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The effects of a microbial inoculant and formic acid as silage additives on chemical composition, ruminal degradability and nutrient digestibility of corn silage in sheep

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Summary

The effects of a microbial inoculant (containing propionic and lactic acid bacteria) and formic acid on chemical composition, ruminal degradability of dry matter (DM) and nutrient digestibility of corn silage were examined. Whole-plant corn was ensiled for 60 days in plastic polyethylene bags, and three treatments were compared, 1: control (no additive), 2: *Propionibacterium acidipropionici* plus *Lactobacillus plantarum* at 3×10^{10} cfu/g of fresh forage, and 3: formic acid (98%) at 2.41/t fresh forage. The silages were subjected to chemical analysis, DM degradability and nutrients digestibility in sheep. At the end of ensiling period, treatment 3 had significantly higher ($P<0.05$) content of crude protein (CP), lactic acid, total acids, DM recovery and pH values than other treatments. Treatment 2 had the lowest pH value, the highest level of propionic acid, and the lowest level of butyric and total acids ($P<0.05$). No traces of ethanol were detected for neither of silages. CP digestibility was higher ($P<0.05$) for treatment 1 compared with others, while ether extract (EE) digestibility was higher ($P<0.05$) for treatments 1 and 3 compared with treatment 2. Effective DM degradability was higher for treatment 3. All silages went under rapid fermentation and were well-preserved and treatment 2 was more stable after opening. The degree of improvement in fermentation using microbial inoculant was lower than formic acid but expected to improve the aerobic stability by inhibition of yeast activity, especially in warm climates.

Key words: Silage additives, Formic acid, Propionic acid bacteria, Digestibility

Introduction

Ensiling is a conservation method for moist forage crops and the major goal in silage making is to preserve silage material with minimum nutrient loss (Adesogan, 2006). It is based on natural fermentation under anaerobic condition in which epiphytic lactic acid bacteria (LAB) convert water-soluble carbohydrates (WSC) into organic acid, as a result, pH decreases and the forage is preserved. To improve the ensiling process, various chemical and biological additives have been developed (Adesogan and Salawu, 2004; Adesogan *et al*., 2007). The biological additives are advantageous because they are safe and easy to use, no corrosive to machinery, do not pollute the environment and are regarded as natural products.

Addition of formic acid to silage material has been reported to have positive effects on fermentation (Haigh, 1988; Snyman and Joubert, 1996). Formic acid has anti-bacterial effect on many bacterial species, including LAB. Therefore, it results in limited fermentation and reduction in organic acid (Kennedy, 1990; Spoelstra *et al*., 1990) but a greater amount of WSC content of silage which is a better source of energy for rumen microorganisms than lactic acid (Bosch *et al*., 1991). According to the calculations made by Chamberlain (1987), the use of silage with a high content of fermentation acids may result in a substantially lower energy yield for rumen microorganisms than the use of silage of restricted fermentation. In terms of animal performance, total dry matter (DM) intake and live weight gain were significantly

higher with formic acid-treated silage than untreated or inoculant-treated silages (Haigh *et al*., 1987).

Bacterial inoculants have positive effects on pH and lactic acid levels, an indication of good fermentation (Kung *et al*., 1987; Sanderson, 1993; Kennedy, 1994; Wrobel and Zastawny, 2004). When major bacterial population is lactic acid bacteria, fermentation products are mainly lactic acid, and acetic acid, and ethanol are at low levels (O'kiely *et al*., 1989; Baskay *et al*., 1999; Jatkauskas and Vrotniakiene, 2004). It has been reported that this type of silage increases DMI (Bolsen *et al*., 1996), nutrient digestibility and net energy for lactation (Ilakova *et al*., 1998), and better animal performance (Wheeler and Mulcahy, 1989; Havilah and Kaiser, 1992). Differences in DM digestibility of silages with or without inoculant have been reported (Kung *et al*., 1993; Rooke *et al*., 1998).

Propionic acid bacteria can ferment sugars and lactate to acetate and propionate. These short-chain aliphatic acids inhibit the growth of yeasts and moulds in silage (Woolford, 1975; Moon, 1983). DM, acid detergent fiber (ADF), neutral detergent fiber (NDF), WSC, total nitrogen (N), pH, lactic acid and ammonia-N content of corn silage with 22.6% DM were not affected by treatment with propionibacteria and LAB across 90-d fermentation in the study of Higginbotham *et al*. (1998). However, in a study by Higginbotham *et al*. (1996) propionibacteria at 1×10^6 cfu/g of forage lowered pH after 30 days of fermentation compared with untreated corn silage but no differences were observed for concentrations of WSC or lactic, acetic and propionic acids.

