

Full Length Research Paper

Effect of adding sour yoghurt and dough as bacterial inoculant on quality of corn silage

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Thirty-six mini nylon-bags-silos, with about 4 kg corn forage were inoculated with 5% sour yoghurt (Yoghurt) and 5% sourdough (Dough) on a dry matter basis. Silos were kept for 70 days during which four silos of each group were evaluated for their apparent properties (smell, colour and texture) and chemical composition at days 10, 30 and 70 of the experiment. Neither Yoghurt nor Dough had any effect on physical properties of ensiled materials. Inoculation of whole-crop corn with sourdough and sour yoghurt significantly decreased pH, ash content and ammonia nitrogen, while dry matter determined using toluene distillation (DMT), Flieg point, crude protein (CP), and total nitrogen increased ($P < 0.05$).

Key words: Silage, sour yoghurt, sourdough, corn forage.

INTRODUCTION

Whole-crop corn silage is a rather cheap feedstuff for dairy cattle nutrition (Kristensen et al., 2010). The spoilage of corn silage and any other undesirable microbial activity may deteriorate its nutritive value and may have a great impact on overall profitability of dairy cattle production systems. Many techniques have been used to avoid undesired microbial activities in corn silage. The use of microbial inoculants is one of such technique which has been implemented for a long time (McDonald et al., 1991). Two major microbial inoculants have been used for corn silage: homofermentative inoculants often based on *Lactobacillus plantarum* and heterofermentative inoculants based on *Lactobacillus buchneri*. Homo-fermentative inoculants have been used to increase the rate of pH reduction in early fermentative phase of ensiling (Muck, 1988), while heterofermentative inoculants have been mainly applied to improve aerobic stability of corn silage upon exposure to air (Kleinschmit and Kung, 2006). Although application of homo-

fermentative as well as heterofermentative silage inoculants may have several beneficial effects, it may also exert an extra financial burden on dairy farmers. Alternatively, naturally containing live bacteria materials such as sourdough and sour yoghurt are available to Iranian dairy farmers at relatively low prices, and could be used as silage microbial inoculants. More than 50 species of lactic acid bacteria (LAB), mostly species of the genus *Lactobacillus* are present in sour dough. The prominent *Lactobacillus* species in sourdough are *Lactobacillus paralimentarius*, *Lactobacillus sanfranciscensis*, *Lactobacillus fermentum* and *Lactobacillus brevis* (Roecken, 1996). The key bacteria in sour yoghurt are *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Tharmaraj and Shah, 2003). To the best of our knowledge, no research has been conducted to test the efficacy of sourdough and sour yoghurt as silage microbial inoculants. Therefore, the current study aimed to investigate the effects of adding sour yoghurt and sourdough as natural sources of lactic acid bacteria on quality and nutritional value of whole-crop corn silage.

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Abbreviations: DM, Dry matter; DMO, dry matter measured with oven drying; DMT, dry matter determined using toluene distillation; N-NH₃, ammonia nitrogen; CP, crude protein; TN, total nitrogen.

MATERIALS AND METHODS

Experimental setup

This experiment was carried out at Animal Nutrition Group of

Table 1. Physical properties of whole-crop corn silage with or without inoculation with sourdough and sour yoghurt.

Class	Apparent property				
	Smell	Touch	Colour	Total score	
Treatment	Control	12.7	3.1	1.4	17.2
	Sourdough	12.8	3.2	1.6	17.6
	Sour yoghurt	12.8	3.2	1.8	17.7
	SEM	0.18	0.11	0.10	0.21
Sampling day	10	12.9	3.2	1.6	17.6
	30	12.8	3.2	1.7	17.7
	70	12.6	3.2	1.5	17.2
	SEM	0.18	0.11	0.10	0.21
Significance (<i>P</i>)	Treatment (T)	NS	NS	NS	NS
	Day (D)	NS	NS	NS	NS
	T × D	NS	NS	NS	NS

NS, Non-significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; SEM, standard error of means. Means with different superscripts within class and column differ significantly ($P < 0.05$). Multi-treatment comparison method: Fisher's Protected LSD.

Lorestan University, Khorramabad, Iran. Thirty-six small nylon-bags silos (Khoraman, Khorramabad, Iran) were filled with approximately 4 kg of whole-corn (single cross 704) and inoculated with 5% of sour yoghurt (Yoghurt; Shahid Eshaghi Dairy production Co, Khorramabad, Iran) and 5% of sourdough (Dough; ZagrosBio, Khorramabad, Iran) on a dry matter basis. The control silos (Control) were only filled by whole-corn. Whole-crop corn was chopped to about 2 to 5 cm particles. The filled nylon-bags were then vacuum sealed (Zagros Indus, Broujerd, Iran) and kept away from light at 15°C for 70 days. From each group, four bags were randomly opened at days 10, 30 and 70 of the experiment.

