Influence of Ensiling Temperature, Simulated Rainfall, and Delayed Sealing on Fermentation Characteristics and Aerobic Stability of Corn Silage

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ABSTRACT

The aim of this study was to determine how delayed silo sealing, high ensiling temperatures, and rainfall at harvest affect the fermentation and aerobic stability of corn silage. One-half of each of 4 replicated, 6×1.5 m plots of a corn hybrid was harvested at 35% dry matter (Dry), and each of the other halves was harvested after they were sprinkled with sufficient water to simulate 4 mm of rainfall (Wet). Six representative (2 kg) subsamples were taken from the Wet and Dry forage piles and ensiled immediately (Prompt). Three hours later, 6 additional representative (2 kg) samples were taken from each pile and ensiled (Delay). Half of the bags from each moisture $\times$ sealing time treatment combination were stored for 82 d in a 40°C incubator (Hot) and the other half were stored in a 20°C air-conditioned room (Cool). A 2 (moisture treatments) $\times$ 2 (sealing times) $\times$ 2 (ensiling temperatures) factorial design with 3 replicates per treatment was used for the study. Wetting the corn silage increased concentrations of $\text{NH}_3\text{-N}$, ethanol, and acetic acid. Ensiling at 40 instead of 20°C increased pH, in vitro digestibility, and concentrations of $\text{NH}_3\text{-N}$, residual water-soluble carbohydrates and acid detergent insoluble crude protein. The higher ensiling temperature also reduced concentrations of neutral and acid detergent fiber and lactic and acetic acid. Delayed sealing reduced concentrations of $\text{NH}_3\text{-N}$ and total volatile fatty acids. Wetting, high temperature ensiling, and delayed sealing each reduced yeast counts slightly, and marginally (8 h) increased aerobic stability. Hot-Wet-Delay silages were more stable than other silages but had the lowest lactic to acetic acid ratio, and total volatile fatty acid concentration. This study indicates that the fermentation of corn silage is adversely affected by wet conditions at harvest and high ensiling temperatures, whereas delayed silo sealing for 3 h caused no adverse effects.

Key words: temperature, moisture, delayed sealing, wilting

INTRODUCTION

Dairy farmers in many parts of the world rely on corn silage as a source of digestible fiber and readily fermentable energy for their cattle. In Florida, Georgia, and many tropical countries, such farmers face several climatic challenges that can complicate corn silage production, particularly temperatures that reduce the rate of photosynthesis (Crafts-Brandner and Salvucci, 2002) and reduce yields (Park and Sinclair, 1993). The hot, humid conditions that prevail during the corn-growing season in these states is partly responsible for the production of 4 generations per year of European corn borer (*Ostrinia nubilalis*) compared with 1 generation per year in Northern states. These climatic conditions are also conducive for proliferation of many bacterial and fungal pathogens that cause stalk rot, smut, leaf blight, and southern rust, and predispose to growth of mycotoxin-producing *Penicillium*, *Aspergillus*, and *Fusarium* molds (Ono et al., 1999; Raid and Kucharek, 2005; Samapundo et al., 2005). In addition to affecting crop growth and disease incidence, previous studies showed that rainfall at harvest and high temperatures during ensiling adversely affect silage fermentation and aerobic stability (Dewar et al., 1963; McDonald et al., 1966; Muck, 1987; Garcia et al., 1989). Rainfall at harvest can increase proteolysis (McDonald et al., 1991) and effluent production (Fransen and Strubi, 1998), thereby reducing DM recovery. Ensiling at high temperatures reduces lactic acid concentration and aerobic stability, and increases pH and DM losses (Weinberg et al., 2001; Ashbell et al., 2002). Most of these discoveries were made on grass and alfalfa silages, and temperature and moisture effects were not simultaneously examined. The few studies found on corn silage only examined temperature effects on silage fermentation and aerobic stability. Therefore, little is known about the concurrent effects of high ensiling temperatures and surface moisture due to rainfall at harvest on the fermentation of corn silage.

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Most of the corn silage produced in Florida is grown by custom operators and hauled to dairies on journeys that can take up to 3 h. Little is known about the effect this delay (before sealing) has on the quality of silage. Because the silage is often left exposed to air and sunlight during hauling, the effect on silage quality may be comparable to that of wilting, which improves the fermentation of corn silage (Mahmoud et al., 1979; Kamra et al., 1983). Short wilting periods (1 to 4 h) are effective in Florida due to the high temperatures that prevail (Umana et al., 1991; Kunkle, 2003). However, the extent to which delayed sealing or wilting can reduce potential adverse effects of temperature and rainfall on the fermentation of corn silage is not known. Therefore, the objective of this experiment was to determine the main effects of simulated rainfall at harvest, high temperatures during ensiling, and delayed sealing for 3 h, and the interaction of these factors on the fermentation and aerobic stability of corn silage.

