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Case Study: Microbial inoculant and ensiling time effects on fermentation profile, nitrogen fractions, and ruminal in vitro and in situ starch digestibility in corn shredlage and late-maturity corn silage

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ABSTRACT

Two experiments were conducted to evaluate the effects of ensiling time and microbial inoculation on N fractions and starch digestibility in either well-processed corn shredlage (SHRD; Exp. 1; 76% of starch passing through a 4.75-mm screen, 39.3% DM, and 32.2% amylase-treated NDF) or late-maturity corn silage (Exp. 2; 48.0% DM and 22.9% amylase-treated NDF). For Exp. 1, unfermented SHRD was allocated into 24 samples of 600 g each and randomly assigned to 6 treatments in quadruplicate. Treatments were a combination of SHRD noninoculated (CON) or inoculated at the recommended inoculation rate (1X; 5 × 10⁴ cfu of Lactobacillus plantarum, Lactobacillus casei, Streptococcus faecium, and Pediococcus per gram of fresh whole-plant corn) or twice the recommended inoculation rate (2X; 10 × 10⁴ cfu/g of fresh whole-plant corn) of a microbial inoculant and ensiled for 30 or 120 d. Exp. 2 used the same experimental methodology except for evaluating treatments within late-maturity corn silage rather than SHRD. In Exp. 1, DM and starch concentrations were unaffected by treatments. Although not affected by inoculation, content of CP increased from 30 to 120 d of ensiling. Measurements of pH were reduced from 3.96 at 30 d to 3.88 after 120 d. Concentrations of lactate and ethanol were similar but acetate and total acids were greater after 120 d. Ammonia-N concentration and starch digestibility increased from 30 to 120 d. Fermentation profile, including ammonia-N, and starch digestibility of SHRD were unaffected by inoculation. In Exp. 2, ensiling time did not affect concentrations of DM, CP, and starch. However, DM and starch contents were 2.5 and 3.4 percentage units greater for 2X than other treatments. Concentrations of lactate and total acids were greater for CON and 1X than 2X. Propionate and ethanol concentrations tended to be greater for CON than other treatments. Despite the lower ammonia-N concentration for 2X, starch digestibility was unaffected by microbial inoculation. Greater lactate, acetate, and total acid concentrations after 120 d of ensiling were observed. Reductions in pH and ethanol concentration were also observed for 120 d compared with 30 d. Late-maturity corn silage fermented for 120 d had greater ammonia-N (5.4 vs. 4.0% of CP) and starch digestibility (66.7 vs. 61.7% of starch) compared with 30 d. Ammonia-N concentration and starch digestibility were greater after 120 d of fermentation in both experiments, suggesting that extended ensiling time is

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advantageous in both scenarios. Inoculation with lactate-producing bacteria, however, did not improve starch digestibility in either experiment.

Key words: storage length, microbial inoculant, starch digestibility, corn shredlage

INTRODUCTION

Benefits of prolonged ensiling time on ruminal in vitro starch digestibility have been observed in whole-plant corn silage (**WPCS**; Der Bedrosian et al., 2012; Ferraretto et al., 2015b). Moreover, prolonged ensiling time increased concentrations of ammonia-N and soluble CP in these trials, implying proteolysis or solubilization of zein proteins (Hoffman et al., 2011). Although the overall benefits of prolonged ensiling time are well established, its effect on starch digestibility in specific scenarios, such as when WPCS is harvested with excellent kernel processing or at late maturity, remains unknown.

Corn shredlage (**SHRD**), a new method of harvesting WPCS, increased kernel breakage at harvest compared with conventionally processed WPCS (Ferraretto and Shaver, 2012a; Vanderwerff et al., 2015). Hence, greater total-tract starch digestibility and corresponding lactation performance by high-producing dairy cows were observed in these trials. Overall, reduction in kernel particle size attenuates the effects of zein proteins on starch digestibility of WPCS and dry or high-moisture corn grain (Johnson et al., 2002; Ferraretto et al., 2013). Effects of fermentation, measured as concentration of ammonia-N (% CP), on starch digestibility in high-moisture corn are also lower when mean particle size is reduced, as underscored in the model of Hoffman et al. (2012). Thus, the potential for additive effects of prolonged ensiling time when excellent kernel processing is achieved at harvest remains uncertain.