Propionic acid-based additives have been used to inhibit yeasts that assimilate lactic acid when silages are exposed to air and thus improve aerobic stability (Woolford, 1975; Dawson, 1994; Weinberg *et al*., 1995). However, these products were not designed to increase the efficiency of fermentation. Thus producers are often faced with a decision to use one or the other types of additives, realizing that each of them may have a shortcoming. Feedback from the field suggests some producers have applied buffered propionic acid additives and microbial inoculants on the same forage, but there is insufficient data to support this practice.

The purpose of the present study was to compare the effects of formic acid and a bacterial inoculant containing both lactic and propionic acid bacteria as additives on corn silage quality, ruminal degradability and nutrients digestibility in sheep.

Materials and Methods

Silage preparation

Whole-plant corn (CS) was harvested at the early dent stage of maturity with approximately 30% DM from a corn field of College of Agriculture, Shiraz University, Iran. The commercial inoculant (I) (LALSILMSOI, Lallemand SA, Saint-Simon, France) used in the experiments consisted of *Lactobacillus plantarum* MA18/5U and *Propionibacterium acidi-propionici* MA26/4U. Three treatments were used in the experiments, 1: control (untreated CS), 2: CS + I at the rate of 3×10^{10} colony forming units (cfu)/g of fresh forage and 3: CS + formic acid (98%) at 2.41/t fresh forage. Bacterial counts were based on the manufacturer's recommendation. For preparation of each treatment, sufficient chopped (3-5 mm length) forage was placed on a polyethylene sheet and sprayed with the solutions of the inoculant and formic acid, followed by thorough mixing. The same volume of water which was used to dissolve the additives was added to the control treatment to maintain equal moisture. Three samples were taken from the preensiled forages of each treatment, and the samples were placed on ice in the field, and transported to the laboratory for chemical analysis. Dark polyethylene bags were packed with 20 kg of each treated forage. Ten silo bags were used per treatment, kept indoor and opened after 60 days of ensiling. Triplicate mini-silos (70-g capacity plastic cylinder) were packed with each treatment and were opened at 60 days after ensiling for studying the dry matter recovery. Dry matter recovery during the fermentation and storage phases were estimated by weighing the mini-silos before ensiling and again on day 60 postfilling period.

At the end of the ensiling period, a 500-g

silage sample from each silo bag was taken for chemical analysis.

Chemical analysis

Chemical composition of forage, silages, and feces were determined following the procedures of AOAC (2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the method of Georing and Van Soest (1970). The pH of each sample was determined in triplicates using 25 grams wet material added to 100 ml of distilled water. After homogenizing for 10 min in a blender, the pH was determined using a digital pH meter (Polan *et al*., 1998). An aliquot of the homogenized sample was then strained through 2 layers of cheesecloth, and the liquid fraction was centrifuged at 2000 g for 20 min and stored frozen at -20°C for organic acid analysis. Water-soluble carbohydrates were determined by phenol sulfuric acid method (Dubois *et al*., 1956). The silage organic acids were determined using gas chromatography (Apparatus: Crompack, Model CP 9002, Netherlands) as described by Playne (1985).

In situ rumen degradability of DM

Rumen degradability was estimated *in sacco* (Orskov and McDonald, 1979). The dry samples from each treatment were ground using a grinder with a 2-mm sieve. Approximately, 5 g (DM) of each sample was transferred into polyester bags (12 × 19 cm) with 50 µm pore size. Four bags per treatment and inoculation time, were incubated in the rumen of two fistulated Sistani cattle (450 kg BW) for 2, 4, 8, 12, 24, 48 and 72 h. The cattle were fed with a diet consisting of 90% mixture of wheat bran and alfalfa hay (50:50) and 10% pistachio hull. The ration was fed in equal portions every 12 h to maintain a relatively stable rumen environment.

Four bags were also washed with tap water to estimate zero time washout. After each incubation time (including the zero h bags), the bags were removed and hand-washed with cold water until the water remained clear. Samples were then dried in an oven at 55°C until constant weight was achieved before determination of DM disappearance. Loss of DM at various incubation intervals was fitted to the non-linear equation $p = a + b\ (1\text{-}e^{-ct})$, where p is the amount degraded at time, "a" is the fraction that is soluble or immediately degraded, "b" is the fraction that is potentially degradable but insoluble, and "c" is the fractional rate constant at which the fraction "b" will degrade per hour. Data (a, b and c) were analyzed by one way analysis of variance.