MATERIALS AND METHODS

Forage Production

Pioneer corn hybrid 31R87RR (Pioneer Hi-Bred International, Inc., Des Moines, IA) was grown on 4 replicated 6×1.5 m plots at the Plant Science and Education Research Unit, University of Florida (Gainesville) and harvested at 35% DM (2/3 milk line). Half of each plot was harvested and chopped (3 cm) with a 2-row forage harvester into a single dry pile (**Dry**) on a tarp, and the remaining halves were sprinkled with 4 mm of water as indicated by a rain gauge, and then harvested into a wet pile (**Wet**). After thorough mixing, six 2-kg representative subsamples from each pile were weighed into plastic bags, which were compacted to exclude air and achieve a density of about 400 kg/m^3 , sealed, and stored under a covered barn (**Prompt**). Three hours later, 6 additional 2-kg representative subsamples were taken from the remaining forage that had been left uncovered in the field in each pile, and these were similarly sealed in plastic bags (**Delay**). Three of the bags from each moisture $\times$ sealing time treatment combination were stored in a 40°C incubator (**Hot**) for 82 d, and the other 3 were stored in a 20°C air-conditioned room (**Cool**). All treatments were ensiled in triplicate to give a total of 24 ensiled samples. Weights of the empty and filled silos were recorded at the beginning and end of the ensiling period. To monitor changes in pH, water-soluble carbohydrates (**WSC**), and $\text{NH}_3\text{-N}$ during fermentation, additional samples (0.7 kg) representing each of the treatment combinations were also ensiled in triplicate for 2, 5, and 7 d. This gave 72 additional ensiled samples.

Sampling

Samples of the preensiled forage were analyzed for water activity (a_w), buffering capacity, concentrations of DM, CP, NDF, and ADF and in vitro true (**IVTD**), and apparent (**IVDMD**) digestibility. Forage samples that were ensiled for 2, 5, and 7 d were representatively subsampled (200 g) for analysis of residual WSC, $\text{NH}_3\text{-N}$, and pH, whereas those that were ensiled for 82 d were representatively sampled for silage juice extraction (20 g), chemical analysis (400 g), microbial enumeration (100 g), and aerobic stability (800 g). Silage samples destined for microbial analysis were heat-sealed within gas-impermeable bags (Kapak/Scotch Pak, Kapak Corp., Minneapolis, MN), and analyzed for yeast and mold counts (AOAC, 2005) at Dairyland Laboratories, Inc. (Sauk Rapids, MN).

Analytical Methods

Buffering capacity was measured using the Playne and McDonald (1966) method, and a_w was measured with an Aqualab CX2 analyzer (Decagon Devices, Pullman, WA). Aerobic stability was measured by placing thermocouple wires at the center of a bag containing 800 g of silage, within an open-top polystyrene box that was covered with 2 layers of cheesecloth to prevent drying of the silages. The thermocouple wires were connected to data loggers (Campbell Scientific Inc., North Logan, UT) that recorded the temperature every 30 min for 30 d. Aerobic stability was denoted by the time (h) before silage temperatures rose by 2°C above ambient temperature (23 to 27°C). Deoxynivalenol (vomitoxin) was measured with an ELISA test kit (Neogen Corporation, Lansing, MI). Oven DM concentration was determined in a forced-draft oven set at 60°C for 48 h. Ash concentration was determined in a muffle furnace at 550°C for 5 h. Silage juice was extracted by blending 20 g of silage in 200 mL of distilled water for 30 s and filtering the slurry through 2 layers of cheesecloth. The pH was measured using a pH meter (Corning model 12, Corning Scientific Instruments, Medfield, MA). The filtrate was centrifuged at 4°C and $21,500 \times g$ for 20 min, and the supernatant was used to analyze lactic acid, VFA, and ethanol with the method of Muck and Dickerson (1988) using HPLC (Hitachi, FL 7485, Tokyo, Japan) and a UV detector (Spectroflow 757, ABI Analytical Kratos Division, Ramsey, NJ). The HPLC protocol used was described by Adesogan et al. (2004). Acetoin and 2,3 butanediol were measured using HPLC (HP 1090, Agilent Technologies, Cheshire, UK) and a refractive index detector (HP 1047A). Twenty microliters of supernatant was injected into a BioRad Aminex HPX-87H (BioRad Laboratories, Hercules, CA) column with 4 mM sulfuric acid mobile phase and a flow rate of 0.4