Delayed harvest and the corresponding increase in DM content of WPCS reduced apparent total-tract starch digestibility and impaired lactation performance by dairy cows in a recent meta-analysis of published studies (Ferraretto and Shaver, 2012b). This has been attributed to an increase in the proportion of vitreous endosperm in the kernel associated with greater maturity (Correa et al., 2002; Ngonyamo-Majee et al., 2009). Furthermore, pH decline is slower and accumulation of organic acids reduced for late-maturity WPCS (Der Bedrosian et al., 2012), presumably due to decreased bacterial growth related to lower water availability (Muck, 1988). Reduced bacterial growth may lessen both proteolysis and solubilization of zein proteins (Simpson, 2001; Lawton, 2002; Hoffman et al., 2011). Perhaps reduced pH and greater concentrations of organic acids associated with the addition of microbial inoculants at ensiling (Muck, 2010) may increase zein-protein solubilization and thereby starch digestibility (Simpson, 2001; Lawton, 2002).

Therefore, the objective of this study was to evaluate the effect of a microbial inoculant and ensiling time on fermentation profile, N fractions, and starch digestibility of (1) SHRD or (2) late-maturity WPCS. We hypothesized that extended ensiling time will increase soluble CP, ammonia-N, and starch digestibility in SHRD and late-maturity WPCS, and microbial inoculation will improve fermentation profile and starch digestibility in SHRD and late-maturity WPCS.

MATERIALS AND METHODS

Two experiments were conducted simultaneously to evaluate either well-processed SHRD (Exp. 1) or late-maturity WPCS (Exp. 2).

Silage Production and Treatments

For Exp. 1, 20 kg of unfermented SHRD was obtained at harvest from the University of Wisconsin–Madison Agricultural Research Station (Arlington, WI) on September 6, 2012. A self-propelled forage harvester (Claas Jaguar, Claas of America Inc., Omaha, NE) equipped with SHRD processing rolls (Shredlage LLC; http://www.shredlage.com/) set for 26-mm theoretical length of cut and 2-mm roll gap was used to harvest a conventional corn hybrid as shredlage. Samples were homogenized and allocated into 24 samples of 600 g each using a quartering technique: homogeneous samples were divided into 4 equal subsamples. Two subsamples were saved for later treatment, whereas the 2 other subsamples were rehomogenized and redivided. The process was repeated until 24 subsamples of approximately 600 g were prepared. The remainder was frozen at −20°C until it was processed for analysis to characterize the material (Table 1). Samples were randomly assigned to 6 treatments with 4 replications per treatment. The 6 treatments were a combination of corn shredlage noninoculated (**CON**) or inoculated at the recommended inoculation rate (**1X**; 5×10^4 cfu/g of fresh whole-plant corn) or twice the recommended inoculation rate (**2X**; 10×10^4 cfu/g of fresh whole-plant corn) with a microbial inoculant and ensiled for either 30 or 120 d. The microbial inoculant contained *Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus faecium*, and *Pediococcus* sp. (Silo Charger "D," NU-AG Bosko Inc., Oskaloosa, IA). All 24 samples, including the noninoculated corn shredlage samples, received the same amount of double distilled water to ensure protocol similarity among all samples. Immediately after microbial inoculant treatment application, samples were placed in nylon-polyethylene standard barrier vacuum pouches (0.09-mm thickness, 25.4 × 35.6 cm; Doug Care Equipment Inc., Springville, CA) and vacuum heat sealed using an external clamp vacuum machine (Bestvac; distributed by Doug Care Equipment Inc.) and stored at room temperature (approximately 20°C) in the dark. After reaching the targeted ensiling time (30 or 120 d), samples were immediately frozen at −20°C to stop fermentation and stored until being processed for analysis.

