	32.20	21.46	2.19
FA + 2%M	4.59	298.56	148.45	318.21	619.30	31.03	20.68	2.82
FA + 4%M	4.52	298.29	152.49	312.19	615.65	37.18	23.92	2.95
FA + 6%M	4.66	312.34	148.42	304.41	628.72	35.40	26.58	3.15
				Experiment (E) III				
Untreated	5.36	332.66	104.62	342.55	668.75	15.21	11.28	6.68
FA	4.54	340.44	112.61	336.84	642.57	28.54	20.65	1.43
FA + 2%M	4.71	340.27	128.53	330.49	630.23	32.27	23.42	1.89
FA + 4%M	4.47	336.46	136.41	326.30	624.81	32.74	21.78	1.24
FA + 6%M	4.38	340.49	126.64	326.47	635.57	37.99	26.60	1.50
s.e	0.32	4.54	5.19	3.22	0.97	2.07	0.96	0.44
A	***	***	***	***	***	***	***	***
E	***	***	***	***	NS	*	NS	NS
AxE	NS	NS	NS	**	NS	NS	*	***

Significance: NS (non-significant); * (P < 0.05); ** (P < 0.01); ***(P < 0.001) M: Molasses, FA:Formic acid contents. Maturing increased ADF and NDF contents, but decreased CP content. In experiments I and III, increasing levels of molasses were added, or not added, to formic acid groups, and this had no effect on the DM as compared with untreated silages, although this increased ($p < 0.001$) in experiment II. In experiment I, II and III the concentration of CP increased ($p < 0.001$) when using formic acid plus molasses compared with untreated and formic acid silages. In experiments I, II and III, as compared with untreated, 2% molasses + formic acid and formic acid silages, the content of ADF decreased ($p < 0.001$) with the use of 4 and 6% molasses and formic acid applications. Increasing levels of molasses and formic acid decreased ($p < 0.001$) the content of NDF, but this effect was variable in experiments I, II and III.

Fermentation characteristics are given in Table 2. The untreated silages had a mean pH value of 5.12, 5.35 and 5.36 in experiments I, II and III, respectively, a low lactic acid and acetic acid content and a high butyric acid content, indicating inadequate levels for silage quality. In experiments I, II and III treatments had significant ($p < 0.001$) effects on the pH and lactic acid content compared with the untreated silage, but in experiment II, formic acid or formic acid plus molasses treatments had no significant effects on butyric acid content compared with untreated silage, while in experiment I it had no effect in terms of formic acid only. In experiment III both formic acid and formic acid plus molasses reduced ($p < 0.001$) butyric acid concentrations. However, the content of acetic acid was significantly ($p < 0.001$) higher in the formic acid and formic acid plus molasses silages than in the untreated silages.

There were differences among the untreated groups and the others in lactic acid concentrations in experiments I, II and III. No significant differences were noticed in butyric acid between formic acid and formic acid plus molasses treatment silages in experiments II and III. Formic acid plus molasses decreased ($p < 0.001$) silage butyric acid in experiment I. The concentration of acetic acid was increased ($p < 0.001$) by 6% formic acid plus molasses only in experiments I and II compared with formic acid treatments, but there were no significant differences between formic acid and formic acid plus molasses in experiment III.

The mean pH, lactic acid, acetic acid and butyric acid contents in the silages were linearly related to the formic acid plus molasses treatments. The pH decreased linearly, and lactic acid and acetic acid concentrations increased linearly. Concentrations of butyric acid decreased linearly with increasing formic acid plus molasses treatments in experiments I, II and III. However, in experiments I, II and III, the pH, lactic acid, acetic acid and butyric acid concentrations were not at an optimal level in all treatments, but 4 and 6% formic acid plus molasses treatments were closer to the optimal level than other added treatments and untreated silages.