mL/min at 45°C with a 37-min run time. Water-soluble carbohydrate concentration was measured with the MAFF (Ministry of Agriculture, Fisheries and Food, 1986) anthrone reaction assay, and $\text{NH}_3\text{-N}$ was analyzed with the Noel and Hambleton (1976) procedure and an AlpKem Auto Analyzer (Alpkem Corporation, Clackamas, OR). Crude protein was calculated as $\text{N} \times 6.25$, using N measurements obtained with a Vario Max CN Elemental N analyzer (Elementar Americas, Inc., Mt. Laurel, NJ). Starch was determined using the procedure of Holm et al. (1986). Adaptations of the respective methods of Van Soest et al. (1966) and Tilley and Terry (1963) for Ankom^{II} Daisy Incubators (Ankom Technology, Macedon, NY) were used to measure IVTD and IVDMD of DM. The rumen fluid was obtained before feeding from 2 nonlactating, fistulated cows fed 9 kg of Coastal bermudagrass (*Cynodon dactylon*) hay and 400 g of soybean meal daily. The NDF and ADF concentrations of the samples were determined (Van Soest et al., 1991) using an Ankom²⁰⁰ Fiber Analyzer (Ankom Technology). The NDF procedure included amylase pretreatment; ADF residues were analyzed for acid detergent insoluble CP (ADICP) using the Elemental N analyzer.

Statistical Analyses

A $2 \times 2 \times 2 \times 5$ (ensiling temperature $\times$ sealing time $\times$ moisture treatment $\times$ ensiling duration) factorial design with 3 replicates per treatment was used to analyze WSC, $\text{NH}_3\text{-N}$, and pH data because these analytes were measured on samples from 5 ensiling durations (0, 2, 5, 7, and 82 d). The GLM procedure of SAS version 8 (SAS Institute, Inc., Cary, NC) was used for the analysis and the model included main effects of moisture, temperature, sealing time, and ensiling duration and all interactions of these factors. Polynomial contrasts were used to determine trends during ensiling and the Tukey test was used to differentiate between interaction means. For all other analytes, which were only measured on samples ensiled for 82 d, a 2 (moisture treatments) $\times 2$ (sealing times) $\times 2$ (ensiling temperatures) factorial design was used. The model was similar to that described above except that ensiling duration was excluded. A 2 (moisture treatments) $\times 2$ (sealing times) design was used for the other analytes (Table 1) that were measured on preensiled samples. Significance was declared at $P < 0.05$ and tendencies at $P < 0.10$. Results are expressed on a DM basis unless otherwise stated.

RESULTS

Chemical Composition of Corn Forage Before Ensiling

Wet forages had lower ($P < 0.05$) DM concentration (32.8 vs. 36.9%) and greater ($P = 0.001$) a_w (0.998 vs.

0.992) and $\text{NH}_3\text{-N}$ concentration (1.10 vs. 0.99%) than Dry forages (Table 1). Delay forages had lower ($P = 0.046$) ash concentrations (5.3 vs. 6.7%) and slightly greater DM concentration (35.5 vs. 34.3%, $P = 0.086$) and a_w (0.997 vs. 0.993, $P = 0.006$) than Prompt forages. The concentration of NDF was unaffected by treatment, but delayed sealing reduced ADF concentration to a greater extent in Wet silages than in Dry silages (wetting $\times$ sealing time interaction, $P = 0.003$). Delayed sealing also increased IVTD and IVDMD values to a greater extent in Wet silages than in Dry silages (wetting $\times$ sealing time interaction, $P < 0.05$).

Changes in pH, WSC, and $\text{NH}_3\text{-N}$ During Fermentation

Silage pH decreased (quadratic, $P < 0.01$) as ensiling progressed, and delayed sealing and the lower temperature enhanced the rate and extent of this decline (Figure 1). Residual WSC concentration also decreased (quadratic, $P < 0.001$) as ensiling progressed, but wetting and the higher temperature reduced the rate and extent of the decline (Figure 2). Ammonia N production increased (linear, $P < 0.001$) as ensiling progressed and prompt sealing, wetting, and the higher temperature exacerbated the production (Figure 3).

Chemical Composition of Corn Silage After 82 d of Ensiling

Dry matter concentration was reduced ($P < 0.05$) by wetting (32.8 vs. 35.9%; Table 2). Delayed sealing increased DM concentrations to a greater extent in Hot silages than in Cool silages (temperature $\times$ sealing time interaction, $P = 0.037$). Delayed sealing decreased ($P < 0.01$) DM losses (1.7 vs. 11.0%) and tended ($P = 0.051$) to increase CP concentration (6.8 vs. 6.3%), but these effects depended on ensiling temperature (sealing time $\times$ temperature interaction, $P < 0.05$) and wetting (sealing time $\times$ wetting interaction, $P = 0.008$). Ash concentration was greater ($P < 0.05$) in Prompt (4.37 vs. 3.92%) and Wet (4.41 vs. 3.71%) silages, than Delay and Dry silages, respectively. Starch concentration was greater ($P = 0.003$) in Hot silages than Cool silages (42.7 vs. 36.2%) and this difference was more pronounced in Delay than Prompt silages (temperature $\times$ sealing time interaction, $P = 0.034$). Wet-Hot-Delay silages had more ($P < 0.05$) residual WSC (1.95 vs. 0.55%) than the other silages (temperature $\times$ sealing time $\times$ wetting interaction, $P = 0.008$).