Table 1. Nutrient composition and physical characteristics of unfermented whole-plant corn silage[1]

Item	Corn shredlage	Late-maturity corn silage
Nutrient		
DM, % of as fed	39.3	50.5
CP, % of DM	6.7	7.3
Soluble CP, % of CP	12.1	7.7
Ether extract, % of DM	2.5	3.1
aNDF,[2] % of DM	32.2	22.9
ADF, % of DM	16.2	12.1
Lignin, % of DM	3.0	2.6
Starch, % of DM	40.3	50.1
7-h ivSD,[3] % of starch	60.7	54.0
Ash, % of DM	2.9	2.7
Particle size sieves,[4] % as fed retained		
19 mm	18.0	4.8
8 mm	48.1	52.3
1.18 mm	30.9	41.1
Bottom pan	3.0	1.8
Processing score,[5] % passing 4,750-µm sieve		
Starch	76.0	70.7

[1]Harvested as corn shredlage (Exp. 1) or late-maturity conventional-processed corn silage (Exp. 2).

[2]aNDF = amylase-treated NDF.

[3]Ruminal in vitro starch digestibility (ivSD) at 7 h.

[4]Particle size was measured using the Penn State Particle Size Separator as described by Kononoff et al. (2003).

[5]Processing score was measured as described by Ferreira and Mertens (2005).

Experiment 2 used the same experimental methodology except for evaluating treatments within late-maturity WPCS (black layer stage) rather than SHRD. Harvest of the late-maturity WPCS was done on September 17, 2012, using a self-propelled forage harvester (JD 6910; John Deere, Moline, IL) set for 19-mm theoretical length of cut and equipped with conventional processing rolls set for 2-mm gap spacing.

Fermentation Profile, Physical Characteristics, Nutrient and Digestibility Analysis

Unfermented samples were analyzed for corn silage processing score (Ferreira and Mertens, 2005), particle size (Kononoff et al., 2003), and DM (method 930.15, AOAC International, 2012) at the University of Wisconsin–Madison. Samples were dried at 60°C for 48 h in a forced-air oven and ground to pass a 1-mm Wiley mill screen (Thomas Scientific, Swedesboro, NJ) before being sent to Dairyland Laboratories Inc. (Arcadia, WI) for CP (method 990.03; AOAC International, 2012), borate-phosphate buffer soluble CP (Krishnamoorthy et al., 1982), NDF (method 2002.04; AOAC International, 2012), ADF (method 973.18; AOAC, 2012), lignin (method 973.18; AOAC International, 2012), ether extract (method 2003.05; AOAC International, 2012), ash (method 942.05; AOAC International, 2012), and starch (Bach Knudsen, 1997; YSI Biochemistry Analyzer, YSI Inc., Yellow Springs, OH).

All fermented samples from both experiments were analyzed in duplicate for DM (% as fed), CP (% DM), borate-phosphate buffer soluble CP (% CP), ammonia-N (% CP), starch (% DM), pH, fermentation profile, ruminal in vitro starch digestibility at 7 h (% of starch; **ivSD**), and ruminal in situ starch digestibility at 12 h (% of starch; **isSD**). For fermentation profile analysis, 20 g of wet sample was diluted 10-fold (mass basis) in double distilled water, blended for 30 s in a high-speed blender, and filtered through 4 layers of cheesecloth. The extract was collected and used for determination of pH, ammonia-N, lactic acid, and other organic acids. The pH was measured immediately in duplicate using 2 Cardy Twin pH meters (Model #B-213, Spectrum Technologies Inc., Plainfield, IL). Two 20-mL aliquots of extract were separated. The first aliquot was centrifuged (25,100 × *g*) for 20 min at 4°C, and the supernatant was frozen for later organic acids analysis. The second aliquot was treated with 5 mL of a 25% trichloroacetic acid solution, allowed to stand 1 h at 4°C, and centrifuged as described previously, with the supernatant frozen for later ammonia-N analysis. Organic acids were determined by HPLC as described by Muck and Dickerson (1988) with a refractive index detector (RID-6A, Shimadzu Corp., Kyoto, Japan) and a Bio-Rad Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA) at 42°C. Measurements of ammonia-N were performed as described by Broderick et al. (2004) using flow-injection (Lachat Quik-Chem 8000 FIA; Lachat Instruments, Milwaukee, WI). Samples were dried at 60°C for 48 h in a forced-air oven and ground to pass a 1-mm Wiley mill screen (Thomas Scientific) before be analyzed for soluble CP (Krishnamoorthy et al., 1982) and CP using a Leco FP-2000A nitrogen analyzer (Leco Corp., St. Joseph, MI). Dried and 1-mm or 4-mm ground samples were sent to Dairyland Laboratories Inc. for starch and ivSD (Richards et al., 1995) analysis, respectively.