Digestibility experiment

Twelve Mehraban male lambs (mean BW 39.93 ± 2 kg) were used in a complete randomized design digestibility experiment. They were divided into three equal groups with similar mean body weight and similar variation between lambs within group. Lambs were housed individually in crates and allowed 16 days of adaptation to the experimental diets and 8 days collection periods, during which separate collections of total feces was made. They had free access to fresh water. Lambs were offered 30% of a pelleted commercial concentrate mixture (DM = 98, NDF = 56.50, ADF = 10.88 and CP = 16.00%), and 70% of experimental silages, *ad libitum* intake. They were fed diets (DM basis) on 4% BW in two equal meals at 8:00 and 16:00 h. Each day, 10% of daily feces samples were collected for each sheep and kept frozen until chemical analysis as described above.

Statistical analysis

All data were subjected to analysis of variance using general linear model (GLM) (SAS, 1996). Mean treatment differences were obtained by Duncan's multiple range tests with a level of statistical significant of 5%.

Results

The DM, crude protein (CP), ether extract (EE), WSC, ADF and pH of the corn silages as affected by treatments are presented in Table 1. The DM content of the forage averaged 29.14% at the beginning and 22.74% at the end of the 60-d fermentation period. Dry matter content of treatment 1 was significantly ($P<0.05$) lower

Table 1: Effects of additives and time postfilling (60 days) on chemical composition (%DM) of corn silages

Days	Treatments	DM g/kg	CP	OM	EE	WSC	NDF	ADF	pH
0	FF	291.40	7.48	95.17	3.50	15.64	67.70	22.64	5.90
60	1	256.30^{b}	6.27^{b}	94.50^{a}	3.00^{a}	3.91^{a}	63.37^{a}	23.80^{b}	4.22^{b}
	2	279.10^{a}	6.40^{b}	95.50^{a}	1.67^{b}	3.69^{a}	67.66^{a}	26.60^{a}	4.00^{c}
	3	286.40^{a}	7.37^{a}	95.50^{a}	2.50^{ab}	5.65^{a}	66.12^{a}	25.86^{ab}	4.51^{a}
SEM		53.58	0.17	0.41	0.34	0.71	1.81	0.61	0.02

1: Control, 2: Inoculant-treated corn silage and 3: Formic acid-treated corn silage. FF: Fresh forage. Means within a column with similar superscript are not significantly different (Duncan's test; P<0.05)

than those of treatments 2 and 3.

Crude protein content was affected (P<0.05) by treatments and was higher in formic acid-treated corn silage. Table 2 presents the fermentation end products of the silages. Fermentation acids were affected by treatments. The concentration of acetic acid was significantly (P<0.05) higher for treatments 1 and 3 than treatment 2. The concentrations of lactic and butyric acids were significantly (P<0.05) higher for treatment 3 and lower for treatment 2. The concentration of propionic acid was significantly (P<0.05) higher in treatment 2. Total acid concentration was significantly (P<0.05) higher and lower in treatments 3 and 2, respectively. Formic acid treatment significantly (P<0.05) increased DM recovery compared with other treatments. No differences (P>0.05) existed among treatments for the degradability of DM in fractions "a", "c" or "a+b" (Table 3).

Fraction "b" was significantly lower (P<0.05) for treatment 1 and effective degradability (ED) of treatment 3 was higher than those of other treatments.

Table 2: The fermentation characteristics (DM basis) and dry matter recovery (% of DM ensiled) of the corn silages treated with additives

Items	Treatments			SEM
	1	2	3	
Acetic acid	1.74^{a}	1.11^{b}	1.91^{a}	0.07
Lactic acid	7.30^{b}	4.37^{c}	8.83^{a}	0.28
Propionic acid	0.35^{b}	0.44^{a}	0.12^{c}	0.01
Butyric acid	0.34^{b}	0.22^{c}	0.39^{a}	0.02
Total acids	9.72^{b}	6.13^{c}	11.25^{a}	0.23
Lactic: acetic ratio	4.24^{a}	4.01^{a}	4.64^{a}	0.42
DM recovery	94.43^{c}	96.70^{b}	98.90^{a}	0.22

1: Control, 2: Inoculant-treated corn silage and 3: Formic acid-treated corn silage. Means within a row with similar superscript are not significantly different (Duncan's test; P<0.05)

Table 3: Parameters of ruminal *in situ* degradation of dry matter of corn silages treated with additives

Items	Treatments			SEM
	1	2	3	
a	39.29^{a}	38.03^{a}	38.53^{a}	1.24
b	43.87^{b}	47.73^{a}	48.07^{a}	1.10
C (h-1)	0.051^{a}	0.041^{a}	0.059^{a}	0.011
a+b	83.16^{a}	85.76^{a}	86.60^{a}	1.14
ED	85.36	88.14	89.00	