Hot silages had lower ($P < 0.1$) NDF (42.0 vs. 44.2%) and ADF (21.3 vs. 22.9%) concentrations and greater ($P < 0.05$) IVDMD (65.8 vs. 62.2%) and IVTD (67.6 vs. 63.5%) values and ADICP (4.30 vs. 2.52%, $P = 0.058$)

Figure 1. Effect of sealing time (0 h, prompt vs. 3 h, delay), ensiling temperature (20°C, cool vs. 40°C, hot), and wetting (4 mm, wet vs. 0 mm, dry) on the pH of corn silage during ensiling. No main effect interactions were detected, $P > 0.05$; ensiling duration effect was quadratic, $P < 0.001$.

concentrations than Cool silages. Delayed sealing enhanced the effect of the higher temperature on NDF concentration (sealing time $\times$ temperature interaction, $P = 0.046$).

Fermentation Characteristics of Corn Silage After 82 d of Ensiling

Wet silages had greater ($P < 0.05$) acetic acid (2.27 vs. 1.83%), $\text{NH}_3\text{-N}$ (2.19 vs. 1.94%; 17.4 vs. 15.0% total

N) and ethanol (1.27 vs. 1.06%) concentrations than Dry silages (Table 3). Compared with Cool silages, Hot silages had lower ($P < 0.05$) concentrations of lactic acid (3.92 vs. 6.76%), acetic acid (1.71 vs. 2.38%), propionic acid (0.0 vs. 0.68%), and total VFA (11.1 vs. 14.6%), and lower ($P = 0.001$) lactic to acetic acid ratios (2.08 vs. 2.84) and Flieg's (1938) homolactic fermentation scores representing homolactic fermentation (84.6 vs. 90.4). Hot silages also had greater ($P < 0.001$) pH values (4.23

Table 1. Effect of wetting (W; 4 mm, wet vs. 0 mm, dry) and sealing time (S; 0 h, prompt vs. 3 h, delay) on the chemical composition and in vitro digestibility of corn forage before ensiling (% of DM or as stated)

	Dry		Wet		SEM	Effects		
	Prompt	Delay	Prompt	Delay		W	S	W × S
DM, %	36.3	37.5	32.2	33.4	0.47	0.001	0.086	NS
CP	7.2	6.6	6.7	6.9	0.15	NS	NS	0.061
Water activity (a_w)	0.991	0.994	0.996	1.000	0.001	0.001	0.006	NS
NDF	49.8	47.6	49.8	47.9	1.22	NS	NS	NS
ADF	23.8	22.5	26.9	21.2	0.46	NS	0.001	0.003
Ash	6.1	5.0	7.3	5.6	0.46	NS	0.046	NS
IVDMD, ¹ %	62.1	62.0	56.2	62.6	0.72	0.017	0.006	0.005
IVTD, ² %	63.3	64.6	60.3	66.6	0.80	NS	0.003	0.038
Buffering capacity, mEq/kg of DM	78.5	92.9	93.0	88.2	4.2	NS	NS	NS
NH ₃ -N	1.00	0.97	1.13	1.07	0.03	0.001	NS	NS
NH ₃ -N, % of total N	8.0	8.4	9.7	8.8	0.29	0.022	NS	NS

¹IVDMD = In vitro apparent dry matter digestibility.²IVTD = In vitro true dry matter digestibility. **Figure 2.** Effect of sealing time (0 h, prompt vs. 3 h, delay), ensiling temperature (20°C, cool vs. 40°C, hot), and wetting (4 mm, wet vs. 0 mm, dry) on water-soluble carbohydrate (WSC) concentration (% of DM) of corn silage during ensiling. No main effect interactions were detected, $P > 0.05$; ensiling duration effect was quadratic, $P < 0.001$.

Figure 3. Effect of sealing time (0 h, prompt vs. 3 h, delay), ensiling temperature (20°C, cool vs. 40°C, hot), and wetting (4 mm, wet vs. 0 mm, dry) on ammonia N ($\text{NH}_3\text{-N}$) concentration (% of DM) of corn silage during ensiling. No main effect interactions were detected, $P > 0.05$; ensiling duration effect was linear, $P < 0.001$.

vs. 3.76) and greater ($P < 0.05$) concentrations of $\text{NH}_3\text{-N}$ (2.30 vs. 1.82%; 18.0 vs. 14.3% total N), ethanol (1.25 vs. 1.08%), and isovaleric acid (1.53 vs. 1.20%) than Cool silages. Delay silages tended to have lower ($P = 0.09$) pH (3.96 vs. 4.03) and lower ($P < 0.05$) concentrations of $\text{NH}_3\text{-N}$ (1.87 vs. 2.24%; 14.9 vs. 17.5 % total N), isobutyric acid (3.31 vs. 4.27%), isovaleric acid (1.19 vs. 1.54%), and total VFA (11.7 vs. 14.0%) than Prompt silages. Wet-Hot-Delay silages had the lowest ($P < 0.1$) lactic:acetic acid ratio and concentrations of ethanol and total VFA (temperature $\times$ sealing time $\times$ moisture interaction, $P < 0.001$). No butyric acid or deoxynivalenol was detected in the silages.