Measurements of isSD were conducted at the University of Wisconsin

campus tie-stall barn (Dairy Cattle Center, Madison, WI) under a protocol approved by the Institutional Animal Care and Use Committee of the College of Agricultural and Life Sciences. Three ruminally cannulated mid-lactation multiparous Holstein cows fed a TMR containing (DM basis) alfalfa silage (44.5%), corn silage (26.8%), alfalfa hay (10.7%), wheat straw (6.5%), and concentrate mixture (11.5%) were used. Dacron polyester cloth bags (R510, 10 × 20 cm and 50-μm pores; Ankom Techonology, Macedon, NY) containing samples of 5 g of DM (approximately 15 g as fed) of each mini-silo were incubated in duplicate within each cow using undried and unground samples. The in situ bags were placed in a nylon laundry bag (30 × 40 cm) and then positioned in the ventral rumen. Each laundry bag was attached to the inside of the rumen cannula with a 75-cm-long nylon rope and contained a rubber weight to ensure they remained submerged in the ruminal contents. Bags were moistened in warm water for 30 s before incubation. Each laundry bag contained a blank bag to allow correction for infiltration of DM into sample bags. After removal, samples were soaked in cold water for 15 min to stop microbial activity before being washed twice in a commercial washing machine (Whirlpool #3347019A, Whirlpool Corp., Benton Harbor, MI) using the regular cycle rinse setting with cold water for 12-min cycles (Cherney et al., 1990). Two bags for each mini-silo (0-h bags) were soaked for 30 min in warm water and washed with the rest of the sample bags. The bags were dried in a forced-air oven at 60°C for 48 h. Residues were ground through a 1-mm Udy mill screen (Udy Corp., Boulder, CO) for starch analysis. Duplicates within cows were composited into 1 sample before nutrient analysis. The mean starch disappearance value of the 2 bags on a treatment per incubation time was calculated for each cow. Samples were sent to Dairyland Laboratories Inc. and analyzed for starch as described previously.

Statistical Analysis

Data for both experiments were analyzed as a completely randomized design in a 3 × 2 factorial arrangement of treatments using Proc Mixed of SAS (SAS Institute Inc., Cary, NC) with inoculation, ensiling time, and their interaction as fixed effects. Mini-silo was used as the experimental unit. Means were determined using the LSMEANS statement and were compared using the PDIFF option. Statistical significance and trends were declared at $P \leq 0.05$ and $P > 0.05$ to $P \leq 0.10$, respectively. Because interactions were not observed unless stated otherwise in text, data are presented and discussed only as main effects.