In experiments I, II and III the degradability of DM was significantly affected by silage treatment (Table 3). In experiments I and II degradability of DM increased ($p < 0.001$) with formic acid plus molasses application after 16 h. As compared with untreated silage, the use of formic acid and formic acid plus molasses had no effects on the degradability of ADF at 4, 16, and 24 h (Table 4).

Discussion

The DM content was similar in the untreated, formic acid and formic acid plus molasses groups in experiments I and III. However, the DM content had no effect on increasing formic acid plus molasses treatments. This may probably be associated with ensilage conditions, ensilage structure and the collection of silage samples. On the other hand, the DM content rose significantly in formic acid and formic acid plus molasses treatments compared with untreated in experiment II. These results are in agreement with Chamberlain et al. (11). In experiments I, II and III no significant differences were noticed in ADF and CP between untreated and formic acid silages (Table 2). However, NDF decreased with the formic acid application in experiments II and III. In several experiments (21,22) these results were similar. ADF and NDF contents were lower with added increasing formic acid plus molasses in experiments I, II and III, because of the low concentrations of ADF and NDF in the molasses. These results are in agreement with those of Luis and Ramirez (23), Castle and Watson (24) and De Visser et al. (25).

Formic acid plus molasses had increased lactic acid production over the control in experiments I, II and III. However, in experiments I, II and III, the pH, lactic acid, acetic acid and butyric acid concentrations on all formic acid plus molasses treatments were not at an optimal level (Table 2). Where these formic acid additions have been associated with adverse effects on fermentation, the

Table 3. Ruminal in situ dry matter degradation (%) of silages in experiments I, II and III (n = 12).

Additives (A)	Incubation time (h)				
	4	8	16	24	48
			Experiment (E) I		
Untreated	26.60	33.27	40.70	47.41	66.20
FA	18.70	23.46	35.98	42.74	66.12
FA + 2%M	20.43	26.70	34.93	40.97	70.83
FA + 4%M	18.36	20.61	29.58	38.12	73.91
FA + 6%M	20.23	24.53	33.42	34.26	72.50
			Experiment (E) II		
Untreated	27.19	32.86	35.49	51.12	66.03
FA	20.52	34.64	39.95	53.69	73.27
FA + 2%M	18.98	33.14	37.94	52.23	72.72
FA + 4%M	26.06	34.61	43.43	53.67	71.86
FA + 6%M	26.75	30.94	37.69	55.23	72.36
			Experiment (E) III		
Untreated	30.48	36.00	37.75	46.81	69.49
FA	28.26	30.18	35.36	46.57	67.69
FA + 2%M	30.49	36.66	42.29	49.78	69.94
FA + 4%M	35.00	41.81	43.26	53.39	73.56
FA + 6%M	31.09	34.14	39.77	52.97	71.95
s.e	0.91	0.70	0.91	0.64	0.53
A	***	***	***	*	***
E	***	***	***	***	**
AxE	***	***	***	***	***

Significance: NS (non-significant); * (P < 0.05); ** (P < 0.01); ***(P < 0.001) M: Molasses, FA:Formic acid

Table 4. Ruminal in situ ADF degradation (%) of silages in experiments I, II and III (n = 12).

Additives (A)	Incubation time (h)				
	4	8	16	24	48
			Experiment (E) I		
Untreated	11.37	18.58	25.99	33.35	42.39
FA	11.49	19.90	26.00	33.74	41.86
FA + 2%M	11.39	20.41	26.15	34.13	41.76
FA + 4%M	12.23	20.11	26.39	33.77	41.06
FA + 6%M	11.92	21.35	26.33	34.22	40.83
			Experiment (E) II		
Untreated	11.16	13.38	24.88	31.26	39.94
FA	10.93	17.53	24.52	30.79	39.28
FA + 2%M	11.42	18.35	24.89	30.38	39.49
FA + 4%M	11.27	18.36	24.79	30.91	39.44
FA + 6%M	11.34	19.49	24.53	30.93	39.41
			Experiment (E) III		
Untreated	9.67	14.15	22.00	28.79	38.84
FA	9.91	16.24	22.10	28.08	37.60
FA + 2%M	10.26	15.94	22.10	28.10	37.56
FA + 4%M	10.00	16.32	22.48	28.25	37.94
FA + 6%M	11.07	16.63	22.06	28.64	37.06
s.e	0.39	0.40	0.39	0.48	0.38
A	NS	***	NS	NS	**
E	***	***	***	***	***
AxE	NS	NS	NS	NS	NS