Microbial Counts and Aerobic Stability of Corn Silages After 82 d of Ensiling

Wetting, high temperature, and delayed sealing each slightly reduced yeast counts and marginally (8 h in-

creased aerobic stability ($P < 0.05$; Table 4), but these effects were additive. Therefore, Wet-Hot-Delay silages were more aerobically stable (45.66 vs. 13.35 h), had fewer yeasts (4.42 vs. 6.26 log cfu/g), and lower aeration DM losses (8.2 vs. 13.8%) than the other silages (temperature $\times$ sealing time $\times$ wetting interaction, $P < 0.05$). Delayed sealing tended ($P = 0.063$) to reduce mold counts (3.11 vs. 4.00 log cfu/g). Postaeration pH values were unaffected by treatment, but they were greater than pH values obtained at silo opening.

DISCUSSION

Treatment Effects on the Forage

The corn forage had typical concentrations of most nutritional components except for relatively low buffering capacity and WSC concentration, and high starch concentration. These factors are partly maturity related

Table 2. Effect of wetting (W; 4 mm, wet vs. 0 mm, dry), ensiling temperature (T; 20°C, cool vs. 40°C, hot), and sealing time (S; 0 h, prompt, vs. 3 h, delay) on the chemical composition and in vitro digestibility of corn silage ensiled for 82 d (% of DM or as stated)

Item ¹	Dry				Wet				SEM	Effects						
	Cool		Hot		Cool		Hot			W	T	S	W × T	W × S	T × S	W × S
	Prompt	Delay	Prompt	Delay	Prompt	Delay	Prompt	Delay								
DM, %	34.1 ^{bc}	36.2 ^{ab}	33.8 ^{bc}	39.4 ^a	30.4 ^c	33.1 ^{bc}	31.6 ^c	35.9 ^{ab}	0.90	0.001	0.009	0.001	NS	NS	0.037	NS
DM loss, %	12.0	3.9	11.6	0.0	7.4	1.2	13.3	1.6	1.52	NS	NS	0.001	0.045	NS	0.031	NS
CP	5.6 ^b	7.4 ^a	6.4 ^{ab}	6.8 ^{ab}	6.0 ^b	6.8 ^{ab}	7.0 ^{ab}	6.1 ^{ab}	0.35	NS	NS	0.051	NS	0.044	0.008	NS
Ash	3.9 ^{bc}	3.8 ^{bc}	3.8 ^{bc}	3.4 ^c	4.4 ^{ab}	4.5 ^{ab}	4.7 ^a	4.0 ^{abc}	0.16	0.001	0.112	0.012	NS	NS	0.022	NS
NDF	42.5 ^{ab}	46.9 ^a	42.8 ^{ab}	40.6 ^{ab}	45.2 ^{ab}	42.4 ^{ab}	44.6 ^{ab}	39.9 ^b	1.40	NS	0.039	NS	NS	0.027	0.046	NS
ADF	21.7	24.5	20.8	20.9	23.0	22.3	22.6	20.8	1.14	NS	0.064	NS	NS	NS	NS	NS
WSC	0.5 ^b	0.4 ^b	0.7 ^b	0.7 ^b	0.5 ^b	0.5 ^b	0.6 ^b	2.0 ^a	0.13	0.002	0.001	0.003	0.011	0.001	0.001	0.008
Starch	37.0 ^{ab}	30.9 ^b	44.7 ^a	43.4 ^a	42.3 ^{ab}	34.7 ^{ab}	39.1 ^{ab}	43.4 ^a	2.53	NS	0.003	NS	0.058	NS	0.034	NS
ADICP	3.3	1.6	3.6	2.3	2.0	3.2	5.8	5.5	1.23	NS	0.058	NS	NS	NS	NS	NS
IVDMD, %	63.6	61.9	66.9	66.0	60.5	62.9	62.4	68.0	1.97	NS	0.020	NS	NS	0.072	NS	NS
IVTD, %	65.7 ^{ab}	61.5 ^b	69.3 ^a	68.5 ^{ab}	62.6 ^{ab}	64.5 ^{ab}	66.2 ^{ab}	66.4 ^{ab}	1.51	NS	0.002	NS	NS	NS	NS	NS

^{a-c}Means in rows with unlike superscripts differ ($P < 0.05$); NS = not significant.

¹WSC = Water-soluble carbohydrates; ADICP = acid detergent insoluble CP; IVDMD = in vitro apparent DM digestibility; IVTD = in vitro true DM digestibility.