RESULTS AND DISCUSSION

Exp. 1—Corn Shredlage

Effects of microbial inoculation on fermentation profile are in Table 2. Overall, inoculation with lactate-producing bacteria reduces pH and shifts fermentation toward lactic rather than acetic acid in varied ensiled feeds (Muck, 2010). In Exp. 1, however, no effect of microbial inoculation was observed ($P > 0.10$) for pH of SHRD, which averaged 3.92. This is likely related to the lack of effect ($P > 0.10$) of microbial inoculation on organic acids, except for greater ($P = 0.02$) 1,2-propanediol for CON compared with 2X at 120 d. A microbial inoculation × ensiling time interaction was observed for 1,2-propanediol ($P = 0.02$; data not presented), which is related to the lack of 1,2-propanediol on d 30 for any of the 3 inoculant treatments. Lactate, acetate, propionate, butyrate, ethanol, and total acids were unaffected by inoculation ($P > 0.10$) and averaged 4.52, 1.10, 0.02, 0.01, 1.28, and 5.65% of DM, respectively. In contrast, microbial inoculation with the 1X dosage of the same microbial inoculant reduced pH and acetate concentration while increasing lactate concentration in rehydrated corn ensiled for 30 d (Ferraretto et al., 2015a). The review of Muck and Kung (1997) highlighted that homofermentative inoculants successfully altered WPCS fermentation patterns in less than 50% of the studies. Muck (2010) listed several factors that may contribute to lack of fermentation profile effects when inoculating WPCS with lactate-producing bacteria, including WPCS fermentation pattern, low sugar concentration at ensiling, and level of epiphytic homofermentative bacteria population at ensiling. Fermentation pattern of WPCS usually shifts from acetic to lactic acid and reduces pH to levels below 4.2 (Kung, 2010), and although a faster decline in pH may occur in inoculated silage, the pH differences may be minimal after opening the silo (Muck, 2010). Similarly, lack of adequate sugar content at ensiling may mask possible benefits of inoculating with lactate-producing bacteria (Muck, 2010). Alternatively, epiphytic bacteria counts at ensiling may attenuate inoculant response (Muck, 2010). Epiphytic bacterial counts were not performed in the present study. Contreras-Govea et al. (2011) inoculated alfalfa, as well as brown midrib and conventional WPCS, with 4 different homofermentative bacterial inoculants. They discussed that despite the appropriate levels of sugars, levels of epiphytic lactate-producing bacteria at ensiling were high and may have attenuated inoculant effects on silage fermentation.

Effects of microbial inoculation on N fractions, ivSD, and isSD of SHRD are in Table 2. Microbial inoculation did not affect ($P > 0.10$) concentrations of DM, CP, and starch (39.6, 7.5, and 39.1%, respectively). Similarly, soluble CP and ammonia-N were similar ($P > 0.10$) among treatments and averaged 40.9 and 3.9% of CP, respectively. Starch digestibility was unaffected ($P > 0.10$) by microbial inoculation for both in vitro and in situ measurements. Breakdown of pericarp, which is highly resistant to microbial attachment, exposes the endosperm (McAllister et al., 1994) and thereby increases starch digestibility of WPCS (Ferraretto and Shaver, 2012a,b). However, a starch–protein

matrix formed by the chemical bonding of zein proteins with starch granules inhibits starch hydrolysis even with endosperm exposed (Giuberti et al., 2014). As previously discussed, microbial inoculation did not affect fermentation profile, which in turn attenuated possible effects of microbial inoculation on N fractions, ivSD, and isSD (Table 2). However, the ivSD of rehydrated and high-moisture corn was not altered by microbial inoculation in the study by Ferraretto et al. (2015a) despite changes in fermentation profile.

Effects of ensiling time on fermentation profile, N fractions, ivSD, and isSD of corn shredlage are in Table 3. Measurements of pH were reduced from 3.95 at 30 d to 3.88 after 120 d of ensiling. This was related to increased ($P < 0.05$) acetate, 1,2-propanediol, and total acid concentrations. However, concentrations of lactate, propionate, butyrate, and ethanol did not differ ($P > 0.10$). Continuous shifts in fermentation profile and nutritive value of WPCS occur (Kleinschmit and Kung, 2006; Der Bedrosian et al., 2012) even after silage reaches the stable phase of the fermentation, typically between 7 to 45 d of fermentation (Pahlow et al., 2003). Windle et al. (2014) reported a pH decline across time in storage, whereas Der Bedrosian et al. (2012) observed a pH decrease only after 180 d of ensiling. Der Bedrosian et al. (2012) and Windle et al. (2014) observed a gradual increase in acetate, but concentration of 1,2-propanediol was not reported. Furthermore, this fermentation pattern is similar for silage inoculated with *Lactobacillus buchneri*, even though lactate concentration was maintained (Kleinschmit and Kung, 2006).