1: Control, 2: Inoculant-treated corn silage and 3: Formic acid-treated corn silage. Means within a row with similar superscript are not significantly different (Duncan's test; P<0.05). a: Fraction soluble in water, b: Fraction degraded at a measurable rate and c: the rate at which the "b" fraction is degraded, "a+b": Potential degradability and ED: Effective degradability values at 0.05 per h outflow rate

Digestibilities of DM, OM, NDF and ADF were not affected by treatments (Table 4). There was a significant increase (P<0.05) in CP digestibility in treatment 1 compared with treatment 3 with no differences between treatment 2 and treatments 1 and 3. EE digestibility was higher (P<0.05) in treatments 1 and 3 compared with treatment 2.

Table 4: Effects of treatments on nutrients digestibility of corn silage (%)

Parameters	Treatments			SEM
	1	2	3	
Dry matter	66.80	68.59	64.64	1.39
Organic matter	68.65	70.52	66.45	1.35
Crude protein	70.08^{a}	65.20^{b}	62.21^{b}	1.74
Neutral detergent fiber	61.89	65.03	60.04	1.80
Acid detergent fiber	41.10	51.17	41.38	3.22
Ether extract	79.21^{a}	39.74^{b}	77.13^{a}	4.39

1: Control, 2: Inoculant-treated corn silage and 3: Formic acid-treated corn silage. Means within a row with similar superscript are not significantly different (Duncan's test; P<0.05)

Discussion

The reduction in the forage DM between day 0 and day 60, might be due to the fermentation process. The lower DM content of treatment 1 (Table 1) was reflected in lower DM recovery and higher total fermentation acids compared with treatment 2, which can be attributed partially to the more extensive fermentation in treatment 1. The non-significant higher DM in treatment 3 might be due to the fermentation restriction by formic acid (Kennedy, 1990; Spoelstra *et al*., 1990; Jaakkola *et al*., 1991). The higher CP content of treatment 3 could be due to the restriction of fermentation, deamination and decarboxylation of proteins (Chamberlain *et al*., 1990; Rooke *et al*., 1998). Silage WSC decreased with time postfilling, and the use of formic acid restricted fermentation, as indicated by higher ($P>0.05$) residual WSC than other treatments (Haigh *et al*., 1987; Kennedy, 1990). Inoculation with propionibacteria and LAB did not affect silage NDF and ADF concentrations at 60-d postfilling, which demonstrates that these bacteria lack fibrolytic activity (Higginbotham *et al*., 1998). The lower ($P<0.05$) ADF content in treatment 1 compared with treatment 2 might be the result of increased cell wall digestion due to increased silage fermentation (Bolsen *et al*., 1996) as shown by higher ($P<0.05$) total acids in this treatment than in treatment 2 (Table 2). All silages (except treatment 3) had a pH less than 4.22, indicating successful preservation and fermentation. This condition was able to minimize the growth of clostridia as indicated by very low butyric acid concentrations of all silages (McDonald, 1981). Forage pH decreased (data not shown) immediately with adding formic acid as expected. Final pH of treatment 2 (4.00) was significantly ($P<0.05$) lower than others (Table 1), which is in the line of the findings of Higginbotham *et al*. (1996) with corn forage treated with propionibacteria at 1×10^6 cfu/g of fresh forage after 30-d postfilling. The higher ($P<0.05$) pH of treatment 3 compared with treatment 1 shows more extensive fermentation in treatment 1 and fermentation restriction in treatment 3 (McDonald *et al*., 1991).

Some researchers reported a decrease (Kennedy, 1990) while others reported no changes (Spoelstra *et al*., 1990) in concentration of acetic acid with addition of formic acid into silages. Final lactic acid concentrations for all silages were within expected ranges for silages containing greater than 65% moisture (6-8% DM) (Higginbotham *et al*., 1998). Lower lactic acid concentration of treatment 2 might be due to the fermentation of sugars and lactate to propionate by propionic acid bacteria which resulted in higher ($P<0.05$) propionic acid of treatment 2. Many researchers have reported that addition of formic acid into silage decreased silage lactic acid content by limiting silage fermentation (Kennedy, 1990; Spoelstra *et al*., 1990), however, there are some data indicating that formic acid increases silage lactic acid concentrations (Chamberlain *et al*., 1982; Charmley *et al*., 1990). 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 microorganisms, it enters into cells by active transport, which requires two-fold energy; thus it is not a good source of energy for rumen microorganisms. Thereby, silages with high lactic acid content may result in low microbial protein synthesis in the rumen (Bosch *et al*., 1988), so higher ($P<0.05$) lactic acid content of treatment 3 might be less efficient for rumen microorganisms than other treatments.