Table 3. Effect of wetting (W; 4 mm, wet vs. 0 mm, dry), ensiling temperature (T; 20°C, cool vs. 40°C, hot), and sealing time (S; 0 h, prompt, vs. 3 h, delay) on fermentation indices in corn silage ensiled for 82 d (% of DM or as stated)

Item	Dry				Wet				SEM	Effects						
	Cool		Hot		Cool		Hot			W	T	S	W	W	T	W
	Prompt	Delay	Prompt	Delay	Prompt	Delay	Prompt	Delay					× T	× S	× S	× T × S
pH	3.82 ^b	3.76 ^b	4.30 ^a	4.11 ^a	3.73 ^b	3.72 ^b	4.27 ^a	4.25 ^a	0.05	NS	<0.001	0.090	NS	NS	NS	NS
NH ₃ -N	1.82 ^{bc}	1.53 ^c	2.40 ^{ab}	1.99 ^{bc}	2.04 ^{bc}	1.88 ^{bc}	2.74 ^a	2.09 ^{bc}	0.12	0.012	0.001	0.001	NS	NS	NS	NS
NH ₃ -N, % of total N	13.8 ^{ab}	11.4 ^b	19.0 ^{ab}	15.8 ^{ab}	17.6 ^{ab}	14.8 ^{ab}	19.7 ^a	17.7 ^{ab}	1.61	0.048	0.006	0.038	NS	NS	NS	NS
Total VFA	15.41 ^a	11.63 ^{ab}	11.29 ^{ab}	10.59 ^{ab}	16.22 ^a	15.41 ^a	13.08 ^{ab}	9.46 ^b	1.18	NS	0.001	0.017	NS	NS	NS	0.098
Lactate	6.78	5.42	3.97	3.95	7.53	7.31	4.53	3.22	0.61	NS	0.001	NS	NS	NS	NS	NS
Acetate	2.35	1.89	1.44	1.66	2.63	2.68	1.9	1.86	0.28	0.045	0.004	NS	NS	NS	NS	NS
Propionate	0.83 ^a	0.60 ^a	0.00 ^b	0.00 ^b	0.79 ^a	0.51 ^{ab}	0.00 ^b	0.00 ^b	0.11	NS	0.001	NS	NS	NS	NS	NS
Isobutyrate	4.12 ^{ab}	2.80 ^b	4.21 ^{ab}	3.58 ^{ab}	3.94 ^{ab}	3.68 ^{ab}	4.81 ^a	3.16 ^b	0.32	NS	NS	0.001	NS	NS	NS	NS
Isovalerate	1.33 ^{ab}	0.91 ^b	1.68 ^a	1.40 ^{ab}	1.34 ^{ab}	1.22 ^{ab}	1.83 ^a	1.22 ^{ab}	0.16	NS	0.008	0.005	NS	NS	NS	NS
Ethanol	1.16 ^{ab}	0.82 ^b	1.07 ^{ab}	1.22 ^{ab}	0.87 ^b	1.48 ^a	1.50 ^a	1.22 ^a	0.09	0.006	0.015	NS	NS	0.065	NS	0.001
La:Ac ¹	2.90 ^a	2.90 ^a	1.81 ^{bc}	2.40 ^{abc}	2.84 ^{ab}	2.72 ^{abc}	2.41 ^{abc}	1.73 ^c	0.217	NS	0.001	NS	NS	0.038	NS	0.078
Flieg's score ²	90.00 ^a	90.00 ^a	83.33 ^b	85.00 ^b	90.00 ^a	91.67 ^a	85.00 ^b	85.00 ^b	0.833	NS	0.001	NS	NS	NS	NS	NS
Acetoin	0.00	0.08	0.26	0.64	0.08	0.10	0.27	0.11	1.627	NS	0.041	NS	NS	NS	NS	NS
2,3 Butanediol	0.27	0.00	0.06	0.11	0.05	0.00	0.29	0.02	1.465	NS	NS	NS	NS	NS	NS	NS

^{a-c}Means in rows with unlike superscripts differ ($P < 0.05$); NS = not significant.

¹Lactic acid to acetic acid ratio.

²Homolactic fermentation index score (Flieg, 1938).

because buffering capacity decreases with maturity (Wilkinson and Phipps, 1979) and soluble sugars are replaced with starch as the plant transitions between vegetative and reproductive stages. The main effects of the treatments on the preensiled forage were that wetting increased water activity and proteolysis, and reduced DM concentration, whereas delayed sealing increased water activity and reduced ADF concentration, thereby increasing digestibility. In agreement, Muck (1987) showed that proteolysis rate increases with plant moisture concentration, presumably because moisture stimulates plant and bacterial proteolytic enzymes, and Williams et al. (1995) reported that wilting reduced fiber concentration by 2.1 to 3.2% in wheat forage and increased fiber digestibility in wheat silage. McDonald et al. (1991) also noted that pentoses could be released from hemicelluloses during the preensiling, aerobic phase. The similar effect of delayed sealing on DM concentration and a_w is unusual and inexplicable, but the differences between the respective values for Delay and Prompt forages had little practical significance.