Concentrations of DM and starch were unaffected ($P > 0.10$) by ensiling time and averaged 39.6% of as fed and 39.0% of DM, respectively. Content of CP increased from 30 to 120 d of ensiling ($P = 0.01$; 7.2 vs. 7.8% of DM, respectively). Minimal or no changes in DM, CP, and starch concentration are in agreement with previous literature (Der Bedrosian et al., 2012; Windle et al., 2014; Ferraretto et al., 2015b). Ammonia-N ($P = 0.001$; 3.3 vs. 4.5% of CP) and soluble CP ($P = 0.001$; 35.5 vs. 46.2% of CP) concentrations increased from 30 to 120 d of ensiling. Likewise, ivSD and isSD increased ($P < 0.05$) 2.7 and 5.3 percentage units, respectively, when ensiling time was extended from 30 to 120 d. Greater ivSD and isSD for 120 d compared with 30 d of ensiling is related to the breakdown of zein proteins as suggested by increased concentrations of soluble CP and ammonia-N (Hoffman et al., 2011). Positive effects of storage length on ivSD and soluble N fractions are consistent with the reports of Der Bedrosian et al. (2012), Windle et al. (2014), and Ferraretto et al. (2015b). Our results emphasize that variance in starch digestibility throughout the year may be observed even in farms that properly process WPCS. These findings suggest that monitoring and understanding starch digestibility variance in SHRD as storage progresses may be essential to optimize diets to circumvent decreased milk and protein yields with low starch digestibility or increased subacute rumen acidosis and milk fat depression with high starch digestibility (Krause and Oetzel, 2006; Ferraretto et al., 2013).

Table 2. Effect of microbial inoculation on fermentation profile, nitrogen fractions, and ruminal in vitro and in situ starch digestibility of corn shredlage[1,2]

Item	CON	1X	2X	SEM	*P* <
Fermentation profile					
pH	3.90	3.92	3.93	0.02	0.46
Lactate, % of DM	4.49	4.50	4.56	0.17	0.96
Acetate, % of DM	1.23	1.05	1.02	0.08	0.15
Propionate, % of DM	0.04	0.03	<0.01	0.03	0.50
Butyrate, % of DM	<0.01	0.02	<0.01	0.01	0.41
1,2-Propanediol, % of DM	0.38[a]	0.24[ab]	0.16[b]	0.05	0.02
Ethanol, % of DM	1.15	1.22	1.48	0.17	0.35
Total acids, % of DM	5.77	5.59	5.59	0.19	0.76
Nutrient					
DM, %	39.2	39.7	39.9	0.3	0.34
CP, % of DM	7.4	7.6	7.6	0.1	0.41
Soluble CP, % of CP	40.4	40.5	41.8	1.5	0.57
Ammonia, % of CP	4.1	3.9	3.8	0.2	0.29
Starch, % DM	38.0	39.9	39.3	3.3	0.53
7-h ivSD,[3] % of starch	71.5	69.8	70.8	1.1	0.57
12-h isSD,[4] % of starch	82.1	83.6	84.1	1.4	0.61

[a,b]Means in the same row with different superscripts differ ($P \leq 0.05$).

[1]CON = control; 1X = 5.0 × 10^4 cfu/g of fresh forage; 2X = 10.0 × 10^4 cfu of *Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus faecium*, and *Pediococcus* sp. per gram of fresh forage.

[2]Ensiled from 30 to 120 d.

[3]Ruminal in vitro starch digestibility (ivSD) at 7 h.

[4]Ruminal in situ starch digestibility (isSD) at 12 h.

Exp. 2—Late-Maturity Corn Silage

Effects of microbial inoculation on fermentation profile, N fractions, ivSD, and isSD of late-maturity corn silage are in Table 4. Concentrations of lactate ($P = 0.001$; 4.52 vs. 3.99% of DM) and total acids ($P = 0.01$; 5.39 vs. 4.82% of DM) were greater for CON and 1X compared with 2X. However, pH and acetate concentration did not differ ($P > 0.10$) among treatments and averaged 3.97 and 0.84% of DM, respectively. Differ-

ences in DM content (Table 4) may partially explain the lower concentration of lactate and total acids for 2X. Ethanol concentration tended ($P < 0.10$) to be greater for CON than the other treatments. Contents of DM and starch were ($P < 0.05$) 2.5 and 3.4 percentage units greater for 2X than other treatments, but CP and soluble CP contents did not differ ($P > 0.10$). Despite the lower ($P = 0.01$; 4.3 vs. 5.0% of CP) ammonia-N concentration for 2X compared with other treatments, ivSD and isSD were unaffected ($P > 0.10$) by microbial inoculation. Inoculation with lactate-producing bacteria was previously reported to decrease ammonia-N concentration (Contreras-Govea et al., 2011; Ferraretto et al., 2015a), but differences in DM content between treatments are a more likely explanation based on fermentation profile data in the present study.