It has been reported that production of propionic acid by propionibacteria ceases below pH values of 4.80 (Pahlow and Hoing, 1994). The final pH of treatment 2 was 4.00 (Table 1) which shows maintaining metabolic activity of added propionibacteria at this low pH in the present study. Weinberg *et al*. (1995) reported that growth of propionibacteria was not sustained under the ensiling conditions in pearl millet and corn silages but in the present study, it was active under ensiling conditions.

In the study of Weinberg *et al*. (1995) no butyric acid was detected in corn silage treated with *P. acidipropionici*. The higher butyric acid concentration with treatment 3 might be related to the higher WSC content of silage since sugar supplements have increased the proportion of butyrate (Sutton,

1968; Huhtanen, 1988). The concentration of butyric acid in treatment 1 was intermediate which might show the activity of clostridia under the condition of this silage.

In the study by Higginbotham *et al*. (1996) propionibacteria lowered silage pH after 30 days of fermentation compared with untreated corn silage with no differences for concentrations of WSC, lactic, acetic and propionic acids, which is in contrast to the findings of the present study. In the present study, no beneficial interaction between LAB and propionic acid bacteria has been shown (lower total acids), which is in agreement with the findings of Parker and Moon (1982). It has been reported that propionic acid bacteria produce metabolites that benefit the growth of LAB (Parker and Moon, 1982) through producing vitamins and other cofactors that might increase silage fermentation (Bullerman and Berry, 1965; Hettinga and Reinbold, 1972). Propionic acid is a fungicidal agent and high concentration of propionate inhibits yeast and mould growth (Huber and Soejono, 1976). Propionibacterium can produce propionic acid from sugars and lactate. This was true for treatment 2 which had lowest lactic acid but higher propionic acid compared with other treatments that can increase aerobic stability of treatment 2 after opening the silo. The higher propionic acid in treatment 2 is not in agreement with the results reported by Weinberg *et al*. (1995) with adding a propionic acid bacterial inoculant to corn silage. The lower acetic, lactic and butyric acids concentrations in treatment 2 reflected in lower ($P<0.05$) total acids concentration (Table 2).

The higher amounts of fermentation acids in treatment 3 compared with other treatments indicated that the added formic acid degraded cell walls and that more fermentable substrate was available.

The higher DM recovery of treatment 3 is in agreement with Haigh *et al*. (1987). This was expected as formic acid is anti-bacterial to many species including LAB and thus, results in limited fermentation and less nutrient loss. However, the addition of microbial inoculant increased ($P<0.05$) DM recovery compared with the DM recovery from untreated silage which is in contrast to the findings of Higginbotham *et al*. (1996). This result can be attributed partially to the more extensive fermentation of treatment 1 which supported by higher ($P<0.05$) total acids (McDonald, 1981) with treatment 1 (9.72% of DM) compared with treatment 2 (6.13% of DM) (Table 2).

The insignificant differences for the degradability of DM in the fractions “a”, “c” or “a+b” were in agreement with the previous findings (Filya *et al*., 2002; Filya, 2003) with corn silage treated with *L. buchneri* alone or in combination with *L. plantarum*. Fractions “b”, “c”, “a+b” ED were numerically higher in treatment 3 which might be due to the effect of formic acid on the cell wall components, specially hemicellulose (Bolsen *et al*., 1996; Adesogan and Salawu, 2004).

Organic matter, NDF and ADF digestibilities were numerically higher in treatment 2 which can be attributed to the effect of inoculant on whole tract digestibility (Ilakova *et al*., 1998). Raeth-Knight *et al*. (2007) reported higher DM and NDF digestibility when dairy cows were direct-fed *Lactobacillus acidophilus* and *Propionibacteria freudenreichii*. Pahlow and Hoing (1994) found that the inoculation treatment of silage led to an improvement in silage quality and nutrients digestibility. Davies *et al*. (1998) concluded that the degree of improvement in nutrient digestibilities of inoculated silage to a large extent depends on the amount of WSC in the biomass at silage fermentation phase.

The addition of microbial inoculant containing propionic acid producing bacteria plus *L. plantarum* did not affect acetic acid, lactic acid, total acids, WSC, lactic:acetic ratio, DM degradability and nutrients digestibility but had lowest final pH value and highest propionic acid and preserved well the whole-plant corn silage. Further studies are needed to determine the proper kind and level of microbial inoculants containing propionic acid producing bacteria for improving silage quality, aerobic stability due to antimycotic properties of propionic acid (specially in warm climates) and animal performance.

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