Treatment Effects on the Silage

As ensiling progressed, the typical reductions in WSC and pH and increase in $\text{NH}_3\text{-N}$ occurred, reflecting fermentation of WSC by silage bacteria, leading to bacterial proteolysis, organic acid accumulation, and pH depression.

Wetting Effects on the Fermentation

The moisture effects in this study should not be compared with those in studies in which differences in treatment moisture contents were maturity-related, because this study examined effects of surface moisture, whereas the others demonstrate effects of cell-bound moisture. There was more secondary, heterolactic fermentation in Wet silages than in Dry silages, as shown by the greater concentrations of acetic acid and ethanol in Wet silages. Greater residual WSC and $\text{NH}_3\text{-N}$ concentrations in Wet silages suggest that wetting decreased the extent of fermentation while increasing proteolysis. Muck et al. (2003) also noted that high moisture levels at ensiling could restore enzyme activity in dry forages and facilitate the growth of proteolytic bacteria in the silo. Clostridial proliferation often results in proteolysis in silages that contain low DM and WSC concentrations, and high a_w (≥ 0.94), particularly at high (37°C) temperatures (McDonald et al., 1991). However, the high DM at harvest, low terminal pH, and absence of butyric acid production in this study suggest that the wetting-induced proteolysis was not caused by clostridia. Rather, enterobacteria, which also

Table 4. Effect of wetting (W; 4 mm, wet vs. 0 mm, dry), ensiling temperature (T; 20°C , cool vs. 40°C , hot), and sealing time (S; 0 h, prompt, vs. 3 h, delay) on fungal counts, aerobic stability, and postaeration pH and DM losses of corn silage ensiled for 82 d

Item	Dry						Wet						Effects					
	Cool			Hot			Cool			Hot			SEM	T		S		T $\times$ S
	Prompt	Delay		Prompt	Delay		Prompt	Delay		Prompt	Delay			W	$\times$	W	$\times$	
Yeasts ¹	7.68 ^a	6.72 ^a		6.44 ^a	7.00 ^a		7.30 ^a	6.83 ^a		6.07 ^{ab}	4.42 ^b		0.37	0.007	0.001	0.029	0.021	NS
Molds ¹	4.33	2.77		2.53	3.4		4.16	2.93		4.99	3.33		0.63	NS	NS	NS	NS	NS
pH	7.1	6.98		6.79	6.78		7.02	6.91		6.38	6.77		0.28	NS	NS	NS	NS	NS
DM loss, %	16.8	6.04		13.25	13.68		14.67	18.34		13.48	8.15		3.08	NS	NS	NS	NS	0.035
Aerobic stability, h	13.0 ^b	15.3 ^b		12.5 ^b	12.0 ^b		10.7 ^b	14.0 ^b		16.0 ^b	45.7 ^a		2.7	0.001	0.001	0.001	0.002	0.004

^{a,b}Means in rows with unlike superscripts differ $P < 0.05$.

¹Log cfu/g.

thrive under warm, humid ensiling conditions, (Woolford, 1984) probably caused the proteolysis.

Temperature Effects on the Fermentation

The higher pH, residual WSC, and $\text{NH}_3\text{-N}$ concentration, and lower lactic to acetic acid ratio and Flieg's score of silages ensiled at the higher temperature indicate curtailed fermentation, increased proteolysis, and secondary heterolactic fermentation, all of which reflect reduced silage quality. The higher residual WSC and starch concentrations of Hot vs. Cool silages agrees with the results of Colombatto et al. (2004), and are partly due to reduction in NDF and ADF concentrations caused by the higher ensiling temperature. Others have also shown that high ensiling temperatures reduce numbers of certain lactic acid bacteria (Weinberg et al., 1998, 2001), enhance proteolysis (Muck and Dickerson, 1988; Weinberg et al., 2001), and make the fermentation less homolactic (McDonald et al., 1966; Weinberg et al., 2001).

The reduction in fiber content and increase in digestibility associated with the higher ensiling temperature concurs with observations that ensiling ryegrass at 37 instead of 22°C increased hemicellulose hydrolysis to xylose and arabinose because the temperature optima for hemicellulase enzymes is 30 to 40°C (Dewar et al., 1963). Cellulose hydrolysis was also found to be greater at 35°C than at 25°C due to greater cellulase activity at the higher temperature (Pitt, 1990). Such increased fibrolysis results in seepage-related nutrient losses and decreased digestibility in grass silages (McDonald et al., 1991). The contrasting effect of the higher temperature on digestibility in this study probably reflects the higher DM concentrations of the corn silages and the absence of seepage losses from the mini-silos used. Colombatto et al. (2004) also demonstrated that higher ensiling temperatures (40 vs. 20°C) increased the fine particulate and soluble fraction of corn silages in mini silos but also increased fermentation gas production and OM degradation, particularly within the first 48 h of incubation in rumen fluid. Therefore, it is unlikely that high temperatures increase the digestibility of corn silage in practical situations due to seepage-related losses, particularly in low DM silages.