Effects of ensiling time on fermentation profile, N fractions, ivSD, and isSD of late-maturity corn silage are in Table 5. Ensiling time influenced fermentation profile, with greater ($P < 0.01$) lactate, acetate, and total acid concentrations after 120 d of ensiling. Similar to our results, Der Bedrosian et al. (2012) reported a gradual increase in lactate concentration in late-maturity WPCS as storage length progressed. Furthermore, reductions in pH and ethanol concentration were also observed ($P < 0.05$) for 120 d compared with 30 d. Ensiling time did not affect ($P > 0.10$) concentrations of DM, CP, and starch (47.7, 7.5, and 43.0%, respectively). Late-maturity corn silage fermented for 120 d had greater ammonia-N ($P = 0.001$; 5.4 vs. 4.0% of CP) and soluble CP ($P = 0.02$; 49.1 vs. 45.5% of CP) concentrations compared with 30 d. Extended ensiling time increased ($P < 0.05$) ivSD and isSD by 5.0 and 6.6 percentage units, respectively. Late-maturity WPCS typically impairs starch digestibility in lactating dairy cows (Ferraretto and Shaver, 2012b), which is often explained by the increase in kernel vitreousness (Correa et al., 2002; Ngonyamo-Majee et al., 2009) and the ratio of vitreous to floury endosperm, which may be associated with increased zein proteins cross-linked to starch granules (Giuberti et al., 2014). Alternatively, fermentation is reduced with lower moisture availability, which in turn may reduce the benefits of ensiling on starch digestibility (Windle et al., 2014). Ensiling time, however, was effective in increasing starch digestibility in late-maturity WPCS through the breakdown of zein proteins surrounding starch granules as implied by soluble CP and ammonia-N measurements (Hoffman et al., 2011). Similar results were observed by Der Bedrosian et al. (2012) and Windle et al. (2014). In these reports, however, DM content was almost 10.0 percentage units lower than in the present study. Overall, our results suggest that prolonged storage may be beneficial even when harvest is delayed and DM content is high. These findings suggest that delayed harvest may be an option to achieve higher yields of starch when inventory allows for prolonged storage and corresponding increase in starch digestibility. However, several other factors should be taken into account when harvesting high DM WPCS, including fiber particle uniformity, degree of kernel processing, difficulty in achieving adequate packing density, and predisposition for impaired aerobic stability.

IMPLICATIONS

Under the conditions of the present study, organic acid production and corresponding pH decline with extended ensiling time occurred in both corn shredlage and late-maturity corn silage. Ammonia-N concentration and starch digestibility were greater after 120 d of fermentation in both experiments, suggesting that extended ensiling time is advantageous in both scenarios. Inoculation with lactate-producing bacteria, however, was not beneficial in altering silage fermentation profile and did not increase starch digestibility in either experiment.

Table 3. Effect of storage length on fermentation profile, nitrogen fractions, and ruminal in vitro and in situ starch digestibility of corn shredlage[1]

Item	30 d	120 d	SEM	*P* <
Fermentation profile				
pH	3.95	3.88	0.02	0.02
Lactate, % of DM	4.57	4.47	0.15	0.67
Acetate, % of DM	0.76	1.45	0.06	0.001
Propionate, % of DM	0.02	0.03	0.02	0.71
Butyrate, % of DM	<0.01	0.02	0.01	0.11
1,2-Propanediol, % of DM	<0.01	0.53	0.04	0.001
Ethanol, % of DM	1.35	1.21	0.14	0.50
Total acids, % of DM	5.34	5.96	0.15	0.02
Nutrient				
DM, %	39.7	39.4	0.3	0.44
CP, % of DM	7.2	7.8	0.1	0.01
Soluble CP, % of CP	35.5	46.2	1.4	0.001
Ammonia, % of CP	3.3	4.5	0.2	0.001
Starch, % DM	39.6	38.5	3.3	0.73
7-h ivSD,[2] % of starch	69.3	72.0	0.9	0.05
12-h isSD,[3] % of starch	80.6	85.9	1.1	0.01

[1]Corn shredlage ensiled for 30 or 120 d.