Physical evaluations and chemical analyses

Physical properties of silages were evaluated according to a twenty points scoring system (Nikpourtehrani et al., 1987). Maximum score for smell, texture and colour of silage were 14, 4 and 2, respectively. The pH of ensiled samples were determined using a digital pH meter (CRISON Alella 08-328) according to the procedure described by Kaiser and Piltz (2003). Buffering capacity of whole-crop corn was determined by titration with 0.1 M lactic acid (Merck, Darmstadt, Germany) at the beginning of the experiment (Fisher and Burns, 1987). Dry matter (DM) content of fresh whole-crop corn as well as ensiled samples were determined by drying at 60°C for 4 h in a forced air oven (DMO). True DM contents were then calculated using the following equation as described by Kaiser and Kerr, (2003):

$$\text{True DM} = [4.686 + (0.89 \times \text{DMO content})]$$

Additionally, the DM content of ensiled samples were determined using toluen distillation (DMT) (AOAC, 1990). Ash and organic matter contents were determined according to AOAC (1990). Flieg point was calculated using the following equation (Kara et al., 2009):

$$\text{Flieg point} = 220 + (2 \times \text{DM}-15) - (40 \times \text{pH})$$

Total nitrogen, crude protein and ammonia nitrogen contents of

samples were determined according to AOAC (1990). Dry matter losses were calculated using the following equation (Miller and Clifton, 1965):

$$\text{Dry Matter Losses} = 17.614 - 0.538 X$$

Where, X is the dry matter content of ensiled material.

Statistical analyses

Statistical analyses were carried out using the MIXED procedure of SAS 9.2 (2003). The model used was:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$$

Where, Y_{ijk} is the dependent variable under examination; μ is the population mean for the variable; A_i is the treatment effect ($i = 3$; Control, Yoghurt and Dough); B_j is the sampling time ($j = 3$; days 10, 30 and 70) and e_{ijk} is the random error associated with observation ij . For all statistical analyses, significance was shown at $P < 0.05$. The Fisher's protected least significant difference (LSD) test was used for multiple treatment comparisons using the LSMEANS of SAS 9.2 (2003) with letter grouping obtained using SAS pdmix800 macro (Saxton, 1998). The residual analysis was carried out to test the model assumptions using the UNIVARIATE procedure of SAS 9.2 (2003) with NORMAL and PLOT options.

RESULTS AND DISCUSSION

Buffering capacity of the whole-crop corn was 81.1 meq/kg DM. The physical properties of ensiled materials were not affected by treatment, sampling time and their interaction (Table 1).

Not only treatment and sampling time, but also their interaction significantly affected the measured chemical properties of ensiled materials (Table 2). Inoculation of

Table 2. Chemical properties of ensiled whole-crop corn with or without inoculation with sourdough and sour yoghurt.

Class		Chemical property									
		pH	DMO (g/kg)	True DM (g/kg)	DMT (g/kg)	Ash (g/kg DMT)	Flieg point	DM losses (g/kg)	CP (g/kg DMT)	TN (g/kg DMT)	N-NH ₃ (g/kg TN)
Treatment	Control	3.9 ^a	220.0	243.0	247.0 ^b	76.0 ^a	96.7 ^b	45.4	87.5 ^c	14.0 ^c	74.0 ^a
	Sourdough	3.8 ^b	228.0	249.0	256.0 ^a	55.6 ^b	102.1 ^a	41.7	92.0 ^b	14.7 ^b	67.0 ^b
	Sour yoghurt	3.8 ^b	226.0	251.0	249.0 ^b	55.2 ^b	101.6 ^a	41.3	97.7 ^a	15.6 ^a	59.0 ^c
	SEM	0.01	3.20	2.90	1.70	1.50	1.20	1.47	1.05	0.20	0.90
Sampling day	10	3.9	221.0 ^b	247.0 ^b	250.0 ^b	63.3 ^a	98.4	43.4 ^a	89.9 ^b	14.3 ^b	64.0 ^b
	30	3.9	216.0 ^b	239.0 ^b	247.0 ^b	63.6 ^a	100.9	47.2 ^a	92.4 ^{ab}	14.7 ^{ab}	67.0 ^a
	70	3.9	235.0 ^a	257.0 ^a	256.0 ^a	56.8 ^b	101.1	37.9 ^b	95.0 ^a	15.2 ^a	69.0 ^a
	SEM	0.01	3.20	2.90	1.70	1.50	1.20	1.47	1.05	0.20	0.90
Significance (P)	Treatment (T)	***	NS	NS	**	***	*	NS	***	***	***
	Day (D)	NS	***	***	**	**	NS	***	**	**	**
	T × D	***	NS	NS	*	NS	*	NS	NS	NS	NS

DMO, Dry matter measured with oven drying; True DM calculated as $4.686 + (0.89 \times \text{DMO})$; DMT, dry matter determined using toluen distillation; DM losses calculated as dry matter losses = $17.614 - 0.538 \times \text{DMO}$; N-NH₃, ammonia nitrogen; TN, total nitrogen; NS, non-significant; *P<0.05; **P<0.01; ***P<0.001; SEM, standard error of means. Means with different superscripts within class and column differ significantly (P<0.05). Multi-treatment comparison method: Fisher's Protected LSD.

whole-crop corn with sourdough and sour yoghurt significantly decreased pH, ash content and N-NH₃, while DMT, Flieg point, CP and total nitrogen (TN) were increased (Table 2). The lower pH and the higher Flieg point in sourdough and sour yoghurt inoculated silages as compared to the control silage was most likely due to higher production of lactic acid which may have inhibited the activity of opportunistic microorganisms. Indeed, our results show that inoculation with sourdough and sour yoghurt lowered organic matter spoilage in the ensiled material as the inoculated silages had a lower ash content when compared with the control silages (Table 2). Inoculation of whole-crop corn with sourdough and sour yoghurt inhibited the proteolysis as indicated by a higher CP and a lower N-NH₃

content in inoculated silages when compared with the control (Table 2).

In conclusion, the results of the present study showed that inoculating the whole-crop corn with sourdough and sour yoghurt prevented organic matter spoilage and proteolysis. However, the effects of sourdough and sour yoghurt on detailed chemical properties of whole-crop corn silage as well as on animal production performance should be further investigated.

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