Significance: NS (non-significant); ** (P < 0.01); ***(P < 0.001) M: Molasses, FA:Formic acid silages have been characterised by low concentrations of lactic acid and high concentrations of butyric acid. It appears that it could be due to either the inhibition of lactic acid production and the fermentation of WSC to butyric acid or the conversion of lactic acid to butyric acid in the lactic acid metabolism cycle without the inhibition of lactic acid production, and these reactions could be carried out by saccharolytic clostridia (6). There are different opinions as to whether formic acid should be added to silage or not. Some researchers have reported that formic acid addition had a comprehensive antimicrobial effect including the inhibition of lactic acid bacteria and hence caused the death of lactic acid bacteria (1,26-30).

Molasses and formic acid had more positive effects than formic acid on silage fermentation in experiments I, II and III. This would suggest that the molasses enriches the fresh material with carbohydrates. As the rate of formic acid plus molasses application increased (Table 2) there were progressive increases in the concentration of lactic acid, decreases in the pH values and higher concentrations of acetic acid than in the untreated silage. This would suggest that the fermentation was increasingly modified by the heterofermentative lactic acid bacteria as the rate of formic acid plus molasses application was increased (24). In agreement with the results of Castle and Watson (24) increasing formic acid plus molasses increased fermentation quality in silage containing formic acid and untreated silage. In contrast, no increase in fermentation quality with formic acid plus molasses was found by Lattemae et al. (15). In general, the effect of the molasses application in experiments I, II and III was similar to that in the large number of trials reviewed by McDonald (26).

The results show that either the formic acid or the formic acid plus molasses produced an alteration in silage fermentation quality compared with untreated silage in experiments I, II and III. However, silage fermentation was not at an optimal level.

The use of formic acid and formic acid plus molasses increased the degradability of DM in experiments I, II and III, indicating a change in the composition of the ADF fraction during ensiling (Table 3). No literature has been encountered relating to the DM degradability in the rumen of the molasses and/or formic acid added silages by using the nylon bag technique. Consequently, results obtained from the nylon bag experiment were compared with in vivo digestibility experiment results. Some workers (13,14) have reported that formic acid addition to silage had an increasing effect on the digestibility of the DM. On the other hand, in studies where formic acid was used as the only additive to silage, the effect of formic acid on the DM digestibility has not been reported (10-12). Lattemae et al. (15) reported that the digestibility of 10% molasses plus formic acid-added red-clover silage increased up to the 4^{th} hour compared with the untreated and 4% molasses plus formic acid-added groups, whereas no differences were observed after the 4^{th} hour. No literature was encountered relating to the degradability of ADF with the nylon bag technique. In addition, classical digestibility experiments relating to ADF degradability were not frequently encountered. However, researchers have reported that the degradability of ADF was not affected (31) by the addition of formic acid, and that ADF degradability increased with low dry matter content (21%), and decreased with high dry matter content (25%) (10). In general, ADF degradability in the rumen was not affected by molasses and/or formic acid additives in this study.

In conclusion, the use of formic acid and increasing the rate of formic acid plus molasses as an additive improved the silage fermentation quality of grass silage as compared with untreated silage in experiments I, II and III. In this study, however, the best silage was obtained with 6% formic acid plus molasses in addition to grass. Degradability of DM improved by the level of 2, 4 and 6% formic acid plus molasses application over a long incubation time.

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