[2]Ruminal in vitro starch digestibility (ivSD) at 7 h.

[3]Ruminal in situ starch digestibility (isSD) at 12 h.

Table 4. Effect of microbial inoculation on fermentation profile, nitrogen fractions, and ruminal in vitro and in situ starch digestibility of late-maturity corn silage[1,2]

Item	CON	1X	2X	SEM	*P* <
Fermentation profile					
pH	3.97	3.97	3.97	0.01	0.82
Lactate, % of DM	4.55[a]	4.48[a]	3.99[b]	0.09	0.001
Acetate, % of DM	0.89	0.80	0.82	0.05	0.38
Propionate, % of DM	0.05	<0.01	<0.01	0.02	0.08
Butyrate, % of DM	<0.01	<0.01	<0.01	<0.01	0.99
1,2-Propanediol, % of DM	<0.01	<0.01	<0.01	<0.01	0.99
Ethanol, % of DM	1.31	1.02	0.94	0.12	0.09
Total acids, % of DM	5.49[a]	5.29[a]	4.82[b]	0.11	0.01
Nutrient					
DM, %	47.0[b]	46.8[b]	49.4[a]	0.4	0.01
CP, % of DM	7.7	7.4	7.6	0.1	0.34
Soluble CP, % of CP	48.6	45.6	46.1	1.5	0.36
Ammonia, % of CP	5.1[a]	5.0[a]	4.3[b]	0.1	0.01
Starch, % DM	40.9[b]	42.9[b]	45.3[a]	1.1	0.04
7-h ivSD,[3] % of starch	64.2	63.8	61.5	4.9	0.69
12-h isSD,[4] % of starch	89.6	89.9	89.4	0.8	0.90

[a,b]Means in the same row with different superscripts differ ($P \leq 0.05$).

[1]CON = control; 1X = 5.0 × 10^4 cfu/g of fresh forage; 2X = 10.0 × 10^4 cfu of *Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus faecium*, and *Pediococcus* sp. per gram of fresh forage.

[2]Ensiled from 30 to 120 d.

[3]Ruminal in vitro starch digestibility (ivSD) at 7 h.

[4]Ruminal in situ starch digestibility (isSD) at 12 h.

Table 5. Effect of storage length on fermentation profile, nitrogen fractions, and ruminal in vitro and in situ starch digestibility of late-maturity corn silage[1]

Item	30 d	120 d	SEM	*P* <
Fermentation profile				
pH	3.98	3.96	0.01	0.03
Lactate, % of DM	4.02	4.67	0.08	0.001
Acetate, % of DM	0.76	0.92	0.04	0.01
Propionate, % of DM	0.03	<0.01	0.01	0.11
Butyrate, % of DM	<0.01	<0.01	<0.01	0.99
1,2-Propanediol, % of DM	<0.01	<0.01	<0.01	0.99
Ethanol, % of DM	1.30	0.88	0.09	0.01
Total acids, % of DM	4.81	5.58	0.09	0.001
Nutrient				
DM, %	47.6	47.8	0.4	0.74
CP, % of DM	7.5	7.6	0.1	0.65
Soluble CP, % of CP	44.5	49.1	1.2	0.02
Ammonia, % of CP	4.0	5.4	0.1	0.001
Starch, % DM	43.0	43.1	0.9	0.93
7-h ivSD,[2] % of starch	61.7	66.7	2.8	0.04
12-h isSD,[3] % of starch	86.3	92.9	0.7	0.001

[1]Late-maturity corn silage ensiled for 30 or 120 d.

[2]Ruminal in vitro starch digestibility (ivSD) at 7 h.

[3]Ruminal in situ starch digestibility (isSD) at 12 h.

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