As in the study of Garcia et al. (1989), the higher ensiling temperature increased ADICP concentration. This reflects the formation of heat-damaged proteins through the Maillard reaction, which involves complexing of WSC or hemicellulose and amino acids under high temperatures. This occurred because the higher ensiling temperature falls within the range (35 to 40°C) at which the Maillard reaction occurs in silages (Muck et al., 2003).

High temperatures increased production of acetoin, which can be oxidized or reduced into undesirable compounds like diacetyl and 2,3 butanediol, respectively. These products are typically formed by *Streptococci*, *Pediococci*, and *Lactobacilli* spp. when glucose is limiting, such as in these low-WSC corn forages, due to inadequate fructose 1,6 biphosphatase for activating lactate dehydrogenase (McDonald et al., 1991).

Delayed Sealing Effects on the Fermentation

The 3-h delayed sealing duration was examined in this study because it represents the maximum time it takes to haul corn forage from custom growers' fields to dairies in Florida. In accord with Adogla-Bessa et al. (1999), delayed sealing reduced DM losses and proteolysis as shown by lower concentrations of NH_3N and isobutyric and isovaleric acids, which are degradation products of valine and leucine, respectively. These effects are similar to the reduced fermentation and curtailed in-silo proteolysis in wilted grass, wheat, and corn silages (Mahmoud et al., 1979; Morgan et al., 1980; Kamra et al., 1983; Williams et al., 1995). They are respectively caused by WSC depletion arising from continued plant and microbial respiration, and reduced amino acid deamination due to decreased activity of clostridia and enterobacteria (McDonald et al., 1991). The beneficial effects of delayed sealing in this study should not be confused with effects of prolonged (>10 h) delays that tend to render inoculant or additive treatment less effective, increase silage pH, proteolysis, and heat-damaged protein concentrations, and hasten spoilage (Nia and Whittenberg, 2000; Uriarte, 2001; Mills and Kung, 2002). Therefore, a prolonged delay before sealing is detrimental to silage quality, whereas a short delay may be beneficial. The importance of limiting the duration of delayed sealing is further indicated by the lower total VFA concentration, and greater residual WSC concentration caused by this treatment, which indicate a less extensive fermentation. The latter reflects the longer respiratory phase that occurred before anaerobiosis commenced, which may have depleted fermentable substrates needed for microbial growth. In such cases, total WSC concentration is relatively unchanged (27.0 vs. 27.3%, $P > 0.05$ in this study), but sucrose and fructose catabolism occurs (McDonald et al., 1991).

Treatment Effects on Fungal Growth and Aerobic Stability

Wetting, delayed sealing, and the higher temperature increased aerobic stability by 8 h, which is of limited practical benefit. These effects were largely due to

reductions in yeast counts that resulted from increased acetic acid production. Although delayed sealing only increased acetic acid concentration numerically, the high pKa (4.78) of the acid implies that this increase would have conferred greater antifungal effects due to greater dissociation of the acid. In agreement with this study, Ashbell et al. (2002) found that corn silage was less stable at 30°C than at 40°C, but Weinberg et al. (2001) found that aerobic stability was unaffected by ensiling temperature in corn silage. This discrepancy is because, unlike the silages in this study, those in Weinberg's study lacked residual WSC and had low lactic acid concentrations, which implied insufficient substrates for yeast growth and deterioration. In this study, lactic acid concentrations were normal and yeast counts were sufficient ($>10^5$ cfu/g) to predispose to deterioration and lactate fermentation by lactate-assimilating yeasts. Consequently, postaeration pH values were much greater than those obtained at silo opening.

Wet-Hot-Delay silages were more aerobically stable (45.66 vs. 13.35 h) than other silages because their yeast counts were low (4.42 log cfu/g), whereas those of other silages (mean 6.26 log cfu/g) exceeded the threshold (1×10^5 log cfu/g) that results in aerobic spoilage. The lower yeast counts in Wet-Hot-Delay silages are partly due to the cumulative inhibition of yeast growth by excess moisture, high temperatures, and delayed sealing. However, Wet-Hot-Delay silages had a lower lactic:acetic acid ratio and total VFA concentration than the other silages. They also had greater pH, DM losses, and concentrations of ADICP than most of the other silages and a distinctive dark color and tobacco odor presumably due to the presence of products of the Mailard reaction. Therefore, such silages would be undesirable for feeding in spite of their high aerobic stability.

CONCLUSIONS

This study shows that high ensiling temperatures and simulated rainfall had detrimental effects on the fermentation process and silage quality, but delayed silo sealing for 3 h did not. The beneficial effects of delayed sealing in this study should not be confused with effects of longer (>10 h) delay periods, which tend to reduce fermentation quality and enhance spoilage. Corn silage producers in hot, humid regions need to adhere strictly to excellent silage-making practices to overcome the adverse effects of moisture and temperature on corn silage production.

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